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Palma ORLOVIĆ-LEKO, *Irena CIGLENEČKI*, *Velibor SPALEVIĆ*¹

CLIMATE CHANGES AND SOIL WATER REGIME

SUMMARY

The research objective was to determine the duration of dry season based on the frequency of precipitation occurrence both upon 25% ($F_a=25\%$) and 50% ($F_a=50\%$) probability, respectively, for three major climate regions in Croatia (Mediterranean, mountainous and continental). In the Mediterranean region, soil water deficit was 246 mm upon 25% probability of precipitation occurrence. Upon 50% probability of precipitation occurrence, soil water deficit was lower, standing at 191 mm. In both cases, soil water deficit was determined during the summer months and it lasted for three months. In the mountainous region, there was a slight soil moisture deficit, which was 32 mm upon 25% probability of precipitation occurrence and 22 mm upon 50% probability of precipitation occurrence. Soil moisture deficit was determined in July or August. In the continental region, upon 25% probability of precipitation occurrence, soil moisture deficit was 230 mm and the dryness lasted for four months during the summer period, while upon 50% probability of precipitation occurrence, soil moisture deficit was 82 mm. Soil moisture deficit lasted for two months in the summer period (July and August). Higher soil water deficit with longer duration could be predicted in the future against the backdrop of the trend of increasing average annual air temperature in all the regions.

Keywords: Climate changes, soil water deficit, dry season duration

INTRODUCTION

Climate changes are altering the statistics of temperature and precipitation. Global climate changes and associated impacts on water resources are the most urgent challenges faced by mankind today and will have enduring social implications for generations to come. Potential impacts may include the change in hydrologic processes and watershed response, including timing and magnitude of surface discharge, stream discharge, evapotranspiration, and flood events, all of which would influence other environmental variables, such as nutrient and sediment flux on water sources (Simonovic and Li, 2004). Changes in

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precipitation are the prime drivers of change in the availability of both surface water and groundwater resources (Beare and Heaney, 2002). 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). The effects of climate change have become increasingly apparent over the past decades (Patt and Schröter, 2008). The average temperature increased by 1.1–1.3°C in 100 years in Central Europe (Kutilek and Nielsen, 2010). More frequent and severe extreme weather events are anticipated to cause greater damage to ecosystems and agricultural systems (Wigley, 2009; Choi *et al.*, 2015). Climate change (temperature increase, precipitation decrease) may be related to environmental pollution. In case of low temperature and low moisture, assimilation of nutrients is considerably worse. Consequently, they are leached from the soil with a more intensive drainage runoff (Root *et al.*, 2003; Soussana and Luscher, 2007). Precipitation distribution in the territory and their changes within a year have a huge impact on hydrological phenomena, soil formation and plant growing seasons (Bukantis, 1994). Climate change impact on flora has been receiving increasing attention throughout the world (Fuhrer, 2003). Climate characteristics and soil water regime, as well as their variable and complex interrelations, define the efficiency of plant production. Each climate element participates, to a greater or lesser extent, in plant development. Nevertheless, water and temperature play dominant roles, the water status in soil being greatly influenced by precipitation and evaporation, and both by surface and groundwater. 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. All the participants in agricultural activities need to bear more responsibility for alleviating the consequences of climate changes although they cannot be fully eliminated (Šimunić *et al.*, 2013). Climate change projections suggest a more variable climate with higher vulnerabilities in the lower income countries (Easterling *et al.*, 2000).

The research objective was:

– to determine the duration of dry season based on the frequency of precipitation occurrence both upon 25% ($F_a=25\%$) and 50% ($F_a=50\%$) probability, respectively, for three major climate regions in Croatia (Mediterranean, mountainous and continental).

MATERIAL AND METHODS

The meteorological stations (MS) Poreč, Gospić and Virovitica belong to the meteorological station network of the Croatian Meteorological and Hydrological Service. The data from the previously mentioned MS for the period 1986–2015 has been used for analysis of agro–hydrological balance components for three major climate regions in Croatia (Mediterranean–Poreč, mountainous–Gospić and continental–Virovitica, Table 1). Thornthwaite-Mather method (TM, Thornthwaite and Mather, 1957) has been used for analysis of agro–hydrological balance components. Potential evapotranspiration (ET_o) has

been calculated using Thornthwaite method (Thornthwaite, 1948) and was used as input for water balance calculation. Other input data used included monthly precipitation amount (P) in a year with the amount of precipitation in the frequency of occurrence both in 25% of cases ($F_a=25\%$) and 50% of the cases ($F_a=50\%$) and upon the presumption that water storage in soil is 100 mm up to root zone depth at the beginning of the year.

Table 1. Meteorological stations and their corresponding regions and the details concerning the position of meteorological stations

Meteorological stations	Climate regions	Latitude	Longitude	Elevation (m)
Poreč	Mediterranean	45°14' N	13°36' E	15
Gospić	Mountainous	44°32' N	15°23' E	546
Virovitica	Continental	45°49' N	17°23' E	118

The following equations were used for the calculation of ET_o :

$$ET_o = 16 \left(\frac{10t}{I} \right)^a \cdot k \quad (1)$$

$$i = \left(\frac{t}{5} \right)^{1.5} \quad (2)$$

$$a = \left(\frac{1.6}{100} \right) \cdot I + 0.5 \quad (3)$$

t = monthly air temperature ($^{\circ}\text{C}$); i = monthly thermal index; I = annual thermal index (sum of 12 monthly indices); and the exponent "a" = a function of I . Aridity Index (AI, Table 2) was calculated by dividing the total monthly precipitation (P) by the total monthly potential evapotranspiration (ET_o), as adopted by United Nations Environment Programme (UNEP) (Salvati et al., 2013), as follows:

$$AI = \frac{P}{ET_o} \quad (4)$$

Table 2. Classification of aridity index (AI)

Aridity Index (AI) values	Climate classification
<0.05	Hyper-arid
0.05–0.2	Arid
0.2–0.5	Semi-Arid
0.5–0.65	Dry sub-humid
0.65–0.75	Humid
>0.75	Hyper-humid

Agro–hydropotential (AHP) is the ratio of actual evapotranspiration (AE) and potential evapotranspiration (ET_o) and derived from the water balance model (Petrasovits, 1984), as follows:

$$AHP = \frac{AE}{ET_o} \quad (5)$$

AHP from 1.0–0.8 means that the water supply to the crops is continuous and not limited. The values from 0.8–0.5 indicate that the water supply to crops is still continuous, but it is getting increasingly restricted. The values from 0.5–0.3 show that the water scarcity is becoming high, the water supply to plants is periodical and restricting and water–stress develops. The value <0.3 shows that strong water stress occurs, causing considerably lower level of biomass and yield deficiency, and when this stage lasts long it also implies the death of the plant.

RESULTS AND DISCUSSION

The precipitation amount with the frequency of occurrence both in 25% of the cases and 50% of the cases and the associated air temperature upon the previously mentioned precipitation values have been presented in Table 3.

Table 3. The precipitation amount upon the frequency of occurrence in both 25% of the cases and 50% of the cases and the associated air temperature at the mentioned precipitation values for the period 1986 - 2015

Month	Poreč				Gospić				Virovitica			
	Fa=25%		Fa=50%		Fa=25%		Fa=50%		Fa=25%		Fa=50%	
	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)
I	5.5	25.0	5.3	54.4	3.8	128.2	-0.2	107.0	-1.6	87.4	0.8	51.5
II	5.4	28.3	5.4	54.1	2.0	138.4	0.6	101.1	2.6	39.4	2.2	47.7
III	8.4	11.9	8.2	49.6	3.1	167.2	4.5	88.9	7.1	33.1	6.7	53.5
IV	12.1	34.9	12.3	60.2	8.0	113.4	9.0	106.5	14.3	36.2	11.8	60.8
V	17.4	148.4	17.2	65.6	13.4	82.4	13.8	94.8	17.7	38.8	16.6	80.5
VI	19.2	70.8	21.0	70.2	15.6	109.2	17.4	88.1	18.9	85.1	20.0	94.4
VII	23.5	21.6	23.6	48.9	20.4	4.6	19.6	60.4	21.8	66.1	22.0	71.9
VIII	22.7	77.7	23.1	74.4	18.8	120.4	19.2	73.7	21.7	28.5	21.3	76.2
IX	20.3	21.6	18.6	98.2	13.6	81.1	14.0	150.8	17.5	28.4	16.3	98.3
X	12.8	80.2	14.4	98.7	9.4	73.6	9.8	161.0	11.1	92.5	11.2	77.8
XI	9.2	201.5	10.1	110.9	-0.1	105.8	5.0	185.6	8.3	85.2	5.8	75.6
XII	6.6	32.0	6.4	72.9	-0.2	118.4	0.4	142.0	3.1	115.6	1.7	66.4
Ave.	13.6		13.8		9.0		9.4		11.9		11.4	
Sum		753.9		858.1		1,245.4		1,360.8		736.3		854.6

The highest precipitation rate with the probability of occurrence in 25% of cases and 50% of cases was recorded at the MS Gospić (mountainous region), while the lowest rate was recorded at the MS Virovitica (continental region).

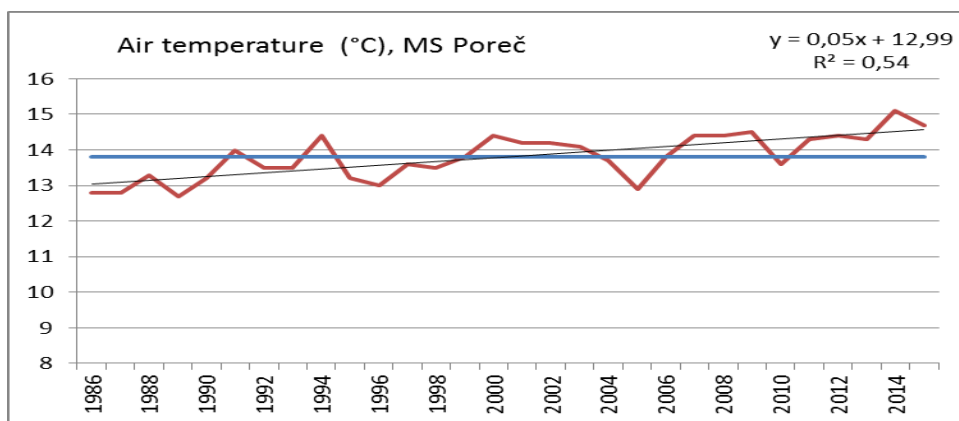


Figure 1a. Average annual air temperature (°C), multi-annual average (°C) and linear trend for the MS Poreč.

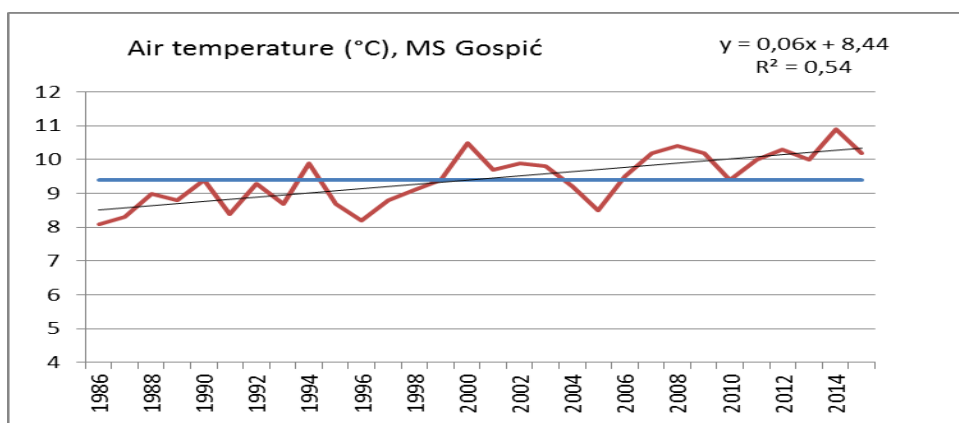


Figure 1b. Average annual air temperature (°C), multi-annual average (°C) and linear trend for the MS Gospić.

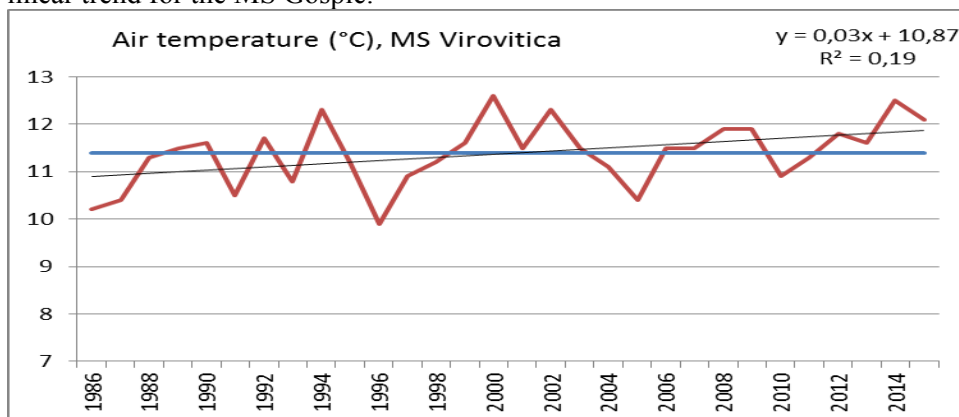


Figure 1c. Average annual air temperature (°C), multi-annual average (°C) and linear trend for the MS Virovitica.

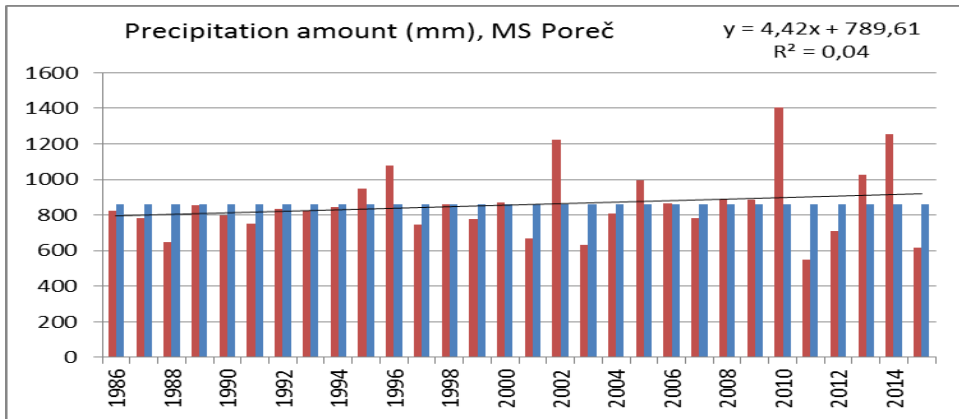


Figure 2a. Annual precipitation amount (mm), multi-annual average (mm) and linear trend for the MS Poreč.

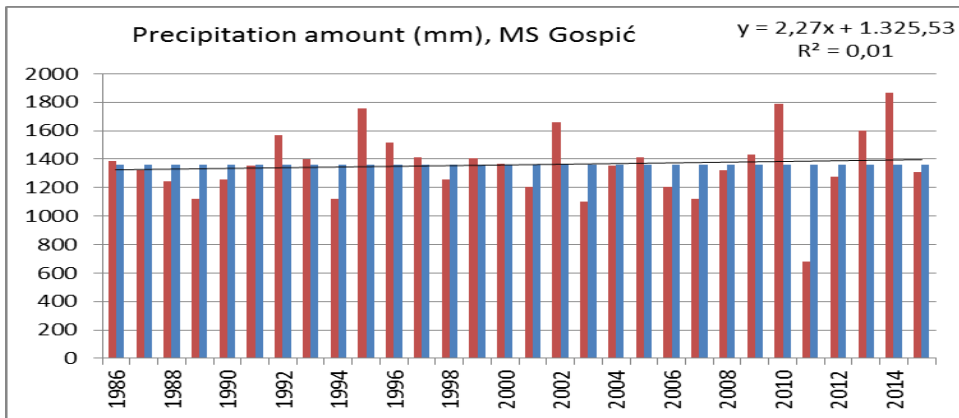


Figure 2b. Annual precipitation amount (mm), multi-annual average (mm) and linear trend for the MS Gospić.

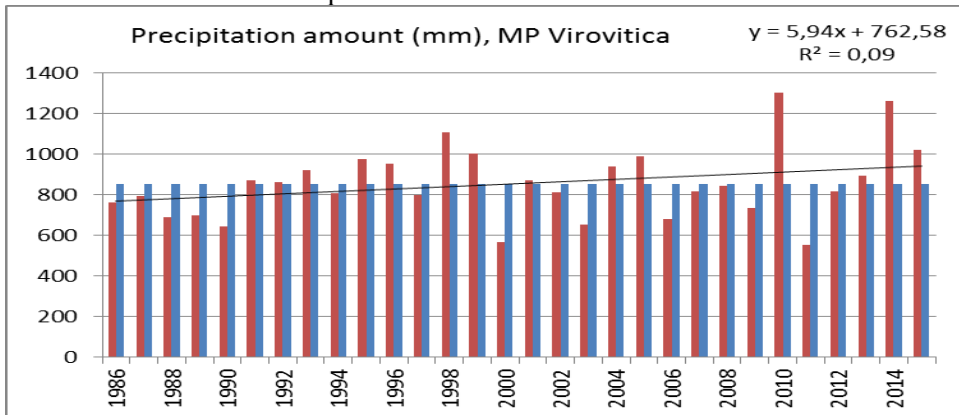


Figure 2c. Annual precipitation amount (mm), multi-annual average (mm) and linear trend for the MS Virovitica.

The highest average air temperature upon the probability of precipitation both in 25% of the cases and 50% of the cases was recorded at the MS Poreč (Mediterranean region), and the lowest at the MS Gospić (mountainous region, Table 3). The precipitation amount with the probability of occurrence in 25% of the cases was lower than upon the probability of occurrence in 50% of the cases, in Poreč about 104 mm, in Gospić 115 mm and Virovitica 118 mm. The average air temperature both in the Mediterranean region and the mountainous region was higher by 0.2°C or 0.4°C when the precipitation occurred in 50% of the cases, while in the continental region it was higher by 0.5°C upon the probability of precipitation in 25% of the cases.

The average annual air temperature (°C), multi-annual average (°C) and linear trends for the period 1986–2015 have been shown in Figure 1a, 1b and 1c. There is a clear tendency of increase in the annual air temperature in all the regions (MS). The highest air temperature in Poreč was 15.1°C (2014), in Gospić 10.7°C (2014) and in Virovitica it was 12.6°C (2000), while the lowest temperature observed was 12.5°C in Poreč (1988), 8.1°C in Gospić (1986) and 9.9°C in Virovitica (1996). Multi-annual average was 13.8°C in Poreč, 9.4°C in Gospić and 11.4°C in Virovitica.

The annual precipitation amount (mm), multi-annual average (mm) and linear trends for the period 1986–2015 have been shown in Figure 2a, 2b and 2c. The annual precipitation has the tendency to increase in all the regions (MS). The wettest year in Poreč was the year 2010 (1403 mm), in Gospić the year 2014 (1866 mm), while in Virovitica it was the year 2010 (1303 mm). The driest year in all the regions was 2011. Moreover, 550 mm precipitation fell in Poreč, as opposed to 683 mm in Gospić and 552 mm in Virovitica. Multi-annual average was 858 mm in Poreč, 1361 mm in Gospić and 855 mm in Virovitica. The linear trends are positive in all the MS (regions).

Positive trends of air temperature and precipitation in their research have been quoted by Šimunić et al. (2013) and Miseckaite et al. (2018). Irrespective of a relatively small increase in air temperature and a relatively short time of monitoring, this could be considered as a mild climate warming (Šimunić et al., 2013)

Soil water balance for all the MS (regions) has been shown in Tables 4a, 4b, 5a, 5b, 6a and 6b. In the Mediterranean region (MS Poreč), upon 25% probability of precipitation occurrence, the annual soil moisture deficit was 246 mm and it was determined during the period in question (July, August and September). The largest monthly soil moisture deficit was in July (94 mm). When the precipitation occurred upon 50% probability, the annual soil moisture deficit was expected to be lower and it was 191 mm, as was determined during the summer months (June, July and August). The largest monthly soil moisture deficit was also determined in July and it was 116 mm. In both cases, the surplus water in the soil was 170 mm upon $F_a = 25\%$ of precipitation probability and 211 mm upon $F_a = 50\%$ of precipitation probability and it was determined during the autumn and winter period.

Table 4a. Soil water balance for the frequency of precipitation occurrence upon 25% probability, MS Poreč.

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
ETo	11	11	27	54	102	121	166	146	106	48	25	14	831
Precipitation	25	28	12	35	148	71	22	78	22	80	202	32	755
Water storage in soil	100	100	85	66	100	50	0	0	0	32	100	100	
AE	11	11	27	54	102	121	72	78	22	48	25	14	585
Surplus	14	17	0	0	12	0	0	0	0	0	109	18	170
Deficit	0	0	0	0	0	0	94	68	84	0	0	0	246
P/ETo	2.27	2.55	0.44	0.65	1.45	0.59	0.13	0.53	0.21	1.67	8.08	2.29	
AE/ETo	1.00	1.00	1.00	1.00	1.00	1.00	0.43	0.53	0.21	1.00	1.00	1.00	

Table 4b. Soil water balance for the frequency of precipitation occurrence upon 50% probability, MS Poreč.

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
ETo	11	11	26	54	101	136	165	148	92	54	28	12	838
Precipitation	54	54	50	60	66	70	49	74	98	99	111	73	858
Water storage in soil	100	100	100	100	65	0	0	0	6	51	100	100	
AE	11	11	26	54	101	135	49	74	92	54	28	12	647
Surplus	43	43	24	6	0	0	0	0	0	0	34	61	211
Deficit	0	0	0	0	0	1	116	74	0	0	0	0	191
P/ETo	4.91	4.91	1.92	1.11	0.65	0.51	0.30	0.50	1.07	1.83	3.96	6.08	
AE/ETo	1.00	1.00	1.00	1.00	1.00	0.99	0.30	0.50	1.00	1.00	1.00	1.00	

Table 5a. Soil water balance for the frequency of precipitation occurrence upon 25% probability, MS Gospić.

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
ETo	13	7	14	43	85	101	137	116	70	43	0	0	629
Precipitation	128	138	167	113	82	109	5	120	81	73	106	118	1,240
Water storage in soil	100	100	100	100	97	100	0	4	15	45	100	100	
AE	13	7	14	43	85	101	105	116	70	43	0	0	597
Surplus	115	131	153	70	0	5	0	0	0	0	51	118	643
Deficit	0	0	0	0	0	0	32	0	0	0	0	0	32
P/ETo	9.85	19.71	11.23	2.63	0.96	1.08	0.04	1.03	1.16	1.70	-	-	
AE/ETo	1.00	1.00	1.00	1.00	1.00	1.00	0.77	1.00	1.00	1.00	0.00	0.00	

Table 5b. Soil water balance for the frequency of precipitation occurrence upon 50% probability, MS Gospić.

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
ET _o	0	2	19	53	82	108	124	112	68	43	17	1	629
Precipitation	107	101	89	107	95	88	60	74	151	161	186	142	1.361
Water storage in soil	100	100	100	100	100	80	16	0	83	100	100	100	
AE	0	2	19	53	82	108	124	90	68	43	17	1	607
Surplus	107	99	70	54	13	0	0	0	0	101	169	141	754
Deficit	0	0	0	0	0	0	0	22	0	0	0	0	22
P/ET _o	-	50.50	4.68	2.02	1.16	0.81	0.48	0.66	2.22	3.74	10.94	142.00	
AE/ET _o	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80	1.00	1.00	1.00	1.00	

In the mountainous region (MS Gospić) there was a slight soil moisture deficit, which was 32 mm concerning precipitation occurrence upon 25% probability and 22 mm in case of precipitation occurrence upon 50% probability. Soil moisture deficit was determined in July or August. Soil moisture surplus was 643 mm and 754 mm, and it was determined from November to April upon Fa = 25% probability and from October to May upon Fa = 50% probability.

Table 6a. Soil water balance for the frequency of precipitation occurrence upon 25% probability, MS Virovitica.

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
ET _o	0	5	24	67	100	109	134	123	80	40	23	6	711
Precipitation	87	39	33	36	39	85	66	29	28	93	85	116	736
Water storage in soil	100	100	100	69	8	0	0	0	0	53	100	100	
AE	0	5	24	67	100	93	66	29	28	40	23	6	481
Surplus	87	34	9	0	0	0	0	0	0	15	110	110	255
Deficit	0	0	0	0	0	16	68	94	52	0	0	0	230
P/ET _o	-	7.80	1.36	0.54	0.39	0.78	0.49	0.24	0.35	2.33	3.70	19.33	
AE/ET _o	0.00	1.00	1.00	1.00	1.00	0.85	0.49	0.24	0.35	1.00	1.00	1.00	

Table 6b. Soil water balance for the frequency of precipitation occurrence upon 50% probability, MS Virovitica.

