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CONTENT

Šimunić, I., Likso, T., Husnjak, S., Orlović-Leko, P., Bubalo Kovačić, M. (2021): Analysis of climate elements in the northeastern region of Croatia for the purpose of determining irrigation requirements of maize and soybean on drained soil. Agriculture and Forestry, 67 (2): 7-20

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ANALYSIS OF CLIMATE ELEMENTS IN THE NORTHEASTERN REGION OF CROATIA FOR THE PURPOSE OF DETERMINING IRRIGATION REQUIREMENTS OF MAIZE AND SOYBEAN ON DRAINED SOIL

SUMMARY

The aim of this research was to determine crop water requirements (soil water deficit) in years with average precipitation amounts and dry years in order to estimate the decline in maize and soybean yields in such years, as well as to determine the actual yields of maize and soybean in years with/almost average precipitation amount and both in dry and wet years.

In the examined 20-year period, annual air temperature in the northeastern region of Croatia increased by 1.7^{o} C, while a very slight negative trend in annual precipitation amount, -0.18 mm/20 yrs, has been identified. The determined soil water deficit in the years with multi–annual mean of precipitation amount ranged from 139.3 mm (soybean) up to 152.7 mm (maize), while in the dry years, water deficit ranged from 299.7 mm (soybean) up to 316.3 mm (maize). The estimation of yield decline (%) in the years with multi–annual mean of precipitation amount ranged from 21.5% (soybean) up to 33.9% (maize), while in the dry years it ranged from 40.5% (soybean) up to 65.0% (maize). In the 5–year period, the lowest yields of both crops were in the year with the lowest annual precipitation amount (maize 5,175 t.ha^{-1} and soybean 2,153 t.ha^{-1}), while the highest crop yields were when the annual precipitation amount was on a par with the average value (maize 9,652 t.ha^{-1} and 9,817 t.ha^{-1} and soybean 3,454 t.ha^{-1} and 3,584 t.ha^{-1}). In the year with the highest precipitation amount, crop yields ranged between the value of the yield aged in the drought and the year with the average precipitation amount (maize 8,875 t.ha^{-1} and 8,929 t.ha^{-1} and soybean 3,188 t.ha^{-1} and 3,202 t.ha^{-1}).

Yield decline problem in the northeastern region of Croatia in dry years can be largely solved through irrigation (need to build/expand the irrigation

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system) and better maintenance of the existing drainage system because the problem can appear during the period with heavy rains.

Keywords: climate elements, irrigation requirements, maize, soybean

INTRODUCTION

Climate change in recent decades is the topic and the major global problem and therefore solutions are being sought to mitigate/prevent its consequences. Climate change can be manifested as a change in climate elements relative to average values or as a change in the distribution of climate events relative to average values. The consequence of that causes more frequent occurrences of floods and droughts which can cause major damage to agriculture and the environment. Climate characteristics and soil water regime, as well as their variable and complex interrelations, define the efficiency of plant production (Šimunić *et al.*, 2007). According to Beltrão *et al.* (1996), the highest yields are obtained at the time of the most favourable air–water ratio in the soil, mainly in the critical periods for each crop. The yields of agricultural crops fluctuate over many years and are influenced by many abiotic and biotic factors. A large number of studies indicate that crop yields primarily vary as a result of extreme climate conditions, although other factors, such as soil fertility, the applied agro–technology measures and plant species may also affect crop yields (Kovačević and Josipović, 2015). Agricultural production is very risky in underdeveloped agricultural areas, especially when surplus and/or deficit of precipitation occurs before or during the growing season. Such conditions make production planning very difficult and/or almost impossible because production and hence yields are dependent on weather conditions, making field crop yields and their quality highly variable (Šimunić, 2016). In Croatia prevention/mitigation of the consequences of climate change in agriculture focuses on the existing (built) hydro–technical facilities, surface and underground drainage systems, as well as irrigation systems that should be adequately used and maintained and the activities for the construction of new hydro–technical facilities and drainage and irrigation systems need to be continued (Šimunić *et al.*, 2020).

The aim of the research was:

– to determine crop water requirements (soil water deficit) in years with average annual precipitation amount and dry years,

– to estimate a decline in yields of maize and soybean in such years,

– to determine actual maize and soybean yields in years with/almost average annual precipitation amount, as well as in dry and wet years.

MATERIAL AND METHODS

For the northeastern region of Croatia, the climate data series from the climatological station Našice (φ=45^o 49' N, λ=18^o 09' E, 150 m above mean sea level) for the 20–year period (2001– 2020) was used.

Based on the climate data, a reference evapotranspiration was calculated for an average and a dry year (probability of precipitation occurrence in 25% cases). The reference evapotranspiration was calculated using the Penman–Monteith method through "Cropwat" software, version 8 (Smith, 1992).

Crop water requirements were determined by soil water balance using the Palmer method, corrected according to Vidaček, using "Hidrokalk" computer program (Širić & Vidaček, 1988). For soil water balance calculation, the corresponding values of effective precipitation for an average and a dry year were used, which was calculated by USDA, SCS method.

The values of soil water constants were taken into account as the average of the values of the represented soil type: drained hypogley soil by means drainage pipes (Husnjak, 2014). The soil studied had the following characteristics: field water capacity was 44 vol% and wilting point was 22 vol%, clay–loam texture in the arable layer. Pipe drainage spacing is 25 m and average depth is 1 m. Drainpipe discharged directly into open channels. The total drained area is 4,800 ha.

Crop water requirements and yield decline were related to two types of crops: maize and soybean. The root depth for the calculation of soil water balance was 0.3 m. Concerning the previously mentioned crops, the vegetation period was considered and phenological phases and their duration were determined. Each phenological phase was corrected by the crop coefficient. Crop yield decline was determined according to the Doorenbos and Kassam method:

$$(1-\frac{Ya}{Ym})=\text{ky}\,(1-\frac{ETa}{ETc}) \quad (1)$$

Ya– Actual yield
Ym–Maximum possible yield
ky – Yield response factor
ETa – Actual evapotranspiration
ETc– Crop (maximum) evapotranspiration

The actual yields for both crops were determined after harvesting and drying up to moisture 13%. Vegetation period for both crops during the research were from the beginning of April to the end of September.

Analysis of variance (ANOVA) was used to test crop yields, while Duncan's Multiple Range test was used to compare the mean values of crop yields depending on the year.

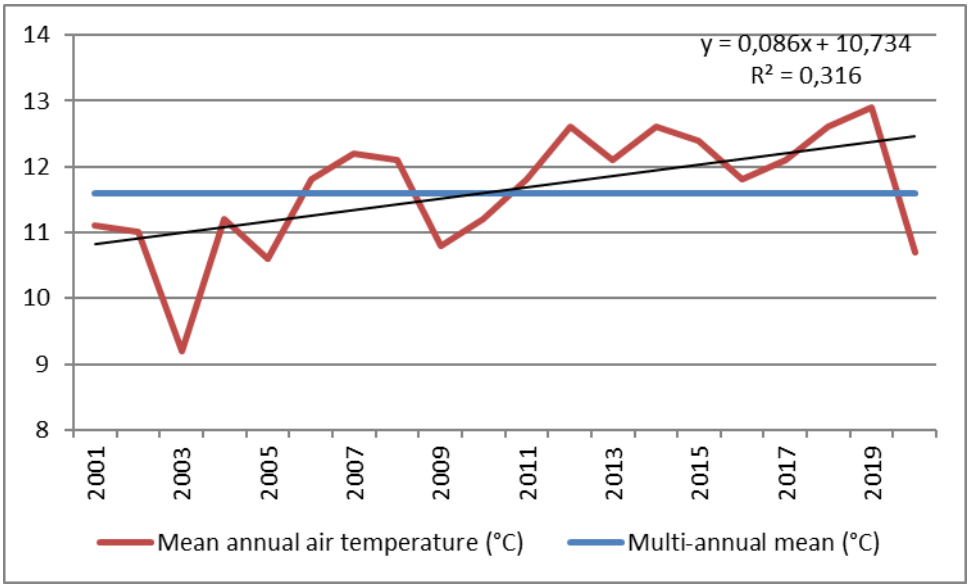
RESULTS AND DISCUSSION

The results obtained have been presented in the following tables and graphs.

Analysis of climate elements

Mean annual air temperature, annual precipitation amounts and corresponding trends for climatological station (CS) Našice have been presented

in Graphs 1 and 2 and annual courses of mean, maximum and minimum monthly precipitation amounts have been presented in Graph 3.

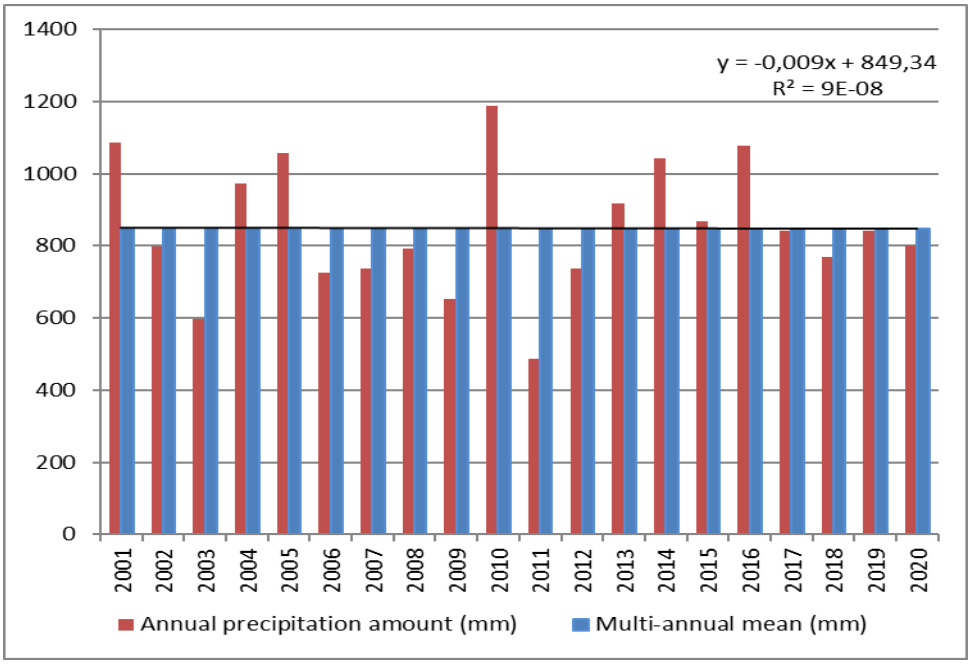


Graph 1. Mean annual air temperature (°C), multi–annual mean (°C) and corresponding linear trend of mean annual air temperature for CS Našice

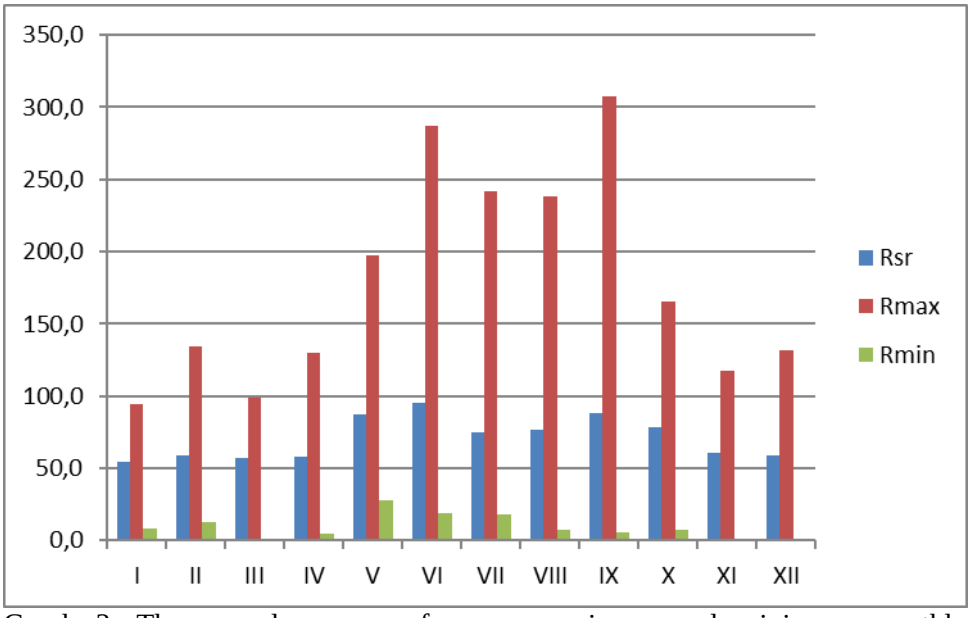
During the analysed period in the northeastern region of Croatia, mean annual air temperatures ranged from 9.2°C to 12.9°C, while the multi–annual mean air temperature was 11.6° C. The corresponding linear trend of mean annual air temperature is 1.7°C/20 yrs, which is evident from Graph 1. During the examined 20-year period, the positive air temperature trend is evident with some inter–annual variations and could be an indicator of climate change. According to Kutilek and Nielsen (2010), the average temperature increased by 1.1–1.3° C in 100 years in Central Europe. The effects of climate change have become increasingly evident over the past decades (Patt and Schröter, 2008). Positive trends of air temperature and precipitation amounts in their research have been quoted by Šimunić *et al.* (2013 and 2019) and Miseckaite *et al.* (2018).

The precipitation regime is one of the most variable climate characteristics of some area, both spatially and temporally (Meteorological and Hydrological Service and Croatian Meteorological Society, page 85, Gajić–Čapka, 2003). This can also be seen in the northeastern region of Croatia.

As shown in Graph 2 for the northeastern region of Croatia, annual precipitation amounts were within the range from 487.3 mm (2011) to 1,188.1 mm (2010), while multi–annual mean of annual precipitation amounts was 855.1 mm. Within the examined 20–year period the difference between the maximum and the minimum value of annual precipitation amount was 700.8 mm.



Graph 2. Annual precipitation amount (mm), multi–annual mean (mm) and corresponding linear trend of annual precipitation amount for CS Našice



Graph 3. The annual courses of mean, maximum and minimum monthly precipitation amounts. The multi–annual mean of annual precipitation amounts is 855.1 mm, CS Našice (2001–2020)

Monthly precipitation amounts can vary significantly from year to year (Graph 3). The variability of monthly precipitation amounts expressed by the coefficient of variation (cv) is larger during the summer months (July, August, September; cv=0.7) than in the cold part of the year (January, November; cv=0.4). The annual course of monthly precipitation amounts in Croatia can be divided into two types (Zaninović *et al*., 2008), depending on the time of the year when the month with the lowest precipitation amount occurs: the maritime type of annual course, with the lowest precipitation amount occurring during the warm period of the year (April to September), and the continental annual course, with the lowest precipitation amount occurring during the cold half of the year (October to March). The annual course of monthly precipitation amount for the northeastern region of Croatia has the characteristics of the continental precipitation regime. From Figure 2 it is evident that there are some differences in annual precipitation amounts from year to year, which is described by the coefficient of variation. In addition, there is a slight negative trend in annual precipitation amounts, –0.18 mm/20 yrs. The trends of precipitation extremes in Europe vary greatly and depend not only on the region, but also on the indicator used to describe an extreme (Groisman *et al.*, 2005). Changes in precipitation are the prime drivers of change in the availability of both surface water and groundwater resources (Beare and Heaney, 2002). Changes in precipitation amount and its distribution have a direct influence on soil water content and affect crop cultivation (Šimunić *et al.*, 2013).

The relationship between reference evapotranspiration and effective precipitation

Reference evapotranspiration that integrates the effects of climate elements and indicates the overall evaporation has been presented in Table 1 and 2, and the relationship between reference evapotranspiration and effective precipitation, both for multi–annual mean and dry year has been presented in Table 3.

Tables 1 and 2 show that multi–annual mean reference evapotranspiration was 2.6 $mm.day^{-1}$ and it was lower than reference evapotranspiration based on the frequency of the occurrence of climate elements upon 25% precipitation probability (2.8 $mm.day^{-1}$). Moreover, with a multi–annual mean of climate elements, the daily evapotranspiration is lower during the vegetation period than in the year with the frequency of the occurrence of climate elements upon 25% precipitation probability. In relation to effective precipitation for the multi–annual mean and reference evapotranspiration calculated on the basis of multi–annual climate elements, Table 3 shows that the difference in water shortage is smaller than in effective precipitation at the frequency of occurrence in 25% of cases and reference evaporation calculated on the basis of associated climatic elements. In both cases, precipitation deficit occurs throughout the growing season. The exact crop water deficit in the focus of this research can be determined by the soil water balance.

Table 1. Reference evapotranspiration based on multi–annual mean of climate elements

Month	Tmin	Tmax	Humidity	Wind	Sun	Radiation	ETo
	°C	°C	%	km.day^{-1}	h.day^{-1}	MJ.m^{-2}.day^{-1}	mm.day^{-1}
Jan	-2,3	5,0	82	233	2,1	4,4	0,6
Feb	-0,8	6,9	79	242	3,1	6,9	0,9
Mar	2,3	12,8	72	268	4,8	11,3	1,8
Apr	6,7	18,4	69	250	6,8	16,7	2,9
May	10,8	22,8	72	276	7,7	20,0	3,9
June	14,7	26,5	72	259	8,9	22,5	4,7
July	16,3	28,7	69	250	9,7	23,0	5,2
Aug	15,9	28,6	71	233	9,1	20,3	4,6
Sep	11,6	23,0	77	250	6,3	13,9	2,9
Oct	7,5	17,9	80	233	4,8	9,2	1,7
Nov	3,4	11,8	84	216	2,9	5,3	0,9
Dec	-0,8	6,1	84	225	1,8	3,7	0,6
Mean	7,1	17,4	76	245	5,7	13,1	2,6

Table 2. Reference evapotranspiration based on the frequency of the occurrence of climate elements upon 25% probability precipitation

Month	Tmin	Tmax	Humidity	Wind	Sun	Radiation	ETo
	°C	°C	%	km.day^{-1}	h.day^{-1}	MJ.m^{-2}.day^{-1}	mm.day^{-1}
Jan	2,5	11,9	71	302	3,4	5,2	1,3
Feb	2,5	11,3	74	216	3,3	7,1	1,2
Mar	3,6	13,6	75	216	4,7	11,2	1,7
Apr	6,0	20,1	67	216	9,7	20,3	3,4
May	11,8	24,2	69	233	7,8	20,2	4,0
June	15,6	28,7	68	233	9,6	23,4	5,2
July	15,6	31,2	59	233	10,8	24,5	6,0
Aug	15,6	28,9	70	233	8,7	19,8	4,7
Sep	9,5	20,8	75	233	6,2	13,8	2,8
Oct	5,8	14,5	80	190	3,8	8,3	1,4
Nov	3,4	11,9	83	207	2,6	5,1	0,9
Dec	-0,9	6,1	83	216	1,0	3,2	0,6
Mean	7,6	18,6	73	227	6,0	13,5	2,8

Crop water requirements in a year with average precipitation amount and in a dry year

The water requirements of maize and soybeans in a year with an average amount of precipitation and a dry year are determined by soil water balance (Tables 4, 5, 6 and 7)

Table 3. Relationship between effective precipitation, multi–annual mean and dry year and reference evapotranspiration based on multi–annual mean of climate elements and based on the frequency of the occurrence of climate elements upon 25% precipitation probability

Month	Multi–annual mean (mm)		Difference	Dry year (mm)		Difference
	Effective precipitation	ETo		Effective precipitation	ETo	
Jan	49.9	18.6	31.3	31.7	40.3	-8.6
Feb	53.1	25.2	27.9	52.2	33.6	18.6
Mar	51,9	55.8	-3.9	75.8	52.7	23.1
Apr	52,2	87.0	-34.8	14.1	102.0	-87.9
May	75.3	120.9	-45.6	74.0	124.0	-50.0
June	80.8	141.0	-60.2	24.0	156.0	-132.0
July	65.9	161.2	-95.3	33.1	186.0	-152.9
Aug	67.5	142.6	-75.1	69.2	145.7	-76.5
Sep	75.6	87.0	-11.4	74.2	84.0	-9.8
Oct	68.6	52.7	15.9	101.2	43.4	57.8
Nov	54.8	27.0	27.8	56.2	27.0	29.2
Dec	53.3	18.6	34.7	49.3	18.6	30.7
Total	749.0	937.6	-188.6	645.3	1.013.3	-368.0

Table 4. The soil water balance for maize for multi–annual mean of precipitation

Month	P	ETo/ETc	L1	L2	R	Roff	AE	S1	S2	S=S1+S2	Deficit
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Jan	44,9	19	0,0	0,0	0,0	26,3	18,6	22,0	44,0	66,0	0,0
Feb	53,1	25	0,0	0,0	0,0	27,9	25,2	22,0	44,0	66,0	0,0
Mar	51,9	56	3,9	0,0	0,0	0,0	55,8	18,1	44,0	62,1	0,0
Apr	52,2	87	18,1	5,6	0,0	0,0	75,9	0,0	38,4	38,4	11,1
May	75,3	36	0,0	0,0	27,6	11,5	36,3	22,0	44,0	66,0	0,0
June	80,8	99	17,9	0,0	0,0	0,0	98,7	4,1	44,0	48,1	0,0
July	65,9	169	4,1	33,1	0,0	0,0	103,1	0,0	10,9	10,9	66,2
Aug	67,5	150	0,0	6,8	0,0	0,0	74,3	0,0	4,1	4,1	75,4
Sep	75,6	70	0,0	0,0	6,0	0,0	69,6	6,0	4,1	10,1	0,0
Oct	68,6	53	0,0	0,0	15,9	0,0	52,7	21,9	4,1	26,0	0,0
Nov	54,8	27	0,0	0,0	27,8	0,0	27,0	22,0	31,8	53,8	0,0
Dec	53,3	19	0,0	0,0	12,2	22,5	18,6	22,0	44,0	66,0	0,0
Year	744	808	44	45	89	88	656				**152,7**

*Palmer's method (1965), calibrated and corrected by Vidaček (1988)

Legend:

P–effective precipitation (mm)
ETo–reference evapotranspiration (mm)
ETc–crop evapotranspiration (mm)
L1–loss of water from the surface layer (0-10 cm)
L2–loss of water from the subsurface layer (10-30 cm)
R–water recharge (mm)
Roff–water runoff (mm)
AE–actual evapotranspiration (mm)
S1–water storage in the surface layer (mm)
S2–water storage in the subsurface layer (mm)
S – total water storage (mm)

Table 5. The soil water balance for soybean for multi–annual mean of precipitation

Month	P	ETo/ETc	L1	L2	R	Roff	AE	S1	S2	S=S1+S2	Deficit
	Mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Jan	44,9	19	0,0	0,0	0,0	26,3	18,6	22,0	44,0	66,0	0,0
Feb	53,1	25	0,0	0,0	0,0	27,9	25,2	22,0	44,0	66,0	0,0
Mar	51,9	56	3,9	0,0	0,0	0,0	55,8	18,1	44,0	62,1	0,0
Apr	52,2	87	18,1	5,6	0,0	0,0	75,9	0,0	38,4	38,4	11,1
May	75,3	36	0,0	0,0	27,6	11,5	36,3	22,0	44,0	66,0	0,0
June	80,8	99	17,9	0,0	0,0	0,0	98,7	4,1	44,0	48,1	0,0
July	65,9	161	4,1	30,4	0,0	0,0	100,4	0,0	13,6	13,6	60,8
Aug	67,5	143	0,0	7,7	0,0	0,0	75,2	0,0	5,9	5,9	67,4
Sep	75,6	70	0,0	0,0	6,0	0,0	69,6	6,0	5,9	11,9	0,0
Oct	68,6	53	0,0	0,0	15,9	0,0	52,7	21,9	5,9	27,8	0,0
Nov	54,8	27	0,0	0,0	27,8	0,0	27,0	22,0	33,6	55,6	0,0
Dec	53,3	19	0,0	0,0	10,4	24,3	18,6	22,0	44,0	66,0	0,0
Year	744	793	44	44	88	90	654				**139,3**

Table 6. The soil water balance for maize in a dry year

Month	P	ETo/ETc	L1	L2	R	Roff	AE	S1	S2	S=S1+S2	Deficit
	Mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Jan	31,7	40	8,6	0,0	0,0	0,0	40,3	13,4	44,0	57,4	0,0
Feb	52,2	34	0,0	0,0	8,6	10,0	33,6	22,0	44,0	66,0	0,0
Mar	75,8	53	0,0	0,0	0,0	23,1	52,7	22,0	44,0	66,0	0,0
Apr	14,1	102	22,0	22,0	0,0	0,0	58,1	0,0	22,0	22,0	43,9
May	74,0	37	0,0	0,0	36,8	0,0	37,2	22,0	36,8	58,8	0,0
June	24,0	109	22,0	17,6	0,0	0,0	63,6	0,0	19,2	19,2	45,6
July	33,1	195	0,0	19,2	0,0	0,0	52,3	0,0	0,0	0,0	143,0
Aug	69,2	153	0,0	0,0	0,0	0,0	69,2	0,0	0,0	0,0	83,8
Sep	74,2	67	0,0	0,0	7,0	0,0	67,2	7,0	0,0	7,0	0,0
Oct	101,2	43	0,0	0,0	57,8	0,0	43,4	22,0	42,8	64,8	0,0
Nov	56,2	27	0,0	0,0	1,2	28,0	27,0	22,0	44,0	66,0	0,0
Dec	49,3	19	0,0	0,0	0,0	30,7	18,6	22,0	44,0	66,0	0,0
Year	655	879	53	59	111	92	563				**316,3**

Tables 4–7 show that soil water deficit occured during the vegetation period both in the year with an average precipitation amount and in the dry year. The water deficit in the year with an average precipitation amount for maize and soybean was 152.7 mm and 139.3 mm, respectively. In dry years deficit of soil water was higher; for maize and soybean it was 316,3 mm and 299.7 mm, respectively. A slightly higher soil water deficit was estimated for maize both in average and dry years. The estimated difference in soil water deficit could be attributed to the different durations of each phenological phase and development of maize and soybean. It is well–known that soil water deficit affects the growth

and development of field crops, which in turn affect their yields and quality. Water deficit is especially harmful if it occurs in the "plant's critical period of water need". This period may have shorter or longer duration and it can occur in different phenological phases of a particular plant. Similar results were obtained in the former studies in connection with soil water deficit in the continental part of Croatia (Šimunić *et al.*, 2007; Kovačević *et al.*, 2013; Šimunić *et al.*, 2020).

Table 7. The soil water balance for soybean in a dry year

Mjesec	P	ETo/ETc	L1	L2	R	Roff	AE	S1	S2	S=S1+S2	Deficit
	Mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Jan	31,7	40	8,6	0,0	0,0	0,0	40,3	13,4	44,0	57,4	0,0
Feb	52,2	34	0,0	0,0	8,6	10,0	33,6	22,0	44,0	66,0	0,0
Mar	75,8	53	0,0	0,0	0,0	23,1	52,7	22,0	44,0	66,0	0,0
Apr	14,1	102	22,0	22,0	0,0	0,0	58,1	0,0	22,0	22,0	43,9
May	74,0	37	0,0	0,0	36,8	0,0	37,2	22,0	36,8	58,8	0,0
June	24,0	109	22,0	17,6	0,0	0,0	63,6	0,0	19,2	19,2	45,6
July	33,1	186	0,0	19,2	0,0	0,0	52,3	0,0	0,0	0,0	133,7
Aug	69,2	146	0,0	0,0	0,0	0,0	69,2	0,0	0,0	0,0	76,5
Sep	74,2	59	0,0	0,0	15,4	0,0	58,8	15,4	0,0	15,4	0,0
Oct	101,2	43	0,0	0,0	50,6	7,2	43,4	22,0	44,0	66,0	0,0
Nov	56,2	27	0,0	0,0	0,0	29,2	27,0	22,0	44,0	66,0	0,0
Dec	49,3	19	0,0	0,0	0,0	30,7	18,6	22,0	44,0	66,0	0,0
Year	655	855	53	59	111	100	555				**299,7**

Estimation of decline in maize and soybean yields due to water deficit

This section describes the reaction of maize and soybean to water deficit and an estimation of a decline in yields of examined crops. Any deficit of soil water causes “stress” in plants and some decrease in yields.

Table 8. Estimation of decreased yields (%)

Crop	Estimation of a decline in yield (%)	
	Average years	Dry years
Maize	33.9	65.0
Soybean	21.5	40.5

According to Table 8, it is obvious that there was a decline in the yields of both crops. The estimation of the decline in maize yields was higher than for soybean. The reason for the latter could be that maize has a slightly higher soil water deficit than soybean and maize reacts more stressfully to a lack of soil water than soybean. In the northern part of Croatia, Šimunić et al. (2020) estimated the reduction of maize and soybean yields, which varied from 45% to 70% and from 28% to 44%, respectively. Table 9 shows crop yields for a 5–year period in the northeastern region of Croatia.

Table 9. Crop yields in the northeastern region of Croatia

Year	Total precipitation amount (mm)		Yield (t.ha^{-1})	
	Year	May–September (vegetation period)	Maize	Soybean
2012	736.7	262.9	5,175a	2,153a
2014	1,042.4	546.0	8,929b	3,188b
2016	1,076.0	620.5	8,875b	3,202b
2019	841.2	465.9	9,817c	3,584b
2020	801.4	464.7	9,652c	3,454b
The difference between the highest and the lowest yield	t.ha^{-1}		4,142	1,431
	%		47.3%	40%

Source about yield: Company Ratarstvo Orahovica

Source: Central Bureau of Statistics of the Republic of Croatia

Source of variation-maize	DF	Sum of squares	Mean squares	F	Pr > F
Between groups	2	14.426	7.213	957.252	**0.001**
Within groups	2	0.015	0.008		
Total	4	14.441			

Source of variation -soybean	DF	Sum of squares	Mean squares	F	Pr > F
Between groups	2	1.265	0.632	147.949	**0.007**
Within groups	2	0.009	0.004		
Total	4	1.273			

Table 9 shows the yield variation of the previously mentioned crops during the examined period. As expected, the lowest yield of both crops was in the year with the lowest precipitation amount (dry year), while the highest yield both for maize and soybean was in the years when annual precipitation amount was on a par with the multi–annual average of annual precipitation amounts. In the years with higher precipitation amounts, the yields of maize and soybean were lower than in the years with average precipitation amount, but the yield of soybean was not statistically justified, while for maize it was statistically justified. High crop yields in hydrologically unfavorable years can be attributed to the effect of drainage, which was confirmed in the research by Šimunić, 1995 and Tomić *et al.*, 2002. The difference between the highest and the lowest yield of maize was 4.142 t.ha^{-1} (47.3%) and for soybean 1.431 t.ha^{-1} (40%). In comparison with the

estimated values of decreased yield (Table 8), the actual yield of maize in a dry year was higher, while the actual and estimated yield of soybean was almost the same. Numerous studies indicate that crop yields vary the most as a result of adverse climate conditions despite the applied standard agricultural techniques (Mađar *et al.*, 1998; Šimunić *et al.*, 2007 and 2013; Dragovic *et al.*, 2012; Kovačević *et al.*, 2012; Kovačević and Josipović, 2015; Josipović *et al.*, 2016; Moteva *et al.*, 2016; Hafiane *et al.*, 2020). Therefore, planned and stable yields of agricultural crops in conditions caused by climate change can be achieved by building hydro–technical objects for drainage and irrigation.

CONCLUSIONS

Several conclusions can be reached based on the obtained results:

1.During the examined 20–year period, the mean annual air temperature in the northeastern region of Croatia increased by 1.7oC, while a slight negative trend in annual precipitation amounts is –0.18 mm/20 yrs.

2.The determined soil water deficit in the years with multi–annual mean of precipitation ranged from 139.3 mm (soybean) up to 152.7 mm (maize), while in the dry years water deficit ranged from 299.7 mm (soybean) up to 316.3 mm (maize).

3.The estimation of yield decline (%) in the years with multi–annual mean of precipitation ranged from 21.5% (soybean) up to 33.9% (maize), while in the dry years it ranged from 40.5% (soybean) up to 65.0% (maize).

4.In the 5–year period the lowest yields of both crops were in the year with the lowest annual precipitation amount, while the highest crop yields were when the annual precipitation amount was on a par with the average value. In the year with the highest precipitation amount, crop yields ranged between the value of the yield aged in the drought and the year with the average annual precipitation amount.

5.The problem of yield decline in the northeastern region of Croatia in dry years can be largely solved through irrigation (need to build/expand the irrigation system), as well as through better maintenance of the existing drainage system because the problem can appear during the period with heavy rains.

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THE INFLUENCE OF GREEN MARKETING ON CONSUMER ENVIRONMENTAL AWARENESS

SUMMARY

Due to increasing environmental problems, environmental management is becoming a challenge and a necessity in the modern world today. Negative consequences of human behavior are increasingly being observed. These reasons have led to environmental changes and consumer awareness of the impact they may have on environmental conservation. Green marketing has raised consumer awareness of how their behavior can have a positive impact on the environment. Various environmental changes affect consumer behavior, so it is imperative that all factors be monitored continuously to meet consumer demands. The main aim of the paper is to research the attitudes and opinions of the respondents on the importance of green food, how green marketing affects the sustainability and use of green food products. The survey was completed in 2019 on a sample of 100 respondents exclusively on the territory of the Republic of Serbia. Research results generally show that consumers are not sufficiently familiar with the term green marketing. The analysis shows that they know what a green product is, but that they cannot easily recognize it in the market, and that they do not buy it sufficiently to preserve the environment.

Keywords: Green Marketing, Consumers, Food Products, Environmental Awareness

INTRODUCTION

Corporate social responsibility is a business concept where business organizations incorporate the care about society and the environment into their business operations. Being socially responsible means not only to fulfill legal

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obligations but to invest in human capital, environment, and relations with customers (Kotler and Lee, 2009).

Green marketing refers to the practice of development and promotion of products based on their actual or perceived environmental sustainability (Mickovic *et al.*, 2020; Pejanovic *et al.*., 2020; Palevic *et al.*., 2019; Parsipour *et al.*., 2019). The emergence of a consumer population that is becoming increasingly concerned with environmental and social factors has led to green marketing becoming an important component of corporate public relations (Fernando, 2020). Green marketing is a form of social marketing in which products, services, and all marketing activities are planned and implemented taking into account the activities and impacts they may have on the environment and society in general (Lackovic and Andrlic 2007).

Green marketing is a form of social, environmental marketing where products, services, and all marketing activities are designed and implemented taking having regard of the actions and impacts they may have on the environment and society in general (Previšić and Ozretić, 2004). According to a simple definition: Green Marketing is a name for marketing efforts aimed at meeting consumer preferences regarding environmental protection (Skataric *et al.*, 2018).

Green marketing is a process of designing, manufacturing, and selling products or services based on environmental benefits, such as using recycled raw materials to manufacture products, using filters in production. Green marketing is no longer a trend but a major change in the way businesses operate. This is a form of social marketing that entails cooperation with suppliers, traders, partners, and competitors to achieve environmentally sustainable development throughout the value chain and cooperation of all business functions to achieve the best solutions that lead to profit and positive contribution to the environment.

Green marketing can involve a number of different activities, such as creating an environmentally friendly product, using eco-friendly packaging, adopting sustainable business practices, or focusing marketing efforts on messages that communicate a product's green benefits (www.shopify.com).

Green marketing is based on the accepted 3R formula: reduce-reuse-recycle, (Ham and Forjan, 2009), and thus, through these three steps, a significant contribution is made to the preservation of the environment:

- Reduce – reduced use of natural resources (replacement of natural resources by synthetic and/or replacing non-renewable sources with renewable ones), reduced energy consumption in the production process, and other business processes.
- Reuse – reuse of packaging or parts thereof (multi-use plastic pallets are introduced for transport instead of wooden ones).
- Recycle – manufacturers collect, in an organized manner, the used products and/or their packaging for the recycling process; recycling of waste generated in the production process.

The adoption of green marketing will provide a more competitive advantage and greater profits for businesses along with environmental protection. Adoption and implementation of green marketing is an opportunity for the company to contribute to environmental protection and thus provide assistance to society achieving its profit goal simultaneously. Thus, the balance between environmental and economic impact is achieved (Nefat, 2015; Nacka *et al.*, 2019).

The above definitions suggest that green marketing is a complex term and serves the following objectives (Ham and Forjan, 2009):

- developing products that balance consumer needs for quality, convenience, performance, and acceptable price, with environmental acceptability in terms of minimum environmental impact.
- creating a high-quality image, including environmental consideration relating to both product characteristics and the manufacturer and its achievements in the field of environmental protection.

MATERIAL AND METHODS

The data on the preferences and attitudes of respondents about the notion of green marketing, green product and sustainability concept itself were collected through a survey questionnaire. The survey was conducted in 2019 on a sample of 100 respondents on the territory of the Republic of Serbia. Descriptive statistics methods were used in the analysis.

The demographic characteristics of the respondents are given in the following section: the survey was conducted on a simple random sample. In the structure of the respondents, the female population had a higher share of 53%, while the share of the male population was 47%. By age, the respondents were divided into interval groups (up to 25, 26-35, 36-45, 46-55, 55+). The most represented respondents belonged to the age group up to 25 years (26%); this group is very significant because the younger population, forming its habits, should take the utmost environmental care. It is followed by the interval group of 46 to 55 years (23%), which will largely influence the younger population with its awareness and care for the environment. The conclusion drawn from the processed questionnaire data is that most of the respondents have secondary education 64%, followed by respondents with a university degree, 22%. In accordance with the basic characteristics of the respondents that are of relevance, their employment status was analyzed. Respondents having permanent employment (56%) prevail.

RESULTS AND DISCUSSION

The main objective of the research was to identify the level of consumer awareness of green marketing and environmental awareness of consumers. The research should be able to answer whether respondents know what sustainable development and green marketing are, whether they know what green products

are and whether they buy them. The task is also to compare the results obtained with similar research in countries of the region.

The first segment of the investigated issue concerned the awareness of the respondents of the term green marketing and its impact on sustainability. The literature uses different terms as synonymous with the term green marketing such as ecological marketing, environmentally responsible marketing, sustainable marketing, environmentally focused marketing. The analysis showed that 60% of respondents are aware that green marketing is the marketing taking care of environmental protection, and environmental consideration, while 40% of respondents were not familiar with this term. However, the results obtained show that a large number of respondents are not yet familiar with the term and do not know what green marketing is. When these results were compared to the results of the research in Croatia (Roguljić, 2015) it can be seen that the results obtained in Serbia were slightly better. Namely, in the research of the abovementioned author, 42% of respondents in Croatia were familiar with the term green marketing. More attention needs to be paid to green marketing and it should be properly introduced to consumers. Better education is needed, especially with regard to younger consumer categories.

The analysis of the results revealed that 45% of respondents were informed of green marketing via the internet, 27% of respondents received the information via TV. These results are expected, as it is well known that most of the information nowadays is provided by the Internet and TV (Figure 1).

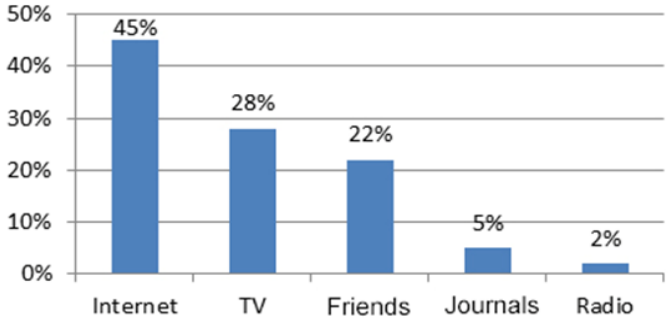

Figure 1. Ways of receiving information about the Green Marketing concept

The green consumer is the consumer who, when satisfying his/her wishes and needs, favors a product with a minimum harmful impact on the human environment (Lacković and Andrlić, 2007). It is therefore important to get the results of the awareness of the term sustainability as well as the impact of green marketing on it. The analysis showed that 69% of respondents were aware that sustainable development represents the development of a society that meets human needs with the resources available, without jeopardizing natural systems and the environment, ensuring the long-term existence of human society and its environment. At the same time, 31% of respondents were not familiar with the

term sustainability. Also, most of the respondents (65%) were aware that the task of the green marketing is to promote the ideas that would increase the consumer awareness of the importance of taking care of the physical environment in which they live and work, indicating their awareness of the impact on sustainable development (Figure 2).

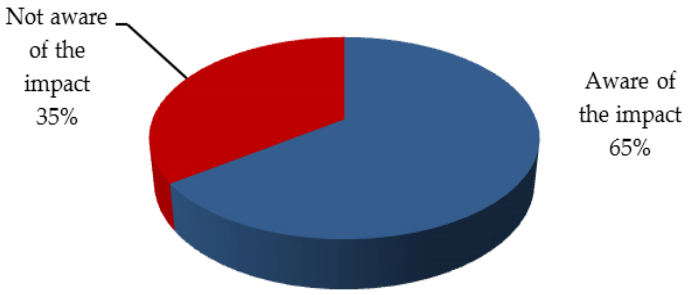

Figure 2. Respondents' awareness of green marketing's impact on sustainability

The results of a survey by Širola and Rosandić (2019) in Croatia show that awareness of the importance of environmental protection has a positive impact on the intention of the purchase and use of ecological products and services.

When asked whether they would propose introducing environmental education as a mandatory school subject, as many as 67% of respondents said they would propose introducing such a subject: environmental education as a mandatory school subject because they feel it is good for the children to be informed from a young age how to act when it comes to the environment; whereas, 13% of respondents would not want it as a mandatory school subject because they feel that children are burdened already, while 20% of respondents have no opinion on this idea.

Green marketing has raised consumers' awareness of how their behavior can have a positive impact on the environment. Separation of raw materials following the use of food products is also a significant attitude towards the environment. Most of the respondents (50%) separate raw materials for recycling after use, 36% of respondents do not know how to do this, and 14% do not want to waste their time. These results are not at an enviable level, but they should improve significantly over time. When these results were compared to the results of the research in Croatia (Roguljić, 2015), it can be noted that the results in Croatia are much better. When asked whether they recycle waste 51.5% of respondents replied in the affirmative, 45.5% recycle occasionally, and only 5.1% never, which is a very good result and an indication of consumers' responsibility.

Based on the research conducted by Tolušić *et al.* (2013) in Croatia, it can be concluded that the awareness of the current environmental problems is widespread, but the actual individual actions contributing to the preservation of the environment are relatively few, which is confirmed by the results obtained.

Only 12% of respondents recycle on a regular basis, 61% occasionally, and as many as 27% never. This issue and increasing the consumers' awareness of they themselves can help and develop environmental thinking can be solved in part by green marketing, encouraging the development of ecology, launching appropriate environmental actions, education on the preservation of the environment, but also by strengthening the links between businesses and scientific institutions (Spalevic *et al.*, 2017).

In the Republic of Serbia, the state has not provided the proper conditions for this purpose. Some respondents stated that they would pay more attention and separate the raw materials if it were made possible by the authorities, they would take care of how they would handle waste if the proper conditions for the separation of plastic, paper, glass, etc. were provided. Following the cross-referencing of the answers received with the gender structure of the respondents, it can be noted that more women separate raw materials for recycling after use. In most cases, plastic is separated (46%), followed by paper (21%), 17% of respondents said they separate glass and 18% metal, only 10% of respondents separate electronic waste, and as many as 46% of respondents do not separate raw materials after use. By cross-referencing the answers received with the previous question where as many as 36% of the respondents stated that they did not know how to classify raw materials, it can be assumed that this percentage would decrease if adequate conditions for the waste separation were provided in our country, so it would automatically increase the number of respondents who separate raw materials after use and thus, these results would be improved in future.

The analysis of the responses received shows that 83% of respondents think that plastic bags absolutely pollute the environment as they are very difficult to decompose, 13% of respondents think that plastic bags do not pollute the environment if they are properly disposed of and 4% think that plastic bags do not pollute the environment because they help them to dispose of waste more easily. These results are good because the respondents are aware to what extent plastic bags pollute the environment.

It is evident that 29% of the respondents participated in environmental activities, but most of them stressed that they participated in such campaigns just a few times, some only once, while as many as 67% of respondents said they did not have the opportunity to participate in the campaigns of this type, and 4% of the respondents think it that it is not important at all. Cross-referencing of the answers received with the age categories shows that young people from categories of up to 25 years of age participated most in such campaigns. When the results obtained were compared to the results of the research in Croatia (Roguljić, 2015), it can be noted that the results in Croatia are much better. Namely, in the research of the abovementioned author, 52% of respondents in Croatia participated in various environmental campaigns.

Of the total number, 81% of respondents think waste management is very important because the environment has to be protected, 14% think it is important,

but that authorized persons should take care of it, and 5% think that some other issue is much more important. When the results obtained are compared with the results from the previous responses, it can be noted that despite the significant care for the environment, very few respondents are involved in various environmental campaigns. In most cases, they are younger, male respondents.

The term "green product" is intended for labeling products and services with reduced environmental impact, i.e. products that have a reduced impact on the environment and human health over the entire life cycle compared to other products intended for the same purpose and use (Ottman, 2011). A green product is a sustainable product designed so as to minimize its impact on the environment throughout its life cycle and even after the process of use. Green products are typically identified with two main objectives - waste reduction and maximizing resource efficiency. They are produced using non-toxic ingredients and environmentally friendly procedures and are certified by recognized organizations. Some of the green product features are it is produced without the use of toxic chemicals and in hygienic conditions. It can be recycled, reused, and is biodegradable in nature. It comes with eco-friendly packaging. It uses a small amount of natural resources. It is environmentally efficient. It has a reduced or zero carbon share. It has a reduced or zero plastic footprint. In a typical scenario, brands producing green products use green marketing to transfer their values at the market (Das Prinona, 2019).

The terms "green" or "sustainable" often refer to products, services, or practices that enable economic development along with preservation for future generations. A green product is a product with a lower environmental impact or less harmful to human health than the traditional product equivalent. Some authors report that almost no product can ever be 100% green, as any product development will have a certain impact on the environment. Green products have the following attributes: they are energy-efficient, often low-maintenance. Contain no ozone-depleting substances, toxic compounds and do not produce toxic by-products. They are often made from recycled materials or ingredients or renewable and sustainable sources. Raw materials are obtained from local producers or resources. They are biodegradable and easily reused in part or in whole (Speer 2011).

The question asked was to what extent the respondents were familiar with the term green products. The results of the research indicate that 75% of respondents know that a green product is a product that does not harm the environment, whether it concerns production, consumption or waste - which is a very good result - while 25% of respondents do not know what a green product is. Most of the respondents, 40% of them, are not sure if they can recognize a green product, while 35% claim they manage well and can recognize green products, whereas 25% say they cannot recognize green products. Such products need to be visibly marked with a specific marking. The assumption is that green products need more advertising to raise consumers' interest so they would look for and recognize green products on the supermarket shelves.

Most of the respondents (41%) believe that a green product is different from a conventional one, while 12% think that these two products do not differ, and as many as 47% of respondents are not sure if a green product differs from a conventional one. Promotional activities are needed to inform consumers so that in the future they would be able to recognize the products more easily and select the products that will have a lower environmental impact. There is a need for green products in Serbia to be marked specifically so that customers can easily identify them and distinguish them from conventional products. That way, they would not be in a dilemma whether these were really green products. Such is the practice in developed countries of the world.

A bit more than half of the respondents (57%) are familiar with the labels on the packaging. One-third of the respondents are not sure if they are fully familiar with the labels, 10% are not familiar with the eco-labels at all. According to the results of the survey, 10% of respondents always pay attention to the packaging labels when buying a product, whereas the majority of respondents, 60%, pay very little attention, and one-third of respondents never pay attention to the labels on the green product. Therefore, better consumer education is needed so they would pay attention to product labels during shopping.

The packaging contains all the information on the product contained in it, as well as the information relating to it. Some of the information is also eco-labeling, which shows what the packaging is made of and how it is used and how it behaves after the consumption of the product that was inside. The labels also provide information on whether the packaging is suitable for recycling and how it can be recycled. Today, consumers and businesses are very focused on PET packaging and its recycling, taking into account its harmful environmental impact. Because of such materials, eco-materials have been used as of recently that are less harmful or are not harmful to the environment at all. With such materials and with sending eco-messages from businesses to consumers, the consumer's environmental awareness is growing (https://energis.ba/).

The desirable benefits of green products can be as follows (Ottman *et al.*, 2006):

–Efficiency and cost efficiency - when buying green products is often mentioned by producers when buying green products. Resources are used again through waste and waste material recycling.

–Health and safety - it is very important to show to green consumers the care for their health and the health of their families.

–Quality - many green products offer better quality than standard products, i.e. they are practical because they save energy through work.

–Symbolism and status - the goal is to create a symbol with the benefits of a green product.

–Convenience - Green products provide benefits to green consumers through work.

A green positioning strategy can also be considered through functional and emotional benefits. The functional benefit includes the brand of the product and

its environmental impact. Green consumers explore the product and each element is important to them; thus, in this situation, it is important whether the product is degradable, i.e. functional benefit may be related to the product manufacturing process and its disposal.

A green consumer may be defined as a consumer who, in satisfying his/her needs and desires, seeks a product with a minimum negative environmental impact. These are, as a rule, educated consumers, and very interesting as a target market, even though consumers who are passive towards environmental protection form a far larger market (Ham, 2009). Such consumers intensively consider environmental issues when buying and using products. They are often very active in their environment, accepting and supporting environmental actions and objectives, and are ready to spend more for green agri-food products. For all these reasons, they expect full information on the product or packaging of the product, as they are motivated by the desire to protect themselves, their family members and to safeguard their future.

The first market segmentation in green marketing was carried out in 1990 by then Roper Starch, GfK Roper Consulting today. The following consumer segments were identified at that time:

True Blue Greens - they are characterized by a high level of environmental awareness. They are very much concerned about the environment and they actively want to make a positive change. It is four times more likely they will boycott products or organizations that are not environmentally responsible. This group is comprised of high-income and higher education consumers.

Greenback Greens – not too active, but more likely to buy an eco-friendly product than average consumers. They do not have enough time to think about environmental issues but are ready to buy green products and support environmental programs. They also have a high income and a higher education level, but not as high as the previously mentioned consumer category.

Sprouts - consumers who believe in the care for the environment in theory but not in practice. They rarely buy green products if they should spend much more money on such a product than for conventional ones. Marketing activities have a significant impact on this consumer category in the sense that it is easy to persuade them to start purchasing these products.

Grousers – skeptical and uneducated with regard to the environment and cynical toward the positive changes they can bring. They are not sufficiently educated on environmental issues and are characterized by a high disinterest in this area. They feel that as individuals they cannot contribute much to solving environmental issues, so they are very passive in this regard. They consider green products to be overrated and worse than classical products and that their price is unrealistically high.

Basic Browns – they are characterized by lower education levels and lower income. They are burdened with everyday problems and do not care about social issues and the environment. They are particularly indifferent towards

environmental issues and belong to the segment that is least interested in global environmental problems and their resolution (Krstić and Jovanović, 2007).

The Natural Marketing Institute (NMI) conducted a survey based on consumer behavior and attitudes. According to the results, consumers are divided into the following groups (Kamenar Sara 2018, according to Dahlstrom, 2011):

a) consumers focused on health, environmental protection, personal development, social fairness, and sustainability - Lifestyles of Health and Sustainability (LOHAS) - this consumer group comprises educated individuals whose decisions on purchasing green products are based on their own environmental awareness and cultural and social values. The main motives include the need for personal participation in environmental protection and care about their health. These consumers are often willing to spend more money on green products. According to the survey, 17% of adult consumers in the United States are considered to be part of this group. NMI divides these consumers into two subgroups – LOHAS leaders and LOHAS followers. The former is quick to adopt green products and influence the shaping of general opinion on green companies and products. They are often the first potential buyers and users of a particular green product. The second group comprises people who are more moderate with regard to green product preferences, but just like leaders, they are often the first buyers of a particular green product.

b) Naturalites – consumers who are slightly less engaged in active environmental protection than the previous group and their main incentive is caring for psychophysical health. Their focus on health is guiding them toward green products. These consumers are generally not environmentally active. They represent approximately 17% of US consumers.

c) Conventionals - consumers who are mostly not driven by the need for active preservation of the environment, but rather by frugality and convenience, that is, they are driven by their own objectives. These consumers need to see the result of their activities, and therefore, they tend to save energy resources and recycle. They recognize the value of buying products that save money in the long term, but the environmental segment is not a decisive factor in the decision-making process. They make up 26% of the population.

d) Drifters – consumers who are younger people limited by financial difficulties. Their views on environmental protection are not fully formed, but they are generally not overly concerned about environmental issues and feel that such issues will probably be resolved. They show a tendency to support environmental protection, but usually for those issues influence of which they feel directly. The choice between green or other products is in most cases dictated by the price. Make up 24% of adult US consumers.

e) Unconcerned – consumers who are neither interested in environmental protection nor are involved in related activities as long as they are not directly affected. They usually do not pay attention to green products and they do not tend to search for information about them. Consumers from this group choose products based on price, availability, quality, and value, but not based on the

environmental impact of the production process or the product itself. This group comprises 16% of the respondents (Dahlstrom, 2011).

Research results show that only 13% of respondents in Serbia stated that they buy green products, while 57% of them buy them occasionally, 30% of respondents do not buy green products, a few respondents stated they may be buying green products at times, but they are not aware of it. It is evident that green products are generally not bought by the consumers, which indicates that a small percentage of respondents (2%) are those who buy them on a daily basis (Figure 3). When the results obtained were compared to the results of the research in Croatia (Roguljić, 2015), it can be noted that the results in Croatia are better. When asked how often they buy green products, 46.5% of consumers buy 2-3 products per month, 37.4% buy 2-3 products a week, 9.1% buy on a daily basis, and 7.1% of consumers do not buy and do not consume green products.

According to the results of the research conducted by Tolušić *et al.*, (2013), 69% of respondents in Croatia stated they bought a product motivated by a promotional message stating that the product is environmentally safe, which is seen as a result of promotion, one of green marketing activities, while 38% responded to this question in the negative. Such responses suggest that consumers are directly influenced by advertising and that distinctive eco-labels should be highlighted and referred to through promotion. Consumers of Vukovar-Srijem County are aware of the problem and are ready to buy ecological products and products of business organizations - companies implementing the sustainable development business policy.

Consumers will act in a more environmentally friendly manner if they realize that an environmentally friendly way can provide sufficient benefits to make up for the cost caused by higher prices of green products. Further to this, consumers can feel individual benefits as a result of environmentally conscious behavior, but these consequences are not necessarily related to a significant improvement in the quality of the environment, but, for example, with emotional benefits, based on psychological factors (Fraj and Martinez, 2006).

Consumers are often more motivated by the so-called economic rather than by the altruistic reasons when choosing a product. One of such essential motives that drive consumers to choose and buy a green product is also their personal interest – they want a product whose basic function will be achieved in entirety or will result in savings. According to Pike and Makower (2009), consumers will be willing to buy a green product – if it comes from a brand they know and trust, if it is at least as good as the product they usually buy if they can buy it at the usual place of purchase, if this does not require a change in purchasing habits if its price is not higher, and, ideally, if it has some other additional benefit (which is not green) - it lasts longer, looks better or saves money. Hence, companies should produce such green products whose green marketing impact on consumers are functional characteristics that are at least equal to the existing products on the market, if not better. Another important reason is the care for health, which primarily concerns food products.

Among the main reasons for the low purchase of green food products are, above all, low income of consumers, the higher price of green products compared to products from conventional production, low market availability, unsatisfactory range of green products and low consumer awareness of green products (Willer, Lernoud, 2014).

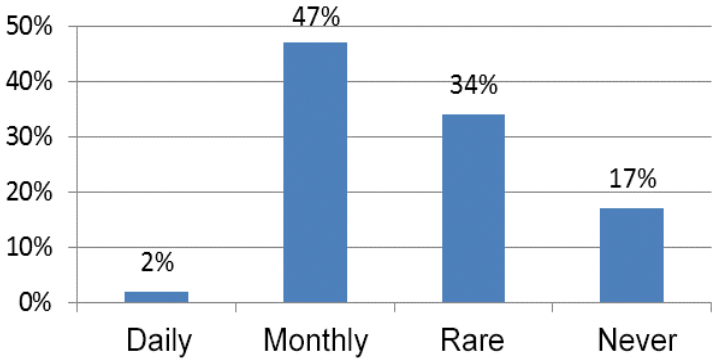

Figure 3: Purchase frequency of green products

A number of claims were proposed to the participants in the survey, to which they were to state their level of agreement. They could respond to each claim with one of 4 responses offered: Strongly disagree, disagree, agree, and strongly agree. Most of the respondents, 50%, answered they agree with the statement that green products appear to be as good or better than ordinary ones and are therefore worthy of a higher price, 10% strongly agree, 30% disagree with this statement, and 10% strongly disagree (Table 1).

The results obtained in this research show that green consumers are the leaders who should influence consumer behavior and that consumers nowadays do not buy products based only on price, performance, and product convenience. Respondents believe that habits influence consumers to buy a particular product. Green consumers are strongly influenced by the recommendations given by friends, family, and acquaintances, and 46% of respondents agree with this statement. Products that do not harm the environment or have a minimum negative environmental impact, resulting from market-responsible thinking and behavior (Table 1).

Adoption of the concept of green marketing as a whole, or only in some of its parts, demonstrates a good approach to business, deliberation, and efforts of the company to distinguish itself from the competition and meet better the needs and wishes of the market segments. There are also situations when green marketing is declared a strategy for achieving competitive advantages and a possible overall quality management strategy (). A successful green marketing strategy has to satisfy four conditions (Roguljić, 2015):

–Companies must not count on the willingness of consumers to pay more for products that do not have harmful environmental effects.

- Marketing claims, concerning environmental aspects, should be clear and understandable, they must not be general and have to meet strict environmental standards.
- Access to green marketing products has to respect the duration of their production and use cycle. Raw materials and resources needed for production and the period of use by consumers are taken account of.
- The green marketing strategy should be proactive, not reactive. This means that businesses should seek to improve environmental awareness before they are forced to do so by law and to offer consumers products and services whose standards, in terms of environmental protection, go beyond the ones currently laid down by the law.

Table 1 Level of agreement of respondents with specific claims (%)

	Strongly disagree	*Disagree*	*Agree*	*Strongly agree*
Green products are worthy of a higher price	10	30	50	10
Green consumers are the leaders who influence consumer behavior	12	44	40	4
Consumers are no longer buying based solely on the price and convenience of products.	5	37	48	10
Habits affect the process of manufacturing, packaging, and the ethical values of a company.	8	12	65	15
The recommendations given by friends, family, and acquaintances have a strong influence on green consumers.	5	34	46	15
Products that do not harm the environment or have a minimum negative environmental impact are considered to be green products.	2	6	58	34

Source: Data processed by the authors

CONCLUSIONS

The analysis of the survey in Serbia showed that 60% of respondents are aware that green marketing is the marketing taking care of the environmental protection, and environmental consideration, while 40% of respondents were not familiar with this term. The results of the survey show unambiguously that respondents are not yet sufficiently familiar with the term green marketing and its role in the preservation of the environment. The analysis also shows that they know what a green product is, but they cannot easily recognize it on the market,

and they do not buy it to the extent necessary to preserve the environment in such a way. It is evident from the research that respondents are aware of the importance of environmental concerns but are not sufficiently familiar with the concept of corporate social responsibility. The social community and companies have the task and responsibility to educate their consumers about the importance of green products and their effect on environmental protection.

In the Republic of Serbia, the state has not yet provided the proper conditions for waste sorting; respondents stated that they would pay more attention and separate the raw materials if it were made possible by the authorities, they would take care of how they would handle waste if the proper conditions for the separation of plastic, paper, glass, etc. were provided. Following the cross-referencing of the answers received with the gender structure of the respondents, it can be noted that more women separate raw materials for recycling after use. The survey showed that respondents know how important proper waste management is, but that they do not have the conditions to handle it properly, since containers for sorting and disposal have not been made available by the competent authorities.

While poor economic situation and low standard of living directly affect the low level of green product purchase and consumption, awareness of environmental protection and preservation has been developed. Respondents with higher education and the female population are more aware. There is a need for green products in Serbia to be marked specifically so that customers can easily identify them and distinguish them from conventional products. That way, they would not be in a dilemma whether these were really green products. In our country, true green consumers have not yet become distinct, as in developed countries of the world. All actors in society (schools, faculties, Ministry, business organizations) should continue working even more intensively on socially responsible business and the implementation of green marketing.

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ANALYSIS OF THE IMPACT OF FRUIT GROWING DEVELOPMENT ON THE INTENSITY OF SOIL EROSION AND RUNOFF: CASE STUDY OF KRUSEVO, BIJELO POLJE, MONTENEGRO

SUMMARY

The research has been conducted to analyse the effects of land use change of the impact of fruit growing development on the intensity of soil erosion and runoff in the Study area of Krusevo, Bijelo Polje, Montenegro by using the Intensity of Erosion and Outflow – IntErO model of Spalevic. The required spatial maps, land use, soil and geology were prepared and analysed in GIS environment. The climatic data such as the volume of the torrential rain, average annual air temperature and average annual precipitation were calculated based on meteorological data received from the State Hydrological Institute for the region of Bijelo Polje (Montenegro). The results of land use change between these two periods (2011-2020) shown that the forest increased in the studied region by 1.57%. Specifically, degraded forests increased by 1.02%; Well-constituted forests increased by 0.55%. For the studied area we calculated forested area on 57.26% (2011), and 58.83% (2020). The values for Meadows in the studied area decreased from 2011 to 2020 for 1.67%; and for Pastures for 1.40%. Ploughlands decreased for the observed period for 1.59%. On the other hand, the surface under the Orchards increased by 3.09%, and that represented the shift from the Meadows to the Orchards; the shift from the Pastures to the Forests. This denser vegetation at the studied region for the observed period (increase of the forests and orchards) has led to higher water infiltration rate into the soil and at the same time to decrease of the sediment yield. The value of Z coefficient of 0.462 (2011); 0.461 (2020) indicates slight decrease of erosion processes because of the fruit growing development with shifting the meadows to orchards categorising the processes to the 3rd destruction category. The strength of the erosion process

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is medium, and according to the erosion type, it is surface erosion. Production of erosion material in the river basin, W year, is calculated on 11327 m^3year^{-1} for 2011; and 11278 m^3year^{-1} for 2020, what shown the decrease of erosion processes because of the subject fruit growing development. Coefficient of the deposit retention (sediment delivery ratio) is calculated as 0.299 what means that 30% of the total eroded material reaches to the outlet point. Real soil losses, G year, are calculated on 3392 m^3year^{-1} (2011), and 3377 (2020); Real soil losses per km^2, G year km^{-2}, are 262 $m^3km^{-2}year^{-1}$ (2011), and 261 $m^3km^{-2}year^{-1}$ (2020), with the same conclusion in relation to the fruit growing initiatives and the values indicates that the river basin belongs to 5^{th} destruction category; it is a region of very weak erosion. The results showed that the appropriate land management and planning with implementing fruit growing in this area decreases maximum flow rate and also sediment yield. The application of the IntErO model may also be further used to understand the effect of land use change with new establishing of the fruit growing in the river basins on hydrological behaviour, soil erosion and sediment yield process and can be used as a useful tool in similar for fruit growing and soil conservation research.

Keywords: IntErO model; Land management; Fruit growing; Sediment yield; Montenegro.

INTRODUCTION

Soil erosion is one of the most significant causes of land degradation and an important environmental hazard throughout the world, especially in developing countries.

Sediment yield and soil erosion are two main constraints on sustainable management of water resources and soil. The quantification of these processes is crucial to design any scientifically based soil and water conservation plan and integrated land management. The acceleration of soil erosion due to human activities on a global scale has led to an increased sediment flow in many parts of the world. Unwanted complementary effects of soil erosion, such as loss of soil fertility, reduced water quality, alteration of the hydrological systems, and environmental contaminations, have been identified as a serious problem for human sustainability (Turner *et al*, 1990; Eswaran *et al*, 2001; Dabral *et al*, 2008.; Wang *et al*, 2013; Efthimiou *et al*, 2016; Khaledi Darvishan *et al*, 2016; Ferreira *et al*, 2016; Li *et al*, 2016; Kavian *et al*, 2018).

Many studies have shown that there is a significant relationship between land use change and soil erosion. Land use change may result in an increase of sediment and nutrient supply to rivers and may affect the water balance in the watershed and its variability, which must be assessed on a local scale (IntErO model, www.geasci.org/IntErO).

The main purpose of this study is the application of the IntErO model to evaluate the effects of fruit growing development on the intensity of soil erosion and runoff in the studied area of Krusevo, Bijelo Polje, Montenegro. With this study we try to create one of the sustainable forms of modelling that would be

calibrated and validated in the close region of the studied catchment and afterwards used to evaluate how fruit growing is influencing local community, eco sectors, and agricultural production in relation to this subject matter.

MATERIAL AND METHODS

Study area

North Montenegro is mainly mountainous, with the presence of deep valleys incised into limestone ranges, and some parts are hilly and underlain by Palaeozoic rocks. The area is densely populated. The rivers in this region belongs to the Danube watershed and drain to the Black Sea. The location of the studied area is presented in the Figure 1 and panoramic view on the Figure 2 and 3.

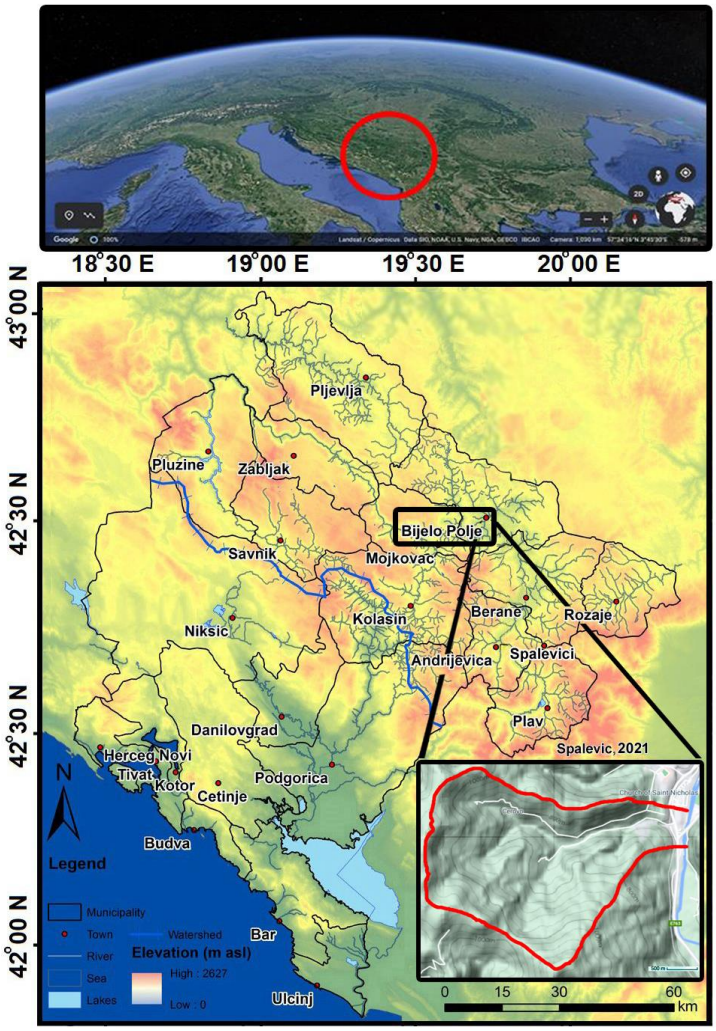

Figure 1: Location of the study area in Krusevo (43°00'12.1"; N 19°44'28.8"E)

Figure 2. Panoramic view on the studied area of Krusevo, Bijelo Polje

Figure 3. Some details on Land Use patterns of the studied area (2021)

The studied area of Krusevo (Bijelo Polje, North Montenegro) is a part of the Pepica River Basin, a left-hand tributary of the river Lim (43°00'12.1"; N 19°44'28.8"E; 43.003358, 19.741339). The basin stretches from its confluence with the Lim (lowest elevation, H min, of 578 m a.s.l) up to the tops where the highest elevation (Hmax) is 1264 m a.s.l. This watershed encompasses an area of 8.1 km^2, and the natural length of the main watercourse, Lv, is 4.4 km.

A flat area occurs around the village of Krusevo in the lower reach, where the fruit growing is established, and steep slopes make up the upper part of the watershed. The average gradient of the catchment, Isr, is 37.53 % and indicates that very steep slopes are present in the river basin. The average river basin altitude, Hsr, is 857.46 m a.s.l; the average elevation difference of the basin, D, is 279.46 m. The drainage density is low indicating a rather permeable substrate.

Climate

The study area is characterized by a mountain Mediterranean climate with rainy autumns and springs, cold winters, and a deficit of precipitation in the summer months. Basic data on the area needed for the calculation of soil erosion intensity and runoff are presented in Table 1.

Table 1. Precipitation and temperature for the period 1948–2020, Bijelo Polje.

	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Max. daily precipitation in mm												
Max.	68.6	92.8	73	93.3	42.6	58.5	97.8	55.8	95.6	157.6	101.6	79.4
Aver.	23.4	22.8	21.5	24	21.9	20.6	21.7	21.1	25.3	29	29	23.5
St.D.	15.6	18.6	13	15	9.9	12.4	15.2	11.9	17.2	24.6	16.2	14.7
Mean monthly temperatures in °C												
Max.	2.9	5.8	7.8	12.6	15.8	18.2	20.8	20.9	17.7	12.6	8.6	4.4
Min.	−5.6	−5.2	−0.7	6.1	9.8	14.1	16.2	14.3	11.3	6.2	−1.6	−4.7
Aver.	−1.6	0.8	4.6	8.9	13.3	16.3	18.1	17.7	14.3	9.4	4.5	0.1
St.D.	2.2	2.7	2.1	1.3	1.3	1	1.1	1.4	1.5	1.4	2.1	2.2
Max. daily temperatures in °C												
Max.	15.4	20.9	25.6	28.1	32.4	35.5	36.8	39.2	36	29.5	23	19.2
Aver.	11.7	14.5	20.1	23.6	27.6	30.4	32.8	32.8	29.4	24.8	18.6	13.6
St.D.	2.8	3	3.1	2.3	2.2	2.5	2	2.5	2.6	2.6	2.8	3.3
Min. daily temperatures in °C												
Min.	−27.6	−24.5	−16.5	−7.5	−4	0	1.2	2.6	−4	−7.2	−15.4	−21.7
Aver.	−15.1	−13	−8.4	−2.8	0.9	4.8	6.5	6.1	2.3	−2.5	−7.3	−12.6
St.D.	5.3	4.7	4.1	1.8	2	1.8	2.1	1.5	2.5	2.3	3.7	4.6

Source: Data from the Hydrometeorological Institute of Montenegro and the Biotechnical faculty of the University of Montenegro (Spalevic *et al*, 2020).

The absolute maximum air temperature ever recorded was 39.2°C. Winters are severe, with negative temperatures as low as −27.6°C. The average annual air temperature, t0, was 8.9°C. The average annual precipitation, H year, was 873 mm. The temperature coefficient for the region, T, was calculated at 0.99. The torrential rain, hb, was calculated at 84.7 mm. (Spalevic *et al*, 2020).

Geology and soils

The broader study area consists of various types of sediment, magmatic and metamorphic rocks generated in the long, Palaeozoic to Quaternary, interval. Most of the terrain is underlain by Mesozoic formations of carbonate composition, while magmatic and silico-clastic rocks are substantially less present. The main rocks outcropping in the area are clastic and subordinate carbonate rocks from the Paleozoic, Triassic clastites, volcanites, tuffs, limestone and dolomites, Jurassic clastic rocks with diabasic effusions and metamorphic rocks and Quaternary, mainly alluvial and colluvial deposits.

In order to define the permeability of the rocks of the study area, we used the Geological Atlas of Serbia (Dimitrijevic, 1992) and extracted a geological map of the studied region from the Geological map of Montenegro (Zivaljevic,

1989). The region consists of Devonian-Carboniferous (D+C) and Permian (P) phyllites, argyllo-phyllites, metasandstones, and conglomerates.

The coefficient of the region's permeability, S1, was calculated to be 0.96, having semi permeable class of rocks, fpp, of 13%, with predominant rocks of poor permeability (class fo, 87%). According to the results of the field visits and supplementary laboratory analysis, but also using the previous research data of the project Soils of Montenegro (1964–1988) carried out by the team of the Biotechnical institute of the University of Montenegro (Fustic & Djuretic, 2000) and Spalevic (2011), the most common soil types in the study basin were: Dystric Cambisols, Fluvisols and Colluvial Fluvisols in the lower alluvial plain.

Vegetation and Land Use

Forests dominate this river basin accounting for 57% of the total vegetation cover and beech forests (*Fagetum montanum*) prevail. The degraded forests are located near settlements and roads because of the firewood harvesting. These forests are characterized by a terminate canopy and by a large number of species of ground flora, shrubs, and lower trees. They differ from beech forests in the inner parts of the basin, characterized by a dense canopy which is the main characteristic especially of the sub-association *Fagetum montanum typicum*, (Milošević *et al*. 2019). On some positions forests of Sessile oak and Turkish oak (*Quercetum petraeae cerridis* Lak.) is recorded. In the lower part of the basin, a narrow belt along the river channel is covered with hydrophilic forest (*Alnetea glutinosae, Salicetea herbacea*).

IntErO model application

The Intensity of Erosion and Outflow - IntErO model (Spalevic, 2011) was used for the analysis of the impact of fruit growing development on the intensity of soil erosion and runoff in the region of Krusevo, Bijelo Polje, Montenegro. The IntErO model is based on Erosion Potential Method – EPM (Gavrilovic, 1962; Gavrilovic, 1972) and is widely used in different environments of the Balkans (Gavrilovic, 1988; Globevnik *et al*, 2003; Blinkov & Kostadinov, 2010; Spalevic *et al*, 2012; Kostadinov *et al*, 2014; Barovic *et al*, 2015; Vujacic *et al*, 2015; Vujacic *et al*, 2017; Dragicevic, 2017; Spalevic *et al*, 2017; Kostadinov *et al*, 2018; Spalevic *et al*, 2019; Gocic *et al*, 2020; Spalevic *et al*, 2020) and internationally: Brazil (Tavares *et al*., 2019; Ayer *et al*, 2020; Bolleli *et al*., 2020; Sakuno *et al*., 2020); Greece (Efthimiou *et al*, 2016); Iran (Behzadfar *et al*, 2014; Gholami *et al*, 2016; Khaledi Darvishan *et al*, 2019; Khaledi Darvishan *et al*, 2018; Mohammadi *et al*, 2021); Italy (Milanesi *et al*, 2015); Morocco (El Mouatassime *et al*, 2019; Ouallali *et al*, 2020); Nepal (Chalise *et al*, 2019)...

The IntErO model, an upgrading of the programs "Surface and Distance Measuring" and "River Basins" of Spalevic and can be used for handling a large number of data with the processing of 27 inputs, returning, after the calculations, 22 final result parameters (Coefficient of the river basin form, A; Coefficient of the watershed development, m; Average river basin width, B; (A)symmetry of the

river basin, a; Density of the river network of the basin, G; Coefficient of the river basin tortuousness, K; Average river basin altitude, Hsr; Average elevation difference of the river basin, D; Average river basin decline, Isr; The height of the local erosion base of the river basin, Hleb; Coefficient of the erosion energy of the river basin's relief, Er; Coefficient of the region's permeability, S1; Coefficient of the vegetation cover, S2; Analytical presentation of the water retention in inflow, W; Energetic potential of water flow during torrent rains, 2×gDF^½; Maximal outflow from the river basin, Qmax; Temperature coefficient of the region, T; Coefficient of the river basin erosion, Z; Production of erosion material in the river basin, W year; Coefficient of the deposit retention, Ru; Real soil losses, Gsp; Real soil losses per km^2. The input data and maps prepared for the IntErO model application are presented in the Table 2.

Table 2. Input data need for the IntErO analysis

Inputs	Amount / Unit
River basin areas (F)	12.92 km²
The length of the watershed (O)	16.3 km
The area of the bigger river basin part (Fv)	8.9 km²
The area of the smaller river basin part (Fm)	4.02 km²
Natural length of the main watercourse (Lv)	3.82 km
The shortest distance between the fountainhead and mouth (Lm)	3.46 km
The lowest river basin elevation	578 m
The highest river basin elevation	1264 m
A part of the basin area consisted of medium permeable rocks (fpp)	0.13
A part of the basin consisted of poor water permeability rocks (f0)	0.87
The volume of the torrent rain (hb)	157.6 mm
Average annual air temperature (t0)	8.9 °C
Average annual precipitation (H year)	893.3 mm

RESULTS AND DISCUSSION

Land use changes (2011-2020)

Previous field research from 2011 and recent Landsat satellite images and maximum likelihood method were used to prepare land use map for the studied area. The study area consists of six (of seven) classes (Tab. 3, Fig. 4) including: (2) Plough-lands, (3) Orchards, (4) Pastures, (5) Meadows, (6) Degraded forests, and (7) Well-constituted forests (No bare land recorded – class 1).

Table 3: Land use classes of the studied area in % and km^2

Land use 2011	**%**	**km^2**	**Land use 2021**	**%**	**km^2**
Degraded forests	37.22	4.80	Degraded forests	38.24	4.93
Well-constituted forests	20.04	2.59	Well-constituted forests	20.59	2.66
Meadows	19.32	2.49	Meadows	17.65	2.28
Pastures	9.24	1.19	Pastures	7.84	1.01
Orchards	8.67	1.12	Orchards	11.76	1.52
Plough-lands	5.51	0.71	Plough-lands	3.92	0.51
Total	**100**	**12.92**	**Total**	**100**	**12.92**

Table 4: Trends of Land use classes' changes of the studied area (2011-2020)

Land use class	%	km^2
Degraded forests	1.02	0.13
Well-constituted forests	0.55	0.07
Pastures	(1.40)	(0.18)
Plough-lands	(1.59)	(0.21)
Meadows	(1.67)	(0.22)
Orchards	3.09	0.40

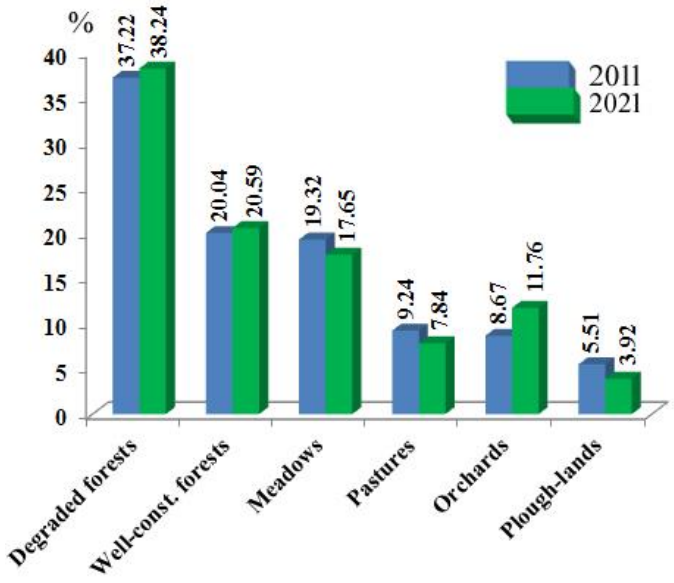

Figure 4. Graphical presentation on land use changes for the period 2011-2020

The results of land use change between these two periods (2011-2020) shown that the forest increased in the studied region by 1.57% (0.2 km^2) for a decade. Specifically, degraded forests increased by 1.02% (0.13 km^2); Well-constituted forests increased by 0.55% (0.07 km^2) for the period 2011-2020. That is in line with findings of Nyssen *et al* (2014) for Montenegro who stated that in the mountainous region of Montenegro, the wooded areas increased slightly, where dense vegetation increased from about 35% in the early-20^{th} century to 56%. Our results for forest land of the studied region were about 1.7% (2011) to 2.8 (2020) percent's higher than those of Nyssen *et al* (2014); for the studied area; we calculated forested area on 57.26% (2011), and 58.83% (2020).

The values for Meadows in the studied area decreased from 2011 to 2020 for 1.67% (0.22 km^2); and for Pastures for 1.40% (0.18 km^2). Plough-lands decreased for the observed period for 1.59% (0.21 km^2).

On the other hand, the surface under the Orchards increased by 3.09% (0.40 km^2), and that represented the shift from the Meadows to the Orchards; but also shift from the Pastures to the Forests.

This denser vegetation at the studied region for the observed period (increase of the forests and orchards) has led to higher water infiltration rate into the soil, and at the same time to decrease of the sediment yield (Tab. 5, Figure 5).

Table 5. The outputs of IntErO model for the Studied are of the watershed

Output variables		**2011**	**2020**	**Unit**
Coefficient of the river basin form	A	0.83	0.83	
Coeff. of the watershed development	m	0.30	0.30	
Average river basin width	B	1.96	1.96	km
(A)symmetry of the river basin	a	0.76	0.76	
Density of the river network of the basin	G	0.30	0.30	
Coeff. of the river basin tortuousness	K	1.11	1.11	
Average river basin altitude	Hsr	840.84	840.84	m
Average elevation difference of basin	D	262.84	262.84	m
Average river basin decline	Isr	31.73	31.73	%
The height of the local erosion base	Hleb	686	686	m
Coefficient of the erosion energy	Er	115.18	115.18	
Coefficient of the region's permeability	S1	0.96	0.96	
Coefficient of the vegetation cover	**S2**	**0.70**	**0.69**	
Analytical present. of the water retention	W	1.7251	1.7251	m
Energetic potential of water flow	2gDF^½	258.08	258.08	m km s
Maximal outflow from the river basin	**Qmax**	**247.37**	**245.07**	**m^3/s**
Temperature coefficient of the region	T	0.99	0.99	
Coefficient of the river basin erosion	**Z**	**0.462**	**0.461**	
Erosion production in the basin	**Wyear**	**11327**	**11278**	m^3/year
Coefficient of the deposit retention	Ru	0.299	0.299	
Real soil losses	**G year**	**3392**	**3377**	**m^3/year**
Real soil losses per km^2	**G yr/km^2**	**262.6**	**261.5**	**m^3/km^2 yr**

Coefficient of the river basin form (A), Coefficient of the watershed development (m) and Average river basin width (B) calculated 0.83, 0.30 and 1.96 km, respectively. The (a)Symmetry of the river basin calculated 0.76 indicate that there is a possibility for large flood waves to appear in the river basin. In the recent decades, anthropogenic influence such as urban development and agricultural land use changes have increased the hazard of flood events and watershed vulnerability to rainfalls and rain storms that lead to Peak flow (Spalevic *et al*, 2011; Chalise *et al*, 2019; Mohammadi *et al*, 2021).

The Density of the river network of the basin (G) obtained 0.3. The G index indicates there is a low density of the hydrographic network. Drainage density as an important factor affects erosion process. Therefore, its management can cause erosion control in the region. This index depends on soil type and amount of flow through the channel (Mohammadi *et al*, 2021; Ouallali *et al*, 2020). The index of average river basin decline calculated 31.73%. The value of this index indicates that in the river basin prevail very steep slopes.

The height of the local erosion base of the river basin and Coefficient of the erosion energy of the river basin's relief obtained 686.

Coefficient of the river basin erosion, Z, is 0.462 (2011); 0.461 (2020). The values of Z coefficient indicates that the river basin belongs to 3rd destruction category. The strength of the erosion process is medium, and according to the erosion type, it is surface erosion. Figure 5 shows decrease of production of erosion material and real soil losses, but also decrease of runoff under the land use change in the period from 2011 to 2020.

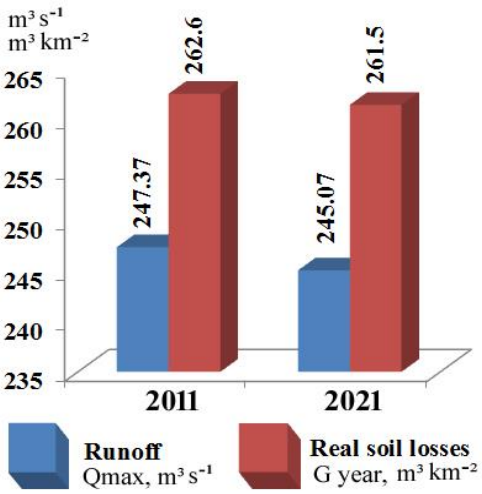

Figure 5. Graphical presentation of the Runoff and Soil losses (2011-2020)

Production of erosion material in the river basin, Wyear, is 11327 $m^3 year^{-1}$ (2011); 11278 $m^3 year^{-1}$ (2020). Coefficient of the deposit retention (sediment delivery ration) is calculated as 0.299. It means that 30% of the total eroded material reaches to the outlet point and deposit on slope and hydrological drainage system.

Real soil losses, G year, are 3392 $m^3 year^{-1}$ (2011); 3377 $m^3 year^{-1}$ (2020). Real soil losses per km^2, G year km^{-2}, are 262.6 $m^3 km^{-2} year^{-1}$ (2011); 261.5 $m^3 km^{-2} year^{-1}$ (2020) what indicates that the river basin belongs to 5th destruction category; it is a region of very weak erosion.

According to Grimes *et al* (2005), some marginal lands were cultivated again in the first decade of this century, as a consequence of the economic crisis in the region, but also on new initiatives of establishing new orchards on the places where we had before meadows. This trend was also confirmed by our research on land use in the small region in the North Montenegro.

CONCLUSIONS

In this study, the IntErO model was used to predict the effect of land use change on soil erosion and sediment yield in Krusevo from the Pepica watershed, from Bijelo Polje region of Montenegro. In the last decade, some parts of the meadows area of the studied region has shift to the orchards due to the new initiatives on fruit growing. Special attention was taken in Raspberry (*Rubus idaeus* L.) growing as one of the most important berry fruits in Montenegro.

The results of land use change between these two periods (2011-2020) shown that the forest increased in the studied region by 1.57%. Specifically, degraded forests increased by 1.02%; Well-constituted forests increased by

0.55%. For the studied area we calculated forested area on 57.26% (2011), and 58.83% (2020). The values for Meadows in the studied area decreased from 2011 to 2020 for 1.67%; and for Pastures for 1.40%. Plough-lands decreased for the observed period for 1.59%. On the other hand, the surface under the Orchards increased by 3.09%, and that represented the shift from the Meadows to the Orchards; the shift from the Pastures to the Forests. This denser vegetation at the studied region for the observed period (increase of the forests and orchards) has led to higher water infiltration rate into the soil and at the same time to decrease of the sediment yield. The value of Z coefficient of 0.462 (2011); 0.461 (2020) indicates slight decrease of erosion processes because of the fruit growing development with shifting the meadows to orchards categorising the processes to the 3rd destruction category. The strength of the erosion process is medium, and according to the erosion type, it is surface erosion. Production of erosion material in the river basin, W year, is calculated on 11327 m^3year^{-1} for 2011; and 11278 m^3year^{-1} for 2020, what shown the decrease of erosion processes because of the subject fruit growing development. Coefficient of the deposit retention (sediment delivery ratio) is calculated as 0.299 what means that 30% of the total eroded material reaches to the outlet point. Real soil losses, G year, are calculated on 3392 m^3year^{-1} (2011), and 3377 (2020); Real soil losses per km^2, G year km^{-2}, are 262 $m^3km^{-2}year^{-1}$ (2011), and 261 $m^3km^{-2}year^{-1}$ (2020), with the same conclusion in relation to the fruit growing initiatives and the values indicates that the river basin belongs to 5th destruction category; it is a region of very weak erosion.

The results showed that the appropriate land management and planning with implementing fruit growing in this area decreases maximum flow rate and also sediment yield. The application of the IntErO model may also be further used to understand the effect of land use change with new establishing of the fruit growing in the river basins on hydrological behaviour, soil erosion and sediment yield process and can be used as a useful tool in similar for fruit growing and soil conservation research.

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ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF SELECTED PLANT SPECIES OF THE BORAGINACEAE FAMILY

SUMMARY

Antioxidant activity is one of the most important properties of plant extracts. Antioxidants from natural sources have been intensively studied in the last few decades. The antioxidant contents of medicinal plants may contribute to the protection of diseases. Bioactive components of plants have a potential role in chemoprevention and inhibition of different phases of the malignant transformation process. Therefore, plant extracts and essential oils are in the focus of research, and in recent decades have been tested on a large number of malignant cell lines. The aim of this study was to examine antioxidant and cytotoxic potential of selected plant species from the Boraginaceae family. Determination of antioxidant activity was performed by ammonium-thiocyanate method. Testing citotoxic activity was performed by MTT test on cancer cell lines: HEP 2c (human larynx carcinoma), RD (human cell line-rhabdomyosarcoma) and L2OB (mouse tumor fibroblast line). The best antioxidant activity showed ethanol, acetone and chloroform extracts of *Anchusa officinalis, Echium vulgare* and *Echium italicum.* The tested extracts showed an inhibitory effect on cancer cells, but chloroform and acetone extracts of all three plant had the most effective effect on L2OB cells. Isolation of individual active components from this plants and their testing for cancer cells would be of great importance for this field of research.

Keywords: antioxidans, cytotoxic potential, plant, extracts, Boraginaceae

INTRODUCTION

The use of plants in the treatment and prevention of various diseases dates back to ancient times. In the last few decades, plants and essential oils have been used studied for their antioxidant properties. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities (Osawa *et al*., 1990). The antioxidant contents of medicinal plants may contribute

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to the protection they offer from disease (Saeed *et al*., 2012). Also, natural-based antioxidants from a plant origin are seen as a promising approach as they are less toxic and more efective (Mishra *et al*., 2014).

Bioactive components of plants have a potential role in chemoprevention and inhibition of different phases of the malignant transformation process. Cancer continues to be a major health challenge, constituting the second- leading cause of death worldwide, despite intensive research that has revealed much about its biology in last few decades (Tariq *et. al*., 2017). The main carcinogens include smoking, unbalanced diet, hormones, chronic infections, genetic mutations, free radicals and UV radiation. According to research by the World Cancer Research Fund, as many as one-third of cancer causes in economically developed countries are related to overweight or obesity, physical inactivity and poor nutrition (American Cancer Society, 2015).

Plant-based bioactive phytochemicals are capable of inhibiting tumor cytogenesis through various means by inhibition or modification of epigenetic processes which suppresses gene initiation, suppression and progression (Greenwell and Rahman, 2015). Therefore, plant extracts and essential oils are in the focus of research, and in recent decades have been tested on a large number of malignant cell lines.

Research by Dai and Mumper (2010) confirmed that a diet rich in phenolic compounds significantly reduces the risk of developing malignancies. Thus, phenolic extracts of berries (blueberries, blackberries, raspberries, cranberries and strawberries), which contain anthocyanins, camphor, quercetin and esters of coumaric and ellagic acid, have been shown to inhibit the growth of colon cancer cell lines (Seeram *et al*., 2006; Zhang and Demain, 2005). Good cytotoxic potential of the cancer cells showed extracts of wine, black and green tea, citrus fruits, olive oil, apples and legumes (Dai and Mumper, 2010).

The anticancer potential of plant components is based on the ability to reduce free radicals, regulate carcinogen-activating and carcinogen-detoxifying enzymes, as well as the ability to inhibit inflammatory cytokines, then on the ability to lead to changes in the regulation of growth factors and target cell pathways. proliferation and apoptosis, as well as angiogenesis, invasion and metastasis of malignant cells (Surh, 2003; Amin *et al*., 2009; Mehta *et al*., 2010; Neergheen *et al*., 2010).

The Boraginaceae family includes approximately 2000 species worldwide, mainly in Europe and Asia (Dresler *et al*., 2017). Plants from the family Boraginaceae are traditionally used in the treatment of fever, asthma, kidney stones, as diuretics and for wound healing (Al-Snafi, 2014). Numerous studies have confirmed anticancer, antioxidant and antimicrobial potential of plant extracts from the family Boraginaceae (Bošković, 2018; Khurm *et al*., 2016; Erdogan *et al*., 2020; Paun *et al*., 2020). Further research and finding new bioactive components with antioxidant and anticancer potential and minimally

harmful effects on healthy untransformed cells is one of the challenges of science today.

Therefore, the aim of this study was to examine the antioxidant and cytotoxic potential of selected plant species from the Boraginaceae family.

MATERIAL AND METHODS

Plant material. Plants of *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. were harvested in the period May-June 2013 in flowering phenophase in area of Brđanska gorge near Gornji Milanovac in Serbia. The plant material was ground and degreased with petroleum and then extracted with a series of solvents (chloroform, ethyl-acetate, ethanol, acetone, petroleum) in a Soxlet apparatus. After cooling, they were evaporated on a rotary vacuum evaporator at a temperature of 40°C.

Determination of inhibition lipid peroxidation by ammonium thiocyanate method. The method is based on initiating lipid autooxidation at elevated temperature (Hsu *et al*., 2008). A series of solutions of extracts, ascorbic acid standards and butyl hydroxytoluene with a concentration of 1000 μg/ml in methanol is prepared. The linoleic acid emulsion is prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 emulsion agent and 50 ml of 0.2 M phosphate buffer to pH=7. Homogenization is carried out by vigorous stirring of the solution. A normal container with a prepared linoleic acid emulsion is coated with aluminum foil and left in the refrigerator.

In the series of eight tubes is weighed 0.5 ml of methanol.To the first tube is added 0.5 ml of the stock solution of the test substrate, ie standard, ascorbic acid and butylated hydroxytoluene. Pipette 0.5 ml of the mixed solution from the first tube and transfer to the second tube. By further successive dilution, batches of all solutions with a concentration of 3,901 - 500 μg/ml are prepared. After dilution, linoleic acid emulsion (2.5 ml, 0.02 M, pH = 7) and phosphate buffer (2.5 ml, 0.2 M and pH=7) were added to the extracts and standar solutions. After mixing, the solutions are incubated in the dark, at a temperature of 37ºC to accelerate the peroxidation process.

During the incubation process, aliquots of 100 μl solution are taken at different time intervals (after 24 h, 48 h, 72 h and 96 h) and the degree of autooxidation is determined by adding 4.7 ml of 75% ethanol solution, 100 μl of 30% ammonium thiocyanate solution and 100 μl of a 0.02 M solution of ferric chloride in 3.5% hydrochloric acid solution. After three minutes, by measuring the absorbance of the solution at 500 nm, the degree of inhibition of linoleic acid peroxidation is determined. Ethanol is taken as a test solution in the control sample. The percentage of inhibition is calculated by the formula:

IC (%) = [(Control sample - Sample) / Control test] x 100.

IC50 values are calculated using the formula already mentioned.

Determination of cytotoxic activity by MTT method. Cytotoxic activity of plant extracts was performed by variable MTT test in vitro. In the experiment were used cancer cells grown on nutrient media: Hep2 (medium: MEM Eagle/5%

FCS) human cell line (human larynx carcinoma), RD (medium: MEM Eagle/10% FCS)- (human cell line-rhabdomyosarcoma) and L2OB (medium: MEM Eagle/10% FCS) - (mouse tumor fibroblast line). Cell suspensions, density 10^4, were seeded in a 96-well microtiter plate and allowed to incubate at 37ºC and 5% CO_2 in a thermostat, and the medium was replaced with 100 µl of medium with different concentrations of extracts of plant extracts (concentrations 25, 50, 100, 250, 500, 750 and 1000 µg/ml). Fresh medium without extract was added to the control cells. After 48 h of treatment, cell viability was determined by the MTT cytotoxicity test based on the stained reaction of the mitochondrial enzyme dehydrogenase from living cells with MTT (Mosmann, 1983). After the incubation, MTT (at a final concentration of 5 mg/ml PBS) was added to each well and the plate was incubated for 2-4 h at 37°C. The colored crystals of the formed formazan were dissolved with 150 µl of DMSO. Absorbance was measured at 570 nm on a Microplate Reader. The percentage of viable cells was determined as the absorbance ratio of treated cells and control cells multiplied by 100. The results were obtained from three independent experiments. According to the American National Cancer Institute (NCI), the criterion for cytotoxic activity of plant extracts is IC_{50}<30 µg/ml (Itharat et al., 2004). Experiments using MTT [3-(4,5-dimethylthiazol-2-ol)-2,5-diphenyltetrazolium bromide] were based on the ability of viable cells to degrade tetrazolium with MTT mitochondrial succinate dehydrogenase to give a blue-colored product formazan.

Statistical processing of the obtained results was performed by analyzing the variance of the two-factorial experiment. Multiple mean comparisons were determined using the least significant difference (LSD) test. A probability value of 0.05 was considered significant. All calculations were performed using a statistical program (SPSS, version 11.0). The results of antioxidant activities are presented as the mean±standard deviation of the three determinations.

RESULTS AND DISCUSSION

The results of antioxidant activity of extracts of *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. are shown in Table 1. The results showed that ethanol extract of *Anchusa officinalis* had the highest lipid peroxidation inhibition (35.45±1.34 IC_{50} µg/ml), followed by chloroform (37.39±1.26 IC_{50} µg/ml) and acetone extract (37.51±1.11 IC_{50} µg/ml). Ethanol extract of *Echium vulgare* showed the highest lipid peroxidation inhibition (49.48 ±1.33 IC_{50} µg/ml), followed by acetone (50.50 ±1.10 IC_{50} µg/ml) and chloroform extract (51.34 ± 1.06 IC_{50} µg/ml). The acetone extract of *Echium italicum* showed the strongest ability to inhibit lipid peroxidation (42.54±1.13 IC_{50} µg/ml), followed by chloroform (43.29±1.20 IC_{50} µg/ml) and ethanol extract (44.56±1.29 IC_{50} µg/ml). The results of our research showed that plants from family Boraginaceae have good antioxidant potential. Also, good antioxidant potential of selected plant species from the family Boraginaceae confirmed Gharib and Godarzee (2016), Bošković (2018), Zemmouri *et al.* (2019) and Paun *et al.* (2020) in their study. Accordint to Paun *et al*. (2020) extracts of *A. officinalis* had

the excellent scavenging activity (IC50 = 0.0032 mg/ml), comparable with ascorbic acid as the reference (IC50 =0.0036 mg/ml). Our results indicate that polar solvents, such as acetone and ethanol, had better antioxidant potential than non-polar solvents, which is in line with the results obtained by Barchan *et al*. (2014).

Table 1. Inhibition of lipid peroxidation (IC_{50}) of extracts of plant *Anchusa officinalis, Echium vulgare* L. and *Echium italicum* L.

Extract/ Plant	*Anchusa officinalis*	*Echium vulgare L.*	*Echium italicum L.*
Chloroform	37.39 ± 1.26	51.34±1.06	43.29±1.20
Ethyl acetate	40.28 ± 1.23	55.22±1.27	47.26±1.12
Ethanol	35.45 ± 1.34	49.48±1.33	44.56±1.29
Acetone	37.51 ± 1.11	50.50±1.10	42.54±1.13
Petroleum	41.32 ± 1.08	56.38±1.02	49.36±1.10

The values of inhibition of lipid peroxidation are expressed in μg/ml

The results of cytotoxic activity of extracts of *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. on Hep 2c, RD and L2OB tumor cells in vitro are shown in Table 2. The cytotoxic effect of the extracts is expressed as IC_{50} μg/ml (concentration that inhibits 50% of cell growth), and the degree of inhibition depended on the type of plant and the solvent used. The tested plant extracts showed an inhibitory effect on cancer cells. But, chloroform (102.28 μg/ml) and acetone extracts (105.54 μg/ml) of *Anchusa officinalis*, chloroform (77.32 μg/ml) and acetone extracts (80.59μg/ml) of *Echium vulgare* and chloroform (87.30 μg/ml) and acetone extracts (91.56 μg/ml) of *Echium italicum* had the most effective effect on L2OB cells. The results of this study are consistent with the studies of Pehlivan-Caracas *et al*. (2012), which proved that plant extracts of *E. vulgare* cause significant inhibition of bile tumors (82%, 63% and 96%). The cytotoxic potential of plant extracts from the family Boraginaceae on the cancer cell line has been reported by other authors (Bošković, 2018; Poma *et al*. 2018; Erdogan *et al*. 2020). Paun *et al*. (2020) pointed out that *A. officinalis* extract caused moderate cytotoxicity on the on the cell line of mouse fibroblast cells line.

Statistical significance of the differences was observed between the plants *Anchusa officinalis* and *Echium vulgare*, *Anchusa officinalis* and *Echium italicum*, as well as between the plants *Echium italicum* and *Echium vulgare* on all cancer cells (factor A). Statistical significance of differences was also observed between ethyl acetate and petroleum extract compared to other tested extracts for Hep 2c cells and L2OB cancer cells (factor B). Chloroform and acetone extracts showed the most effective cytotoxic effect compared to other tested extracts, which can be related to their pronounced antioxidant capacity and

the presence of many pharmacologically active substances, which are in agreement with the results by Boskovic (2018).

Tabela br. 2. Citotoxic activity IC_{50} (µg/ml) tested extracts *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. on cancer cell

		Hep 2c	RD	L2OB
Plant	*Ancusa officinalis*	129.51	141.91	111.96
	Echium vulgare	108.69	121.1	91.16
	Echium italicum	117.37	129.76	100.47
Extract	Chloroform	131.34	163.23	88.97
	ethyl-acetate	160.08	175.25	137.67
	Ethanol	122.45	174.18	134.21
	Acetone	124.03	136.35	92.56
	Petroleum	163.79	107.59	146.69
Anchusa officinalis	Chloroform	144.67	176.53	102.28
	ethyl-acetate	173.39	188.59	150.34
	Ethanol	135.44	187.49	147.17
	Acetone	137.34	176.33	105.54
	Petroleum	176.77	120.57	159.35
Echium vulgare	Chloroform	119.65	151.59	77.32
	ethyl-acetate	148.43	163.56	125.30
	Ethanol	110.42	162.54	122.25
	Acetone	112.39	151.37	80.59
	Petroleum	151.81	95.61	134.38
Echium italicum	Chloroform	129.7	161.56	87.30
	ethyl-acetate	158.42	173.61	137.36
	Ethanol	121.48	111	133.20
	Acetone	122.37	162.35	91.56
	Petroleum	162.8	106.59	146.33
LSD 0,05	Factor A	1.676	1.504	2.308
	Factor B	2.37	2.126	3.263
	Factor AxB	4.105	3.683	5.652

Based on the results of antioxidant activity, it is possible to assume that extracts with pronounced antioxidant activity can affect the redox state of cells and thus lead to a decrease in cell proliferation. According to research by Robinson *et al.,* (2017) antioxidant and free radical scavenging activity of the extract may be the reason behind its anti-cancer property.

Finding new anticancer agents that would show a pronounced selective antitumor effect against malignant cells, as well as minimal toxic effect against healthy untransformed cells, and especially against healthy immunocompetent cells, which are involved in immune control of tumor suppression, is extremely important for the development of new drugs in oncology.

CONCLUSIONS

The plant world is an inexhaustible source of pharmacologically active components. Numerous studies in the past few decades have confirmed that these components have remarkable antioxidant potential, and are used in medicine in the prevention and treatment of many diseases, among them cancer. Therefore, the aim of this study was to examine the antioxidant and citotoxic potential of plant from the family Boraginaceae.

Research has indicate that ethanol, acetone and chloroform extracts of *Anchusa officinalis*, *Echium vulgare* and *Echium italicum* showed the best antioxidant activity.

The tested plant extracts showed an inhibitory effect on cancer cells, but chloroform and acetone extracts of the tested plants had the most effective effect on L2OB cells.

This study showed that plant extracts from the Boraginaceae family have cytotoxic potential on cancer cells, and these plants are a source of antioxidants. Isolation of individual active components from plants and their testing for cancer cells would be of great importance for this field of research.

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A QUALITATIVE AND QUANTITATIVE ANALYSIS OF EXTRACTIVES FROM THE SPECIES *Trifolium pratense* L. IN THREE DIFFERENT SOLVENTS

SUMMARY

This research focuses on the relative quantitative and qualitative analysis of extractives from the species *Trifolium pratense* L. samples that were collected from flowers, stems and whole plants. Extractions were carried out with a Soxhlet device and three different solvents (water, ethanol, dichloromethane) were used. Chemical analyses were conducted with gas chromatography and mass spectrometry. The results revealed significant amounts of chemical compounds, such as megastigmatrienone, phytol, squalene, carenol, borneol etc, found in the specimens. The identification of red clover genotypes containing high concentration of these compounds could have multiple applications in chemical, food and pharmaceutical industry.

Keywords: *red clover, extractions, gas chromatography, mass spectrometry*

INTRODUCTION

Red clover (*Trifolium pratense* L.) is a perennial forage legume, mainly used for agriculture (cutting in grass–clover leys of 2–4 years of duration), but also occurring naturally in permanent grasslands and meadows in temperate zones (Figure 1). Although red clover is one of the most important livestock plants, nowadays is showing tremendous potential upon analysis (Ball *et al*., 2015).

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Species of the genus *Trifolium* have been used in traditional medicine for many centuries. Some *Trifolium* species have biological properties such as antioxidants, anti-inflammatory, antiparasitic (stomach), estrogenic, cytostatic, cytotoxic and are used in cases of cancer or cardiovascular disease. They are also important sources of phytoestrogens in nature, mainly due to isoflavones. (Sabudak and Guler, 2009; Beck *et al*., 2005; Fugh-Berman and Kronenberg, 2001). The high concentration of quercetin, soyasaponin, flavonoids and isoflavones, make the seeds of some *Trifolium* species an ideal source of beneficial phytochemicals for the human diet (Kledjus *et al*., 2001; Polasek *et al*., 2007; Figheiredo *et al*., 2007; Pare, 2000).

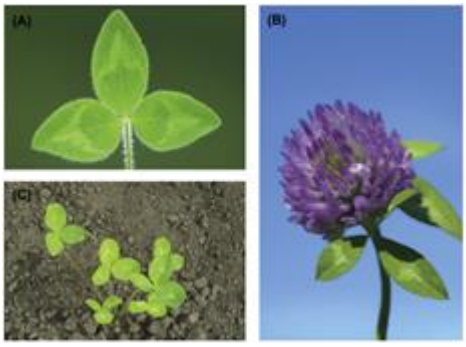

Figure 1. Red clover (A) leaf, (B) flower and (C) a seedling (Ball *et al*., 2015).

A study by Oleszek and Stochmal (2002) found that most species contain quercetin as the main flavonoid (0.05-0.3 mg/g) or with small admixtures of other, unrecognizable flavonoids. The components like biochanin A and isoflavanoides, genistein, daidzein, irilone etc. have been also isolated from the flowering tops, roots and leaves (Rehman, 2019).

In a research conducted by Booth *et al*. (2006), the chemical and biologic profile of red clover extract was studied, by identifying and measuring the major and minor components using visible HPLC-UV chromatogram and evaluating each compound for estrogenic and antioxidant activity. The preformulated extract was approximately composed of 35.54% isoflavones, 1.11% flavonoids, 0.06% pterocarpans, 0.03% coumarins,0.03% tyramine.

Ma *et al*. (2005), at their study, isolated the chemical constituents by chromatography and spectral analysis. The results showed that 8 compounds were obtained and identified as 1-octadecanol, 5-hydroxy-7,4′-dimethoxyisoflavone, biochanin A, prunetin, formononetin, genistein, ononin and daidzein. Compounds 1-octadecanol and 5-hydroxy-7,4′-dimethoxyisoflavone were found in this plant for the first time (Ma et al., 2005).

Moreover, Vlaisavljević *et al*. (2014), investigated the chemical constituents of the essential oils coming from *Trifolium pratense* in three different growth stages to test them for their antioxidant and antimicrobial activities, with the use of gas chromatography-mass spectrometry. As it is shown in Table 1,

numerous chemical compounds were found, mainly consisted of monoterpenes, sesquiterpenes, diterpenoids, aliphatic compounds and aromatic compounds.

At another research, Saviranta et al (2010), Vlaisavljević *et al*. (2017), aimed to investigate the phenolic content of red clover and its biological activity at various growth stages (30 cm growth, 50 cm growth and bud phase) of the plant. Isoflavonoids, genistein, and daidzein, as well as other phenols, p-hydroxybenzoic and caffeic acids, kaempferol 3-O-glucoside, quercetin 3-O-glucoside, and hyperoside were found in all the extracts, but the content of these compounds was the highest in the extract of the plant at the lowest growth stage (30 cm, vegetative). These results indicated that red clover has potential health benefits, and that growth phase affects its biological activity. The extract of red clover at the growth stage of 30 cm is a great source of bioactive compounds and could be used in phytotherapy and nutrition.

Table 1. Chemical composition of Trifolium pratense L. essential oil (Vlaisavljević *et al*., 2014)

No	Component	No	Component
1	Hexane	19	Decane
2	2-Pentanone	20	Undecane
3	Methylbenzene	21	Dihydrocarvone
4	1,3-Dimethylbenzene	22	Beta-ionone
5	1,4-Dimethylbenzene	23	10-Methylnonadecane
6	Pentanoic acid	24	Megastigmatrienone
7	7-Octen-4-ol	25	Hexadecane
8	Beta-myrcene	26	Dodecanoic acid
9	Cyclopropane	27	2,6-Diisopropylnaphthalene
10	Nonanal	28	Tetradecane
11	2,4-Heptadienal	29	Pentadecane
12	1-Bromocycloexane	30	Isopropyl myristate
13	Fenchyl alcohol	31	Tetrahydroionone
14	1,2,6-Hexanetriol	32	Hexahydrofarnesyl acetone
15	p-Cymene	33	Ocenol
16	L-Limonene	34	Phytol
17	Benzaldehyde	35	n-Hexadecanoic acid
18	Isobornyl thiocyanoacetate	36	Pentacosane

Tundis *et al*. (2015), studied the chemical profile and health properties of *T. pratense* (red clover) and *T. repens* (white clover). Furthermore, edible flowers were investigated for quercetin, kaempferol, luteolin, rutin, and myricetin that were used as markers and quantified by HPLC. The results support the use of *Trifolium* flowers as healthy food ingredients. Rehman (2019), at his review about *T. pratense* and its biological activities, indicated that red clover is a rich source of plant secondary metabolites isoflavonoids, which belong to the group of phenylpropanoids.

Saviranta *et al*. (2008) conducted at their study the quantification of three isoflavones in various clover species and their aerial plant parts by a high-

performance liquid chromatography. Isoflavone contents were quantified in the plants of 11 perennial and 4 annual species of genus *Trifolium*, among them the species *T. pratense*. HPLC revealed the concentration of biochanin A, isoflavones, daidzein, genistein, coumarin and other cardiac glycosides was high in *T. pratense*, among the rest of *Trifolium* species.

The aim of this research was to analyze the extractives of the species *Trifolium pretense* of Greek origin, in quantity as well as in quality, in order to obtain data that can promote the multiple uses of this species in various industrial sectors. The methodology applied differed from other studies, since Soxhlet device was used for the extractions. The interest of pharmaceutical and food industries for substances originating from plants is constantly rising and this essay wishes to contribute to that field.

MATERIAL AND METHODS

The material under investigation originated from Northern Greece (Holomontas, Chalkidiki) (Figure 2). The research took place during the period 06/2015-11/2015.

Figure 2. Map of the area where sample collection was conducted.

At first, the plant samples were carefully cleaned to remove soil and foreign particles, then the roots were removed and finally left to dry. (Figure 3). The flowers were separated from the stems at most samples. Then, all samples were cut into smaller pieces by hand with a sharp blade and were grinded with a mill (Wiley's mill) (Figure 3), in order to create particles with approximately the same dimensions, smaller than 0.1mm. The analysis was conducted on two samples in each case and a third one when needed, according to the standards. The total number of the collected plants was approximately 80-100 plants. Their average height was 31.32 cm (22,5-43,5 cm).

The quantitative estimation of extractives soluble in hot water, ethanol and dichloromethane were conducted according to ASTM Standards (ASTM D1107-

96, D1108-96, D1110-84). The specimens used for the extractions originated from the flowers, the stem or the whole plant. The stages of the extraction of the samples are illustrated in Figure 4.

For the extractions, a glass Soxhlet type device with the appropriate size was used, so as a 2,0g specimen and glass filter with medium porosity to be fit. Each hot water extraction lasted almost 6 hours and almost 4 hours for each of the other two solvents and 4 cycles of the solvent were repeated per hour.

Figure 3. Samples of the T. pratense plants collected, left to dry and grinded with Wiley's mill.

Figure 4. T. pratense samples after grinded in the mill and stages of extraction.

After the procedure, the specimens were removed from the Soxhlet device and left at normal conditions of temperature and humidity (approximately 25^{o}C and 55%) for 24h, before they were put in the oven at 103±2^{o}C for another 24 hours, until the total drying of the material. In the end, they were weighed to determine the dry weight of the extracted (wood) material, after the removal of the extractives (Chavenetidou, 2009).

Qualitative analysis of extractives was conducted with gas chromatography and mass spectrometry. The solvents containing the extractives after extraction was reduced with the use of rotary evaporator up to 1-2mL, in order to trace very small amounts of the chemical compounds of interest. In most cases, at the final stage of the procedure the reduction was reached with the use of nitrogen gas stream. In all cases, specimens were filtered in a chromatographic column (clean-up step) with the use of the following materials: Florisil (MgO_3Si) 2.5g, Al_2O_3 3.5g and Na_2SO_4 1.5g to absorb moisture or other materials that would damage the chromatographic column.

For the identification of the compounds and the quantification of the results, gas chromatograph Agilent 7890A was used, provided with non-polar capillary column DB-5ms, 30m length and 0.25mm internal diameter, film thickness 0.25μm and as a filler 5% phenyl polysiloxane, 95% methyl polysiloxane, using Helium as a carrier gas (flow 0.99333 mL/min, pressure 11.656 psi) and mass spectrometer with quadrupole Agilent 5975C). Finally, the mass spectrometer with quadrupole Agilent 5975C was also implemented and 1-bromo-2-nitrobenzene was used as internal standard for the estimation of the quantity (Tziouvalekas, 2011).

Two temperature programs were applied, in order to succeed better analyses. The temperature programs which were applied where:

1.Initial temperature: 60^{o}C – for 4 minutes. Final temperature: 240^{o}C for 5 minutes. Raising rate 50^{o}C/min

2.Initial temperature: 70^{o}C – for 4 minutes. Final temperature: 280^{o}C for 10 minutes. Raising rate 50^{o}C/min.

RESULTS AND DISCUSSION

In the following tables 2 and 3 the results from the statistical analysis are presented. According to the analysis of variance (Table 3), statistically significant differences were revealed between the amount of extracts released in the three different solvents that were used. Moreover, the part of the plant that was used for the analysis also played a crucial role as the differences between them were also statistically significant, although there was also statistically significant interaction between the two factors evaluated. The mean percentages of the extracts are presented in table 3.

Table 2. Analysis of variance for the three solvents and samples that was used for the analysis.

Source	df	F	Sig.
Solvent	2	384.800	0.000
Sample	2	13.571	0.010
Solvent * Sample	3	6.463	0.036
Error	5		
Total	12		

Table 3. Mean extract percentages of T. pratense samples soluble in water, ethanol and dichloromethane

Solvent	Mean %Extracts	Sample	Mean %Extracts
Dichloromethane	10.797a	Stem	18.707a
Ethanol	16.392b	Flower	20.539a
Water	30.433c	Whole Plant	26.704b

Means that differ statistically significantly ($p<0.05$) according to Tukey's multiple comparison test are followed by different letters.

As figure 4 shows, the percentage of the extractives was higher in the case of water used as a solvent, then ethanol and at last dichloromethane. In the cases that the whole plant was used for the extraction, the percentages of the extractives' content were higher than the rest. Both flowers and stems contain significant amounts of extractives, in all cases.

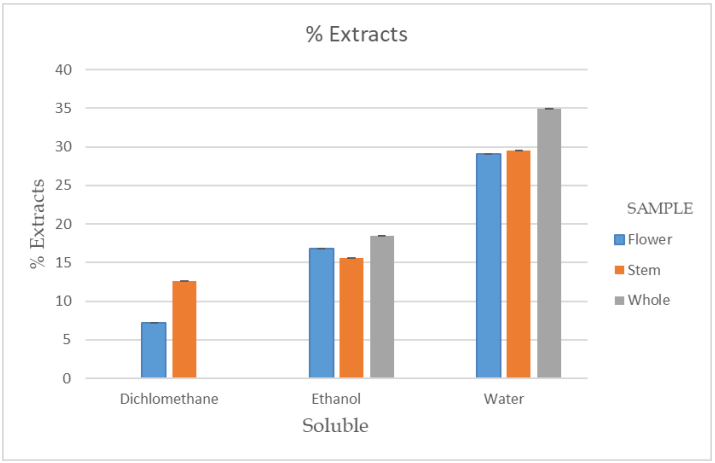

Figure 4. Extract percentage in three solvents (water, ethanol & dichlomethane).

The results of the qualitative analysis are presented in detail in the following tables (Tables 4, 5 and 6), which contain data from all the three solvents applied. The approximate amount of each substance was calculated by the fraction Integration area/internal standard area, with the use of **Benzene-1-bromo-2-nitro-** used as a standard area. From the detailed processing of the data it is obvious that:

-Specimens from different parts of the plant appeared to contain a few widely used chemical compounds, some of them in significant amounts, such as megastigmatrienone (stem-0.155), phytol (stem-3.324, flower-.0.72), squalene (stem-0.842, flower-0.140), linoleic acid (stem-0.783, flower-0.296), carenol (stem-0.107), borneol (stem-0.236), tetradecane (stem-0.587, flower-0.445), pentadecane (stem-0.094, flower-0.205), hexadecane (stem-0.151, flower-0.763), 7-tetradecene (stem-1.846, flower-0.123), hinesol (stem-0.575).

-Stem contained greater amount of pyrrolidine, 1-acetyl-, benzyl alcohol, tributyl acetylcitrate, squalene, 7-tetradecene, tetradecane and linoleic acid than flower

-Phytol appeared in much larger quantity at the stem than the flower

-Flower contained larger amount of benzeneamine, 4-bromo-, phenol, 2,5-bis (1,1-dimethylethyl) and bacchotricuneatin C than the stem

-Specimens appeared to contain some widely used chemical compounds, some of them in significant amounts, such as phytol (0.503), butyl citrate (0.792), oleanitrile (0.120), tetradecane (0.587), 7-tetradecene (1.846) and benzenamine-bromo (0.198)

-The samples extracted with the use of C_2H_5OH contained significantly larger amounts of phytol, 7-tetradecene and squalene than those extracted with the use of CH_2Cl_2

-Samples extracted with the use of CH_2Cl_2 contained, in most cases, different chemical compounds than those extracted with the use of C_2H_5OH

Table 4. Chemical compounds at stem after extraction with C2H5OH

Trifolium pratense stem			
Chemical compounds	Integration area/internal standard area	Chemical compounds	Integration area/internal standard area
Pyrazine, methyl	0.020	Megastigmatrienone	0.155
Benzaldehyde	0.135	Diethyl phthalate	2.464
Benzyl alcohol	0.137	n-capric acid	0.047
.gamma.dodecalactone	0.057	Hinesol.beta.panasinsene	0.575
Carenol	0.107	Isopropyl myristate	0.191
Pyrrolidine, 1-acetyl-	0.262	Thiophene	0.224
Benzeneamine, 4-bromo-	0.086	Phytol	3.324
benzothiazole	0.214	Linoleic acid	0.783
Benzene-1-bromo-2-nitro-	**1.000**	Tributyl acetylcitrate	1.047
2-(3-hydroxypropyloamino) pyrimidine	0.019	Triphenylene	0.484
Benzoic acid	0.016	Squalene	0.842
Phenol, 2,5-bis (1,1-dimethylethyl)	0.240	Piperidine	0.622
Bacchotricuneatin C	0.148	Benzopyranone	2.312
Borneol	0.236	Silane	0.183
1-Heptanol	0.244	7-Tetradecane	1.846
1-Octen-3-ol	0.137	Hexadecane	0.151
1-Octanol	0.176	Pentadecane	0.094
Hinesol	0.575	Heptadecane	0.153
Tetradecane	0.493	Nonedecane	0.307

The previous tables show that compounds mainly consisted of acids, phenols, aldehydes, ketones, alcohols, esters and hydrocarbons, findings in agreement with those of Kami (1978) and Vlaisavljevic *et al*. (2014). Moreover,

the chemical compounds found at this research agree with those of Vlaisavljević *et al*. (2014) as it is shown in Table 1. Compounds such as limonene, phytol, megastigmatrienone benzaldehyde, undecane, hexane, tetradecane, pentadecane, hexadecane were found in both essays.

Table 5. Chemical compounds at flowers after extraction with C2H5OH

Trifolium pratense flower			
Chemical compounds	Integration area/internal standard area	Chemical compounds	Integration area/internal standard area
Benzaldehyde	0.050	**Benzene-1-bromo-2-nitro-**	**1.000**
Piperidinone,1-methyl-	0.145	Phenol, 2,5-bis (1,1-dimethylethyl)	0.343
Silane	0.207	Benzophenone	0.196
Benzyl alcohol	0.081	Triallysilane	0.229
Phenylethyl alcohol	0.034	Phytol	0.072
Benzeneamine, 4-bromo-	0.379	Phthalic acid	0.046
Benzothiazole	0.083	Linoleic acid	0.296
Caprolactam	1.394	Tributyl acetylcitrate	0.099
Bacchotricouneatin c	0.964	Squalene	0.140
Pyrrolidine, 1-acetyl-	0.061	Undecane	0.042
Hexadecane	0.763	7-Tetradecane	0.123
Pentadecane	0.205	Carbonic acid	0.180
Hexane	0.288	1-Octanol	0.075
Oleic acid	0.067		

Table 6. Chemical compounds at stem after extraction with CH2Cl2

Trifolium pratense stem			
Chemical compounds	Integration area/internal standard area	Chemical compounds	Integration area/internal standard area
D- Limonene	0.009	Squalene	0.019
Limonene	0.032	Benzophenone	0.050
Piperazineethinamine	0.053	Isopulegol	0.004
Benzenamine-bromo	0.198	Oleic acid	0.007
Benzene-1-bromo-2-nitro-	**1.000**	Oleanitrile	0.120
Vanillin	0.010	Phytol	0.503
Naphthaelne,2,6-dimethyl-	0.006	Butyl citrate	0.792
m-Nitroaniline	0.012	Hexadecane	0.744
Tetradecane, 2,5-dimethyl	0.016	Tetradecane	0.587
3-Tetradecene	0.006	Dodecane	0.014
Hexadecanoid acid	0.330	N-Acetylpyrrolidone	0.032

CONCLUSIONS

Overall, results demonstrate a strong effect of the solvent that was used on the percentage of the extractives. As depicted in figure 4, the percentage of the extractives was higher when water was used as a solvent, followed by ethanol and at last dichloromethane. Additionally, when the whole plant was used for the extraction, the percentages of the extractives' content were higher than the rest.

Both flowers and stems contain significant amounts of extractives, in all cases. The findings of the chemical analysis demonstrated that specimens from different parts of the plant appeared to contain some widely used chemical compounds, some of them in significant amounts, such as megastigmatrienone, phytol, squalene, linoleic acid, carenol, caprolactam, bacchotricuneatin C and borneol. Furthermore, samples appeared to contain some widely used chemical compounds, some of them in significant amounts, such as phytol, linoleic acid, butyl citrate, oleanitrile and benzenamine-bromo, hexadecane. Overall, results show a great potential of the species *Trifolium pratense* for the use of their chemical compounds by various industrial sectors.

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ESTIMATE OF WATER EROSION IN COFFEE GROWING AREAS IN SERRA DA MANTIQUEIRA, MINAS GERAIS STATE, BRAZIL

Abstract

Water erosion is a major cause of soil degradation worldwide. This natural phenomenon has been continually accelerated by anthropogenic activities, with constants increase of soil losses. The main objective of this study was to apply the Modified Universal Soil Loss Equation (MUSLE) model to estimate soil loss in rainy events during one year period. The total study was conducted between August 2017 and July 2019 in coffee growing areas located in the Serra da Mantiqueira, Southern Minas Gerais, Brazil. Most of the factors used for the MUSLE equation were determined from Geographic Information Systems. The results showed that soil losses ranged from 53.40 to 28.37 Mg in both areas depending on the land use and 33.12 and 23.82 Mg related to the soil classes. The largest soil losses were estimated to exposed soils in eucalyptus without conservationist practices, in the highest slopes and in Haplic Cambisol (CX). It was concluded that the conservation management practices correlated to anthropic activities adopted in the coffee crop contributed to the reduction of soil losses and maintenance of edaphic conditions.

Keywords: Soil losses, Prediction models, Modified Universal Soil Loss Equation, MUSLE, Brazil.

INTRODUCTION

Coffee is one of the most important natural products of international trade. About 63% is produced in Central and Southern America, 30% in Asia and 7% in

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Africa (Potts, 2014). In Brazil, Minas Gerais State accounts for most of the productive area, with about 1.23 million hectares (CONAB, 2019) and gross production of 30,735,800 bags of coffee. Minas Gerais Southern is responsible for a large part of this production, with about 655,221 hectares destined for planting (CONAB, 2019).

Despite its socioeconomic relevance, coffee production in the Southern Minas Gerais is also characterized by extensive land use, inadequate edaphic management, inefficient crop rotation, misuse and lack of herbicide alternation and non-application of related plans the Integrated Management of Pests and Diseases - MIPD (Giunti, 2017). These elements are responsible for gradually intensifying erosion processes (Bertoni and Lombardi Neto, 2014, Dechen *et al*., 2015), increasing losses of soil, water, organic matter, nutrients and agrochemicals, which deplete arable land and harm the sustainability of agricultural systems (Oliveira *et al*., 2012).

Over time, simulation models of erosive processes have been developed and improved. One of these models is the Modified Universal Soil Loss Equation - MUSLE (Williams, 1975), capable of estimating the soil losses that occur in a given period or after isolated rain events. The MUSLE data entry is simple and the results can be obtained quickly, based on the runoff, the peak flow, the edaphic properties, the terrain characteristics and the adopted management practices.

This study estimated the soil losses in Latosols and Cambisols, with a predominance of coffee cultivation, in high altitudes of the Serra da Mantiqueira, Southern Minas Gerais, aiming to identify the areas with the greatest soil losses and to indicate measures mitigates of erosive processes.

MATERIAL AND METHODS

The study area is the Rio Verde Farm, located at Ribeirão José Lúcio hydrographic Subbasin, a tributary of the Rio Verde, located in the Conceição do Rio Verde Municipality, Minas Gerais State, Brazil. The area has 1,355.39 ha and is located at coordinates 7575500 to 7570000 N and 479000 to 483800 E, Datum SIRGAS 2000, Zone 23 K (Figure 1).

Figure 1: Study area location map

The maps were produced in ArcGIS 10.2 (ESRI, 2014). The digital land use and occupation map was produced from images Landsat-8 TM (Thematic Mapper) satellite, bands TM6, TM5, and TM4, orbit 219/75, obtained from the United States Geological Survey (USGS, 2017); images from Google Earth and maps provided by Ipanema Coffees were also used. The classification of land use and occupation was based on mappings by Ipanema Coffees, visually by analyzing satellite images and subsequent verification and correction in the field (Figure 2).