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
ET _o	1	5	24	57	100	128	147	130	79	44	16	3	734
Precipitation	52	48	54	61	81	94	72	76	98	78	76	66	856
Water storage in soil	100	100	100	100	81	47	0	0	19	53	100	100	
AE	1	5	24	57	100	128	119	76	79	44	16	3	652
Surplus	51	43	30	4	0	0	0	0	0	0	13	63	204
Deficit	0	0	0	0	0	0	28	54	0	0	0	0	82
P/ET _o	52.00	9.60	2.25	1.07	0.81	0.73	0.49	0.58	1.24	1.77	4.75	22.00	
AE/ET _o	1.00	1.00	1.00	1.00	1.00	1.00	0.81	0.58	1.00	1.00	1.00	1.00	

In the continental region (MS Virovitica) in case of precipitation occurrence upon 25% probability soil moisture deficit was 230 mm, while in case of precipitation occurrence upon 50% probability soil moisture deficit was 82 mm. Soil moisture deficit upon $Fa = 25\%$ was determined from June to September, and $Fa = 50\%$ was determined in July and August. In both cases, the largest monthly soil moisture deficit was determined in August and it was 94 mm and 54 mm. Soil moisture surplus was 255 mm and 204 mm, and it was determined from November to March upon $Fa = 25\%$ probability and from November to April upon $Fa = 50\%$ probability.

In all the regions soil moisture deficit was determined in the summer months, during the vegetation period. The largest soil moisture deficiency was determined in the Mediterranean region, followed by the continental region, and the lowest soil moisture deficiency (insignificant) was determined in the mountainous region, which has the greatest precipitation amount in comparison with the other two regions. By comparing the precipitation amount in the Mediterranean and continental region upon $Fa = 25\%$ and $Fa = 50\%$, it is evident from the tables (4a, 4b, 6a and 6b) that the precipitation amount is equal, but because of the higher air temperature there is a greater evaporation of water from soil in Mediterranean region, whose consequence is soil moisture deficit.

Soil water deficit affects the growth and development of field crops, which in turn affects both their yield and quality. Hence, efficient agricultural production requires provision of water through an adequate irrigation system to compensate the estimated water deficit for plant production. Water deficit is especially harmful for plants if it occurs during the "period of critical development of the plant" (Šimunić *et al.*, 2013).

According to aridity index (AI), in the Mediterranean region (Table 4a and Table 4b) in case of the frequency of precipitation upon $Fa = 25\%$ probability, one month was arid (July), two months were semi-arid (March and September), two were dry sub-humid (June and August) and the other seven months were in the humid or hyper-humid climate category. The strongest drought was determined in July when aridity index was 0.13. In case of the frequency of precipitation upon $Fa = 50\%$ probability, one month was semi-arid (July), two were dry sub-humid (June and August), one humid (May) and all the other months were hyper-humid. The strongest drought was determined also in July when aridity index was 0.30. According to aridity index (AI), in the mountainous region in case of the frequency of precipitation upon $Fa = 25\%$ probability, one month was hyper-arid (July) and the others were in the hyper-humid category. The strongest drought was determined in July when aridity index was 0.04. In case of the frequency of precipitation upon $Fa = 50\%$ probability, one month was semi-arid (July), one was in the humid category of climate (August) and the other months were hyper-humid. The strongest drought was determined also in July with aridity index reaching 0.48. In the continental region in case of the frequency of precipitation upon $Fa = 25\%$ probability, four months were semi-arid (May, July, August and September), one was dry sub-humid (April),

while the other months were in the hyper-humid category. The strongest drought was determined in May when aridity index stood at 0.39, and in case of the frequency of precipitation upon $Fa = 50\%$ probability, one month was semi-arid (July), one was dry sub-humid (August), one humid (June) and the other months were hyper-humid. The strongest drought was determined also in July, with aridity index reaching 0.49. As had been assumed, the dry period lasted longer, with less precipitation compared with the highest precipitation. The duration of the dry season was equal in the Mediterranean region and in the continental region (five months) and the shortest (one month) in the mountainous region upon $Fa = 25\%$ probability, i.e. three months in the Mediterranean region, one month in the mountainous region and two months in the continental region upon $Fa = 50\%$ probability.

According to the agro-hydropotential (AHP), in the Mediterranean region, in case of the frequency of precipitation upon $Fa = 25\%$ probability, three months showed water scarcity in the soil (July, August and September), where strong water stress occurred (0.21) in September. In all the other months the supply of water to crops was not limited. In case of the frequency of precipitation upon $Fa = 50\%$ probability, two months (July and August) showed a soil water deficit. In July AHP was 0.30 and it also showed the occurrence of strong water stress. In the mountainous region, in case of the frequency of precipitation upon $Fa = 25\%$ probability, one month (July) was dry, AHP was 0.77 and it indicated that the water supply to crops was still continuous, but increasingly restricted. The same applied in case of the frequency of precipitation upon $Fa=50\%$ probability. One month was also dry, AHP was 0.80 and it was determined in August. In the continental region in case of the frequency of precipitation upon $Fa = 25\%$ probability, four months showed soil water deficit (June, July, August and September). In June AHP was 0.85 and it showed that the water supply to crops was not limited, while AHP during the three months in question (July, August and September) was below 0.5, of which September showed 0.24 and it implied a strong water stress. In case of the frequency of precipitation upon $Fa = 50\%$ probability, one month showed 0.81 (July) and in August AHP was 0.58. Based on the agro-hydro potential as a ratio of actual and potential evapotranspiration it is evident that there was a higher soil water deficit in case of the frequency of precipitation upon $Fa = 25\%$ compared with the frequency of precipitation upon $Fa = 50\%$. Comparing the regions, the longest dry season was defined in the continental region (four months) upon $Fa = 25\%$, followed by the Mediterranean region (three months) and the shortest (one month) in the mountainous region. This may be due to the fact that the continental region had slightly less precipitation than the Mediterranean region (19 mm difference), while the highest rainfall was recorded in the mountainous region. Upon $Fa=50\%$ the same applied concerning the drought duration in the Mediterranean region and in the continental region. The drought lasted for two months. Almost the same amount of precipitation (858 mm or 856 mm) fell in both of these regions. A milder form of drought was recorded in the mountainous region over one month.

CONCLUSIONS

In the period 1986-2015 annual air temperature and annual precipitation showed a positive trend in all the three regions (MS).

In all the regions in question soil moisture deficit was determined over the summer months, during the vegetation period. The largest soil moisture deficiency was determined in the Mediterranean region (246 mm upon $F_a=25\%$ and 191 mm upon $F_a=50\%$), followed by the continental region (230 mm upon $F_a=25\%$ and 82 mm upon $F_a=50\%$) and the lowest soil moisture deficiency (insignificant) was determined in the mountainous region (32 mm upon $F_a=25\%$ and 22 mm upon $F_a=50\%$).

The duration of dryness in the Mediterranean region was three months upon $F_a=25\%$ and also three months upon $F_a=50\%$, in the continental region it was four months upon $F_a=25\%$ and two months upon $F_a=50\%$, while in the mountainous region the duration of dryness was one month in both cases of probability of precipitation occurrence.

According to aridity index, in the Mediterranean region, in case of the frequency of precipitation upon $F_a = 25\%$ probability, one month was arid, two months were semi-arid, two were dry sub-humid and the other seven months were in the humid or hyper-humid climate category, upon $F_a = 50\%$ probability, one month was semi-arid, two were dry sub-humid, one humid and all the other months were hyper-humid. In the continental region, in case of precipitation upon $F_a = 25\%$ probability, four months were semi-arid, one was dry sub-humid, while the other months were in the hyper-humid category and in case of the frequency of precipitation upon $F_a = 50\%$ probability, one month was semi-arid, one was dry sub-humid, one humid and the other months were hyper-humid. In the mountainous region, in case of precipitation upon $F_a = 25\%$ probability, one month was hyper-arid and all the other months were in the hyper-humid category. In case of the frequency of precipitation upon $F_a = 50\%$ probability, one month was semi-arid, one was in the humid category and the other months were hyper-humid.

According to the agro-hydropotential, in the Mediterranean region in case of precipitation upon $F_a = 25\%$ probability, three months showed water scarcity in the soil (July, August and September), where September saw the occurrence of strong water stress (0.21). In all the other months, water supply to crops was not limited. In case of the precipitation upon $F_a = 50\%$ probability, three months (June, July and August) showed soil water deficit. In July, AHP was 0.30 and it also showed a strong water stress. In the continental region, in case of the frequency of precipitation upon $F_a = 25\%$ probability, four months showed soil water deficit (June, July, August and September). In June, AHP was 0.85 and it indicated that the water supply to crops was not limited, while AHP during the three months in question (July, August and September) was below 0.5, where September saw 0.24, which indicated a strong water stress. In case of the frequency of precipitation upon $F_a = 50\%$ probability, one month showed 0.81 (July) and, in August, AHP was 0.58, indicating that water supply to crops was

still continuous, yet it was getting increasingly restricted. In the mountainous region in case of the precipitation upon $F_a = 25\%$ probability, one month (July) was dry, AHP was 0.77. In case of the frequency of precipitation upon $F_a = 50\%$ probability, one month (August) was dry, AHP was 0.80, yet it showed that water supply to crops was not limited.

An even higher soil water deficit with longer duration could be predicted in the future against the backdrop of upward trend in annual air temperature in all the regions.

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CHANGES IN THE WATER BALANCE OF BOSNIA AND HERZEGOVINA AS A RESULT OF CLIMATE CHANGE

SUMMARY

The analysis of meteorological data from the period 1961–2014 show the rise in the mean annual temperature in the entire territory of Bosnia and Herzegovina. The changes are more pronounced in the central – hilly part of the country. The increase in annual air temperature ranges from 0.4 to 1.0°C per decade, whereas temperature increases during vegetation period were up to 1.2°C per decade. Additionally, increases in air temperature over the last fourteen years are even more pronounced. Changed distribution of precipitation, significant variations and the increasing soil moisture deficit during vegetation period (April – September) are also evident in Bosnia and Herzegovina. The increase in air temperature combined with changes in the distribution of precipitation has resulted in a change of evapotranspiration and annual water balance. The main objective of this study was to determine and compare the severity of changes in mean annual water balance components between different regions in Bosnia and Herzegovina. Monthly weather data from 26 weather stations in Bosnia and Herzegovina, for the time period of 50 years (1967 – 2016) were used to determine and analyze impact of climate change on the following water balance components: temperature, precipitation, reference evapotranspiration, actual evapotranspiration, total runoff, soil moisture deficit and amount of snow. The results indicate that climate change has a substantial effect on the all water balance components. Air temperature (0.21 - 0.7 °C per decade), reference evapotranspiration (0.61 - 42.81 mm per decade) and soil moisture deficit (1.35 - 27.71 mm per decade) show an increasing trend over the entire territory of Bosnia and Herzegovina with the strongest increase in the north-west part of the country.

Keywords: climate change, soil water balance, soil moisture deficit, evapotranspiration

INTRODUCTION

Based on the analysis of meteorological data from the period 1961–2016, the mean annual temperature is showing a continuous rise on the entire territory of Bosnia and Herzegovina (BiH). The increase in annual air temperature ranges

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from 0.4 to 1.0°C, with more pronounced changes in the continental part (Radusin *et al.*, 2016). There is a positive trend in the mean (0.2-0.5 °C per decade), the maximum (0.3-0.6 °C per decade) and the minimum temperatures (0.3-0.5 °C per decade) throughout the year (Popov *et al.*, 2018b; Trbic *et al.*, 2017), with a significant increase in the frequency of warm extremes (Popov *et al.*, 2018a).

The average annual precipitation is 1,255 mm and it is characterized with the high variation in spatial distribution, which ranges between 706 mm to 3,259 mm (Drešković & Mirić, 2013). In the period 1961–2016 most of the territory of BiH is characterized by a slight increase in the amount of annual sum of precipitation (Popov *et al.*, 2019; Radusin *et al.*, 2016; Vucijak *et al.*, 2014). However, due to the increased intensity and variability of precipitation as well as the increased share of heavy rains in the total amount of rainfall, there is the increased risk of flooding, landslides, hail and soil erosion especially in the north-eastern part of BiH (Radin *et al.*, 2016). Thus, the most vulnerable municipalities to climate change in BiH can be found in this area (Zurovec *et al.*, 2017).

The changes in the air temperature and the amount of precipitation results in changes in evapotranspiration (Cadro *et al.*, 2019; Cadro *et al.*, 2017) and the values of different soil water balance elements (Giugliano *et al.*, 2013). Understanding the spatial and temporal variability of soil water balance elements such as evapotranspiration, water surplus, runoff, soil moisture deficit is essential for many hydrological, agricultural and environmental models (Guler, 2014; Huntington, 2006), especially in assessing regional climate change scenarios and natural hazards (landslides, floods, droughts, wildfires, disease epidemics, insect/animal plagues).

Changes in the soil water balance in the territory of BiH are until now analyzed only for a specific area using a smaller number of weather stations. Increase of potential evapotranspiration (PET) and soil moisture deficit (SMD) was found for Banja Luka, Tuzla, Zenica and Mostar (Čadro *et al.*, 2016). Increasing trends in reference evapotranspiration, runoff and soil moisture deficit and decreasing trend in the amount of the snow were found for the Sarajevo located in central part of BiH (Čadro *et al.*, 2018; Miseckaite *et al.*, 2018).

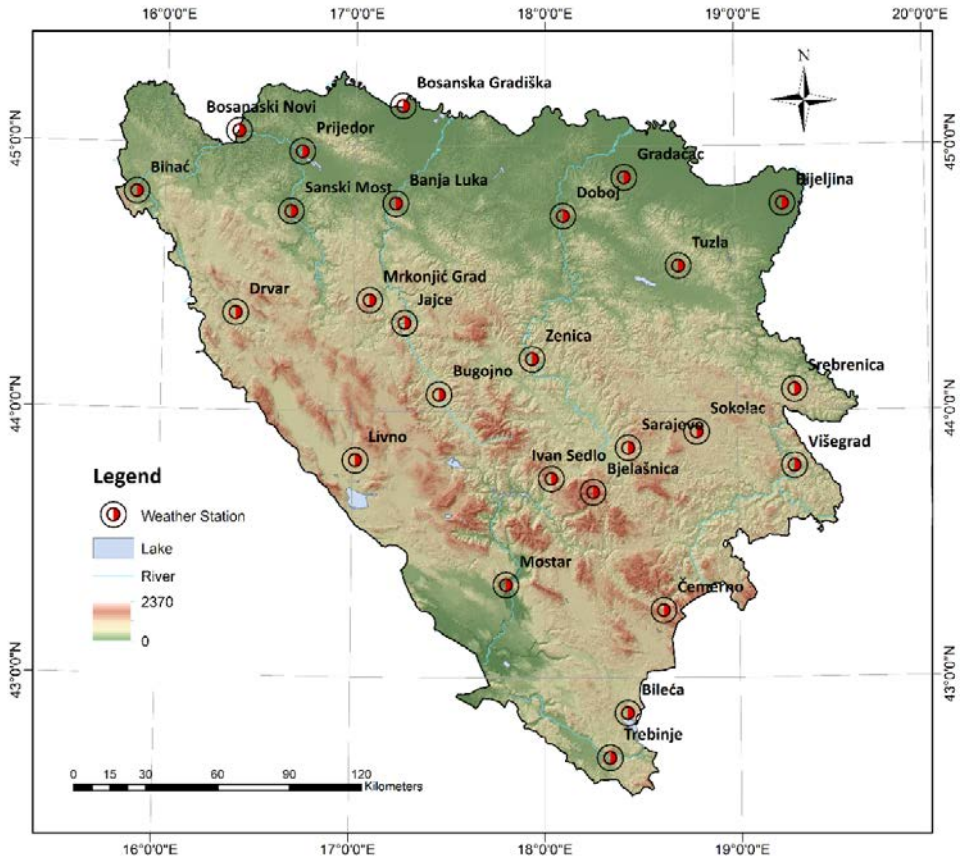
The main challenges when performing this kind of research in BiH is limited data availability, discontinuity of data records and low station density (Cadro *et al.*, 2019) that combined with complex interactions between the nature of the climate parameters and topographical features (Mardikis *et al.*, 2005) especially in areas with the complex terrain such as BiH, additionally makes it difficult to obtain precise results. For all these reasons, when analyzing soil water balance, it is necessary to include as many weather stations (WS) as possible.

Based on the above, the main objective of this study was a precise calculation of all the soil water balance elements in BiH and analysis of their changes in time as a result of climate change taken into consideration long period of time and as many weather stations as possible.

MATERIAL AND METHODS

Study area and data availability

BiH is a country located in south-eastern Europe. It covers an area of around 51,209 km², that is equivalent to 0.5 % of the Europe. Based on the climate regionalization, temperate warm and humid climate has a dominant surface share (64.62 %), followed by *Df* - humid boreal (24.53 %), and Mediterranean climates (10.71 %) (Drešković & Mirić, 2013). The land is mainly hilly to mountainous, with 5% is lowlands, 24% hills, 42% mountains, and 29% of karst area (Čadro et al., 2012; Radusin et al., 2016; Žurovec et al., 2017).



Map 1. Location of 26 selected weather stations in BiH.

Twenty-six weather stations (WS), relatively regularly distributed throughout BiH, were selected for this study (Table 1, Map 1). These WS are collecting all daily climate data required for evapotranspiration and soil water balance calculation and almost all have historical data records for a period of at least 30 years (360 months). Exception are Bosanska Gradiška (240), Trebinje (192), Srebrenica (216), Višegrad (228) and Mrkonjić Grad (264) that are included for a better spatial coverage.

Daily climatic data, including mean (T_{mean}), maximum (T_{max}) and minimum (T_{min}) air temperature, sum of precipitation (P), mean relative humidity (RH), wind speed (u) and sunshine hours (n) for the period 1967 - 2016 (50 years) were collected and averaged over each month. Data were provided by the *Federal Hydrometeorological Institute Sarajevo* and the *Republic Hydrometeorological Service of the Republic of Srpska*. Basic location characteristics and number of months used for each location are shown in Table 1.

Table 1. Location, observation periods and climate characteristics of 26 selected weather stations (WS) in BiH.

WS	z (m)	$^{\circ}E$	$^{\circ}N$	Time period	P (mm)	T_{mean} ($^{\circ}C$)	T_{max} ($^{\circ}C$)	T_{min} ($^{\circ}C$)	RH (%)	u ($m\ s^{-1}$)	n (h)
Bijeljina	90	19.250	44.783	1967-2016	756	11.56	17.50	6.41	79	1.23	4.69
Banja Luka	160	17.216	44.783	1967-2016	1043	11.29	17.34	5.89	76	1.51	5.01
Doboj	147	18.095	44.739	1967-2016	931	11.12	17.05	6.16	79	1.41	4.55
Gradačac	225	18.417	44.883	1981-2016	851	11.90	16.79	7.63	74	2.35	5.59
Bosanska G.	94	17.250	45.150	1986-2016	800	12.30	17.72	7.44	74	2.15	-
Bosanski N.	134	16.384	45.051	1967-2016	1027	10.72	16.66	5.64	78	1.66	-
Prijedor	141	16.721	44.976	1967-2016	960	11.28	16.82	6.19	77	1.1	5.02
Tuzla	305	18.700	44.550	1967-2016	911	10.46	16.80	5.47	76	1.19	4.9
Bileća	443	18.425	42.868	1967-2016	1642	12.24	18.10	6.82	72	1.77	6.52
Mostar	99	17.800	43.350	1967-2016	1474	15.03	20.39	10.48	62	2.51	6.42
Trebinje	280	18.333	42.700	1986-2016	1728	14.48	19.84	10.48	62	-	-
Bihać	246	15.850	44.816	1967-2016	1341	11.10	16.49	6.03	74	1.65	5.04
Drvar	485	16.383	44.367	1967-2016	1134	9.56	16.28	3.71	77	2.98	5.01
Livno	724	17.016	43.816	1967-2016	1147	9.46	15.77	3.54	71	1.71	6.32
Sanski Most	158	16.666	44.750	1967-2016	1044	10.62	17.07	5.16	79	1.85	5.07
Sokolac	913	18.789	43.926	1967-2016	867	7.10	13.21	1.13	79	1.63	4.9
Srebrenica	377	19.300	44.083	1967-2016	985	10.41	15.72	5.77	80	1.2	3.9
Višegrad	416	19.295	43.796	1986-2016	751	11.14	18.26	5.65	76	1.44	-
Čemerno	1304	18.612	43.254	1967-2016	1790	6.21	10.41	2.79	79	2.52	5.35
Ivan Sedlo	968	18.033	43.750	1967-2016	1471	7.58	12.22	3.7	78	2.16	4.5
Jajce	440	17.267	44.333	1967-2016	907	10.27	16.06	4.9	77	1.56	4.05
Bugojno	562	17.450	44.066	1967-2016	839	9.37	15.7	3.79	75	1.99	4.61
Bjelašnica	2067	18.250	43.700	1967-2016	1233	1.49	3.68	-0.66	84	7.87	4.77
Mrkonjić G.	575	17.084	44.419	1986-2016	1053	9.71	15.5	5.06	77	2.27	4.54
Sarajevo	630	18.433	43.866	1967-2016	938	10.01	15.62	5.31	71	1.63	4.96
Zenica	344	17.933	44.200	1967-2016	812	10.68	17.13	5.38	74	1.54	4.48

Reference evapotranspiration (ET_o)

Reference evapotranspiration (ET_o) required for the soil water balance calculation, was calculated using standard FAO-PM equation, given by (Allen et al., 1998):

$$ET_0 = \frac{0.408\Delta \cdot (R_n - G) + \gamma \cdot \frac{900}{T_{mean} + 273} \cdot u_2 \cdot (e_s - e_a)}{\Delta + \gamma \cdot (1 + 0.34 \cdot u_2)} \quad (1)$$

where ET_o is the reference evapotranspiration (mm day^{-1}), R_n the net radiation at the crop surface ($\text{MJ m}^{-2} \text{day}^{-1}$), G the soil heat flux density ($\text{MJ m}^{-2} \text{day}^{-1}$), T_{mean} the mean daily air temperature at 2 m height ($^{\circ}\text{C}$), u_2 the wind speed at 2 m height (m s^{-1}), e_s the saturation vapor pressure, e_a the actual vapor pressure, $e_s - e_a$ the saturation vapor pressure deficit, Δ the slope of the vapor pressure curve ($\text{kPa } ^{\circ}\text{C}^{-1}$) and γ is the psychrometric constant ($\text{kPa } ^{\circ}\text{C}^{-1}$).

All necessary parameters required for calculation of ET_o where computed following the procedure developed in FAO-56 (Allen et al., 1998) via *REF-ET: reference Evapotranspiration Calculator* (Allen & Zhenguli, 2016) software.

Since reflected solar radiation (R_s) is required for R_n calculation and this parameter is not measured on WS in BiH, it was estimated from the measured sunshine hours data (The Campbell–Stokes sunshine recorder) with the Ångström (1924) equation:

$$R_s = (a_s + b_s \cdot n/N) \cdot R_a \quad (2)$$

where R_a is the extraterrestrial radiation ($\text{MJ m}^{-2} \text{day}^{-1}$) calculated for each day of the year and for different latitudes, from the solar constant ($G_{sc} = 0.0820 \text{ MJ m}^{-2} \text{min}^{-1}$), the solar declination (δ) and the time of the year (J) and then by selecting the R_a for 15th day of each month converted to monthly values, n is the actual duration of sunshine (h), N is the maximum possible duration of sunshine or daylight hours (h), as is the regression constant, expressing the fraction of extraterrestrial radiation reaching the earth on overcast days ($n = 0$) and $a_s + b_s$ is the fraction of extraterrestrial radiation reaching the earth on clear days ($n = N$). In the absence of actual solar radiation (R_s) measurements, the values $a_s = 0.25$ and $b_s = 0.5$ were used as suggested by Allen et al. (1998).

For the four WS (Bosanska Gradiška, Bosanski Novi, Trebinje and Višegrad) where measured solar radiation data (R_s) or sunshine hours data were missing for a certain month, solar radiation was estimated using Hargreaves' formula (Hargreaves & Samani, 1985) (Eq. 3), as suggested in Allen et al. (1998):

$$R_s = k_{R_s} \sqrt{(T_{max} - T_{min})} \times R_a \quad (3)$$

where R_a is extra-terrestrial radiation (MJ m⁻² d⁻¹), T_{max} maximum air temperature (°C), T_{min} minimum air temperature (°C), k_{Rs} adjustment coefficient (°C^{-0.5}). Value of $k_{Rs} = 0.16$ was used for Trebinje, $k_{Rs} = 0.14$ for Višegrad and Bosanski Novi and $k_{Rs} = 0.13$ for Bosanska Gradiška as suggested by Cadro *et al.* (2019) and Čadro *et al.* (2017a).