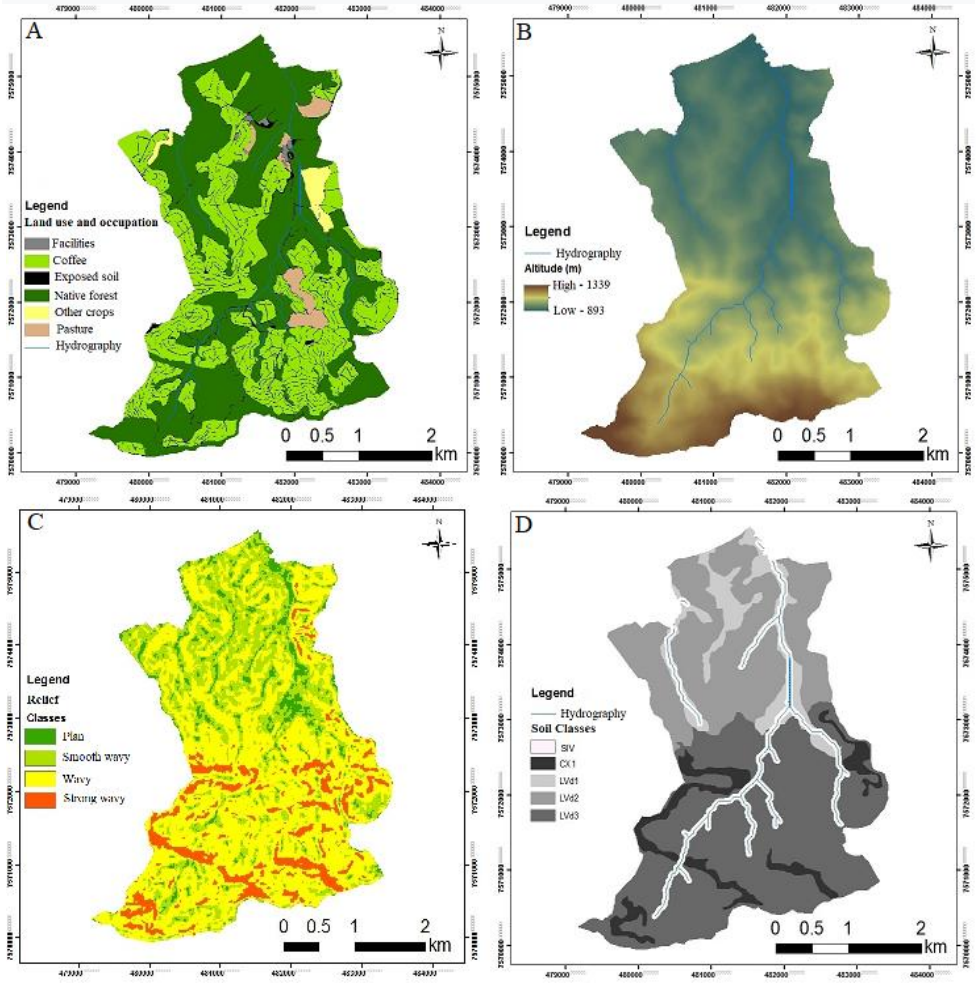

Figure 2: Land use and occupation map in the Ribeirão José Lúcio Subbasin (A), Digital Elevation Model (DEM) (B), Relief (C) and Soil Classes (D).

Notes: Other crops refer to eucalyptus, mahogany and macadamia.

SIV = Indiscriminate Lowland Soils; CX1 = Haplic Cambisol; LVd1 = Dystrophic Red Latosol in flat and smooth undulated relief; LVd2 = Dystrophic Red Latosol in undulated relief; LVd3 = Dystrophic Red Latosol in strong undulated relief.

The digital elevation model (DEM) was generated from the interpolation of the contour lines of the Varginha Topographic Chart (IBGE, 1979). The value of each cell (pixel) was 12.5 x 12.5 m.

The terrain slope map was generated by the Slope tool applied in the MDE (ESRI, 2014). The relief of the Subbasin was classified according to EMBRAPA (2018).

The digital map of soil classes was produced based on the Minas Gerais State Soil Map, scale 1:650,000 (UFV *et al*., 2010). The relief classes present in the slope map guided the definition of the soil classes (McBratney *et al*., 2003), together with the morphological field descriptions and physical and chemical analyzes carried out in the Soil Laboratories of the Department of Soil Science of the Federal University of Lavras.

From the maps, 18 points were selected for soil collections. At each sampling point, collections were carried out at depths 0 – 20 cm and 20 – 40 cm. Deformed, undisturbed clod-shaped and undisturbed samples were collected with a cylindrical sampler with a volume of 92.53 cm³ and a height of 5 cm. The soils were described according to Santos *et al*. (2005).

Soil loss in watersheds was based on the MUSLE (Williams, 1975) (Eq. 1).

$$A = 11.8 \times (D \times Q_p)^{0.56} \times K \times LS \times C \times P \quad (1)$$

where: A - represents soil losses (Mg); 11.8 and 0.56 - original MUSLE adjustment coefficients (dimensionless); D - runoff or surface runoff (mm); Qp - peak flow ($m^3\ s^{-1}$); K - average erodibility of the basin soil (Mg h MJ^{-1} mm^{-1}); LS - topographic factor (dimensionless); C - land use and management factor (dimensionless); P - conservation practices factor (dimensionless).

To estimate the K factor of soils, the model 3 (Silva *et al*., 1999) was adopted to classified as Latosols, which has the r^2 de 0.91 and adopts easily measured variables. The K factor of model 3 was estimated by the equation below: (Eq. 2).

$$Y = -3.89 \times 10^{-2} + 5.11 \times 10^{-3} X_{14} - 1.25 \times 10^{-2.} X_{15} + 5.41 \times 10^{-3} X_{16} - 7.27 \times 10^{-3} X_{18} + 5.33 \times 10^{-2} X_{33} + 3.21 \times 10^{-5} X_{34} - 5.66 \times 10^{-5} X_{36} + 8.33 \times 10^{-4} X_2 - 1.17 \times 10^{-2} X_4 + 1.53 \times 10^{-2} X_{13}$$
$$r^2 = 0.91^{**} \quad (2)$$

The K factor for soils classified as Cambisols was estimated by the indirect method for the two depths (0 - 20 cm and 20 - 40 cm) through the expression of Bouyoucos (Bertoni and Lombardi Neto, 2014). The model is described below: (Eq. 3).

$$K = [(\%sand + \%silt)/(\%clay)]/100 \quad (3)$$

where: K - average soil erodibility (Mg h MJ^{-1} mm^{-1}); % sand, % silt and % clay - percentages of the respective fractions for each depth (Table 1 and 2).

Table 1. Erodibility values (K) and the variables used

Variable	Description	LVd1	LVd2	LVd3	CX1
K	Erodibility (Mg ha MJ^{-1} mm^{-1})	0.0227	0.0149	0.0218	-
$\mathbf{X_2}$	Soil cation exchange capacity at pH 7.0 (cmolc kg^{-1})	6.49	9.17	8.23	8.18
$\mathbf{X_4}$	ΔpH = pH KCl – pH H_2O (adimensional)	-1.43	-0.71	-0.90	-0.95
$\mathbf{X_{13}}$	Ki Relation (adimensional)	1.33	1.33	1.33	-
$\mathbf{X_{14}}$	MSM Munsell (adimensional)	2.00	3.00	3.00	3.00
$\mathbf{X_{15}}$	Soil profile drainage code (dimensionless)	4.00	4.00	4.00	4.00
$\mathbf{X_{16}}$	Structure grade code (adimensional)	2.00	3.00	2.00	2.00
$\mathbf{X_{18}}$	Structure shape code (adimensional)	3.00	3.00	3.00	3.00
$\mathbf{X_{33}}$	Total pore volume (dm^3 dm^{-3})	0.62	0.60	0.60	0.61
$\mathbf{X_{34}}$	Flocculation index (dimensionless)	0.54	0.61	0.50	0.42
$\mathbf{X_{36}}$	Aggregate instability index (g kg^{-1})	97.60	93.60	93.60	92.05

Notes: ΔPh - difference between pH KCl and pH H_2O; pH KCl - pH determined in potassium chloride solution; pH H_2O - pH determined in water solution; Ki Relation - molecular ratio between SiO_2 and Al_2O_3. Source: Silva *et al*. (1999).

Table 2. Erodibility values (K) of Cambisols and the variables used

Variable	Description	Class	%sand	%silt	%clay	K
K	Erodibility	CX1	47	26	27	0.0270
K	Erodibility	CX2	35	30	35	0.0185

Notes: CX1 - Haplic Cambisol in depth 0 – 20cm; CX2 - Haplic Cambisol in depth 20 – 40cm. Source: Mannigel *et al*. (2002).

The topographic factor LS was estimated according to the methodology of Moore and Burch (1986) in ArcGIS 10.2. from the DEM Topodata, with 12.5 m resolution. The expression of Moore and Burch (1986) was inserted in the Raster Calculator function (Eq. 4).

$$LS = (\text{Slope Length} * 12.5/22.13)^{0.4} \times (0.01745 * \text{Slope Degree}/0.0896)^{1.4} \times 1.4 \quad (4)$$

where: LS - topographic factor (dimensionless); Slope Length - flow accumulation (dimensionless); 12.5 - DEM cell size; Slope Degree - slope in

degrees. C Factor and P values were obtained from the specialized literature (Table 3).

Table 3. C Factor and P values for verified uses and managements

Land use and occupation	C Factor	Source C Factor	P Factor
Coffee (3,95 x 0,55 m)	0.13	Prochnow *et al*. (2005)	0.50
Degraded pasture	0.10	Panagos *et al*.(2015a)	1.00
Native forest	0.01	Silva *et al*. (2016)	0.01
Facilities	0.01	Panagos *et al*. (2015a)	1.00
Eucalyptus down the hill	0.30	Martins *et al*. (2010)	1.00
Exposed soil	1.00		1.00

Notes: P values obtained from Bertoni and Lombardi Neto (2014) and Panagos *et al*. (2015b). Only data referring to eucalyptus were used for the land use and occupation class "other crops".

To calculate the runoff (D) the abstraction method (SCS, 1972) was used through Equation 5 below: (Eq. 5).

$$D_{ij} = \frac{(P - 0.2 \times Sa)^2}{(P + 0.08 \times Sa)} \quad (5)$$

where: Dij - runoff volume (per pixel) for a given rainy event (mm); P - maximum precipitation volume (mm); Sa - soil water retention parameter obtained from the land surface characteristics.

The parameter Sa (Equation 6) was determined as a function of the relationship between the parameters: (a) soil class, land use and occupation, slope and adopted management; (b) time, as a function of soil water content. The time is derived from the Curve Number (CN) index (Eq. 6).

$$\mathrm{Sa} = 254\left(\frac{100}{\mathrm{CN}-1}\right) \quad (6)$$

Soil hydrological groups were obtained from Sartori *et al*. (2005). LVd belongs to Hydrological Group A (very deep, high infiltration rate, more resistant to erosion and clayey texture); CX1 belongs to Group C (deep or shallow, reduced infiltration rate, less resistant to erosion and sandy texture).

Peak flow and sediment rate (Qp) were quantified for one year. Peak flow (Qp) was measured directly in the Parshall gutter installed in the watercourse of the Ribeirão José Lúcio Subbasin. For surface runoff, values were assigned for each pixel in the area. Soil loss maps were produced every 3 months.

RESULTS AND DISCUSSION

The values of the areas referring to land use and occupation, soil classes and relief are presented in Table 4.

Table 4. Land use and occupation, soil classes and relief values

Land use and occupation

Type	Area (ha)	Area (%)
Exposed soil	80.20	5.91
Facilities	18.18	1.22
Coffee	543.94	40.26
Native forest	669.01	49.35
Pasture	34.05	2.52
Other crops	23.18	1.72
TOTAL	**1,355.39**	**100**

Soil classes

Tipe	Area (ha)	Area (%)
CX1	94.10	6.94
LVd1	121.16	8.88
LVd2	411.90	30.43
LVd3	676.03	49.87
SIV	52.20	3.90
TOTAL	**1,355.39**	**100**

Relief classes

Relief	Slope (%)	Area (ha)	Area (%)
Plan	0 – 3	80.22	5.97
SW	3 – 8	359.78	26.78
Wavy	8 – 20	771.09	57.40
StW	20 – 45	132.10	9.45
TOTAL		**1,355.39**	**100**

Notes: SW – smooth wavy relief ; StW – strong wavy relief.

CX1 obtained the highest erodibility values due to the high sand fraction indexes. LVd2 had the lowest erodibility values due to the high content of organic matter (X_3), the lowest coarse sand content dispersed in water (X_{27}), the highest clay content dispersed in water (X_{32}) and the second highest value of the index of aggregate stability (X_{36}).

K values were lower than those found by Demarchi and Zimback (2014) for LVd. This fact is due to the difference in the attributes of each soil and also to the methodology used to obtain the K factor, since these authors used the methodology of Mannigel *et al*. (2002), which considers only the texture to obtain erodibility and overestimates the final values of K.

The K values for CX1 were higher than the values found by Silva *et al*. (1999), which was determined to be 0.0355. In the Ribeirão José Lúcio Subbasin the soils are derived from quartzites and metavolcanosedimentary sequences.

Quartzites produce sandy soils and this is visible in steep reliefs. In the lower altitudes, with the metavulcanosedimentary sequences, it is more clayey. This variation is due to the different procedures for obtaining the factor and the distinction of sand values can be highlighted, having lower contents in the work by Silva *et al*. (1999).

The LS factor ranged between 0 and 617.29 and an average value of 7.28. The model proved to be efficient in determining the LS factor, as the highest values were found in areas with higher slopes and more intense flows. In the areas where the ramp length is greater, the average was high, however the values were close. The highest value of C was obtained in the exposed soil, (C = 1.00), followed by eucalyptus downhill (C = 0.30). Coffee obtained a value of 0,13. The lowest value of C was obtained in the urbanized area; however, erosion is not calculated at this location, only surface runoff. The native forest had a value of 0.01. Regarding the P factor, the highest values were obtained in degraded pasture, exposed soil and eucalyptus downhill (P = 1.00). The lowest value was found in native forest (0.01), while coffee planted in contour lines had an intermediate value (0.50).

The native forest has the lowest CN values, while the highest values were obtained in the exposed soil. This fact occurs due to the vegetation cover, which attenuates runoff, while the impermeability of the soil increases runoff.

Table 5. Rainfall events analyzed over a one-year period

Moment	Date	Precipitation Index (mm)	Q (m^3)	Qp (m^3/s)	Y – MUSLE (Mg)
1	02/22/2018	33 mm	1,860.68	0.040	13.876
	03/17/2018	46 mm	2,398.96	0.083	22.915
	03/28/2018	30 mm	1,759.33	0.039	12.134
2	05/02/2018	17 mm	989.11	0.022	6.239
	06/28/2018	9 mm	443.45	0.009	6.641
	07/07/2018	11 mm	556.97	0.013	12.898
3	09/03/2018	26 mm	1,429.08	0.026	21.337
	11/11/2018	19 mm	1,397.65	0.020	16.032
	11/25/2018	38 mm	2,289.06	0.045	20.430
4	12/02/2018	51 mm	2,464.21	0.052	31.038
	01/05/2019	40 mm	2,105.73	0.041	24.872
	01/23/2019	28 mm	2,090.08	0.033	25.631

where: Q – surface runoff or runoff; Qp - peak flow and Y - soil loss

The runoff map is showed in Figure 3 and the estimated values of soil losses according to land use and occupation are listed in Table 6.

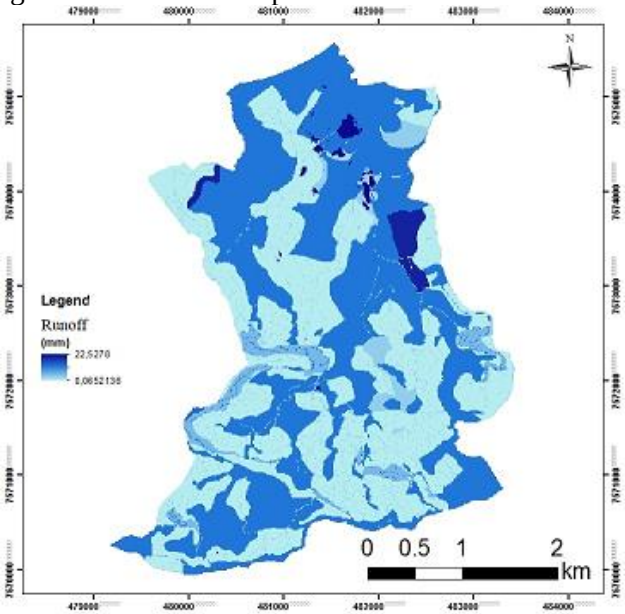

Figure 3. Runoff map in the Ribeirão José Lúcio Subbasin

Table 6. Subbasin soil losses according to land use and occupation

	Moment 1		Moment 2	
Land use and occupation	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**
Exposed soil	10.66	0.15	7.94	0.09
Coffee	13.71	0.02	8.33	0.01
Native forest	4.09	0.01	2.54	0.01
Pasture	10.91	0.37	6.66	0.19
Other crops	19.88	0.88	10.07	0.43
TOTAL	53.40	0.29	35.54	0.15
	Moment 3		Moment 4	
Land use and occupation	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**
Exposed soil	9.34	0.14	12.75	0.17
Coffee	10.98	0.02	15.02	0.03
Native forest	3.66	0.01	4.44	0.01
Pasture	9.03	0.25	11.96	0.40
Other crops	17.55	0.60	20.22	0.95
TOTAL	50.56	0.26	64.39	0.35

According to land use and occupation, the total land loss estimated by MUSLE in the Ribeirão José Lúcio Subbasin was variable in the 4 moments. The highest soil losses were obtained at time 4, which corresponds to the season with the highest rainfall. The highest average soil losses were obtained in other crops. This fact is due to the large spacing between the eucalyptus trees, as they are at an early stage of development, the high slope at the cultivation site and mainly due to the absence of conservation management.

The native forest favored infiltration and reduced surface runoff, attenuating the carrying of soil particles and consequent transport of sediments. This result is evidenced in another study (Bolleli *et al*., 2020), since the organic matter in the edaphic composition attenuates surface runoff and favors water infiltration. Coffee cultivation also had low soil loss values, demonstrating the efficiency of crops in mitigating erosive processes and maintaining soil properties, as demonstrated in Tavares *et al*. (2019).

The native forest occupies approximately 49.35% of the area, and has very low estimated soil loss values, stressing once again the importance of vegetation cover in reducing the erosive process (Lense *et al*., 2020). Compared to other works, the average soil loss values were higher than those obtained by Silva *et al*. (2016) due to the steeper relief. Soil losses related to the exposed soil were low, as this is located mainly between the coffee planting rows, a fact that contributes to the reduction of runoff. Soil loss values according to soil classes are shown in Table 7.

Table 7. Soil losses according to soil classes

	Moment 1		Moment 2	
Soil Classes	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**
CX1	13.90	0.17	10.55	0.13
Lvd1	12.98	0.12	9.61	0.10
Lvd2	7.77	0.02	5.73	0.01
LVd3	5.08	0.01	4.17	0.01
*SIV	-	-	-	-
TOTAL	37.73	0.38	28.46	0.25
	Moment 3		Moment 4	
Soil Classes	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**	**Total Loss (Mg)**	**Average Loss (Mg ha^{-1})**
CX1	12.46	0.13	15.99	0.16
Lvd1	11.55	0.11	14.21	0.13
Lvd2	6.90	0.02	7.81	0.02
LVd3	5.85	0.01	6.19	0.01
*SIV	-	-	-	-
TOTAL	34.76	0.33	41.20	0.42

Notes: SIV was considered sediment deposition area.

According to soil classes, the greatest total and average soil losses occurred in CX1 and the smallest in LVd3. Class CX1 is more susceptible to the erosive process due to the intrinsic attributes of this soil, such as high sand content, low clay content, and is associated with higher slopes and the presence of greater trails.

According to Gelagay and Minale (2016), the highest average soil losses were also found in high slope locations for the Koga watershed in Ethiopia. This fact can be verified in Oliveira *et al*. (2012), who demonstrated the direct relationship between precipitation, runoff and sediment transport. These authors used the ArcMUSLE (Zhang *et al*., 2009) to assess the production of sediment and consequent loss of soil. Dechen *et al*. (2015) also found that the higher the percentage of soil cover, the lower the runoff and the lower the losses of water, soil, organic matter and soil nutrients. The final maps of soil loss estimates are shown in Figure 4.

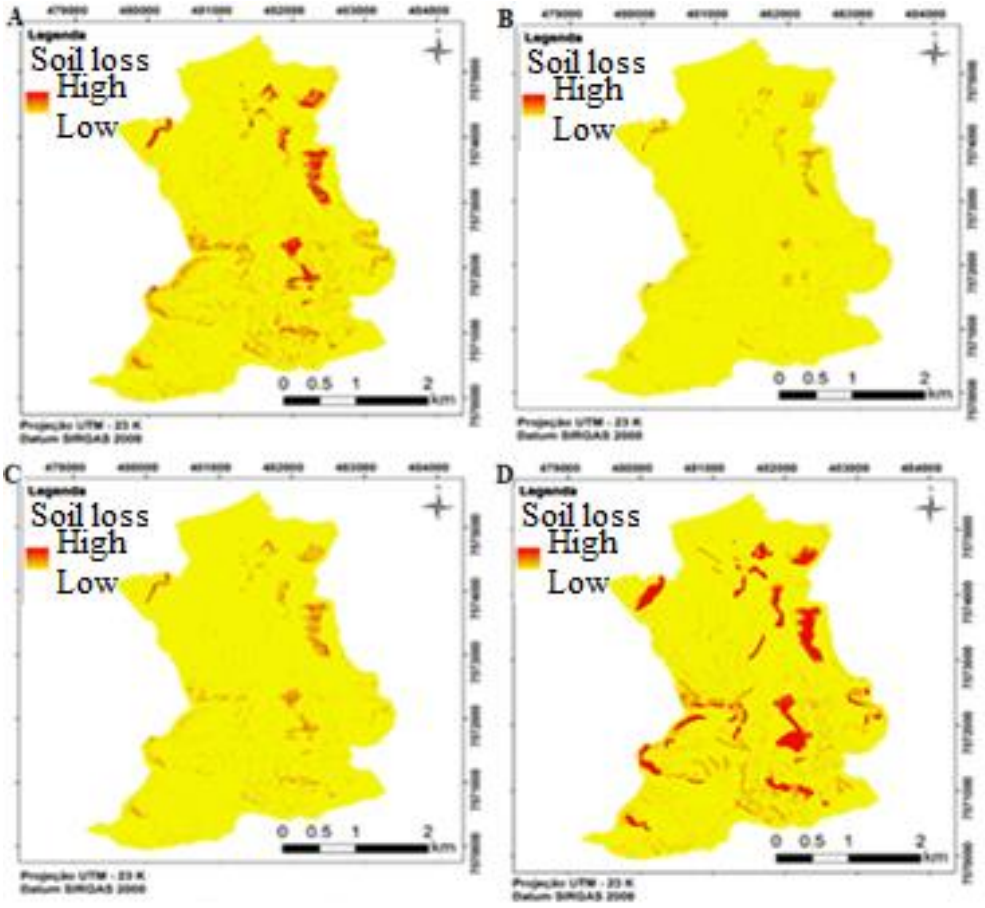

Figure 4. Estimative soil losses maps in the Ribeirão José Lúcio Subbasin, (A) Moment 1; (B) Moment 2; (C) Moment 3 and (D) Moment 4

CONCLUSIONS

The lowest estimates of total and average soil loss occurred in native forest, demonstrating the vegetation's efficiency in mitigating erosive processes. Coffee has also been shown to be directly efficient in mitigating erosive processes, especially in steeper terrain.

Class CX1 was the one that presented the highest estimates of average and total soil loss as a function of the more sandy content, the high K value and also for being inserted in the highest slopes and under the planting of other crops.

MUSLE proved to be efficient in the spatial identification of soil losses, revealing the efficiency of native forest and conservation practices adopted in the coffee plantation, such as contour and contour planting, reducing surface runoff and consequently soil losses.

Finally, it is concluded that the influence that anthropic interventions exert on the landscape is directly responsible for environmental susceptibility and the quality of resources. Because of this, studies on this bias are essential to understand and monitor soil erosion processes and the consequent sustainable management of resources.

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FRENCH WINTER WHEAT VARIETIES UNDER UKRAINIAN NORTH STEPPE CONDITION

SUMMARY

The main purpose of research was to assess the variety material of bread winter wheat of the Western European ecotype (variety type), obtained from the Laboratory of Ecophysiology and Biodiversity of Cereals INRA (France). The parameters of evolution are winter resistance, grain quality, yield and its structure under the North Steppe of Ukraine conditions (at compare with the national standard and leading local variety) and make a phenological description of the material. In the context of climate change and more moderate winter conditions at the Steppe zone of Ukraine, the issue of priorities in estimation of new genotypes of winter wheat was of interest. Early maturity as a way to avoid early drought at key development stages was found to be no longer as a key priority. The advantage of some western varieties concerning local ones by grain production has been revealed, which is due to a fundamentally new approach to yield formation by individual components of the yield structure. The disadvantages (mainly by grain quality) for these samples are also shown. New sources of genetic material for breeding programs by grain quality in winter wheat have been identified. Structural differences in improving winter wheat as a crop between local and foreign approaches are characterized.

Key words: winter wheat, variety, grain productivity, quality.

INTRODUCTION

With an annual production of about 764.5million tons (2019) (USDA, 2020), bread wheat (*Triticum aestivum* L.) is one of the most important crops in the world. Winter wheat is the leading grain crop in terms of total yield and

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cultivation in the world and the most important, top one in Ukraine food grain crop. Bread wheat occupies 48% of the area under cereal crops and gives 38% of total grain production (Nazarenko and Bezus, 2018).

The territory of Ukraine is characterized by diversity on climatic zones and extremely unstable meteorological conditions by years and seasons. The presence of different natural-contrast zones leads to the creation of varieties genetically diverse by at least three main agroecotypes of winter wheat (Li *et al*., 2019). Varieties of semi-intensive and intensive winter wheat are characterized by high or above average tilling capacity, demands in precipitations during development critical phases, a fairly high frost and winter resistance. In the phase of full tilling, the plants tolerate well low negative temperatures up to -20°C, form large, well-grained spike with grains above than average or high quality (Mba *et al*., 2012; Nazarenko *et al*., 2019b).

Many years of breeding and genetic research have created a significant number of valuable hybrid populations. Tests of these materials in contrasting ecological zones contributed to the creation of adapted agroecotypes of high-yielding varieties of winter wheat. Over the last 15 years, new complex varieties of intensive winter wheat have been created and zoned, which are characterized by high yields, adaptability, disease resistance, as well as high grain quality parameters and fairly high winter resistance and drought resistance (Tuberosa and Salvi, 2006).

Agroclimatic steppe conditions are characterized by significant variability in weather conditions over the years, growing seasons, areas, which determine the existing size and variability of winter wheat yield in the region (Bhutta *et al*., 2005).

Along with the improvement of cultivation technology, an important role in increasing yields and reducing its variability by the years in the region belongs to the variety genotype. Regarding this, the key priority is to gain yield through breeding winter wheat varieties able to use environmental resources and peculiarities of the ecological zone most efficiently, to resist abiotic and biotic stressors (Andrusevich *et al*., 2018).

It is established that moisture supply above than average long-term level at critical stages of development ensures positive correlation of the yield with plant survival, with the number of plants per 1 m^2 before harvest, weight of 1000 grains, grain weight of main spike. Under highly intensive complex abiotic stress (freezing, drought), grain yield was positively correlated with winter resistance and grain nature. It is important to winter wheat breeding in the steppe to increase the resistance of varieties to abiotic stresses (drought, freezing, rotting) (Tester and Langridge, 2010).

The development of synthetic breeding for quality was less efficient than for yield, although better product quality is more important than just increased yield. For example, it is believed that increasing the protein content in the grain by 0.1% is equivalent to producing an additional 0.6-0.7 t ha^{-1} of grain (Bordes *et al.*, 2008; Bordes *et al*., 2011).

The main achievements in the breeding of wheat for grain quality belong to scientists in the southern regions, where there are appropriate agroclimatic conditions (temperature, moisture, soil fertility) (Mba *et al*., 2012).

Product quality parameters (protein, gluten, fat, sugars, vitamins, etc.), as a rule, positively correlate with the resistance of cultivated plants to abiotic and biotic stressors and negatively with high yields (Tribo *et al*., 2003). The greatest importance in the formation of high yield and grain quality has stability of weather conditions in the critical stages of growth and development of plants (Rangare *et al*., 2010).

The most important factors in increasing yield parameters are: collection, identification, conservation, and study of genetic diversity of plants for their wide inclusion of economic and valuable traits and adaptive reactions in the breeding process (Gepts and Hancock, 2006); selection of varieties that combine high potential yield and quality with resistance to toxic and negative environmental factors (Tester and Langridge, 2010); use of the main mechanism of stability of cultivated plant species – avoidance of the action of stressors in time and space due to adaptive macro-, meso- and micro-zoning of crops, as well as optimization of their species and varietal structure (Andrusevich *et al*., 2018); design of highly productive and ecologically sustainable agroecosystems and agrolandscapes based on the use of greater biological diversity of cultivated species and varieties (Nazarenko *et al*., 2018; Nazarenko *et al*., 2019a); the use of biologically active substances to optimize the growth and development of plants in accordance with the weather and other environmental conditions (Prabhu, 2019); more time- and space-differentiated (high-precision) use of natural, artificial, biological, labor, and other resources (Shewry *et al*., 2012); development of standards of parameters of fodder grain, requirements for technological and breeding aspects of its quality management (Oury and Godin, 2007); use of mechanisms and structures of potential productivity, ecological stability, and yield quality (Li *et al*., 2019); efficient use of anthropogenic energy (fertilizers, pesticides, irrigation, regulated microclimate, etc.) (Andrusevich *et al*., 2018; Datcu *et al*., 2020).

The bread-making qualities of wheat are determined by the content of some components of glutenins and gliadins. The start of the formation of the same components is depends on variety peculiarities. Twenty days after pollination, the rapid accumulation of glutenins begins, which peaks on the 28th-31st day after pollination, and the accumulation of high-molecular glutenins significantly prevents the accumulation of gliadins (Bordes *et al*., 2011).

One of the most multifunctional stress metabolites of plants is the concentration of soluble sugars. In free form, these compounds can exhibit a multifunctional biological effect, which is evident in the osmoregulatory and protective function. The stress-protective effect of sugars is their ability to interact with macromolecules directly or indirectly and thus contribute to the preservation of their native conformation. Undoubtedly, concentrations of soluble sugars are one of the components of the stress response. This is evidenced by their high rate of accumulation, as a corresponding effect on the action of extreme

factors, and the relative non-specificity of a number of biological effects (Shah *et al*., 2018; Khalili *et al*., 2018).

MATERIAL AND METHODS

Experiments were conducted at the experimental fields of Dnipro State Agrarian and Economic University (DSAEU). The field`s geographic coordinates are: 48°50'N lat. and 35°25' E long. The experimental field is laid on 59 meters above the sea level. Weather conditions for hydrothermal indicators in the years of research (2017 – 2020) varied, which made possible to obtain objective results, but in general, they were typical. Air temperature during winter wheat growing season (September - July) is 7.4 °C, the average rainfall is 458 mm for the location of the research fields, air temperature during winter wheat growing season 2018 (September - July) was 10.5 °C, the average rainfall is about 543.8 mm; air temperature in season 2019 was 9.6 °C, the average rainfall is about 476.2 mm; air temperature in season 2020 was 10.9 °C, the average rainfall is about 552.3 mm.

The field station of DSAEU has been used for many years (start from 60^{th} years of XX century) as a field for intensive agricultural farming and researches. It is located far away from the city Dnipro (about 28 km) which is enough to avoid industrial or town air pollution effects. The research fields occupy an area of 60 hectares. Special attention was paid to the differences on several agronomical-value traits (grain yield, yield structure, protein and main protein components content in grains).

We evaluated two varieties of national breeding – Podolyanka (national standard) and Komerciyna (DSAEU, the variety was created at and for the Steppe zone), 12 varieties of INRA breeding (Institute of National Studies in Agronomy, France) obtained from the Laboratory of Ecophysiology and Biodiversity of Cereals (Clermont-Ferrand, France) Courtiot, Flamenco, Gallixe, Geo, Ghayta, Gotik, Grapeli, Koreli, Lyrik, Musik, Renan and Skerzzo.

Sowing plots of winter wheat varieties were placed according to a randomized scheme with a plot area of 5 m^2 in 3 repetitions, and the seeding rate depended on the weight of a thousand grains. Yield assessment was performed by continuous threshing, yield structure was determined by standard parameters in triplicate, the sample was 25 - 30 plants including the marginal effects (plant height, parameters of the main ear, plant yield, thousand grains weight (TGW)).

During the growing season, phenological observations were made, germination and survival after the winter period was determined, crop conditions were visually evaluated, dates and character of main critical stages were determined.

Winter resistance was evaluated by the concentration of soluble sugars, determined at the tillering nods of varieties according to generally accepted GOST 26176-91 (GOST, 1993).

Agrochemical analysis of soils for content of nutrient elements was provided too ($N\text{-}NO_3$, mg kg^{-1} 18.7 – 32.8, P_2O_5 14.8 – 27.1, K_2O 134 – 235).

The protein content and contents of gliadin and glutenin were identified on device Spectran RT (for protein content) and RP-HPLS (for gliadins and glutenins).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, factor analyses were conducted by module ANOVA, cluster analysis by module of multivariate statistic. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSION

Table 1 presents the general characteristics of the main traits of the cultivated varieties of winter wheat obtained in the terms of phenological observations during the growing seasons of 2017 - 2020.

Table 1. General characteristic of winter wheat varieties phenotype

Variety	Plant height	Maturing (2018/2019)		Awns
		Date	Classification	
Podolyanka	high stem	22-23.05	medium	awnless
Komerciyna	high stem	20.05	early medium	awnless
Courtiot	semi-dwarf	17.05	early	awn
Flamenko	short stem	26.05	late	awn
Gallixe	short stem	26.05	late	awnless
Geo	short stem	26.05	late	awn
Ghayta	short stem	25-26.05	late	awn
Gotik	medium	26.05	late	awnless
Grapeli	medium	26.05	late	awnless
Koreli	medium	26-27.05	late	awn
Lyrik	short stem	26.05	late	awnless
Musik	short stem	26.05	late	awn
Renan	medium	26.05	late	awn
Skerzzo	medium	26-27.05	late	awn

The earliness as trait was previously considered as the key for Steppe zone. As we can see from the data, only one variety of French breeding was early-maturing, an early-medium variety of local breeding, the national standard was the medium by maturity; all other foreign varieties were at the late-maturing group. It is believed that such trait is negative for our zone because critical periods in the formation of grain yield with such development fall on periods with insufficient precipitation and higher air temperature. However, the study further proves this concept as outdated, which does not correspond to the late changes in the weather and climatic conditions of the North Steppe of Ukraine.

As for the traits that characterize the structure of the plant (plant height), the varieties of foreign breeding are shorter, with even one semi-dwarf. This is an absolute advantage in comparison with medium-height national varieties. Also, the varieties of French breeding have a higher economic suitability coefficient

(defined as the ratio of grain mass to the total formed biomass) (0.27 - 0.31) (Nazarenko *et al.*, 2019b).

The work also considered such a trait as spike awns. It is believed that awnless plants are more resistant to pests. However, as we can see, both awn and awnless forms are equally present among foreign varieties. However, in the context of this study, this was not decisive.

Table 2 shows the results of an examination of winter wheat growth and development at winter period, starting from germination and until the resumption of vegetation after the period of low temperatures. As we can see, in general, domestic varieties have higher winter resistance in comparison with INRA varieties.

Table 2. Winter wheat samples parameters during winter period (2017/2020 periods of vegetation dates)

N	Variety	G	BW	CS				S	AW
				11	02	03	04		
1	Podolyanka	98.3	5	32.2	26.3	20.2	19.9	97.9	5
2	Komerciyna	98.9	5	28.4	22.1	19.8	19.0	98.4	5
3	Courtiot	93.4	4	22.1	17.9	16.9	14.0	92.8	4
4	Flamenko	90.6	4	20.2	18.0	14.8	12.9	89.9	4
5	Gallixe	91.2	3.5	16.4	14.6	12.1	11.6	90.1	3.5
6	Geo	92.3	4	23.0	19.2	16.8	15.4	90.1	4
7	Ghayta	89.2	3.5	15.9	11.7	10.2	10.0	85.1	3.5
8	Gotik	88.7	4	20.7	17.2	14.1	13.2	86.9	4
9	Grapeli	91.2	4	21.0	19.1	16.5	13.4	90.1	4
10	Koreli	90.7	4	22.3	19.7	14,9	12.7	88.9	4
11	Lyrik	90.2	4	18.6	12.6	11,1	10.5	86.1	4
12	Musik	88.4	3.5	13.4	10.4	10.1	9.5	85.0	3.5
13	Renan	92.3	4.5	21.0	19.8	14.9	12.7	90.8	4.5
14	Skerzzo	91.9	4	25.1	23.1	19.2	18.0	90.7	4

G – germination [%]; BW – evaluation before winter period [balls]; CS – content of sugars in tillering nod [%]; S – surviving of plants after winter period [%]; AW – evaluation after winter period [balls].

* indicate significant differences from standard at $P < 0.05$

The standard made was especially developed due to its high ecological flexibility and adaptability (the ability to ensure a stable yield and satisfactory grain quality in a wide range of weather conditions). According to winter resistance, foreign varieties in terms of sugar content in the root node were seriously inferior to the standard. The content was especially low in Ghayta, Lyrik, and Musik. However, other varieties also did not reach the required level. Only domestic varieties scored 5 points in winter tolerance estimation, the visual evaluation of other genotypes was at the level of 3.5 - 4 points, except for one variety Renan. Their germination was lower. The best among INRA varieties in terms of sugar content (winter tolerance) was Geo.

Since 2016, winter has been getting significantly more moderate, for example, the winter wheat plant growth at winter season from 2017 did not stop until the end of December, and, in some years, it took place in early January. The periods with sharp reduction in temperature (down to -15 - -18°C) have significantly decreased by duration and at the winter period of 2019 - 2020 were practically absent, with the exception of a few days in February. Therefore, the lower winter tolerance of these samples did not lead to any negative consequences. That is, the idea of this property as a key one is somewhat outdated, and varieties of the Western European ecotype no longer suffer from our weather conditions as much as in the 1990s - 2000s.

Table 3 presents data on grain productivity. A three-year field test showed that, according to averaged data, such varieties as Komerciyna, Gallixe, Ghayta, and Koreli had overcome the standard. All the years of testing, Gallixe, Ghayta, and Koreli have been consistently surpassed the standard, the conditions of 2019/2020 (higher precipitation in late May - early June, less arid conditions) led to negative consequences for the Komerciyna local variety, which formed the yield at the standard level. However, this also had a positive effect on the overall yield of all genotypes.

Table 3. Grain productivity of winter wheat genotypes (2018-2020 years)

N	Variety	Yield, t ha^{-1}			Average	St. Dev
		2018	2019	2020		
1	Podolyanka	5.23	5.42	7.89	6.18	0.48
2	Komerciyna	6.61*	6.40*	7.27	6.76*	0.45
3	Courtiot	4.24	4.79	5.75	4.93	0.56
4	Flamenko	4.45	4.74	9.76*	6.32	0.59
5	Gallixe	7.33*	7.69*	9.36*	8.13*	0.08
6	Geo	4.00	4.10	7.06	5.05	0.54
7	Ghayta	7.12*	7.76*	9.58*	8.15*	0.48
8	Gotik	5.63	5.79	7.22	6.21	0.52
9	Grapeli	5.64	6.09*	7.47	6.40	0.56
10	Koreli	7.02*	6.79*	9.13*	7.65*	0.29
11	Lyrik	5.63	5.78	7.64	6.35	0.12
12	Musik	5.02	5.23	7.77	6.01	0.53
13	Renan	4.91	5.04	7.80	5.92	0.53
14	Skerzzo	5.26	5.59	7.87	6.24	0.42

* - difference is statistically significance from check at $P_{0.05}$

In some years, Grapeli (2019) and Flamenko (2020) also surpassed the standard by yield. In general, as we can see, late maturing only contributed to better productivity of foreign varieties. Low winter resistance did not have a significant effect.

Table 4 presents the results of the analysis of variance by factors variety and year (climatic conditions in a given period) for all genotypes. It was found that both factors influenced on grain productivity - the factor year was more powerful ($F = 64.59$; $F_{critical} = 3.36$; $p = 0.01$), the factor variety was less

significant, but sufficient ($F = 7.44$; $F_{critical} = 2.11$; $p = 0.01$). Thus, the statistically significance of both factors that underlie the experiment was confirmed.

Table 4. Results of factor analysis

Source of variation	SS	df	MS	F	P-value	F critical
Variety	36,58848	13	2,814498	7,440355	8,06E-06	2,119166
Year	48,86606	2	24,43303	64,5907	8,2E-11	3,369016
Error	9,835143	26	0,378275			
Summary	95,28968	41				

The obtained material was classified according to grain productivity by cluster analysis (Fig. 1). It showed that the material was divided into six groups. The first group included varieties that consistently demonstrated the standard-level productivity, without significant variations. It consisted of Podolyanka, Skerzzo, Musik, Renan, Gotik, Grapeli, and Lyrik.

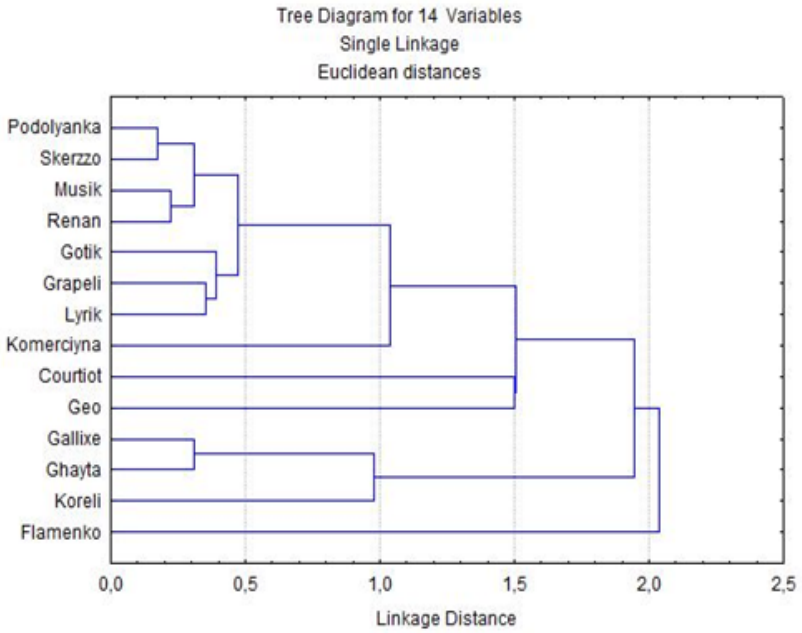

Figure 1. Cluster analysis of genotypes grain production

In the second group, the variety Komerciyna surpassed the standard in general, but in 2020 came less of yield. In the third group only one variety, Courtiot, the only semi-dwarf and at the same time earlyness variety in the experiment, showed yields consistently below the standard in all the years of the study.

The fourth group consists of one Geo variety, which for two years showed yields consistently below the standard variety, but in the last year, which was more favorable in terms of hydrothermal conditions, form yield at the standard level.

The fifth group contains several foreign varieties – Gallixe, Ghayta, and Koreli. These are the most perspective varieties for grain productivity, which definitely exceeded the standard in any conditions and formed a high yield under any conditions during the experiment period. The last group is represented by the only variety – Flamenko, which for two years gave yields below the standard. However, in the last year, it exceeded it.

Thus, as the cluster analysis shows, drought in the 2nd-3rd decade of May - the 1st decade of June is still important for Steppe conditions. Less drought-susceptible varieties produce consistently high yields that are above or at the standard level. These are the first (Podolyanka, Skerzzo, Musik, Renan, Gotik, Grapeli, Lyrik), the second (Komerciyna), and especially the fifth (Gallixe, Ghayta, Koreli) groups. The main part of the varieties of the Western European ecotype is quite suitable for North Steppe conditions (which, apparently, are increasingly shifting towards the conditions of the region of creation). The cluster analysis has significantly underlined the results of the initial grain yield analysis.

The appropriate analysis to establish the influence of the yield structure parameters of (Table 5) on total grain productivity. The higher-yielding varieties exceeded the standard in terms of such parameters as the weight of a thousand grains (and they were the only ones who exceeded the standard in this parameter), and for two out of three cases, superiority was observed in grain weight per plant (all other genotypes did not show it either) and in the same two cases exceeded the standard in terms of grain weight per main spike. The number of grains per spike parameter is rather contradictory. Thus, the key parameters were the thousand grains weight and the weight of the grain from the main spike and plant.

That is if previously noted that the investigated form high yields on the basis of good productive tillering, then in the case of French varieties, both the high productive grain content of the main spike and good productive tillering affect the synthesis.

Our early researches showed that varieties are usually focused on one of two parameters, and cases when it was possible to effectively achieve their combination haven't been observed. This is a qualitatively new fact in our long-term studies of various genotypes of winter wheat.

However, modern agriculture practice demands on the quality and nutritional value of food in a higher priority than the total grain production. Therefore, the investigation cannot be considered complete without an analysis of quality parameters.

Table 6 presents the results for such parameters as the content of protein, gluten, glutenins (high and low molecular weight and total), and gliadins.

Table 5. Parameters of main components of yield structure (at average)

Variety	PH	Per main spike		WGP	TGW
		GN	GW		
Podolyanka	103.0±2.0	36.5±3.6	1.9±0.5	4.3±0.7	51.2±6.8
Komerciyna	103.8±1.7	33.0±4.8	1.5±0.3	4.4±0.8	43.5±5.8
Courtiot	60.2±1.7*	35.8±2.9	1.5±0.2	3.1±0.2	41.7±2.8
Flamenko	78.0±2.3*	31.6±3.0	1.1±0.1	3.8±0.3	35.0±2.0
Gallixe	74.4±1.9*	42.2±7.4*	2.5±0.2*	5.2±0.3*	54.1±2.9*
Geo	75.2±1.5*	37.5±5.4	1.6±0.2	4.2±0.2	41.5±2.1
Ghayta	77.8±0.4*	48.8±2.7*	2.4±0.3*	5.7±0.4*	53.6±2.3*
Gotik	88.0±0.6*	48.2±3.7*	1.9±0.3	3.7±0.3	39.2±2.0
Grapeli	85.2±2.8*	49.3±11.0*	1.4±0.3	3.8±0.3	30.3±2.3
Koreli	94.2±1.2*	44.0±9.7*	1.3±0.2	4.6±0.3	54.7±2.0*
Lyrik	74.8±1.2*	35.6±5.5	1.3±0.2	3.0±0.2	36.3±2.1
Musik	68.6±6.4*	42.3±4.1*	1.6±0.3	3.2±0.3	38.5±2.6
Renan	82.2±3.4*	28.3±3.9	1.1±0.1	3.4±02	39.6±2.9
Skerzzo	85.2±1.9*	44.3±8.6*	1.8±0.2	3.5±0.3	40.5±2.9

PH – plant height[cm]; GN – grain number[piece]; GW – grain weight[g]; TGW – thousand grain weight [g]; WGP – weight of grain per plant [g]
* indicate significant differences from standard at $P < 0.05$

Table 6. Grain quality parameters (at average)

Variety	PC	GC	Glutenins			Gliadins
			HMW	LMW	Total	
Podolyanka	13.99	25.59	0,16003	0,46485	0,62890	0,4598
Komerciyna	13.48	24.98	0,18522	0,54343	0,73268*	0,4248
Courtiot	14.55*	27.14*	0,19321*	0,54839	0,74160*	0,4352
Flamenko	10.92	17.51	0,15465	0,71101*	0,86566*	0,4564
Gallixe	11.46	20.21	0,15736	0,65789*	0,81525*	0,4894*
Geo	14.57*	27.69*	0,20483*	0,43901	0,64384	0,3345
Ghayta	13.42	24.72	0,22938*	0,58478*	0,81416*	0,4001
Gotik	11.02	18.33	0,17022	0,64633*	0,81655*	0,4325
Grapeli	11.71	19.85	0,16660	0,64457*	0,81117*	0,4536
Koreli	12.66	22.14	0,15337	0,70987*	0,86324*	0,4234
Lyrik	12.11	19.02	0,17446	0,66748*	0,84194*	0,4326
Musik	12.46	21.78	0,16747	0,70093*	0,86840*	0,4445
Renan	14.41*	26.01	0,23984*	0,45888	0,69872	0,3909
Skerzzo	13.94	26.81	0,16094	0,54091	0,70185	0,4385
Average	12.90	22.98	0,17982	0,59416*	0,77456*	0,4297
St.D, %	0.40	1.01	0,02763	0,09600	0,08228	0,0368

PC – protein content [%]; GC – gluten content [%]; HMW – high molecular weight clutenins [%]; LMW – low molecular weight clutenins [%].
* indicate significant differences from standard at $P < 0.05$

The results reaffirmed that high quality still does poorly and sometimes contradicts increased yields. Although it will receive a genotype with an acceptable level of this trait and higher yield is quite common. Protein levels at or above standard level, content of high-molecular-weight glutenins above standard, high content of gliadins is acceptable.

Among the most perspective high-yielding varieties, Ghayta was the only to show an acceptable level of quality by all traits (acceptable protein content, high content of high molecular weight glutenins and gliadins). The rest of the high-yielding varieties generally performed poorly. In the first group (Skerzzo, Musik, Renan, Gotik, Grapeli, Lyrik), the Renan variety deserves attention in terms of protein and high-molecular-weight glutenins, gliadins at the standard level. Komerciyna does not differ statistically from the standard in terms of quality.

In general, Courtiot, Geo, and Renan are donors of the protein content trait; Courtiot, Geo, Renan, and Ghayta – the content of high-molecular-weight glutenins; Gallixe – the content of gliadins. In a complex, varieties Courtiot, Geo, and Renan are the most perspective.

French varieties have a common problem in terms of the high content of low-molecular-weight glutenins. Also, only one genotype had high gliadin content.

Generally, the period investigations were characterized by moderate weather conditions than those obtained as a result of long-term observations (table 1-3). However, recent global climate changes have led to significantly more moderate conditions in region. First of all, this refers to an increase in temperature in winter, shifts in periods with insufficient precipitation, and an increase in the average monthly and decades precipitation rate at critical phases for winter wheat growth and development (Chope *et al.*, 2014; Shah *et al.*, 2018).

This led to the fact that the Varieties of Western European breeding, previously less adapted to North Steppe conditions, began not only to explicitly compete with local varieties but also - as shown in these studies - significantly surpass in yield parameters both the standard and the newest local variety, which is no longer well suited to local conditions and starts to lose (Essam *et al.*, 2019; Hongjie *et al.*, 2019). At the same time, the lower winter resistance of French varieties has no longer become a key problem. Despite the significantly lower sugar content, overwintering as a whole can be considered quite successful for the subsequent formation of grain productivity at and above than the standard level (Nazarenko and Bezus, 2018; Shah *et al.*, 2018).

The earliness (previously, earlier maturing forms had been considered as advantageous) also can no longer be considered a key point for obtaining a competitive advantage (Tsenov *et al.*, 2015; Nazarenko *et al.*, 2019b). Moreover, apparently, the French samples have a significantly higher ability to use the increase in precipitations just at later stages of development, which local varieties do not have. As for the parameters of the yield structure, the weight of a thousand grains became key as in previous studies (Essam *et al.*, 2019). However, the local

varieties showed to form a higher yield with a significantly lower this parameter. All this makes the research data somewhat contradictory (Nazarenko *et al.*, 2019a; Li *et al.*, 2019).

As for quality, several more perspective forms with a high content of both protein and high-molecular-weight glutenins among foreign varieties were identified. At the same time, local varieties also demonstrated enough grain quality. Thus, although the level of the local sources in terms of grain quality should be recognized as satisfactory, the use of European forms for its improvement is not only quite promising but also necessary both as components in crossing systems and directly - since local forms now lack the previously observed advantage such as higher adaptability (Bordes *et al.*, 2008; Katyal *et al.*, 2016; Li *et al.*, 2019).

Both local genetic resources and the widespread use (both as varieties for cultivation or sources for breeding) of the variety sources of the EU countries provide new opportunities to increase the yield and quality of grain (Žofajová *et al.*, 2017).

CONCLUSIONS

Thus, as a result of a complex of investigations of winter resistance, grain productivity and quality, it was possible not only to identify perspective genotypes by each trait but also to show the possibilities of genotypes with a set of such traits that even surpass the already used under Steppe conditions winter wheat varieties. The combination of climatic factors and critical stages of development has become more favorable for the use of varieties of intensive (western-europe) ecotype and allows less focus on local varietal resources. The key parameters for the existing mechanism of yield formation and the possibility of combining high yield with the equally high-quality grain were showed. The cultivation of varieties of Western European breeding for the agriculture of the region should be recognized as possible and desirable, as well as the fact of the growing lag of local varieties, which is no longer compensated by the less favorable conditions for growing winter wheat under local conditions. This cannot be considered as a positive trend, and local breeding of crops requires a substantial increase in efforts. The more perspective varieties for the conditions of the region were identified. Further research will focus on key parameters such as drought tolerance, photosynthetic activity, peculiarities in nutrient elements utilization and accumulation for the investigated varieties to confirm the parameters that ensure the revealed superiority in terms of grain yield and quality parameters.

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EXOGENOUSLY APPLIED 24-EPIBRASSINOLIDE MODULATES PHYSIOLOGICAL AND BIOCHEMICAL CONSTITUENTS IN LAVENDER (*Lavandula angustifolia*) PLANTS UNDER DROUGHT STRESS CONDITIONS

SUMMARY

Drought is one of the most important environmental constraints that negatively affect the growth and yield of plants worldwide. Application of plant growth regulators such as brassinosteroids is a practical and alternative approach to ameliorate the drought-induced damages in plants. In the present research, the effect of exogenously applied EBL and various drought stress intensities on lavender plants were studied in a factorial field experiment at two locations in northeast of Iran. The results revealed that drought stress had negative impacts on shoot dry matter, and pigments content of lavender plants in both regions, however, EBL application led to improve in plant characteristics under drought stress. The greatest essential oils content and free proline content were observed in plants sprayed with EBL under drought stress conditions. Drought stress resulted in a significant increase in H_2O_2 and MDA content in plants, but the content of these oxidative stress markers was lower in plants sprayed with EBL compared to the non-sprayed plants. Drought stress increased the total phenol in plants. The major compounds of lavender EOs were α-thujene, γ-terpinene, linalool oxide, α-terpinolene, α-thujone, camphor and α- humulene. The foliar EBL application partially ameliorated the negative impacts of drought stress and improved the growth and morpho-physiological attributes of lavender plants along with their essential oil content and major constituents.

Keywords: Brassinolide, Drought stress, Essential oil, *Lavandula angustifolia*.

INTRODUCTION

Lavender (*Lavandula angustifolia* Mill., Lamiaceae) is an aromatic medicinal plant recommended in traditional medicine for pregnancy, relief of

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convulsion, insomnia, anxiety, skin issues and treatment of several neurological disorders (Babar *et al*., 2015). The origin of Lavender is believed to be from the Mediterranean, Middle East and India. The vegetative body of *Lavandula angustifolia* has a pleasant odor that is caused by its essential oils (EOs), which synthesized and stored in lavender leaves and flowers and special pores (Haig *et al*., 2009). Lavender EOs are widely used in perfume and cosmetics production, hygiene, medicine, aromatherapy, food industry, beverages, and eco-friendly pesticides and herbicides, etc. (Tonutti and Liddle, 2010). The genus Lavandula is exclusively distributed across the Mediterranean areas and encompasses about twenty species of small evergreen shrubs with aromatic foliage and flowers. Linalool, linalyl acetate, camphor, terpinen-4-ol, β-o-cymene and 1, 8-cineole were identified as the major components of lavender essential oils (EOs) (Price, 1993; Koulivand *et al*., 2013; Brailko *et al*., 2017).

Drought is a major threat to crop yields in arid and semi-arid areas of the world, limiting plant growth, development and productivity (Hussain *et al*., 2018). Drought stress leads to lower plant yield through disrupting various plant metabolic/physiological processes such as turgor maintenance, carbon fixation rate, CO_2 exchange, ultimately leading to increased oxidative damage (Farooq *et al*., 2009; Hussain *et al*., 2018). In other words, when plants are under stress, different types of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen ($^1O_2$) are generated, especially within the chloroplasts. ROS reacts with proteins and lipids, and cause damage to cellular structures and metabolism, especially those related to photosynthesis (Lawlor and Tezara, 2009). Plants counter drought stress by adopting various strategies including activating antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, and peroxidase) and non-enzymatic antioxidant systems (*e.g.* ascorbic acid, glutathione, carotenoid, and *a*-tocopherol) for ROS scavenging. In response to drought, the osmotic adjustment occurs through the accumulation of compatible solutes such as soluble sugars, organic acids, proline, amino acids, alcoholsugars, and ions, leading to the maintenance of higher turgor potential (Chaves *et al*., 2009; Zhang *et al*., 1999).

Biological, chemical and agronomical methods have been applied to decrease the negative effects of drought stress in plants. Among these, the use of plant hormones is a promising and practical strategy to promote crop yield under drought stress (Tanveer *et al.*, 2019, Mohammadi *et al*., 2020). Exogenous application of growth regulators has been shown to improve plant tolerance to various abiotic stresses such as drought, heavy metal, and salinity stresses (Shahzad *et al*., 2018; Tanveer *et al*., 2019; Mohammadi and Moradi, 2013). Brasinosteroids (BLs) constitute a class of phytohormones that play several roles in the growth of plants. 24-Epibrassinolide (EBL) is an active byproduct of biosynthesis of brassinolide that is able to stimulate various plant metabolic processes including photosynthesis, and biosynthesis of proteins and nucleic acids.

Environmental perturbations, particularly drought, are the main obstacles to agricultural and horticultural production in many regions of the world, especially in arid and semi-arid regions such as Iran. Hence, it is central to develop drought-tolerant cultivars and explore the potential of diverse tolerance-inducing materials to reduce the adverse effects of drought stress in order to achieve acceptable economic threshold yields for crop and medicinal plants. In this regard, application of plant growth regulators such as brasinosteroids is a potentially useful tool in alleviating the detrimental effects of drought in plants, as well as enhancing the yield of medicinally important secondary metabolites. There is currently no research on the effects of drought stress and EBL on the growth and EOs yield in lavender. Given the pharmacological importance of the plant, the present study was conducted to identify the effect of EBL on growth, and EOs yield of lavender plants under different drought stress conditions.

MATERIAL AND METHODS

Plant materials and experimental set up

Field experiments were conducted to investigate the effects of EBL (the plant metabolic inducer and activator) and various drought levels (irrigation regimes corresponding to FC, 100, 60, and 30% FC) on growth, physiological parameters, and essential oil production of *Lavandula angustifolia* plants, during 2018 (mid-April to late-October) in two fields, Ahar and Kaleybar, located in East Azarbaijan province, Iran. The climatic data of both regions in the period of field trials are presented in Figure 1. The chemical and physical characteristics of the soils in both regions are given in Table 1.

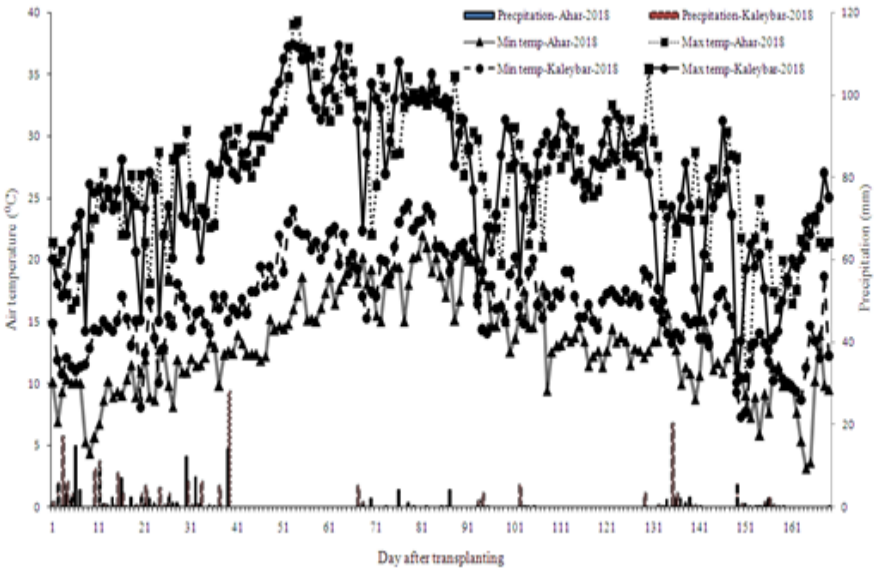


Figure 1: Air temperature (°C) and precipitation (mm) of the cultivated locations (Kaleybar and Ahar) during the plant growth period.

Table 1. The physical and chemical characteristics of soils of the cultivation regions.

Region	Texture	Sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (mg/kg)	N (%)	EC (ms)	pH
Ahar	Clay loam	30.15	25	35.84	392	10.16	0.23	1.05	7.53
Kaleybar	Clay	29.15	17.5	53.34	280	7.75	0.14	2.28	7.5

This experiment was carried out in a factorial arrangement in two regions namely Ahar and Kaleybar located at East Azarbaijan province of Iran with three replications. The first factor was spraying of EBL at two levels (0, 100 mg L^{-1}) and the second factor was different irrigation regimes (corresponding to FC, 100, 60, and 30% FC) on lavender plants. The treatments (irrigation regimes, and EBL application) were applied three weeks before flowering. TDR was used to determine the stress level.

The EBL (24-Epibrassinolide) was obtained from Sigma-Aldrich (St. Louis, USA). The EBL powder was completely dissolved in deionized water and dispensed into flask. Thereafter, the volume was made up with distilled water and mixed thoroughly to achieve a homogeneous solution. Then, the volumetric flask was completely wrapped with aluminum freezer foil to keep out light, and kept at 6 °C for at least 24 hours. We chose foliar spraying due to the absorption capacity of the solutions by leaves and aerial organs of the plants and ease of application, and the produced concentrations (0 and 100 mg L^{-1}) were sprayed on the canopy.

Lavender plants were harvested at the full flowering stage. A subsample of the plants was immediately put into the liquid nitrogen container, and then placed in an ice box containing dry ice with a lid and transferred to the laboratory and stored in -20 °C freezer. The remaining samples were dried in clean facilities in the shade for a week.

Photosynthetic Pigment Measurements

The fresh plant leaf (0.1 g each) samples were ground in a mortar with 20 mL of distilled acetone. The extract was centrifuged at 2800 g for 10 minutes. The clear supernatant was made up to 10 mL with 80% acetone. The absorbance of the extract was read at 470 (carotenoids)), 645 (chlorophyll a), and 663 (chlorophyll b) nm for measuring total chlorophyll and carotenoids (Lichtenthaler and Wellburn, 1983). Photosynthetic contents were estimated according to the following equations and expressed as mg g^{-1} fresh weight (fw).

Chl *a* (mg g^{-1} fw^{-1}) = $12.25 \times A_{663.2} - 2.79 \times A_{646.8}$

Chl *b* (mg g^{-1} fw^{-1}) = $21.50 \times A_{646.8} - 5.10 \times A_{663.2}$

Cl *a* + *b* (mg g^{-1} fw^{-1}) = $7.15 \times A_{663.2} + 18.71 \times A_{646.8}$

Carotenoids (mg g^{-1} fw^{-1}) = $(1000 \times A_{470} - 1.82 \times$ Chl *a* $- 85.02 \times$ Chl *b*)/198

Determination of H_2O_2, MDA and Proline Content

The levels of H_2O_2 in leaves of lavender plants were determined by following the method of Velikova *et al*. (2000), using an extinction coefficient of

0.28 μM^{-1} cm^{-1}. The samples absorbance was noted at 390 nm, and the H_2O_2 content was expressed as μmol g^{-1} FW, based on the standard calibration curve.

The lipid peroxidation status of leaf samples was analyzed through quantifying their malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction. The MDA content was measured in lavender leaves according to the method of Heath and Packer (1968), using an extinction coefficient of 155 mM^{-1} cm^{-1}. Free proline was extracted from lavender leaf tissues using aqueous sulphosalicylic acid and quantified using the ninhydrin method (Bates *et al*., 1973).

Total Phenols Estimation

The content of total phenols in lavender plant extracts was determined using Folin-Ciocalteu test. In brief, to every, 25 μl of the sample, 125 μl of Folin–Ciocalteu's reagent (10 % v/v in distilled water), 100 μl Na_2CO_3 (7.5% w/v) were pipetted. The amount of reaction absorbance was noted at 765 nm against a blank sample, after 90 min. Measurements were compared with a standard curve made from gallic acid solutions (10, 50, 100, 250, 500, and 1000 mg L^{-1}) and expressed as a microgram of gallic acid equivalents per mL of sample (McDonald *et al*., 2001). The measurements were repeated three times (n=3).

Extraction of Essential Oil (EOs)

The shade-dried lavender leaf samples (40 g) were grounded and hydrodistilled for 3 h using a British Pharmacopoeia model Clevenger-type apparatus (British Pharmacopoeia, 1988). Anhydrous sodium sulfate (Na_2SO_4) was added to each extracted essential oil for removing possible water drops, and then kept at 4℃ before GC and GC/MS analyses. The essential oils content expressed on a dry weight basis (w/w %), and were calculated according to following equation: EOs content (%) = (distilled EOs (g)/ 40 g) ×100

Identification of Essential Oils Constituents

EOs samples were analyzed using gas chromatograph (GC) instrument. GC device of Agilent Technologies model A7890 gas chromatograph was used for the analysis. The device was equipped with a flame ionization detector, and the quantification of materials was performed on Euro Chrom 2000 (KNAUER) using the area normalization technique while dismissing the response factors. The analysis was performed using a HP-5 fused silica capillary column (30 m × 0.32 mm id., film thickness 0.25 μm, J and W Scientific Inc., Rancho Cordova, CA). The operating conditions were designed in a manner that the injector and detector temperatures were 280 °C and 260 °C, respectively. Helium was used as the gas carrier with a linear velocity of 22.73 cm/s. The oven temperature was programmed to increase from 60 °C to 210 °C at a rate of 3 °C/min.

The GC/MS analysis was performed on a Hewlett Packard (HP) 6890 GC/MS system equipped with a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed to increase from 60 °C to 220 °C at a rate of 6°C /min. The transfer line temperature was 280 °C and the injector temperature was 250 °C. The carrier gas was Helium, and the flow rate was 1 ml/min. The ionization energy of MS was 70 eV. The components of

the oils were identified by comparison of their mass spectra with those 130 of a computer libraries (Wiley 275 database) or with authentic compounds. These were confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes. The components were identified by comparing their retention indices (RI, HP-5) with those reported in the literature and also by comparing their mass spectra with the Wiley GC/MS Library, Adams Library, Mass Finder Library data and the existing mass spectra data (Adams, 2007; Davies, 1990).

Statistical Analysis

The SAS statistical software was used to perform the analysis of variance (ANOVA) and statistical analysis of the dataset. The hierarchical cluster analysis (HCA) and heatmap analyses were conducted using MetaboAnalyst platform (http://www.metaboanalyst.ca). Duncan's Multiple Range Test (DMRT) with the probability level of 0.01 ($P \leq 0.01$) was used to statistically compare the means.

RESULTS AND DISCUSSION

Morphological parameters

Combined ANOVA indicated that the interactions of cultivation regime, foliar EBL application, and drought stress on dry matter of lavender plants were significant at $p \leq 0.01$ (Table 2). The maximum plant dry matter was obtained in the Ahar region under 60% FC with foliar EBL application, and the lowest dry matter was observed under 30% FC (Table 2).

Drought stress adversely affects plant cell turgor, stomatal conductance, photosynthesis, respiration and transpiration, and interferes with other metabolic processes that directly depend on the presence of water. Crop growth and fitness decline occurs under drought conditions due to limited photosynthesis efficiency (Taiz and Zeiger, 2006). In our study, the maximum plant dry weight of lavender plants belonged to the EBL application under 60% FC level in Ahar region; and the lowest dry weights were observed for the untreated plants (sprayed with water) under 30% FC level in both studied regions (Table 2). Brasinosteroids can absorb water and maintain cell turgor by stimulating the biosynthesis of osmotic adjustments, causing an increased stomatal conductance and greater intake of CO_2 into plants (Pustovoitova *et al*., 2001). Exogenous application of brasinosteroids was shown to reduce the detrimental effects of mild drought stress in sugar beet (*Beta vulgaris*) plants (Schilling *et al*., 1991). The decreased growth of plants could be due to the reduction of turgor pressure and consequently decrease in cell division under drought stress conditions (Cabuslay *et al*., 2002, Mohammadi and Abdollahi, 2016). Greater growth attributes of plants in the Ahar region compared to the Kaleybar region under all three irrigation regimes can be partially attributed to the superior nutrient status of the soil in the Ahar region (*e.g.* higher soil content of N, P, and K; Table 1). Previous studies indicated that the water potential was maintained in plants under stress with

external application of brasinosteroids (Shahid *et al*., 2015) and it was also observed in our results (data not shown). Therefore, brasinosteroids could help to improve the plant's water relations under stress conditions.

Table 2. Analysis of mean (mean comparison) for some studied traits in lavender (Lavandula angustifolia) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions and both regions.

Region	24-epibrasinolide application	Drought stress level	Total dry matter (g)	Total chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Ahar	Control	Well-watered	21.70±1.91 cd	0.776±0.08 abc	0.802±0.17 a
		60 % FC	24.70±2.78 c	0.830±0.03 abc	0.838±0.025 a
		30 % FC	9.38±1.78 gh	0.541±0.05 de	0.568±0.01 bcd
	100 mg L^{-1}	Well-watered	28.03±2.94 b	0.795±0.22 abc	0.560±0.19 bcd
		60 % FC	38.29±0.56 a	0.852±0.19 a	0.599±0.22 bcd
		30 % FC	12.79±3.02 ef	0.657±0.03 bcd	0.482±0.17 cd
Kaleybar	Control	Well-watered	15.06±1.14 e	0.513±0.04 de	0.555±0.07 bcd
		60 % FC	20.82±1.21 d	0.609±0.08 de	0.710±0.10 ab
		30 % FC	7.16±0.62 h	0.323±0.02 f	0.382±0.02 d
	100 mg L^{-1}	Well-watered	18.49±0.13 d	0.521±0.02 de	0.463±0.02 cd
		60 % FC	24.21±0.75 c	0.628±0.03 cd	0.597±0.13 bc
		30 % FC	10.71±0.92 fg	0.418±0.01 ef	0.390±0.02 d

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test (n=3).

Physiological parameters

The main effects of cultivation region, drought stress and EBL foliar application on chlorophyll content were significant at $p \leq 0.05$ (Table 2). The highest total chlorophyll contents belonged to the EBL application treatment without drought stress or with moderate drought stress (60% FC) in lavender in Ahar (Table 2). The cultivation region had significant effects on chlorophyll content, so that its highest amount was found in the Ahar region; and moderate drought stress (60% FC) also had an additive effect on this trait (Table 2). Drought stress also showed significant effect on carotenoid content (Table 2).

Exogenous application of EBL and cultivation region had significant effects on carotenoid value of lavender plants at $p \leq 0.01$ (Table 2).

Drought stress and cultivation region had significant effects on total chlorophyll content of plants (Table 2). Photosynthetic pigments can be damaged in plants under intense stress conditions that consequently results in the production of ROS (Slama *et al*., 2007). The highest total chlorophyll content belonged to the moderate drought stress (60% FC) treatment in Ahar; while severe drought (30% FC) caused a significant decrease in the chlorophyll content of plants (Table 2). Compared to the severe drought stress (30% FC), the high content of total chlorophyll under moderate drought stress conditions may be due to the preservation of high RWC in leaves, leading to the maintenance of necessary factors for the synthesis of photosynthetic pigments.

Beside their role as adjuvant pigments, carotenoids play antioxidant and free radical scavenging roles in plants exposed to the moderate stress conditions

(Egert and Tevini, 2002). Carotenoids are also likely to decrease under severe stress conditions (*e.g*. 30% FC). Lavender plants grown in the Ahar region had a significantly higher carotenoid content than those of Keleybar (Table 2), because of Ahar (a cold semi-arid) region compared to Kaleybar (a cold semi-humid) site. The lowest carotenoid content was observed under 30% FC (*i.e*., severe drought stress) (Table 2).

The main effect of the cultivation region and the interaction between drought stress and EBL foliar application on lipid peroxidation (MDA content) of plants was significant (Table 3). Interaction of cultivation region, drought stress and foliar EBL application was significant on H_2O_2 content of lavender plants (Table 3). The interaction of cultivation region and drought stress, and foliar EBL application was significant on proline content of plants (Table 3). However, the highest proline content was observed in lavender plants with EBL application under severe drought stress in Ahar (Table 3); and the lowest proline content was obtained in well-watered (100% FC) plants treated with EBL in Kaleybar region (Table 3).

The maximum MDA content was obtained under no EBL application and severe drought stress (30% FC) conditions, whereas non-stress conditions and EBL application significantly reduced the MDA content of plants exposed to moderate and severe drought stress intensities (Table 3). The high MDA content of plants under severe drought conditions could be attributed to increases in ROS, membrane peroxidation, and the leakage of ions from the membrane, eventually leading to cell damage and reduced plant growth (Foyer *et al*., 1994). Here, foliar EBL application led to the improved performance (growth and physiological attributes) of lavender plants under drought stress conditions, which is in line with known capacity of brassinolide to protect plants under stress conditions possibly through changes in membrane characteristics, increase in membrane stability, and the reduced leakage of solutes/ions from membrane (Khripach *et al*., 1998).

The maximum H_2O_2 content belonged to untreated plants under severe drought conditions in Ahar, while the lowest content was obtained in plants undergone foliar EBL application and non-stress conditions in Kaleybar region (Table 3). Drought stress negatively affects plant growth through increasing the generation of ROS such as H_2O_2, reducing photosynthesis and disturbing the water balance, which ultimately lead to declined growth and productivity of plants (Talaat *et al*., 2015). Brasinosteroids have the potential to significantly reduce the ROS in plants by improving their antioxidant system and increasing the overall plant tolerance to stress conditions (Hemmati *et al*., 2018).

Pytochemical parameters

The interaction of cultivation region, drought stress and foliar EBL application was significant on the EOs yield of lavender plants (Table 4). EBL application and severe drought stress in Kaleybar region resulted in the highest EOs yield in Lavender, whereas the lowest EOs yield was observed in untreated

plants under severe drought stress (Table 4). In a study on thyme, drought stress with (70%FC and 90% FC) led to increased and decreased EOs yield, respectively (Mohammadpour Vashvaei *et al.*, 2015). In the present study, the boosted EOs yield could be due to the prevention of intracellular oxidation events under drought stress conditions.

EBL application had a significant impact on total phenol content of the lavender plants; and the interaction between cultivation region and drought stress was statistically significant. The highest total phenol content was obtained with EBL application under severe drought stress (Figure 2). Furthermore, the highest phenol content was obtained under mild drought stress in both regions, and severe drought stress was in Ahar; and the lowest phenol content was seen in Kaleybar region (Figure 2).

Table 3. Analysis of mean (mean comparison) for some studied traits in lavender (*Lavandula angustifolia*) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions and both regions.

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test (n=3)

Region	24-epibrasinolide application	Drought stress level	H_2O_2 content (µmol g^{-1}FW)	MDA content (nmol g^{-1} FW)	Proline ontent (µmol g^{-1}FW)
Ahar	Control	Well-watered	0.27±0.01 cd	0.884±0.11 g	5.700±0.12 ef
		60 % FC	0.39±0.01 b	4.032±0.67 c	8.023±0.35 c
		30 % FC	0.58±0.05 a	6.194±0.77 a	7.071±0.45 d
	100 mg L^{-1}	Well-watered	0.21±0.01 e	0.748±0.16 g	6.205±0.12 de
		60 % FC	0.28±0.03 c	2.116±0.16 ef	9.170±0.96 b
		30 % FC	0.30±0.03 c	3.271±0.15 d	12.294±0.64 a
Kaleybar	Control	Well-watered	0.23±0.02 de	0.716±0.05 g	4.838±0.50 fg
		60 % FC	0.30±0.02 c	3.697±0.21 cd	6.078±0.62 de
		30 % FC	0.37±0.02 b	5.348±0.47 b	4.297±0.71 g
	100 mg L^{-1}	Well-watered	0.17±0.01 f	0.381±0.08 g	4.545±0.10 g
		60 % FC	0.22±0.03 e	1.768±0.26 f	6.854±0.59 d
		30 % FC	0.23±0.02 de	2.380±0.35 e	8.189±0.22 c

The results of the EOs analysis indicated that there were 28 compounds in lavender plants following employed treatments in both locations (Tables 5, 6). The major compounds of lavender EOs were α-thujene, γ-terpinene, linalool oxide, α-terpinolene, α-thujone, camphor and α- humulene. Interactions of cultivation region, drought stress, and EBL application on linalool oxide, β-thujone, lavandulol, terpinene-4-ol, and lavandulyl acetate compounds were significant (Table 5, 6).

Table 4. Analysis of mean (mean comparison) for essential oil contents in lavender (Lavandula angustifolia) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions and both regions.

Region	24-epibrasinolide application	Drought stress level	Essential oil content (w/w) %
Ahar	Control	Well-watered	0.87±0.18 def
		60 % FC	0.70±0.12 efg
		30 % FC	0.46±0.03 g
	100 mg L^{-1}	Well-watered	1.24±0.02 bc
		60 % FC	1.42±0.03 ab
		30 % FC	0.85±0.03 def
Kaleybar	Control	Well-watered	0.64±0.04 fg
		60 % FC	0.93±0.20 def
		30 % FC	0.49±0.09 g
	100 mg L^{-1}	Well-watered	0.96±0.14 cde
		60 % FC	1.12±0.09 cd
		30 % FC	1.57±0.19 a

*: Means followed by the same letter(s) in each column are not significantly different based on Duncan's Multiple Range Test (n=3).

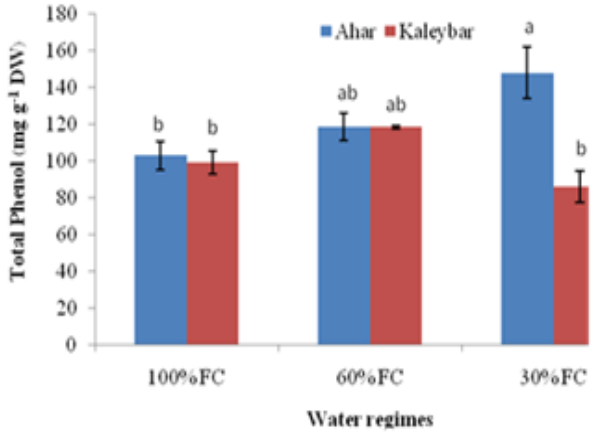


Figure 2: Effect of drought stress on total phenol contents of lavender (Lavandula angustifolia) plants in Ahar and Kaleybar locations.

The results of the EOs analysis indicated that there were 28 compounds in lavender plants following employed treatments in both locations (Tables 5, 6). The major compounds of lavender EOs were α-thujene, γ-terpinene, linalool oxide, α-terpinolene, α-thujone, camphor and α- humulene. Interactions of cultivation region, drought stress, and EBL application on linalool oxide, β-

thujone, lavandulol, terpinene-4-ol, and lavandulyl acetate compounds were significant (Table 5, 6).

The results also indicated that the highest amounts of α-thujene, γ-terpinene, α- terpinolene, α- thujone, camphor, lavandulol, and α-humulene were obtained in no-stressed plants. The highest amounts of α-thujene, linalool oxide, α- thujone, and α- humulene were obtained in the foliar EBL treated plants. Furthermore, the highest amounts of α-terpinolene, sabinol, lavandulol and camphor were observed in the Kaleybar region (Table 5, 6). The correlation matrix among pairs of the 28 terpenoid constituents exist in the EOs of lavender plants upon experimental treatments is represented in Figure 3A. This information could increase our knowledge about changing patterns in the EOs compositions as well as the correlation among these constituents in response to the employed treatments. The hierarchical cluster analysis divided the EOs constituents based on correlation coefficients into two main categories/clusters, each of them grouped into several sub-clusters. According to the correlation matrix analysis (Figure 3A), the highest correlations were found among (+)-4-carene, linalool oxide, α-thujene, trans-caryophyllene, viridiflorene and α-humulene under the experimental treatments. Correlation analysis of the EOs components revealed that there were positive correlations among the number of terpenoids (monoterpenes and sesquiterpenes) constituents and grouped together, however, the other compounds with lower correlations were grouped in another cluster. Therefore, EOs constituents that are classified in the same group/cluster responded similarly to the employed treatments. The obtained correlations may be due to the similar impacts of EBL-mediated regulation of the expression of genes (Sharma *et al*., 2017), post-transcriptional regulation and redox homeostasis (Zhao *et al*., 2017), activities of various enzymatic antioxidants (Bajguz, 2000), signaling compounds (Xi and Yu, 2010), and other factors affecting biosynthesis, regulation and accumulation of secondary metabolites.

The correlation among pairs of the studied traits (*i.e*., plant dry weight, RWC, plant height, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, MDA, H_2O_2, essential oils content, proline, and total phenolics) in response to the EBL application and drought stress levels is shown in Figure 3B. The results obtained by hierarchical cluster analysis technique could be visualized using a color-coded heatmap and dendrograms based on the Pearson correlation coefficient of each trait with other traits, resulting in two main clusters. The various clusters exhibit different response patterns of the studied traits to the reference treatments. Furthermore, our findings suggest that the EBL and drought stress treatments caused to a co-induction of plant height and dry weight, RWC, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, and essential oil content of lavender plants, however, other traits such as MDA, H_2O_2, proline and total phenols have lower correlations with application of EBL and increasing drought stress levels. Heatmap based on the relative levels of studied traits in

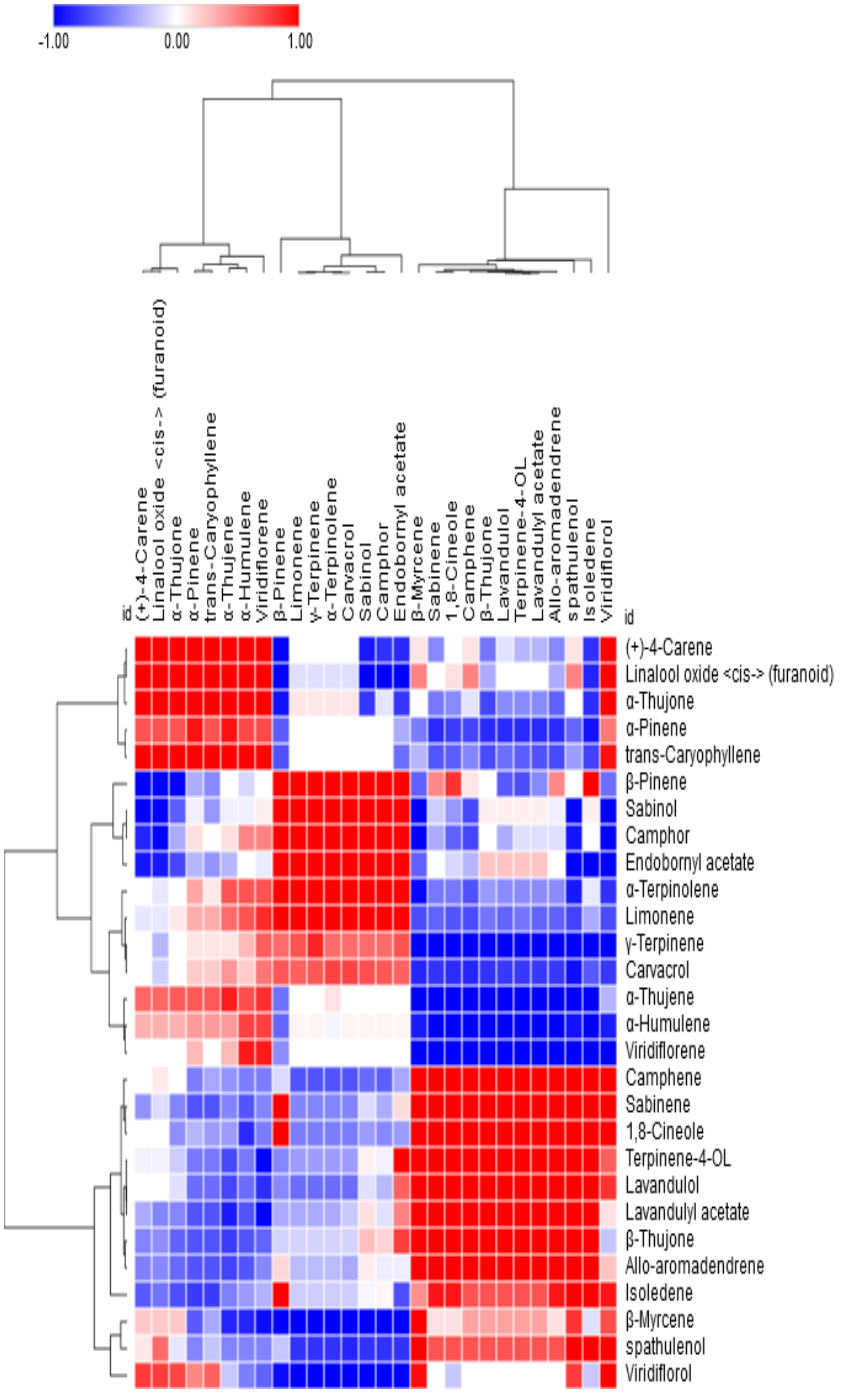
lavender plants in response to the different employed treatments is summarized in Figure 3C.

Table 5. Essential oils constituents in lavender (*Lavandula angustifolia*) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions in Ahar region.

Compounds	RI	LRI	Control			100 mg L^{-1}		
			100% FC	60% FC	30% FC	100% FC	60% FC	30% FC
α-Thujene	925	924	9.23±0.45	6.21±0.54	10.48±0.98	5.337±0.11	5.781±0.16	8.231±0.97
α-Pinene	931	932	4.12±0.11	4.341±0.21	3.999±0.11	2.586±0.07	2.678±0.07	3.894±0.11
Camphene	945	946	1.687±0.04	1.632±0.04	1.612±0.04	1.725±0.06	1.651±0.04	1.712±0.03
Sabinene	971	969	1.121±0.03	0.09±0.02	1.085±0.03	0.073±0.01	0.11±0.01	1.061±0.01
β-Pinene	974	974	2.101±0.08	1.45±0.03	1.945±0.08	1.365±0.06	1.389±0.06	1.73±0.03
β-Myrcene	989	988	1.43±0.05	0.431±0.04	1.62±0.04	0.383±0.04	0.421±0.08	1.54±0.04
(+)-4-Carene	1015	1008	0.823±0.02	6.23±0.26	0.887±0.06	5.196±0.15	5.551±0.14	0.992±0.08
Limonene	1027	1024	0.69±0.01	2.31±0.04	0.65±0.08	2.225±0.09	2.761±0.09	0.61±0.06
1,8-Cineole	1029	1026	1.342±0.07	3.431±0.11	1.373±0.07	2.784±0.14	2.541±0.08	1.421±0.07
γ-Terpinene	1057	1054	7.012±0.1	1.32±0.05	6.809±0.87	1.264±0.07	1.331±0.04	6.912±0.74
Linalool oxide	1065	1067	2.871±0.08	35.651±2.54	2.632±0.14	39.897±2.07	41.12±2.04	2.541±0.06
α-Terpinolene	1087	1086	13.023±1.01	4.651±0.12	12.589±1.04	3.397±0.11	3.441±0.06	12.867±1.03
α-Thujone	1105	1101	10.121±0.81	11.112±0.87	9.538±0.41	12.398±1.11	13.011±1.18	9.876±0.87
β-Thujone	1115	1112	1.321±0.04	0.551±0.04	1.406±0.06	0.699±0.04	0.54±0.08	1.411±0.04
Sabinol	1139	1137	3.87±0.21	0.241±0.02	3.422±0.08	0.256±0.02	0.243±0.01	3.651±0.04
Camphor	1144	1141	16.02±1.21	0.832±0.06	15.008±1.21	0.961±0.08	0.922±0.07	15.78±1.74
Lavandulol	1165	1165	1.672±0.06	0.321±0.04	5.499±0.17	0.338±0.06	0.311±0.03	5.567±0.84
Terpinene-4-ol	1176	1174	0.432±0.03	0.112±0.01	0.502±0.08	0.062±0.01	0.11±0.02	0.511±0.05
Endobornyl acetate	1286	1284	0.421±0.02	0.143±0.02	0.455±0.03	0.124±0.02	0.132±0.01	0.471±0.08
Lavandulyl acetate	1290	1288	0.451±0.04	0.122±0.01	0.479±0.04	0.131±0.03	0.125±0.02	0.311±0.07
Carvacrol	1300	1298	0.911±0.07	1.711±0.08	0.881±0.07	1.62±0.07	1.76±0.04	0.811±0.06
trans-Caryophyllene	1422	1417	1.432±0.06	3.811±0.19	1.388±0.06	3.479±0.14	3.54±0.09	1.365±0.10
Isoledene	1442	1374	4.023±0.15	0.32±0.04	3.976±0.10	0.4±0.07	0.387±0.04	3.211±0.14
α-Humulene	1457	1452	6.981±0.24	4.532±0.14	6.251±0.45	5.818±0.19	5.67±0.12	7.125±0.64
Viridiflorene	1498	1496	0.612±0.07	0.342±0.06	0.506±0.07	0.447±0.07	0.387±0.07	0.451±0.07
Spathulenol	1582	1577	0.476±0.03	0.111±0.02	0.457±0.05	0.01±0.01	0.11±0.02	0.461±0.09
Allo-aromadendrene	1588	1458	0.231±0.02	0.082±0.01	0.199±0.02	0.055±0.02	0.08±0.01	0.176±0.02
Viridiflorol	1597	1592	0.321±0.04	3.421±0.12	0.309±0.06	3.297±0.10	3.421±0.13	0.311±0.03
Total of compounds identified (%)			94.748	95.511	95.957	96.327	99.524	95
Classes of constituents								
Monoterpene hydrocarbons			39.659	35.677	40.031	33.251	35.224	37.969
Oxygenated monoterpenes			41.013	47.215	42.84	49.57	50.705	43.931
Sesquiterpene hydrocarbons			13.279	9.087	12.32	10.199	10.064	12.328
Oxygenated Sesquiterpene			0.797	3.532	0.766	3.307	3.531	0.772

Table 6. Composition of essential oils in lavender (*Lavandula angustifolia*) plants with or without 24-epibrasinolide foliar spray under different drought stress conditions in Kaleybar region.

Compounds	RI	LRI	Control			100 mg L^{-1}		
			100% FC	60% FC	30% FC	100% FC	60% FC	30% FC
α-Thujene	925	924	3.869±0.45	4.12±0.23	3.34±0.69	2.236±0.11	1.346±0.12	1.453±0.23
α-Pinene	931	932	1.029±0.04	1.098±0.08	1.11±0.01	0.121±0.03	0.084±0.02	0.098±0.02
Camphene	945	946	0.667±0.03	0.543±0.03	0.543±0.03	7.321±0.85	6.384±0.41	6.76±0.81
Sabinene	971	969	0.057±0.01	0.032±0.01	0.211±0.01	2.453±0.11	1.566±0.14	1.65±0.08
β-Pinene	974	974	1.784±0.11	1.65±0.04	1.762±0.12	2.231±0.14	1.084±0.08	1.097±0.06
β-Myrcene	989	988	0.076±0.01	0.08±0.02	0.211±0.03	0.543±0.03	0.515±0.07	0.511±0.07
(+)-4-Carene	1015	1008	0.131±0.02	0.143±0.03	0.265±0.02	0.432±0.01	0.364±0.03	0.321±0.05
Limonene	1027	1024	6.513±0.74	4.74±0.21	6.871±0.74	0.267±0.02	0.294±0.05	0.276±0.04
1,8-Cineole	1029	1026	2.851±0.12	2.761±0.41	2.98±0.22	5.45±0.32	4.229±0.19	4.11±0.13
γ-Terpinene	1057	1054	2.674±0.06	1.65±0.08	3.21±0.14	0.211±0.02	0.187±0.03	0.197±0.02
Linalool oxide	1065	1067	3.354±0.08	3.421±0.21	3.87±0.21	8.23±0.89	5.99±0.24	5.12±0.14
α-Terpinolene	1087	1086	12.43±1.1	14.12±1.25	10.21±1.02	1.87±0.07	1.448±0.11	0.55±0.08
α-Thujone	1105	1101	0.558±0.07	0.551±0.04	0.66±0.03	0.321±0.03	0.314±0.03	0.321±0.04
β-Thujone	1115	1112	1.698±0.11	0.654±0.03	2.675±0.11	12.54±1.64	13.657±1.28	14.21±1.27
Sabinol	1139	1137	41.099±2.31	43.78±2.74	37.51±1.79	0.211±0.06	0.179±0.03	0.121±0.07
Camphor	1144	1141	8.126±0.45	9.345±0.68	9.342±0.84	0.211±0.04	0.248±0.04	0.112±0.06
Lavandulol	1165	1165	0.088±0.01	0.098±0.02	0.211±0.03	20.123±2.31	23.536±2.47	26.122±2.64
Terpinene-4-ol	1176	1174	0.158±0.02	0.165±0.01	0.187±0.01	11.098±1.09	13.387±1.28	15.23±1.84
Endobornyl acetate	1286	1284	0.219±0.04	0.211±0.03	0.276±0.03	0.121±0.02	0.118±0.02	0.211±0.03
Lavandulyl acetate	1290	1288	0.362±0.06	0.367±0.04	0.431±0.02	14.54±1.01	16.059±1.05	18.121±1.27
Carvacrol	1300	1298	3.908±0.21	3.76±0.10	3.267±0.41	0.186±0.02	0.193±0.03	0.091±0.02
trans-Caryophyllene	1422	1417	0.556±0.05	0.655±0.03	0.633±0.02	0.121±0.02	0.1±0.02	0.021±0.01
Isoledene	1442	1374	0.353±0.07	0.358±0.04	0.441±0.03	0.765±0.07	0.89±0.04	0.11±0.03
α-Humulene	1457	1452	2.184±0.05	2.32±0.1	4.32±0.11	0.231±0.03	0.391±0.06	0.102±0.02
Viridiflorene	1498	1496	0.263±0.03	0.276±0.03	0.341±0.05	0.111±0.01	0.127±0.02	0.05±0.01
spathulenol	1582	1577	0.05±0.01	0.07±0.01	0.11±0.01	0.167±0.02	0.194±0.01	0.078±0.02
Allo-aromadendrene	1588	1458	0.105±0.04	0.121±0.02	0.211±0.03	1.54±0.11	1.487±0.03	1.21±0.11
Viridiflorol	1597	1592	0.062±0.01	0.076±0.01	0.098±0.02	2.67±0.21	2.338±0.11	0.98±0.14
Total of compounds identified (%)			95.224	97.165	95.296	96.321	96.709	99.233
Classes of constituents								
Monoterpene hydrocarbons			19.056	15.261	20.858	28.676	25.795	26.894
Oxygenated monoterpenes			72.595	78.028	68.284	62.04	65.387	69.788
Sesquiterpene hydrocarbons			3.461	3.73	5.946	2.768	2.995	1.493
Oxygenated Sesquiterpene			0.112	0.146	0.208	2.837	2.532	1.058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Viridiflorene
β-Pinene
Limonene
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α-Terpinolene
Carvacrol
Sabinol
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Sabinene
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Camphene
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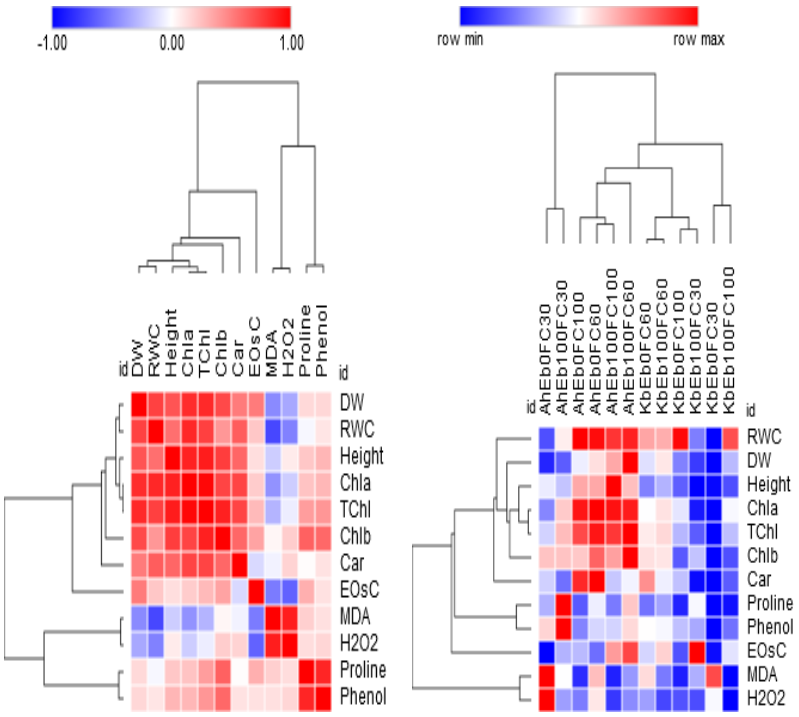

(B) (C)

Figure 3: (A) Classification of the terpenoid compounds [(oxygenated sesquiterpene (OS), sesquiterpene hydrocarbons (SH), oxygenated monoterpenes (OM), monoterpene hydrocarbons (MH)] exist in the essential oils of lavender (Lavandula angustifolia) plants upon exogenous application of 24-epibrassinolide (Eb, 0 and 100 mg/L) under drought stress levels (100, 60, and 30% FC) at two cultivated sites, Ahar (Ah) and Kaleybar (Kb). Hierarchical cluster analysis (HCA) with Pearson correlation on 28 essential oil constituents identified. The colors of the matrix boxes represent the magnitude and direction of the association: intense red and blue indicate strong positive and negative correlations, respectively. (B) The HCA with Pearson correlation coefficient among various examined traits [(plant dry weight (DW), leaf relative water content (RWC), plant height, chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TChl), carotenoids (Car), essential oils content (EOsC), malondialdehyde (MDA), hydrogen peroxide (H2O2), free proline and total phenolics content in the lavender plants foliar sprayed with 24-epibrassinolide under drought stress conditions. (C) HCA with heatmap based on the different morpho-physiological traits measured in lavender plants upon experimental treatments, showing the treatment-variable relationships. Two main clusters were identified at the variable level.

CONCLUSIONS

Drought, in the changing climatic conditions of the world, is a major environmental constraint for plant production and productivity. However, 24-epibrassinolide, a plant hormone and an active by-product produced during brassinolide biosynthesis, has been recognized as an effective drought stress ameliorating approach. In the present study, drought stress adversely affected lavender (*Lavandula angustifolia*) plants performance via interference in primary- and secondary metabolism due to enhanced levels of cellular injury indices such as hydrogen peroxide, malondialdehyde, and decreased levels of plant biomass, photosynthetic pigments (chlorophylls and carotenoids). Moreover, foliar application of 24-epibrassinolide was not only able to mitigate the negative impacts of water deficit stress, but also enhanced dry weight, essential oils production, free proline as well as total phenolics content.

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PERFORMANCES OF REGIONAL TOURISM IN THE AREA OF NORTHERN SLOVAKIA

SUMMARY

Tourism is a dynamically developing segment, belonging to important components of the national and world economy. The Slovak Republic has a suitable starting potential for the development of tourism. The North of the Slovak Republic consists of two self-governing regions, acting as tourism regions, which are the subject of the study. Their offer in terms of tourism is diverse. At present, the development of the tourism phenomenon has been significantly slowed down by the Covid-19 pandemic. The study focuses on the Žilina self-governing region and the Prešov self-governing region, which form a unified whole in the North of Slovakia. The aim of this paper is to use analytical methods to identify and describe the performance of tourism in selected regions that form the North of Slovakia. The analysis of tourism development in the Žilina self-governing region and the Prešov self-governing region on the basis of selected statistical indicators, points to the development situation in the time horizon of 15 years. Despite the adjective most mountainous part of Slovakia, the results of the study confirm that these regions are attractive, and, in the past, they were experiencing an increase of visitors. This fact subsequently requires the expansion of services in tourism in the form of complex services and a higher degree of cooperation of entities in the region.

Keywords: tourism, regions, overnight stays, accommodation facilities, income of accommodation facilities

INTRODUCTION

Tourism contributes to the economic growth of the regions and thus also to the economic growth of the country as a whole. It brings not only economic

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benefits but also the cultural, social, ecological ones with an emphasis on sustainability. The potential of tourism in Slovakia is supported by the fact that Europe is the largest market considering the number of international arrivals each year. Slovakia is a country with a very good, although still not fully exploited potential of tourism.

The geographical division of the territory is seemed as a necessary and important for the preference for the development of types of tourism, thus contributing to the reduction of regional disparities. The partnership between tourism and the region depends on the quality of the offer of the natural environment, cultural potential (human footprint in the region) and on the fulfilment of the dynamic component of tourism - attractive organized events of various kinds and measurements. Subsequently, the offer transformed into a product of networks of entities in tourism can address a visitor who will make some effort and visit the selected region.

By increasing the visitors' rate, it is needed to develop the range of services provided in tourism. These facts are monitored through measurable indicators, summarized in statistical results and reports. Based on their values, it is possible to monitor and respond to changes, evaluate the development of the region and then, if necessary, correct it.

Due to the Covid-19 pandemic, not only economic, social life but also dynamically launched tourism has slowed down. The tourism industry, which has dealt very quickly with the occurrence of various diseases around the world as well as with the economic crisis, is experiencing extremely difficult times during this period. Many experts agree that this is the most affected sector.

Overview of the researched issues in the literature

In the scientific bibliography of the recent period, a relatively large group of authors deals with the issue of both tourism and analysis of regions. From the number of publications, due to the focus of the study, relevant studies of the authors are selected, which focus on the relationship and interaction of tourism and the region.

Gao, Xu a Zhang (2019) examine the relationship between CO2 emissions, energy consumption, economic growth and tourism development in the region of selected countries. Dunets *et al.* (2019) are dedicated to sustainable tourism within mountain regions. A comprehensive analysis of tourism in mountainous areas and determining the strategic priorities for different regions is addressed for instance by Dunets *et al.*, 2019a; Sharafutdinov *et al.*, 2018; Voronkova *et al.*, 2019 and others. The influence of the socio-demographic characteristics of visitors on the visited region and the communities in them is examined by Alrwajfah *et al.*, 2019; Rebollo 2018; Micić *et al.*, 2019. The importance and purpose of accommodation facilities in the region monitor for instance Gabryjończyk and Kułaga 2017; Štefko *et al.*, 2018; Márquez 2018.

J. K. Walton (2020) perceives tourism as an act and a process of spending time away from home in search of recreation, relaxation and pleasure, while

using the commercial provision of services. At the beginning of the 21st century, international tourism became one of the most important economic activities. The Statistical Office of the Slovak Republic (2013) characterizes tourism as the activity of people traveling outside their usual environment, not longer than one continuous year (outbound tourism), for the purpose of rest or business reasons, which, however, are not related to paid activities performed at the visited place.

The region is generally understood as a part of the earth's surface, which differs from the surroundings in a certain defined characteristic (Rajčáková 2009). From a geographical point of view, the region represents a limited area, which differs from the others by the set of natural conditions, the structure of the population, the structure of the economy, etc. From a sociological point of view, the region is a territorial unit within which the basic life functions of the population take place (work, housing, education, health, social security, etc.). The region represents a limited area with approximately the same natural-geographical features, economic base, is characterized by a high integrity of intra-regional links, the ability to reproduce development factors from its own resources and has its own typical culture (Bajanová 2010). Regions can be divided into natural and the administrative ones (Výrostová 2010).

Regional arrangement of Slovak territory from 1945

The communist coup in 1948 marked the introduction of a centralist system of governance. Subsequently, it was necessary to create and adopt an appropriate model of regional organization. The basis of the new system was the regional unit. The regional system was introduced by Act no. 280/1948 Coll. on the regional establishment, which created the national committees of the regional level (Mikuš 2018).

The form of regional establishment and the rules of the system were valid until the adoption of Constitutional Act no. 100/1960 Coll. While the official name of the state is changed to the Czechoslovak Socialist Republic (ČSSR). In the same year, there were changes in regional management. By the Act no. 36/1960 Coll. on the territorial division of the state, which is referred to at the time as the Reduction Act, the number of administrative units was reduced from the original 19 to 10. In the conditions of Slovakia, it was a reduction from 6 to 3 regions in order to create an easier-to-manage system. Since the entry into force of the law, Slovakia has been divided into West Slovakia (headquarters in Bratislava), Central Slovakia (headquarters in Banská Bystrica) and East Slovakia (headquarters in Košice).

With the adoption of the Act on the Czechoslovak Federation (October 1968), real self-government did not exist in Slovakia (Kováč 2007). Another change in the state establishment and regional administration was the assignment of the capital city of Bratislava to the three existing regions. In the regional report, a three-plus-one model was created. This, as a relatively stable component in the construction of public administration, survived until social and political changes in the late eighties (Mikuš 2018).

Figure 1: Regional establishment of Slovakia after 1960 (Source: Slovakiamap.jpg., 2015.)

After 1989, the society had to deal with several urgent tasks. The fall of the communist dictatorship led to the need to focus on the creation of a democratic system, the resolution of the state status of Slovakia and the rebuilding of directive economic planning to market (Čaplovič a kol. 2000). The 3 + 1 model was considered a remnant of the past and was therefore temporarily cancelled and replaced by a new mechanism. From the original three-stage model (region, district, municipality) a two-stage establishment was created. Districts as the higher part remained the same, but in the first stage, consisting of municipalities, 121 territorial districts were created. These were created based on the Regulation of the Government of the Slovak Republic no. 548/1990 Coll. (Mikuš 2018).

Territorial reform itself was complicated and demanding. Discussions at the political level brought several proposals, which included eight resp. twelve regions. The 3 + 1 model of higher territorial units was rejected (Machyniak 2018). The Act no. 302/2001 Coll. on self-government created Upper territorial units represented by self-governing regions. Act no. 416/2001 significantly extended their operation on the transfer of certain scopes from state administration bodies to municipalities and local authorities. Since January 2002, the self-government has consisted of eight local authorities (MV SR 2007).

Gradually, a uniform structure for society was created at the national or international level, known as the common nomenclature of territorial units for statistics - classification NUTS (Nomenclature des unités teritoriales statistique). The essence of the NUTS classification is based on the hierarchical classification of regions. It consists of 5 regions, with NUTS I, NUTS II and NUTS III levels merging with regional and NUTS IV (LAU 1) and NUTS V (LAU 2) associate with the local level. The classification does not take into account the classical divisions of regions, logical rules of regionalization, heterogeneous and incomparable regions, and is therefore unsuitable for two-way comparisons, according to critics from professional circles (Sloboda 2014). NUTS have a legal

basis based on Regulation of the European Parliament and of the Council No 1059/2003. The reason for its adjustment was the enlargement of the EU with new members. The building blocks for the NUTS classification are national authorities made up of EU cities and towns (Kołodziejski 2020).

Figure 2 Regional establishment of Slovakia after 2001 - self-governing regions
Source: Red College, 2015.

Northern Slovakia regions´ potential for the development of tourism

The presented part of the study presents the most significant primary potential of the monitored regions, which is created by man in the static component as well as in its dynamic component. At the same time, it presents the most significant natural potential of the regions.

At the same time, Regional Tourism Organizations and Regional Tourism Organizations in the Regions are also mentioned and described. These types of organizations were established on the basis of Act 91/2010 Coll. on the support of tourism. "A regional tourism organization is a legal entity that supports tourism and creates conditions for its development in the region and protects the interests of its members. According to the law, a local tourism organization is a legal entity that supports the development of tourism, while its goal is to provide groups and individuals with services to satisfy their interests and needs in travel, meetings and entertainment at a specific time and place" (Šenková 2018).

The general goal of the mentioned types of tourism organizations is (according to Act 91/2010 Coll.) to network entities operating in tourism, to encourage them to cooperate, to promote their products and to create packages of tourism products from the given region. One of the goals of regional and local tourism organizations is to extend the stay of visitors in the region. Subsequently, each of the organizations presents itself with a certain specificity, which is part of its region, so pf the territory in which it operates.

Perfect mapping of the offer of regions, determining the significance of potential points of interest for visitors (transnational, national, regional and local)

is possible by monitoring the data of the Statistical Office or creating own, often more detailed database, containing the necessary statistical indicators.

The potential of regions is a summary of conditions and preconditions for tourism in a given area - region. Potential is only an existing supply of factors, an option that may or may not be exploited. It does not yet guarantee the success and good results of a region with a high potential for tourism. The role of the human factor (entrepreneurship, skills, motivation, organization, marketing) and the overall approach of people who often know how to eliminate less favourable territorial assumptions and achieve results even better than in regions with higher potential are important here (Švedová 2013).

The Žilina self-governing region is located in the northwest of Slovakia. The Žilina self-governing region belongs into five regions - Horné Považie, Kysuce, Liptov, Orava and Turiec. Within the regions, a wide range of cultural monuments of international importance dominate (for example, open air museum Vlkolínec - UNESCO) (Szabo 2020), national significance (for example the town of Martin, which is connected with the identity of Slovaks), but also local significance (for example architecture in Podbiel, Čičmany municipality) (Slovakregion 2016). There are many castles such as Orava Castle or Strečno, a rich network of bike paths, or winter sports centers (such as Roháče). Thermal swimming pools in the villages of Bešeňová and Liptovský Ján, Lúčky and Rajecké Teplice (Szabo 2020) are popular. There are 4 national parks (High and Low Tatras, High and Low Fatra), 4 protected landscape areas, 39 natural monuments, 62 national nature reserves and 9 protected natural monuments in the Žilina self-governing region. The natural environment creates suitable conditions for mountain tourism or recreation by the water of the Orava dam, Liptovská Mara, the Žilina waterworks and the Hričovská reservoir (ŽSK 2018).

Numerous information offices provide information for visitors to the Žilina self-governing region about activities and attractions in tourism. The Žilina self-governing region has a total of 27 established information offices in its territory, of which the following can be mentioned:

- Tourist information center Čičmany,
- Tourist information center Žilina,
- Information center Vlkolínec,
- Information center Jased Jasenská dolina,
- Tourist information center of Martin,
- Tourist information center Snowland Valčianska dolina (ŽSK 2016).

There are also Regional tourism organizations (KOCR) and Local tourism organizations (OOCR) in the Žilina self-governing region:

- KOCR Žilinský turistický kraj
- OOCR Malá Fatra
- OOCR Rajecká dolina
- OOCR Región Liptov
- OOCR Klaster Orava
- OOCR Organizácia cestovného ruchu Kysuce

- OOCR Turiec – Kremnicko (ŽSK 2016a).

The regional culture includes the state chamber orchestra, theatres, national libraries and museums. The region is home of the University of Žilina, the Jessenius Faculty of Medicine of Comenius University in Martin, the Academy of the Armed Forces of General Milan Rastislav Štefánik in Liptovský Mikuláš and the Catholic University of Ružomberok (ŽSK 2018). The city of Žilina itself offers visitors various events such as the Carnival Festival (Carneval Slovakia), Medieval Day, Creative Crafts Workshops, Fest Anča, Central European Festival of Concert Art Allegretto (TIK Žilina 2020). Popular is the international folklore festival Jánošík's Days in Terchová, the festival of the amateur theatre Scénická žatva and a show of professional theatres Dotyky a spojenia (Touches and Connections) (Slovakregion 2016d).

The Prešov self-governing region is located in the northeast of Slovakia. It includes the regions of Horný Zemplín, Šariš, Spiš, Zamagurie, Tatry. These regions offer a rich choice and opportunities for tourism development based on their potential. The Prešov self-governing region as a region has world-famous monuments (for example, wooden churches of the Carpathian Arch, Spiš Castle, the towns of Bardejov, Levoča as UNESCO monuments or the A. Warhol Museum of Modern Art) (Slovakregion 2016a). Sights of national significance are for example the Red Monastery Museum or the Dukla, the city of Prešov - a city monument reservation) and with local significance are for example, the pilgrimage site of Litmanová (Slovakregion 2016b). The most attractive and very popular destination of Slovakia is the Tatras Mountain with an unforgettable scenery of the Gerlach, Kriváň and Rysy peaks. The high mountain location creates suitable conditions for winter sports and tourism in the resorts of Štrbské Pleso, Starý Smokovec and Tatranská Lomnica. The Tatra Ice Dome in Hrebienok is another great dominant of the Tatras region and thus also of the Prešov region (Slovakregion 2016c). The Prešov region includes and partially covers five national parks - Tatra National Park, Low Tatras National Park, Poloniny National Park, Slovak Paradise National Park and Pieniny National Park (PSK 2018).

These attractions (as well as other cultural and natural attractions and activities offered in the north of Slovakia) form a significant potential for the development of tourism in the northern regions of Slovakia. They represent not only an attraction for visitors but also a reason for investing to tourism and an attractive environment for business entities in tourism (for example, accommodation facilities, etc.).

The Prešov self-governing region disposes by several information, city and tourist centers (total: 34 registered), of which the following can be mentioned:

- Tourist information center and travel agency Bardejov
- Information center of Levoča
- Information center of Prešov
- Tatranská Information center Tatranská Lomnica
- Tatranská Information center Vysoké Tatry

- Tourist information center of Snina (PSK 2013).

Information activities are provided by Local tourism organizations (OOCR, total 7) and Regional tourism organizations (KOCR, total 1):

- KOCR Severovýchod Slovenska
- OOCR Región Vysoké Tatry
- OOCR Tatry-Spiš- Pieniny
- OOCR Severný Spiš – Pieniny
- OOCR Horný Zemplín – Horný Šariš
- OOCR Vysoké Tatry – Podhorie
- OOCR Región Šariš
- OOCR Šariš – Bardejov (PSK 2013a).

Events with international participation are also held on the territory of the Prešov self-governing region such as the chess tournament of Count Ján Zamoyský Stará Ľubovňa, the cultural festival Days of Master Pavel in Levoča, the club of lace lace in Prešov, the festival of costumed dolls in Poprad and many others (PSK 2015).

MATERIAL AND METHODS

The main goal of the study is to use analytical methods to compare the development and performance of tourism in selected regions that form the north of Slovakia. The Žilina and Prešov self-governing regions form the northern area of Slovakia, which, thanks to the regions, but especially the Tatra Mountains, has significant potential for the development of tourism.

Data from the sources of the Statistical Office of the Slovak Republic were analyzed using the method of secondary data analysis. By comparing selected statistical indicators, the study presents the state of statistical indicators in the tourism sector for the observed period of fifteen years (from 2004 to 2019).

The study evaluates selected relevant statistical indicators on the example of accommodation facilities, length of stay (beds), number of visitors and revenues from accommodation (including VAT).

Accommodation facilities are facilities that regularly provide temporary accommodation to visitors (Šenková 2009). Overnight stays (beds) in accommodation facilities represent all beds provided for the night's rest of the facility's guests, including occasional beds. Beds for the owner and staff of the accommodation facility are not included here (Statistical Office of SR 2018). Šenková (2009) says that each accommodation facility is also linked to the indicator of bed capacity of accommodation facilities, which is determined by multiplying the number of permanent beds and days of accommodation facilities in operation.

A visitor is a person who uses the services of a temporary accommodation facility, regardless of the country of residence. The reason for using the temporary accommodation facility for the visitor is a trip, vacation, business trip, training, course, visit, church ceremony, spa stay. On the contrary, this does not include persons who consider the accommodation facility to be a hostel

(domestic, foreign workers in Slovakia). The visitor may not exceed the period of temporary accommodation exceeding a calendar year (Statistical Office of SR 2018).

Revenue is defined as profit, benefit or total cash income, sales income (Kačala and Pisarčíková, 2003).

Before the research was conducted, a research problem was set, which focused on the mutual influence of selected statistical indicators, the importance of their monitoring and interconnection. The research problem was defined: It can be assumed that the selected statistical indicators influence each other, and it is important to monitor them for better coordination of regional development as two units.

Subsequently, a research question arose: Are the selected statistical indicators influencing each other, and is it important to monitor them for better coordination of the development of regions as two units?

The first part of the research problem examining the interaction (relationship) between selected statistical indicators was formulated into hypotheses. The second part of the research problem assumes positive answers to the formulated hypotheses in order to be able to determine the answer by deduction.

The comparison of the tourism development in the Žilina and Prešov self-governing regions was through established hypotheses:

Hypothesis no. 1: We assume that with the growing number of visitors, there was an increase in the number of accommodation facilities in the Žilina self-governing region in the period under review.

Hypothesis no. 2: We assume that with the growing number of visitors in the monitored period there was an increase in the number of accommodation facilities in the Prešov self-governing region.

Hypothesis no. 3: We assume that with the growing number of overnight stays, the number of accommodation establishments in the Žilina self-governing region increased in the observed period.

Hypothesis no. 4: We assume that with the growing number of overnight stays, the number of accommodation facilities in the Prešov self-governing region increased in the observed period.

Hypothesis no. 5: We assume that with increasing revenues from accommodation, there is a growing number of overnight stays in the Žilina self-governing region

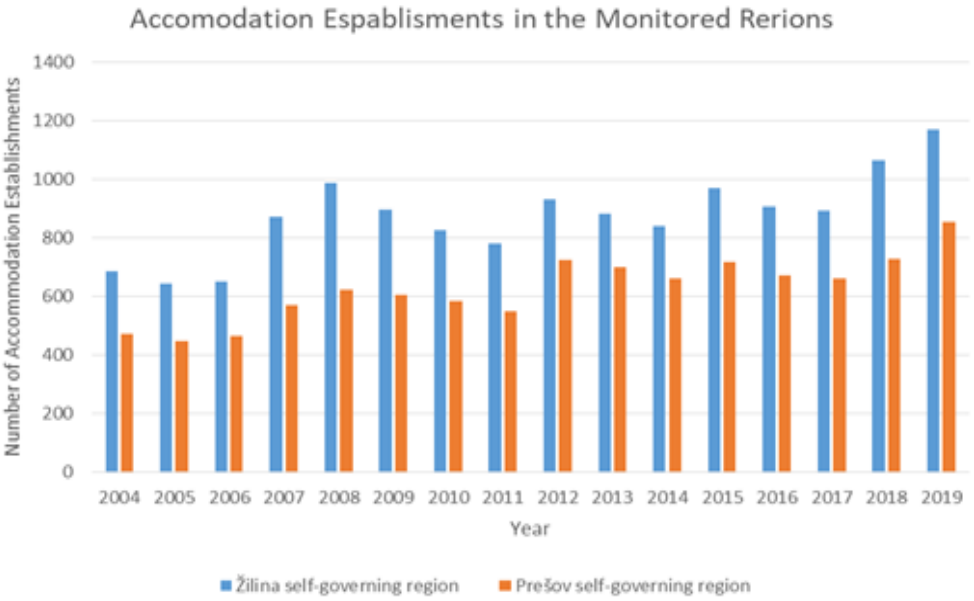
Hypothesis no. 6: We assume that with increasing revenues from accommodation, there is a growing number of overnight stays in the Prešov self-governing region.

In the study methods of regression analysis, correlation analysis - Pearson's correlation coefficient were used. The general form of the regression function has the following form: $y = \beta_0 + \beta_{1*}x+e_i$ i=1,2,...n, where β0 is the locating constant, y is a dependent variable, x is an independent variable and ei is understood as an error term denoting also as u_i. The significance level, the p-value, is 0.05.

RESULTS

The highest number of accommodation establishments in 2019 was the same in both regions, and the lowest number of accommodation establishments was in 2005 (see Graph 1). The highest year - on - year change in the Žilina self - governing region represents an increase in the number of accommodation establishments by 34% in 2007.

The lowest increase in the number of accommodation establishments in the Žilina self-governing region was in 2006 by 1%. The Prešov self-governing region recorded the highest year-on-year increase in the number of accommodation establishments in 2012 by 31% and the lowest year-on-year change in 2006 was by 3%. At the end of the observed period, the number of accommodation establishments multiplied several times compared to the first year of the observed period. Subsequently, it is possible to conclude that the monitored self-governing regions recorded an overall boom in tourism.



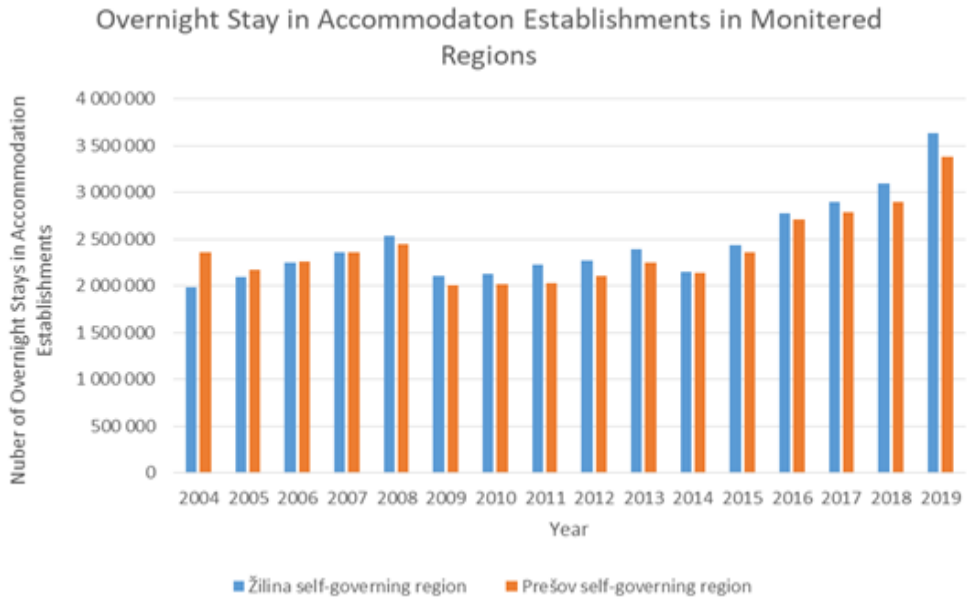
Graph 1. Development of the number of accommodation establishments in the selected regions in the monitored period, (Source: own processing)

As can be seen in Chart 2, in the Žilina self-governing region the highest number of overnight stays of visitors in accommodation establishments was in the last monitored year 2019, the lowest was in 2004. In the Prešov self-governing region the highest number of overnight stays of visitors in accommodation establishments in the same observed year 2019.

On the contrary, the lowest number of overnight stays can be observed in 2009. The highest increase in the year-on-year change in overnight stays in the Žilina self-governing region was 17% in 2019 compared to 2018. The lowest, 1%

increase in the year-on-year change was in 2010. In the Prešov self-governing region, the highest increase in the year-on-year change in overnight stays was in 2019 by 17% and the lowest in 2011 by 0.1%.

From Graph 3 it is possible to read that the largest number of visitors visited the Žilina self-governing region in 2019 and the least in 2004. The Prešov self-governing region also had the highest number of visitors in 2019, the lowest number of visitors was recorded in 2009.

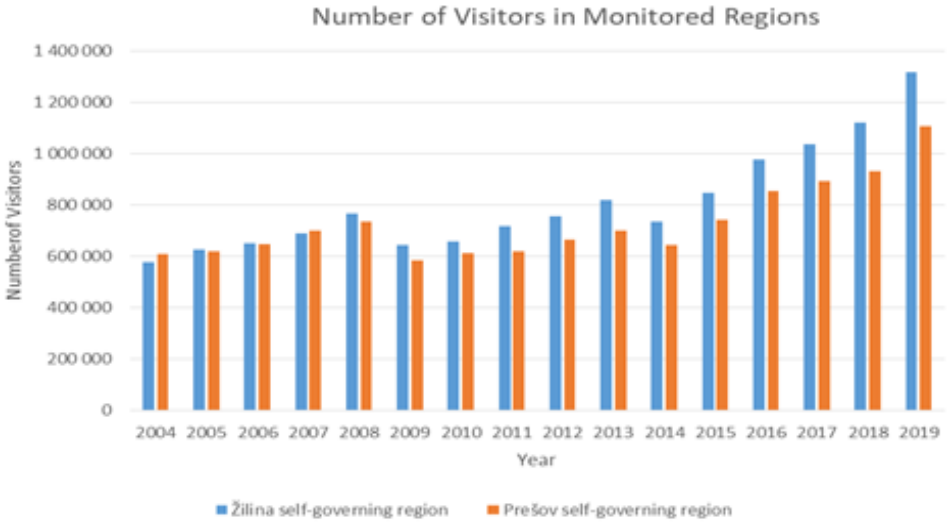


Graph 2. Development of the number of overnight stays in accommodation establishments in the selected regions in the monitored period (Source: own processing)

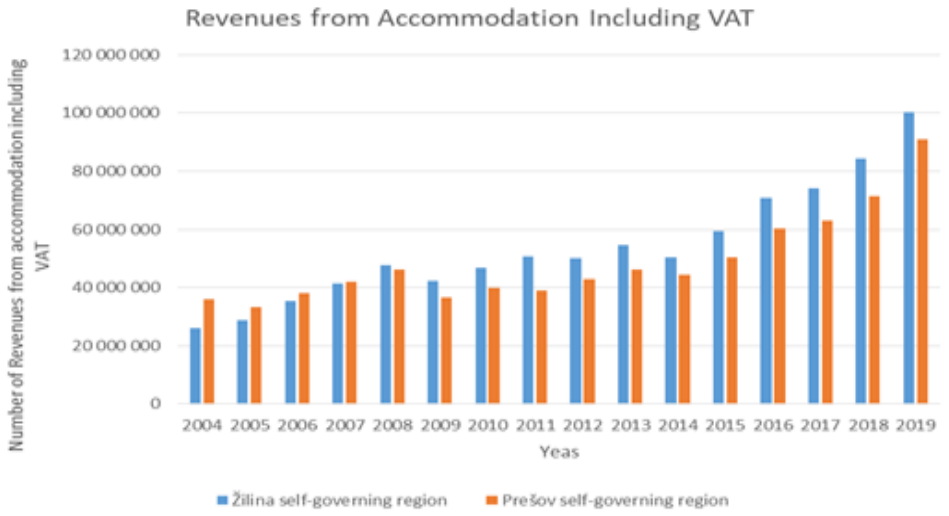
The highest increase in the year-on-year change in the number of visitors in the Žilina self-governing region was by 17% in 2019 and the lowest increase in the year-on-year change in the number of visitors in 2010. In the Prešov self-governing region the increase in the year-on-year change in the number of visitors in 2011 was by 1%. The above data mostly indicate an upward trend in the number of visitors in both regions.