Actual vapor pressure (e_a) was derived from relative humidity data (Allen *et al.*, 1998) as:

$$e_a = \frac{e^{\circ}(T_{min})^{\frac{RH_{max}}{100}} + e^{\circ}(T_{max})^{\frac{RH_{min}}{100}}}{2} \quad (4)$$

where e_a is actual vapor pressure (kPa), $e^{\circ}(T_{min})$ saturation vapor pressure at daily minimum temperature (kPa), $e^{\circ}(T_{max})$ saturation vapor pressure at daily maximum temperature (kPa), RH_{max} maximum relative humidity (%), RH_{min} minimum relative humidity (%). In the absence of relative humidity data, e_a was estimated by assuming that the dew point temperature (T_{dew}) is close to daily minimum temperature (T_{min}) (Allen *et al.*, 1998). When wind speed was not available, the average regional wind speed value was used.

Soil water balance

Monthly water balance was calculated by using Thornthwaite-Mather method (Thornthwaite & Mather, 1955; Thornthwaite & Mather, 1957) that was modified and later described in Dingman (2002). Except data on monthly precipitation (P) and evapotranspiration (ET_o) applied water balance requires data on soil available water content ($SOIL_{max}$). The value $SOIL_{max} = 100$ mm was used (McBean *et al.*, 1995) since it is regionally (BiH, Serbia and Croatia) the most commonly used value (Šimunić, 2013; Vlahinić, 2004).

To detect the trends within time series of water balance components (annual precipitation - P , reference evapotranspiration - ET_o , actual evapotranspiration - AET , soil moisture deficit - SMD , total runoff - TRO and $SNOW$) parametric method of linear regression was used, as shown in following equation:

$$y = a + b \times x \quad (5)$$

where x is the explanatory variable, y the dependent variable, b the slope of the line and a the intercept. The slope (b) indicates the mean temporal change of the studied variable. Positive values of the slope show increasing trends, while negative values of the slope indicate decreasing trends (Gocic & Trajkovic, 2013, 2014).

RESULTS AND DISCUSSION

The descriptive statistics (mean and CV) and the slope (b) for all analyzed climate and soil water balance elements (T_{mean} , P , ET_o , AET , SMD , TRO and

SNOW) at 26 selected WS for the period 1967 – 2016, are summarized in table 2, 3 and 4.

Table 2: Results for the statistical tests for the annual air temperature (T_{mean}) and sum of precipitation (P) - 26 weather stations (WS) in BiH, period 1967 – 2016.

Element Weather station	T_{mean}			P		
	Mean	b	CV	Mean	b	CV
Bijeljina	11.56	0.047	0.255	756	0.535	36.85
Banja Luka	11.29	0.049	0.249	1,042	-0.228	52.50
Doboj	11.12	0.038	0.211	931	2.555	53.77
Gradačac	12.05	0.070	0.272	840	1.465	48.78
Bosanska G.	12.24	0.063	0.224	799	2.111	52.92
Bosanaski Novi	10.72	0.043	0.248	1,027	0.690	49.42
Prijedor	11.28	0.039	0.255	947	-0.994	44.74
Tuzla	10.45	0.036	0.221	919	0.422	48.02
Bileća	12.18	0.021	0.167	1,658	0.733	99.06
Mostar	15.03	0.037	0.191	1,474	-1.021	95.06
Trebinje	14.95	0.037	0.150	1,747	-0.792	107.59
Bihac	11.10	0.036	0.215	1,341	2.804	59.67
Drvar	9.48	0.034	0.189	1,125	2.461	55.28
Livno	9.46	0.043	0.206	1,147	0.826	55.08
Sanski Most	10.62	0.036	0.204	1,045	0.507	45.71
Sokolac	6.89	0.046	0.241	854	3.406	42.08
Srebrenica	10.41	0.033	0.215	985	4.155	51.30
Višegrad	11.15	0.052	0.217	757	4.318	40.54
Čemerno	6.21	0.031	0.183	1,790	-1.914	92.46
Ivan Sedlo	7.57	0.028	0.184	1478	0.648	76.58
Jajce	10.16	0.028	0.177	909	-0.799	39.69
Bugojno	9.37	0.046	0.233	841	-0.382	35.75
Bjelašnica	1.49	0.023	0.154	1,228	10.093	81.96
Mrkonjić Grad	9.71	0.039	0.216	1,053	7.647	58.66
Sarajevo	10.01	0.037	0.202	937	0.824	41.05
Zenica	10.68	0.044	0.218	812	0.842	37.39
BiH	10.28	0.040	0.211	1,094	1.574	57.77

Note: b – slope ($^{\circ}\text{C year}^{-1}$ and mm year^{-1}), CV – Coefficient of variation (%).

The mean annual air temperature in BiH is 10.20°C , ranging from 1.49°C (WS Bjelašnica) to 15.03°C (WS Mostar). The T_{mean} shows increasing trends at all WS, ranging from $0.021^{\circ}\text{C year}^{-1}$ (WS Bileća) to $0.070^{\circ}\text{C year}^{-1}$ (WS Gradačac). In general, the entire territory of BiH shows warming trend of 0.4°C per decade (Table 2). In addition, increases in air temperature over the last ten years are even more pronounced (Radusin et al., 2016). Trend intensity differ

between country regions. It is highest in the north ($0.036\text{-}0.070\text{ }^{\circ}\text{C year}^{-1}$) and lowest in the south - Mediterranean part of the country ($0.021\text{-}0.037\text{ }^{\circ}\text{C year}^{-1}$). The highest variations in annual temperature are determined for the 3 WS (Bijeljina, Gradačac and Prijedor), all located at the north of BiH.

Mean annual precipitation in BiH is 1,094 mm, ranging from 756 mm (WS Bijeljina) to 1,747 mm (WS Trebinje). Trends of the annual amount of precipitation (P) for majority of analyzed WS are increasing ($0.507\text{-}10.093\text{ mm year}^{-1}$). However, there is few locations (Mostar, Trebinje, Bugojno, Mrkonjić Grad), especially in Hercegovina (south BiH), that are showing decreasing trend of precipitation, ranging up to $-1.914\text{ mm per year}$. Similarly, in other studies decrease in total precipitation and the number of days with precipitation occurrence was found, whereas the duration of dry periods is prolonged over the entire East Herzegovina region (Popov *et al.*, 2019).

On the other hand, the highest increasing trend is found for mountain WS Bjelašnica, ranging 10.093 mm of precipitation per year, or a 100 mm over a period of 10 years. The highest variations in annual precipitation was found for WS in south (Trebinje) and WS at higher altitudes (Bjelašnica and Čemerono). Also, as confirmed with other studies, trends are not spatially and temporally coherent (Popov *et al.*, 2017). For the most of WS changes in annual precipitation are not significant, they are more pronounced by seasons (Radusin *et al.*, 2016), especially during the last decade (2007-2016), resulting in increased frequency of months with extreme precipitation (Popov *et al.*, 2017), catastrophic floods landslides and soil erosion.

The air temperature and precipitation change patterns in BiH are consistent with the predominant trends in other areas of East Europe (Branković *et al.*, 2013; Bukantis & Rimkus, 2005a; Bukantis & Rimkus, 2005b; Burić *et al.*, 2013; Jaagus *et al.*, 2009; Rutgersson *et al.*, 2014; Tripolskaja & Pirogovskaja, 2013; Unkasevic & Tosić, 2013) and with trends observed globally (Jacob *et al.*, 2018; Kharin *et al.*, 2013; Popov *et al.*, 2018b; Trenberth *et al.*, 2013).

Based on 26 analyzed WS, the mean ET_0 for BiH is 780 mm, while AET that beside climate conditions depend on the soil moisture, is 140 mm lower, or 640 mm in average, the difference represents SMD (Table 3). In study that included 108 WS in BiH and similar methodology, mean annual SMD of 143 mm was found (Čadro *et al.*, 2019), the small difference in SMD confirms the accuracy of the data obtained in this study. Similarly, to the T_{mean} reference evapotranspiration (ET_0) shows increasing trends at all WS, ranging from $0.061\text{ mm year}^{-1}$ (WS Trebinje) to $4.281\text{ mm year}^{-1}$ (WS Višegrad).

In general, for the entire territory of BiH increasing trend of ET_0 is $20.59\text{ mm per decade}$ (Table 3). The highest trend is found for the area from Drvar in the west to Banja Luka at the north of the country, as well as the area around Višegrad in the east.

Table 3. Results for the statistical tests for the annual reference evapotranspiration (ET_0), actual evapotranspiration (AET) and soil moisture deficit (SMD) - 26 weather stations (WS) in BiH, period 1967 – 2016.

Element Weather station	ET_0			AET			SMD		
	Mean	<i>b</i>	CV	Mean	<i>b</i>	CV	Mean	<i>b</i>	CV
Bijeljina	714	0.549	11.91	578	-0.102	19.50	136	0.651	22.56
Banja Luka	785	3.242	17.86	667	0.502	16.03	118	2.739	25.93
Doboj	725	1.308	13.53	624	-0.025	15.22	101	1.333	21.60
Gradačac	860	2.897	17.32	645	1.367	20.99	215	1.529	32.61
Bosanska G.	798	2.278	17.27	600	1.182	21.52	198	1.096	32.00
Bosanaski N.	763	1.610	15.46	650	-0.195	17.11	113	1.804	23.61
Prijedor	668	3.034	20.01	582	0.597	13.01	87	2.438	21.82
Tuzla	730	1.443	12.54	637	0.195	15.62	92	1.249	22.25
Bileća	896	3.509	20.16	694	0.738	21.52	202	2.771	29.70
Mostar	1,086	1.640	19.61	770	-0.597	24.96	316	2.237	36.94
Trebinje	1,190	0.061	15.52	854	-1.900	26.59	336	1.961	37.05
Bihać	782	1.734	12.99	699	0.044	14.13	83	1.690	19.49
Drvar	827	3.947	23.26	694	2.084	19.49	133	1.863	28.35
Livno	809	0.826	12.13	640	-0.161	16.44	168	1.369	21.91
Sanski Most	777	1.652	14.04	668	-0.278	13.07	109	1.931	21.75
Sokolac	680	1.946	17.23	574	1.946	15.18	106	1.361	20.10
Srebrenica	675	1.780	18.32	617	0.531	15.38	58	1.248	19.83
Višegrad	843	4.281	15.20	614	4.056	25.39	229	0.225	28.81
Čemerno	695	0.664	12.61	607	0.028	12.05	88	0.636	14.20
Ivan Sedlo	694	2.029	14.65	626	1.450	13.03	69	0.579	16.45
Jajce	717	1.805	13.25	623	0.977	15.56	94	0.828	19.96
Bugojno	766	1.597	13.51	620	0.672	14.12	146	0.925	22.13
Bjelašnica	516	1.570	16.94	441	1.435	11.87	75	0.135	11.70
Mrkonjić Grad	749	3.516	17.88	653	3.530	16.16	96	-0.014	19.74
Sarajevo	780	2.009	13.18	641	0.953	16.05	139	1.056	22.45
Zenica	777	2.617	19.91	614	0.743	17.86	163	1.874	29.59
BiH	780	2.059	16.01	640	0.760	17.22	141	1.366	23.94

Note: *b* – slope (mm year^{-1}), CV – Coefficient of variation (%).

AET trend has a similar spatial distribution but higher variation than ET_0 trend. For the south, north-west and north-east part decreasing (from -0.025 to -1.900 mm year^{-1}) or low increasing trend (0.028-0.597 mm year^{-1}) was found. While for the central part, from Drvar to Višegrad, or the mountainous region of the country (Dinarides), high increasing trend were recorded (0.597-4.054 mm year^{-1}). AET can be a measure of agricultural water productivity, these results indicate an improvement of general conditions for agricultural production in the central - mountainous region of BiH. Thus, Climate change may have a positive

effect on the yield in this area (Čadro *et al.*, 2018; Radusin *et al.*, 2016), and in same time negative effect on the rest of the country (north and south). Such positive effect also found in similar studies, especially for the northern Europe (Jacob *et al.*, 2018), while the rest of Europe, especially the Mediterranean region, will mostly be negatively affected (Behrens *et al.*, 2010).

Table 4. Results for the statistical tests for the annual total runoff (*TRO*) and amount of snow soil moisture deficit (*SNOW*) - 26 weather stations (WS) in BiH, period 1967 – 2016.

Element Weather station	<i>TRO</i>			<i>SNOW</i>		
	Mean	<i>b</i>	CV	Mean	<i>b</i>	CV
Bijeljina	178	0.922	18.20	118	-0.920	15.62
Banja Luka	377	-0.497	33.80	170	-1.696	23.25
Doboj	308	2.871	34.97	150	-0.932	19.53
Gradačac	195	1.142	22.79	115	-2.471	17.32
Bosanska Gradiška	207	0.230	32.47	115	-2.469	16.15
Bosanski Novi	380	0.784	33.85	176	-1.590	22.74
Prijedor	368	-1.545	30.02	163	-1.922	20.27
Tuzla	280	0.374	30.06	157	-1.171	20.66
Bileća	963	-0.079	79.66	201	-0.607	32.44
Mostar	711	-0.522	74.67	40	-1.050	13.83
Trebinje	885	5.361	78.62	46	-1.363	17.36
Bihać	640	2.559	45.85	216	-1.035	31.19
Drvar	432	-0.395	42.87	240	-2.295	27.54
Livno	511	0.926	42.94	298	-2.366	36.73
Sanski Most	379	0.950	30.17	180	-0.979	22.67
Sokolac	285	1.560	24.22	266	-0.409	19.42
Srebrenica	361	3.621	36.50	157	-0.965	22.78
Višegrad	149	-0.349	18.04	133	-1.042	17.24
Čemerno	1,190	-1.906	86.51	820	-3.545	62.67
Ivan Sedlo	855	-0.770	61.39	520	-2.284	49.87
Jajce	286	-2.153	26.77	163	-2.363	20.54
Bugojno	223	-1.115	22.85	184	-1.848	22.29
Bjelašnica	783	8.770	75.13	697	6.611	55.53
Mrkonjić Grad	405	4.441	42.30	216	-0.418	30.87
Sarajevo	299	-0.165	26.14	204	-0.917	25.42
Zenica	200	0.196	19.96	148	-1.010	18.23
BiH	456	0.970	41.18	227	-1.194	26.24

Note: *b* – slope (mm year⁻¹), CV – Coefficient of variation (%).

SMD is result of difference between evapotranspiration and available soil moisture (Kos et al., 1993; Žurovec, 2012). The *SMD* shows increasing trends at all WS (0.135-2.771 mm year⁻¹) except Mrkonjić Grad (-0,014 mm year⁻¹). In average the entire BiH territory shows the increasing trend in *SMD*, ranging 13.66 mm per decade (Table 3) that will in the future cause more severe long-lasting and extreme droughts and higher yield reduction. The increasing trends and year to year variations are high at the north-west (1.096-2.739 mm year⁻¹) and south regions (1.961-2.771 mm year⁻¹), while the central and east parts (-0.014-1.361 mm year⁻¹) of BiH are less affected by the changes in *SMD*. These results are in line with previous studies of water scarcity and vulnerability to climate change all over BiH and region (Cindrić et al., 2010; Čadro et al., 2017b; Čadro et al., 2016; Čustović et al., 2015; Hodžić et al., 2013; Miseckaite & Čadro, 2018; Miseckaite et al., 2018; Perčec Tadić et al., 2014; Vlahinić et al., 2006; Whan et al., 2015; Žurovec & Čadro, 2015; Žurovec et al., 2017).

The mean *TRO* for BiH is 465 mm, the highest was found for the WS Čemerno (1190 mm) and the lowest, almost ten time smaller, for the WS Višegrad (149 mm). It is interesting to note that these locations are relatively close to each other (Map 1), and this confirms the statement of the complexity of natural conditions in BiH and the need for as many WS as possible. *TRO* trends are not spatially coherent. The high variations in trend, from decreasing (-2.153 mm year⁻¹) to increasing (8.770 mm year⁻¹) trends were found. In average for BiH there a low positive trend of *TRO*, which amounts 9.70 mm per decade.

The average amount of snow for BiH is 227 mm, and this climate element, comparing to all others, shows decreasing trend for all WS. Exception is mountain WS Bjelašnica (6.611 mm year⁻¹). The average decreasing trend of snow for BiH is -11.94 mm per ten years and the highest decreasing trend was found for WS Čemerno (-35.45 mm per decade). Also, the highest variations were found for mountain WS, Čemerno, Bjelašnica and Ivan Sedlo.

CONCLUSIONS

Linear regression was applied to determine soil water balance response to climate change in BiH. The air temperature, precipitation, FAO-56 PM reference evapotranspiration and water balance components: actual evapotranspiration, total runoff, soil moisture deficit and amount of snow trends were analyzed. Monthly weather data from 26 weather stations, for the time period of 50 years (1967 – 2016) were used.

If observed on the level of BiH, the results obtained show increasing trends in *T*, *P*, *ET₀*, *AET*, *SMD* and *TRO* series and decreasing trend in the amount of the *SNOW*. However, at the regional level, there are a lot of differences between climate elements trends. This is especially evident for the *P*, *AET* and *TRO*.

At the regional level, as the result of climate change, the highest increase in dry conditions was found for the north-west BiH (Bosanski Novi, Prijedor, Sanski Most, Banja Luka, Gradačac), where high increase in temperature (0.36 – 0.70 °C per decade) and *SMD* (18.04 – 27.39 mm per decade) trends were

identified, while the trend of *AET* is showing decline for the most of weather stations. Similarly, high increase of *SMD* and decline of *AET* was found in the south, for the weather stations Mostar, Bileća and Trebinje (19.61 – 27.71 mm per decade), however, there the intensity of the temperature increase is lower (0.21 – 0.37 °C per decade).

When it comes to surplus, the biggest changes or the highest increasing trends are in the area of Bjelašnica, Trebinje and Drina and Bosna river basins. Area already affected by heavy rains cosign soil erosion, landslides and floods (2010, 2014, 2019). In same time, increasing trends of *AET* are indicating an improvement of general conditions for agricultural production in the central - hilly region of BiH, from Drvar in the west to Višegrad in the east.

Based on these results, priority areas for the regionally specific climate change adaptation measures, protection from natural hazards (drought, floods, landslides) as well as actions for the disaster risk reduction could be identified.

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MODELING SNOWMELT-RUNOFF UNDER CLIMATE CHANGE SCENARIOS IN THE BEHESHTABAD WATERSHED

SUMMARY

The aim of this study was to evaluate the variability of time distribution and contribution of runoff from snowmelt under the influence of climate change in the Beheshtabad Watershed, Iran using the Snowmelt-Runoff Model (SRM) and Long Ashton Research Station Weather Generator Model (LARS-WG). The LARS-WG model accuracy in downscaling of GCMHadCM3 output with A1B emissions scenario was evaluated based on data for the base period (1986-2010) and climatic data for the future periods (2011-2030 and 2046-2065) were obtained. The SRM variables and parameters were prepared from the Shahrkord station and Snow Cover Areas (SCAs) were obtained by MODIS satellite images. After the calibration and validation of SRM model, then the SRM model was run with the future data and revealed the effects of climate change on snowmelt runoff. The results show the displacement of the peak flow from April to March, and reducing the contribution of snowmelt runoff from 27.2 to 24.5 and 22.3 percent for two future periods. The present study confirmed the effects of climate change on future climate data and discharge and temporal pattern of snowmelt-runoff.

Keywords: Climate change, Snowmelt-runoff model, LARS-WG model, Temporal pattern of runoff.

INTRODUCTION

The changes in future climate and its implications have always been very important aspect for world's water resources (Adnan *et al.* 2017). The snow is one of the important forms of precipitation in the hydrologic cycle in mountainous regions and plays a valuable role to provide drinking water and agricultural resources. The snow is one of the major sources of water in most parts of the world. The estimation of the equivalent water of snowmelt is considered as one of the most important activities of hydrologists. Because more than 10 percent of the earth's surface is covered permanently by glaciers and 30% of its surface is covered by snow in the northern hemisphere in winter (Sayedi Elmabad *et al.* 2010).

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According to studies conducted in Iran by about 60 percent of surface water and 57 percent of groundwater in the snowy regions feed by snowmelt water (Sayedi Elmabad *et al.* 2010). However, in most watersheds, the required meteorological and hydrologic data for the simulation, similar to snow survey data usually are not available (Barovic *et al.*, 2015; Khaledi Darvishan *et al.*, 2017; Spalevic *et al.*, 2017)). Thus, it can be formulated with factors affecting environmental energy needed to melt and snowmelt (Ferguson 1999). In this field, snowmelt is estimated with various models classified as energy balance, degree-day and radiation-temperature. Cline *et al.* (1998) and Homan *et al.* (2011) calculated snowmelt-runoff using energy balance. Although the energy balance model has strong physically base, but it needs many data and not be used due to lack of data on mountain watersheds (Vafakhah *et al.* 2015). Some models such as snowmelt runoff model (SRM) have been designed to predict the daily snowmelt and applied widely for snowmelt simulation (Martinec *et al.* 2008).

Previous studies show that in most parts of the world, climate change led to increase in temperature, extreme events and entropy and to decrease rainfall. In addition, the amount of snow and snow period will decrease and therefore, the volume of runoff will increase in winter and reduce in the spring due to climate change (Hugo 2003). The investigation of climate change effect on water resources and specifically on the snowmelt runoff can greatly enhance the accuracy of the simulation and regardless of the fact that the climate is changing, we can't carry out realistic planning of exploitation of water and snow resources (Hardy 2003).

Several attempts have been made to investigate the effects of climate change on snowmelt-runoff. A study conducted by Payne *et al.* (2003) in the Columbia River basin for period the 2040-2060 predicted temperature increase of 1.2°C and the average winter precipitation decrease of 3 percent, relative to base time. Ma and Cheng (2003) showed that temperature increase of 4°C, the snow cover area (SCA) and snowmelt season shift towards earlier dates, and the snowmelt runoff using SRM model is changed significantly in the Gongnaisi River basin in the western Tianshan Mountains. Stewart *et al.* (2004) showed that a shift 30–40 days would occur in the timing of springtime snowmelt in Western North America for the 1995–2099 period. Miller *et al.* (2004) for a set of California river basins predicted that late winter snow accumulation decreases by 50 percent toward the end of this century. Jian and Shuo (2005) simulated the changes of snowmelt runoffs in response to a warming of 4°C using SRM on the upper Heihe Watershed in northwestern China. The result of the simulation indicated that a forward shifting of snow melting season, an increase in water flows in earlier melting season, and a decline in flows in later melting season would result. Hreiche *et al.* (2007) simulated the changes of flow characteristics in response to a warming of 2°C on Lebanese catchments. Their results showed that droughts would occur days to one month earlier and snowmelt floods would often replace by rainfall floods. Changchun *et al.* (2007) analyzed annual temperature and precipitation time series and SCA for the 1982–2001 period. The

SCA slowly increased and the effect of precipitation on SCA is larger than that of temperature. Ma *et al.* (2013) analyzed the impact of climate change on snowmelt runoff using Hadley Centre Coupled Model version 3 (HadCM3) and SRM in Kaidu Watershed, Northwest China. The results indicated that the streamflow in spring would increase with the increased mean temperature and the discharge and peak flow in summer would decrease with the decreased precipitation. Khadka *et al.* (2014) investigated the impact of climate change on SCA and snowmelt runoff in the Tamakoshi basin of Nepal. The results showed that temperature, precipitation, streamflow and the number of days with high discharge would increase. A comprehensive study of response of snow basins to climate change in the mountains is still lacking in Iran, mainly because of the inaccessible terrain, lack of observed climatic data, and the fact that response of snow is not uniform throughout the all mountains. The aim of this study is to investigate the effect of climate change on SCA and the snowmelt runoff in the Beheshtabad Watershed as a part of the Karun basin, Iran.

MATERIALS AND METHODS

Study area

The Beheshtabad Watershed is located in the northern part of the Karun basin and Chaharmahal and Bakhtiari Province with an area of about 3905 km² and a geographical position of 50° 23' to 51°25' east and 31°49' to 32°34' north (Fig. 1). The elevation ranges from 1660 m above sea level at the outlet of the watershed to 3620m a.s.l. on Saldaran Mountain. The mean annual temperature and the mean annual precipitation are 11°C and 471 mm, respectively of which 245 mm falls during the winter months, 89 mm during spring, 5 mm during summer and 132 mm during autumn. About 55% of precipitation in the Beheshtabad Watershed falls as snow. Approximately 42% of the watershed is covered by pasture, 12% by rocks and 46% of the land is used for agricultural activities (Rostamian *et al.* 2008).

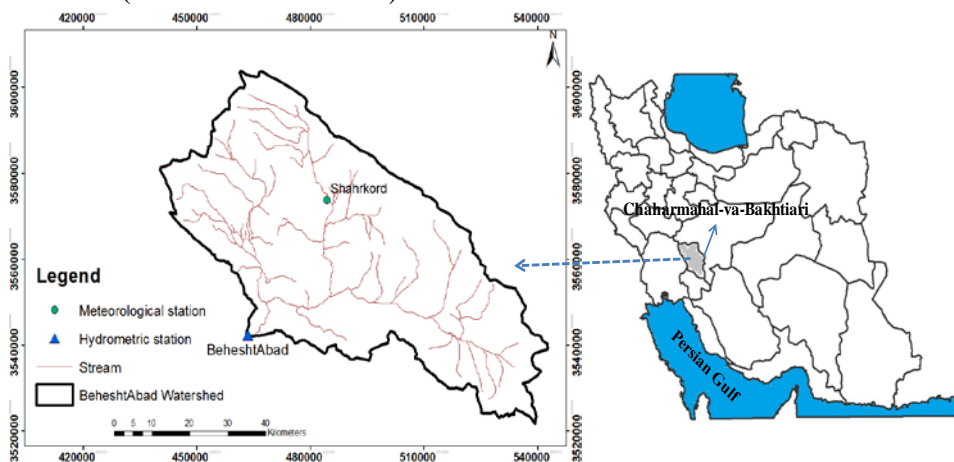


Fig 1. Location of the Beheshtabad Watershed in Iran

Long Ashton Research Station Weather Generator Model (LARS-WG)

LARS-WG is a stochastic weather generator (Semenov 2008), and it is widely used for the climate change assessment. This model is useful for producing the daily precipitation, daily solar radiation, and daily maximum and minimum temperatures at a particulate site under the present and future climate conditions.