Graph 4 shows that in the Žilina self-governing region the highest revenues from accommodation were achieved in 2019 and the lowest in 2004. Data for the years 2004 to 2008 were converted according to the exchange rate of the National Bank of Slovakia from the Slovak koruna to the euro. In the Prešov self-governing region, they were also the highest in the last monitored year 2019 and the lowest in 2005. There is clearly a high difference between the lowest revenues in the compared regions. The fluctuations in the growth and decline in sales in

both regions are worth noting. In the years 2015 to 2019, higher sales prevail compared to the previous year, which is a positive finding. The highest increase in year-on-year change in the Žilina self-governing region was by 23% in 2006, the lowest by 5% in 2017. In the Prešov self-governing region, the highest increase in year-on-year change in 2019 was 27% and the lowest in 2017 by 5%.



Graph 3. Number of visitors development in the selected regions in the monitored period (Source: own processing)



Graph 4. Revenues in accommodation establishments development in selected regions in the monitored period (Source: own processing)

The factual finding is that at the beginning of the observed period (2004) the Prešov self-governing region recorded higher revenues from accommodation

than the Žilina self-governing region. At the end of the observed period (2019), the development was the opposite. Nevertheless, it is possible to observe a growing development trend for both regions.

Subsequently, after evaluating the secondary data, the hypotheses were evaluated. The results for the monitored selected statistical indicators - the number of visitors, the number of accommodation establishments and the number of overnight stays - in the observed period of 2004 - 2019, within the Prešov and Žilina self-governing regions are as follows:

H1: There is a statistically significant relationship between the variables. The number of accommodation facilities depends on the number of visitors in the Žilina self-governing region. There is a statistically significant relationship between the variables. The strength of the Pearson correlation coefficient is above 0.5, which means a strong strength of the relationship between the variables.

H2: There is a statistically significant relationship between the variables. The number of accommodation facilities depends on the number of visitors in the Prešov self-governing region. Pearson's correlation coefficient is statistically significant. There is a statistically significant relationship between the variables. The strength of the Pearson correlation coefficient is above 0.5, which means a strong strength of the relationship between the variables.

H3: There is a statistically significant relationship between the variables. The number of accommodation facilities depends on the number of overnight stays in the Žilina self-governing region. Pearson's correlation coefficient is statistically significant. There is a statistically significant relationship between the variables. The strength of the Pearson correlation coefficient is above 0.5, which means a strong strength of the relationship between the variables.

H4: There is a statistically significant relationship between the variables. The number of accommodation facilities depends on the number of overnight stays in the Prešov self-governing region. Pearson's correlation coefficient is statistically significant and there is a statistically significant relationship between the variables. The strength of the Pearson correlation coefficient is above 0.5, which means a strong strength of the relationship between the variables.

H5: There is a statistically significant relationship between the variables. Revenues from accommodation depend on the number of overnight stays in the Žilina self-governing region. Pearson's correlation coefficient is statistically significant and there is a statistically significant relationship between the variables. The strength of the Pearson correlation coefficient is above 0.5, which means a strong strength of the relationship between the variables.

H6: There is a statistically significant relationship between the variables. Revenues from accommodation depend on the number of overnight stays in the Prešov self-governing region. Pearson's correlation coefficient is statistically significant and there is a statistically significant relationship between the variables. The strength of the Pearson correlation coefficient is above 0.5, which means a strong strength of the relationship between the variables.

Table 1. Results of tested hypotheses (Source: own processing)

Hypothesis	Test / method	Žilina self-governing region		Findings
H1	Regression analysis	Regression line	y = 427 + 0,000554x	increase in visitors by one unit may increase the number of accommodation establishments by 0.000554
		p-value	0,0000	H_0 Rejection - H_1 acceptation
	Correlation analysis	Pearson´s correlation coefficient	0,80433996	strong strength of the relationship between variables
		p-value	0,0002	H_0 Rejection
H2	Regression analysis	Regression line	y = 224+0,000554x	increase in visitors by one unit may increase the number of accommodation establishments by 0.000554
		p-value	0,003	H_0 Rejection - H_2 acceptation
	Correlation analysis	Pearson´s correlation coefficient	0,73174624	strong strength of the relationship between variables
		p-value	0,0013	H_0 Rejection
H3	Regression analysis	Regression line	y = 252 + 0,000253x	increase in visitors by one unit may increase the number of accommodation establishments by 0,000253
		p-value	0,0000	H_0 Rejection – H_3 acceptation
	Correlation analysis	Pearson´s correlation coefficient	0,79118318	strong strength of the relationship between variables
		p-value	0,0003	H_0 Rejection
H4	Regression analysis	Regression line	y = 208 + 0,000175x	increase in visitors by one unit may increase the number of accommodation establishments by 0,000175
		p-value	0,001	H_0 Rejection - H_4 acceptation
	Correlation analysis	Pearson´s correlation coefficient	0,59907265	strong strength of the relationship between variables
		p-value	0,0142	H_0 Rejection
H5	Regression analysis	Regression line	y =1,34e+006 + 0,0208x	increase in visitors by one unit may increase the number of accommodation establishments by 0,0208
		p-value	0, 0000	H_0 Rejection - H_5 acceptation
	Correlation analysis	Pearson´s correlation coefficient	0,93933283	strong strength of the relationship between variables
		p-value	0,0000	H_0 Rejection
H6	Regression analysis	Regression line	y = 1,32e+006 + 0,0219x	increase in visitors by one unit may increase the number of accommodation establishments by 0,0219
		p-value	0, 0000	H_0 Rejection - H_6 acceptation
	Correlation analysis	Pearson´s correlation coefficient	0,94072061	strong strength of the relationship between variables
		p-value	0,0000	H_0 Rejection

Subsequently, based on the evaluation of the established hypotheses, it can be stated that it makes sense to monitor selected statistical indicators for better coordination of regional development as two units. Regional tourism organizations and subsequently local tourism organizations in selected regions must monitor statistical indicators. As stated in the theoretical basis of the study, number of visitors depends on the richness and attractiveness of the offer

(dynamic or static, anthropogenic or natural). Other indicators, such as the number of overnight stays and the number of accommodation establishments in the regions, are linked to the number of visitors. As the above-mentioned, tourism organizations operating in selected regions strive to gain a regular visitor and especially to extend their stay.

The P_2O_5 content slightly increased in 2019 on the NP background and reached the maximum value in the experiment - 432 mg per kg^{-1} of soil, which is higher compared with the control by 70 mg, but within the experimental error. In the other treatments, the difference was even less significant. The same picture was observed in 2020.

The content of K_2O in treatments with traditional mineral fertilizers did not differ significantly from the control variant. The use of cinder, both in spring and autumn, led to a noticeable increase in K_2O in the soil. The raising of K_2O content compared with the control after autumn application was equal to LSD_{05} value (33 mg) in 2019 and reached 183 mg in 2020 (maximum in the experiment). In the treatment with spring application the difference was insignificant (22 mg) in 2019, but next year was reached 37 mg (LSD_{05} value 12 mg). The total K_2O content was higher in 2020 compared with 2019 for both these treatments. This trend can be explained by the slow release of potassium from poorly soluble compounds in the cinder composition.

DISCUSSION AND CONCLUSION

Hypotheses H1, H2, H3, H4, H5 and H6 monitored the behaviour and dependence of variables - visitors, number of accommodation establishments, number of overnight stays and revenues from accommodation (including VAT) in the monitored regions in the observed period (2004 - 2019). The comparison of the results is as follows. Based on the results of the regression analysis, it can be stated that in hypotheses H1, H2, H3, H4, H5 and H6, hypothesis H0 was rejected and subsequently hypothesis H1, H2, H3, H4, H5 and H6 were accepted. In each tested hypothesis, there is a linear relationship between the variables. This means that the established hypotheses are confirmed. There is a statistically significant relationship in the hypotheses. The strength of the Pearson correlation coefficient relationship is strong for the Žilina self-governing region and the Prešov self-governing region. When comparing the monitored regions, it is possible to confirm the increase of one indicator together with another. The results of the tested hypotheses also show that the Žilina self-governing region has a higher value of the Pearson correlation coefficient than the Prešov self-governing region in the case of comparing hypotheses H1 and H2 than in the case of comparing H3 and H4. In the case of comparing hypotheses H5 and H6, the Prešov self-governing region has a higher value than in the case of the Žilina self-governing region. However, as can be seen from Table 1, the strength of the relationship is high in both regions. The pandemic situation in Slovakia since March 2020 has significantly affected the development of tourism in the case of the region - the Žilina self-governing region. In 2019, based on data from the Statistical Office

(Statistical Office SR 2020), the Žilina self-governing region was the third most visited region by foreign visitors (16.8% - after Bratislava and Prešov), but the first with regard to the attendance of the domestic population. Regarding the number of overnight stays, it occupied the second place both in the case of overnight stays by foreign visitors (the first was the Bratislava region) and in the number of overnight stays within domestic tourism (the first was the Prešov self-governing region). The attractiveness and popularity of the region, especially in domestic tourism, is also underlined by the fact that together in the Prešov region they reached a year-on-year increase of 21.8% in 2019. In the case of a year-on-year increase in foreign visitors, the Žilina region took fourth place. The number of overnight stays was also included in the evaluation of the year-on-year increase in the monitored indicator.

Within the first half of 2020, the tourism was hardly tested by a pandemic and various measures (restrictions and bans) associated with it. In this year, the Žilina self-governing region ranked first in tourism statistics (24.7%) within the indicator of the share of domestic visitors. The first position was also within the indicator the share of overnight stays realized by domestic visitors (24.4%) and in the second place (26.6%) in the case of the share of foreign visitors (after the Bratislava region), but in the first place (31.8%) in the case of the indicator the share of the number of overnight stays foreign visitors. Despite the higher, (perhaps optimistic point of view) development of tourism in the Žilina self-governing region, the sad reality drop tourism performance in the first half of 2020 by -45.1% in the number of domestic and -42.7% in the number of foreign visitors compared to the same period in 2019. In other words, it is the second lowest drop within the regions of Slovakia). In the case of the number of overnight stays, this is a decrease of -40.3% of overnight stays realized by domestic participants and -36.8% of overnight stays realized by foreign participants (the second lowest decrease within the regions of Slovakia) (Statistical Office SR 2020a).

The state of tourism within the Prešov self-governing region in the first half of 2020 was as follows. Data were used from Statistical Office of Slovak Republic 2020 (Statistical Office SR 2020b). The indicators of number of visitors as well as the number of overnight stays indicate that the Prešov self-governing region is one of the first three popular regions in Slovakia. The share of domestic visitors was 22.6% in the first half of 2020, which puts the Prešov self-governing region in the second place. In the case of foreign visitors, it ranked third (after the Bratislava and Žilina regions) with a share of 15.9%.

The share of overnight stays of domestic visitors was 23.9%, which secured the Prešov self-governing region the second place (after the Žilina region). The share of overnight stays of foreign visitors was 18.1% (third place after the Žilina and Bratislava regions).

Unfortunately, the decrease in the total monitored indicators did not avoid either the Prešov self-governing region. In percentage terms for the first half of 2020, the decrease in the number of domestic visitors was 41.8% and foreign

visitors 48%. As for the number of overnight stays, as well as in the case of the number of visitors, a decrease was recorded. On the other hand, it is necessary to add that in both cases it was the least significant decrease within the regions of Slovakia. The share of overnight stays for domestic visitors was -39.5% and in the case of the share of overnight stays of foreign visitors, there was a decrease of -44.1%.

Within the research problem, not only hypotheses were solved and evaluated, but also whether it is intended to monitor selected statistical indicators. The answer was possitive. Due to the diversity in the offer but also the geographical similarity of the regions, while it can be stated that together they form a natural whole, it is necessary that the above statistical indicators will be monitored by the subjects of destination management. In the paper, they are listed as regional and local organizations which, unlike information centres, form strategic and promotional activities within the destination (the territory in which they were created by merging tourism entities) and thus contribute to the overall growth of the region. These entities should summarize data for smaller territorial units (for example, the city) as a statistical office (the smallest statistical unit is a district), just for detailed knowledge of the use of the region's potential in the form of service providers (in case of study number of accommodation facilities, beds, sales but also other indicators). Subsequently they should summarize visitors of the region, which indicate the willingness to accept the offer of the region and see its attractions.

In time, it is possible to assume asking about the effectiveness of a certain number of local tourism organizations within a region (we mean as a self-governing region) and therefore whether: is it necessary to combine such a number of local tourism organizations or rather into a few more destination management organizations covering the region?

Another stimulus is not only the networking of tourism entities within smaller regions, which are managed as a destination by a regional / local tourism organization, but also to establish cooperation between regional / local organizations between the monitored regions (Prešov and Žilina) due to their regional proximity and similarity.

In many European countries, tourism is an important part of the national economy. Thanks to the natural potential of the country and the potential created by man, tourism in Slovakia has a great opportunity to take a strategic position in the state.

The north of Slovakia, represented by the Žilina and Prešov self-governing regions, has extremely rich potential and suitable preconditions for this.

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THE INFLUENCE OF VARIETY AND FERTILIZATION ON THE YIELD AND CONTENT OF VITAMIN C IN LEAF OF PARSLEY *(Petroselinum ssp.)*

SUMMARY

In this paper the impact of different varieties and fertilizers on yield and vitamin C content in the leaf of parsley was investigated. In two-year research, three varieties of parsley were used and three variant of fertilization were applied (mineral fertilizers, organic and organomineral fertilizer) as well as control variant. The varieties used in the research are: „Domestic sawmills “, „Berlin semi-long type “, „Mooskrause “. The yield of leaf and vitamin C content of the leaf parsley was significantly influenced by the variety and type of fertilizer. In both years the research the highest yield of leaf was achieved by variety „Domestic sawmills “, with an average yield of 58.69 t/ha, while the highest vitamin C content had a variety „Berlin semi-long “. Two – year research showed that the application of organic fertilizer had a positive effect on yield and vitamin C content in leaf of parsley.

Key words: leaf of parsley, variety, fertilizers, yield, vitamin C.

INTRODUCTION

Parsley (*Petroselinum hortense*) is important vegetable, aromatic and medicinal plant origin in the Mediterranean area. It is growing because of its turnip root and leaves of characteristics, pleasant smell and taste. Both fresh and dried parsley are used in food, cosmetic and pharmaceutical industry, for production of spices, essential oil and medication production as well (Lopez et al. 1999). The production of parsley in BiH is not sufficiently represented and most of our parsley production is in households. Although the climatic conditions for cultivation are favorable, there is no more intensive production, and there is no

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use of modern technology in cultivation, while at the same time import is noticeable.

The growing conditions, including soil type, fertilization, moisture content and insolation, may have had influence on yield and biological value of parsley leaf **(**Jadczak *et al*. 2018). Very important for productivity and quality of parsley is correct selection genotype in certain production conditions. There are three types of parsley: flat leaves parsley (*ssp. neapolitanum*); curly leaves parsley (*ssp. crispum*) which are planted because of their leaves and there is also turnip rooted parsley (*ssp. tuberosum*) that is planted because of its root. Types of parsley differ in morphology and chemical composition, but also in essential oil content and composition (Petropoulus *et al*. 2004).

Vitamin C is an important factor in human nutrition. Although there are many functions of vitamin C, his role in health is discussed mostly in relation to its role as an antioxidant and its effects on cancer, blood pressure, immunity, drug metabolism and urinary excretion of hydroxyproline (Barrita and Sanchez 2013). One is of the most important factor in the nutritional quality many horticultural crops and has many biological activities in human body.

Parsley leaves contain from 96.88 to 312.7 mg / 100 g of vitamin C, far exceeding its content in lemon and orange (Osinska *et al*. 2012). The same authors was found that the content of vitamin C in frozen parsley leaves ranges from 10.06 to 47.10 mg / 100 g.

According to the results of the research carried out by Cauni *et al*. (2010), the leafy parsley contains significantly more vitamin C (133.0 mg/100 g) with compared some other leafy vegetables (celery 85.0 mg/ 100 g, lettuce 24.0 mg/100 g, cabbage 51.0 mg/100 g).

The aim of the research was to determine how the variety and type of fertilizer effects on yield and the content of vitamin C in the leaf of parsley.

MATERIAL AND METHODS

Research of the yield and content of vitamin C in leaves of parsley during two vegetation periods: 2013 and 2014 was done. We perfomed two factorial field experiment in area in the north suburb of Mostar, called Bijelo Polje. We set this in randomized block in 4 replications, the whole parcel was 1 m^2.

Three parsley varieties were used as the subject of the research and three fertilization variants plus a control variant were applied.

The varieties used in the research are: Domestic sawmills "- sawmill type (*P. hortense* ssp. *neapolitanum*), „Berlin semi-long "- root type (*P. hortense* ssp. *Tuberosum*), „Mooskrause "- curlyleaves type (*P. hortense* ssp.*crispum*).

Variants of fertilization are: organic, organo-mineral, mineral and control variant. All types of fertilizers were applied with pre-sowing soil preparation.

For organic fertilization sheep manure was used, in an amount of 20 t/ha. Sheep manure on average contains 0.60% N, 0.3% P_2O_5 and 0.2% K_2O (Lazić *et al*. 2013). The organic matter content is about 29%. As an organomineral fertilizer, we used Phenix fertilizer. It is a combination of organic and mineral

fertilizers whose NPK values are 6: 8: 15 + MgO. The percentage of organic matter is 50%. Organo-mineral fertilizer was applied in the recommended amount for vegetable crops of 1 t/ha. For mineral fertilization, a permanently complex fertilizer NPK 8:16:24 was applied in a quantity of 720 kg/ha and KAN (27% N) fertilizer in an amount of 170 kg/ha to supplement the required quantity N. Quantities of applied fertilizers were within the ranges recommended by different authors (Lešić *et al*. 2004, Lazić *et al.* 2013).

The seed was sown in rows at a distance of 20 cm, in the first decade of March. The rate of sowing is 3 g/m^2.

Before setting up the experiments, soil samples were taken for chemical analysis, from the 0-30 cm depth. The results of the analysis showed that the pH value of the soil determined in water has a slight alkaline reaction (7.56) and in KCl poorly acidic to neutral (6.76). The total N (Kjeldahl) content was 0.21 percent indicating that the soil was well supplied with nitrogen. According to the content of available P_2O_5 (8.20 mg/100 g), determined by the Al-method according to Egner-Riehm, the soil is poorly supplied with phosphorus (class III). The content of K_2O (Al-method according to Egner-Riehm) was 16.80 mg/100 g, which corresponds to a good supply (class II) The content of humus is 1,60 percent, which indicates that the soil has low humus content.

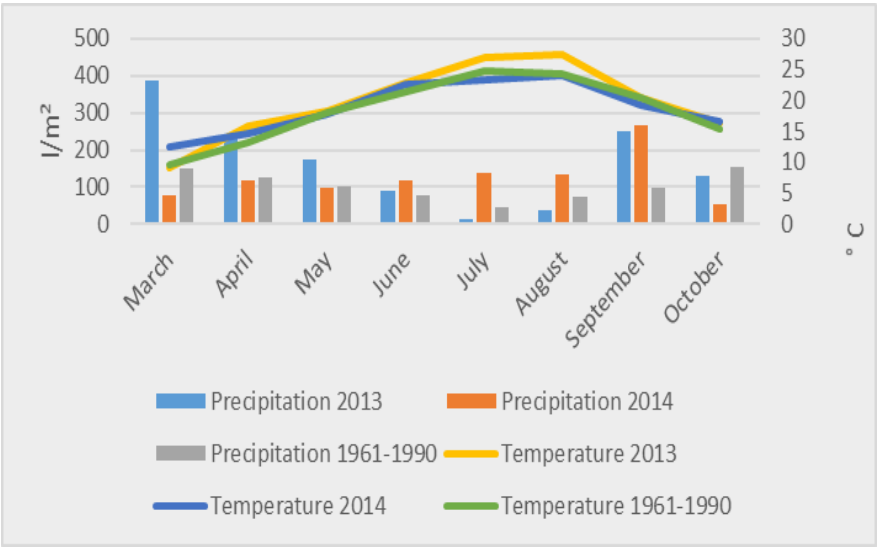
The yield of the leaves (t/ha) was determined by measuring the overhead part of the plant after each cutting (three cuttings), on the basis of that we obtained the yield by the cuttings and the total yield. The first cutting was in the second decade of June, the second in the first decade of September and the third in the last decade of October. The results presented data for the total yield.

Samples for the analysis of plant material were taken after the first cuttings of the leaf. After taking the samples were frozen at -18 ˚C for one month. The content of vitamin C was determined by titration using iodide solution (Skroza *et al*. 2010).

Presented data are statistically processed using the software package program SPSS 20. Two-way ANOVA followed by post hoc Tukey's test for mean separation was performed and the significant differences (p=0.05) were determined. This method was used in order to investigate the influence of interaction of two examined factors (independent variables), fertilizers and genotypes, on the dependent variables, yield and content of vitamin C. Pearson's Correlaton was done in order to measure the strength and direction of linear relationships between pairs of continuous variables.

Climatic factors

During the experiment, the climatic conditions of the area where the experiment was set up were monitored. The data of the Federal Meteorological Institute in Sarajevo, Mostar meteorological station, were used for the analysis of climatic conditions. Graph 1 shows the average air temperatures and precipitation during the growing season in both years of the research, as well as the perennial averages for average air temperatures and precipitation.



Graph 1. Average air temperatures and precipitation during the growing seasons 2013-2014 and perennial averages in the Mostar area

RESULTS AND DISCUSSION

The yield of leaf parsley, depending on variety and used fertilizer variant as well, was from 21.68 to 80.30 t/ha.

Table 1. The Influence of variety and fertilization on yield of parsley leaf (t/ha) in 2013.

Fertilizer	Genotyp			**Average**
	"Domestic sawmills"	„Berlin semi long"	„Mooskrause“	$\bar{x}$ ± SD
Control	61,51	36,40	33,81	**43,91**^{A} ± 3,03
Organic	80,30	46,31	55,45	**60,69**^{B} ± 3.03
Organo-mineral	46,85	29,02	30,44	**35,44**^{C} ± 3,03
Mineral	60,00	33,83	35,40	**43,10**^{AC} ± 3.03
Average	**62,16**^{a}	**36,43**^{b}	**38,77**^{c}	
		Sig.		
Genotype		,000		
Fertilizer		,000		
Genotype*Fertilizer		,388		

^{a,b,c} *– significant differences between genotypes themselves (p<0.05)*
$^{A, B, C}$ *– significant differences between examined fertilizers (p<0.05)*

From data presented in Table 1 can be seen that there is no statistically significant interaction between the effect of variety and fertilizer on parsley yield in 2013 (p=.388), but there is statistically significant difference between varieties

themselves ($p<0.05$). Nevertheless, can be concluded that the yield obtained with organic fertilizer is significant different compared to other examined fertilizers ($p<0.05$).

Table 2. The Influence of variety and fertilization on yield of parsley leaf (t/ha) in 2014.

Fertilizer	Genotyp			**Average**
	"Domestic sawmills"	„Berlin semi long"	„Mooskrause“	$\bar{x}$ ± SD
Control	50,22	26,17	21,68	**32,69**^{A} ± 3,14
Organic	56,51	38,56	24,28	**39,78**^{AB} ± 3.14
Organo-mineral	43,48	24,84	21,72	**30,00**^{C} ± 3,14
Mineral	70,73	27,79	29,53	**42,68**^{B} ± 3.14
Average	**55,23**^{a}	**29,34**^{b}	**24,30**^{c}	
		Sig.		
Genotype		,000		
Fertilizer		,001		
Genotype*Fertilizer		,034		

^{a,b,c} *– significant differences between genotypes themselves ($p<0.05$)*
$^{A, B, C}$ *– significant differences between examined fertilizers ($p<0.05$)*

From data presented in Table 2 can be seen that there is statistically significant interaction between the effect of variety and fertilizer on parsley yield in 2014 ($p=.034$). So, there is statistically significant difference between all of the examined varieties and fertilizers themselves ($p<0.05$). On the other hand, from post hoc tests, can be concluded that the parsley treated with organo-mineral fertilizer has significant difference yield ($p<0.05$) compared to yields obtained with other examined fertilizers.

In both years of research, the highest yield of parsley leaves had variety „Domestic sawmills “. Leafy parsley had higher yield of overhead biomass, and it had more leaves than petals when we compared it with root parsley Kmiecik and Lisiewska (1999). Research results Jadczak *et al*. (2018) showed that the yield of parsley leaves varied significantly between different varieties and years of research and ranged from 57.40 to 166.52 t/ha. According to research Kolota (2011) flat leaf parsley, to which the variety „Domestic sawmills“belongs, depending on fertilization with different amounts of nitrogen, had lower yield of leaves when we compared it with curly parsley, to which the variety „Mooskrause“ belongs. These authors obtained that the yield of the curly parsley type ranged from 55.3 to 60.9 t/ha and flat leafy parsley from 52.5 to 59.6 t/ha.

In terms of fertilization, the lowest leaf yield in both years of the our research was achieved with the use of organo-mineral fertilizer. In 2013, the highest yield of parsley leaves was with the usage of organic fertilizer, and it was significantly higher when we compared it with other variants of fertilizer. In

2014, the highest yield was achieved by application of mineral fertilizer, but the usage of mineral fertilizer was not significant when compared it with the application of organic fertilizer. Lim and Vimala (2012) say that the usage of appropriate amount of organic fertilization of leafy vegetable can give the same yields or even higher yields when we compare it with vegetable fertilized with mineral variant of fertilizer.

The fact that optimal yield of parsley leaves can be achieved with the use of organic fertilizer is significant, since parsley belongs to a group of leafy vegetables that has a tendency to increase the accumulation of harmful nitrates, especially in conditions of inadequate mineral fertilization and excessive use of nitrogen fertilizers (Santamaria 2006). Rahimić *et al*. (2018) found that both leaf and root of parsley accumulated significantly more nitrate with the use of mineral fertilizer compared to organic and organomineral fertilizer, as well as variant without the use of fertilizers. The usage of organic fertilizer (manure) had positive influence and its increased growth and carrot's root yield (Ahmed *et al.* 2014.).

All three parsley varieties had significantly higher leaf yield in 2013, when we compared it with the yield in 2014. This difference may be conditioned by the weather conditions, due to the weather conditions differ between years of research (Graph 1). There was a lot of rainfal during the spring and the summer in 2014. There were also a lot of cloudy days during growing period in 2014, and parsley is a plant that requires a lot of light.

The content of vitamin C in the leaf of parsley, depending on investigated factors, was from 24.00 to 64.67 mg/100 g. As we have already said in material and methods of our work, plant material was frozen for one month before we used it for chemical research. The content of vitamin C in the leaf of curly parsley was from 222.0 to 299.7 mg/100 g, and the content of vitamin C in leafy parsley was from 196.4 to 285.6 mg/100 g (Kolota 2011). In our research, we have found lower values of the content of vitamin C, the reason for that is because our researched plant material was frozen.

According to data presented in Table 3 can be seen that there is no statistically significant interaction between the effect of variety and fertilizer on the content of vitamin C in parsley in 2013 ($p=.481$). There is no statistically significant differences, neither between the examined varieties, nor between examined fertilizers themselves ($p>0.05$).

According to data presented in Table 4 can be seen that there is statistically significant interaction between the effect of variety and fertilizer on the content of vitamin C in parsley in 2014 ($p=.000$). In this case, there is significant differences between all of the examined varieties and fertilizers themselves, too ($p<0.05$). From post hoc tests, can be concluded that the vitamin C in the parsley treated with organic-mineral fertilizer is significant different ($p<0.05$) compared control variant.

Table 3. The Influence of genotype and fertilization on on the vitamin C content in the leaf of parsley (mg/100 g) in 2013.

Fertilizer	Genotyp			**Average**
	"Domestic sawmills"	„Berlin semi long"	„Mooskrause“	$\bar{x}$ ± SD
Control	47,82	48,67	53,47	**49,99**^{A} ± 6.51
Organic	44,33	57,46	39,00	**46,93**^{A} ± 6.51
Organo-mineral	31,00	37,33	35,00	**34,44**^{A} ± 6.51
Mineral	45,52	34,45	24,00	**34,66**^{A} ± 6.51
Average	**42,17**^{a}	**44,48**^{a}	**37,88**^{a}	
		Sig.		
Genotype		,503		
Fertilizer		,045		
Genotype*Fertilizer		,481		

^{a} – *no significant differences between genotypes themselves (p>0.05)*
^{A} – *no significant differences between examined fertilizers (p>0.05)*

Table 4. The Influence of variety and fertilization on the vitamin C content in the leaf of parsley (mg/100 g) in 2014.

Fertilizer	Genotyp			**Average**
	"Domestic sawmills"	„Berlin semi long"	„Mooskrause“	$\bar{x}$ ± SD
Control	37,03	39,33	24,50	**33,62**^{A} ± 3,14
Organic	33,70	35,33	59,67	**42,90**^{AB} ± 3.14
Organo-mineral	26,87	64,67	41,83	**44,46**^{B} ± 3,14
Mineral	51,40	45,00	34,40	**43,60**^{AB} ± 3.14
Average	**37,25**^{a}	**46,08**^{b}	**40,10**^{c}	
		Sig.		
Genotype		,036		
Fertilizer		,028		
Genotype*Fertilizer		,000		

^{a,b,c} – *significant differences between genotypes themselves (p<0.05)*
$^{A, B,}$ – *significant differences between examined fertilizers (p<0.05)*

In both years of our research, the highest content of vitamin C in the leaf of parsley had the variety „Berlin semi-long “. Our results were the same as results of Kmiecik and Lisiewska research (1999), and their research showed that all genotypes of leafy parsley had lower content of vitamin C when compared it with root parsley. The similar results were in Valšikova research *et al.* (2016). In their research, the content of vitamin C in the leaf of parsley was significantly different, depending on the variety, and significantly higher content of vitamin C had variety of root parsley, when we compared it with variety of leafy and curly parsley.

The fertilization of parsley with different type of fertilizer did not have positive influence on the content of vitamin C in the leaf in 2013, because the highest content had the leaf of parsley in controlled variant. However, these differences were not statistically significant. When we compared it with previous years, the lowest content of vitamin C in 2014 was in control variant, significant lower with compare to organo-mineral variant, while compared to the other variants of this difference was not significant. The similar results were in Warman and Havard research (1997), they have researched the influence of organic and mineral fertilizer on the content of vitamin C in carrot and cabbage.

When we calculate the two-year average, we can conclude that the highest content of vitamin C was in organic variant of fertilization (44.91 mg/100 g), and the lowest content of vitamin C was in mineral variant of fertilization (39.13 mg/100 g).

Table 5. Correlation between the yield obtained with several fertilizers and varieties used in this research (two-year average)

		Control	Organic	Organo-mineral	Mineral	Domestic Sawmills	Berlin semi long	Mooskrause
Control	Pearson Correlation	1	,999*	,997*	,988	,070	(,132)	(,293)
	Sig. (2-tailed)		,022	,049	,101	,955	,916	,811
	N		3	3	3	3	3	3
Organic	Pearson Correlation		1	,999*	,992	,036	(,166)	(,326)
	Sig. (2-tailed)			,027	,079	,977	,894	,789
	N			3	3	3	3	3
Organo-mineral	Pearson Correlation			1	,997	(,006)	(,207)	(,365)
	Sig. (2-tailed)				,052	,996	,867	,762
	N				3	3	3	3
Mineral	Pearson Correlation				1	(,088)	(,286)	(,440)
	Sig. (2-tailed)					,944	,815	,710
	N					3	3	3
Domestic Sawmills	Pearson Correlation					1	,781	,883
	Sig. (2-tailed)						,219	,117
	N						4	4
Berlin semi long	Pearson Correlation						1	,940
	Sig. (2-tailed)							,060
	N							4
Mooskrause	Pearson Correlation							1
	Sig. (2-tailed)							
	N							

*. Correlation is significant at the 0.05 level (2-tailed).

In the research of Poubova (2003) about the influence of different variants of fertilization (organic fertilization, mineral fertilization (N) + organic, as nitrogen fertilization itself) on the yield and the content of vitamin C in the fruits of pepper, there was no difference in the content of vitamin C in different variants

of fertilization, while the content of vitamin C was the lowest in the variant without fertilization.

The results of other researches, the influence of fertilization variant on the content of vitamin C in vegetables, were different. The lowest content of vitamin C with the usage of organic fertilizer, when we compared it with mineral variant was found in Sikora research *et al.* (2010), while the Worthington research (2001) showed that the content of vitamin C in average was higher for 27% in vegetables fertilized with organic fertilizer, when we compared it with vegetable fertilized with mineral variant of fertilizer, the exception was with carrot. On the other hand, the research Fjelkner-Modig *et al.* (2000), the content of vitamin C in cabbage, onion, peas and potato was not significantly different in the usage of different variants of fertilizers (organic and mineral). The higher content of vitamin C was in the organic production of carrot and potato, that was found in the research of Hunter *et al*. (2011).

According to the results presented in Table 5, can be seen that there is strong possitive correlation between the parsley yield in untreated variant and variant treated with organic (r= +.999*) and organic-mineral fertilizer (r= +.997*) at level 0.05. On the other hand, there is strong possitive correlation between the analyzed organic and organic-mineral fertilizer (r= +.999*) at level 0.05.

CONCLUSIONS

The variety and the type of fertilization significantly affected on the yield of parsley leaves and the content of the vitamin C in the leaf of parsley. The variety „Domestic sawmills“had significantly higher yield in the leaves when we compared it with other varieties in both years of our research (55.23-62.16 t/ha).

The usage of organic fertilizer had the highest leaf yield in 2013 (60.69 t/ha), significantly it was higher when we compared it with controlled variant and other variants of fertilization, while it had the highest leaf yield with mineral variant of fertilization in 2014 (42.68 t/ha), but it wasn't significantly different from the yield in organic variant (39.78 t/ha). All three varieties had higher leaf yield in 2013 when we compared it with 2014.

In both years of our research the highest content of vitamin C had variety „Berlin semi-long”. In 2013 this content was not significantly different from other two varieties in our research, while it was significant in 2014.

According to two-year average, the highest content of vitamin C in the leaf of parsley was with the usage of organic fertilizer.

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ANALYSIS OF THE EROSION POTENTIAL AND SEDIMENT YIELD USING THE INTERO MODEL IN AN EXPERIMENTAL WATERSHED DOMINATED BY KARST IN BRAZIL

SUMMARY

Soil losses from water erosion jeopardize agricultural sustainability and food security for current and future generations. The research aimed to evaluate the application of the Erosion Potential Method by the Intensity of Erosion and Outflow – IntErO program in a karst watershed in a region with typical savanna climate in the northeast of the State of Goiás, Brazil. Input data were adapted according to the corresponding characteristics of tropical regions. The results indicated that the Extreme watershed has a value of 0.62 in the index (0 to 1) which defined the strength of the intensity of erosion. The river basin belongs to the category 3 of destruction with moderate erosion intensity, which indicates processes of surface erosion in the largest area of the hydrographic basin, and annual soil loss of 480.60 m^3 km^2 yr^{-1}. According to the IntErO model calculations 16% of the eroded material reaches the outflow of the hydrographic basin, and 84% of these sediments are deposited within the Basin, inside the surface and underground caves and galleries of the karst. Calculations by the IntErO model with the Erosion Potential Method in its algorithm proved to be valuable tool in evaluating the production of sediments in tropical soils, especially in evaluating different scenarios after establishing the inputs database for Brazil and will serve as a good starting point for future evaluations.

Key words: Karst Hydrology, Erosion Potential Method, IntErO model, Soil Conservation, Sedimentology.

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INTRODUCTION

Soil is a finite natural resource that takes thousands of years to mature. Its sustainability is essential for the production of food and many other ecosystem goods and services, including climate regulation and nutrient cycling (Greiner *et al*., 2017). However, with the current developments in erosion, urbanization and climate change, impacts that promote the reduction of its surface layers (more fertile) become a risk for current and future generations (Parsipour *et al.*, 2019; Curovic *et al*., 2019; Spalevic *et al.*, 2020).

Water erosion is a natural process, subject to intensification according to the uses and managements adopted in agriculture. The process initiated by the impact of the raindrop breaks particles from the structure of the soil's surface layer, causing loss of arable soils and accumulation of sediments in the lower regions (EMBRAPA, 2013).

The erosion rate generally increases when the volume and velocity of surface water runoff occurs on steeper terrain with longer slope length. The adoption of conservation practices that ensure maximum vegetation cover and reduction in slope length, especially on steeper slopes and intensive cultivation, are essential to ensure the agricultural and environmental sustainability of terrestrial ecosystems (FAO, 2015). Hydrographic basins are ideal for evaluating the impacts of the intensification of water erosion processes, as it is a system with open water inlets and outlets for precipitated water, which can be drained or infiltrated. (Dyonisio, 2010).

In karst basins, Palmer (1984) draws attention to the importance of genetic aspects, especially in the hydrological bias, which shape the surface forms (lapias, canyons), the subsoil (porous medium), the vadose zone (free and gravitational flow), and the water table, with emphasis on recharge aspects (autochthonous, allochthonous). In groundwater systems, the transport of autochthonous and allochthonous sediments through conduits can imply high speeds and ascending water table, depending on the width of the underground channels (shape of the conduits), which can promote rapid flooding above the vadose or limited zone in confined flow networks (Caldeira *et al*., 2019). Among the fine suspended material (silt, clay and sand) the fine sand particles are more easily transported, which explains the presence of sandy sedimentary fans in caves dominated by mud and gravel (Gillieson, 1996).

In this aspect, Karst systems are sensitive to small changes in land use, such as activities that promote soil erosion, siltation of rivers and pollution of the karst aquifer, which must be mitigated in order not to increase the damage caused to karst systems. Thus, the present study aims to quantify and evaluate the application of the indirect model of Intensity of Erosion and Runoff - IntErO (Spalevic, 2011), which uses equations of the Erosion Potential Method of Gavrilovic (1962; 1972, 1988). This model has been applied in basins all over the world: Greece (Efthimiou *et al*, 2016), Iran (Mohammadi *et al*, 2021; Khaledi Darvishan *et al.*, 2019; Gholami *et al.*, 2016; Behzadfar *et al.*, 2014), Morocco (Ouallali *et al.*, 2020; El Mouatassime *et al.*, 2019), Montenegro (Spalevic *et al.*,

2020; Spalevic *et al.*, 2016; Spalevic *et al.*, 2014; Spalevic *et al.*, 2012), Nepal (Chalise *et al.*, 2019) but also in Brazil recently (Sakuno *et al.*, 2020; Tavares *et al.*, 2019; Lense *et al.*, 2019).

Based on the characteristics of the hydrographic basin, the program estimates the production of annual sediments associated with the intensification of water erosion at the basin scale. Such results can be useful as an indication of areas with imminent potential risk of increasing rates of soil loss from arable areas, river siltation and aquifer and surface water pollution.

MATERIAL AND METHODS

Study Area

The karst river basin of the Extrema River has an area of 27.8 km², and a rainfall regime of 1,164 mm yr^{-1}, and is located in the northeast of Goiás State, Midwest Region, Brazil (Figure 1).

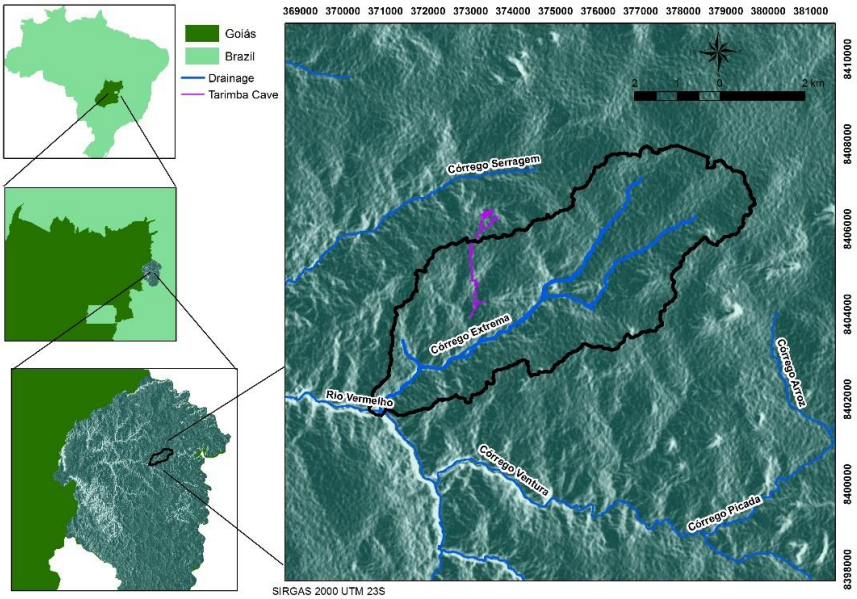

Figure 1: Location of the karstic watershed of the Extrema River with shaded relief effect.

The geomorphology surrounding the Extrema River basin is composed of the Central Chapadão (upper portion), originating from the South American surface that constitutes the Urucuia Group, formed by sandstones that present unconsolidated siliciclastic sediments, and the lower portion (Vão do Paranã) with intercalated pelitic rocks to the carbonates of the Lagoa do Jacaré Formation (Bambuí Group). In the "Lagoa do Jacaré" Formation clastochemical sediments (carbonate rocks) are favorable to karstification.

According to the Köppen-Geiger climatic classification, the climate is type tropical with dry winter (Aw) (Cardoso *et al.*, 2014). Over the past few decades,

tropical savannas worldwide have been among the most affected biomes by the suppression of native vegetation. The dry season and the deficiency of phosphorus and other nutrient minerals in the very old soils do not favor forest development, giving rise to landscapes consisting mainly of grasslands with sparse or isolated trees (Walter and Breckle, 1986). Therefore, environmental changes resulting from human activities in these ecosystems pose threats to both biodiversity and climate.

With the increasing exchange of native vegetation for pastures, added to the natural savanna climate, erosion processes are intensified in rainfall events, resulting in surface runoff with a large volume of sediment that is transported to underground channels and galleries in the karst. As it represents the classical dynamics of the fluviocarstic system in the region, the Extrema River whatershed is an area with densification of karstic features that act as recharge areas through wide and distributed fractures and convections in sinks and underground flows.

In Strahler's (1957) hierarchical classification, the Extrema River configures a first-order level with a pattern of dendritic basin, with quick response to precipitation. At lower altitudes, between the domain of carbonates and siliciclastic sediments, karstic depressions occur with intensified erosive processes, from which there is capture of surface runoff by fractures and/or collapsed sinkholes, generating the accumulation of sediments in some caves above the level of base.

One hypothesis is that upper layers in adjacent caves contribute to the sediment carried by the underground flow in the Extrema cave. Possibly, the main source of sediment production in floods comes from the Tarimba cave (Figure 2), which has thick layers of preserved sedimentary rocks and a permanent flow in its interior. Another hypothesis is that the sediments originate from areas of upstream sinks, with the Tarimba cave acting as an underground stream that transports the sediments.

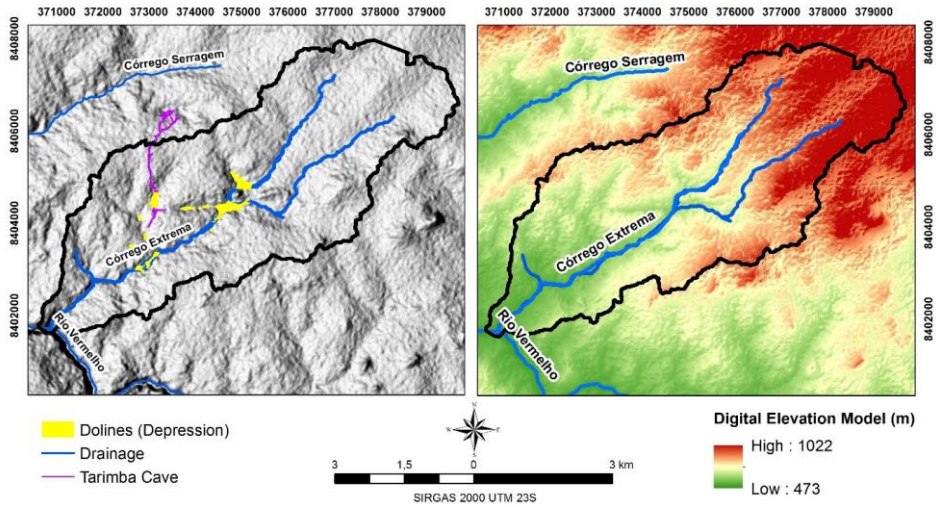

Figure 2: Depressions and connections of underground flows (Tarimba Cave) for the Extreme flow, and digital elevation model (m).

IntErO Model Application and EPM

The Intensity of Erosion and Outflow (IntErO) program package of Spalevic (2011) is based on Erosion Potential Method (EPM) of Gavrilovic (1962; 1972, 1988). The EPM is an empirically-based model that estimates soil loss and water erosion intensity determining factors that directly affect the rates of soil loss from water erosion at the scale of watersheds, such as land slope, soil resistance, field erosion, soil use and management, temperature and precipitation (Gavrilovic, 1988).

The calculations of the EPM model parameters are performed in an automatic form, in the compilation of the input data in the IntErO program. Spalevic (2011) proposed the creation of a database with twenty-six entries, including erosion, geometric, topographical, meteorological data, maximum flow and drainage system characteristics, integrating the EPM model parameters simultaneously (Table 1).

Table 1: Input data for the IntErO in Extrema watershed.

Inputs	**Amount and Unit**
River basin areas (F)	27.80 km²
The length of the watershed (O)	37.09 km
The area of the bigger river basin part (F_v)	14.08 km²
The area of the smaller river basin part (F_m)	13.72 km²
Natural length of the main watercourse (L_v)	7.57 km
Length of the contours/isohyets (L_{iz})	115.34 km
Altitude of the first contour line (h_0)	580 m
Incidence (Up)	100 yr
The lowest river basin elevation (H_{min})	579 m
The highest river basin elevation (H_{max})	854 m
River basin consisted of a very permeable product (f_p)	0.74
A part of the basin consisted of medium permeable rocks (f_{pp})	0.26
A part of the basin with poor water permeability rocks (f_0)	0
A part of the river basin under forests (f_s)	0.30
A part under grass, meadows, pastures, and orchards (f_t)	0.65
A part of the basin under plough-land, and without grass (f_g)	0.05
The length of the main watercourse with tributaries I & II class	9.91 km
The distance between the fountainhead and mouth (L_m)	6.79 km
The volume of the torrent rain (h_b)	50 mm
Average annual air temperature (t_0)	26° C
Average annual precipitation (H_{yr})	1,164 mm

Soil loss (W_{yr}) in the EPM model is estimated by Equation 1. Its algorithms are incorporated in the IntErO application (Spalevic, 2011), which calculates in an automated way, avoiding errors in manual modeling.

$$W_{yr} = T \cdot H_{yr} \cdot \pi \cdot \sqrt[2]{Z^3} \cdot R_u \qquad \text{Equation 1}$$

Where: W_{yr} is total sediment production (m^3 yr^{-1}); T is temperature coefficient (dimensionless); H_{yr} is mean precipitation (mm yr^{-1}); π is the value of 3.14; Z is erosion coefficients (dimensionless); F is watershed area (km^2).

The temperature coefficient (T) is calculated according to Equation 2.

$$T = \sqrt[2]{\frac{t_0}{10}} + 0{,}1 \qquad \text{Equation 2}$$

Where: T is temperature coefficient (dimensionless); t_0 is average air temperature (°C yr^{-1}).

The erosion coefficient (Z) is obtained by Equation 3:

$$Z = Y \cdot X_a \cdot (\varphi + \sqrt[2]{I_{sr}}) \qquad \text{Equation 3}$$

Where: Y is soil resistance to water erosion (dimensionless); X_a is land use and management (dimensionless); φ is degree of erosion on the ground (dimensionless); I_{sr} is average slope of the watershed (%).

The values of the Z coefficient classified according to the degree of erosion intensity (Table 2)

Table 2: The degree of erosion intensity (Z)

Categories	Erosion intensity	Erosion Coefficient (Z)	Average of Z
I	Very severe	Z > 1.0	Z = 1.25
II	Severe	0.71 < Z < 1.00	Z = 0.85
III	Moderate	0.41 < Z < 0.70	Z = 0.55
IV	Weak	0.20 < Z < 0.40	Z = 0.30
V	Very weak	Z < 0.19	Z = 0.10

RESULTS AND DISCUSSION

Gavrilovic (1972) prepared tables with values that represent the attributes (Y, X_a, φ) needed to calculate the erosion coefficient Z (Dragicevic *et al*., 2016; 2017). However, the model was initially applied in temperate climate regions,

being necessary to adapt the values according to the characteristics of Brazilian tropical soils (Sakuno *et al.*, 2020) (Figure 3).

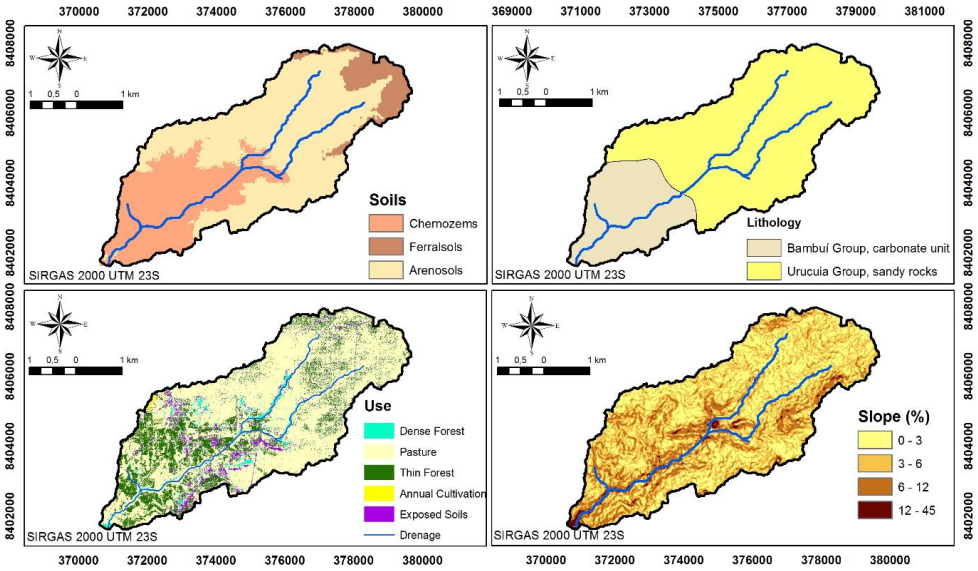

Figure 3: Cartographic base of soils, land use, lithology, and slope of the Extrema River watershed to obtain the Y, X_a and φ factors.

The coefficients of the river basin form (A), average river basin width (B) and watershed development (m) were calculated to be 0.95, 9.32 km, and 0.41, respectively. The value of peak discharge, with a return interval of 100 years (Q_{100}) and for a land use setup of 2021 resulted to 38.94 $m^3 s^{-1}$.

The drainage density of the study river basin (G) we calculated as 0.36, what indicates that there is a low density of the hydrographic network. The factor G is an important affecting the flood hydrograph and erosion process. The index of average decline to be 8.30% shown that in the studied watershed mild slopes prevail. The Z coefficient value of 0.626 indicates that the river basin belongs to destruction category III. The resistance to the erosion process is medium, where the surface erosion is predominant.

The production of erosion material (W yr^{-1}) in the Extrema watershed was calculated to be 81,927.2284 $m^3 yr^{-1}$ and the coefficient of the deposit retention (Ru) resulted in 0.163. This means that 16% of the total eroded material reaches the exit point, while the remaining 84% é deposited in irregularities of the relief inside the watershed, in the hydrological drainage system, caves and underground galleries. Calculated real soil losses per year per square kilometer for the river basin amounts to 480.60 $m^3 km^2 yr^{-1}$, corresponds to the results obtained in 2021.The detailed report for the hydro morphological parameters is shown in Table 3.

Table 3: Outputs data for the IntErO in Extrema watershed.

Outputs		Amount and Unit
Coefficient of the river basin form	A	0.95
Coefficient of the watershed development	m	0.41
Average river basin width	B	9.32 km
(A)symmetry of the river basin	a	0.03
Density of the river network of the basin	G	0.36
Coefficient of the river basin tortuousness	K	1.12
Average river basin altitude	H_{sr}	634.36 m
Average elevation difference of the river basin	D	55.36 m
Average river basin decline	I_{sr}	8.30 %
The height of the local erosion base of the river basin	H_{leb}	275.00 m
Coefficient of the erosion energy of the river basin's relief	E_r	38.12
Coefficient of the region's permeability	S_1	0.48
Coefficient of the vegetation cover	S_2	0.75
Analytical presentation of the water retention in inflow	W	0.6537 m^{-3}
Energetic potential of water flow during torrent rains	$(2gDF)^{1/2}$	173.76 m km s^{-1}
Maximal outflow from the river basin	Q_{100}	38.94 m^3 s^{-1}
Temperature coefficient of the region	T	1.64
Coefficient of the river basin erosion	Z	0.626
Production of erosion material in the river basin	W yr^{-1}	81,927.2284 m^3 yr^{-1}
Coefficient of the sediment retention	Ru	0,163
Real soil losses	G yr^{-1}	13,360.31 m^3 yr^{-1}
Real soil losses per km^2	G yr^{-1} km^2	480.60 m^3 km^2 yr^{-1}

CONCLUSIONS

In the last three decades, the forest area has decreased with the replacement of native vegetation for the production of pastures in the northeast region of Goiás State, Brazil. This change in land cover increases the risks to water erosion, especially in sensitive areas of karstic watersheds. The accumulation of the annual production of sediments is demonstrated through deposits inside the caves of the watersheds in the region. Studies on the origin and fate of these sediments must be carefully monitored to understand the hydrosedimentological behavior of karst systems in tropical climates. This study analyzed some factors and processes that are associated with soil losses and the production of sediments by water erosion, serving as an important indicator of areas at risk of accelerated erosion, and must be constantly evaluated and monitored. The application of the IntErO model demonstrated that the removal of sediments by water erosion in the Extreme River watershed belongs to the 3rd category of destruction (Z = 0.62), which is classified as medium degree. Finally, it is important to emphasize that climate change can increase soil erosion and sediment production processes in extreme rainfall events, which are increasingly common in tropical regions, which requires such processes to be evaluated annually. It is strongly recommended that this approach be considered when planning public monitoring policies.

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MICORRHYZAL COLONISATION ON ARAUCARIA SEEDLING WITH DIFFERENT DOSES OF NITROGEN, PHOSPHORUS AND POTASSIUM

SUMMARY

Arbuscular mycorrhizal fungi (AMF) favours the growth and development of seedlings of various tree species, with consequent reduction in the use of chemical inputs. The present study aimed to evaluate the effect of nitrogen (N), phosphorus (P) and potassium (K) on diversity and sporulation of AMF species in the root colonisation (RC) of seedlings of *Araucaria angustifolia*. The experiments were performed using different doses of N (fixed P and K), P (fixed N and K) and K (fixed N and P) (mixed in pots whit historical soil from *Araucaria angustifolia* as substrate), with each having four treatments and four replicates. Seedlings were transplanted and two years after, the growth parameters, diversity and spore density, diversity indices (Shannon, Margalef's richness and Pielou equity) and mycorrhizal colonisation were evaluated. A total of 14 species of AMF were identified on roots of *Araucaria angustifolia* species from the genus *Glomus* and *Rhizophagus clarus*, regardless of the doses of fertilisers used. P had the greatest effect on diversity of AMF species, since the average doses favour high RC and a greater number of AMF species.

Keywords: Brazilian pine, Plant nutrition, Mycorrhizal diversity and sporulation

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INTRODUCTION

Araucaria angustifolia are coniferous species native to the southern region of Brazil, demonstrating great economic, ecological and social importance (Dos Santos *et al*., 2016; Zanette *et al*., 2017). Its wood is of excellent quality and highly appreciated by the timber industry (Delucis *et al*., 2013; Hackbarth *et al*., 2017). Hence, in the 1950s its area of distribution was considerably reduced due to the exploitation.

Currently, the remaining areas of *Araucaria angustifolia* provide additional income for several families that benefit from the extraction of the fruit called pinion (Danner, 2012), an important product in the southern region of Brazil (Duarte *et al*., 2006). This suggests the need to improve the productive process of this species from production of seedlings to their implantation and field management, with the aim of reforesting these areas originally populated by the species. However, legal factors preventing its usage as wood, as well as its slow growth, hampers its plantation (Carvalho, 2003).

Araucaria angustifolia have the ability to establish a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), which are associated with host plants, thus favouring a greater capacity of water and nutrient absorption from the soil, as well as promoting a greater growth and survival of plants (Nadeem *et al*., 2014).

The choice of substrate and fertilisation are factors that favour the production of vigorous forest seedlings plants. In addition, the presence of AMF in production of seedlings ensures the successful growth and establishment of certain species, so as to reduce the need for application of chemical inputs (Nouri *et al*., 2014).

Therefore, this study aimed to evaluate the effect of different doses of N, P and K, so as to obtain the appropriate doses that favour the diversity and sporulation of AMF species, in addition to root colonisation (RC) for the adequate growth of seedlings of *Araucaria angustifolia*.

MATERIAL AND METHODS

The present study was conducted in an open field in the Agricultural Sciences sector of the Federal University of Paraná, Curitiba-PR, which is located at 25° 25' 47" S, 49° 16' 19" W and 950 m above sea level.

Soils and roots, which were used for inoculation or growing/cultivating of plants, were collected from pots (volume: 16 L) containing poor ravine soil as substrate, which was collected from an area with history of plantations of *Araucaria angustifolia*. According to granulometry analysis (Embrapa, 2011), the soil was composed of 24% sand, 21% silt and 55% clay. According to chemical analysis (Embrapa, 2011), the soil had a pH of 4.0 ($CaCl_2$) and contained 0.90 mg dm^{-3} P, 4.20 g dm^{-3} carbon (C), 1.60 mmol dm^{-3} calcium (Ca^{+2}), 0.80 mmol dm^{-3} magnesium (Mg^{+2}), 0.08 mmol dm^{-3} K^{+}, 2.90 mmol dm^{-3} aluminium (Al^{+3}) and 11 mmol dm^{-3} H^{++}. The extracted soil was sieved, and 3.6 g kg^{-1} dolomitic

lime was added to increase the pH to 6.0, after which incubation was done for one month.

The fertilisation experiments using different doses of N, P and K were performed in three stages, with each having four treatments and four replications (Table 1). Nutrients were applied in soil homogenisation before filling the pots, except for N fertilisation, which was fractionated twice in the following year after the experiment was installed. A total of 48 *Araucaria angustifolia* seedlings (six months old and 20 cm tall) were transplanted in pots, which were randomly distributed and kept on a plastic cover, so as to avoid possible contact with the ground.

Table 1. Experiments on fertilisation of six-month old *Araucaria angustifolia* seedlings with different doses of N, P and K.

Nutrient	Treatments with different doses of NPK (g dm^{-3})											
	---- Experiment I ----				---- Experiment II ----				---- Experiment III ----			
N	0	0.35	0.70	2.80	1.40				1.40			
P_2O_5	1.15				0	0.29	0.58	2.30	1.15			
K_2O	0.78				0.78				0	0.19	0.39	1.56

N: Urea; P_2O_5: triple superphosphate; K_2O: Potassium chloride.

Two years after transplantation, the height (Alt), stem basal diameter (D) and total fresh matter (MFT) of roots (MFR) and aerial part (MFPA), as well as the total dry matter (MST) of roots (MSR) and aerial part (MSPA), which were obtained after drying in a stove at 60°C forced air circulation (Constantino *et al*., 2019).

A soil sample of 100 g was used for the spore extraction. The soil was liquefied for 10 seconds and sieved in overlapping meshes of 500 and 53 µm (Gerdemann and Nicolson, 1963). Then, 70% sucrose solution was added, and the mixture was centrifuged twice for four minutes (Jenkins, 1964) and sieved again using 250 and 50 µm meshes.

For the purpose of identification, spores were separated into groups according to their morphological characteristics and placed on semi-permanent slides prepared with polyvinyl alcohol, lactic acid, glycerol (Morton *et al*., 1993) and Melzer reagent (Koske and Tessier, 1983). The spores were observed with an optical microscope (400x). The spores were identified according to Invam (2018). AMF communities were characterised by the following diversity indices: 1.) Shannon diversity ($H' = -\Sigma\,[(ni / N)ln(ni / N)]$, where *ni* is number of individuals of each species and *N* is total number of individuals in the community; 2.) Margalef's richness ($D_{Mg} = (S - 1) / lnN$, where *S* is total number of species in the community and *N* is total number of individuals in the community; and 3.) the equity of Pielou ($J = H'/ln(S)$, where *H'* is Shannon diversity and *S* is total number of species in the community. R statistical package was used.

The finest roots were selected and kept in 10% KOH for 24 hours and incubated in a water bath at 80° C for one hour. H_2O_2 was added to clean the

roots, which were washed and stained with a blue ink. They were again incubated in a water bath at 80° C for another 5 minutes and lactoglycerol was added. The mycorrhizal colonisation was counted/evaluated under a stereomicroscope in 1 x 1 cm grid plate (Giovanetti and Mosse, 1980).

Data obtained on growth parameters, AMF spore count and percentage radical colonisation (%Col) were treated by analysis of variance supplemented with Tukey post hoc test at 5% of the probability. Redundancy analysis (RDA) of the AMF species was performed using CANOCO programme, version 4.5, considering the chemical attributes of the soil and the growth parameters as explanatory variables.

RESULTS AND DISCUSSION

According to the chemical analysis of the soil (Table 2), the fertilised samples from the three experiments resulted in highly acidic soils, with average pH of 5.19 (I), 4.86 (II) and 4.61 (III), showing the lowest values when the highest dose of N was applied and in the absence of K. Also, the mean values of Ca^{2+} and Mg^{2+} were found for all the experiments, with the highest values being obtained when the intermediate doses of N, P and K were applied.

Table 2. Chemical attributes of the soil at end of the experiments with two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

Soil attribute	Treatments with different doses of NPK (g dm^{-3})											
	------ Experiment I (N) ------				------ Experiment II (P) ------				------ Experiment III (K) ------			
	0	0.35	0.70	2.80	0	0.29	0.58	2.30	0	0.19	0.39	1.56
pH ($CaCl_2$)	5.53a*	5.60a	5.33a	4.33b	4.78a	4.98a	4.90a	4.78a	4.40b	4.68ab	4.88a	4.48b
Ca^{2+} (cmol dm^{-3})	4.18ab	4.33a	4.33a	3.63b	3.65b	3.95b	4.13b	4.75a	3.55b	4.10ab	4.33a	3.68ab
Mg^{2+} (cmol dm^{-3})	3.25a	3.33a	3.15a	2.55b	3.13a	3.15a	3.25a	3.15a	2.78b	3.13a	3.20a	2.60b
K^{+} (cmol dm^{3})	0.50a	0.48a	0.42a	0.21b	0.41b	0.38b	0.45a	0.33b	0.07b	0.11b	0.15b	0.69a
Al^{3+}(cmol dm^{-3})	0.00b	0.00b	0.03b	1.10a	0.35a	0.20a	0.20a	0.30a	0.83a	0.30ab	0.18b	0.53ab
$H+Al^{3+}$ (cmol dm^{-3})	5.58ab	4.30b	4.80b	8.08a	6.20a	5.80a	5.60a	6.75a	7.68a	6.48ab	6.05b	7.38ab
T (cmol dm^{-3})	13.50a	12.4a	12.7a	14.4a	13.3b	13.2b	13.4b	14.98a	14.0a	13.81a	13.72a	14.34a
SB (cmol dm^{-3})	7.92a	8.13a	7.90a	6.39b	7.18a	7.48a	7.83a	8.23a	6.39b	7.33ab	7.67a	6.96ab
m (%)	0.00b	0.00b	0.25b	14.7a	4.75a	2.75a	2.50a	3.50a	11.7a	4.00ab	2.00b	7.25ab
P (mg dm^{-3})	29.90a	35.3a	37.9a	34.7a	1.43b	7.53b	16.3b	135.5a	34.8b	43.63b	42.7b	50.05a
C (g dm^{-3})	6.73a	5.08a	10.5a	10.0a	4.30a	4.68a	5.75a	2.98a	8.90a	8.65a	8.35a	9.45a

Where: pH ($CaCl_2$ 0,01 mol L^{-1}); Ca^{2+}, Mg^{2+}, Al^{3+} (extracted with KCl 1 mol L^{-1}); $H+Al^{3+}$ (extraction by calcium acetate 0.5 mol L^{-1}); K^{+} and P (Mehlich-1 extraction); Cation exchange capacity (T); Base saturation (SB) and Al^{3+} saturation (m).