The LARS-WG uses input observed daily weather data for a station to determine probability distributions of parameters specifying for weather variables as well as correlations between the variables (Semenov and Brooks 1999; Khordadi *et al.* 2015).

Complex statistical distribution model is employed by LARS-WG model for the purpose of modeling meteorological variables. The duration of wet and dry periods, semi-empirical distribution of radiation series and daily precipitation data are the basis for modeling. Calibration of the model, assessment of model, and production of meteorological data are the main parts of this model (Babaiya and Najafinik 2006; Hashmi *et al.* 2011).

HadCM3 is a coupled atmosphere-ocean general circulation model (AOGCM) developed at the Hadley Centre in the United Kingdom with a spatial resolution of $2.5^{\circ} \times 3.75^{\circ}$ (Khadka *et al.* 2014).

In order to downscale using LARS-WG, the ability of LARS-WG for producing the weather time series i.e. daily precipitation, daily maximum and minimum temperatures in the period of 1986–2010 from the Shahrekord station was analyzed. To do this, the weather time series include daily precipitation, daily maximum and minimum temperatures were used as an input to LARS-WG for simulating the weather time series. The statistical properties of the simulated time series were compared to those of the observed time series using t-test, Nash-Sutcliffe coefficient efficiency (NS) (Equation 3) (Nash and Sutcliffe 1970) and coefficient of determination (R^2).

Snowmelt Runoff Model (SRM)

Meteorological and hydrological characteristics of watershed and variables are necessary data to implement SRM model. Model input variables are distributed among elevation zones (each with approximately 500 m of relative relief), and include daily average air temperature, daily total precipitation, and SCA (Vafakhah *et al.* 2015). The following equation is used in SRM to simulate daily streamflow discharge:

$$Q_{n+1} = [c_{S_n} a_n (T_n + \Delta T_{n+1}) S_n + c_{R_n} P_n] \frac{10000}{86400} (1 - k_{n+1}) A + Q_n k_{n+1} \quad (1)$$

where Q (m^3/s) is the average daily discharge, c is runoff coefficient expressing the losses as a ratio (runoff/precipitation), with c_S referring to snowmelt and c_R to rain, a ($cm/^{\circ}C.day$) is the degree-day factor indicating the snowmelt depth resulting from 1 degree-day, T ($^{\circ}C.day$) is number of degree-days, ΔT_{n+1} ($^{\circ}C.day$) is the adjustment by temperature lapse rate when

extrapolating the temperature from the station to the average hypsometric elevation of the basin or zone, $P(\text{cm})$ is the precipitation (rainfall) contributing to runoff, S is ratio of the snow covered area to the total area, A (km^2) is the area of the basin (or elevation zone), k is the recession coefficient indicating the decline of discharge in a period without snowmelt or rainfall, n is the sequence of days during the simulation period, and $10000/86400$ converts $\text{cm.k m}^2/\text{day}$ to m^3/s .

The Beheshtabad Watershed has been divided into four elevation zones using the topography maps at a scale of 1:50000 obtained from geographical organization in Iran (Fig. 2).

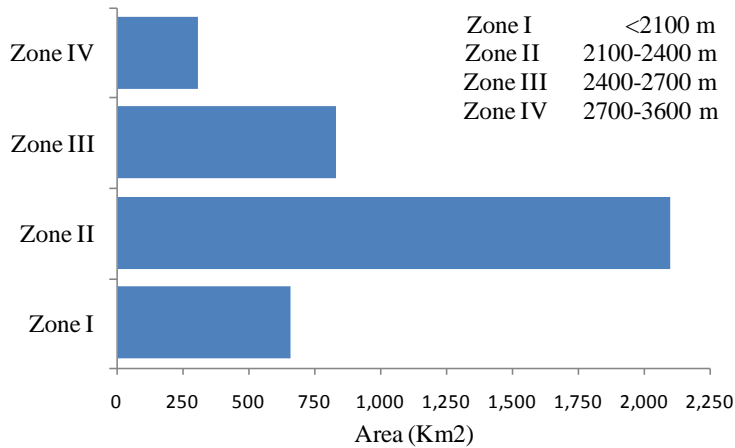


Fig 2. Elevation zones in the Beheshtabad Watershed

In this study, MODIS TERRA satellite with spatial resolution of 250 and 500 m was used to estimate SCA in the watershed. Normalized Difference Snow Index (NDSI) is used as a criterion to separate snow cover from other land covers. Snow has high reflectance of visible radiation and strong absorption in middle infrared wavelength which are used to separate it from other land covers. Reflectance in band 4 (0.545–0.565 μm) and band 6 (1.628–1.652 μm) are used to calculate NDSI as:

$$NDSI = \frac{MODIS_{Band4} - MODIS_{Band6}}{MODIS_{Band4} + MODIS_{Band6}} \quad (2)$$

In the non-forest area, NDSI threshold value of 0.4 is used to delineate snow area along with reflectance in band2 $\geq 11\%$ and reflectance in band4 $> 10\%$ (Hall *et al.*, 1995). MODIS images for water years of 2012-2013 and 2013-2014 were used for SRM calibration and validation periods, respectively in the study.

The statistical properties of the simulated time series were compared to those of the observed data using NS and volume difference (D_v) in order to test the ability of SRM model for reproducing the observed data statistics:

$$NS = 1 - \frac{\sum_{i=1}^n (Q_o - Q_e)^2}{\sum_{i=1}^n (Q_o - \bar{Q}_o)^2} \quad (3)$$

where Q_o and Q_e are the observed and simulated discharge, \bar{Q}_o is average observed discharge.

$$D_V[\%] = \frac{V_o - V_e}{V_o} \times 100 \quad (4)$$

where V_o is the observed yearly runoff volume, V_e is the estimated yearly runoff volume.

RESULTS AND DISCUSSION

LARS-WG model was performed based on the historical climate data obtained from 1986-2010 for verification of the model. For this purpose, a large number of years of simulated daily weather data were generated. In addition, to evaluate the model's ability to simulate meteorological data in observed period, p-value, NS and R^2 were used and the results were analyzed (Table 1). The results of statistical index showed high accuracy of model in production climate data. So that the minimum and maximum temperature values have the highest correlation and the rainfall is the lowest correlation. The model shows a better performance for the maximum and minimum temperatures than precipitation. In general, simulation of precipitation is more complex and difficult to obtain a good agreement between observed and simulated compared with downscaling of temperature (Fowler *et al.* 2007; Hassan *et al.* 2014).

Table 1. Results of LARS-WG evaluation in the Beheshtabad Watershed

Variable	p-value	Nash-Sutcliff	R^2
Precipitation	0.022	0.94	0.98
Minimum temperature	0.29	0.97	0.99
Maximum temperature	0.71	0.99	0.99

Figs. 3 and 4 summarize results of climate change analysis. As can be seen from the Figs. 3 and 4, both temperature and precipitation are predicted to increase in the future periods.

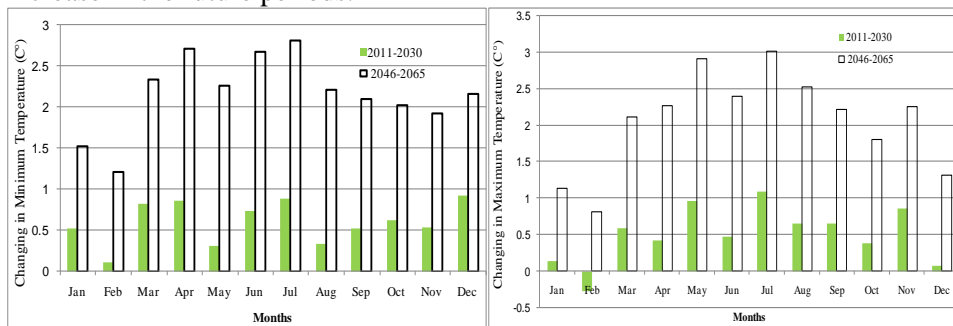


Fig 3. Future changes in Tmax and Tmin with respect to the historical data (1986–2010) under HadCM3 A1B scenario

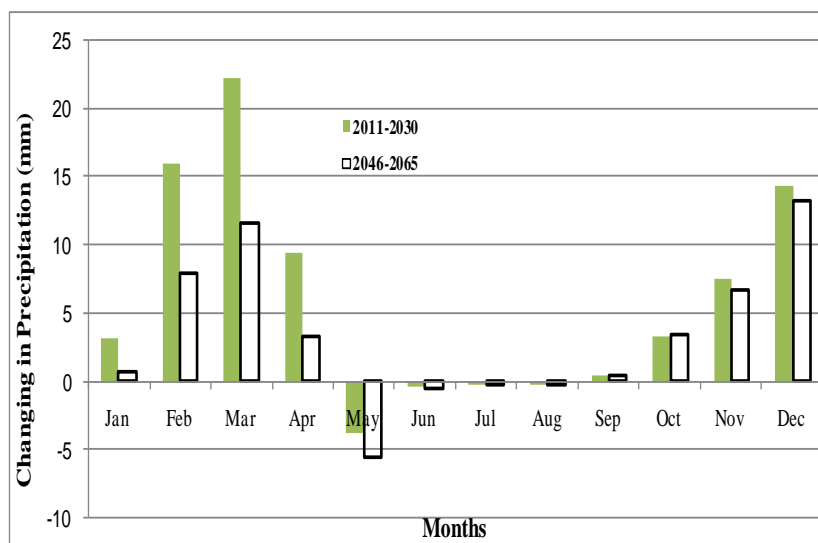


Fig 4. Future changes in precipitation with respect to the historical data (1986–2010) under HadCM3 A1B scenario

As shown in Fig. 3, LARS-WG shows increasing minimum temperature in all months for two study future periods. Results also indicated that maximum temperature will increase except Feb.

In future periods, the greatest increasing of T_{max} is in Jul. about 1 and 3 °C for the 2011-2030 and 2046-2065 periods, respectively. The results showed that the mean annual T_{max} increase from 19.8 °C (in baseline period: 1986-2010) to 20.4 °C and 21.9 °C for the 2011-2030 and 2046-2065 periods, respectively. The increase of temperature in study area is in agreement with previous studies (Ashraf *et al.* 2011; Farzanmanesh *et al.* 2012; Hassan *et al.* 2014; Goodarzi *et al.* 2014).

Future changes in precipitation for the future periods in comparison with the observation period don't follow a uniform trend. In other words, in some months the amounts of future precipitation are more than the observation period and in some months are less than the observation period. As shown in Fig. 4, the future precipitation would increase for the 2011-2030 and 2046-2065 periods, in comparison with the observation period except May, June, July and August.

SCA analysis

SCA for the 2001-2010 period and SCA in each elevation zones for the base period were obtained from the MODIS images. Fig. 5 shows the mean seasonal SCA of zones. Also, based on the relationship between SCA and daily temperature and rainfall variables, regression relations were obtained in each zone for different months (Table 2). These relationships were used to estimate SCA in zones for future periods.

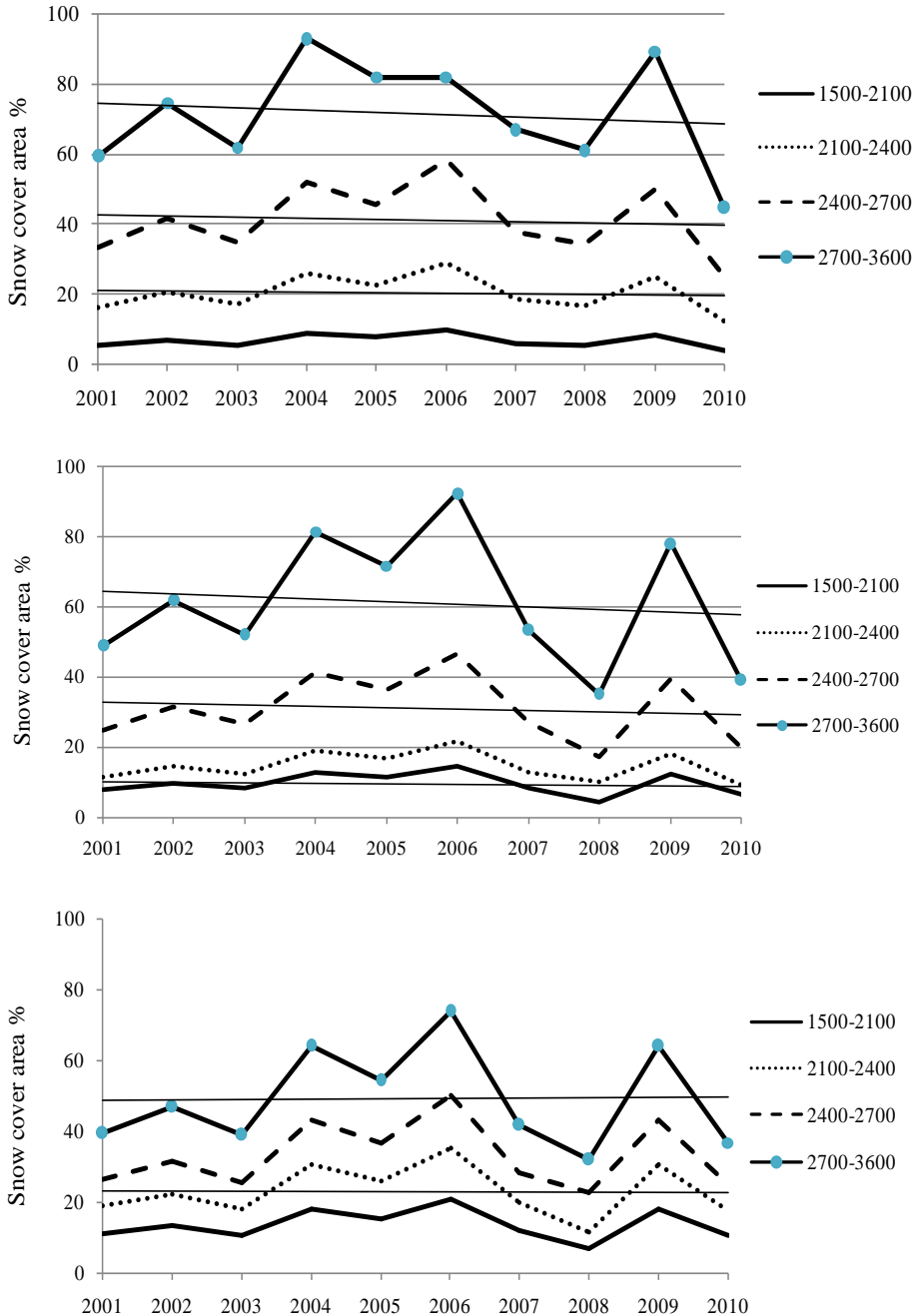


Fig. 5. Average SCA area in elevation zones for autumn, winter and spring (respectively, from top to bottom) in the base period

Table 2. Regression equations coefficients for estimating SCA in different months

Month	elevation zones	Coefficients of independent variables				Constant coefficient	R ²	P-value
		T _{i-1}	P _{i-1}	T _i	P _i			
December	1500-2100	2.78	4.65	-3.90	0.41	1.68	0.91	0.003
	2100-2400	-20.47	47.06	-16.72	1063	44.86	0.91	0.003
	2400-2700	0.00	14.01	-34.75	0.00	127.86	0.69	0.003
	2700-3600	-5.09	1.32	-18.92	-7.03	77.11	0.64	0.091
January	1500-2100	-10.49	12.90	-56.80	0.00	79.67	0.66	0.029
	2100-2400	36.28	59.50	203.62	0.00	383.32	0.84	0.001
	2400-2700	24.61	12.74	-68.85	0.00	45.23	0.92	0.000
	2700-3600	5.92	0.35	-10.65	0.00	189.48	0.81	0.001
February	1500-2100	15.90	9.83	-44.80	385.06	206.00	0.93	0.000
	2100-2400	6.54	3.24	-134.84	702.14	162.73	0.88	0.002
	2400-2700	8.05	3.32	-49.24	140.03	281.86	0.66	0.078
	2700-3600	-1.60	1.81	-12.28	91.82	143.00	0.51	0.036
March	1500-2100	-1.44	0.85	-2.30	0.16	27.07	0.32	0.549
	2100-2400	0.00	17.08	-72.63	5.05	425.38	0.35	0.295
	2400-2700	0.00	12.92	-40.45	1.90	222.98	0.35	0.304
	2700-3600	-2.95	5.78	-11.32	3.28	103.50	0.41	0.382
April	1500-2100	2.92	0.64	-5.80	3.52	30.37	0.54	0.862
	2100-2400	-14.39	1.70	15.80	6.64	8.89	0.26	0.602
	2400-2700	-6.01	0.64	5.70	0.00	13.27	0.21	0.447
	2700-3600	5.18	1.79	-12.01	0.00	47.79	0.57	0.014
Annual	1500-2100	1.10	0.06	-21.57	2.22	189.45	0.46	0.000
	2100-2400	-9.11	11.57	-73.72	4.98	475.98	0.54	0.000
	2400-2700	-1.30	5.45	-35.54	3.33	225.33	0.67	0.000
	2700-3600	-2.58	1.95	-11.37	3.75	108.68	0.68	0.000

* The dependent variable in all relations is SCA in the elevation zone .

**In these equations, P_i, T_i, P_{i-1} and T_{i-1} are precipitation in current day, temperature in current day, precipitation in pervious day and temperature in previous day, respectively .

***In cases where the R² of monthly relations is not acceptable, the annual relationship was used .

Snowmelt runoff estimation

Table 3. Intervals search parameters for SRM calibration and optimal calibrated and sensitive parameters

Parameter name	Symbol	Normal range	Interval change	Optimal value	Sensitive rank
Recession coefficient (k)	x	0.1-1.5	0.01	1.02-1.04	1
	y	0.01-0.1	0.01	0.06-0.1	6
Rain runoff coefficient	C _R	0.01-0.99	0.02	0.70-0.76	3
Snowmelt runoff coefficient	C _S	0.01-0.99	0.02	0.68-0.78	2
Degree-day coefficient*	$a \left(\frac{cm}{^{\circ}Cd} \right)$	0.01-1	0.05	0.20-0.35	4
Critical temperature	T _{crit} (°C)	0-4	0.2	2	5

*From snow density data

The results of SRM calibration and manually sensitivity analysis are given in Table 3. As can be seen from the Table 3, recession coefficient (k) and snowmelt runoff coefficient (c_s) were found to be the most sensitive parameters. After successful daily runoff calibration, validation and sensitivity analysis of the model with $NS=0.60$ and $D_v=-14.72\%$ in calibration period and $NS=0.58$ and $D_v=-28.31\%$ in validation period. SRM model was run to simulate daily runoff for the 2011-2039 and 2046-2065 periods.

Results of the influence of climate change on snowmelt-runoff

Hydrological impacts of climate change in terms of changes in rainfall and temperature in the basin is determined. The resultant effects of climate change on the hydrological Beheshtabad Watershed can be observed at fluctuations in flow rate in the Beheshtabad hydrometric station. For this purpose, the SRM model by anticipated climatic variables of GCM models for specific scenarios was run and compared with changes of runoff for different periods. SRM model was run 40 times for the 2011-2039 and 2046-2065 periods and calculated the daily estimated runoff (as the monthly average) for each period. The monthly average discharge in the 1986-2010 period were used as the base period. Fig. 6 shows the observed hydrograph (for the base period) compared with the predicted hydrographs for the next two periods. In addition to variation in the Beheshtabad discharge, change at the peak time is also clearly visible in this graph.

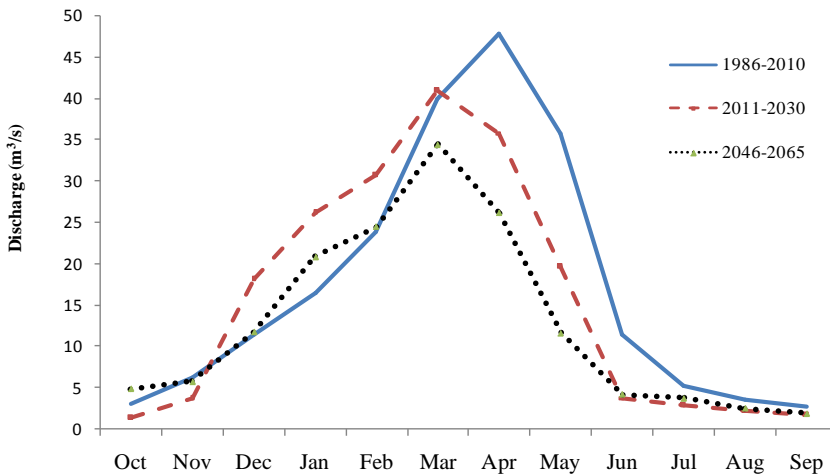


Fig 6. The observed and estimated hydrograph in the Beheshtabad Watershed during three periods

Flow duration curves (FDCs) for future periods were drawn based on discharge data from the SRM and were compared with the base period (Fig. 7). As can be seen from the Fig. 7, FDCs in future periods have the same trend with FDC in the base period, but streamflow value will decrease significantly in

future. This shows that a significant change in the number of days with low flow in about 50% of year will be very low and close to zero (especially in the 2046-2065 period). The more important point is to reduce the annual volume of river flow, reduce low flows during dry period in years that river cant supply agricultural water needs in dry season .

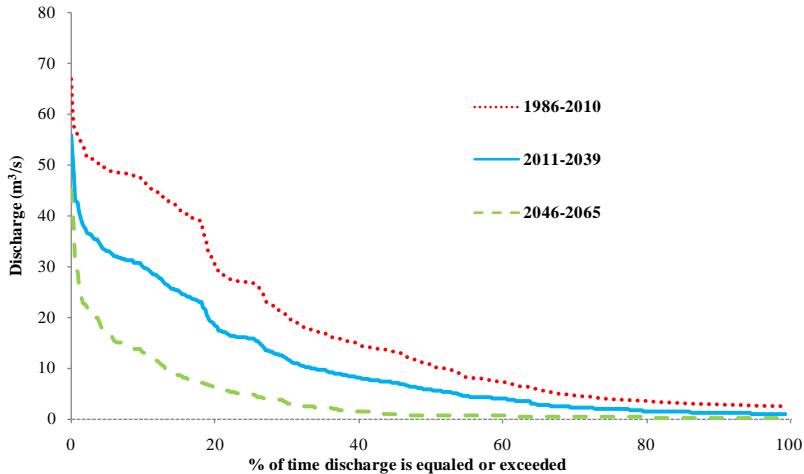


Fig.7. Comparison of exceedance flow in three periods

Impact of climate change on the Beheshtabad River discharge

Fig. 7 shows the general decrease annual runoff in the Beheshtabad River by 10 and 26 percent, respectively, for the periods of 2030-2011 and 2065-2046. This finding is consistent with those of Mansouri *et al.* (2014) in the Zarinerood Watershed who found to reduce in runoff after the significant increase in rainfall. Despite the significant increase rainfall in future periods (Fig. 4), may seem unreasonable decrease in runoff. However, exacerbated the negative effects by increase temperature on water resources by increasing the evaporation, and will reduce the quality and quantity of water resources.

The results also show the relative increase runoff compared with the base period in January and February. This increase due to rising temperatures in future periods and the subsequent change in type of rainfall and will increase snowmelt and runoff. In the other words, as the weather warms one side more precipitation as rain, which is directly converted to runoff and increase the runoff and on the other hand in case of snow, rising temperatures melt faster and prevents the accumulation of snow. Results showed a significant reduction peak flow in the 2011-2030 and 2046-2065 periods respectively 7 and 13 m³/s.

Moreover peak monthly rate in future periods compared with the base period is takes place a month earlier, that's mean moves from April to March. The reason for this is rising temperatures, especially in March. Because as increase in temperatures in March, precipitation turned to rain rather than snow accumulation, and becomes the direct runoff. The results obtained in this study

on the reduction of runoff under climate change, correspond as well as with the results of Massah Bavani (2005), Huang *et al.* (2013), Zarghami *et al.* (2011) and Modaresi *et al.* (2011).

The contribution of snowmelt runoff in the Beheshtabad River

SRM model can separate the snowmelt runoff and precipitation runoff. In addition, the model was run for the future periods (40 years), the model was performed for the 2001-2010 period as the base period. Table 4 shows the separation results of runoff from rain and snowmelt runoff during observation and two next periods (2011-2030 and 2046-2065).