* Averages with same letter on the line do not differ from each other by Tukey's test at 5% of the probability.

In samples from the first two experiments (I and II), the final availability of K was medium to high and, in the third experiment, application of the highest dose of K resulted in a high value for this nutrient (0.69 cmol dm^{-3}). Furthermore, in general, the presence of Al^{3+} in experiments II and III was similar, varying within the range of 0.18–0.83 cmol dm^{-3}; however, in experiment I, as the dose of N increased, the concentration of this nutrient was in the ranged of 0.0–1.10 cmol dm^{-3}.

For P, application of moderate doses of N and K in experiments I and III, respectively, resulted in average values for this nutrient; whereas, in experiment II, a high value was obtained (135.58 mg dm^{-3} P) when the highest dose of P was applied. On the other hand, regardless of the experiments, there were no significant differences in the concentration of C with respect to application of the different doses of N, P and K.

At least 14 AMF species were identified in the different experiments (Table 3). Dominant species on *Araucaria angustifolia* seedlings roots were from the genus *Glomus* and *Rhizophagus clarus*, regardless of the application of N, P and K (during cultivation).

Table 3. Average number of mycorrhizal spores obtained from the experiments with two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

AMF species	Treatments with different doses of NPK (g dm^{-3})											
	------ Experiment I (N) ------				------ Experiment II (P) ------				------ Experiment III (K) ------			
	0	0.35	0.70	2.80	0	0.29	0.58	2.30	0	0.19	0.39	1.56
Acaulospora tuberculata	10a*	9a	6a	2a	9a	12a	14a	14a	2a	5a	6a	4a
Acaulospora scrobiculata	66a	49a	63a	33a	48b	89a	88a	54ab	51a	97a	86a	110a
Acaulospora spinosa	182a	81a	137a	13a	41a	44a	54a	52a	27a	34a	78a	58a
Gigaspora sp.	13a	10ab	6ab	5b	18a	21a	19a	14a	4b	6ab	10a	6ab
Dentiscutata heterogama	9a	5a	4a	1a	14a	16a	13a	13a	1ab	3a	1ab	0b
Ambispora leptoticha	31a	30a	28a	11a	13a	13a	18a	11a	12a	39a	32a	34a
Entrophospora infrequens	0a	0a	1a	0a	8a	10a	9a	6a	0a	0a	0a	0a
Glomus spinuliferum	41b	25b	33b	106a	106a	116a	95a	107a	57a	48a	39a	32a
Glomus macrocarpum	73b	93b	90b	184a	135a	133a	134a	152a	154a	145a	174a	122a
Glomus sp.1	35a	46a	58a	74a	60a	84a	64a	66a	64a	84a	95a	81a
Glomus sp.2	58b	57b	80ab	119a	148a	241a	177a	140a	141a	158a	165a	119a
Glomus sp.3	92a	74a	84a	83a	194a	257a	206a	166a	81b	119ab	152ab	156a
Glomus sp.4	44a	56a	57a	20a	123a	102a	89a	86a	19a	29[a]	29a	34a
Rhizophagus clarus	901a	300b	229b	238b	158b	441ab	618a	345ab	232a	316a	411a	274a
Total Spores	1552a	832b	874b	887b	1070a	1576a	1596a	1223a	841a	1083a	1276a	1029a

* Averages with same letter on the line do not differ from each other by Tukey's test at 5% probability.

There was a greater presence of fungi from the genus *Glomus*, which had numerous populations in experiment II (general average of 59%), which increased until the application of the average doses of P (from 0 to 0.58 g dm^{-3}). The constant application of P and N in experiment I as well as K in experiment II at different concentrations resulted in almost similar populations, with averages of 44% and 55%, respectively. The species *G. macrocarpum*, *G.* sp.2 and *G.* sp.3 dominated in increasing order (Table 3).

A significant number of *Rhizophagus clarus* species was also found, with the highest average observed in experiment I (417 individuals, representing 37% of the population) and being almost similar when compared to experiment II (390 individuals). There was a greater number of individuals of this species in the

absence of N (901 individuals) and when intermediate doses of P were applied (441 and 618 individuals).

A greater number of individuals of the genus *Acaulospora* sp. in experiment I was obtained (15% of the total population) when N was not applied (258 individuals). Larger populations of this genus (*Acaulospora)* were obtained when intermediate amounts of N, P and K were applied in each experiment. Similar results were observed for *R. clarus*. *A. scrobiculata* and *A. spinosa* were the most representatives, highlighting that these species had 89 and 88 individuals when intermediate doses were applied in experiment II and when N was not applied in the experiment I, with an average of 182 spores, which is different from those of the rest.

In all treatments, *Gigaspora* sp. and *D. heterogama* species presented a general average of 1% of the total population and both stood out in experiment II; however, this did not differ significantly when the different doses of P was applied, obtaining averages of 18 *Gigaspora* sp. and 14 *D. heterogama* spores. In contrast, the species *A. leptoticha* obtained a general average of 3% of the total of individuals in experiments I and III, presenting the highest values, regardless of the doses of N and K applied, with 25 and 29 spores obtained, respectively.

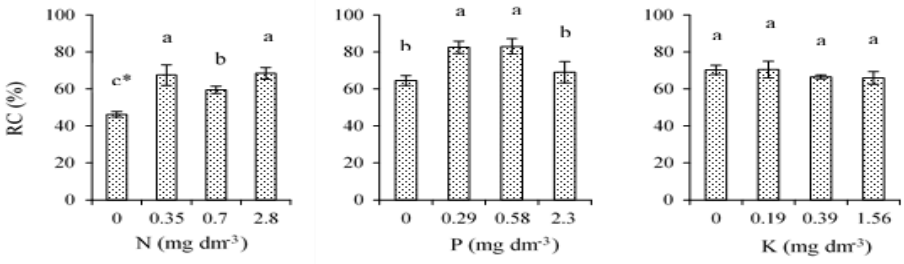
The species *Entrophospora infrequens* had a greater presence in experiment II, with an average of 1% of the population (eight individuals), regardless of the doses of P applied. In general, the AMF population was higher in experiment II, with an average of 1,366 individuals, which increased until the application of intermediate doses of P (from 0 to 0.58 g dm^{-3}). In the case of the other treatments, there was no significant difference between the different doses evaluated and they presented averages of 1,036 (experiment I) and 1,057 (experiment III) spores.

Results of the comparisons between the Shannon diversity index values, Margalef's richness and Pielou equity of the experiments are shown in table 4. The highest values of all 3 indices occurred in experiment II, with minimal differences between the applied doses of P.

Table 4. Mycorrhizal diversity index of two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

Species richness (RE)/Index	Treatments with different doses of NPK (g dm^{-3})											
	----- Experiment I (N) -----				----- Experiment II (P) -----				----- Experiment III (K) -----			
	0	0.35	0.70	2.80	0	0.29	0.58	2.30	0	0.19	0.39	1.56
RE	13	13	14	13	14	14	14	14	13	14	13	12
Shannon	1.60	2.10	2.20	2.00	2.28	2.14	2.03	2.18	2.03	2.10	2.05	2.12
Margalef	1.63	1.79	1.92	1.77	1.86	1.77	1.76	1.83	1.78	1.86	1.68	1.59
Pielou	0.62	0.82	0.83	0.78	0.86	0.81	0.77	0.83	0.79	0.80	0.80	0.85

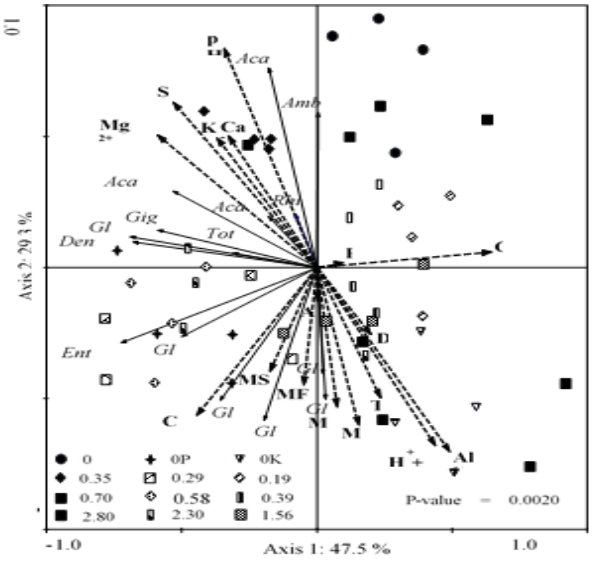
According to percentage RC of araucaria seedlings, the highest values were obtained when intermediate doses of P were applied (0.29 and 0.58 g dm^{-3}), reaching 83% (I experiment); whereas, its lowest value (46%) was obtained in the absence of N (Figure 1).



* Averages with same letter do not differ from each other by Tukey's test at 5% probability.

Figure 1. Root colonisation (RC) of two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

Results of the RDA on effects of the final chemical analysis of the soil, growth responses and percentage colonisation of AMF species in two and half years old araucaria seedlings, relative to different doses of N, P and K (Figure 2), shows that separation of most of the experimental treatments occurs in presence of cations (Ca^{2+}, K^{+}, Mg^{2+} and SB) and at the pH in axis 1 ($p < 0.05$), which explains 47.5% of all data variability. Therefore, this axis was responsible for the grouping of treatments with moderate doses of N, together with the presence of most of the AMF observed in the samples (*Aca Tub, Aca Scr, Aca Spi, Gig Sp., Den Het, Amb Lep, Gl* 4 *and Rhi Cla*). On the other hand, axis 2 explains 29.3% of the variability, mainly in the *Glomus* genus (*Gl Spi*, *Gl M*, *Gl* 1 and *Gl* 2), influencing the seedling growth responses (MFR, MSR, MFT, MST, Alt and D) and percentage colonisation (%Col). In the case of the cations (T, $H^{+}+Al^{3+}$ and $Al^{3+)}$ and C present in the samples, they did not have the least influence on the AMF species community.



Variables	Redundancies	
	Axis 1	Axis 2
pH $Cacl_2$	-0.2524	0.7302
Ca^{2+} (cmol dm^{-3})	-0.2411	0.4396
Mg^{2+} (cmol dm^{-3})	-0.4337	0.4414
K^{+} (cmol dm^{-3})	-0.2717	0.4325
Al^{3+} (cmol dm^{-3})	0.3619	-0.6137
$H^{+}+Al^{3+}$ (cmol dm^{-3})	0.3228	-0.5926
T (cmol dm^{-3})	0.1738	-0.4344
SB (%)	-0.3901	0.5512
P (cmol dm^{-3})	0.0554	0.0110
C (g dm^{-3})	0.4759	0.0521
Alt (cm)	0.0021	-0.1132
D (mm)	0.1472	-0.2236
MFR (g)	-0.0360	-0.3915
MSR (g)	-0.1285	-0.3477
MFT (g)	0.0948	-0.4957
MST (g)	0.0551	-0.4651
Col (%)	-0.3275	-0.4930

Figure 2. Redundancy analysis (RDA) of the AMF species in relation to chemical analysis of the soils and growth responses of two and a half years old *Araucaria angustifolia* seedlings, depending on fertilisation with different doses of N, P and K. *Aca Tub*: *Acaulospora tuberculata*; *Aca Scr: Acaulospora scrobiculata; Aca Spi: Acaulospora spinosa; Gig Sp: Gigaspora sp.; Den Het: Dentiscutata heterogama; Amb Lep: Ambispora leptoticha; Ent Inf: Entrophospora infrequens; Gl Spi: Glomus spinuliferum; Gl M: Glomus macrocarpum; Gl 1: Glomus sp.1; Gl 2: Glomus sp.2; Gl 3: Glomus sp.3; Gl 4: Glomus sp. 4; and Rhi Cla: Rhizophagus clarus.*

There is a difference in the diversity of AMF in seedlings from nurseries in relation to those observed in native areas, or in replanted areas of araucaria that were studied in various regions of São Paulo (SP), Rio Grande do Sul (RS) and Paraná (PR). In this sense, in SP, an average of 43 species was obtained (Bonfim *et al*., 2016; Moreira *et al*., 2007a; Moreira *et al.*, 2009); however, in RS, an average of 12 species was found (Breuninger *et al*., 2000; Zandavalli *et al*., 2008), while in the case of PR, Vilcatoma-Medina *et al*. (2018) obtained an average of eight species of *Araucaria angustifolia* seedlings of different ages. The application of different doses of N, P and K in araucaria seedlings influenced the presence of the 14 species, stimulating symbiosis (both to promote the multiplication of microorganisms and optimal growth of plants), especially when moderate doses of P were applied.

Soil and climatic factors can affect AMF population and nutrient absorption, which can affect seedling growth (Helgason and Fitter, 2009; Tahat and Sijam, 2012; Toljander *et al*., 2008). Araucaria cultivation grows well in soils with a moderately acidic pH. In a previous study, it was shown that when the acidity of the substrate was corrected and moderate doses of P were used, 11 AMF species were identified (Moreira *et al*., 2012). In the present study, application of the different doses of N and K may have influenced the structure of the substrate and contributed to a greater diversity of species and high sporulation.

A strong correlation between P and K and AMF is evident, such that the deficiency of these nutrients can induce the accumulation of K, preventing the transfer of polyphosphates to the plant (García *et al.*, 2014). Although AMF symbiosis improves the absorption of P and N by the host, these nutrients in turn influence the RC of AMF and its symbiotic performance (Nouri *et al*., 2014).

In the present study, fungi of the genera Glomus and Acaulosora were dominant in araucaria seedlings, as well as in most studies performed in different forests in Brazil (Moreira *et al*., 2007a; Bonfim *et al*., 2016; Moreira *et al*., 2016). These genera produce more spores and require less time to reproduce in the same environment when compared to the rest of AMF (Hart *et al*., 2002; Piotrowski *et al*., 2004; Suresh *et al*., 2010).

The species from genus *Glomus* were dominate until the application of moderate doses of P, as reported by Moreira and Cardoso (2002), Moreira *et al*. (2012) and, in *Acacia mearnsii* seedlings (Mello *et al*., 2008), since high concentrations of P inhibit symbiosis with *G. intraradices* (Breuillin *et al*., 2010).

The importance of the presence of the *Glomus* is confirmed by inoculation with *G. intraradices* and *G. clarum*, which influence the growth parameters of seedlings of *A. angustifolia* (Moreira-Souza and Cardoso, 2002). *G. macrocarpum* was confirmed as one of the most important species in young plantations of *Eucalyptus urophylla* (Santos *et al.*, 2017), especially in the moderate presence of N and P, as corroborated in this study on seedlings of *A. angustifolia*. *G. clarum* is also beneficial for annual crops, while it favours plant growth and improves the efficiency of P and N absorption (Cely *et al*., 2016).

Rhizophagus clarus is among the most common species of the ecosystem (Lee and Eom, 2015). It was the second most abundant in araucaria seedlings and its population decreased by high application of P. Ferreira *et al.* (2015) reported a reduction of up to 70% of *R. clarus* in the presence of excess P, while *R. irregularis* was found to be be tolerant to high levels of P in the cultivation of *Eucalyptus marginata* (Kariman *et al*., 2014).

Excess fertilisation with P affects sporulation and consequently reduces the number of individuals of *Acaulospora* genus (Lin *et al*., 2012; Moreira *et al.*, 2012), which was also observed in the present study. In the treatments that had medium-high levels of P, there were greater number of individuals of *Aaulospora* compared to those obtained by treatment with an average P content of 13 mg dm^{-3} (Moreira *et al.*, 2007a). In the samples with average levels of P (0.29 to 0.58

g dm^{-3}) (experiment II), the number of individuals of the species *A. scrobiculata* was 68% higher when compared to araucaria seedlings of different ages with high levels of P (Vilcatoma-Medina *et al*., 2018).

Gigaspora (Gigaspora sp. and *D. heterogama*) was affected by the increase in the doses of nutrients, especially N. A similar result was obtained by Johnson *et al*. (2003) for soils enriched with N and in sandy soils with medium pH (Lekberg *et al.,* 2007). The species *A. leptoticha* and *E. infrequens* were present in different soils of Brazilian forests with acidic reaction, moderate Al^{3+} and low K content (Costa *et al.,* 2016; Silva *et al.,* 2007), which were also recorded in this study. Thus, they are considered to be important in the production of Araucaria seedlings.

Regarding the analysis of AMF diversity index, experiment II had a greater prominence, probably due to the chemical properties of the substrates at end of the experiment, as in the case of the P, which presented different and low concentrations compared to the rest of the experiments, which were always constant and high, as well as for pH and organic matter. Diversity and richness of AMF in a native araucaria forest was greater when compared to those of a replanted forest (Moreira *et al.* 2007b).

The highest *A. angustifolia* seedlings occurred in experiment II (Constantino *et al.* 2019). In the presence of P, an average height of 79 cm was obtained, which is 34% higher when compared to its absence. When moderate and constant doses of P were applied in the different concentrations of N and K, these stood out with average heights of 74 cm and 73 cm, respectively.

When comparing the height and diameter of the three experiments, our results were 50% smaller and 30% thinner than those reported by Zandavalli *et al*. (2004) in two-year-old seedlings. Moreover, Zandavalli *et al*. (2004) inoculated *A. angustifolia* with *G. clarum* in a sterilised natural soil that had a good amount of P, K, organic matter and low content of Al^{3+} (in comparison with the soil used in the present study). When AMF species were inoculated together with the application of moderate doses of P on seedlings of *Khaya senegalensis* and *Acacia mangium*, the growth parameters improved considerably (Jeyanny *et al.* 2011; Jeyanny *et al.*, 2013).

In two-year-old *A. angustifolia* seedlings, the variables MFPA (341 g) and MFR (256 g) were highlighted in the presence of moderate doses of P and N (Constantino *et al*., 2019), which were higher than those found by Zandavalli *et al.* (2004). In a one-and-half-year-old *A. angustifolia* seedlings, MSPA and MSR occurred similarly to those verified for MFPA (140 g) and MFR (122 g), which were twice as high as those found by Moreira-Souza and Cardoso (2002). The presence of cations, such as Al^{3+}, which are predominant in acid soils, can inhibit the absorption of nutrients, while certain species of AMF can facilitate their availability in the soil (Meharg, 2003). This fact was verified in this study, where the final presence of this element did not interfere in the substrates, since its effect was moderately considerable, due to the contribution of the nutrients that facilitated the reproduction of the fungi, as well as due to the fact that it was

corroborated in the seedlings of araucaria of different ages (Vilcatoma-Medina *et al.*, 2018) and in *Tectona grandis* seedlings (Rodrigues., *et al* 2018).

The highest values for mycorrhizal colonisation of *A. angustifolia* seedlings were evident in the moderate concentrations of P (experiment II), which were three times higher than those observed in one-year-old seedlings fertilised with P (Moreira-Souza and Cardoso, 2002; Moreira *et al.*, 2012), as well as compared to those in native, replanted and burned forests of *A. angustifolia* (Moreira *et al*., 2006; Moreira *et al.*, 2007a; Moreira *et al.*, 2016), which is similar to those presented in seedlings aged 2 and 5 years (Vilcatoma-Medina *et al.*, 2018; Zandavalli *et al.*, 2004) and to those of the forests of *A. araucana* (Diehl *et al.*, 2008; Diehl and Fontella, 2010); and much higher than those of *Eucalyptus* sp. plantations (Lima *et al*., 2013).

In the present study, the importance of fertilisation on *A.angustifolia* seedlings in the presence of AMF followed the order: P > N > K. This result is probably due to the fundamental role that P plays in the composition of organic molecules, such as nucleic acids, ATP and phospholipids (Campos, *et al.*, 2018). Therefore, the more limiting phosphorous is, the greater the effect it will have on the behaviour of AMFs. It was also found that the application of moderate doses of P and N and low K resulted in a significant number of AMF.

As already seen in the present study, the population of AMF species were favourably influenced by different concentrations of nutrients and consequently on the growth of *A. angustifolia* seedlings. This result was similar to that observed by Fuentes-Ramírez *et al.* (2018), in which AMF species increased significantly after burning in *Araucaria araucana* plantations, with incorporation of nutrients due to the mineralisation of organic matter and contribution of ash.

CONCLUSIONS

Araucaria angustifolia seedlings are inhabited (or colonised) by a diversity of AMFs, with emphasis on the species from the genus *Glomus* and *Rhizophagus clarus*, regardless of the fertilisation regime carried true its cultivation.

Phosphorus is the element that has the greatest effect on AMF diversity, such that when applied in medium doses, it favours radical colonisation and a highest number of AMF species.

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INFLUENCE OF IRON, ZINC AND BIMETALLIC ZN-FE NANOPARTICLES ON GROWTH AND BIOCHEMICAL CHARACTERISTICS IN CHICKPEA (*Cicer arietinum*) Cultivars

SUMMARY

Nano-fertilizers in agriculture are becoming prevalent because of the unique and outstanding properties of these materials. Bimetallic nanoparticles can simultaneously provide essential elements for plants. A field experiment was conducted on a semi-arid region at Maragheh, northwest of Iran to study effects of foliar application of Fe_3O_4 nanoparticles (Fe-NPs), ZnO nanoparticles (Zn-NPs), mixed monometallic Fe_3O_4 + ZnO nanoparticles, bimetallic Fe-Zn nanoparticles and distilled water (as control) on biochemical attributes of two chickpea cultivars (Gogso and local). The tallest plant was recorded for cv. Gogso by application and Zn-NPs and Fe_3O_4+ ZnO nanoparticles. Evaluation of seed yield component such as 100-seed weight, pod and seed number per plant showed that foliar spry of bimetallic Fe-Zn nanoparticles significantly increased the agro-economic performance especially in cv. Gogso. Assessment of leaf chlorophyll a, b and carotenoids content showed that although the additive effect of Fe-NPs was greater than the Zn-NPs, the highest pigments content was recorded for cv. Gogso by utilization of bimetallic Fe-Zn. Also, antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and guaiacol-peroxidase (GPX) significantly induced by application of micronutrients and highest activity was recorded for prayed by bimetallic Fe–Zn. This was confirmed by a significant reduction in hydrogen peroxide (H_2O_2) concentration. Our results showed that cv. Gogso was more responsive to composite micronutrient nanoparticles than local cultivar. This highlights that the bimetallic Fe-Zn nanoparticles improves plant physiological properties, seed yield, and its utilization is therefore especially beneficial for progressive nano-fertilizer industries.

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INTRODUCTION

Chickpea is an important pulse crop widely used for food and fodder throughout the world. It is one of the main sources of protein for majority of the population in developing countries among all pulse crops. The world's chickpea consumption is third after dry beans and peas (*Pisum sativum* L.) and has shown an increase of 14.2% in area and 27.3% in quantity of production since 2010 (FAOSTAT, 2019). It is a good source of carbohydrates and protein, and also its protein quality is considered to be better than other pulses (Merga and Haji, 2019). Also, it is adaptable to wide climatic variation, has low production cost, and promotes biological fixation of atmospheric nitrogen (Pegoraro *et al*., 2018). Hence it can be included in crop rotations of semi-arid areas.

Arid and semi-arid regions account for more than 30% of the total earth surface (Liu *et al*., 2018). The soils of the arid and semi-arid area are increasing agricultural importance, but their inherent fertility is low. Also, micronutrients availability is much affected by soil pH. So that in alkaline soils the availability of zinc, iron, manganese and copper is reduced and so, plants exhibit strong deficiency symptoms. On the other hand, population growth pressure and agricultural intensification in this region poses a serious threat to agricultural systems and may increase the degree of desertification and decrease soil quality (Mohammed *et al*., 2021). Food security in semi-arid regions is generally influenced by water and nutrient management.

Zinc (Zn) deficiency is mostly recognized from the countries where cereal-based food is dominant (Cakmak, 2008; Hacisalihoglu, 2020). Zn becomes the fourth most important nutrient after nitrogen (N), phosphorus (P), and potassium (K), and it is deficient in the 47% of the Iran soils (Boostani *et al*., 2019). Zn is structural part of many enzymes that are used in the metabolism of auxin and carbohydrates, in the synthesis of proteins and integrity of membrane (Tsonev and Cebola Lidon, 2012; Umair Hassan *et al*., 2020). Furthermore, it also has critical roles in pollen development, fertilization and chlorophyll synthesis (Karim *et al*., 2012; Hacisalihoglu, 2020). Iron (Fe) is an essential micronutrient for almost all living organisms because it plays important role in metabolic processes such as photosynthesis, DNA synthesis and respiration (Schmidt *et al*., 2020). Further, many metabolic pathways are activated by iron, and it is a central component of electron chains and a co-factor of many vital enzymes. Although Fe is the fourth most abundant element in the earth's crust, it is the third-most limiting nutrient for plant growth primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang, 2011). Limited Fe availability in soils is one of the main limiting factors of yield and quality of agricultural productions worldwide, particularly in alkaline and calcareous soils of semi-arid regions (Mohammed *et al*., 2021). This poor availability is closely linked to physical, chemical, and biological processes within the rhizosphere as a result of soil–microorganism–plant interactions. Iron shortage in plants might be

prevented by the foliar application of Fe fertilizers where the soil conditions are unsuitable.

Recently, nano-technology has been widely employed for the production of fertilizers due to the high efficiency and the homogenous distribution of nano-form of the nutrients within the plants. Nanotechnology is the application of beneficial particles at the atomic or molecular level, usually at scales <100 nm. In recent years, research into nano-fertilizers has increased. Nano-fertilizers can be more soluble or more reactive than conventional fertilizers (Konappa *et al*., 2021; Mejías *et al*., 2021). It appears that new generation of fertilizers based on nano-technology has a potential to provide solutions to fundamental agricultural problems caused by conventional fertilizer management (Mastronardi *et al*., 2015).

Nano-fertilizers have been provided a new efficient alternative to normal regular fertilizers. Nano-particles can help in increasing reactive points of these nanoparticles, which increases the absorption of these fertilizers in plants (Sadak and Bakry 2020). According to the type of formulation, nano-fertilizers are classified into three categories: 1) nanoscale fertilizer, which corresponds to the conventional fertilizer reduced in size typically in the form of nanoparticles; 2) nanoscale additive fertilizer, is a traditional fertilizer containing a supplement nanomaterial; 3) nanoscale coating fertilizer, refers to nutrients encapsulated by nano-films or intercalated into nanoscale pores of a host material; and 4) Bimetallic nanoparticles (BNPs), which are formed by the combination of two different metals. Bimetallic nanoparticles have attracted huge attention as compared to monometallic nanoparticles in both technological and scientific view because BNPs shows better properties. (Mastronardi *et al*., 2015; Sharma *et al*., 2019).

However, the evaluation of the exact effects of foliar applying micronutrients nano-fertilizers individually, mix of different monometallic or in the form of bimetallic nanoparticles on chickpea crops in semi-arid region need to be better established. The present investigation was undertaken to improve the understanding about the foliar application of Fe and Zn nanoparticles on the growth and biochemical characteristics of spring chickpea in a Mediterranean semi-arid environment.

MATERIAL AND METHODS

Experimental site and plant material

A field experiment was carried out during 2019−2020 growing season in semiarid highland region of Hashtroud (latitude: 37°28′N; longitude: 447°02′E; altitude: 1815 m) in northwest of Iran. Seeds of two chickpea cultivars (local and Gogso) were obtained from Dryland Agricultural Research Institute (DARI) and during the first-year seeds were propagated in isolated fields under full irrigated condition, according to Sabaghnia *et al*., (2015). The experiment was established on a silt loam soil (75% silt, 15% clay, 10% sand), with pH 7.63, low in organic carbon (0.64%), total nitrogen 0.08%, CaCo3 9.3%, electrical conductivity (EC)

1.62 ds m^{-1}, iron 2.89 ppm, manganese 1.02 ppm, zinc 0.44 ppm and potassium 363 ppm. The previous crop in the experimental field was wheat. The climate of Hashtroud is temperate and the winter months are much rainier than the warm months. According to Köppen-Geiger climate classification, the climate of this region is BS (cold semi-arid). The average annual temperature is 13.8°C in Hashtroud and its mean annual rainfall is 428 mm. Typically, during the spring-sown chickpea crop cycle, the amount of rainfall is usually very low. The precipitation was 79.8 mm during the cropping season. The experimental field was ploughed twice: in early autumn and two weeks before planting, subsequently soil was harrowed twice to bring the soil to fine tilth. After the second primary tillage operation, well-rotten farmyard manure was applied as per the treatment and thoroughly mixed into the topsoil. The recommended dose of fertilizer 60 kg ha^{-1} of nitrogen and 30 kg ha^{-1} of phosphorus was applied in the form of urea and triple superphosphate at the time of seed bed preparation. Cut worm (*Agrotis ipsilon*) was controlled by poison bait (Dipterex [trichlorfon; 200g] + sugar; 200g + wheat bran; 5kg). After opening of furrows, the seeds were hand planted on 11 April 2019.

Experimental design and plant growth measurement

The trial was laid out in randomized complete block design with three replications in split plot arrangement (plot size of 2 × 2 m^2) keeping foliar application of micronutrient fertilizers in main plots and sub-plots allocated to the cultivars (local and Gogso). Micronutrient fertilizers were including Fe_3O_4 nanoparticles (Fe-NPs), ZnO nanoparticles (Zn-NPs), Fe_3O_4+ ZnO nanoparticles, bimetallic Fe-Zn nanoparticles and foliar spray of distilled water as control. The rows were 35 cm apart with the plant-to-plant distance of 5 cm. A sprinkler system was applied for irrigation. Nano metal oxides were obtained from the Iranian Nanomaterials Pioneers Company. The synthesized nano particles were characterized morphologically by transmission electron microscope (Zeiss EM 10 C, Merck, Darmstadt, Germany). The plants were sprayed with the spraying liquids (2mM) until their leaves reached the point of maximum liquid retention, after which runoff occurred. Foliar treatments were applied at inflorescence emergence stage BBCH= 51 (first flower bud visible outside leaves) and repeated every four days for four times. Seed yield and yield component were measured from a 2.0 m^2 harvest area from the central four rows of each plot when the crop reached physiological maturity.

Antioxidant enzyme assay

For biochemical analysis leaf samples was collected at fruit development stage (BBCH= 71) and freshly harvested plant material immediately was freeze in liquid nitrogen and store it at -70°C for later use. For catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and guaiacol-peroxidase (GPX) extraction, leaf samples (0.5g) were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in refrigerated centrifuge for 15 min at 15000×g. The

supernatant was used for enzyme activity assay as described previously by Janmohammadi (2012). CAT activity was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH=7), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H2O2 and decrease in absorbance recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed. Guaiacol peroxidase (GPX) was determined by measuring the oxidation of guaiacol. The assay mixture contained 10 mmol/L potassium phosphate (pH 6.4), 8 mmol/L guaiacol, and 2.75 mmol/L H_2O_2. The increase in absorbance was recorded at 470 nm within 2 min (linear phase) after the addition of H_2O_2.

Hydrogen peroxide (H_2O_2) concentration was determined according to Loreto and Velikova (2001). Leaf samples of 0.5 g were homogenized in 3 mL of 1% (w/v) tri-chloroacetic acid. The homogenate was centrifuged at 10,000 rpm and 4°C for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1M KI. H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H_2O_2 was calculated from a standard curve plotted in the range from 100 to 1000 μmol mL^{-1}. H_2O_2 concentration was expressed as μmol g^{-1} DW. Activities of SOD and APX was calculated according Cakmak and Marschner (1992).

Pigments content

Photosynthetic leaf pigments, chlorophylls a (chl a) and b (chl b) and total carotenoids x+c were quantified by the method of Lichtenthaler (1987) through a spectrophotometer at 661.6 nm, at 644.8nm and at 470 nm. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute 1988), the least significant difference (LSD) being used to compare means of traits ($p < 0.05$).

RESULTS AND DISCUSSION

The results indicated independent significant effects of nano-elements foliar applications and cultivars on plant height as well as significant effects were found by the cultivar × foliar sprays interactions (Table 1). Application of Fe and Zn both individually and in combined form increased plant height. However, combined application of micronutrient in the form of bimetallic structure increased plant height by about 18%. The tallest plant recorded for cv. Gogso by foliar application of bimetallic nanoparticles. In plant systems many metabolic pathways are activated by Fe and Zn, and it is a prosthetic group constituent of many enzymes. They also play some important roles in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants. All mentioned process individually can increase height by increasing the supply of photoassimilates or by cross talking with phytohormones and stimulants of plant growth. Phytohormone signaling plays crucial roles in regulating the response to

variable mineral availability. High pH and precipitation lead to Zn deficiency in soil.

Evaluation of seed yield component showed that there is significant difference between the pod number of the cultivars. The pod number in cv. Gosgo was 19% higher than local cultivar. This indicates that the source-sink relationship in the new cultivar has been qualified to a considerable extent. However, grain yield and grain number of legumes are very sensitive to the assimilate availability during the post-flowering period. Therefore, it seems that the cv. Gosgo with higher plant height, more leaf area and larger canopy width has been able to produce more amounts of photoassimilates in the pre-flowering stages. The higher number of primary branches in Gosgo cultivar compared to the local cultivar also confirms this point (Table 1).

Table 1. Means comparison of morphological characteristics and pigments content of chickpea (Cicer arietinum L.) cultivars as affected by different nano-form micronutrients.

	PH	PB	PN	SP	HSW	Chl a	Chl b	CAR
C	29.00^{bc}	3.50^{b}	50.50^{a}	1.00^{a}	36.33^{d}	90.00^{e}	141.50^{d}	467.33^{cd}
Fe	31.00^{ab}	4.33^{a}	51.83^{a}	1.50^{a}	39.34^{bc}	117.17^{bc}	184.33^{c}	522.67^{c}
ZN	30.50^{b}	3.83^{b}	51.40^{a}	1.50^{a}	40.09^{b}	104.19^{d}	164.33^{c}	509.67^{c}
Fe+Zn	33.66^{a}	4.50^{a}	53.83^{a}	1.50^{a}	41.67^{b}	129.17^{b}	202.33^{b}	683.67^{ab}
Bimetal	34.17^{a}	4.50^{a}	54.33^{a}	1.67^{a}	44.17^{a}	141.79^{a}	317.67^{a}	864.87^{a}
Local	26.20^{b}	3.33^{b}	48.07^{b}	1.4^{a}	39.27^{b}	120.80^{a}	198.67^{a}	645.73^{a}
Gogso	37.13^{a}	5.00^{a}	56.80^{a}	1.4^{a}	42.20^{a}	120.60^{a}	205.27^{a}	623.73^{a}
Micronutrients (M)	**	Ns	Ns	Ns	**	**	**	**
Cultivar (C)	**	**	**	Ns	**	Ns	Ns	Ns
M×C	*	Ns	Ns	Ns	Ns	Ns	Ns	Ns

PH: plant height (cm), PB: primary branch, PN: number of pods per plant, HSW: 100-seeds weight (g), Chl a: chlorophyll a content (mg g^{-1} FW), Chl b: chlorophyll b content (mg g^{-1} FW), CAR: carotenoids content (mg g^{-1} FW). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe_3O_4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe_3O_4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. In each ccolumns rows with different letters are statistically different at the 5% level. Ns: non-significant, *: Significant difference at P ≤ 5%, following two-way ANOVA, **: Significant difference at P ≤ 1%, following two-way ANOVA.

Assessment of 100-seed weight showed that this component affected by foliar treatment and heaviest seed was obtained by application of mixed Zn+Fe and bimetallic nanoparticles which improved seed weight by 14% and 21% over the control. Also seed weight affected by cultivar effects and in cv. Gosgo was higher than local cultivar. However, the interaction of cultivar × foliar sprays was non-significant (Table 1). Interaction effects of cultivar and foliar spray was significant for number of seed per plants. Although the use of micronutrients

could improve number of the seeds in both cultivars, the rate of increase with the use of mixed Zn+Fe and bimetallic nanoparticles in Gosgo cultivar was more prominent than the local cultivar (Figure 1). So that foliar application of bimetallic nanoparticles in cv. Gosgo and local increased number of seed per plants by 34% and 22% over control. Better response cv. Gosgo to foliar application of micronutrient can be attributed to better canopy growth, taller stem, more leaf area and better micronutrient use efficiency. Growing micronutrient-efficient genotypes contributes to environmentally-benign agriculture by lowering the input of chemicals and energy. The greater rates of short-term micronutrient uptake and their transport is important factor in genotypic response. However, mechanisms conferring micronutrient efficiency are diverse, complex and still poorly understood (Khoshgoftarmanesh *et al*., 2010).

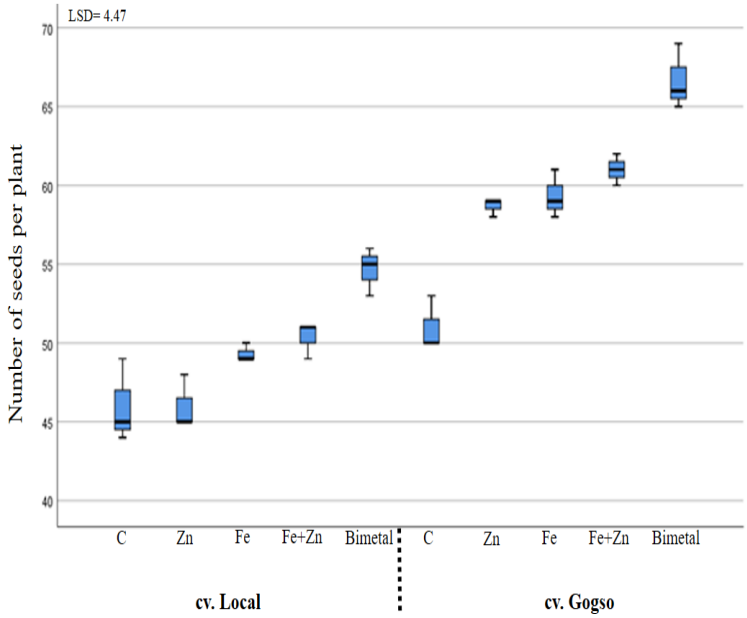

Figure 1. Effects of foliar application of different nano-form micronutrients on number of seeds per plant in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result ($P \leq 0.05$).

A similar trend also was recorded for seed yield (Figure 2). In addition to the effectiveness of binary compounds, grain yield in cv. Gosgo was significantly increased by the use of Fe nanoparticles. Contrary to the findings of Niyigaba *et al.*, (2019) we did not find any antagonist effects between applied micronutrient. Our finding showed that applying these micronutrients in combined form was better than separately utilization to increase seed yield. The result emphasized

that high-yielding varieties need more micronutrients, which should be supplied through the combination of micronutrients fertilizers. Nano-pores and stomatal openings in plant leaves facilitate nanomaterial uptake and their penetration deep inside leaves leading to higher nutrient use efficiency. Nano-fertilizers have higher transport and delivery of nutrients through plasmodesmata, which are nanosized (50–60 nm) channels between cells. The higher NUE and significantly lesser nutrient losses of nano-fertilizers lead to higher productivity (6–17%) and nutritional quality of field crops (Iqbal, 2019).

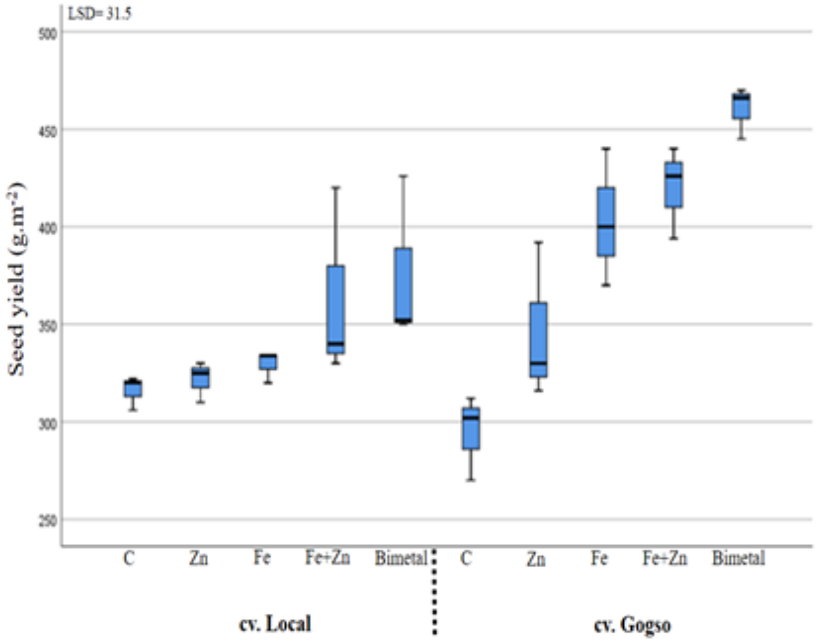

Figure 2. Effects of foliar application of different nano-form micronutrients on seed yield of two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result ($P \leq 0.05$).

Fe and Zn foliar application, irrespective to applied source, significantly increased the chlorophyll *a* and *b* and total carotenoids content compared with the control (Table 1). Also, based on the findings it was indicated that the effect of Zn sources on these characteristics was different. The maximum and minimum chlorophyll and carotenoids were detected in foliar sprayed by bimetal nanoparticles and control plants. Carotenoid content was more affected by foliar application than chlorophyll. Our results confirmed other findings regarding significant role of Zn and Fe on leaf pigments content (Janmohammadi *et al*., 2012; Raliya *et al*., 2016; Roosta *et al*., 2018).

However, there was no difference in pigment content between cultivars and also the interaction effects of cultivar × foliar application was insignificant. Chlorophyll is one of the major components of chloroplast and is positively related to plant photosynthetic rate. Chlorophyll content could be negatively affected by hot and dry spell at end of spring in Mediterranean region. Thus, a change in chlorophyll contents can directly depict the health of plants as well as plant response to the change in environment. As demonstrated in the results, there was increase in chlorophyll contents in leaves with NPs and it can be resulted in improved photosynthetic parameters and higher seed yield.

Evaluation of APX activity showed that although in both cultivar foliar application of Fe+Zn and bimetal nanoparticles significantly increased the APX activity, cv. Gosgo was more responsive to Fe, Fe+Zn and bimetal nanoparticles than local cultivar (Figure 3). Foliar application of bimetal nanoparticles increased APX activity by 75% and 211% over control in local and Gosgo cultivars, respectively.

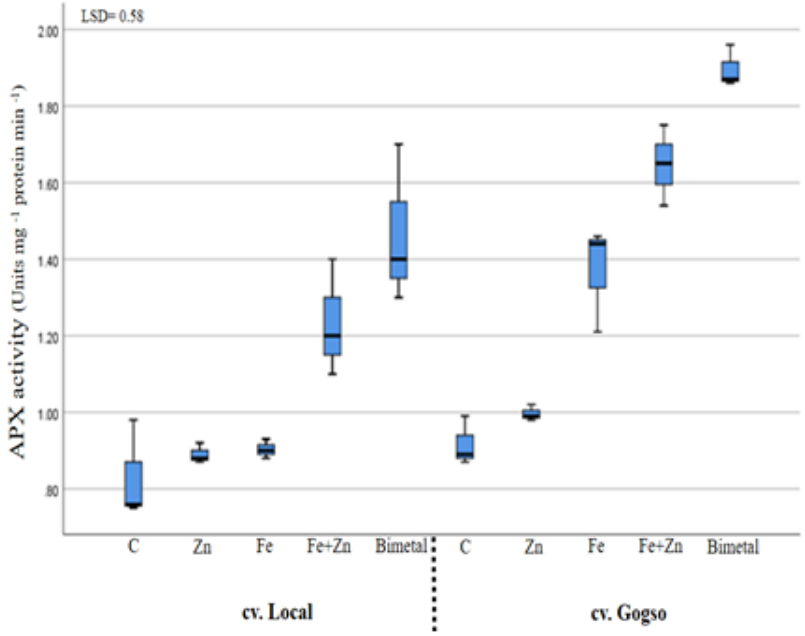

Figure 3. Effects of foliar application of different nano-form micronutrients on ascorbate peroxidase activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result ($P \leq 0.05$).

Assessment of CAT activity showed that both micronutrient NPs significantly enhanced the CAT activity .However, the intensity of the effect of

iron, zinc and their mixed form in the local cultivar was not very high and cv. Gosgo was more responsive than local cultivar to all nano-particles treatments (Figure 4).

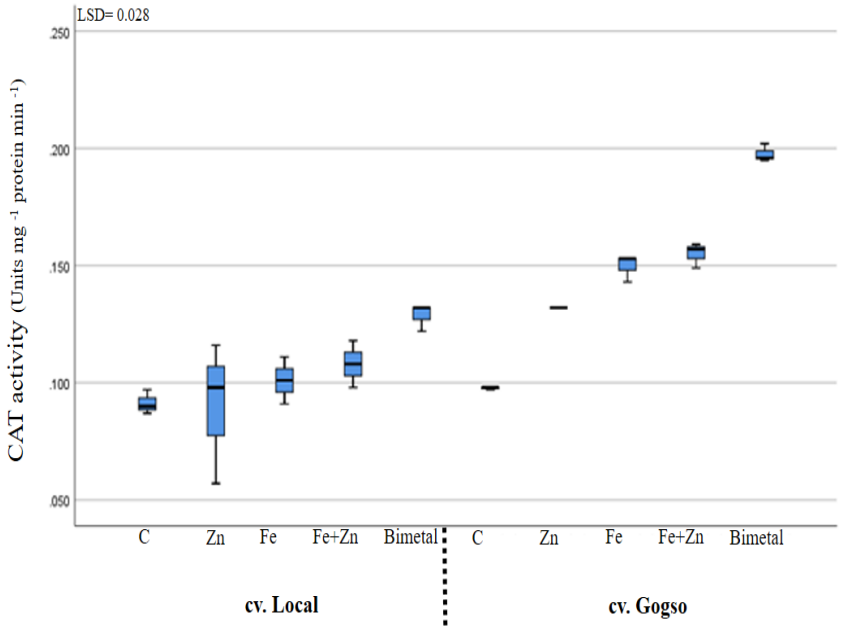

Figure 4. Effects of foliar application of different nano-form micronutrients on catalse activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result ($P \leq 0.05$).

The additive effect of bimetal on CAT activity in cv. Gosgo was about 50% higher than the local cultivar. A similar situation was recorded for GPX activity so that the highest activity was recorded for cv. Gosgo sprayed with bimetal, mixed Fe+Zn and Fe (Figure 5). Interestingly, GPX activity in cv. Gosgo under control conditions (without foliar application) was higher than foliar sprayed by monometallic nanoparticles in local cultivar.

Evaluation of SOD activity showed that the positive effects of foliar spray of monometallic nanoparticles was lower than bimetal and mixed Fe+Zn in both cultivars (Figure 6). Although the use of bi-metal significantly increased the activity of this enzyme in the local cultivar, the highest level of activity was recorded in cv. Gosgo with the use of bi-metal and mixed Fe+Zn.

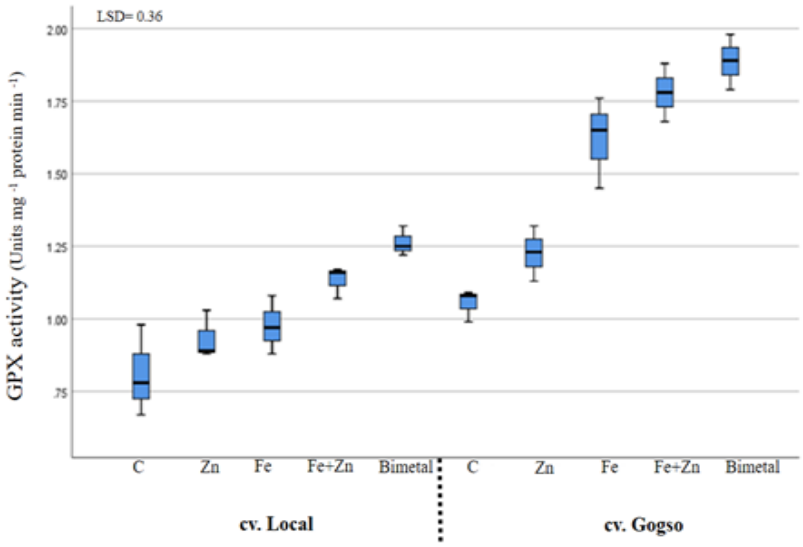

Figure 5. Effects of foliar application of different nano-form micronutrients on guaiacol-peroxidase activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

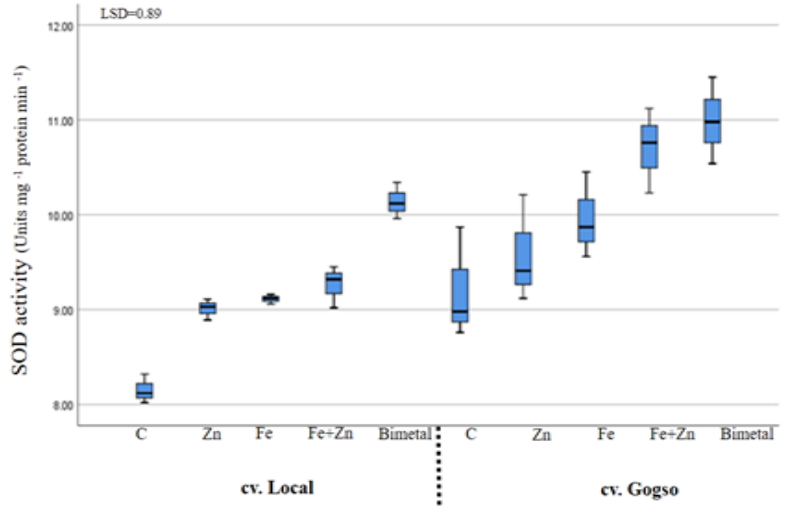

Figure 6. Effects of foliar application of different nano-form micronutrients on superoxide dismutase activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

Assessment of H_2O_2 concentration revealed that concentration this reactive oxygen species is very high in local cultivar (Figure 7). Although the use of micronutrients, especially the mixed Fe+Zn and bimetallic nanoparticles, could reduce the concentration H_2O_2 in both cultivars, the percentage of H_2O_2 reduction due to foliar spray of micronutrient was much higher in the local cultivar when compared with cv. Gosgo. This indicates the weakness of antioxidant systems and low capacity of ROS scavenging in local cultivar. The results obtained from the study of H_2O_2 concentration are largely consistent with the results of the antioxidant enzymes activity.

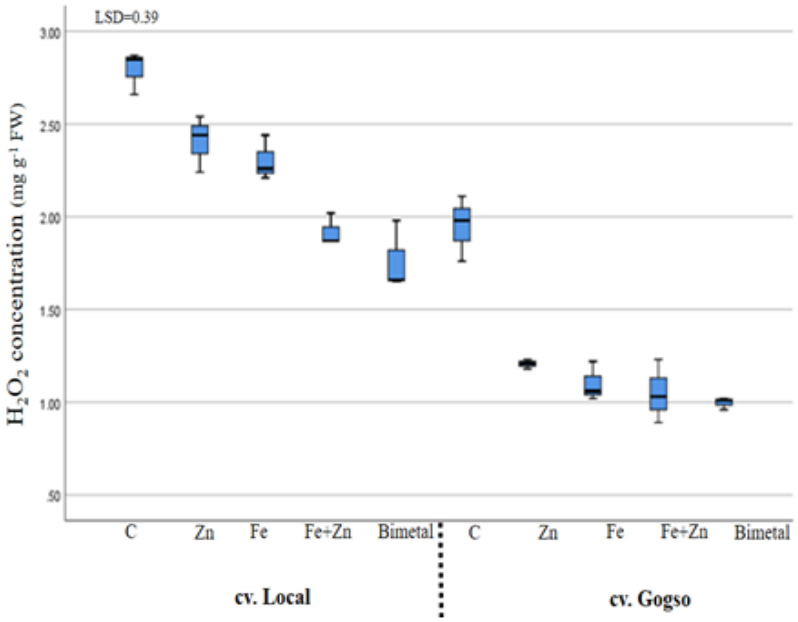

Figure 7. Effects of foliar application of different nano-form micronutrients on H2O2 concentration in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result ($P \leq 0.05$).

It seems that nano-Zn and nano-Fe application, especially in bimetallic form, reduced H2O2 levels, and as a fact, nano-spraying alleviated the adverse effects of abiotic stress. Altogether, application of nano-fertilizers alone is not a totally successful strategy in to improve chickpea production in semi-arid region. A more efficient and sustainable solution to micronutrient deficiency limitations to crop production is the development and use of micronutrient-efficient plant genotypes that can more effectively grow on soil with low phytoavailable micronutrient contents, which would reduce fertilizer inputs and protect the environment. Selection of plant genotypes that can tolerate low nutrient supply

may increase productivity on low fertility soils and reduce fertilizer requirements. By selecting suitable genotypes applying a new generation of bi-metallic micronutrient fertilizers can be more successful.

CONCLUSIONS

Finding revealed that both Zn and Fe should be applied in studied site. Study results depicted that separate and individual consumption of both Zn and Fe NPs increased growth and seed yield components in both local and new introduced cultivars. However, results showed that with the combined consumption of these elements extensive positive effects on antioxidant activity, scavenging of reactive oxygen species and pigment content. This indicates the deficiency of both elements in studied site and their synergistic effects in improving growth and physiological processes. Our finding showed that the best plant performance obtained by application of bimetallic Fe-Zn nanoparticles especially in new introduced cultivar. The use of bimetallic fertilizers can be promising in the production of nano composite fertilizers. In bimetallic nanoparticles, two different metals combine to show novel properties which are the combination of the two metals present in it. Despite the fact that some knowledge has been acquired through present and previous studies, many questions still remain unanswered such as the fate and behavior of bimetallic nanoparticles in plant systems and its effects on seed quality and human health.

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WHAT IS SMART WATERSHED MANAGEMENT?

SUMMARY

Smart management (SM) in different aspects of our planet earth is an emergence tool for today's world. Since the watershed is a basic unit for all agricultural, environmental, and socioeconomic researches and developments, so SM practicing at the watershed scale is critical. In this sense, SM in a watershed scale could be introduced as connectedness of the new Information and Communication Technologies (ICT) into watershed management practices to provide added value in the better decision making or more efficient exploitation operations and management. In this way, different ICT solutions such as precision equipment, the Internet of Things (IoT), sensors, geo-positioning systems, Big Data, unmanned aerial vehicles, and robotics need to be adapted by watershed stakeholders viz. the residents, farmers, experts, land planners, and managers, as well as the decision- and policy-makers. The smart watershed management (SWM) is associated with almost every luxury in our life and included all principles and concepts of smart water management, smart farming, smart city development, etc. It is essential to choose appropriate smart technologies that could be possible by comparing regular and novel technologies, balancing customary with green infrastructures, combining regional and universal knowledge, customizing options from abroad to regional situations, allocating with ecological and societal impressions of the substitute technologies. Altogether these decisions have need of technology appraisal and assessment tools and sound watershed governance to ensure pellucidity and

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comprehensiveness. It is challenging to ensure the proper utilization of watershed resources. However, proper implementation of smart technologies in the watershed will surely make our lives convenient and conserve our valuable resources. SWM can provide significant environmental and economic benefits.

Key words: Intelligence techniques; Market dynamic; Precise adjustments; SMART data.

INTRODUCTION

Watershed management is defined as the conserving, regulating, and managing the process of land, water, and other natural resources that are available in a watershed. Providing the proper goods and services without adverse effects on the ecological balance is the main target of the watershed management lately established by integrated watershed management [Wang *et al*., 2016]. Acknowledging the existing land use, soil, water, flora, fauna, and human communities in the watershed and their inter- and intra-relationships at the upland and downstream areas is the first step to achieve integrated watershed management [Ffolliott *et al*., 2002, Zardari *et al*., 2014]. Table 1 shows a list of targets that are more important and need to be taken into account while preparing a watershed restoration and conservation plan.

Table 1. Watershed management (WM) targets with their description and sources

WM targets	Source	WM targets	Source
Providing good water quality	[Haidary *et al*., 2013; Mirzaei & Jafari., 2019]	Protecting habitat for fish and aquatic species	[McConnaughey *et al.*, 2020]
Controlling soil erosion	[Fernández & Vega., 2016]	Protecting the natural/cultural heritage and forests	[Powell, 2000]
Flood controlling	[Brooks *et al.*, 2013; Tang *et al.*, 2020]	Improving and managing the nutrient	[Lee *et al.*, 2019]
Protecting woodlands, wetlands and habitats	[Markle et al., 2018; Barbieri *et al*., 2019; Fang et al., 2020]	Improving the quality and quantity of groundwater	[Gupta *et al.*, 2019]
Balancing ecological, economic and social interests of watershed	[Zardari *et al*., 2014]	Conserving rivers, lakes and streams	[Surasinghe *et al.*, 2020]
Promoting co - operation among countries	[Dieperink., 2000]	Upgrading and reconstructing ecosystem	[Loucks *et al.*, 2005]
Managing, evaluating and Increasing productivity	[Guangyu *et al.*, 2016]	Provisioning the ecotourism and entertainment	[Frijters & Leentvaar., 2003]
Strengthening vegetation	[Mosaffaie *et al*, 2019]	Monitoring the migration of birds	[Global Nature Fund, 2016]

With the complexity of all aspects of watersheds, including human activities, climatic, hydrology, social, and economic variables, the use of traditional watershed management tools and methods have become obsolete and needs to be upgraded. Today the smart management recognized as a new

approach that uses very practical tools that applicable for different managerial subjects such as water management, ecosystem management, smart home forensics, irrigation, smart city initiatives, medical, etc. [Mulla & Mote, 2016; Shahanas & Sivakumar, 2016; Tadokoro et al., 2016; Do et al., 2018; Kamienski et al., 2019, Saiz-Rubio & Rovira-Más, 2020]. Towards this, we integrated this subject with watershed management and introduced an innovative framework, i.e., smart watershed management (SWM). Center for Watershed Protection [Center for Watershed Protection, 2006] integrated and aligned 14 municipal programs under Smart Watershed for the United States to manage urban watersheds, smartly. The used programs were:

"(1) Sub-watershed restoration planning,
(2) Stream and sub-watershed field assessment,
(3) Sub-watershed monitoring and reporting,
(4) Watershed restoration financing,
(5) Management of natural area remnants,
(6) Storm-water retrofitting,
(7) Urban stream repair/restoration,
(8) Illicit discharge detection and elimination,
(9) Maintenance, inspection, and enforcement,
(10) Smart site practices during the redevelopment,
(11) Watershed education and personal stewardship,
(12) Public involvement and neighbourhood consultation,
(13) Pollution prevention at storm-water hotspots
(14) Pollution prevention at municipal operations"

There are also some websites and organizations that are focused on smart approaches, especially. For example, "Smart Growth" that "covers a range of development and conservation strategies that help protect our health and natural environment and make our communities more attractive, economically more robust, and more socially diverse" (http://smartgrowth.org/). Different projects and tools were developed by Smart Growth under EPA support, as reported on the website. "EverBlue" is another example that works on the SWM. They believe that "conventional watershed management methods are expensive, inadequate, and challenging to implement. Towards this, "EverBlue" introduced SWM because of its cost-effective, easy-to-deploy filtration tools.

Information and Communication Technologies (ICTs) play a significant role in smart management. SWM can make the most of the social and economic well-being of composition ICT products and provides added values in better decision making or more efficient exploitation operations and management. In our idea, SWM is the use of integrated and real-time ICT. Different ICT solutions such as precision equipment, the Internet of Things (IoT), Hardware (Sensors and Sensors Adapters), geo-positioning systems, Big Data (mapping and information systems viz., GIS, RS, and GPS), unmanned aerial vehicles, and robotics operating systems need to be adapted by watershed stakeholders. Data collecting

and integrating need to be adapted by means of "sensor networks or smart meter, data distribution using WiFi or internet, data processing, and storage using cloud technologies, modelling and analytics, and visualization and decision support using web-based tools" [64] at a watershed scale. Figure 1 shows an overview of the SWM schematic. In the following sections, we explained the potential tools that could help in the realization of SWM.

Figure 1**.** A schematic view of smart watershed management (SWM)

1. Internet of Things (IoT): Collecting Information

The term Internet of Things (IoT) was first thought by Kevin Ashton in 1999 in the framework of supply chain management [Ashton, 2009]. It is referred to as electrical apparatus of any dimension or competence linked to the internet for sharing information [Ansari *et al*., 2020]. IoT means broad communication between objects in order to create a smart environment. IoT is the future of the internet for the new generation, including a variety of technology [Shahanas & Sivakumar, 2016]. Every physical object on the IoT can interact without the aid of human intervention remains the same. The IoT is made up of a global network of objects connected to each other. Where each tool has a unique identification address, and these tools communicate based on standard communication rules and protocols [Gubbi et al., 2013]. A smart environment is also including the information and communication technologies that make the infrastructural and service components of an environment more knowledgeable, more interactive, and more efficient. IoT is a network of connected tools. The tools can be a sensor, cell phone, actuator, Radio Frequency Identification (RFID) systems, or anything that can transmit and receive information over a communication channel.

In order to activate comprehensive calculations on the IoTs, the basic three-tier construction needs to be discussed [Pande & Padwalkar, 2014]. "The first tier consists of a variety of 'things' or 'objects' such as sensors, mobile phones, actuators, etc. The middle tier is the network tier which can be wireless or wired for a reliable transfer of information generated/collected by the 'things' and technology uses protocols that exhibit high three-tier security" [Atzori *et al*., 2010; Mutchek & Williams, 2014]. The abilities of IoT tools provide numerous advantageous applications to ordinary people, companies, industries, and governments (Figure 2) [Yaqoob *et al*., 2019]. The IoT, alongside predictive analytics, supports the concept of SWM, practically. To this end, an example of an information-based SWM cycle is shown in Figure 3.