Table 4. Percent of snowmelt runoff in the Beheshtabad Watershed

period season	2001 -2010	2011-2030	2046-2065
Spring	33.0	25.1	20.3
Summer	15.9	13.5	12.8
Autumn	14.1	14.2	16.0
Winter	24.6	29.0	29.3
Annual	27.2	24.5	22.3

Table 3 shows that snowmelt runoff has a relatively large contribution in the Beheshtabad River runoff that is different in seasons of the year. During the observation period, the highest contribution of snowmelt runoff is in spring. While contribution of snowmelt has changed in future periods and the highest contribution of this will happen in winter. In other words, snow stored during January and February would be quickly melted due to increase in temperature in March, and increases the snowmelt runoff contribution of winter.

Also changes in land use and land cover increased absorption of temperature and accelerating the snow melting. On the other hand, decrease the snow accumulation for spring and decrease snowmelt runoff contribution from the rainfalls. So disturbed the balance between rain and snow and then decrease contribution of snowmelt runoff from the total. The results of Khadka *et al.* (2014) shows as well as changes in the contribution of snowmelt runoff during the decades of 2000 and 2050, but the changes are not significant.

CONCLUSIONS

The results obtained in this study indicate that the SRM model performed successfully for snowmelt-runoff simulation. The results of the study also indicate that rainfall would increase with 2.17 and 9.7 percent for the 2011-2030 and 2046-2065 periods, but rainfall would decrease in May, which is very essential for agriculture activates especially dry farming in the study area. Finally, monthly peak discharge under climate change scenarios in the

Beheshtabad watershed would decrease 10 and 26 percent for future periods (2011-2030 and 2046-2065) compared with base period (2001-2010). Therefore, all annual runoff would reduce during spring, which is crucial period for irrigation. In addition, monthly peak discharge in future periods compared with the base period is takes place a month earlier (from April to March) due to increase in temperature. These findings indicate impact of climate change on water resources and temporal distribution of water availability in the study area, which is important for water resources management planning.

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**NEW RECORDS OF MITES (TROMBIDIFORMES:
ERYTHRAEIDAE, MICROTROMBIDIIDAE, TROMBIDIIDAE)
FROM GREECE AND HUNGARY AND THE LIST OF
TERRESTRIAL PARASITENGONA
FOUND IN BOTH COUNTRIES**

SUMMARY

Abrolophus anzelmi, *A. petanovicae*, *A. wratislaviensis*, *Allothrombium clavatum* and *Iranitrombium miandoabicum* are new to the fauna of Greece. *Charletonia cardinalis*, *Moldoustium baltiense* and *A. clavatum* are new to the fauna of Hungary. New measurements for *A. anzelmi* and *I. miandoabicum* are given. The list of terrestrial Parasitengona found in Greece and Hungary is provided.

Keywords: Prostigmata, new records, new measurements, Greece, Hungary

INTRODUCTION

In Greece and Hungary the mites of the terrestrial Parasitengona are sufficiently known. In Greece these mites were studied by Berlese (1910), Krausse (1916), Cooreman (1960), Beron (1988, 1990), Southcott (1993), Haitlinger (1993, 1999a, 2003, 2006a, b, 2015, 2016), Haitlinger & Šundić (2016, 2018) and Antonatos & Emmanouel (2014). They described a many new species from Greece or new for the fauna of Greece.

The list of 65 species known from the country is given below. At least the same species should still be found. In Hungary the mites of the terrestrial Parasitengona were mainly studied by Gabryś & Mąkol (1991, 1996), Fain & Ripka (1998a, b), Haitlinger (2007 b), Ripka & Szabo (2010). In this country hitherto were found 64 species (which list is given below) and knowledge of mite fauna is incomplete. Now we found 5 new species to the fauna of Greece: *Abrolophus anzelmi* Haitlinger & Łupicki, 2013, *A. petanovicae* Saboori, Šundić & Pešić, 2012, *A. wratislaviensis* (Haitlinger, 1986), *Allothrombium clavatum* Saboori, Pešić & Hakimitabar, 2010 and *Iranitrombium miandoabicum* Saboori & Hajiqanbar, 2002 and 3 species new to the fauna of Hungary: *Charletonia cardinalis* (C. L. Koch, 1937), *Moldoustium baltiense* Haitlinger, 2008 and *Allothrombium clavatum*.

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Moreover, new measurements for *A. anzelmi*, based on specimens from continental Italy and Greece and for *Iranitrombium miandoabicum*, based on specimens from Europe are given in Table 1, 2.

MATERIAL AND METHODS

In May 2018, 53 larvae belonging to 11 species were collected in Greece and in May 2012 and June 2018, 7 larvae belonging to 5 species were collected in Hungary. All specimens were obtained from herbaceous plants. The measurements below are expressed in micrometers (μm). The terminology of setal notation are adapted from Haitlinger (1999 b, 2013). Specimens from Greece were collected by M. Šundić and specimens from Hungary were collected mR. Haitlinger.

RESULTS AND DISCUSSION

Family Erythraeidae Robineau-Desvoidy, 1828

Genus *Abrolophus* Berlese, 1891

Abrolophus anzelmi Haitlinger & Łupicki, 2013

Material: one larva from herbaceous plants from Kalavryta 758 m, Peloponnese, Greece, May 25, 2018. First record from Greece. *A. anzelmi* was known only from Sicily and recently was collected in Toscana prov., Italy (Haitlinger and Łupicki 2015, Haitlinger and Šundić 2018). Measurements were given only for holotype and two other specimens from Sicily (Haitlinger and Łupicki 2015). Further measurements for specimens from Sicily, continental Italy and Greece are given in Table 1.

A. petanovicae Saboori, Šundić & Pešić, 2012

Material: Two larvae from herbaceous plants in Kalavryta, Peloponnese, Greece, May 25, 2018. First record from Greece. Hitherto this species was known only from Montenegro and Serbia (Saboori *et al.* 2017).

A. podorasensis (Haitlinger, 2007)

Material: Five larvae from herbaceous plants in Kalavryta, Peloponnese, Greece, May 25, 2018. Up to now was known from the area Kotas n. Kastoria and Antrako n. Gravena (Haitlinger, 2006 a, b).

A. quisquiliarus (Hermann, 1804)

Material: One larva from Püspökladány, two larvae from Hajdúszoboszló, all from herbaceous plants, June 9, 2018. In Hungary it earlier was noted from Bátorliget-láp (Gabryś and Mąkol 1991).

A. silesiacus (Haitlinger, 1986)

Material: Seven larvae from Ioannina, May 20, 2018 and six larvae from Messatida, Peloponnese, Greece, May 25, 2018, all from herbaceous plants. In Greece this species was known only from Samos (Haitlinger, 2006b).

Table 1. Metric data for *Abrolophus anzelmi* Haitlinger & Łupicki, 2011 from Sicily (S), Italy (Toscana prov.) (I) and Greece (G).

Character	H	S n=3	I n=1	G n=1	Range
IL	745	345-761	443	-	345-761
IW	535	230-513	290	-	230-535
L	81	79-93	103	93	79-103
W	75	89-92	77	88	75-92
AW	58	43-54	48	51	43-58
PW	68	53-67	65	70	53-70
ISD	45	49-59	54	61	45-61
AL	72	59-69	70	67	59-72
PL	77	68-73	75	71	68-77
AP	25	20-30	28	26	20-30
ASE	40	31-35	42	35	31-42
PSE	70	73-74	75	68	68-75
AA	14	13-14	13	14	13-14
SB	17	14-16	15	15	14-17
GL	183	166-185	181	-	166-185
DS	43-71	35-68	42-67	45-61	35-71
PsFd	-	29-51	57	57	29-57
PsFv	-	86	86	83	83-86
PsGd*	-	27-32	29	46	27-46
<i>1a</i>	125	128-139	135	118	118-139
<i>2a</i>	-	59-68	68	53	53-68
<i>3a</i>	-	38-40	44	48	38-48
<i>1b</i>	71	61-70	54	62	54-71
<i>2b</i>	48	39-59	42	43	39-59
<i>3b</i>	53	44-51	48	45	44-53
PaFe (L)	-	52	48	50	48-52
PaFe (W)	-	40	42	48	40-48
PaGe (L)	-	23	20	21	20-23
PsGe (W)	-	25	33	36	25-36
OD	-	30-38	32	33	30-38
Prd (L)	-	14-20	14	-	14-20
ω_1	-	35	36	35	35-36
<i>as1</i>	-	30	-	-	
<i>as2</i>	-	47	39	-	39-47
<i>bs</i>	-	40-51	38	-	38-51
<i>cs</i>	-	27	-	28	27-28
Ta I	106	82-94	94	93	82-106
Ti I	156	131-149	142	149	131-156
Ge I	119	101-117	115	114	101-119
Tf I	63	54-70	57	63	54-63

Bf I	86	70-83	81	74	70-86
Tr I	64	35-52	46	49	35-64
Cx I	79	59-63	65	74	59-79
Ta II	91	76-79	75	82	76-91
Ti II	143	110-134	125	131	110-143
Ge II	98	85-87	84	90	84-98
Tf II	53	44-57	47	48	44-57
Bf II	72	47-63	58	64	47-72
Tr II	58	46-50	44	52	44-58
Cx II	95	75-80	77	93	75-95
Ta III	100	81-90	88	80	81-100
Ti III	190	167-182	176	177	167-190
Ge III	132	109-132	124	126	109-132
Tf III	81	68-81	74	75	68-81
Bf III	80	57-81	77	78	57-81
Tr III	72	43-64	58	59	43-72
Cx III	89	68-79	75	91	68-91
Leg I	673	563-622	600	616	563-673
Leg II	610	490-614	540	560	490-614
Leg III	744	609-709	672	686	609-744
IP	2007	1662-1945	1812	1862	1662-2007

* longest dorsal seta

***A. wratislaviensis* (Haitlinger, 1986)**

Material: One larva from herbaceous plants in Messatida, Peloponnese, Greece, May 25, 2018. First record in Greece.

Genus *Balaustium* von Heyden, 1826

***Balaustium nikae* Haitlinger, 1996**

Material: Three larvae from herbaceous plants in Kalavryta, Peloponnese, Greece, May 25, 2018 and one larva from Tiszavárkony, Hungary, June 12, 2018, all from herbaceous plants. Hitherto this species was known in Greece from Mouries n. Kilkis, Gorgopi n. Polikastro and 11,6 km north of Antrako n. Grevena (Haitlinger, 2006a) and in Hungary only from Poroszló n. Tiszafüred (Haitlinger, 2007b).

Genus *Charletonia* Oudemans, 1910

***Charletonia cardinalis* (C. L. Koch, 1837)**

Material: One larva from herbaceous plants, 3 km north of Szentes, Hungary, May 23, 2012. First record from Hungary. A species widely distributed in Europe.

Table 2. Metric data for *Iranitrombium miandoabicum* Saboori & Haiqanbar, 2003 from Iran (H – holotype, P – paratypes), Albania, Greece and Montenegro (AGM).

Character	H	P	AGM n= 8	Range
IL	245	185-225	124-227	124-245
IW	112	92-97	69-133	69-133
L	87	61-75	76-84	61-87
W	70	63-70	76-84	63-84
AW	51	49	48-53	48-53
PW	56	56	55-58	55-58
AA	36	32-34	28-35	28-36
SB	34	34	31-36	31-36
ASB	65	49-51	53-67	49-67
PSB	22	22-24	24-32	22-32
MA	32	27	24-33	24-33
AP	15	15	16-24	15-24
AL	27	22-25	19-26	19-27
PL	41	44-46	30-43	30-46
AM	27	27-29	21-27	21-29
S	37	34-37	27-40	27-40
GL	-	-	54-65	54-65
DS	37-78	32-76	23-75	23-78
LSS	75	65-80	70-81	65-81
HS	36	37-39	32-42	32-42
SL	46	36-44	35-45	35-46
SS	18	22-23	17-22	17-23
LSS ₂	-	-	27-35	27-35
HS ₂	-	-	14-21	14-21
SL ₂	-	-	31-44	31-44
SS ₂	-	-	15-21	15-21
1a	27	22-24	13-30	13-30
2a	27	26-34	22-31	22-34
3a	36	34-41	25-37	25-41
1b	25	20-24	22-35	20-35
2b	46	34	27-33	27-46
3b	40	34-36	30-41	30-41
cs	-	-	4-12	4-12
bs	-	-	17-29	17-29
OD	-	-	9-16	9-16
ω_1	-	-	11-14	
Ta I	46	46	46-51	46-51
Ti I	29	29	26-32	26-32
Ge I	24	23-24	19-26	19-26

Fe I	34	32-36	30-39	30-39
Tr I	25	22-25	22-25	22-25
Cx I	36	36	35-44	35-44
Ta II	41	39	39-46	39-46
Ti II	27	25-29	25-30	25-30
Ge II	19	19	16-21	16-21
Fe II	32	30-34	28-35	28-35
Tr II	25	24-25	24-27	24-27
Cx II	41	39	43-49	39-49
Ta III	46	44-49	42-48	42-49
Ti III	32	29-34	27-36	27-36
Ge III	22	19	17-22	17-22
Fe III	36	29-36	33-37	29-37
Tr III	27	24-29	24-29	24-29
Cx III	41	39-40	41-45	39-45
Leg I	194	191-193	183-215	183-215
Leg II	185	176-185	178-203	176-203
Leg III	204	185-206	178-211	178-211
IP	583	552-584	539-629	539-629

Genus *Erythraeus* Latreille, 1806

Erythraeus (Erythraeus) sicilicus Haitlinger, 2011

Material: Five larvae from herbaceous plants, Messatida, Peloponnese, Greece, May 25, 2018. This species was known from Sicily and Kos, (Greece) (Haitlinger, 2011, 2016). First record from continental Greece.

Genus *Marantelophus* Haitlinger, 2011

Marantelophus iranicus (Haitlinger & Saboori, 1996)

Material: 23 larvae from herbaceous plants, Kalavryta, Peloponnese, Greece, May 25, 2018. Earlier it was known from Panagia n. Metsovo and Samos (as *Grandjeanella multisetosa* or *Marantelophus multisetosus*) (Haitlinger, 2006a, b).

Genus *Moldoustium* Haitlinger, 2008

Moldoustium baltiense Haitlinger, 2008

Material: Two larvae from herbaceous plants, Hortobágy, June 10, 2018 and 4 km west of Földes, June 11, 2018. First record from Hungary. Hitherto this species was known only from Moldova and Ukraine (Haitlinger, 2008).

Family Trombidiidae Leach, 1815

Genus *Allothrombium* Berlese, 1903

Allothrombium clavatum Saboori, Pešić & Hakimitabar, 2010

Material: One larva from Messatida, Peloponnese, Greece, May 25, 2018 and one larva from Nemti, Hungary, June 8, 2018, all from herbaceous plants. First

record from Greece and Hungary. Hitherto this species was known only from Montenegro and Serbia (Saboori *et al.* 2010, Šundić *et al.* 2016).

***A. fuliginosum* (Hermann, 1804)**

Material: one larva from herbaceous plants, Hajdúszoboszló, Hungary, June 9, 2018. In Hungary this species was known from Kisharsány Villany, Budapest, Bátorliget: Fényi-erdő and Miskolc (Gabryś and Małkol 1991, 1996, Haitlinger, 2007b, Ripka and Szabo 2010).

***Iranitrombium miandoabicum* Saboori & Hajiqanbar, 2003**

Material: 4 larvae from herbaceous plants, Kalavryta 758 m, Peloponnese, Greece, May 25, 2018. First record from Greece. This species was known only from Albania, Iran and Montenegro (Saboori *et al.* 2003, Haitlinger and Šundić 2015, 2018). Measurements for this species was based only on Iranian specimens. Measurements for European specimens are given in Table 2.

List of species (terrestrial Parasitengona) found in continental Greece and on some islands

Continental Greece

Erythraeidae Robineau-Desvoidy, 1828

Abrolophus anzelmi Haitlinger and Łupicki 2013, *A. norvegicus* (Thor, 1900), *A. petanovicae* Saboori *et al.* 2012, *A. podorasensis* (Haitlinger, 2007), *A. quisquiliarus* (Hermann, 1804), *A. rhopalicus* (C. L. Koch, 1837), *A. silesiacus* (Haitlinger, 1986), *A. wratislaviensis* (Haitlinger, 1986), *Balaustium madeirense* Willmann, 1939, *B. murorum* (Hermann, 1804), *B. nikaie* Haitlinger, 1996, *Bursaustium gaspari* Haitlinger, 2000, *Charletonia bucephalia* Beron, 1975, *C. cavannae* (Berlese, 1885), *C. dalegori* Haitlinger, 2003, *C. krendowskyi* (Feider, 1954), *C. venus* Southcott, 1961, *Erythraeus (Zaracarus) budapestensis* Fain and Ripka 1998, *Leptus (Leptus) josifovi* Beron, 1975, *Marantelophus iranicus* (Haitlinger and Saboori 1996), *Myrmicotrombium (Graecotrombium) mirum* Beron, 1990, *Phanolophus oedipodarum* (Frauenfeld, 1868)

Smarididae Vitzthum, 1929

Smaris squamata (Hermann, 1804), *Fessonnia papillosa* (Hermann, 1804)

Microtrombidiidae Thor, 1935

Eutrombidium feldmanmuhsamae Feider, 1977, *E. robauxi* Southcott, 1993, *E. sorbasensis* Mayoral and Barranco 2004, *E. trigonum* (Hermann, 1804)

Trombidiidae Leach, 1815

Allothrombium clavatum Saboori *et al.* 2010, *A. fuliginosum* (Hermann, 1804), *A. meridionale* Berlese, 1910, *A. triticium* Zhang, 1995, *Dolichothrombium grandjeani* Andre, 1954, *Iranitrombium miandoabicum* Saboori and Hajiqanbar 2003, *Paratrombium insulare* (Berlese, 1910), *Trombidium holosericeum* (Linnaeus, 1759), *T. mediterraneum* (Berlese, 1910), *T. rimosum* C. L. Koch, 1837.

Podothrombiidae Thor, 1935

Podothrombium macrocarpum Berlese, 1910.

Chyzeridae Womersley, 1954

Parawenhoekia dectici Paoli, 1937.

Neothrombiidae Feider, 1959

Giftitrombium skalaensis Haitlinger, 2007.

Neotrombidiidae Feider, 1955

Neotrombidium indosinensis Andre, 1960 (= *N. helladicum* Cooreman, 1960)

(Cooreman, 1960, Beron, 1990, 2008, Haitlinger, 1993, 2006a, 2007a, b, 2015, Małkol and Wohltmann 2012, Antonatos and Emmanouel 2014).

Corfu

Erythraeidae: *Charletonia krendowskyi*, *Leptus* (L.) *villosus* (Berlese, 1910).

Trombidiidae: *Allothrombium gracile* Berlese, 1910, *Eutrombidium diecki* Krausse, 1916, *E. robaxi*, *Trombidium mediterraneum*.

Microtrombidiidae: *Dimorphothrombium corycraeum* (Berlese, 1912) (Berlese, 1910, Krausse, 1916, Southcott, 1993, Beron, 2008, Haitlinger, 2006a).

Crete

Erythraeidae: *Curteria graeca* Beron, 1988, *Leptus* (L.) *trimaculatus* (Rossi, 1794)

Smarididae: *Smaris squamata*.

Chyzeriidae Womersley, 1954

Cretessenia leoni Haitlinger, 1999 (Haitlinger, 1999a, Beron, 1988, 2008,).

Kefallonia

Erythraeidae: *Balaustium murorum* (Beron, 2008, Małkol and Wohltmann 2012).

Kythnos

Erythraeidae: *Curteria graeca* (Beron, 1988, 2008, Małkol and Wohltmann 2012).

Kos

Erythraeidae: *Abrolophus silesiacus* (Haitlinger, 1986), *Charletonia kosensis* (Haitlinger and Šundić 2016, *Erythraeus* (E.) *sicilicus* Haitlinger, 2011

Trombidiidae: *Allothrombium polikarpi* Haitlinger, 2006 (Haitlinger, 2016, Haitlinger and Šundić 2016).

Lesbos

Erythraeidae: *Abrolophus silesiacus*, *Charletonia krendowskyi*, *Erythraeus* (Z.) *budapestensis*, *E. (E.) regalis* (Haitlinger and Šundić 2018).

Lefkas

Erythraeidae: *Abrolophus rhopalicus*; *Charletonia bucephalia*, *C. dalegori*, *Leptus* (L.) *josifovi*, *Phanolophus oedipedarum*.

Smarididae: *Smaris squamata*

Microtrombidiidae: *Eutrombidium trigonum* (Beron, 2008, Haitlinger, 2006a).

Rhodes

Erythraeidae: *Charletonia dalegori*, *C. glifadaensis* Haitlinger, 2003, *C. kaliksti* Haitlinger, 2003, *C. krendowskyi*, *Curteria graeca*, *Erythraeus (E.) rutgeri* Haitlinger, 2003, *Helladoerythraeus pachytibialis* (Beron, 1988), *Leptus (L.) andae* Haitlinger, 2003, *L. (L.) gennadicus* Haitlinger, 2003, *L. (L.) monolithesiscus* Haitlinger, 2003, *Marantolephus rudaensis* (Haitlinger, 1986).

Smarididae: *Smaris squamata*

Microtrombidiidae: *Eutrombidium trigonum*

Trombidiidae: *Allothrombium fuliginosum* (Haitlinger, 2003, Beron, 1988, 2008, Małkol and Wohltmann 2012).

Samos

Erythraeidae: *Abrolophus silesiacus*, *Charletonia kalithensis*, *C. samosensis* Haitlinger, 2006, *Erythraeus (E.) kastaniensis* Haitlinger, 2006, *E. (E.) passidonicus* Haitlinger, 2006, *Leptus (L.) josifovi*, *Marantelophus iranicus*.

Trombidiidae: *Allothrombium polikarpi*

Podothrombiidae: *Podothrombium monolatesicus* Haitlinger, 2006 (Haitlinger, 2006b, Beron, 2008, Małkol and Wohltmann 2012).

List of species (terrestrial Parasitengona) found in Hungary

Calyptostomatidae Oudemans, 1923

Calyptostoma velutinum (Müller, 1776)

Erythraeidae

Abrolophus artemisiae (Schrank, 1803), *A. crassitarsus* (Schweizer, 1951), *A. miniatus* (Hermann, 1804), *A. norvegicus*, *A. quisquiliarus*, *A. strojnyi* Gabryś, 1992, *Balaustium murorum*, *B. nikae*, *Curteria southcotti* Gabryś, 1992, *Charletonia cardinalis* Oudemans, 1910, *Erythraeus (E.) acis* (Berlese, 1882), *E. (E.) adpendiculatus* (Schrank, 1781), *E. (E.) cinereus* (Dugès, 1834), *E. (E.) crocatus* (C. L. Koch, 1837), *E. (E.) jowitae* Haitlinger, 1987, *E. (E.) opilionoides* (C. L. Koch, 1837), *E. (E.) regalis* (C. L. Koch, 1837), *E. (Z.) budapestensis*, *Leptus (L.) clethrionomydis* Haitlinger, 1987, *L. (L.) mariae* Haitlinger, 1987, *L. (L.) longipilis* (Berlese, 1910), *L. (L.) molochinus* (C. L. Koch, 1837), *L. (L.) phalangii* (de Geer, 1778), *L. (L.) rubricatus* (C. L. Koch, 1837), *L. (L.) trimaculatus*, *L. (L.) vertex*, *Marantelophus multisetosus* (Zhang & Goldsarazena, 1996)

Smarididae

Hirstiosoma latreillei (Grandjean, 1947), *Fessonnia papillosa* (Hermann, 1804).

Trombidiidae

Allothrombium clavatum, *A. fuliginosum*, *A. incornatum* Oudemans, 1905, *A. meridionale*, *A. pulvinum* Ewing, 1917, *Trombidium holosericeum*, *T.*

hungaricum Gabryś and Małkol 1991, *T. kneissli* (Krausse, 1915), *T. mediterraneum*, *T. rimosum*.

Podothrombiidae

Podothrombium bicolor (Hermann, 1804), *P. exiguum* Fain and Ripka 1998, *P. filipes* (C. L. Koch, 1837), *P. hispanicum* Robaux, 1967, *P. macrocarpum* Berlese, 1910, *P. pannonicum* Fain and Ripka 1998.

Microtrombidiidae

Atractothrombium sylvaticum (C. L. Koch, 1837), *Campylothrombium clavatum* (George, 1909), *Dactylothrombium pulcherimum* (Haller, 1882), *Dromeothrombium coifatti* Robaux, 1966, *Echinothrombium spinosum* (Canestrini, 1885), *Microtrombidium pusillum* (Hermann, 1804), *Milandanielia intermedia* (Feider, 1950), *Platytrombidium fasciatum* (C. L. Koch, 1836), *Trichotrombidium muscarum* (Riley, 1878), *Triscidothrombium discrepans* (Willmann, 1950), *Willmnella racovitzai* (Feider, 1948), *Enemothrombium bifoliosum* (Canestrini, 1884), *Eothrombium siculum*.

Tanaupododae Thor, 1935

Rhinothrombium nemoricola (Berlese, 1886), *Tanaupodus passimpilosus* Berlese, 1910.

Johnstonianidae.

Diplothrombium longipalpe (Berlese, 1887), *Johnstoniana errans* (Johnston, 1852), *J. eximia* (Berlese, 1910).

Trombellidae Thor, 1935

Trombella mahunkorum (Gabryś and Małkol 1991)

(Gabryś and Małkol 1991, 1996, Fain and Ripka 1998a, b, Haitlinger, 2007b, Beron, 2008, Ripka and Szabo 2010, Małkol and Wohltmann 2012).

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EGG QUALITY INFLUENCE ON INCUBATION RESULTS AND THEIR PHENOTYPE CORRELATION

SUMMARY

This study presents research results of quality and incubation values for Isa Brown hybrid eggs. Eggs were produced in the 33rd week of age at the production peak. Research was conducted on the sample of 252 incubation eggs (240 fertilized eggs). Chick hatchability compared to the number of inserted eggs was 86.51%, and compared to the number of fertilized eggs 90.83%. Average values for quality parameters of brooding eggs and incubation results were as follows: egg weight before insertion 58.41 g, egg length 5.49 cm, egg width 4.29 cm, egg shape index 78.16% and egg volume 53.14 cm³, one day old chick weight was 39.15 g, absolute egg weight loss was 6.60 g, relative egg weight loss was 11.31% and relative chick share in the egg weight was 67,03%.

Statistically justified ($P < 0.001$; $P < 0.01$; $P < 0.05$) correlation (complete one) was determined between egg weight and egg width (0.908), egg weight and egg volume (0.923) and egg weight and chick weight (0.918), very strong correlation was determined between egg weight and egg length (0.870), strong correlation was determined between egg weight and absolute egg weight loss (0.690), weak correlation was determined between egg shape index and egg width (0.395), and very weak correlation was determined between egg weight and egg shape index (0.188), egg weight and relative egg weight loss (-0.166), egg shape index and egg length (-0.147) and egg shape index and chick weight (0.189). Between egg weight and relative chick share in the egg weight, egg shape index and absolute egg weight loss, egg shape index and relative chick share in the egg weight no statistically justified ($P > 0.05$) correlation was determined.

Key words: egg quality, incubation parameters, correlation.

INTRODUCTION

The success of industrial production of poultry and eggs is based on the breeding of various parent flocks of heavy and light line hybrids of hens. Keeping and exploiting parental flocks, as well as incubating eggs for brooding chicken, comprises of specific, highly specialized and complex phases in production processes. It should be kept in mind that the average production of

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one parent flock in the egg production for brooding hens and day-old chicks in practice is a combined result of the genetic potential of the breeds that are kept as well as breeding technology during production, and the egg incubation technology. Very often, all the merit or guilt for the level of production achieved is attributed to the genetic potential of the flock, but in practice a great share goes to non-genetic factors e.g. breeding technology, usage period – flock age, parent body weight, gender correlation, egg weight as well as technological procedures during the chick hatching.

Parent flock age at the start of egg laying is one of the major factors that have direct influence on incubation egg production success as well as on day old chick production. It is well known that laying intensity of fertilized eggs and chick hatchability from the number of incubated eggs gradually increases until it reaches its maximum, then it gradually decreases. Length of the time for achieving the maximum, apart from genotype, depends on a large number of non-genetic factors. The most important non-genetic factors are proper parent flock breeding technology, adequate storing of incubation eggs as well as storing time. Moreover, the proper and optimal technological procedures during artificial hatching of chicks is very important factor.

In the recent research results it can be seen that most of the authors in their research, have examined influence of genetic and non-genetic factors to laying intensity of fertile eggs during the production cycle, and especially to fertilization rate and hatchability of incubation eggs. These are the most significant factors that have direct influence on production success in poultry offspring production. However, most of the authors present research results which are related to the influence of specific factors (age, laying hen weight, laying period, season, incubation conditions, egg weight, shell permeability, weight of day old chicks) and their correlations to fertility success, hatchability and egg weight loss dynamics (correlation between egg weight and embryo growth during incubation period) for different chicken hybrids.

Because of the above mentioned, in the production of poultry eggs and in the order to achieve better reproductive results, it is necessary to examine the quality, i.e. the basic physical characteristics of the eggs for brooding hens. (Baboo *et al.*, 2013; Đermanović and Mitrović, 2013; Đermanović *et al.*, 2012, 2013, 2015; Kabir *et al.*, 2014a,b; Mitrović *et al.*, 2011; Usman *et al.*, 2014). Therefore, the aim of the paper was to determine the basic external quality characteristics of the light line hybrid Isa Brown breeding eggs, that were laid at the peak of production when highest egg fertility is expected as well as chick hatching.

MATERIALS AND METHOD

Egg incubation for parent flock light line hybrid Isa Brown was done in incubation station which is within the poultry farm “Jugokoka” Belgrade. The eggs that were produced at the production peak (33rd week of age) were placed in the incubator. The number and percentage of fertilized eggs was determined, the

number and percentage of hatched chicks compared to the number of incubated (fertilized eggs), the number and percentage of eggs with dead embryo as well as dynamics of egg weight loss up to 18th day of incubation period were also determined. Before being placed into the incubator each egg was weighed and measured (length and width), and marked on husk. In addition to individual egg measuring before inserting in to incubator and after 18 days of incubation, each day old chick was measured too. Special attention in this study was given to the egg category from which healthy and vital chicks hatched.

Egg shape index (ESI) was determined using the formula: $ESI = (EW/EL) \times 100$, EW – egg width and EL – egg length, while for egg volume we used following formula: $V = (\pi/6) \times L \times W^2$, V – egg volume; W – egg width; L – egg length; π – constant (3.1416).

On 25th day eggs were transferred to the hatchery where they were individually placed in the special separators in order to easily determine from which egg each hatched chick originated. At the end of the incubation period the weight of day old chicks was measured. Based on egg weight and chick weight relative chick share in the egg was determined using the following formula: $PC = (CW/EWe) \times 100$.

For most of the monitored parameters arithmetic mean, arithmetic mean error, standard deviation and deviation coefficient were determined. In addition, using special formula phenotype correlation coefficients were determined for monitored traits.

$$r_{xy} = \frac{\sum x_i y_i - \frac{(\sum x \sum y)}{nxy}}{\sqrt{(\sum x_i^2 - \frac{(\sum x)^2}{n}) (\sum y_i^2 - \frac{(\sum y)^2}{n})}}$$

$i = 1, 2, \dots, k$

Main data rendering was done using statistic software IBM SPSS statistics Version 22 (2013).

RESULTS AND DISCUSSION

The goal of the study was to determine incubation results for eggs of light line hybrid Isa Brown. Using random sample technology 252 eggs were chosen and they were individually measured and marked. After determining egg weight, absolute and relative parameters of the total number of incubated fertilized and non-fertilized eggs, same parameters for eggs with dead embryo were also determined, as well as relative hatchability parameters for chicks of both genders compared to the number of inserted and fertilized eggs, (Table 1).

From the data in the table 1 it can be seen that for parent flock analysis of light line hybrid Isa Brown, total number of 252 incubation eggs were taken. Egg fertility in the research period was 95.24%, i.e. 240 eggs while 12 eggs were not fertilized (4.76%). After the incubation process was finished it was determined that from 22 eggs (8.73% from total inserted number, 9.17% from fertilized eggs) did not hatch, i.e. embryo death was determined. The calculated hatchability of day old chicks of both genders compared to total number of inserted eggs was 86.51%, and compared to fertilized eggs was 90.83%.

Table 1. Egg fertility and hatchability of the parental flock in the 33rd week of age

Category of eggs	Eggs	Percentage
Total number of eggs inserted	252	100.00
Fertility of eggs	240	95.24
Non fertility of eggs	12	4.76
Egg with a dead embryo (A)	22	8.73
Egg with a dead embryo (B)	22	9.17
Hatchability chickens of both sexes (A)	218	86.51
Hatchability chickens of both sexes (B)	218	90.83

(A) From the number of inserted eggs; (B) From the number of fertilized eggs.

Depending on the age, i.e. parent flock laying period, when studying productive and reproductive traits of parent flocks of different line hybrids and chicken breeds, different results were obtained by many authors: Đermanović *et al.* (2008; 2009), Mitrović *et al.* (2005; 2009; 2010), Abanikannda and Leigh (2015), Aşçı and Durmuş (2015), Nikolova *et al.* (2011) i Denli *et al.* (2018).

One of the significant parameters for egg quality is egg weight. Egg weight increases with age of the flock - if proper diet, healthcare and breeding technology of parental flocks is provided. Average values and variability of basic egg quality parameters and incubation values in the period of analysis are shown in table 2.

Table 2. Variability and average values of egg traits from which chicks hatched

Indicators	\bar{x}	$S\bar{x}$	S	C.V.
Weight of eggs before incubation (g)	58.41	0.22	3.29	5.63
Length of the eggs (cm)	5.49	0.01	0.13	2.37
Width of the eggs (cm)	4.29	0.01	0.11	2.56
Egg shape index (%)	78.16	0.07	1.05	1.34
Volume of eggs (cm ³)	53.14	0.26	3.78	7.11
Loss of egg weight up to 18 days of incubation (g)	6.60	0.03	0.50	7.58
Loss of egg weight up to 18 days of incubation (%)	11.31	0.05	0.69	6.10
Weight of day-old chicks (g)	39.15	0.17	2.46	6.28
Relative share of chicken in the egg weight (%)	67.03	0.11	1.67	2.49

From the data in table 2 it is clear that average egg weight upon inserting was 58.41 g, egg length 5.49 cm, egg width 4.29 cm. Based on these parameters average shape index value was determined 78.16%, and egg volume of 53.14 cm³ was determined. It is well known that during the incubation process eggs lose a certain part of their weight. Above mentioned shows that average absolute egg weight loss up to 18th day of incubation was 6.60 g, and relative 11.31%. The data in table 2 shows that average weight of hatched day old chicks of both genders was 39.15 g, and that average relative chick share in egg weight was 67.03%. If absolute and relative results are observed it can be concluded that obtained results were satisfactory, even though some unsatisfactory results for

egg volume and absolute loss of egg mass up to 18th day of incubation (C.V. – 7.11 and 7.58) were found.

Most of the authors have conducted similar researches but mainly for heavy line hybrids. Therefore, Abanikannda and Leigh (2015), determined for three genotypes average egg weight of 61.33 g, length 58.02 mm, width 43.87 mm, egg shape index 75.73%, volume 59.03 mm³. Similar egg weight values (60.3 g and 57.1 g) in the same production period for two genotypes of light type were determined by Denli et al. (2018). However, when it comes to egg shape index authors of both researched genotypes determined lower values (77.5% and 75.8%). Moreover, significant difference ($P \leq 0.05$) for researched parameters at the laying peak depending on genotype Lohmann Brown and Atak-S) was determined by Denli et al. (2018). Kocevski et al. (2011) in their research determined that egg weight at the laying peak was under significant ($P \leq 0.05$) influence of age, but not under the influence of genotype, even though eggs from Isa Brown were slightly heavier than DeKalb White hybrid.

By incubation egg value reference is made to it refers to the fertilization percent, i.e. percent of hatched chicks compared to the number of inserted eggs. Therefore, research in the field of egg weight loss during incubation and day old chick weight can give significant contribution for quality assessment. Determining correlation between researched parameters has special significance for determining incubation egg quality and achieved incubation results (Table 3).

Table 3. Coefficients of phenotypic correlation between egg weight, egg shape index and quality of eggs and chicks

Traits	n	r_{xy}	Relationship	$t_{exp.}$
EWe:EL	218	0.870 ^{***}	Very strong	22.665
EWe:EWi	218	0.908 ^{***}	Complete	31.998
EWe:ESI	218	0.188 ^{**}	Very weak	2.826
EWe:EV	218	0.923 ^{***}	Complete	35.253
EWe:ALEWe	218	0.609 ^{***}	Strong	11.336
EWe:RLEWe	218	-0.166 [*]	Very weak	2.485
EWe:CW	218	0.918 ^{***}	Complete	34.177
EWe:RSC	218	0.053 ^{ns}	Non	0.783
ESI:EWe	218	0.188 ^{**}	Very weak	2.813
ESI:EL	218	-0.147 [*]	Very weak	2.184
ESI:EWi	218	0.395 ^{***}	Weak	6.319
ESI:ALEWe	218	0.121 ^{ns}	Very weak	1.791
ESI:RLEWe	218	-0.022 ^{ns}	Non	0.323
ESI:CW	218	0.189 ^{**}	Very weak	2.829
ESI:RSC	218	0.061 ^{ns}	Non	0.898

EWe – egg weight (g); EL – egg length (cm); EWi – egg width (cm); ESI – Egg shape index (%); EV – egg volume (cm³); ALEWe – absolute loss of egg weight up to 18th day of incubation (g); RLEWe – relative egg weight loss up to 18th day of incubation (%); CW – chick weight (g); RSC – Relative chick share in the egg weight (%). ^{ns} $P > 0.05$; ^{*} $P < 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$.

Based on obtained values for phenotype coefficient correlation and their significance (table 3) it can be determined that egg weight has some influence to physical traits and incubation results during the maximal egg production period (laying peak). Therefore, between egg weight and egg length, egg weight and egg width, egg weight and egg volume, egg weight and absolute egg weight loss and day old chick weight phenotype correlation coefficients were determined and they were at the level $P < 0.001$, which means that strong, very strong and total correlations were determined. In addition, positive but very weak ($P < 0.01$) correlation was determined between egg weight and egg shape index, and negative also very weak ($P < 0.05$) correlation was determined between egg weight and relative egg weight up to 18th day of incubation. However, between egg weight and relative chick share in the egg no statistically significant ($P > 0.05$) correlation was determined.

Unlike for egg weight, data from table 3 shows that between egg shape index and egg width, egg shape index and chick weight positive weak and very weak correlation was determined at the levels of $P < 0.001$ and $P < 0.01$, while negative very weak ($P < 0.05$) correlation was determined between egg shape index and egg length. However, between egg shape index and other studied parameters determined correlations were not statistically confirmed ($P > 0.05$).

In relation with egg shape index and egg weight correlation Duman *et al.* (2016) determined same values, but weaker correlation ($P < 0.05$). Above mentioned results are in accordance with results obtained by Aygun and Yetisir (2010), while Olawumi and Ogunlade (2008) obtained completely opposite results and determined negative significant correlation between egg shape index and egg weight.

CONCLUSION

Based on this study it can be concluded that next to the egg weight other physical traits (length, width, shape index and egg volume) have a higher or lower rate influence to incubation results, during entire production cycle and especially during the peak egg production period. Average egg weight, during the insertion at studied period, was 58.41 g, length 5.49 cm, width 4.29 cm, egg shape index 78.16%, volume 53.14 cm³. It is well known that during the incubation process eggs lose a certain amount of their weight. Above mentioned shows that average absolute egg weight loss up to 18th day of incubation was 6.60 g, and relative 11.31%. Average weight of hatched day old chicks of both genders was 39.15 g, and that average relative chick share in egg weight was 67.03%.

Based on the values of phenotype correlation coefficient and their significance it can be concluded that egg weight has some influence to physical traits and incubation results. Therefore, between egg weight and egg length, egg weight and egg width, egg weight and egg volume, egg weight and absolute egg weight loss and day old chick weight phenotype correlation coefficients were determined and they were at the level $P < 0.001$, which means that strong, very

strong and total correlations were determined. Between egg weight and egg length, egg weight and egg width, egg weight and egg volume, egg weight and absolute egg weight loss, as well as between egg weight and chick weight determined phenotype correlation coefficients were determined at the level $P < 0.001$, i.e. strong, very strong and complete correlation was determined. Positive very weak ($P < 0.01$) correlation was determined between egg weight and shape index, and negative also very weak ($P < 0.05$) correlation was determined between egg weight and relative egg weight loss up to 18th day of incubation. However, between egg weight and relative chick share in egg weight no statistically justified correlation was determined ($P > 0.05$). Unlike for egg weight, between shape index and egg width, and shape index and chick weight positive weak and very weak correlation was confirmed at the levels $P < 0.001$ and $P < 0.01$, while negative very weak ($P < 0.05$) correlation was determined between egg shape index and egg length. However, between egg shape index and other researched parameters no statistically significant correlations were determined ($P > 0.05$).

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INDUCED MUTATIONS OF WINTER WHEAT CAUSED BY GAMMA-RAYS FIXED ON PLANT HEIGHT AND STEM STRUCTURE

SUMMARY

The objectives of our investigations are to describe the variation by mutations of stem architecture of the main groups of modern Ukrainian winter wheat varieties (8 varieties) due to their interactions with dose specific. Agronomic-value traits like as general short stem, dwarfs and semi-dwarfs have been investigated too. New perspective mutant lines have been obtained in terms of investigation. Main components for mutation breeding successful was genotype-mutagen interaction (due to factor analyses). By sensibility (in sense of number of mutations and type of mutations) genotypes can be subdivided on two groups. At the first group only varieties, which created with gamma-rays were observed. Gamma-rays were not useful for obtaining mutations by plant architecture for radiomutants varieties. Higher level of short-stem and semi-dwarfs mutation were inducted by 200 – 250 Gy doses, while for dwarfs in spite of dwarfs, which were observed more after 250 Gy. Semi-dwarfs as mutations significance responded to gamma-rays action by changes with doses.

Key words: winter wheat, mutation breeding, plant height and structure, gamma-rays.

INTRODUCTION

Mutations have been used successful in several crops for breeding agronomical important traits. Induced mutations in wheat have been obtained for morphological and quantitative characters by treatment with different mutagens (Nazarenko et al, 2018). The main purpose of using mutagens has been to induce genetic variation, which is the first step in a breeding programmer. Grain yield, a complex polygenic trait is highly affected due to environmental stresses (Nazarenko and Kharitonov, 2016). Improvement of various complex traits can be possible through different breeding approaches. Induced mutations have significant impact along with conventional breeding approaches in cereals. Mutagenesis has become an established tool in plant breeding to supplement existing germplasm and to improve several specific morphological traits. More than 3500 varieties of plants obtained either as direct mutants or derived from their crosses and 2700 mutant varieties of different plants including cereal crops

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have been released throughout the world through direct or indirect use of mutation breeding (IAEA, 2018).

Bread wheat (*Triticum aestivum* L.) with the annual production of about 757 million tons (in 2017) (USDA, 2018), is one of the world's most important cereal crops. Winter wheat is the world's leading cereal grain and the most important food crop, occupying first position in Ukraine. Ukrainian agriculture takes about 48% area under cereals and contributing 38% of the total food grain production in the country (Nazarenko, 2015).

The improvement of grain yield and yield components of wheat through application of mutagens leads towards improvement of new cultivars with improved traits. The use of induced mutations has thus become an important approach to plant breeding for the improvement of crop plants (Naveed *et al.*, 2015).

The present studies were therefore undertaken to investigate the effects of gamma rays on various yield associated traits and to evaluate the value of induced mutations in wheat improvement.

Plant height is important agronomic traits related to plant architecture and grain yield in wheat. Tiller number and plant height are pointed out as two major agronomic traits in cereal crops affecting plant architecture and grain yield (Ellis *et al.*, 2004). In investigation of Chinese researches of NAUH167, a new mutant of common wheat landrace induced by ethylmethyl sulfide treatment, exhibits higher tiller number and reduced plant height was attributed to the decrease in the number of cells and their length. Genetic analysis showed that the high-tillering number and dwarf phenotype were related and controlled by a partial recessive gene (Xu *et al.*, 2017).

Dwarfing and semi-dwarfing mutations have mutual effects. As for example, dwarfing gene Rht-5 was associated with a plant height reduction, delaying heading date by 1 day, increasing the number of fertile tillers plant⁻¹, while reducing the number of spikelets spike⁻¹ and number of grains spike⁻¹. The results of this study could be useful for proper use of Rht-5 dwarfing gene in breeding programs to improve lodging tolerance, yield potential in wheat and increase efficiency of marker assisted selection for agronomic traits (Daoura *et al.*, 2014).

Over the next decade, wheat grain production must increase to meet the demand of a fast growing human population. One strategy to meet this challenge is to raise wheat productivity by optimizing plant stature. As a sample of this investigation, the reduced height 8 (Rht8) semi-dwarfing gene is one of the few, together with the Green Revolution genes, to reduce stature of wheat (*Triticum aestivum* L.), and improve lodging resistance, without compromising grain yield. Rht8 is widely used in dry environments such as Mediterranean countries where it increases plant adaptability. With recent climate change, its use could become increasingly important even in more northern latitudes. Morphological analyses show that the semi-dwarf phenotype of Rht8 lines is due to shorter internodal

segments along the wheat culm, achieved through reduced cell elongation and associated with stem mutations in our investigations (Gasperini et al, 2012).

The development of wheat mutants not only provided new genetic resources for wheat improvement, but also facilitated our understanding of the regulation of these traits at the molecular level. Identification of a dwarf mutant with a compact spike, NAUH164, produced from ethyl methyl sulfonate treatment of wheat variety Sumai 3, has reduced plant height and shortened spike length. Dwarfness and compact spike were controlled by a single dominant gene that was designated Rht23 (Chen et al, 2015).

Focused on only yield traits we have to understand that on high grain yield influence at complex many difference trait.

The objectives of our many-years investigations are to describe the genotypic variation of new mutant winter wheat lines by all spectra of the agronomic-value traits, investigation of consequences of main groups of breeding-useful mutagens treatment in interaction with modern winter wheat varieties. The most target objects are developing relations between genotype and nature of mutagen, mutagen dose, which determining the succeed of modern mutation breeding. Second our purpose to estimate new lines and their suitability as direct new varieties or components for future breeding crosses.

MATERIAL AND METHODS

Dried wheat grains (approx. 14% moisture content, in brackets method of obtaining varieties or used mutagens) of 'Favoritka', 'Lasunya', 'Hurtovina' (irradiation of initial material by gamma rays), line 418, 'Kolos Mironovschiny' (field hybridization), 'Sonechko' (chemical mutagenesis, nitrosodimethylurea (NDMU) 0.005%) and 'Kalinova' (chemical mutagenesis, 1,4-bisdiazotsetilbutan DAB 0.1%), 'Voloshkova' (termomutagenesis – low plus temperature at plant development stage of vernalizaion has been used as mutagen factor) of winter wheat (*Triticum aestivum L.*) were subjected to 100, 150, 200, 250 Gy gamma irradiation (rapid dose, Co⁶⁰, 0.048 Gy/s). Each treatment was comprised of 1,000 wheat seeds. Non-treated varieties were used as a control for mutation identified purpose (Nazarenko, 2015).

Treated seeds were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, to raise the M1 population. The untreated seeds of mother varieties (parental line/variety) were also planted after every ten rows as control for comparison with the M1 population. M1 plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval (Nazarenko, 2017).

In M₂ – M₃ generations productive and other value families have been selected via visual estimation. The sowing was done by hand, at the end of September, at a depth of 4-5 cm and with a rate of 100 viable seeds to a row (length 1.5 m), interrow was 15 cm, between samples 30 cm, 1 - 2 rows for sample with control-rows of untreated varieties in every twenty-sample interval.

Estimation of total yield per plot and its components was conducted from 2014 to 2018 years ($M_4 - M_8$ generations). The controls were national standard by productivity 'Podolyanka' and initial variety. The working-methods in the breeding trials are satisfied to state variety exam requests. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 20 m² in 2 – 3 replications. (Shu *et al.*, 2013).

Experiments were conducted on the experiment field of Dnipropetrovsk State Agrarian-Economic University (village Aleksandryvka, Dnipropetrovsk district, Dnipropetrovsk region, Ukraine). Normal cultural practices including fertilization were done whenever it is necessary. Evolution was conducted during 2011 – 2018 years.

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, the grouping mutants cases was performed by cluster analysis, factor analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSION

Total size of population 17600 families at second-third generation (include controls) and represented by variants of mutagen treatment at table 1. Investigators ran on with trivial problem for high doses limited number of material for next stapes of breeding process (Nazarenko, 2016a; Nazarenko, 2017b).

From $M_2 - M_3$ generations 1,482 potential productivity winter wheat mutation lines and 5,862 lines with mutation changes were determined overall (table 1).

The main purpose of our investigation was to determine rate and spectra of winter wheat mutations by plant height and stem structure (high stem (more than 1 m), short stem (0.6 – 0.8 m), semi-dwarf (0.2 – 0.4 m) and dwarf (0.2 – 0.4 m), thick and thin stem) after gamma-rays action and develop relations between number and type of mutations and gamma-rays doses, genotypes of mutation object.

General spectra of mutations have been grouped on six groups (38 traits) according to general practice. In this investigation part of first group (mutations of stem structure) has been analyzed. Visible mutation changes and their heredity in the course of several generations have been summarized.

From previous investigations fact of decreasing general mutation rates and number of mutation traits (level of changeability) for radiomutants after gamma-rays action has been developed. Regarding dates of table 2 – 4 any statistically reliable difference between rates in this group between three types of genotypes hasn't been observed.