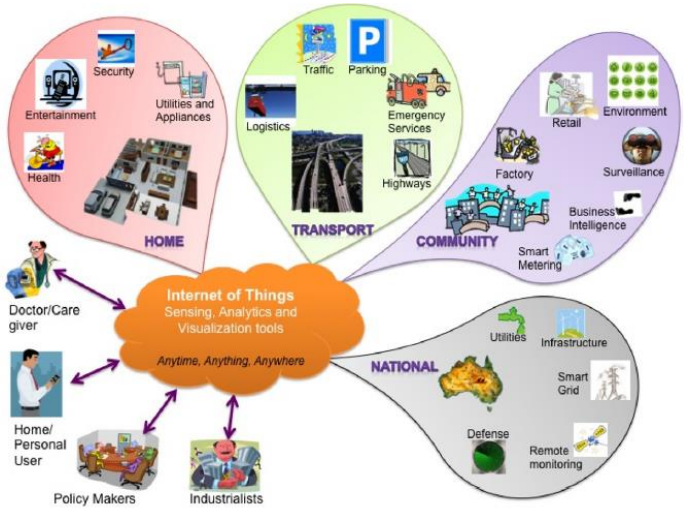

Figure 2**.** Potential Internet of Things (IoTs; [Gubbi *et al*., 2013]) for smart watershed management (SWM)

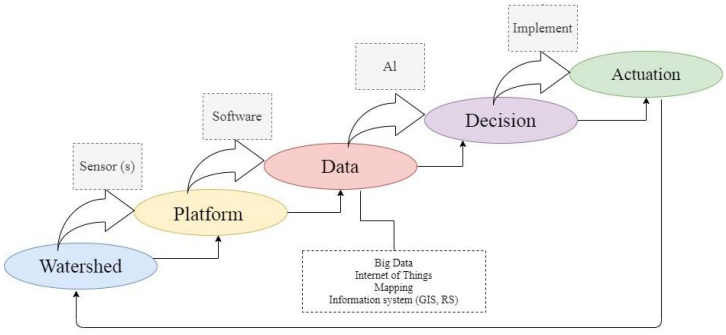

Figure 3. Information-based smart watershed management (SWM) cycle (Derived from [Saiz-Rubio & Rovira-Más., 2020])

2.Hardware: Sensoring Technologies

This layer of smart management could be named as the "eyes" of SWM. It used the elements of systems to record and transmits regarding watershed management data [Vlasov *et al*., 2018]. A sensor is a device that responds to physical, electrical, or magnetic input signals (with wired or wireless). It produces an output of measurement information in a form that is suitable for transmission, supplementary conversion, processing and/or storing, but which cannot be directly perceived by the observer [Freyden, 2005; Kozlova, 2009; Kabalci & Kabalci, 2019]. Extensively, systems that equipped with wired communication channels delivered a trustworthy transmission medium and an extraordinary speediness with long service life. A wireless sensor network involves a set of spatially-distributed intelligent sensors planned to monitor physical factors [Muyeen & Rahman, 2017; Vlasov *et al*., 2018]. Sensors placed in watershed units allow experts to obtain detailed maps of both topography and resources in the area, as well as dynamic monitoring of vital variables such as moisture, temperature, precipitation, etc.

3.Smart Data

Nowadays, information is essential for the prosperous supervision and operation of SWM. There is much information from the past, part of which is due to its public use of computers, communication networks, and sensors. In this age of Big Data, it has become likely to obtain, distribute, and process data close to real-time [Li *et al*., 2020]. According to the US National Institute of Standards and Technology (NIST), Big Data that is also useful for SWM consists of four dimensions of volume, velocity, and variety [Manyica *et al*., 2011; Sun & Scanlon, 2019]:

–Volume talks about Big Data size. The data volume is considered substantial if it is at a scale afar usual database software programs to collect, storage, accomplish, and process information, timely [Manyica *et al*., 2011; Saiz-Rubio & Rovira-Más, 2020]. In the past decade, using of Big Data was raised with the rapid development of ICT and IoT [Sun *et al*., 2019].

–Velocity discusses the facility to obtain, apprehend, and interpret events as they take place [Saiz-Rubio & Rovira-Más, 2020].

–Variety denotes the diverse formats of data used that could be as videos, text, voice, images, and the various complexity grades [Saiz-Rubio & Rovira-Más, 2020].

–Variability mentions the variations in entire other characteristics of the Big Data, such as, changes in data flow rates or dissimilarities in data meaning particularly in crowd-sourced data [Sun *et al*., 2019].

4.Mapping and information systems based data (GIS, RS, and GPS)

Maps visualize complex properties of our surroundings in a modest and clear manner for adapting different targets. The maps consider simplifications of reality, and it is not possible to represent the entire complications and minutiae. However, modern achievements of data acquisition, analysis, process, and visual depiction have enhanced that leading to better temporal and spatial resolutions of maps [DeLorme, 2001]. Map format has been recognized as the furthermost method to display watershed management data, because mapping is expedient to delineate spatial patterns and trends as well as homogeneous zones. Maps are also very useful to make efficient decisions; answer land management questions, provide and interpret the spatial information [Saiz-Rubio & Rovira-Más, 2020].

The geographic information systems (GIS) and remote sensing (RS) play an essential role in the SWM. GIS comprises a collection of hardware and software that captures geographic data efficiently, stores it for analysis and displays as per requirement [Martin *et al*., 2020]. GIS plays a significant role in the creation of SWM by utilizing geospatial data generated by various techniques. GIS-based planning of SWM can help in identifying alternative options and their potential impacts when required. The planning tasks could be also performed through functions of the GIS in terms of spatial analysis (Figure 4).

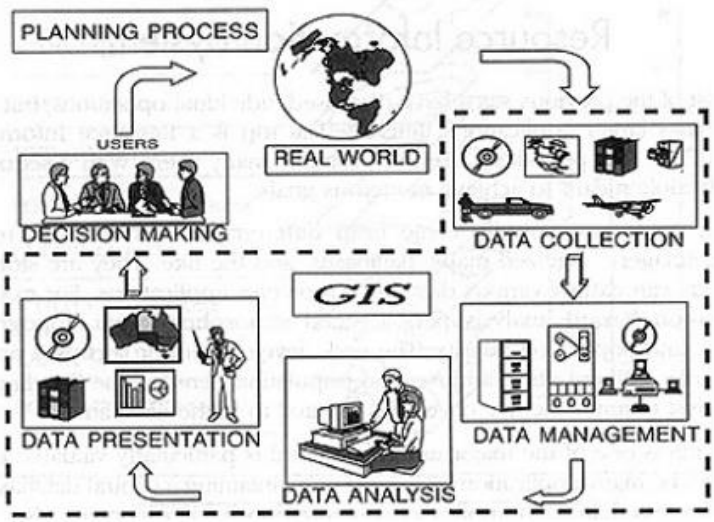

Figure 4. GIS role in the planning development
(http://rst.gsfc.nasa.gov/Front/tofc.html)

The RS technique implicates information mining in respect to diverse object of the earth, indirectly. Geo-spatial data collecting from remote devices (such as sensors) has been used for analysis. Traditionally, acquiring data typically by optical and radar sensors based on the satellite and airborne platforms was referred to RS [Schowengerdt, 2006]. Whilst, currently, any

acquisition means of image and spatial figures (such as airborne surveying and photogrammetry) has been considered as RS [Mikhail et al., 2001], though the satellite-based RS and mobile mapping for terrestrial platforms, are still sporadically applied [Toth & Jóźków, 2016]. RS is useful in numerous SWM targets, for instance sensing moistness, evaporation, and transpiration of soil, condition evaluation (land cover, snow cover), trend changes (temperature, precipitation, etc.) classification of land use, watershed health, and compliance of agriculture monitoring.

The RS process encompasses the relations concerning incident radiation and the targets of interest. Figure 5 shows the imaging systems where the following seven elements (visible light, radio-waves, micro-waves, infrared, UV rays, x-rays, and gamma rays) are involved. Note, however, that RS also involves the sensing of an emitted energy and the use of non-imaging sensors (Figure 6). RS can be broadly classified into three types concerning the wavelength region and type of sensor involved for data acquisition; viz. optical (Visible and Reflective Infrared), thermal infrared and microwave [Gao *et al*., 1998; Murino *et al*., 2014; Lei *et al*., 2020].

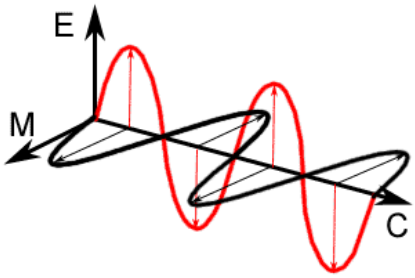

Figure 5. Electric wave (E) and magnetic wave (M)
materializing the electromagnetic wave
(Source: http://www.ccrs.nrcan.gc.ca/resource/tutor/fundam/index_e.php)

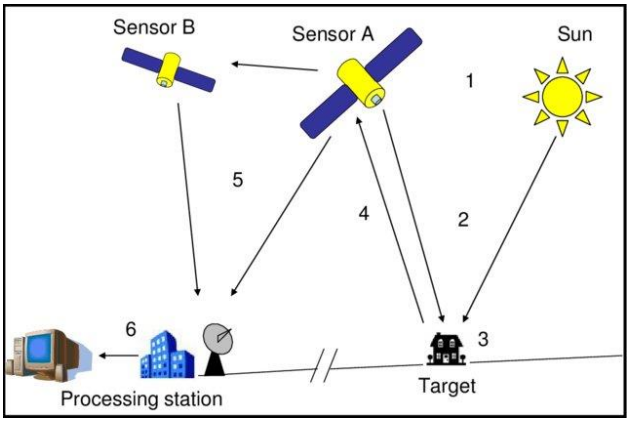

Figure 6. RS process stages
(http://www.ansp.org/museum/kye/tech_environment/2001_remote_sensing.php)

One way to determine the geo-positioning is Global Positioning System (GPS). GPS is a satellite-based navigation system applied to locate a geo position anywhere on earth, using its longitude and latitude. It fixes the precise geodetic location and elevation on the earth's surface. The marvellous applications were known for GIS data collection, monitoring, and plotting using GPS technology [Hanna *et al*., 2012]. Figure 7 shows a view of satellites orbiting the earth.

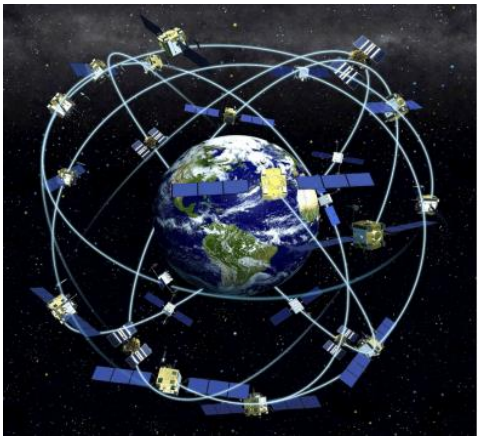

Figure 7. Arrangement of NAVSTAR satellites circling earth
(Source: www.linuxjournal.com)

5.Unmanned aerial vehicles systems

Using of an unmanned aerial system (UAS) as a communication platform and an innovative technology has a marvellous prospective in watershed management [Boursianis *et al*., 2020]. UAS is an aircraft without a human pilot on board, which can experts autonomously or controlled telemetrically to carry out a special mission [Boukoberine *et al*., 2019]. Low cost and high mobility are two benefits of UAVs when capturing aerial snapshots [Liu *et al*., 2014]. UAS can provide flexible, fast, and cost-effective tools for inspecting watershed management [Mota *et al*., 2014]. Figure 8 shows a prototype UAS platform.

Figure 8. A prototype UAS platform [Guerra *et al*., 2020]

6.Robotics Operating System

Studies on sensory feedback control for robotic operating systems (ROCs) in subject of smart management have recently risen [Fitzpatrick *et al*., 2008; Wang *et al*., 2016]. The ROCs can interact, assimilate motion, and handle functions to promote or substitute human beings in diurnal responsibilities [Boursianis *et al*., 2020]. To produce these kind of ROCs, there are several engineering methods [Niazi & Hussain, 2011] and support technologies [Iñigo-Blasco *et al*., 2012] available, with the multi-agent system (MAS) being one of the most successful [Guerra *et al*., 2020].

7.The necessity of smart management adaptation

Smart management provides many applications for easy human access to a better life, which can be referred to as some of these:

Smart City: The literature showed that the percentage of the city inhabitants will continue to rise. Therefore, the urban development and planning could be promoted through a smart digital city [Batty, 2007]. For example, the use of smart management in India has been very efficient. Smart parking, intelligent traffic management, and integrated multi-faceted transportation have brought smart transportation to the city. Further values could be added to the smart environments using adaptation of smart waste management, air quality monitoring, and noise monitoring systems [Shahanas & Sivakumar, 2016].

Smart Climate: Climate is the major uncontrollable factor that influences crop yield and has been accepted as one of the factors contributing to yield stagnation globally [You, 2009; Gichenje & Godinho, 2019]. Improvements in crop yields have slowed since the 1990s [Rosegrant & Cline, 2003] and even stagnated in much of the world since the last century [Ray, 2012]. Climate-smart soil management incorporates total sustainable management practices and associated environmental, cultural, social, and economic activities within the framework of a changing climate [Garcia-Franco *et al.,* 2018].

Smart Water Management: Water is vital components of our earth to our life style, and universal water-related concerns consist of short supply and uneven distribution. To this end, smart water analytics provide a change by dint of bringing real-time data in front. Therefore, the analysts will spend short time and do quick actions on the data analysis at a low cost [Shahanas & Sivakumar, 2016; Nguyen *et al*., 2018]. According to many studies [Choi & Lee, 2013; Byeon *et al.*, 2015; Choi *et al.*, 2016], it can be stated that using a smart system can be managed. Smart water management can be a useful and effective method to improve water quality and quantity.

Smart Farming towards Agriculture: Growing population and rushed climate changeability worldwide has placed immense pressure on agricultural foodstuffs [Virk *et al*., 2020]. According to the result, smart management can provide helpful knowledge for farmers to elevate their living ideals, through high yield, and income and also can be a sound food security index [Rodríguez *et al*., 2019, Kumari & Iqbal, 2020].

CONCLUSION

Being smart is a vital step for our earth management. As the researchers conclude that everything on this planet is created smartly, so smart tools are needed to use, save, and manage the earth's planet resources and even developing new ones. Smart Watershed management (SWM) is one of the interdisciplinary subjects that need to establish, globally. Because the watershed is known as a basic unit for land planning, then achieving smart management is essential at a watershed scale. In this regard, linking the recent human achievements in the field of Information and Communication Technologies (ICT) with watershed management strategies is very useful. According to the literature review, various programs are suggested, but undoubtedly there are strengths and weaknesses for each program. Therefore, selecting the best one based on the governing situation of the watershed and mixing some of them, also need smart thinking. SWM is direct links smart tools to watershed restoration practices and creates a robust practical basis to advance a comprehensive and action-oriented rehabilitation plans. SWM, with the help of digital technology, improves the data collection methods and analytics to support proactive decisions and increase the efficiency of watershed utilities.

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DETERMINATION OF FOREST DECLINE DUE TO THE ACTION OF DOMINANT STRESS FACTOR THROUGH MONITORING OF DEFOLIATION - CASE STUDY OF MALJEN, SERBIA

SUMMARY

The paper presents the results of research on the influence of climatic factors on the occurrence of defoliation and decline of forests as a final outcome. The research was conducted in a mixed beech and fir forest, in the period from 2004 to 2019 at sample plot 415 - Maljen, as a representative example of forest decline, which is part of regular monitoring conducted on the territory of the Republic of Serbia. Due to the fact that defoliation may be due to the influence of various factors, research, analysis and results are focused on long-term trends. Special attention is paid to the analysis of the degree of influence of climatic parameters on the occurrence of defoliation. Data on temperatures and precipitation in the period 2004-2019 were processed and analyzed on a monthly and annual basis, as well as in the vegetation period, for the main meteorological station Valjevo, which is closest to sample plot 415. Also, drought assessment was performed during this research period based on Lang's rain factor, de Martonne aridity index and climate diagram by Walter. In order to confirm these methods, the Standardized Precipitation Index was calculated as one of the method that has been mostly used in drought identification lately. It was stated that the defoliation of trees was initiated by extreme climatic events during three consecutive drought years (2011-2013), after which there was the largest decline of forests in the researched area.

Keywords: defoliation, abiotic factors, drought, forest decline, sample plots, Maljen.

INTRODUCTION

Forest decline is a natural process due to which old and physiologically weakened trees are more susceptible to decay. However, when a large number of

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trees in one stand or in a wider area begin to decline, and their number increases from year to year, it is clear that there has been some disturbance at the site or in the entire environment. Decline is not a consequence of a sudden event, but a process that progressively increases from year to year until it reaches proportions noticeable to the naked eye. When it comes to individual trees, it is usually easy to determine the cause of death, although it can be very diverse (physiological age limit, damage from biotic or abiotic factors, the struggle for light or other living conditions, etc.). However, when it comes to the decline of trees of one or more species, especially in wide territories, the causes of tree decay change successively.

The ICP Forests program represents an international cooperative program for monitoring the impact of air pollution on forests while monitoring of forest is performed within this program as one of the most diverse approaches to researching the impact of various environmental factors on forest ecosystems. Defoliation was adopted by ICP Forests in many European countries in the 1980s as the main indicator of the forest condition. It represents a visual assessment of the crown of a tree and is an indicator of the missing amount of leaves/needles in relation to the reference tree in the immediate vicinity and is the main parameter when assessing the condition of individual trees (ICP Forests Manual and Criteria).

There are discourses of many researchers who deal with this issue, which is the dominant factor (abiotic, biotic or anthropogenic), which negatively affects forest ecosystems and leads to defoliation. Sometimes the same factor can be predisposing, stimulating and contributing to defoliation. On the one hand, the primary factor of defoliation and decline of forests is considered to be climate change, because they cause constant stress in the environment that strongly affects forests (Aber, J. *et al.*, 2001; Dale, H.V., *et al.*, 2001; Seidling, W 2007; Kadović, R. and Medarević M. 2007; Bašanac-Bosanac, Lj. *et al.*, 2011, 2014, 2015; Češljar, G. *et al.*, 2013, 2014; Ćirković-Mitrović, T. *et al.*, 2013; De la Cruz, A. *et al.*, 2014; Sánchez-Salguero, R. *et al.* 2017). The mentioned authors find a connection between the increase in defoliation and climatic conditions, emphasizing, above all, the frequent droughts followed by high air temperatures. Schuldt B. *et al.* (2020) state that even species that are considered drought-resistant, including beech, suffer long-term damage, while increased mortality is also recorded on fir.

Beech and fir forests, mainly on smaller areas on several lower mountains in Serbia, among them on Maljen, were described by Gajić, M. *et al.* (1954). According to these authors, these are mountain beech-fir forests, which occur at lower altitudes (descending to about 700 m).

A high degree of defoliation on beech trees, as a consequence of severe drought, was stated by Seletković, I. *et al.* (2009) on beech trees on the Medvednica mountain massif in Croatia. Popa, I. *et al.* (2017) state that, in the network of Level I sample plots in Romania, there are significant negative correlation between defoliation of beech trees and mean temperature from 1997

onwards, in the monitoring period 1992-2013. The widespread occurrence of crown defoliation on beech trees after drought and heat waves in the beech forests of Tuscany in central Italy was also noted by Pollastrini, M. *et al.* (2019).

Also, it is known that fir has a very narrow ecological valence and that it is difficult to tolerate the extremes of various factors of abiotic nature, and that in the previous period it was affected by more intensive decline processes in the entire area. Androic and Klepac (1969) also stated that an extremely wet or dry year is unfavorable for fir, when the trees lose the ability of normal assimilation, so decline occurs in the still partially green canopy. According to the annual reports of the ICP of the Republic of Croatia, fir is the most damaged species of forest trees in this country, with a very high percentage of significant damage (Seletković, I. and Potočić, N., 2004). Research has established that decline is a consequence of the synergistic action of several unfavorable habitat factors, such as drought, frost, large changes in air temperature, etc. (Potočić, N. *et al.*, 2008; Tikvić, I., 2008; Anić, I. *et al.*, 2009).

Based on all the above, defoliation can be influenced by various biotic, abiotic and anthropogenic factors that can act individually or in interaction. One-sided interpretation of these very complex phenomena and support for the decisive role of only one factor does not answer many questions. Therefore, the aim of this paper is to describe in detail the conditions that prevailed during the research period at the selected site in order to provide answers to the forest decline in this area, which was also found in the entire territory of the Republic of Serbia.

MATERIAL AND METHODS

Data collection was conducted as part of monitoring the condition of forests at the sample plot of Level I during the vegetation period when the leaf was fully formed (June-August), in the period from 2004-2019. As a good indicator of the impact of drought, i.e. abiotic factor on forest ecosystems during the research period (2004-2019), an example of sample plot 415 - Maljen I (Figure 1), in the state forest, on the mountain Maljen (coordinate network 4x4 km, coordinates: Y7431000 and X4886959) was singled out. This sample plot is located at 630 m above sea level, on the northern exposure, on eutric cambisol on serpentinite , with sufficient water availability. The average age of the stand is 61-80 years. In terms of phytocenological affiliation, the investigated locality is located in a beech and fir forest (A*bieti-Fagetum serpentinicum* Beus 1980).

According to the ICP Forests methodology (ICP Forests Manual and Criteria), during sampling of tress for monitor their condition in 2004, 24 trees were sampled, of which 15 beech trees and 9 fir trees, which were without mechanical or any other damage that could affect their vitality in the coming period.

The method of work is based on the criteria of the International Forest Program (ICP Forests Manual and Criteria), where, as the main parameter of the

forest condition, visually assessment of defoliation is conducted, by classes 0-4, degrees and percentage of leaf/needle loss (Table 1).

Figure 1. Position of sample plot 415 on the map of the Republic of Serbia

Table 1: Categorization of defoliation according to *ICP Forest Manual and Criteria*

Classes of defoliation	**Leaf /needle loss (%)**	**Degree of defoliation**
0	0-10	None
1	> 10-25	slight (warning phase)
2	>25-60	moderate
3	>60<100	Severe
4	100	Dead

From climatic factors, the average annual air temperatures, the average annual air temperatures in the vegetation period (April-September), the sum of precipitation at the annual level and the sum of precipitation at the annual level in the vegetation period (April-September) were analyzed for the main meteorological station (MS) Valjevo for the period 2004-2019.The data were

taken from the website of the Republic Hydrometeorological Service of Serbia (RHSS). The non-reactive method was used as a special scientific method for collecting data on climatic and meteorological conditions (Neuman W.L. 2006). This method includes research that does not involve direct data collection and in that sense is the opposite of research techniques such as interviews, surveys and experiments. Its basic techniques, such as content analysis or observation of documents and the use of existing statistics, documents and their secondary analysis, were used to assess the impact of temperature extremes (high and low temperatures) on forest ecosystems and identify possible changes over the years.

For the purpose of analyzing the height of the water layer in mm (if there were no evaporation, surface runoff and sinking into the soil), the Lang (1920) rain factor (IL) was calculated, which represents the ratio between the annual sum of precipitation and the average annual air temperature. According to the size of the IL, Lang characterized the following bioclimatic types of areas: 0-20 deserts, 20-40 semi-deserts, 40-60 steppes and savannas, 60-100 weak forests, 100-160 high forests, > 160 perhumid types.

Also, the annual aridity index according to De Martonne (1926) was calculated, which is used to determine the type of water runoff and the need for irrigation. The classification of drought according to the aridity index according to De Martonne (DMI) was performed as follows (Hrnjak, I. *et al.*, 2014): <10 - arid climate; 10-20 – semi-arid, 20-24 - mediterranean, 24-28 – semi-humid, 28-35 - humid, 35-55 - very humid and > 55 - extremely humid climate.

In order to consider the relationship between the movement of annual temperatures and precipitation as the most important factors of the climate of a region, climate diagrams were calculated and presented by Walter H. *et al*. (1975).To confirm the previously described methods for drought detection, the Standardized Precipitation Index (SPI) was calculated by McKee *et al.* (1993) as one of the methods most recently used in drought identification. SPI was calculated at intervals of 6 and 12 months (SPI-6 and SPI-12) and the assessment of the dry period was performed based on the categorization shown in Table 2.

Table 2. Categorization of moisture condition by SPI – Source: RHSS

Category of moisture conditions	**SPI values**
Exceptional drought	SPI ≤ -2.326
Extreme drought	-2.326 < SPI ≤ -1.645
Severe drought	-1.645 < SPI ≤ -1.282
Moderate drought	-1.282 < SPI ≤ -0.935
Minor drought	-0.935 < SPI ≤ -0.524
Near normal	-0.524 < SPI< +0.524
Slightly increased moisture	+0.524 ≤SPI< +0.935
Moderately increased moisture	+0.935 ≤SPI< +1.282
Considerably increased moisture	+1.282 ≤SPI< +1.645
Extremely wet	+1.645 ≤SPI< +2.326
Exceptionally wet	SPI ≥ +2.326

RESULTS AND DISCUSSION

Sample plot 415 at the Maljen locality was chosen as a good indicator of the primary cause of drought-induced decline, because during the research period at this locality gipsy moth (*Lymantria dispar* L.) was not found as the primary cause of damage, nor any other insect (eg. bark beetles), or a fungus that could affect the normal functioning of the plant organism and cause a stressful situation. Also, not a single tree was replaced at this site during the entire research period, which could have happened due to numerous other reasons such as regular felling, windbreaks, snowdrifts, forest theft, fire, etc. This means, that the same trees that go through identical favorable or unfavorable environmental conditions (microclimatic conditions), were monitored throughout the period. There are no industrial facilities or any other sources of pollution in the vicinity of the investigated site. For these reasons, as the only parameter, we can compare defoliation during the entire research period, which is shown in Table3.

From the presented data it can be concluded that all trees during the research period had defoliation within class 0 (0-10%) and no class 1 (> 10-25%) weak, which indicates the fact that it is a stable and healthy stand (2004-2010). However, the first symptoms of the beginning of decline were observed during 2011, when defoliations were observed on some trees, which significantly deviate from those observed until then. In the following 2012, this trend continued, and in 2013 the first decline was observed, which continued in 2014 and 2015. Table 3 shows that 30% of the trees that were continuously monitored in 2014 had symptoms of gradual to complete decline. In addition to the trees that have been isolated and monitored for many years, the researchers noted in their field records, that the same symptoms appear in the entire stand with the final outcome of decline. Also, in this period, very small leaves were noticed on the beach, which was a consequence of many years of drought, which the trees tried to fight against in that way, because less water is lost over a smaller leaf area, so it was the first line of defense against drought. As a consequence of a long period without precipitation with high temperatures, there was a premature leaf fall, which occurred well before the usual autumn leaf fall, which was observed in a large number of other investigated localities.

The beginning of intensive defoliation of beech trees in the research area, with a defoliation class of as much as 80%, was observed two years after the first extremely warm and dry year (2012). At the end of the research period, there were 20% of dead beech trees, while the most decline were in the period 2013-2015.

Concerning fir trees, the sensitivity to climatic extremes was higher in relation to beech. In the driest year of 2011, defoliation of 80% was found on one tree, and in the following years there was a sudden decline, so that in 2015, more than 2/3 of fir trees were dead. In the literature, a significant number of authors estimate that the occurrence of forest decline is largely conditioned by high temperatures and precipitation (Martinez-Vilalta J. *et al.* 2011; Fan Z. *et al.*, 2012; Zhang Q. *et al.*, 2017; Romagnoli M. *et al.*, 2018; Brèteau-Amores S. *et al.*,

2019). In addition to the amount of precipitation and their distribution during the vegetation period, the process of forest decline is greatly influenced by the deficit of soil moisture (Speich M.J.R., 2019). Fighting severe drought, forest trees slow down transpiration, and thus absorb less nutrients from the soil and slow down all other physiological processes. In such conditions, if the drought lasts for a long time, the trees are physiologically weak and become less resistant to other anthropogenic, abiotic and biotic causes of forest decline.

Air temperature is one of the most important climatic factors that affect vegetation. Analysis of average values of annual temperatures for MS Valjevo for the period 2004-2019, indicates that the warmest two periods stand out, namely 2012-2015 and 2017-2019 (Table 4). When it comes to positive temperature deviations from normal, 2018 was the year of climate records in Serbia: the warmest in the history of meteorological measurements; the warmest spring; the warmest April and the warmest summer according to the minimum temperature. According to the obtained data on the average annual temperature, in the research period, the highest average value was registered in 2019 (13.4°C).

During the vegetation period (April-September), the highest average annual values of air temperature in the area of Valjevo were registered in 2012 and 2018 (Table5). According to Kolić, B. (1988) with the increase of air temperature in the beginning, the intensity of photosynthesis increases sharply. However, the greater the increase in temperature, the intensity of photosynthesis does not follow the increase in air temperature, because with the increase in temperature begins the process of respiration, which is by its energy characteristics opposite to photosynthesis. The maximum intensity of photosynthesis in the vegetation period occurs at air temperatures of 15.5°C". Taking into account the above, it can be concluded that the air temperature in the investigated locality in the vegetation period during the entire research period was higher than necessary.

Based on the obtained data for the research period (2004-2019) and data for the reference period (1961-1990), their comparison was performed both on an annual level (Figure 2) and during the vegetation period (Figure 3). On both previously mentioned figures, a deviation of the mean monthly temperatures during the research period in relation to the normal 1961-1990 is noticeable. This trend is especially pronounced in the vegetation period, and the largest deviations were recorded during 2012. According to the data shown in Tables 6 and 7, the lowest average amounts of precipitation in the area of MS Valjevo were registered in the period 2011-2013. During the summer of 2011, longer periods without precipitation were recorded, which make this year one of the driest in the entire territory of Serbia since the measurements began. This year can be considered extremely dry because the average amount of precipitation was below 500 mm, which according to Rakićević T. (1980) is the limit for declaring drought. The deficit of moisture in the soil was intensified by the long-term high air temperature, which was about 3 degrees higher during the summer months compared to the several year average.

Table3: State of defoliation at sample plot 415 in the period 2004-2019.

SP Level I	No. and label trees	Tree Species	Defoliation in percentages (%) per year															
			2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
415	1N	Beech	30	30	10	10	0	10	10	5	0	10	**100**	0	0	0	0	0
415	2N	Fir	20	10	0	20	20	10	10	**30**	**80**	**90**	**100**					
415	3N	Beech	20	20	0	10	0	10	0	0	0	20	10	10	10	10	10	0
415	4N	Beech	10	10	0	10	10	0	0	0	0	0	10	0	10	0	0	0
415	5N	Beech	0	20	10	10	0	0	10	0	0	10	0	0	0	0	0	0
415	6N	Beech	10	20	10	10	0	10	0	0	0	10	0	0	0	0	0	0
415	1E	Beech	0	0	10	10	0	0	0	20	0	10	10	10	10	10	0	0
415	2E	Beech	10	0	0	10	10	10	0	10	10	20	10	10	10	10	0	0
415	3E	Beech	10	0	10	10	0	10	0	0	10	0	10	0	0	10	15	10
415	4E	Fir	0	10	0	10	25	10	0	5	0	0	10	15	10	15	0	0
415	5E	Fir	20	10	10	10	20	0	0	5	0	0	**100**					
415	6E	Beech	0	20	10	20	0	10	0	20	10	10	10	10	0	10	0	0
415	1W	Beech	10	10	0	10	10	10	0	0	10	10	10	0	0	0	10	10
415	2W	Beech	10	30	10	10	0	10	0	10	0	10	10	20	15	0	15	10
415	3W	Beech	0	10	10	10	10	20	0	5	0	0	0	0	0	0	15	0
415	4W	Fir	0	0	0	20	10	10	10	0	0	0	0	15	10	0	0	0
415	5W	Beech	0	20	0	10	10	10	5	10	0	10	0	0	0	0	0	0
415	6W	Beech	10	30	10	10	0	10	5	0	0	10	0	40	75	80	**100**	
415	1S	Fir	0	10	10	10	10	10	0	0	0	10	0	10	10	10	0	0
415	2S	Fir	0	10	0	10	20	10	10	10	0	**30**	**70**	**100**				
415	3S	Fir	0	20	10	20	30	10	0	**80**	**80**	**100**						
415	4S	Fir	0	10	10	0	10	0	5	0	0	20	**60**	**100**				
415	5S	Beech	20	20	20	10	10	10	5	0	0	**80**	**100**					
415	6S	Fir	0	10	0	10	10	20	0	10	10	**40**	**80**	**100**				

Table 4: Average values of annual air temperature (°C) for MS Valjevo in the period 2004-2019.

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
$t_{sr.}$	11,5	10,7	11,5	12,7	12,8	12,5	11,9	11,9	**12,7**	**12,7**	**12,8**	**12,9**	12,5	**12,7**	**13,0**	**13,4**

Table5: Average values of annual air temperatures (°C) for MS Valjevo in the vegetation period (2004-2019)

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
$t_{sr.}$	17,7	17,7	18,3	19,3	18,9	19,4	18,5	19,5	**20,4**	19,2	18,1	19,8	18,9	19,6	**20,2**	19,5

Table 6: Average precipitation amounts (mm) for MS Valjevo in the period 2004-2019

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Σ	846,4	843,9	821,7	843,3	656,1	916,3	1062,1	**601,0**	**611,0**	**681,7**	**1332,4**	765,9	980,4	737,7	791,9	741,1

Table 7: Average precipitation amounts (mm) for MS Valjevo in the vegetation period (2004-2019)

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Σ	497,2	533,4	465,5	393,2	360,1	427,0	677,7	**378,1**	**276,1**	**344,7**	**1066,6**	378,7	547,4	377,8	465,5	460,3

After the dry period, 2014 came with an extremely large amount of precipitation (1332.4 mm). In the period from April 14 to May 5, 2014, between 120 and 170 l/m² of precipitation was measured in most places in Serbia, while in

some places in the vicinity of Valjevo, the amount of precipitation exceeded 300 l/m².

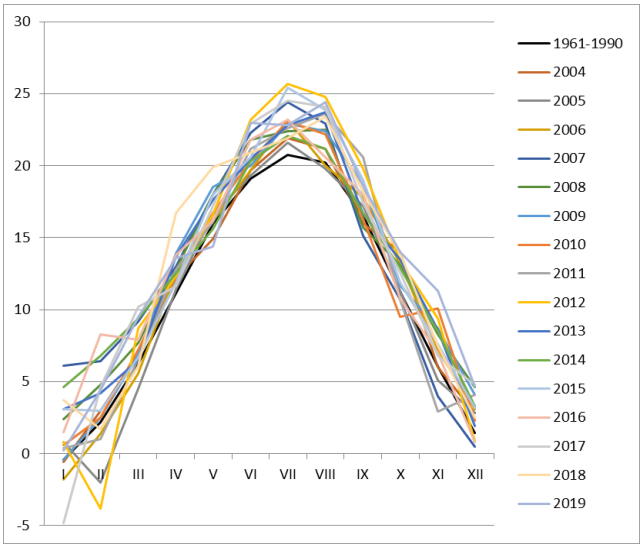

Figure 2. Mean monthly air temperature values (2004-2019) in relation to the normal for the MS Valjevo

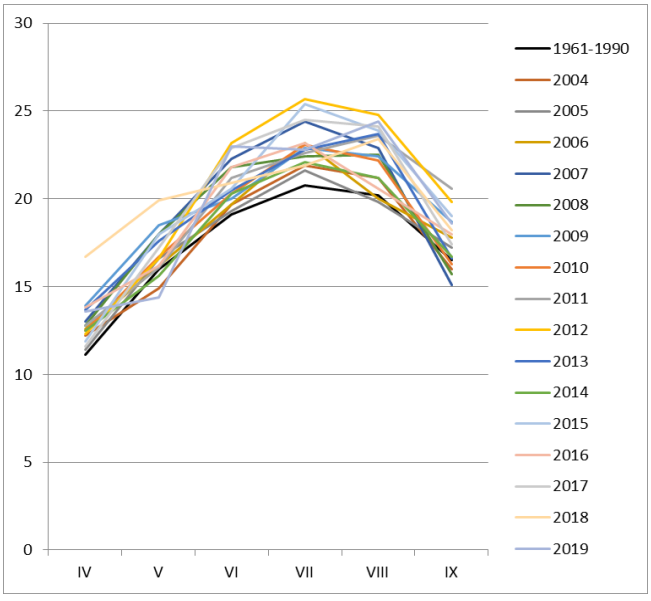

Figure 3. Mean monthly values of air temperature in the vegetation period (2004-2019) in relation to the normal for the MS Valjevo

To confirm the impact of the several year drought registered in the period 2011-2013 in the entire territory of Serbia, Figure 4 shows climate diagram by H.

Walter *et al.* (1975) for the main MS Valjevo, which is closest to the sample plot 415 locality.

These diagrams represent the ratios of annual temperatures and precipitation as the most important factors of the climate of a locality and give a visual representation of the monthly movement of drought, i.e. the distribution of the humid and arid periods during the year. On the climate diagram for 2011 (Figure 4a) it can be seen that some months (June, August) were dry and extremely dry (November). In summary, 2011 had the lowest amount of precipitation in relation to the next two observed years, but also for the entire research period. Climatologically, 2012 (Figure 4b) is also characterized by the lowest recorded temperatures at the beginning of the year, absolute maximums, absolute minimums of air temperatures and strong spring frost. The month of February 2012 was the coldest month since the beginning of meteorological measurements and the summer of 2012 was the warmest since meteorological measurements in Serbia is conducted. Also, for 2012, it can be said that the summer was extremely dry, because the precipitation was significantly below normal for several consecutive months. Extremely hot was also 2013 (Figure 4c), the seventh warmest in the period from 1951 to 2013. Six heat waves were registered, and according to the data of RHSS (2013), the strongest intensity of heat waves registered during 2013 was recorded in the area of Valjevo. In 2013, higher amounts of precipitation were recorded, compared to the previous two years, but this year stands out due to the length of the heat waves that characterized it.

Based on the analysed data for the MS Valjevo for the period 2004-2019, I_L is calculated according to Lang and aridity index (DMI) by de Martonne (1926) (Tables 8 and 9).

Table 8: I_L according to Lang for MS Valjevo

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
I_L	73,6	78,9	71,3	66,3	51,1	73,4	88,9	50,6	48,3	53,6	103,9	59,6	78,5	58,3	60,8	55,3

By analyzing the values of the rain factor according to Lang's bioclimatic classification, it can be concluded that the investigated area is at the transition from semi-arid bioclimatic type of area to semi-humid type. Stands out the period from 2011-2013, which is in the arid bioclimatic type. The exception is 2014 (I_L 103.9), when the humid bioclimatic type (climatic type of high forest area) prevailed.

Table 9: DMI according to de Martonne for MS Valjevo

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
DMI	39,4	40,8	38,2	37,1	28,7	40,7	48,4	27,5	27,0	30,0	58,4	33,5	43,6	32,6	34,4	31,7

By analyzing the values of the DMI according to de Martonne's classification of climate and area, it can be concluded that the research area is

characterized by humid and very humid climate (DMI 35-55). With the characteristics of the semihumid climate (DMI 24-28), 2008, 2011 and 2012 stand out, while 2014 is characterized by an extremely humid climate (DMI 58.4).

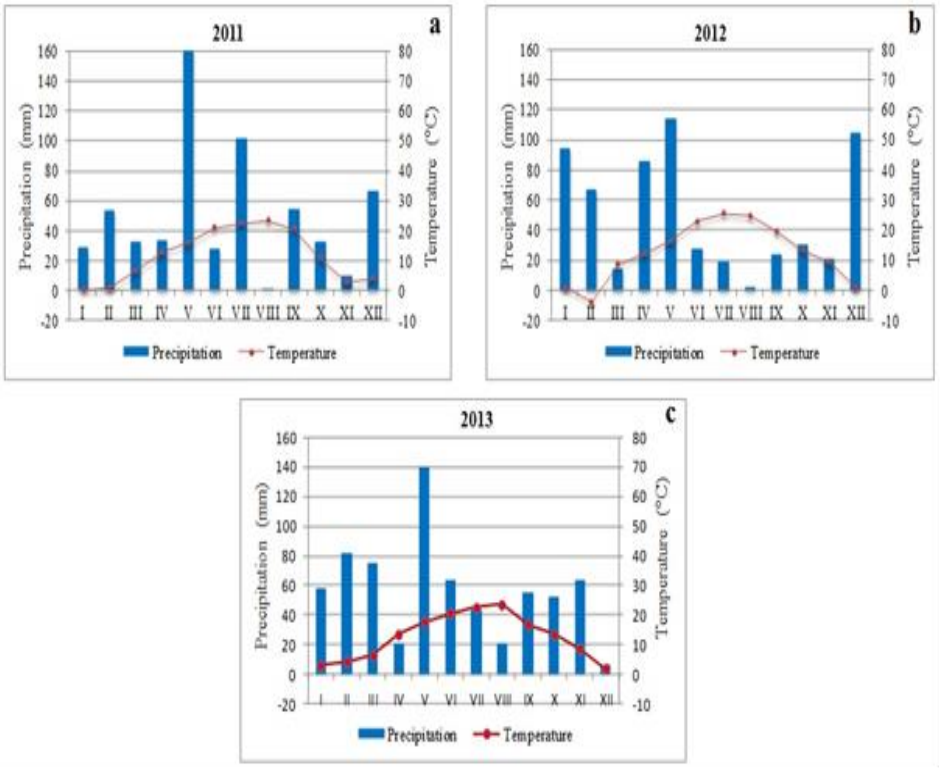

Figure 4. Ratio of annual temperatures and precipitation change during the driest several year period for MS Valjevo. (a) 2011, (b) 2012 and (c) 2013.

In order to confirm the impact of the drought period and its impact on individual trees and forests in general, the state of drought intensity is given based on the SPI during the growing season (SPI-6, Figure 5) and annually (SPI-12, Figure 6).

It can be noticed that this index in the vegetation period shows three consecutive years of intense drought, where 2011 is the driest year. However, considering the humidity conditions on an annual level, it can be noticed that 2011 was by far the driest during the entire research period and goes to the level of extreme drought. Which means that the trees reacted to such a long-lasting drought, which not only affected the vegetation period, when moisture is essential for plants for undisturbed growth and development, but also throughout the year

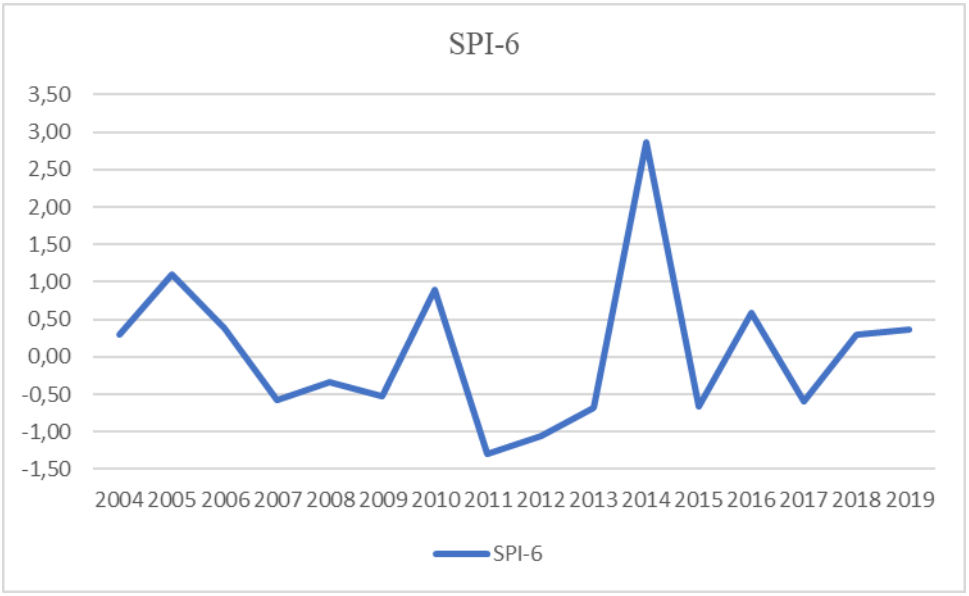

Figure 5. Humidity conditions in the vegetation period

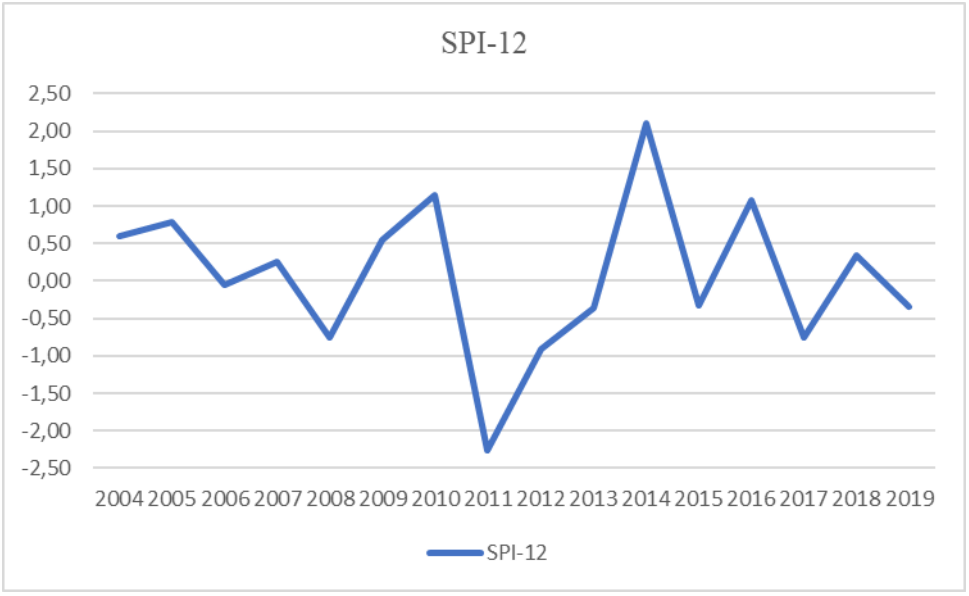

Figure 6. Humidity conditions on an annual basis

CONCLUSIONS

The phenomenon of forest decline exceeds the boundaries of a narrow professional, forestry issue, represents a problem of the broadest social interest and makes it one of the most significant social problems. Solving the problem of

forest decline has specifics due to the diversity of climate, orographic conditions, habitat types, vulnerability by defoliators, tree species and forest ecosystems, as well as inequalities in the degree of industrialization of individual regions, the degree of measures and actions to protect the environment.

Although the research is defined as a case study, after three consecutive drought years (2011-2013), forest decline was found on the entire territory of the Republic of Serbia, so it can be concluded that they are unfavorable climatic conditions were the primary and dominant stress factor. In fir trees, the sensitivity to climatic extremes was higher in relation to beech. More than 65% of the trees of this species were dead at the end of the research period. In the case of beech, the percentage of dead trees was 20%. Also, the drought assessment based on the Lang rain factor (I_L), the de Martonne Drought Index (DMI) and the Walter climate diagram clearly distinguishes the years of drought (2011-2013). These observations were confirmed by the application of SPI both in the vegetation period and throughout the year, after which the drying of individual trees in a researched area begins to be recorded.

By continuous monitoring for a longer period of time, it is possible to determine the cause of decline of a certain tree and bring it into connection with a certain ecological factor. Intensive decline of trees occurs in the dry year, or in the year immediately after the drought, so it is possible to predict this phenomenon based on monitoring climatic parameters, if there is no action of other biotic and abiotic factors that would negatively affect the forest. If the symptoms that appeared on the isolated trees can be diagnosed in the immediate environment, then it is even easier to draw a conclusion about the cause.

The advantage of this research, in relation to other researches that are performed only after the appearance of decline as the final cause of the influence of a this factor, can be seen when these researches find decline of individual trees or larger forest areas in a locality, the cause is that moment is very difficult to determine, because it can be initiated by various factors that have occurred in the past to which some other factors have been added. Therefore, this type of continuous monitoring can accurately determine the initial stages of the impact of a specific factor.

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TYPOLOGY OF RURAL AREAS ON THE TERRITORY OF MRKONJIĆ GRAD MUNICIPALITY

SUMMARY

This paper analyses the typology of rural areas in the municipality of Mrkonjić Grad. Rural areas represent a significant natural resource that needs to be maintained, properly used, and carefully managed, for the benefit of present and future generations. In addition to the previously determined geographical, demographic, traffic, climatic, geological, social, economic, infrastructural, agrarian and ecological characteristics of the area, the focus is on the research and analysis of the typology of rural areas from the standpoint of socio-professional structure and dynamics of demographic changes in Mrkonjić Grad municipality. Considering the great importance, but also the numerous problems that were observed during the research, the question of the future development and progress of the mentioned rural areas is actualized.

Keywords: rural areas, classification, socio-professional structure and demographic change, types of areas

INTRODUCTION

Rural development is largely determined by the available resources and competitiveness of agriculture, but is also a result of geographical position, which has a great influence on vicinity or isolation in relation to the economic centres (Cvijanovic *et al*., 2020; Despotović *et al*., 2016). The transition process has also affected changes in the development of rural areas and their exposure to different physical, ecological, economic and socio-cultural pressures which contravene its value and qualities (Kosanovic *et al*., 2016; Cvijanovic *et al*., 2020). However, not all areas are equally affected by the transition process nor do they have the same basic features of rurality. Rurality is a regional geographical

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characterization that defines the nature of the external economic environment. However, the concept of rurality comprises a heterogeneous group of areas and may be too broad a concept to categorize the economic situation of "non-urban" regions (Laurin *et al*., 2020). There are many classifications that define rural areas such as geographical classification, classification based on indicators of economic activity, classification depending on the degree of integration into the national economy, as well as new concepts in rural typologies and classification which are related to external pressures. By combining different indicators of rural development and their crossing, it is possible to observe specific characteristics of rural communities, on the basis of which they are classified into certain types. One of the starting points is the non-acceptance of the synonym that rural must mean agricultural area (Zakić and Stojanović, 2008), although, in the opposite sense - it certainly is. The key dilemma in the process of allocating rural areas refers to the different understanding in giving importance to the natural, economic, social, cultural and other aspects of rural phenomena. The key criteria for the separation of rural areas can be classified in (Simonović and Ribar, 1993): Demographic, starting from population thresholds; Physiognomic, which start from the degree of closure, ie population density or building density; Functional, given the necessary threshold values of the active population in non-agricultural activities for the settlement to have the status of a city; Legal, by applying legal criteria according to which certain rural areas have the status of a city and Combined, by applying a number of criteria.

Bosnia and Herzegovina is certainly one of the most rural countries in Europe, with between 40 and 60% of the population living in rural areas according to the definition used (UNDP, 2013). In the Republika Srpska, so far, no official division into urban and rural areas has been made using the OECD or some other methodology[2](EC, 2014). If a modified OECD criterion for assessing rurality, population density of 100 inhabitants per square kilometre were applied to the territory of Republika Srpska, it would be obtained that 95% of the territory in RS is rural with 83% of its population. The average population density in Republika Srpska is 60 inhabitants per m2 (Ministry of Agriculture, 2015). All previous activities in the field of rural development in Bosnia and Herzegovina and Republika Srpska deserve attention, but it is still insufficient, given the level of development of rural areas. The activities of rural development must be dealt with not only by those who program and create conditions for development, but also by the entire development population to which development refers (Mirjanic *et al*., 2010).

The aim of this paper is to identify and classify rural areas in the municipality of Mrkonjic Grad based on socio-professional structure and dynamics of demographic change classified as (Bogdanov, 2015; Kayser, 1990): remote rural areas, semi-agrarian rural communities, stagnant rural areas and

[2] As part of the OECD method to identify predominantly urban, intermediate and predominantly rural regions, all local administration units (LAU) with a population density below 150 inhabitants per km2 were classified as rural.

transitional rural regions. Defining the types of rural areas on the example of one local community, would provide a basis for tailor-made innovation policy for lagging regions including economic, social, and political factors for the analysis of regions (Copus, 2001, p. 544)**.**

MATERIAL AND METHODS

Method of comparison of rural areas was applied on the territory of the municipality of Mrkonjić Grad according to the socio-professional structure and dynamics of demographic changes in the studied area. Transitional rural areas are characterized by generally favourable demographic characteristics. Such areas are in the transition phase from agriculture to industry, with a large number of employees outside the primary sector (Bilozor *et al*., 2019; Djanibekov *et al*., 2019). Semi-agrarian rural areas are characterized by a demographic decline, but with large agricultural resources that are actively used, there are also profitable farms that coexist with poor farms of small size and modest resources (Bogdanov, 2015). Remote rural area are areas in which more than half of the inhabited place is facing a large demographic decline, and which relies heavily on agriculture as a source of income (Brezzi *et al*., 2011).

The paper selects three types of rural areas in the municipality of Mrkonjić Grad, namely Podrašnica as transitional rural area, Donji Baraći as semi-agrarian rural area and Ubovića Brdo as remote rural area. All three types of rural areas differ in their geographical, traffic, social, demographic, tourist and other characteristics. This method serves us to compare the mentioned rural areas and show their differences.

The survey method was conducted in three settlements. The method was performed using a semi-structured questionnaire. The questionnaire contained 16 questions, open and closed, on the basis of which the results of the current situation and the situation in the 90s in the mentioned rural areas were obtained, which refer to the criteria of socio-professional structure and dynamics of demographic changes. The households in which the survey was conducted were selected by the snowball method (Goodman, 1961), so that one household recommends another household and so on until a survey of a given number of respondents is performed. The sample was selected based on the size of the population or the number of households, 20% in relation to the total number of households in the settlement of Podrašnica, 30% in relation to the total number of households in Donji Baraći and 50% of the total number of households in Ubovića Brdo. In total 300 households were surveyed.

Historical method was also applied. The time determinant is the situation today and the situation in the 90s, i.e. just before the events that had a drastic impact on the demographic, economic and social picture of the Municipality as a whole and even different types of rural areas.

RESULTS AND DISCUSSION

The municipality of Mrkonjić Grad belongs to the middle-developed municipalities, it is located in the south-western part of Republika Srpska, and in the western part of Bosnia and Herzegovina (Spatial plan of Municipality of

Mrkonjic Grad, 2016-2036). The area of the municipality of Mrkonjić Grad is located in the southern part of the Banja Luka region. In the last few years, as a consequence of the civil war, there have been sudden changes in the structure of the population on the territory of the municipality of Mrkonjić Grad. The events of the war initiated the processes of forced migration and displacement of the population. Population migrations resulted in changes in age, economic, gender and other structures, which directly affected the economy and further development of the municipality. Analysing the existing network in the system of settlements, a large degree of concentration of population and activities in the municipal centre Mrkonjić Grad and settlements around Mrkonjić Grad was noticed, while in the northern, western and south-western part of the municipality it is much rarer. The network of settlements can be defined as monocentric resulting from pronounced dominance of Mrkonjić Grad, the only settlement of urban character. Apart from Mrkonjić Grad, which has a dominant role as a municipal centre, a number of settlements appear, whose development and position in the area of the municipality indicate the formation of secondary centres (village community centres) in the hierarchy of settlements. Each of the three rural areas covered by the research belongs to a certain type of settlement classified as: remote rural areas, semi-agrarian rural communities and transitional rural regions.

Demographically, all three types of areas are characterized by a pronounced obsolescence of the population, which in remote rural areas leads to the extinction of the population in these areas. These areas have lost over 60% of the population in the observed period, and currently do not have any inhabitants belonging to the younger category of the population under 19. Depopulation of rural areas has been confirmed as a process that occurs through most counties in rural America (Johnson and Lichter., 2019) and in number of research papers across EU and non-EU countries, Italy, Spain, Sweden, Montenegro and other (Osti, 2010; Paniagua, 2017; Syssner J., 2020; Rodríguez *et al*., 2021; Mickovic *et al*., 2020)

Table 1: Change in total population between two Census years, per type of the area

Type of the rural area	1991 Census	2013 Census	Difference
Remote rural areas	213	81	61,97%
Semi agrarian rural areas	525	287	45,33%
Transitional rural areas	1096	733	33,12%

Source: Republic of Srpska Institute of Statistic

The aging index is over 40 in all three types of the regions, which is considered the situation when the population has entered the phase of pronounced aging. The largest demographic decline is in deeply rural areas where the participation of those under 19 has dropped to zero. The dependency index, which indicates the ratio of dependent categories (old and young) in relation to

the working active population, also shows unfavorable characteristics, i.e. that there are over 100 dependent people per 100 working able people, and over 300 in deeply rural areas. For remote rural areas it means the threat of demographic extinction. The pronounced aging process in rural areas is observed and confirmed by numerous research across Europe and the World. Pantic and Miljkovic in their research on regional differences between rural areas of Serbia also concludes the problem od depopulation and population aging as a dominant demographic issues (Pantic and Zivanovic Miljkovic, 2010).

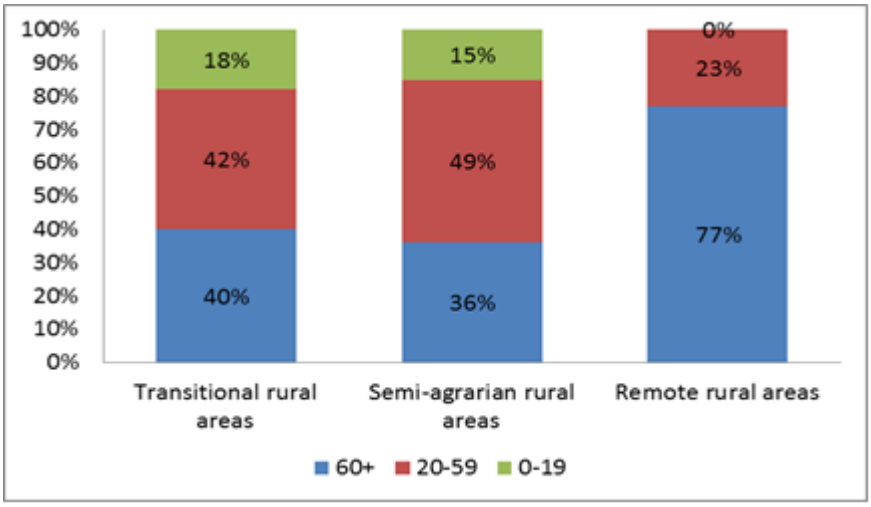

Figure 1: Age structure of rural household members, per type of the rural area, 2019 (Source: results of the survey)

Table 2: Changes in dependency ratio 2020 and 1991 Census

	Aged Dependency Ratio[3]		Dependency ratio[4]	
	1991	2020	1991	2020
Transitional rural areas	100,00	220,00	114,28	139,13
Semi agrarian rural areas	36,36	240,00	75,00	106,25
Remote rural areas	28,57	333,33	100,00	333,33

(Source: authors own calculation based on surveyed data)

The size of the rural household also shows a trend of population obsolescence. Namely, in contrast to the situation recorded in 1991, when there were the most numerous households with 4 or more members in all types of

[3] (number of people aged 65+) / (number of people aged 15-64) *100
[4] (number of people aged 0-14 + number of people aged 65+) /(number of people aged 15-64) *100

areas, today such households are most numerous only in semi-agrarian areas, while in remote rural areas the most numerous are two-member households, and in transitional areas the household with three members. Smaller the household, more affected it is in social and economic terms. There is a range of research on different needs and challenges in accessing public services for certain types of households: couples and single people, working-age and pensioners, and households with and without children (Smith *et al*., 2012) as well as size-related farms poverty problems (Smith *et al*., 2012, Petrović and Milić, 2015, Grujić *et al*., 2014).

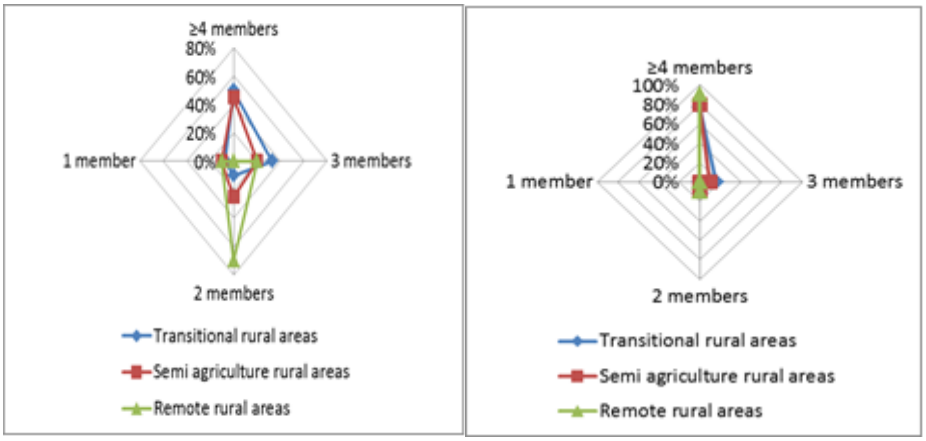

Figure 2: Changes in size of the household per type of the rural area, nowadays a) and 1991 b) (Source: authors own research)

The working status of household members is determined by changes in the demographic structure, so the most significant difference compared to the period before the 90s is in the remote rural area where the share of pensioners increased from 15% to 53%, while the agricultural population disappeared as well as the temporary residence population working abroad. It is also interesting that in these areas, agriculture activity on others farm is not present nowadays, and a new source of income was introduced, and this is unearned income such as rents, dividends or donations.

In the semi-agrarian rural community, it is interesting to note that sources of income in the non-agricultural sector were and remain dominant in total sources of income, which could even classify these areas as transitional rural areas, but other indicators don't prove such categorization. Enterprenuership activity in these areas, however, is not developed in the non-agricultural sector, and the importance of agriculture is unchanged.

For transitional rural areas, a drastic decline in the importance of agricultural in relation to non-agricultural activities can be observed. The development of entrepreneurship is also noticeable, i.e. the number of private own companies in the non-agricultural sector.

Other research also confirms the tendency of rural households to diversify their income and thus provide a useful strategy in terms of managing disaster risk and improving social welfare but may also offer a new perspective for the research of vulnerability, resilience, and adaptive ability of rural social ecosystem. (Jinhong *et al*. 2016, Zrakić *et al*. 2019).

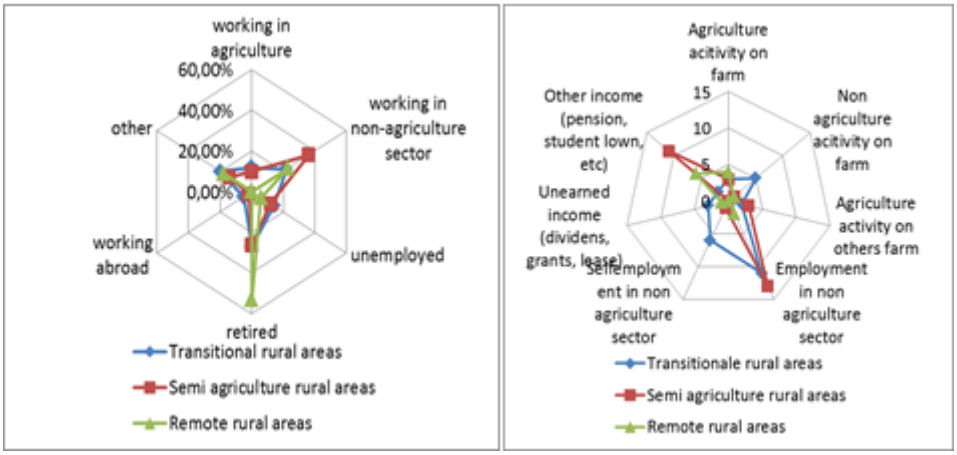

Figure 3: Working status a) and main sources of income of rural households b), per type of rural area (Source: authors own research)

Tourism activity on the farm as a form of income diversification is not developed, although all areas have the potential for tourism development. The infrastructure of the area has a potential for further development of both tourism and other entrepreneurial activities. According to Desić (2012), the basic problem of untapped potentials in rural tourism in Bosnia and Hercegovina is poor, and in some places non-existent infrastructure, low environmental and tourist awareness, both among the population and local authorities to solve problems and improve rural tourism. The same author believes that there is a lack of appropriate administrative and professional organization and training of staff, people are not educated to make the most of the potential they have, and that there is also a lack of developed information system, financial mechanisms, education, research and professional institutions, legislation and many other factors. Sehic-Krslak (Sehic-Krslak, 2018) states that the biggest problem faced by individuals who contribute to the development of rural tourism is primarily the legal framework that in Bosnia and Hercegovina is still not sufficiently defined for this area.

Dominant production system is in providing self-sufficiency, regardless of the type of area. A small part of the produced market surpluses of households are primarily sold directly on farm, while sales on the local or regional market are almost completely lost. According to the views of the respondents limiting factors for a higher level of resource use are insufficient level of farm investment, high fuel prices, lack of labour force, low purchase prices of their products. For the

inhabitants of deeply rural areas, the limiting factors are first of all age and illness, then poor infrastructure and lack of labour and mechanization.