General mutation rate to all types mutation has been increased until level of 200 Gy dose. After reached this dose, number of mutation up to 250 Gy was significantly lower. Just the same to the level of changeability (parameter to

overall score rate of mutations and number of mutations types). High level of changeability was corresponded to dose 150 – 200 Gy.

Table 1. Number of mutant families at second – third generations

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Control	500	500	500	500	500	500	500	500
Gamma-rays, 100 Gy	500	500	500	500	500	500	500	500
Gamma-rays, 150 Gy	500	500	500	400	500	500	500	500
Gamma-rays, 200 Gy	500	350	500	250	450	500	450	400
Gamma-rays, 250 Gy	300	350	500	100	400	400	350	400

Table 2. Spectrum of mutations under gamma-rays action (radiomutants)

N	Trait	Check		100 Gy		150 Gy		200 Gy		250 Gy	
		lines	%	lines	%	lines	%	lines	%	lines	%
variety Favoritka											
1	high stem	0	0.0	2	0.4	3	0.6	4	0.9	4	1.0
2	short stem	1	0.2	2	0.4	2	0.4	2	0.4	3	0.75
3	semi-dwarf	0	0.0	0	0.0	0	0.0	2	0.4	1	0.25
4	dwarf	0	0.0	0	0.0	0	0.0	1	0.2	1	0.25
variety Hurtovina											
1	high stem	0	0.0	5	1.0	5	1.0	4	0.8	3	0.8
2	short stem	0	0.0	3	0.6	3	0.6	4	0.8	2	0.5
3	semi-dwarf	0	0.0	0	0.0	0	0.0	2	0.4	1	0.3
4	dwarf	0	0.0	0	0.0	0	0.0	2	0.4	1	0.3
variety Lasunya											
1	high stem	2	0.4	1	0.2	2	0.4	3	0.7	1	0.3
2	short stem	2	0.4	4	0.8	2	0.4	3	0.7	3	0.9
3	semi-dwarf	0	0.0	0	0.0	1	0.2	2	0.4	2	0.6
4	dwarf	0	0.0	0	0.0	0	0.0	1	0.2	2	0.6

The same tendentious was expected from this group of mutations, but dates were not so clearly and sometimes a little contradictory.

Table 3. Spectrum of mutations under gamma-rays action (chemomutants)

N	Trait	Check		100 Gy		150 Gy		200 Gy		250 Gy	
		lines	%	lines	%	lines	%	lines	%	lines	%
variety Kalinova											
1	high stem	4	0.8	4	0.8	6	1.2	6	1.2	0	0.0
2	short stem	1	0.2	5	1.0	13	2.6	13	2.6	4	0.8
3	semi-dwarf	0	0.0	0	0.0	1	0.2	3	0.6	4	0.8
4	dwarf	0	0.0	0	0.0	0	0.0	1	0.2	5	1.0
variety Sonechko											
1	thick stem	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0
2	thin stem	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0
3	high stem	0	0.0	7	1.4	8	2.0	17	6.8	0	0.0
4	short stem	0	0.0	4	0.8	6	1.5	4	1.6	1	1.0
5	semi-dwarf	0	0.0	0	0.0	4	1.0	2	0.8	1	1.0
6	dwarf	0	0.0	0	0.0	1	0.25	1	0.4	1	1.0

For first group similar number of mutations was characterized to all dose, but partly lower for 250 Gy. Rates of mutations are not high. Only for dwarf mutations are caused by 200 – 250 Gy doses only. For variety Lasunya more quantity of short-stem mutation was characterized.

Seldom mutations of stem thickness cannot be observed at all cases, and were appeared only in other groups and only for genotypes Sonechko, Kolos Mironivschini, Voloshkova. Rate of this type of changes was not high and only after 150 – 250 (more prevalent 200 – 250 Gy) action.

For second group (table 3) higher rate of mutations was developed. The same direction was saved, but doses 150 – 200 Gy significance preferable to this type of mutations (especially for short stem and semi-dwarf mutations, for dwarfs 250 Gy more suitable).

Regarding table 4 the same situation was observed with peak at doses 150 – 200 Gy and lower number of mutations at 250 Gy. Generally, mutation rate was varied from 0,2 to 1,6 % (line 418, 100 Gy) for high steam, from 0,2 to 2,8 % (line 418, 200 Gy) for short steam, from 0,2to 1,0 % (variety Sonechko, line 418, 150 – 200 Gy) for semi-dwarfs and from absence to the 100 -150 Gy doses for some genotypes to 0,6 % (line 418, 200 Gy) for dwarfs forms (table 2 – 4).

Mutations types thick and thin stem are characterized only for three varieties (at 100 – 200 Gy) and for no one radiomutants. Rates by these traits are very lower at all three cases. It's in accordance to generally direction in decreasing type of mutations and variability level for these types of genotypes.

We can subdivided initial material by the method of breeding as radiomutants (Favoritka, Hurtovina, Lasunya), chemomutants (Kalinova and Sonechko), thermomutants (low plus temperature at plant development stage of

vernalization has been used as mutagen factor) (Voloshkova) and forms, obtained after hybridization (Kolos Mironivschini, line 418).

Table 4. Spectrum of mutations under gamma-rays action (hybrid varieties)

N	Trait	Check		100 Gy		150 Gy		200 Gy		250 Gy	
		lines	%	lines	%	lines	%	lines	%	lines	%
variety Kolos Mironivschini											
1	thick stem	0	0.0	0	0.0	0	0.0	2	0.4	0	0
2	thin stem	0	0.0	0	0.0	1	0.2	0	0.0	0	0
3	high stem	1	0.2	6	1.2	11	2.2	8	1.6	3	1.0
4	short stem	1	0.2	9	1.8	4	0.8	4	0.8	9	3.0
5	semi-dwarf	0	0.0	0	0.0	2	0.4	3	0.6	3	1.0
6	dwarf	0	0.0	0	0.0	0	0.0	3	0.6	4	1.3
variety Voloshkova											
1	thick stem	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0
3	thin stem	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2
4	high stem	3	0.6	1	0.2	4	0.8	1	0.2	0	0.0
5	short stem	4	0.8	4	0.8	3	0.6	8	1.6	4	0.8
6	semi-dwarf	0	0.0	1	0.2	1	0.2	1	0.2	3	0.6
line 418											
1	high stem	1	0.2	8	1.6	6	1.2	4	1.0	1	0.3
2	short stem	0	0.0	4	0.8	5	1.0	11	2.8	4	1.0
3	semi-dwarf	0	0.0	1	0.2	2	0.4	4	1.0	1	0.3
4	dwarf	0	0.0	0	0.0	1	0.2	3	0.8	1	0.3

Thus, mutants with thick stem - rate of occurrence from 0 to 0.4%, probability of occurrence - low (only for 3 varieties), patterns of occurrence for doses and genotypes were not found; thin stems from 0 to 0.2% are unlikely to occur predominantly in the same variants (and exclusively in the same varieties) as the previous mutation; high-stem mutations - the frequency of occurrence in the average of 0.9%, high-frequency mutation, occurring in any variants; low-stem - high probability of occurrence, but more rarely than high-stem, an average of 0.7%, the frequency in some variants of up to 3%; half-dwarfs is also highly probable, but less than the previous one, up to 1%, on average - 0.3%, typical for up to 150 - 200 Gy. The dwarf is also a highly probable mutation, but less than the previous one, to 1.3%, an average of 0.2%, preferably at the action of 200-250 Gy.

According to our investigations more effectiveness at mutation induction were for high stem form doses 100 – 150 Gy, for short stem mutants 100 – 150 Gy, for both semi-dwarf and dwarf forms 150 – 250 Gy with peak for most part

of genotypes at 200 Gy dose. Part of genotypes (preferable radiomutants) hasn't been shown these kinds of changes at 100 – 150 Gy doses at all.

Regarding analyze of these groups it has been developed that rate of these types of mutations was significantly lower for first group, than for others. According to ANOVA analyses number of mutations was depended on dose at all cases, relation with genotype and mutation rate has been identified with significance reliability for only one case short stem mutations ($F_{2,49}$, $F_{critical}$ 2,36). In spite of this fact, genotype and mutagen interaction are statistically reliable for all cases and traits.

Due to the discriminant analyses only fact of semi-dwarfs mutants appearance can be used as indicator of gamma-ray action for initial material classification (Wilks Lambda 0.305508, F-remove 2.786654)

CONCLUSIONS

In the present study, a mutagenized wheat population is developed using gamma-rays. Plant height mutations as well as mutants by stem are identified. These stable mutants are developed as lines at the next generations. The generated information would be useful in developing new winter wheat cultivars from mutants lines either direct or through hybridization program.

Due to results of our investigations gamma-rays irradiated as a method for creation new variation material on plant height and stem structure has been shown as very successful. Large number of material has been obtained both as for perspective new varieties and as the sources for future winter wheat breeding program for changing plant architecture. Only one trait appeared significant influence of genotype as a key component for mutation breeding success but at all times genotype-mutagen interaction regarding results of ANOVA analyze was significance in its influence on mutation rates. The same situation was for factor dose of gamma-rays, but the first-place component was interaction of gamma-rays and genotype.

Gamma-rays were not effective to radiomutants for mutations by stem high. Doses of 200 – 250 Gy were more preferable to short-stem and semi-dwarfs mutation obtaining, in spite of dwarfs, which were observed more after 250 Gy.

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OVERVIEW OF THE FSC CHAIN OF CUSTODY CERTIFIED VENEER COMPANIES IN ROMANIA

SUMMARY

Forest Stewardship Council (FSC) represents one of the most common certification schemes in forestry worldwide. The aim of this paper was to highlight the profile of the FSC Chain of Custody (CoC) certified veneer companies from Romania. Data available in January 2019 on the official website of FSC (*i.e.* www.info.fsc.org) were taken into consideration.

Based on the official records, at the beginning of 2019, 43 organizations based in Romania had a FSC CoC certificate for the product category W7 Veneer, most of them being located in the central and western parts of the country.

As regards the certification bodies active in Romania, more than half of the valid FSC CoC certificates were issued only by two, namely Soil Association Certification Limited (SA) and SGS Société Générale de Surveillance SA (SGS).

By taking into consideration the worldwide demand of certified veneers, it is expected that this sector would gain an increasing attention in the future, meaning that more and more resources should be allocated, including specialized people.

Keywords: chain of custody, forest certification, FSC, Romania, veneer

INTRODUCTION

Worldwide, it is estimated that every year around 3,000 million cubic meters of wood are harvested, more than half being used in various branches of wood industry (Oprea et al., 2018), veneers being among the main outputs (Buongiorno, 2018). In many countries with large forest areas, wood industry includes a significant number of employees in its numerous branches. For example, in the United States of America, in forest products and paper industry more than one million people are working (Quesada et al., 2018).

Along with technological developments in the wood industry, in general, and furniture sector, in particular, veneer has been used in many different and unique ways, such as laminated veneer lumber (Silva et al., 2019) or as a

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component of the flexible furniture for the new generation offices (Tuncel and Kayan, 2018).

In Romania, woodworking still represents one of the main activities, numerous industrial branches, such as furniture, boards industry or veneer industry having a long tradition (Ciupan et al., 2018). This is mainly due to the rich forest cover, which accounts for around 7 million hectares, according to the results of the second cycle of the National Forest Inventory (IFN, 2018). The Romanian forests are dominated by common beech (*Fagus sylvatica* L.), Norway spruce [*Picea abies* (L.) H. Karst.] and oak species (Genus *Quercus* L.), especially sessile oak [*Q. petraea* (Matt.) Liebl.] and pedunculate oak (*Q. robur* L.). As regards the most used species in veneer industry the list is composed by oak species (the two above-mentioned species), but also the most common non-native species - red oak (Nicolescu et al., 2018), wild cherry (*Prunus avium* L.), walnut (*Juglans regia* L.) (Dumitrascu et al., 2018; Tofan and Niță 2018) and the common beech.

Nowadays, as a consequence of the restitution process which started in 1991 (Scriban et al., 2017), the forest sector is characterized by a high number of private owners (*i.e.* 800.000) who have small and very small forest lands (Popa et al., 2019). Moreover, due to the current normative acts, especially the Forest Code (Law no. 46/2008), the forest owners from Romania are confronted with considerably more restrictions in comparison with other countries across Europe (Nichiforel et al., 2018), generating in this way a negative impact to the overall forest management. As regards the forest management units, according to the most recent data provided by the Ministry of Waters and Forests (MAP, 2019), in Romania there are 147 private forest districts and 327 state-owned forest districts, out of which 320 represent territorial branches of the National Forest Administration ROMSILVA that is managing 3.2 million hectares of state-owned forests plus around one million hectares of different owners (Enescu, 2018).

In the last two-three decades, the forest started to be regarded not only as an important renewable resource, but also as an important indicator of environmental responsibility (Palaghianu and Dutcă, 2017). This is in line with the international concerns of the general public regarding the importance of the management of the forest ecosystems in a sustainable manner. In this context, the first international forest certification initiatives appeared in the early '90s (Măciucă and Diaconescu, 2013). One of them is Forest Stewardship Council (FSC), which is following a global template (Buzogány, 2016; Hălălișan et al., 2018), being designated for large-scale forests (Frey et al., 2018). FSC is the widely adopted forest standard (Blumroeder et al., 2018) on voluntary basis (Jaung et al., 2016; Matarazzo et al., 2016). It is based on ten principles and it includes three decision-making chambers with stakeholders from social, economic and environmental fields (Niedzialkowski and Shkaruba, 2018), the decisions being made when all of them reach a consensus (Teder and Kaimre, 2018). FSC promotes the concept of Chain of Custody (CoC), which guarantees that the wood or wood-based products originate from properly managed forests

(Hălălișan et al., 2013; Yamamoto et al., 2014), being recently reported that CoC certification has a significant influence on wood products market worldwide (Gilani et al., 2017).

As regards the situation of the FSC forest management (FM) certification in Romania, nowadays there are more than 2.7 million hectares certified, the vast majority of them (92%) bellowing to the state and being managed by the National Forest Administration ROMSILVA (Ilie et al., 2018), while the rest is managed by 28 private-owned forest districts (FSC, 2019). The situation is more complex when it comes to the industry sector, where nowadays hundreds of companies received a CoC certificate, Romania being a country with tradition in this field, the first FSC CoC certificates being issued in 2001 (Gavrilit et al., 2016).

The aim of this paper was to provide an overview of the FSC CoC certified veneer companies in Romania.

MATERIAL AND METHODS

The list of FSC CoC certified veneer companies in Romania was made by centralizing the data available on the official website of FSC (FSC, 2019). The information available at the beginning of 2019 (January, 6) was taken into account. Special attention was given to the country of origin, primary and secondary activities and the main output categories of the certified organizations (Figure 1).

The image shows a search interface with three main sections: Organization, Certificate, and Product. Each section contains several filters. In the Organization section, the 'Country or Area' dropdown is set to 'Romania'. In the Certificate section, the 'Status' dropdown is set to 'Valid' and the 'Certificate Code' dropdown is set to 'All'. In the Product section, the 'Level 1' dropdown is set to 'W7 Veneer'. Other filters include 'Name', 'State/County', 'Show Sites/Member', 'FSC Controlled Wood', 'CW Risk Assessment', 'Level 2', 'Level 3', and 'Species'.

Organization

Name Legal name of the organization

Country or Area Romania

State/County The state or county of the organization

Show Sites/Member

Certificate

Status Valid

Certificate Code All | COC

FSC Controlled Wood

CW Risk Assessment

Product

Level 1 W7 Veneer

Level 2 All

Level 3 All

Species The scientific name of a species

Figure 1. Search section from FSC official website

RESULTS AND DISCUSSION

At the beginning of 2019 (January), 811 companies across Romania were FSC CoC certified, most of them being located in the north of the country (Bîtcă, 2019). The most common FSC certified products consisted in rough wood (60%), roundwood (57%), fuel wood (55%), solid wood (50%) and planks (44%), respectively (Bîtcă, 2019). Among the 811 companies, only 43 organizations (5%) had a FSC CoC certificate for the product category W7 Veneer, according to the product classification available on the official website of FSC (FSC, 2019). The counties with the highest number of certified FSC CoC companies for veneer production were Braşov, Ilfov (including Bucharest), Caraş-Severin, Timiş and Vâlcea. In the southern-eastern part of Romania, with the exception of Ilfov County (including the capital city), no certified company for processing or trading veneers exists (Figure 2).

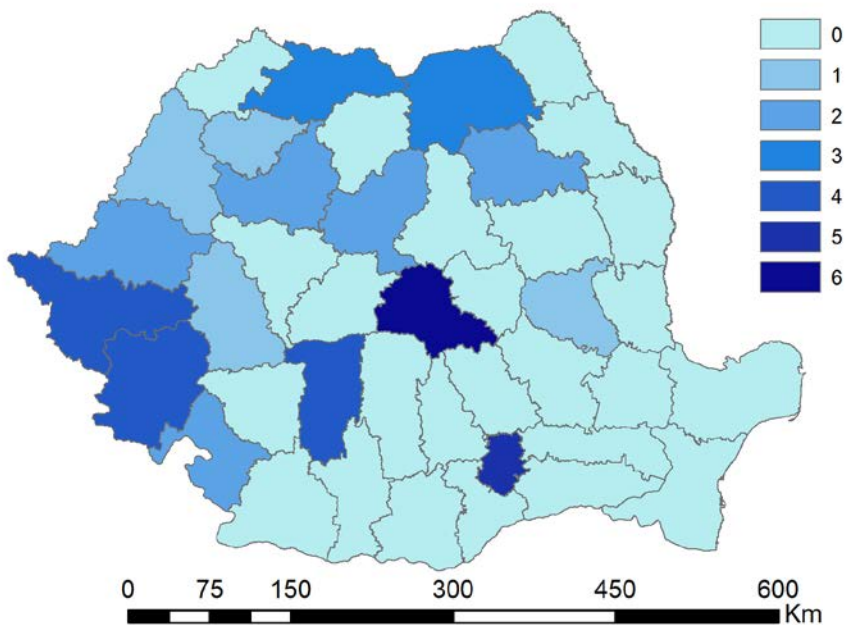


Figure 2. Distribution of the certified companies across Romania

As regards their primary activity, almost half of the certified companies were primary processors and only five of them were brokers/traders without physical possession (Figure 3). Two out of the eighteen companies that were classified as primary processors as main activity were also certified for logging, as secondary activity, and another three out of the same eighteen were certified as distributor/wholesaler, as secondary activity.

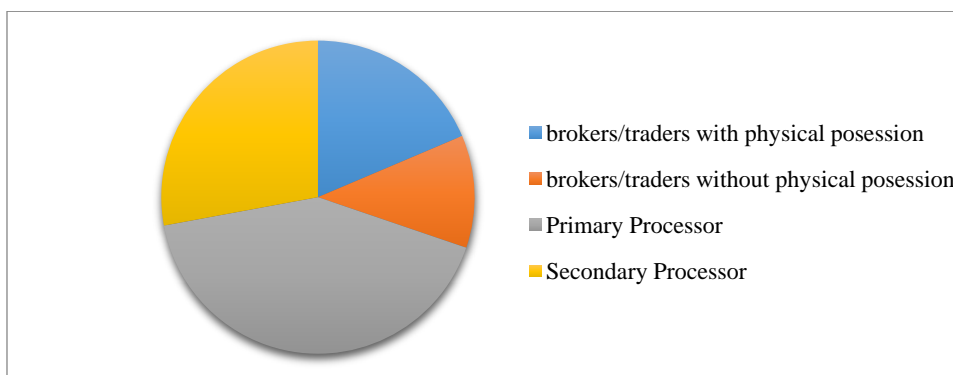


Figure 3. Classification of the organizations according to their primary activity

The main outputs in the case of half of the certified companies were FSC 100% certified, while the combination of FSC Controlled Wood and FSC Mix was recorded only in one case (Figure 4).

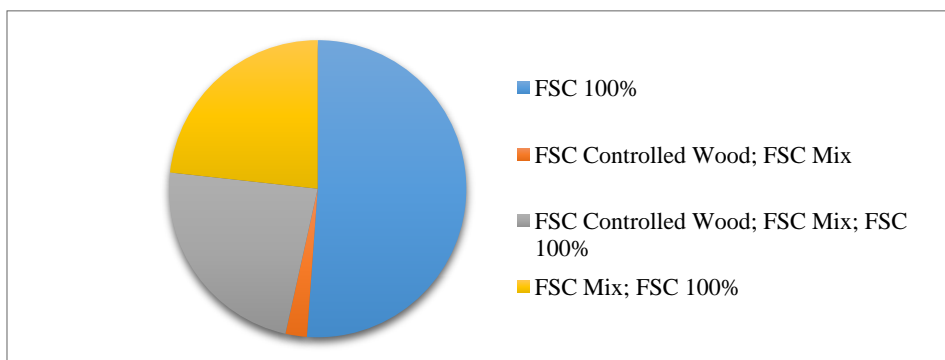


Figure 4. Main outputs of the certified companies

More than half of the valid FSC CoC certificates were issued only by two certification bodies, namely Soil Association Certification Limited (SA), based in United Kingdom for fifteen companies and SGS Société Générale de Surveillance SA (SGS), based in Switzerland for ten organizations, respectively.

In comparison with FSC certification, PEFC certification scheme is less common in Romania. Currently, across the country there are forty three PEFC CoC certified companies (Enescu et al., 2019), out of which only two (namely J.F. Furnir S.R.L. and S.C. Wood Industries S.R.L.) are CoC certified for veneer products, such as veneer sheets or veneer logs and laminated veneer lumber (PEFC, 2019). This situation might change soon due to the fact that the PEFC national certification system was recently (midsummer 2018) endorsed, and it is expected that both PEFC FM and CoC will gain more interest in Romania (Enescu et al., 2019).

As regards the global market, veneer-based market is characterized by an intense trade, countries from Africa, Europe or Oceania being, in general, net

exporters of veneer sheets, while most of the Asian countries are importers (Guan et al., 2019). The veneer market in North America is dominated by the United States of America, which, in 2016, placed on the first place in the worldwide top of the countries regarding the exported and imported quantities of veneer sheets, holding shares of 11.2% and 11.3%, respectively (Guan et al., 2019). Top five of the biggest exporters at global level is completed by China, Canada, Ukraine and Germany, while in top five of the biggest importers, except USA, Germany and China, India and Italy hold important shares (Guan et al., 2019). Worldwide, the trade is amplified also by the high diversity of tree species suitable for veneer-based products, such as shining gum (*Eucalyptus nitens* H.Deane & Maiden), in Australia (Blackburn et al., 2018), red oak (*Quercus rubra* L.), in the United States of America (Paletto and Notaro, 2018), hybrid poplar (*Populus x canadensis* Moench), in Italy (Pra and Pettenella, 2019) or acacia (Genus *Acacia* Martius) plantations, in Vietnam (Frey et al., 2018).

CONCLUSIONS

FSC is the most common forest certification scheme (for both forest management and chain of custody) in Romania, several forest owners and hundreds of companies being certified. Among the most common certified wood products, the veneer represents a special category due to its particularities, such as production, market and price.

CoC certification is regarded as an important element for the companies that are present on international markets, especially on the specialized ones, such as the veneer market, the certificates being regarded as a proof that the wood originates from a sustainable managed forest.

Last but not least, by taking into account the increasing worldwide demand of certified veneers, it is expected that this sector would gain more and more attention in the future in Romania, meaning that additional resources should be allocated by the companies, including specialized people.

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CONTEMPORARY AND TRADITIONAL IN THE LIFE OF A RURAL WOMAN

SUMMARY

Given the importance of the woman on the farm, her role in the countryside is often unfairly neglected. The aim of this paper is to examine the social and socio-economic status of women in rural areas, based on the conducted research. The paper is dedicated to October 15 - Day of Rural Women.

Surveys were conducted in the municipalities of Pljevlja and Kolašin. An interview method was used in order to collect data. Survey results show that rural women play a role both as housewives and as workers on their own farms. It originates from the nature of rural family, which simultaneously emerges both as a consumer unit-household and a production unit-farm. Respondents' answers testify to small but visible improvements in the position of rural women in the surveyed area. Due to the traditional gender roles that involve caring for children and the elderly, solely by doing housework, women are left with little room for independent activities, education and employment outside the home. The survey showed that the respondents were not familiar with the celebration of International Day of Rural Women - October 15th. In this regard, it is necessary to work on its popularization in order to strengthen the social and economic position of rural women.

Key words: rural woman, farm, household, agriculture.

INTRODUCTION

Within the concept of neo-endogenous rural development, special attention is paid to empowering the so-called vulnerable social groups. Due to the nature of family and social roles and positions, they also include rural women (Cvejić, Babović, Petrović, Bogdanov, Vuković, 2010). Social vulnerability of rural women arises both from the characteristics of the rural social structure and features of the rural gender regime, which is manifested in gender-defined roles, positions, relationships and (self) perceptions of both women and men (Čikić, 2016). Therefore, it is very important to raise a question what are the opportunities of rural women to participate in all three forms of reproduction of family farms, and which factors shape them (Cikic, 2016).