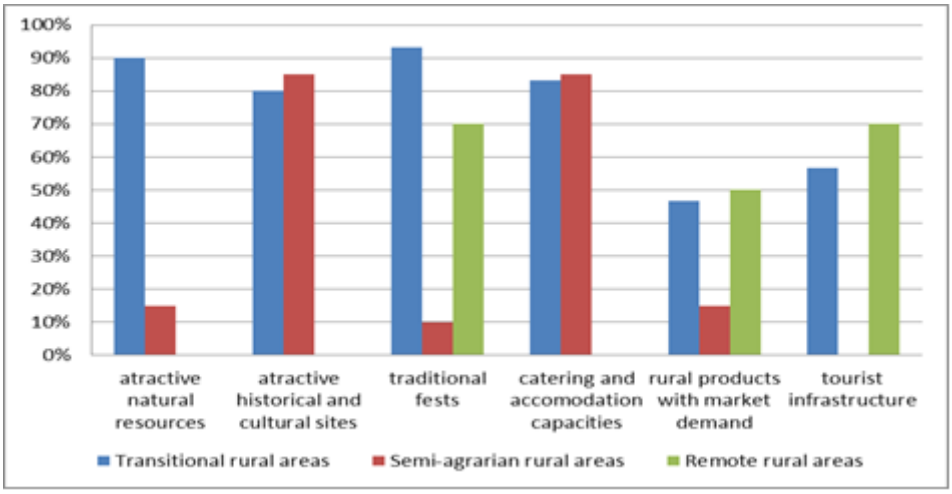

Figure 4: The most important tourist resources per type of rural area (Source: authors own research)

Table 3: Access to rural infrastructure and public services, per type of rural area (Source: authors own research)

	Transitional rural areas		Semi agarian rural areas		Remote rural areas	
	today	1990	today	1990	today	1990
Water supply	+	+	+	+	+	
Electricity	+	+	+	+	+	+
Internet and cable network						
Lighting	+		+			
Sewage	+	+	+	+		
Garbage collection	+		+			
Public transport	+	+	+	+		
Primary school	+	+	+	+		+
Ambulance	+	+	+	+		
Facilities for cultural and sports facilities	+		+	+		
Local shop	+	+	+	+	+	+
Local market				+		

This is the picture with all less developed countries in Europe, which is confirmed by studies by the European Parliament (2013): The relative rural poverty in some member states, and the hardship stemming from the economic recession, are factors contributing to the relative stability and in some cases the

proliferation of self-suficient farms. The main economic role of self suficient farms is a welfare one, alleviating poverty by acting as a "social buffer" for households with few other assets and minimal other income sources. In five major member states, subsistence production contributes between 20% and 50% to the incomes of households at the risk of poverty. In the current recession, this welfare role has also been noted in Italy, Greece and Portugal.

In all three types of rural areas that are the subject of research, a significant improvments are noticed in the access to infrastructural facilities today and in comparison, to the period of the 90s. However, there are significant differences between different types of areas in infrastructure development (Table 3).

Infrastructure development according to the results of other research is determined by various factors, the degree of development of the country as a whole (GDP value), population density, urban versus rural area, and then depending on the typology of the rural area (Steckel *et al*., 2017). On the other hand, the package of basic infrastructure today, apart from water, electricity, sewage, etc. has shifted to personal computer ownership and accessibility to the Internet. As a result, information infrastructure is becoming one of the factor endowments that determines the competitive advantage of rural areas (Fox and S Porca, 2001).

CONCLUSIONS

The research results show a different level of economic activity in relation to the type of rural area, different levels of resource use, as well as different structure of available resources. There are also differences in the participation of primary activities (agriculture and forestry) in relation to sources of income from the non-agricultural sector. Agriculture as a source of employment in all rural areas is losing importance, and forestry and non-agricultural activities are taking precedence. Market channels for the placement of agricultural products are underdeveloped in all areas, and the share of direct sales on the farm is the dominant form of product placement. High dependence in the form of a relationship between the dependent and the working population leads to a low economic standard, a decline in the quality of life and weak motivation for further work and development. In remote rural areas, in terms of living conditions, lack of infrastructure and public services, there are no conditions for demographic renewal, while in other types of areas, demographic and population policy measures can still have an effect if applied in a short period of time. Compared to the period before the 1990s, we can say that the rural area of Mrkonjić Grad has been underdeveloped in the last 30 years, and that apart from investing in infrastructure, there are no examples of good practices of village modernization, income diversification, protection of rural heritage and other measures for development of rural areas. Urban areas have become saturated with migrations from remote rural areas wich leads to increasing unemployment rate in suburban areas.

Tourism activity on the farm as a form of income diversification is not developed, although all areas have the potential for tourism development. The infrastructural equipment of the area is good for further development of both tourism and other entrepreneurial activities.

The solution for further economic development of these areas is in adapting interventions and development programs to different types of areas, where transitional areas should be directed towards the development of entrepreneurship activities, self-employment and new employment. Semi-agrarian communities require modernization of agricultural production and marketing, and remote rural areas are in need of outside intervention activities towards activation of resources either by investing in tourism, forestry or some other activity.

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BASIC PARAMETERS OF ANGULARITY AND BIOSTATIC MODEL OF THE BOSNIAN BROKEN-HAIRED HOUND – BARAK

SUMMARY

Bosnian broken-haired hound called Barak belongs to a large group of hound dogs. Barak is indigenous breed of hound in Bosnia and Herzegovina, and it is widespread among hunters as working hunting dog. In order for the dog to act appropriately while standing or moving, its body needs to be built in such way to enable appropriate response to the impact of various external forces. All parts of dog`s body stands in certain correlation that are specific to each group of dogs. Besides the the size of individual parts of extremities, or the body itself, the angles between the bones in joints are extremely important.

The aim of this paper was to determine the basic parameters of angularity of Bosnian broken-haired hound – Barak by conducting zootechnical measurements. The study involved 120 dogs, including 64 males and 56 females, aged from 9 months to 10 years, from entire territory of Bosnia and Herzegovina. The total of 6 parameters of angularity were measured on each dog. This is the first analysis of the angularity for the Bosnian broken-haired hound Barak, and the results can be used for further detailed description of this breed, as FCI standard for Barak does not describe any angle except for shoulder and pastern.

The results indicates almost equal values of angles in both males and females, and except for the angles of shoulder and croup, no statistical significance was observed in other angles between the genders.

Keywords: angularity, Barak, hound, movement

INTRODUCTION

Bosnia and Herzegovina, and thus Republic of Srpska, is considered as area of great biodiversity and characterized by large number of indigenous breeds

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(Nikitovic *et al*., 2015). Bosnian broken-haired hound called Barak is indigenous breed of hound in Bosnia and Herzegovina, and it is widespread among hunters as working hunting dog. Along with Barak, a large number of hounds used for hunting in Bosnia and Herzegovina belongs to breeds Serbian Hound, Serbian Tricolour Hound and Posavatz Hound (Nikitovic *et al*., 2020). The body of Barak, as a working hound dog, should meet the basic measures and principles that applied to this group of dogs. Official FCI Standard describes that Barak has rectangular body which length should be 10% longer than height at withers (FCI, 2019). As for angles, the standard suggests that angle of shoulder joint should be 90°, and pastern should form the angle up to 10° with a vertical, while there are no values in the standard for other angles of joints, neither for forelegs nor hind legs.

In order for the dog to act appropriately while standing or moving, its body needs to be built in such way to enable appropriate, positive response to the impact of various forces from the external environment. The body is able to, by contractions of the muscles, produce biokinetic energy which, with appropriate transmission system, enables certain parts of body to move. The movement of the body, or its parts, is achieved by the mutual action of muscular system and adequate response from the skeleton. The dog`s body must be built to stand in balance. In order to maintain that balance, it is necessary that resultant of all forces, which act on the body at any moment, should equal to zero (Nikolic and Hudec, 1988).

The body remains in balance, during rest or movement, only if the biostatic principles of the body structure are met. All parts of dog`s body stands in certain correlation that are specific to each group of dogs. Besides the the size of individual parts of extremities, or the body itself, the angles between the bones in joints are very important. The typical movement of hound is trot, which enables the longest movement with minimum spend of biokinetic energy. Hind legs are the producers of biokinetic energy that enables the dog to move forward. In this system, the knee joint has very important role, and therefore must be adequately angled. Moving by trot implies synchronised movement of forelgs and hind legs in rhythm: hind right – front left, hind left – front right (Urosevic and Drobnjak 2018). Angularity of the forelegs must be adequate as well, since the forelegs enable the maximum use of kinetic energy produced by hind legs.

The hind legs signicantly contributes and affects the balance of the body. Certain components of the hind legs must be in the appropriate proportion. The longer the femur, the longer are the muscles attached to it, and they provide more biokinetic energy that moves the body forward. If the angle of the hock, and therefore the angle of pastern, is more closed, the hind legs are more under the body. This possition is not good for the movement proccess and increases the pressure on the hock. The movement is not harmonious (Urosevic and Drobnjak, 2018).

With a good biostatic model, the length of the forelegs steps are equal to the steps made by hind legs. With good angularity, the height reached by forelegs

is equal to height reached by hind legs. In the case of open angles on the forelegs and hind legs, the length of the steps is reduced, and thus the movement is slow and difficult (Urosevic and Drobnjak, 2018).

The aim of this paper was to determine the six basic parameters of angularity of Bosnian broken-haired hound – Barak, as there are no official standard values for the joint angles, but only for the bones position.

MATERIAL AND METHODS

The research involved 120 dogs, including 64 males and 56 females, from entire territory of Bosnia and Herzegovina. All dogs were older than 9 months, and this age is considered in the cynology to be the youngest when dogs can be judged as the adults for the measurement of exterior parameters. The oldest dog was 10 years old.

The angles of shoulder joint and elbow joint were measured on the forelegs, while the angles of knee joint and hock joint were measured on the hind legs. Additionaly, the angles of neck and croup were also measured on each dog. Values of the measured angles were determined using protractor.

The obtained results were processed using the software *Statistical Package for the Social Sciences (SPSS) for Windows Release 17.0.0*. Descriptive statistic values were calculated: coefficient of variation (CV), standard error (SE), standard deviation (SD), minimum value (MIN), maximum value (MAX) and mean value ($\bar{x}$). Using the T test, statistical differences were calculated for each of the measured parameters between the genders. As statistically significant were taken differences $P < 0.05$.

RESULTS AND DISCUSSION

Values of angularity parameters in examined Barak dogs are shown in Table 1. The angle of the shoulder joint in males ranged from 90° to 125°. The average value of the male`s shoulder joint was 109.64°. The standard deviation (SD) was 8.13, while the coefficient of variation (CV) was 7.42%. In females, the minimum of shoulder joint angle was 92°, and it was measured in only one individual, while the maximum value of 120° was measured in two females. The mean value of this parameter in females was 106.23° with a standard deviation (SD) of 7.75. There is statistical difference ($P < 0.05$) between the genders for this parameter.

The angle of the elbow joint in males' averages at 128.23° with standard deviation of 7.81. Variation interval ranges from 110° to 145°. One dog has the minimum value of this angle, and also one dog has the maximum value. The coefficient of variation was 6.10%. In females, a large interval of variation was noticed in the elbow joint angle and ranged from 100° to 149°. Most females, 11 of them, had the value of this angle that amounts 135°. The mean value of females` elbow joint was 128.39°, with standard deviation of 11.62. No statistical difference between the genders ($P > 0.05$) was found for this exterior parameter.

Table 1. Values of angularity parameters in examined Barak dogs

Parameter	Gender	N	Min	Max	CV	SE	$\bar{x}$ ± SD	t
Shoulder joint angle	♂	64	90.00	125.00	7.42	1.01	109.64±8.13	2.339*
	♀	56	92.00	120.00	7.30	1.03	106.23±7.75	
Elbow joint angle	♂	64	110.00	145.00	6.10	0.97	128.23±7.81	0.692ns
	♀	56	100.00	149.00	9.05	1.55	128.39±11.62	
Knee joint angle	♂	64	92.00	145.00	7.70	1.10	114.75±8.83	0.334ns
	♀	56	95.00	139.00	9.18	1.40	114.16±10.48	
Hock joint angle	♂	64	110.00	155.00	7.74	1.29	133.59±10.34	0.533ns
	♀	56	107.00	155.00	6.78	1.20	132.64±8.99	
Neck angle	♂	64	15.00	55.00	32.33	1.31	32.44±10.49	1.512ns
	♀	56	15.00	55.00	30.98	1.22	29.70±9.19	
Croup angle	♂	64	20.00	53.00	20.96	0.94	35.91±7.52	2.086*
	♀	56	20.00	49.00	20.82	0.92	33.14±6.90	

In the examined population of males, the knee joint angle ranged from 92° to 145°, with coefficient of variation of 7.70%. The mean value was 114.75° with standard deviation of 8.83. The largest number of male dogs (68.76%) has the knee joint angle in the interval between 110° and 120°. The mean value of this parameter in females was 114.16°, with standard deviation of 10.48. Interval of variation ranged from 95° to 139°. Both minimum and maximum values were recorded in two female dogs. No statistical difference between the genders ($P > 0.05$) was found for this parameter.

The mean value of hock joint angle in males was 133.59°. The minimum value of 110° was recorded in one male dog, while the maximum value of 155° was recorded in two male dogs. The coefficient of variation was 7.74% and standard deviation (SD) was 10.34. In females, both minimum (107°) and maximum values (155°) were recorded in one dog. Most females had the angle of hock joint at 130°, while the mean value was 132.64°, with standard deviation of 8.99. No statistical difference between the genders ($P > 0.05$) was found for this exterior parameter.

When it comes to the angle of the neck, a large interval of variation is noticable. Both males and females has minimum value of 15°, and maximum value of 55°. Males has mean value of 32.44°, with standard deviation of 10.49. Mean value in females was 29.70°, with standard deviation of 9.19. No statistical difference between the genders ($P > 0.05$) was found for this exterior parameter.

As for the angle of the croup, interval of variation in males ranged from 20° to 53°. The mean value for this angle was 35.91°, with a standard deviation of

7.52. Based on the obtained results, it can be concluded that there are dogs with straight croup, which is desirable feature, and also dogs with lowered or steep croup, which is not a good characteristic for the hound. In females, the mean value of the croup angle was 33.14°, with standard deviation of 6.90 and coefficient of variation of 20.82%. For this exterior parameter, it was determined that there is a statistical difference ($P < 0.05$) between the genders.

Angularity of the legs, as well as position of the neck and croup, is very important for the hounds, in terms of adequate movement and accomplishment of work tasks. The length of individual parts of the forelegs and hind legs must be balanced, which means they must be approximately the same, in order for dogs to move properly. Also, there must be the harmony between the angles, which means that shoulder joint angle should be approximately equal to hip angle, and the elbow joint angle should correspond to knee joint angle. If the value of the shoulder angle increases (becomes more open), the ability of the forelegs to step out decreases (Urosevic and Drobnjak, 2018).

In the examined population of Barak dogs, the elbow angle in males averages at 128.23°, while the knee angle averages at 114.75°, which means the difference between the angles is bigger than desirable. The same situation is in females, where corresponding angles of elbow and knee averages at 128.39° and 114.16° respectively. It is noticable that the values of the angles are almost the same in males and females, and except for the angle of shoulder joint, no statistical significance was observed in other angles between the genders.

The FCI standard for Bosnian broken-haired hound called Barak suggests that angle of shoulder joint should be 90°. It is noticeable that the mean value of this parameter is higher than standard by 19.60° in males, and 16.20° in females. The minimum values of this angle are within the values prescribed by the standard for both males and females.

The angles of the neck and croup in relation to the horizontal plane are also very important (Nikitovic *et al.*, 2020). The angle of the neck averages at 32.44° in males, and 29.70° in females. The croup angle averages at 35.91° and 33.14° in males and females respectively.

Since there is no research of angularity in other breeds of hounds from the Balkans, adequate comparison is not possible. The shepherd dog Tornjak is the closest breed to Barak that is also autochthonous breed in Bosnia and Herzegovina (Nikitovic, 2020). Since both breeds originated and developed in the same area, it would be interesting to compare the angles of these breeds, though not belonging to the same group of dogs. The mean value of the Tornjak`s neck angle is 23.50°, while the shoulder angle averages at 122.75°, and elbow angle at 149.62°. Average knee angle is 121.00°, hock joint angle is 141.50°, and croup angle 32.00°. The mean value of the Tornjak females` neck angle is 25.71°, shoulder angle averages at 118.86°, and elbow angle at 142.57°. When it comes to the hind legs in females, the average value of the knee angle is 124.57° and hock joint angle is 138.00°. The females` croup are bent at average angle of 33.00° (Urosevic *et al*., 2014).

Comparing the results of examined population of Barak and corresponding research for Tornjak in Bosnia and Herzegovina, we can see that all measured angles of Barak dogs is smaller than Tornjak`s, for both males and females, except for the neck angle. When it comes to the croup angle, the Barak has slightly lower croup, which means the angle is higher than Tornjak, while in females, there is almost no difference between the two breeds for this parameter.

Although both breeds originated and developed in the same area and under approximately the same ambient conditions, there are large differences in angularity, the forelegs and neck in particular. This difference in angularity results in different biostatic model between these two breeds, as well as different ways of how they work.

CONCLUSIONS

Bosnian broken-haired hound called Barak belogs to the group of hounds, with a rectangular body shape. Important exterior indicators of hound dogs include the size and ratio of joint angles, though not the common method of measurement. This is the first analysis of the angularity for the Bosnian broken-haired hound Barak, and the results can be used for further detailed description of this breed.

As for the results, we can conclude from this research that the angle of the shoulder is higher than the value prescribed by FCI standard, in both males and females. A quite large interval of variation is noticable in females` elbow joint angle, as well as for neck angle in both males and females. No statistical differance was found in angles between the genders, except for shoulder joint angle and croup angle. This research shows us a great heterogeneity in the examined population.

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PRODUCTION OF PLANTING MATERIAL OF RASPBERRY VARIETY "GLEN AMPLE" IN THE NORTH MONTENEGRO

SUMMARY

The objective of the paper is to present the method of production, quantity, and quality of obtained planting material per unit area of continental fruit within the period of three years (2018-2020), and, in particular, the nursery of raspberry variety "Glen Ample" in Polimlje, Bijelo Polje municipality, northern Montenegro. The paper shows the initial condition, preparation for the formation of nurseries, production, and quality of raspberry seedlings, which are among the most important berry fruit species grown in northern Montenegro. The production of nurseries was within the limits for the production of raspberry planting material. The number of plants per square metre (m^2) at the end of the season was 20.2, and 202,000 per hectare (ha). The height of the seedlings at the end of the vegetation was 1.06 m, and the diameter above the root neck was 9.5 mm. The planting material met the prescribed standards. Based on the standard, seedlings were classified into three classes, and most seedlings of class I (73%) were obtained, followed by class II (24%), while only 3% of seedlings were out of class. Raising raspberry plantations with quality planting material is one of the most important factors for intensifying the production of this type of fruit in Montenegro.

Keywords: planting material, *Rubus idaeus* L., fruit growing, Montenegro.

INTRODUCTION

Raspberry (*Rubus idaeus* L.) is one of the most important berry fruit types in Montenegro (Jovancevic, 1970). Among fruit trees, it has been ranked at the 24th place in terms of production in the world, so that, after strawberries and blackcurrant, it is the most important berry fruit tree (Nikolić and Milivojević, 2010). Raspberry is a fruit of the northern hemisphere. The largest gene centres of

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this plant are located in Asia and North America (Mišić, 1998). The first varieties of raspberries were created at the beginning of the 17th century. They were formed by somatic mutations, selection, natural and planned crossing of wild species. It is known that there have been more than 1100 varieties of raspberries of different biological and production characteristics (Petrović and Milošević, 2002). Regardless of the fact that there have been a large number of varieties of raspberries, we have still been working on its breeding to create new varieties with improved production characteristics (Petrovic and Leposavić, 2009). According to Mišić (1998), the main goals of raspberry breeding are to create new, better varieties (more fertile, larger, firmer fruits), resistant to unfavourable abiotic environmental factors and pathogens, as well as to create varieties of good quality fruits, suitable for mechanized harvesting and freezing.

The production of healthy and quality planting material is the basis of successful, modern nursery production (Lučić *et al*., 1996; Nikolić *et al*., 2006, Ivić and Fazinić, 2011). One of the factors for achieving high yields and good quality of raspberry fruits is the use of quality, varietal and healthy planting material. Raspberries are propagated in the following ways: 1) from rootstocks, 2) by root cuttings and 3) by tissue culture ("*in vitro*"). The rootstocks should be formed in an open field that is spatially isolated from other rootstocks or native raspberry plantations. The advantage of using root cuttings is the accelerated multiplication of newly created varieties. Root cuttings are cut so that they have developed at least one underground bud, and 2-3 buds are optimal. Cuttings produced in this way should have well-developed veins, with at least 3-4 mm in diameter.

The most modern and safest way of producing raspberry planting material is "*in vitro*" propagation (tissue culture) performed in laboratory conditions which results in virus-free, healthy and varietal pure planting material of high gender potential (Petrović and Leposavić, 2016). In practice, raspberries are propagated *en masse* with mature and green shoots. It is not recommended to use raspberry shoots from native plantations for raising new plantations, which is prohibited by the law on seeds and planting material. Such way of production and use of shoots can lead to several dangers such as the spread of fungal diseases and viruses and multiplication of clones (seedlings) that have lost the positive characteristics of the variety and have become germinating from the seeds of fallen fruits.

For these reasons, only raspberry planting material produced in a modern way, which is under the professional and health control of authorized persons by the competent Ministry of Agriculture (in Montenegro), should be used for raising new plantations. The highest quality raspberry shoots are obtained from rootstocks that are raised from varietal pure and healthy planting material, in separate and closed spaces (Lolić, 2018).

The introduction of new varieties and the use of healthy planting material is the key to the rapid and proper development of fruit growing. Therefore, this study aimed to examine the possibility of producing planting material of raspberry cultivar "Glen Ample" in agro ecological conditions of northern Montenegro.

MATERIAL AND METHODS

The location of the studied area of Loznice (Municipality of Bijelo Polje-Montenegro) is presented in the Figure 1.

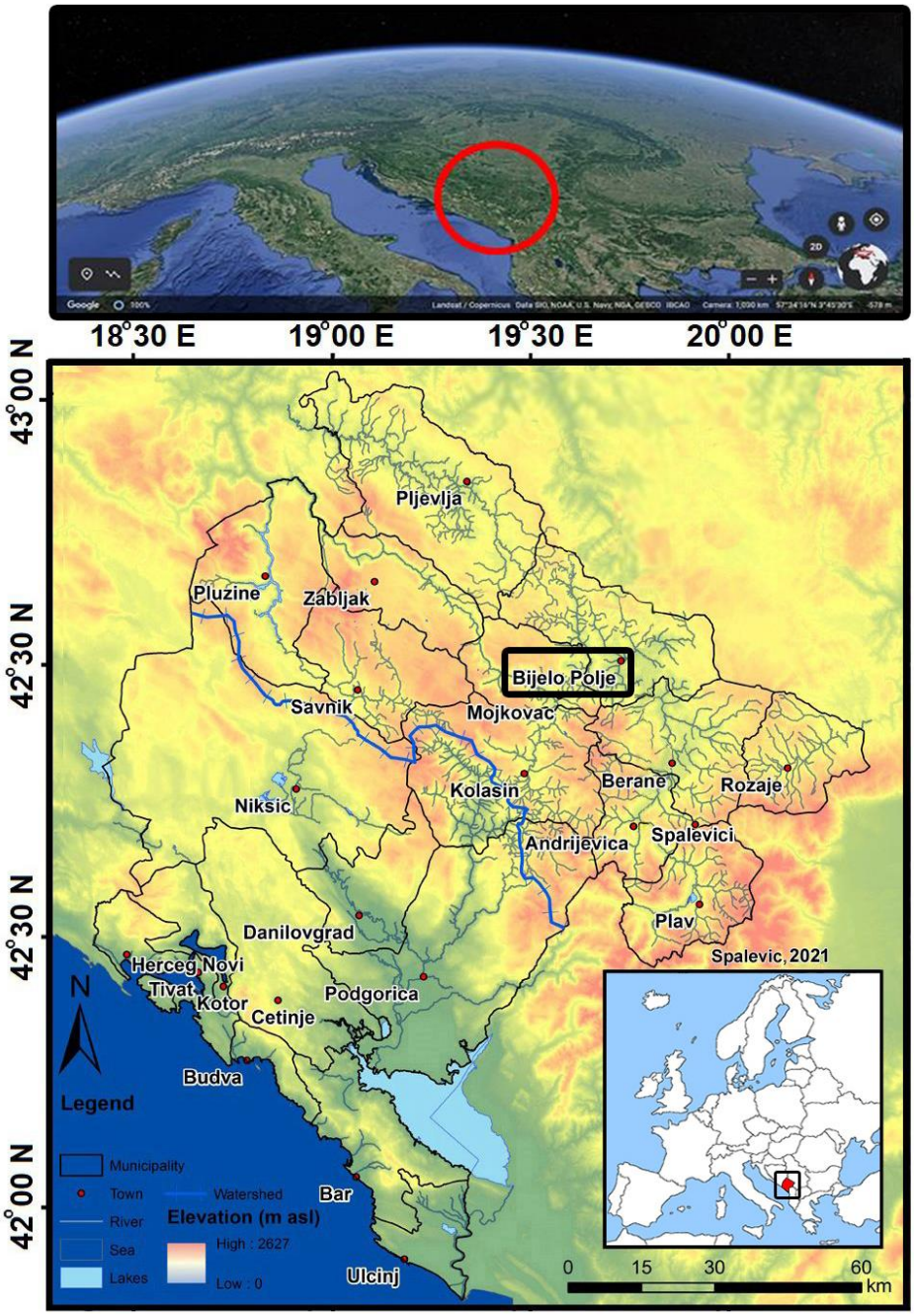

Figure 1: Location of the study area of Loznice, Bijelo Polje, North Montenegro

The municipality of Bijelo Polje, located in the north Montenegro along the main road and railway Belgrade-Bar, has been set at the position of 43.04° N 19.75° E. It has been framed on the south by the mountain Bjelasica and on the north by Lisa at the area of 924 km^2. The climate is moderate continental, since it has been located at the valley-mountainous area with very favorable conditions for the development of many branches of agriculture and tourism (https://www.bijelopolje.co.me).

Raspberry nursery was formed on an area of 0.5 ha, where the previous crop was a perennial meadow with the brown acid soil (*District Cambisol*), plots with a slight slope of about 3%, located at an altitude of 650 m a.s.l. in the village of Loznice, 43°02'08.8"N, 19°45'47.3"E (Figure 1-4).

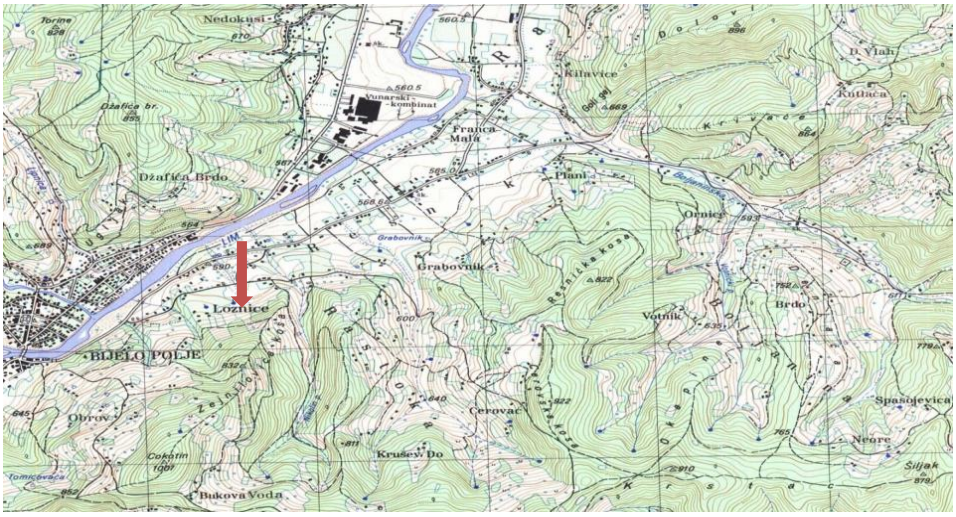

Figure 2: Location of the Raspberry nursery, Loznica, Bijelo Polje, Montenegro (Source: Topographic map 1: 25000, Bijelo Polje-East: 131-4-3, JNA, 1980)

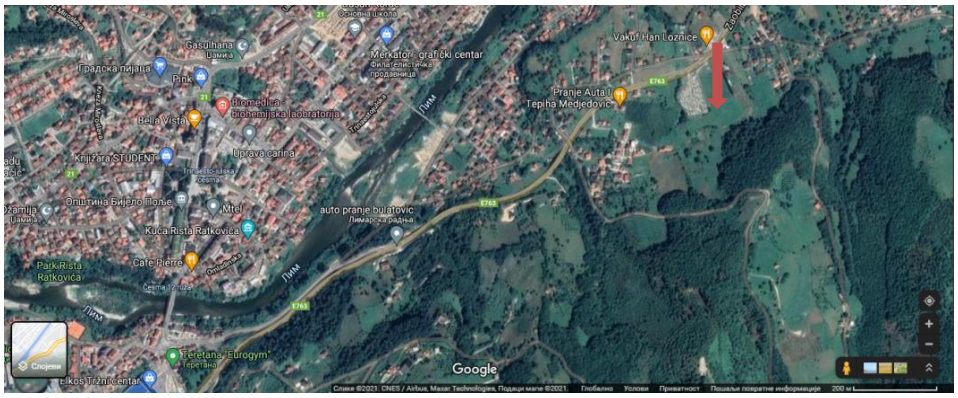

Figure 3: Nursery in Loznice, Bijelo Polje, (Source: Google earth, June 2021)

Figure 4: Nursery in Loznice, Bijelo Polje, (Source: Google earth, June 2021)

Preparatory works were performed during 2016-2017, and the organized process of production of "certified" planting material was carried out by the Private Institution Biotechnical Center, Bijelo Polje, following the Law on Planting Material (*Official Gazette of the Republic of Montenegro* N^{o}28/6, N^{o}73, N^{o}40/11, N^{o} 61/11, and 48/15) and constant control for food safety, veterinary and phytosanitary services from Podgorica and Phytosanitary Laboratories of the Biotechnical Faculty of the University of Montenegro. At the end of each of these years, the seedlings received the necessary phytosanitary documentation, certificate, label, and plant passport, and then marketed locally and internationally, which proves that the process was set up correctly and had the goal achieved, which is a healthy and well-developed seedling of raspberries.

Glen Ample is from Scotland (1994, SCRI - Scottish Crop Research Institute, Dundee). The varieties "Glen Prosen" and "Meeker" took part in their creation, and the hybrid is created from the same parents as the variety "Glen Rosa". It has been very common in the United Kingdom (Knight & Fernandez, 2008), and has been rapidly spreading to other European countries. The shoots are moderately lush and smooth, with very few thorns. It has high and stable fertility. The fruits have a very nice exposure on medium to long fruiting branches (Figures 5 and 6) with good quality. It is suitable for growing both in the open field and in various types of protected and semi-protected spaces. The primary purpose of the fruits of this variety is fresh consumption, but also for various types of processing. It has been moderately resistant to the most significant diseases, (Petrović *et al*., 2020).

The variety "Glen Ample" has been registered in Montenegro since November 2016, (Decision N^{o} 060-320 / 14-0422-1123 of 28.11.2016, license holder from Scotland James Hutton Limited, and authorized propagandist PU Biotechnical Center from Bijelo Polje, in Montenegro, agreed on the multiplication of the variety on December 2, 2016, when the legal conditions were met for it to be grown for the first time in a nursery in Montenegro).

Figure 5: Raspberry “Glen Ample” fruiting twig

Figure 6: Raspberry "Glen Ample" fruit

The precise assessment of the suitability of the site for the construction of the raspberry nursery was performed during the construction of the nursery.

Agrochemical analysis of soil samples was carried out at two depths of 0-30 cm and 0-50 cm before the establishment of nurseries in the laboratory of the Center for Soil Studies and Land Reclamation of the Biotechnical Faculty, University of Montenegro, Podgorica (Table 1).

Table. 1. Agrochemical analysis of soil samples from the site "Loznice I"

Depth (cm)	pH u H_2O	pH u KCL	$CaCO_3$ (%)	IDG (%)	Humus (%)	Available P_2O_5 (mg/100g)	Available K_2O (mg/100g)
0-30	4,78	4,1	0	0	4,22	5,7	4,6
0-50	4,63	4,05	0	0	2,61	3,7	2,7

The Table 1 shows that the soils are strongly acidic (pH in water is 4.78 and 4.73, pH in KCl 4.10 and 4.05), carbonate-free, poor in easily accessible phosphorus and potassium. In the layer 0-30 cm there is a lot of humus (4.22%), while in the layer of 0-50 cm the humus content is significantly lower (2.61%).

The soil was sampled and analyzed for the presence of nematodes. Report N^o 04-2913 dated of 8 July 2016 of the Phytosanitary Laboratory of the Biotechnical Faculty, University of Montenegro did not confirm the presence of nematodes in the soil. The performed analyses have shown that this location is suitable for the production of raspberry planting material. After assessing the suitability of the site for nursery production, the land was prepared for the nursery. First, the treatment with total herbicides was performed during the summer of 2016 (one month before tillage), to clear the land of weeds to make the optimal condition for raspberry growth. These measures included:

calcification (200 kg/ha), humus - the application of well-burned manure (20 t/ha), as well as the application of complex NPK fertilizer (10:20:30, 2 t/ha). After spreading lime, organic, and mineral fertilizers on the surface, they were ploughed to a depth of 30 cm. In the spring, before planting the parent plants, the soil was finely prepared by milling to a depth of 5-10 cm.

Planting of mother plants of the variety "Glen Ample" was done manually, in rows at a depth of about 10-15 cm in April 2017, using the planting material imported from the nursery Hengartner Pflanzen GmbH (Zurich, Switzerland).

The distance between the rows was 1.6 m and the distance between the rows in the row was 0.6 m. The amount of planted plants was 10,416 per hectare. The plant uptake index was measured in August 2017 by counting plants that did not have the potential for further growth. However, it was rather tall (97%). Having been planted, the plants were irrigated and fertilized with a system of dripping perforated strips "drop by drop" with a diameter of Ø16 ("Hirro 10"), whose droppers were perforated at a distance of 10 cm (dropper from a dropper). The row spacing was cultivated and milled 2 to 3 times with a motor cultivator, while the application of plant protection products was performed with a back petrol motor atomizer. The application of plant protection products was performed following the forecast and assessment of the possible occurrence of diseases and pests on average 6 to 7 treatments during the vegetation period (March, October) during 2017, 2018, 2019 and 2020.

During 2017, a system of 18 sprayers with a diameter of Ø 25.44 mm with a wetting operation of about R = 40 m per sprayer was used as a nursery watering system. Since herbicides are still not allowed to be used in the production of raspberry planting material, weeds were removed by hand (plucking) and taken out of the plot. The seedlings were taken out by hand in the autumn when the above-ground part of the plant's tree was woody and a large part of the leaf mass fell from the plant. After the seedlings were removed, they were processed. The seedlings were first shortened to a length of about 0.3 m, tied in bundles of 50 pieces each, labelled and as such traded on the market. Part of the seedlings was furrowed in a drained place so that they would be ready for the spring market offer. After the autumn removal of the seedlings, the nursery was cleaned of the remains of plants, leaves and branches, which were taken out of the plot and cut. In the spring of the following year, before the movement of vegetation (February-March), shallow tillage was started with a frost to a depth of 10 cm.

After controlling the percentage of the admission of mother plants in the nursery during 2017, it was found that it was rather high (97%). The production of new shoots was measured during the next three vegetation years, on two occasions: in summer during the vegetation (June / July) and in autumn before the end of vegetation (October, November). The procedure was such that the number of plants in the nursery in 2018, 2019, and 2020 was measured on a surface of 1 m^2 by the method of a random sample in 5 repetitions. The number of plants per ha was obtained by calculation. The tested plants had to have the prescribed height, the thickness of the root neck, properly branched aboveground part, and

without the presence of symptoms of diseases and pests on the tree and leaves. The control of the presence of diseases and pests in the nursery was performed by an expert from the Biotechnical Faculty, who was hired by the Phytosanitary Administration. The development of the aboveground part of the plant was analyzed by field observations following the valid UPOV TG/43/7 (April 9, 2003) descriptor.

The production of raspberry planting material was performed in accordance to the Law on Planting Material (Official Gazette of the Republic of Montenegro, No. 28/6, No. 73/10, No. 40/11, No. 61/11, and 48/15). The law stipulates that the raspberry seedlings should meet the following criteria: the length of the aboveground part should be at least 20 cm and have at least 2 well-developed buds and a diameter immediately above the root neck from 5 to 12 mm.

RESULTS AND DISCUSSION

Quality planting material is a key factor in the successful cultivation of all types of fruit, including raspberries. The Figures 7-22 present the most significant technological processes in the production of raspberry planting material of the "Glen Ample" variety.

The results of testing the number of plants per m^2 and per ha have been shown in the Tables 2 and 3. The number of plants per m^2 of the variety "Glen Ample" was measured in two terms. The first measurement was performed in the summer period (June-July), and the second in the autumn period (October-November). Measurement data have been presented by repetitions, years and as an average for three years.

Figure 7: Mother Plant planted in April 2017

Figure 8: Mother plant condition as of June 13, 2017

Figure 9: Mother Plant,
(July 6, 2017)

Figure 10: Inter row cultivation in the nursery, after planting (August 20, 2017)

Figure 11: Plant health control
(June 2018)

Figure 12: Plant health control
(September 2018)

Figure 13: After the autumn extraction of seedlings, the spring cultivation of the nursery (March 2019)

Figure 14: Manual weeding
(April 2018)

Figure 15: Nursery, condition of plants (September 2018)

Figure 16: Nursery, condition of plants (November 2018)

Figure 17: Application of plant protection products in the nursery

Figure 18: Manual extraction of raspberry seedlings from nurseries in Bijelo Polje

Figure 19: Seedlings after removal and tying

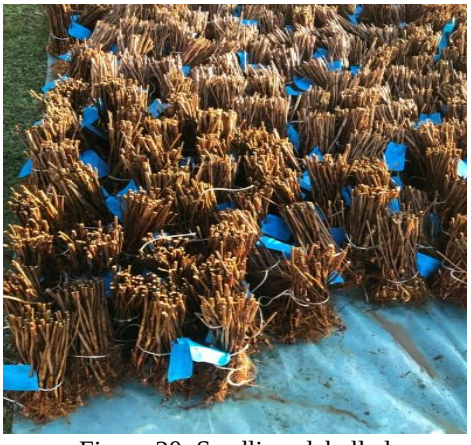

Figure 20: Seedlings labelled in bunches of 50 pieces

Figure 21: Seedlings in a dedicated box (November 2020)

Figure 22: Seedlings ready for delivery to the buyer (November 2020)

Table 2 indicates that the number of plants per m^2 in the summer period (June-July) was 19.3. By years small variations in this trait can be observed. The calculation determined the number of plants per ha, which in the summer period (June-July) amounted to 202,000. The lowest number of plants per ha was determined in 2019 (198,000), and the largest in 2020 (206,000).

Table 2. Number of plants per m^2 and ha in the summer period (June-July) in the nursery "Loznice I", Bijelo Polje (period 2018-2020)

Year	Number of plants per m^2					Average	Number of plants per ha					Average
2018.	18	19	19	20	20	**19,2**	180.000	190.000	190.000	200.000	200.000	**202.000**
2019.	19	17	19	21	20	**19,2**	190.000	170.000	190.000	210.000	200.000	**198.000**
2020.	17	18	20	22	20	**19,4**	170.000	180.000	200.000	220.000	200.000	**206.000**
Average						**19,3**						**202.000**

Analysis of the number of plants per m^2 in the autumn period (October-November) shows that it was higher and averaged 20.2 (Table 3). Conversion to ha showed that the number of plants averaged 202,000. The highest number of plants per ha was in 2020 (206,000), and the lowest in 2019 (198,000).

Table 3. Number of plants in autumn (October-November) in the nursery "Loznice I", Bijelo Polje (period 2018-2020)

Year	Number of plants per m2					Average	Number of plants per ha					Average
2018.	20	21	21	20	19	**20,2**	200.000	210.000	210.000	200.000	190.000	**202.000**
2019.	19	18	21	20	21	**19,8**	190.000	180.000	210.000	200.000	210.000	**198.000**
2020.	18	22	22	21	20	**20,6**	180.000	220.000	220.000	210.000	200.000	**206.000**
Average						**20,2**						**202.00**

As the raspberry variety "Glen Ample" is new in Montenegro only in this nursery is proliferated, and due to the fact that the situation is similar in the environment where there are only nurseries that produce older varieties "Willamette" and "Meeker", it is rather difficult to make a good comparison with yield of seedlings per unit area with other works. Depending on the variety and care conditions, a quality lemon balm can have an annual production of 200,000 or even 300,000 raspberry seedlings. In the Institute for Fruit Growing in Čačak, in the rootstock of certified material on the hill "Grad" near Kosjerić, near the variety "Villamette" in 2008, the production of 400,000 raspberry seedlings per 1 hectare was achieved (Leposavić 2016). Considering that "Glen Ample" gave an average of 202,000 pcs / ha in the examined three-year period, it can be stated that this is a satisfactory yield of seedlings and is close to the results found in practice.

Table 4 shows the data regarding the height of raspberry seedlings of the "Glen Ample" variety, measured in two terms: summer period (June-July) and autumn period (October-November).

Table 4. Height of seedlings (m) in the nursery "Loznice I", Bijelo Polje (period 2018-2020)

Year	Summer (June-July)					Average	Autumn (October-November)					Average
2018.	0.63	0.51	0.69	0.66	0.50	**0.60**	1.10	0.98	0.92	1.05	1.12	1.03
2019.	0.69	0.61	0.71	0.70	0.58	**0.66**	1.04	0.93	0.97	1.15	1.21	1.06
2020.	0.61	0.55	0.70	0.67	0.60	**0.63**	1.03	1.10	0.98	1.12	1.25	1.10
Average						**0.63**						**1.06**

By measuring the height of plants during the summer (June-July), it was determined that they had a good growth, which averaged 0.63 m, with a small variation over the years, from 0.60 m in 2018 to 0.66 m in 2019. However, concerning these measurements, the height of seedlings at the end of vegetation (October-November) was higher by about 40%. The average height of seedlings at the end of the vegetation was 1.06 m. The seedlings had the highest height in 2020 (1.10 m), and the lowest in 2018 (1.03 m). The height of the seedlings was following the Law on Planting Material.

Seedling diameter is one of the most important parameters for classifying seedlings. Table 5 shows changes in the diameter of seedlings, which is shown in two terms: summer period (June-July) and autumn period (October-November).

Table 5. Diameter of seedlings (mm) above the root neck in the nursery "Loznice I", Bijelo Polje (period 2018-2020)

Year	**Summer (June-July)**					**Average**	**Autumn (October-November)**					**Average**
2018.	5.0	4.0	4.0	6.0	4.0	**4.6**	7.0	7.0	9.0	12.0	10.0	**9.0**
2019.	6.0	4.0	6.0	7.0	6.0	**5.8**	7.0	8.0	12.0	13.0	11.0	**10.2**
2020.	6.0	5.0	7.0	6.0	4.0	**5.6**	8.0	6.0	11.0	12.0	9.0	**9.2**
Average						**5.3**						**9.5**

In summer, the diameter of seedlings above the root collar averaged 5.3 mm. The smallest diameter of seedlings was in 2018 (4.6 mm), and the largest in 2019 (5.8 mm).

Based on the data from Table 5, it can be seen that the diameter of the seedlings above the root collar at the end of the vegetation was about 45% higher compared to the summer period. This indicates that in the second part of the vegetation in raspberries, I express the growth of shoots in thickness. The diameter of the seedlings at the end of the vegetation was high. It ranged from 9.0 mm in 2018 to 10.2 mm in 2019. The average diameter of seedlings for all three examined years was 9.5 mm.

According to the prescribed standards on the categorization of planting material, seedlings were classified into three classes: Class I, Class II and class outside the category (Figure 23).

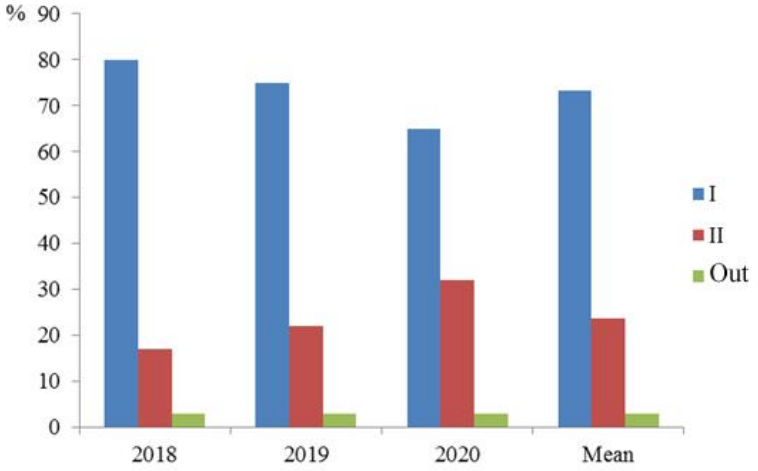

Figure 23: Class of raspberry planting material in the nursery "Loznice I", Bijelo Polje (period 2018-2020)

The obtained results indicate that the largest number of seedlings is those of class I (73%), then class II (24%), while only 3% of seedlings were out of class. Observed by the parameter *years*, the largest number of seedlings of class I was in 2018 (80%), and the lowest in 2020 (65%). In 2020, the largest number of Class II seedlings was obtained (32%), and the least in 2018 (17%). The number of seedlings outside the class was very small in all three examined years and amounted to 3%.

CONCLUSIONS

The production of raspberry planting material of the variety "Glen Ample" can be successfully organized in the municipality of Bijelo Polje and northern Montenegro due to the corresponding agro-ecological conditions. At the end of the season, the number of plants was 20.2 per m^2, or 202,000 per ha. The quality of the seedlings met the required standards and took place in accordance with the

Law and regulations. According to these criteria, the largest number of seedlings belonged to the category I class (73%), so it can be concluded that quality of planting material produced in local agro ecological conditions gives an advantage to future raspberry planters to establish new plantations faster, more efficiently and cheaper with such seedlings.

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IMPLEMENTATION OF INTERNAL CONTROL WITH REFERENCE TO THE APPLICATION OF "IT" IN COMPANIES OPERATING ON THE PRINCIPLES OF THE GREEN ECONOMY

SUMMARY

The process of introducing internal control in the operations of companies that operate with the introduced principles of environmental protection and principles based on the green economy is inextricably linked. The process of introducing internal control depends on the decision made and the will of the company's top management to implement the control system in its regular operations. The implementation of the internal control system is a long process. Its practical application should be carried out by motivated and professional staff who are maximally determined to use the achievements of the IT sector, as well as the application of international accounting standards, financial reporting standards and external and internal audit standards. In this paper, the authors pointed out that only the connection of several sectors in the company is subject to the work of internal control from the beginning to the end of the process of making business decisions and decisions related to management at all levels of management in these companies. Internal control should use new knowledge in its work and make the most of the advantages of the application of the dynamically developing IT sector, especially in the last decade in all economies.

Keywords: internal control, green economy, company sectors, IT.

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INTRODUCTION

The main players in rural development are for instance, factors as impact of technologies, development of domestic and foreign markets, benefits in communication and transport and population migration (Dudić et al., 2020). Modern management of companies, regardless of the activity in which the company operates, requires top management to apply and introduce internal control mechanisms (Wyatt, 2004; Wu et al., 2016, Popović, 2015; Popović et al., 2015; Rodriguez et al., 2019; Radović et al., 2019) into the regular business of the company.

The functioning of the work of an internal auditor in a company that functions according to the adopted principles of green economy in companies in the Republic of Serbia should be viewed as a process (Popović et al., 2020). Management based on the application of internal control requires the engagement of motivated workers in the control sector who will use modern technology and especially the application of IT (Toković, 2011) to organize internal control (Radović et al., 2011) in all parts of the company, to improve control of all parts of the company, i.e. with the aim of improving control in all sectors of the company.

In addition to the application of new innovative solutions in the establishment of internal control, companies strive to make important business decisions by top management, the same and introduce the practical application of already adopted standards in business, such as international accounting and other standards (Daske et al, 2008; Han, 2017; Popović et al., 2017).

The application of standards in production is particularly pronounced in agricultural production (Bojović et al., 2019; Terzić et al., 2019; Rajičić et al, 2020; Ljubičić et al., 2021; Ugrenović et al., 2021; Jovović et al., 2021). Within agricultural production, the goal of introducing internal control is to reduce all risks in production (Popović, 2015) in order to achieve the best possible business, financial and other business goals (Cheng et al., 2013).

It is important to point out that the entire process of implementing the introduction of internal control in companies is a long and comprehensive process (Damodaran, 2007; Cantino, 2009; Lee, 2019) which requires the application of long-term solutions that will adopt top management in business and implementation in future production and business periods business.

Viewed in this way, the top management of the company, i.e. top management that has a clear vision of long-term development of a modern company with the application of internal control has a chance to achieve general business success.

Top management should adopt general and important postulates of basic company management (Williams, 2010) in order to achieve the best possible business results in business. The connection between internal controls should be viewed in the context of linking internal control and internal audit (Radović et al., 2019a) as a factor that will contribute to increasing the business security of a large number of very heterogeneous companies.

In this paper, the authors draw attention to the importance of observing internal control, the application of which can improve performance, especially in agriculture, but also in companies that operate according to the adopted principles of the green economy.

The aim of the author was to present internal control as a mechanism by the application of which top management will be able to significantly improve the business of companies in companies in which it performs the function of management.

MATERIAL AND METHODS

In preparing this study, the authors used the already stated views of the authors on issues of corporate governance. The previously stated views of the authors especially referred to those companies that had introduced some form of internal control mechanisms in their regular business (Banaszevska, 2017; Chen et al., 2017).

The essential authors of this study have already accepted the so-called generally accepted attitudes of the authors regarding the use of primarily internal control in the regular operations of the company (this referred to the discovery of the benefits of using internal control, especially regarding achieving positive overall business results in the company and reducing the overall risk to the company). Using the methods of collecting real data from the top management of the company, they are classified into units that correspond to the established functions in the company.

RESULTS

Successful top management is looking for finding a model of internal control organization that will be close to optimal management in companies. Respecting the above, the authors gave an overview of a possible model in which the key phases of internal control mechanisms are given, which is important for business decision-making in companies in a transition country such as the Republic of Serbia (Figure 1).

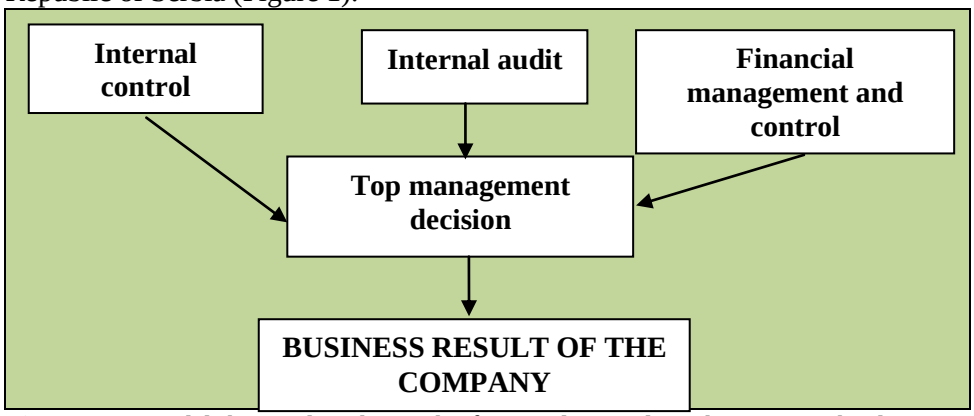

Figure 1. A model that applies the work of internal control mechanisms in the decision-making process of top management in companies that have accepted the principles of the green economy. **Source:** Authors (2021).

Improvements in companies 'operations in terms of implementation of internal control mechanisms and internal control depend on the existence of the application of numerous standards in companies' operations. The authors presented the possible application of standards in business and work of internal control mechanisms in companies that accept the principles of green economy in the Republic of Serbia (Figure 2).

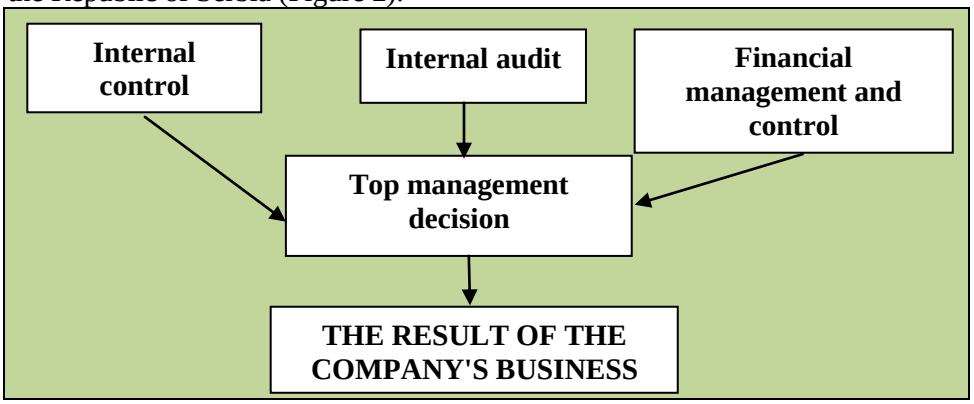

Figure 2. A model based on top management with the use of standards in the management of companies that have accepted the principles of the green economy.
Source: Authors (2021).

In the following, the authors gave a possible schematic presentation of the impact on the adoption and implementation of the policy of implementation of internal control in a company that wants to operate on the principles of green economy, which is shown in Figure 3.

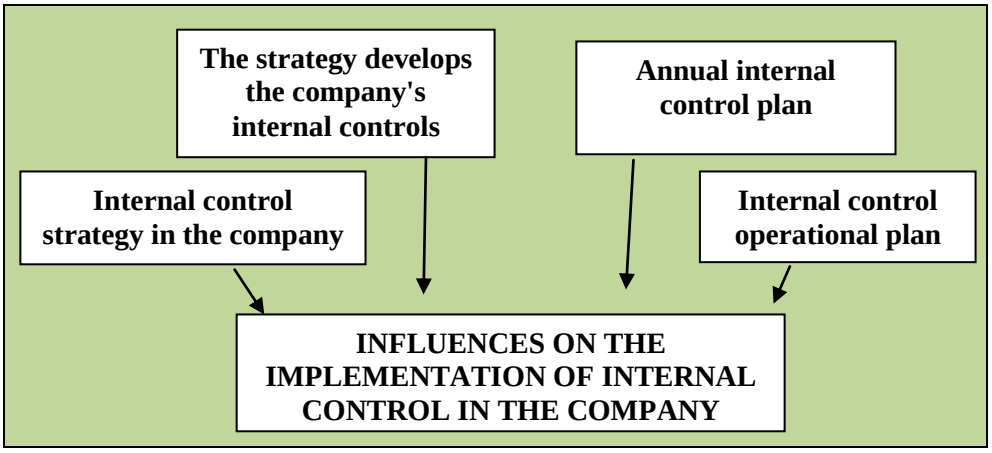

Figure 3. Model based on respecting the influence of external and internal factors on the decision-making process of internal control in the management of companies that have accepted the principles of the green economy.
Source: Authors (2021).

The application of software in companies that mainly perform activities in the field of environmental protection and the so-called green economy should be viewed as a dynamic system. Such a system in which the key components change dynamically is constantly evolving and is subject to change and adaptation to the requirements of users and top management.

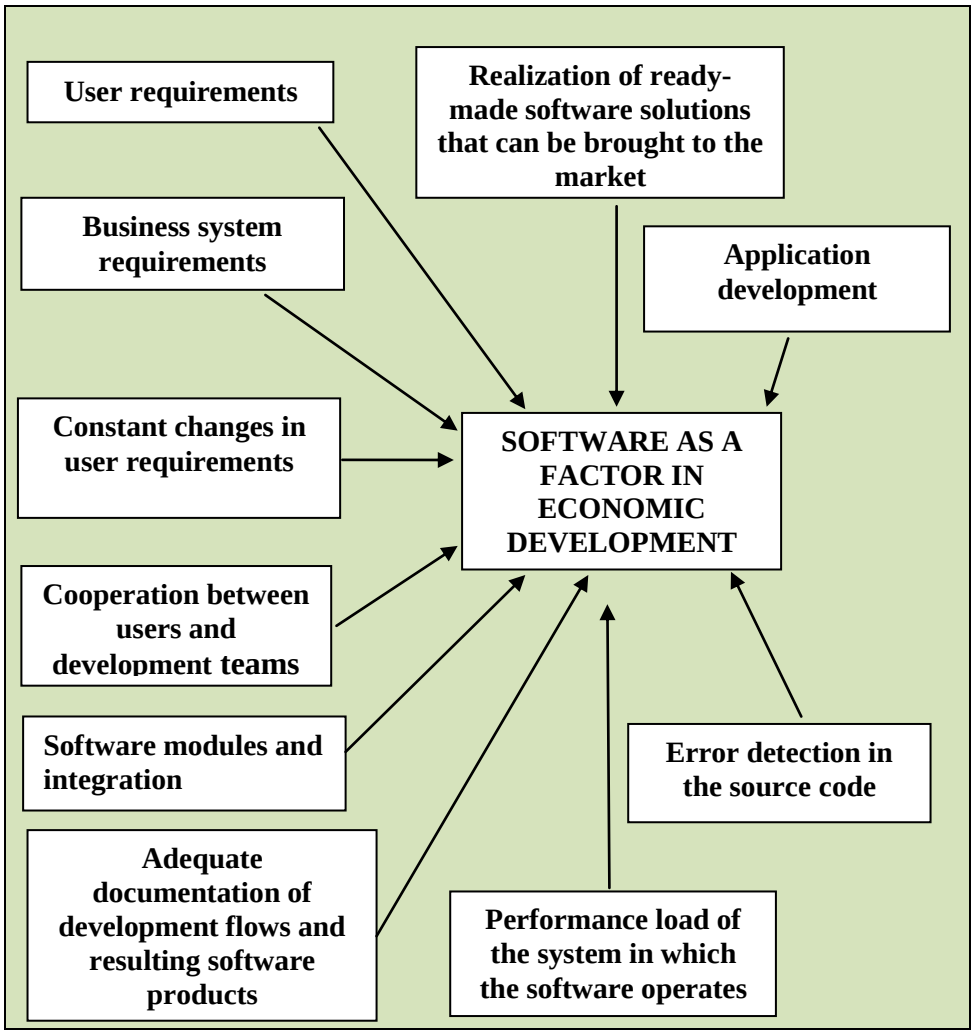

Figure 4. An overview of the possible impacts of software on the economic development of companies operating in the field of green economy.
Source: Authors (2021).

Software development implies the development of continuous processes that are essentially innovative and development for the entire company. If they develop in a large number of such and other companies, there is a better life in the

country and the economic development of the country moves in the direction of rapid development that is dynamic and fast.

The authors reinforced the above on the basis of the presentation in Figure 4, where they gave an overview of possible impacts on the development of the economy, i.e. the observation was done primarily by observing the development of companies that mainly perform activities in the field of green economy.

In the following, the authors provide an overview that explains the connection between software development and practical requirements of software users or buyers, which in companies dealing with the green economy practically means that the requirements of top management of these companies are shown in Figure 5.

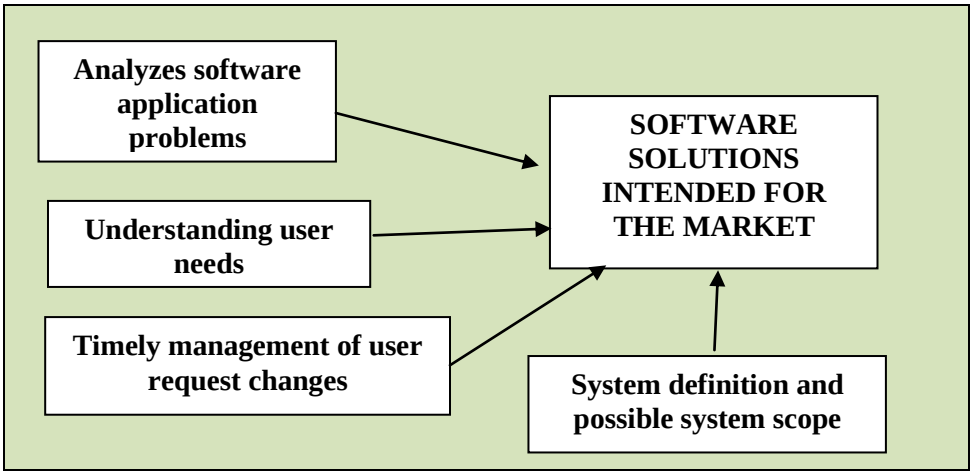

Figure 5. An overview of possible user requirements, ie requirements sent by top managers of companies that perform the predominant activity in the field of green economy.
Source: Authors (2021).

Based on the overview given in Figure 5, it is clear that there are a large number of heterogeneous requirements that can be placed by top managers of companies before software development companies.

DISCUSSION

The obtained basic results obtained by the authors of the study are presented in the form of possible models that could be practically used primarily in terms of the implementation of internal control in the operations of companies operating on the principles of green economy.

The results obtained by the authors of the study are presented in the form shown in Figures 1-3. They are compatible with the already published views (Alibegović et al., 2018) because they essentially group the control functions in the company within the real possible model of behavior of the top management of the company.

The stated views were strengthened with the obtained research results, which referred to the presentation of the software and its practical application for the business of the mentioned companies.

The presentation of the results regarding the application of software obtained by the authors of the study are given in the form of the presentation in Figures 4-5. The results obtained by the authors of the study coincide with the already published views (Toković, 2011) and essentially reflect the expression of the importance of the practical application of software in the business of companies that operate according to the principles of the green economy.

It is important to note that these companies must develop the required software performance in accordance with user requirements at the optimally required level. Software development should be viewed as a continuous process that is constantly in the process of finding the best innovative solutions. The development of software in companies that base their existence on the principles of the green economy should be aimed at meeting the essential needs of companies that will be able to compensate the costs of creating a software solution for the company as a customer.

CONCLUSIONS

The functioning of internal control mechanisms, primarily internal control in companies that operate according to the adopted principles of green economy in companies in the Republic of Serbia, should be exclusively in the form of a continuous process mechanism introduced by top management in companies.

Top management companies have an interest in developing a system of internal control mechanisms.

The first conclusion would be that the authors emphasized the importance of implementing internal control as a system that will be able to improve the business of companies operating in the field of green economy.

Another conclusion would be that the authors in this paper pointed out the existence of increasing importance of the implementation of internal control as a mechanism that can give positive results in the regular business of companies.

The third conclusion that can be pointed out after the presentation in the paper would be that companies based on the requirements of top management in their business should fulfill the objectivity, expertise and responsibility of business where the development of software solutions will certainly contribute as a key auxiliary mechanism of overall management.

Finally, the authors emphasize the comprehensive position arising from this paper that companies that have not yet conducted internal control should create the conditions for its introduction, because there are essentially many benefits within which top management will have as an imperative to introduce internal control mechanisms, especially internal controls as a factor by which it will be possible to develop better, safer and generally acceptable management in companies that predominate in the field of green economy.

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The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

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Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the

experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used etc.

- RESULTS followed by **DISCUSSION**

Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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