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A rural woman performs the function of a housewife in difficult circumstances because she is burdened with work in agricultural production. It has two economic functions: in the area of consumption and in the field of production. This arises from the nature of the rural family, which at the same time appears as a consumer unit-household and as a production unit-farm (Dimković, 1979). Due to traditionally gender roles that involve raising children and helping the elderly, exclusively doing housework, not owning land or other financial resources, and performing agricultural and additional activities to increase the home budget, women are left with little room for independent economic activities, education and employment outside the home. (Barada, Čop, Kučer, 2012). Some authors consider that ordinary women's activities could be a basis of rural local economy development, because the existing way of life has provided women with the knowledge of their own community needs and skills that could be used in local service industries, food production intended for the market, tourism, work from home, environmental protection, etc. (Hazl *et al.* 2011). The women in the villages declare themselves to be unemployed despite the fact that they contribute greatly to the household budget by participating in both permanent and occasional farm jobs. Their daily lives encompassed with economic work activities and their employment exceeds the limits of formal employment becoming daily activities of women (Šikić-Mičanović, 2012). Most of the researches to date confirm the continuity of disadvantaged family and social status of rural women. It is reflected in the lack of chances to manage their own lives and participate in the political and social life of the community (Korać, 1991; Rajković, 2009). Unfavourable social status is reflected in the daily struggle with a low material standard (Rajković, 2009; Babović, Vuković, 2008), in addition to the difficulties in harmonizing family and work responsibilities due to an underdeveloped institutional support system (Blagojević, 2010). Therefore, it is not surprising that women in the countryside still see the meaning of their existence mainly in the family, even more closely caring for children (Korać, 1991). The most recent researches indicate the diversification of the family and social position of rural women depending on their education, work activity, age (Čikić, 2016). Women graduated from high and higher school are significantly more satisfied with the quality of their lives, and among younger rural women, there are ones who see their interest and show a willingness to enter into entrepreneurship and association (Bogdanov *et al.*, 2011).

Family farms are a specific element of rural social structure, as they are characterized by the unity of family life, production, consumption and housing (Čikić, 2016). Farming and farm life are not considered to be a desirable lifestyle (Bendin, 2009; Sokić, 2005), few girls from farms are ready to stay and even fewer are ready to come to the farms (First-Dilić, 1973). High and non-agricultural education is considered to be the best ticket to escape from agriculture and countryside, which in the conditions of limited social vitality and social unattractiveness of the village appears as one of the life aspirations among young people in the village (Marković-Krstić, 2005; Stojanov, 1988). The

education of young (rural) women is also a prerequisite for their better positioning in the labour market, therefore it can be considered as a modern form of dowry (Čikić, 2016).

The remains of the classic Montenegrin patriarchal family still exist in the Montenegrin village today. The woman is still sought after somewhere as a „workforce“, as an „economic unit“, as a „reproductive unit“ for the production of children, especially sons („happiness“) (Vujačić, 1973). Rural areas are in many ways specific to urban areas and deserve special attention. In the process of transition, the village, which was insufficiently in the focus of development policies even in socialism, becomes even more marginalized and women in the village have become invisible (Blagojević, 2009).

The position of rural women who form a special social category is subject to all general international and national documents regulating women's rights. Recognizing the crucial role and contribution of rural women, on December 18, 2007, the UN General Assembly adopted Resolution 62/136 establishing the International Day of Rural Women, i.e. International Day of Countryside Women. The aim was to emphasize the importance of the role of rural women in the production of food and the fight against poverty in the world. Given the importance of the issue, the Commission on the Status of Women (CSW) dedicated its 56th session in 2012 to empowering rural women and their role in reducing poverty and hunger (Barada, Chop, Kučer, 2012). The first International Day for Rural Women was celebrated on October 15, 2008.

Bearing in mind that October 15th - Day of Rural Women is approaching, this work is dedicated to that day and should contribute to its popularization, as well as to the improvement of the position of women in rural areas of Montenegro.

At the national level, obeying women's rights in rural areas is guaranteed by the highest legal act - the Constitution of Montenegro. The international framework includes: the Beijing Declaration and Platform for Action, the United Nations Convention on the Elimination of All Forms of Discrimination against Women (CEDAW), the UN General Assembly Resolution on the Advancement of Women in Rural Areas, Recommendation of the Council of Europe Parliamentary Assembly on the Advancement of Women in the Country (Program for improving the employability of women in rural areas of Montenegro, 2013).

The aim of the paper is to examine the social and socio-economic status of women in rural areas on the basis of the conducted research.

MATERIAL AND METHODS

An interview method was used to collect the data. The purpose of the interview is to gather very clear, precise and quantitative answers to the given topic (Janičijević, 2014). A few interview questions form a Likert-type scale. The Likert scale stands out for its simplicity, economy of construction and frequency of application (Prpić, 2005). The focus of the interview is on the role and position

of the female population in the household economy viewed through the prism of social and ethical norms. The official data of the Statistical Office of Montenegro (MONSTAT) in addition to scientific and professional papers dealt with this issue were used in drafting this paper. Statistical tables and area charts were used for displaying the data. Desk research method and a comparison method were used for displaying data in the paper. The survey was conducted in the municipalities of Pljevlja and Kolašin. In a sample of 20 respondents, 50% of them were located in Kolašin and 50% in Pljevlja.

RESULTS AND DISCUSSION

Agricultural employment of women in Montenegro accounts for a small fraction of their total employment (less than 4%), and women make up one third of the agricultural workforce. The position of women in the countryside is a result of the position of women in society as a whole, the position of agriculture as an economic branch, the demographic situation, and position of rural areas in relation to urban areas (Blagojević, 2009). Considering that engaging in agriculture is an important basis for the existence of a still significant number of women, it is necessary to improve their agricultural employment. The reasons for the low interest of women to remain in rural communities are as follows: women in rural areas are rarely owners of property, they are rarely in the position of head of the household, ie. less often they have the role of deciding on agricultural production and the role of responsibility for the economic risks of doing business on the farm (Despotović *et al.* 2015). According to the Agriculture Census, women account for only 12.87% of the holders of family farms. Out of 48,824 holders of family farms, 16,228 holders are over 65 years of age or older, with a share of 33.24% (Table 1.). Holders of farms are usually older men.

Table 1. Workforce on family farms according to the age and gender

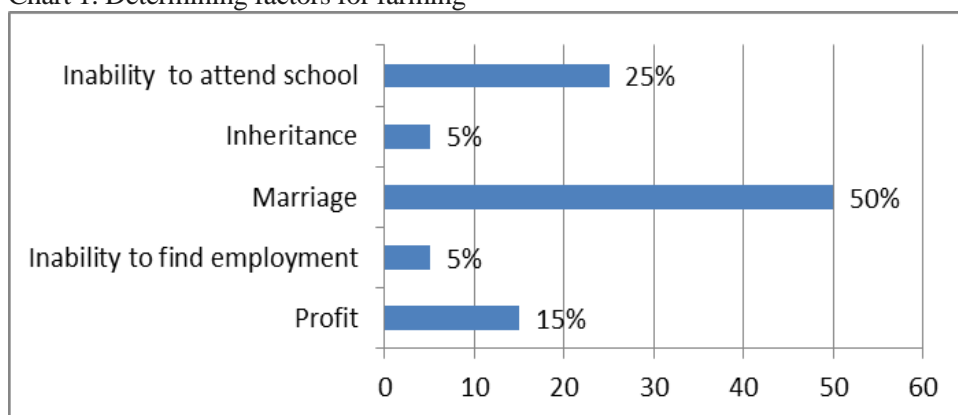
Age	Total workforce		Farm holders		
	members	share	share	male	female
TOTAL	98341	100,0	48824	42538	6286
Under the age of 24	6717	6,80	344	307	37
Between the age of 24 and 34	11340	11,50	2387	2228	159
Between the age 35 and 44	15675	15,90	5993	5540	453
Between the age 45 and 54	21562	21,90	11675	10769	906
Between the age 55 and 64	19849	20,20	12197	10657	1540
65 and elder	23198	23,60	16228	13037	3191

Source: Agricultural Census 2010, Farm structure

In order to maximize human resources in rural communities and revitalize the local economy, participation of women is necessary. Many studies show that the inclusion of women in the local economy on an equal footing is rapidly reducing poverty and contributing to development (Gender and Sustainable Development, 2008).

The study involved interviewees from the age of twenty to over 65. In this regard, an attempt was made to cover a wide range of working age. Interviewees at the level of 40% were equally interviewed between the ages of 36-50 and 51-65. Participation of interviewees aged 26 to 35 and over 65, was at the level of 10% each. The interviewees mostly graduated primary school (55%), while 45% completed high school. Rural elementary schools played a major role in educating farmers. With the reform of 1952, an elementary school in the period of 8 years became a compulsory basic school (Petak, 1988). The marital status of interviewees in the households surveyed is as follows: 70% married, 20% widowed, 5% unmarried and 5% divorced. They mainly live in two- and five-member households (60%), in households with six or more members 20%, four-member 10%, three-member 5% and one-member 5%. Most of the respondents 86% live independently with their family, while 40% live in a community with the extended family.² Only 25% of the interviewees are owners of the house where they live, while 75% do not own real estate. The reasons why the interviewees decided to engage in agriculture are presented in the Chart 1.

Chart 1. Determining factors for farming



Source: Results of the interviews performed by the author

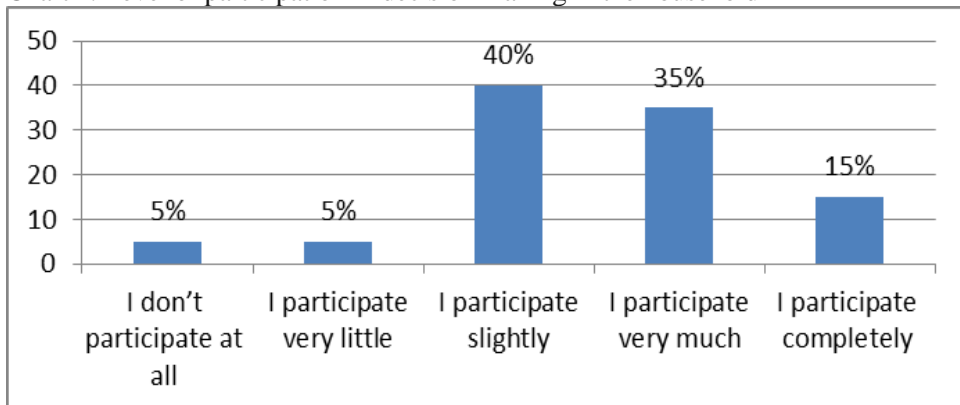
Marriages are cited by interviewees between the ages of 36 and 65 as the most important reason for farming. Of the total number of respondents who are married, the most significant reason for farming is 44% of those aged 36 to 50, 33% of the ages 51-65, and 22.22% of those over 65 years old. Inability to attend school is the second most important reason for farming, for respondents aged between 36 to 50 years, as well as for respondents aged 51 to 65. Education states as another important socio-cultural factor for participation of women in biological reproduction of family farms (Čikić, 2017). The characteristics of the

² Independent family implies life in the community of spouses and their children, while the extended family refers to the life in the community where beside the closest relatives also reside (father in-law, mother in-law, brother and sister in-law and etc.)

educational structure of rural women are one of the main indicators of a change in their family and social status, that is, the transformation of the rural gender regime. This particularly refers to the category of young women, given that their educational structure has significantly approximated to the educational structure of young women in cities (Bubalo-Živković, Lukić, 2015). The lower level of formal qualifications makes women less competitive in the labour market, which hinders their employment opportunities (Čikić, 2017).

When asked if they were the owners of the land, 60% of the interviewees stated that they did not own it, while 40% replied positively. Ownership is an important socio-economic factor because it "implies" a position and role of a woman in the economic and social reproduction of farms (Čikić, 2017). The respondents who answered that they were the owners of the land were the widows and younger respondents who inherited the land. Research conducted by him (Čikić, 2017), he states that a woman as the owner of the land and the formal title holder of the estate appears most often in the case of absenteeism of the male heirs. 85% of respondents have their own income from (selling cheese, fruit, handicrafts, etc.). Respondents are mostly health insured through their husbands, while 35% have their own agricultural insurance. Each of the respondents would not advise daughters to stay in the village. The views expressed by the respondents indicate that they are still in a subordinate position in the family, especially from the standpoint of decision making and business decision making. Farming as an economic and business unit is a source of income, the amount of which depends on making adequate business decisions. The level of participation of the respondents in the business decisions in the households is shown in Chart 2.

Chart 2. Level of participation in decision making in the household



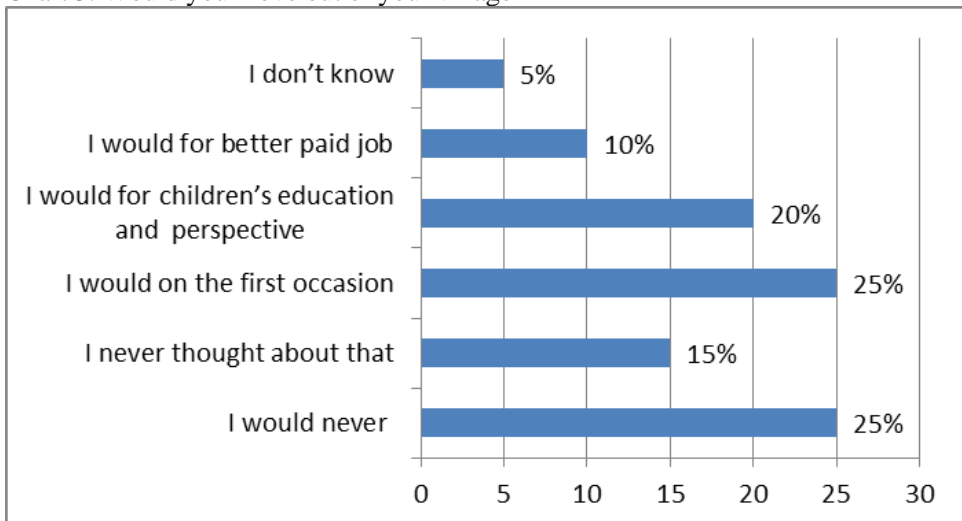
Source: Results of the interviews performed by the author

The majority of respondents (40%) participate slightly in making business decisions, although in reality a woman is the main and basic "work force" on the farm, with many duties and obligations, without adequate rights and decision-

making (Dimković, 1979). 85% of female respondents participate in the allocation of the home budget, yet 15% do not. The result obtained is consistent with the fact that the rural woman performs the function of housewife. The influence of the rural woman in determining the structure of expenditures contributes to the reconstruction of single-family homes, the purchase of furniture, etc., as well as the improvement of living conditions (Dimković, 1979).

Impossibility of finding an employment in rural areas and limited availability of public services (education, social care, health care, etc.) are the causes of migration from rural to urban areas (Bradeš et al., 2018). A woman as an agricultural producer is most often employed on a farm that is attached to her household and forms the basis of her family's economic existence (Grebo, 1966). Chart 3. shows the respondents' answers to the question "Would you move out of your village"?

Chart 3. Would you move out of your village

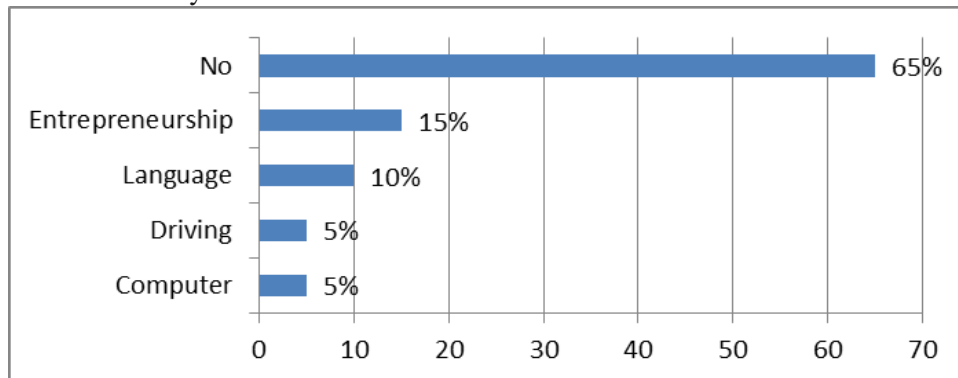


Source: Results of the interviews performed by the author

A quarter of the respondents want to move out of the village on the first occasion, while the same number would never leave the village. The education of rural women is a prerequisite for their better positioning in the labour market, therefore leaving the countryside appears as one of their life aspirations (Čikić, 2017).

Most interviewees have completed elementary and high school. Economic empowerment of rural women involves investing in oneself or developing skills to open up new business opportunities. However, the length of working days of rural women leaves them no room for further training (Bradeš et al., 2018). Chart 4. shows the respondents' answers to the question if they would have liked to attend the courses.

Chart 4. Would you like to attend courses



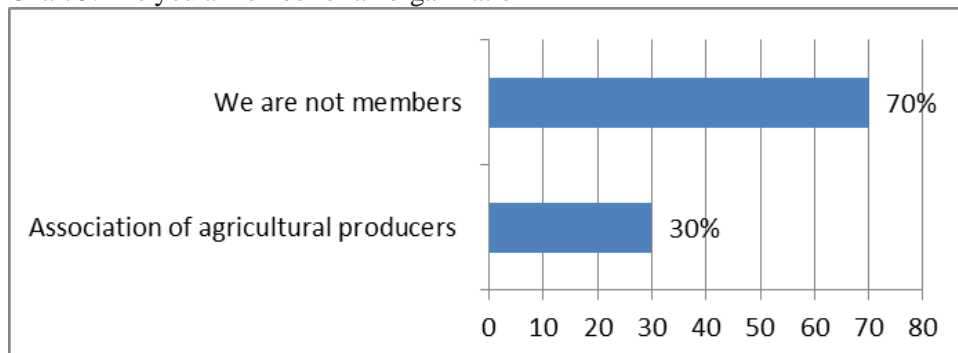
Source: Results of the interviews performed by the author

Theoretically, during the working day or week, after the completion of work and fulfilment of obligations of wife, mother, housewife and farmer, the rural woman has some time to freely dispose of it. Its rational spending raises the level of education, thus leisure and spare time contribute to mental and physical recreation of an exhausted organism. Celebration and seasonality characterize opportunities for leisure activities. There are factors that reduce a woman's chances of meaningful leisure use. These are: tradition and real opportunities for the use of certain educational and cultural content (Dimković, 1979).

Rural areas where road infrastructure surveys were conducted are favourable, as 60% of respondents stated that the village has an asphalt road, while telecommunications infrastructure (mobile telephony, TV signal, cable signal) is fully represented on all farms.

Social inclusion was explored through a question relating to membership of agricultural producers' associations, cultural arts societies, sports societies and non-governmental organizations, Chart 5.

Chart 5. Are you a member of an organization

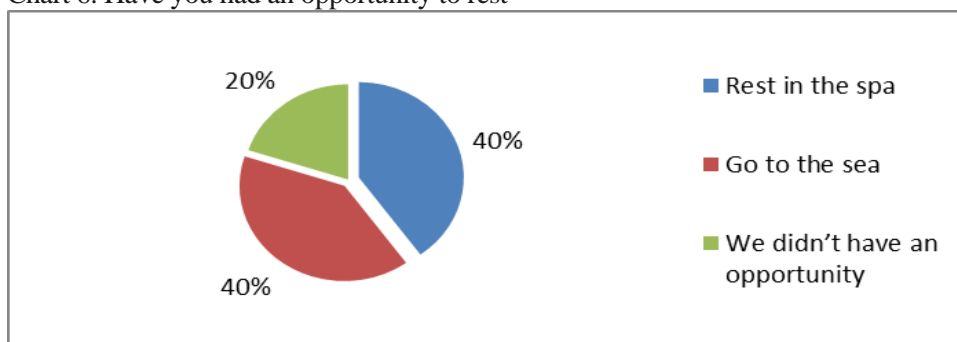


Source: Results of the interviews performed by the author

Interviewees stated that they were not members of any association (70%), while 30% stated that they were members of an association of agricultural producers. Social norms are the pressure that rural women obey. Customs of the past and habits do not allow a rural woman to enjoy social activities. Knitting, embroidery, and similar jobs are considered jobs that give a woman rest from hard work, and visits to relatives, neighbours, and friends are still the primary forms of leisure (Dimković, 1979).

In order to observe the position of the respondents regarding the use of vacations, the question was asked: "Have you had an opportunity to rest in: a spa, at sea, in the mountains and to visit some farms in the surrounding area?" Answers to the questions are shown in the Chart 6.

Chart 6. Have you had an opportunity to rest



Source: Results of the interviews performed by the author

The answers show that 80% of the interviewees had the opportunity to use the holiday (40% at sea, 40% in the spa), while 20% of the respondents did not have the opportunity to take the time to rest. None of the respondents visited the mountain or any farm in the area.

Some of the respondents' personal views on the position of women in their village were originally conveyed and read as follows:

"If you work you will earn"; " It is hard, especially for elderly"; "Difficult and troublesome"; "Work from dawn to dark"; "Better than my mother, but still difficult - no rest"; "Much easier than before"; "Monotonous - all the same"; "Solitude kills"; "Desolation"; "There is no one else"; "Day and night work";	"We are respected "; "We are appreciated"; "It's enough work," "Commitment to work"; "Solid"; "Neglected"; "Subordinate"; "Good"; "From time to time"; "Average"; "Currently good"; "Bad before"; "Pretty good".
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These views are the result of different experiences of the respondents. Regardless of the generational affiliation of the interviewees, they notice an improvement in the position of women compared to earlier. Respondents' answers testify to small but visible improvements in the position of rural women in the surveyed area.

When asked "do they know what October 15 is celebrating", no respondent knew the answer. The purpose of celebrating International Day of Rural Women is to remind all actors of society of the large but still unacknowledged role of rural women, as well as the need to constantly improve their living conditions. New sustainable development goals include gender equality and women's empowerment. The aim is to double agricultural productivity and the income of small producers, especially women. Women are important for the success of almost all sustainable development goals (Ban Ki Mun, 2013). In order to improve the role of women in rural areas, the Ministry of Agriculture and Rural Development of Montenegro is actively participating in the celebration of October 15th - International Day of Rural Women.

CONCLUSION

The factors that determine a position of a woman in the countryside are the following: level of education, income level of the farm, level of agricultural development and position of woman in society, and in general, etc. The role of a woman is often unjustifiably reduced to a role in biological reproduction. The village woman represents the pillar of the farm, without which the transformation and modernization of the village cannot be carried out. Particular progress would be made by raising the general education of the rural woman.

The results of the research show that position of the woman on the farm is changing and that women have a certain power in the business decision-making process. Marriage is still cited as the most important reason for farming by women aged 36 to 65 and at the same age state the inability to attend school. The characteristics of the educational structure of rural women are one of the main indicators of a change in their family and social status, that is, a transformation of the rural gender regime. Education arises as a prerequisite for introduction and expanding of innovation on the farms, which would result in creating favourable socio – economic conditions, thereby indirectly improving the position of rural women.

Socio-social inclusion of the respondents was assessed through a question related to membership of agricultural associations, cultural arts societies, sports societies and non-governmental organizations. Most of the respondents, 70%, are not involved in any organization or in social activities.

When asked about the perception of one's position, the answers are very diverse and the result of their personal experiences. Nevertheless, it is noted that, regardless of their generational affiliation, the respondents notice an improvement in the position of women in relation to older generations.

Respondents' answers testify to small but visible improvement in the position of rural women in the surveyed area.

Bearing in mind that International Day of Rural Women is celebrated on October 15th, the respondents were asked "Do you know what is celebrated on October 15th"? None of the respondents knew what was being celebrated that day. Negative responses indicate the need to take action to affirm this date, which would have broader societal relevance and be one of a number of necessary steps for positioning rural women in society.

Obtained data show that the position of the rural woman is better today than in the previous period, but that it is necessary to work on providing the necessary knowledge and skills of rural women, promoting, networking and advocating their interests.

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RESERVES FOR INCREASING THE YIELD OF SUNFLOWER SEEDS IN THE UKRAINIAN STEPPE

SUMMARY

The highest yields of sunflower hybrids are provided when plants are placed after surface plowing and chiseling at a depth of 25-27 cm. Reducing the depth of cultivation to 10-12 cm and direct sowing in untreated soils leads to a significant decline in sunflower yield by 20-30%. An important factor in increasing the crop yield is the use of fertilizers (N₄₀P₆₀K₃₀), which is better during the basic soil treatment in the autumn or under pre-sowing cultivation, which provides an increase in the yield of seeds at a level of 0,2-0,4 t / ha. High efficiency in the technology of sunflower growing is also provided by the use of physiologically active substances. The yield increasing from their application varies within 0,09-0,32 t / ha or 3,2-10,4%.

Keywords: sunflower, seeds, mineral fertilizers, soil cultivation, herbicides, yield.

INTRODUCTION

The level of weed infestation directly affects the intensity of competitive relationship between sunflower crops and weeds. Weed suppression in the sunflower crop has to be done with adequate herbicides and in due time in order to suppress a significant reduction in morphological and yield parameters (Simić et al, 2011). For the control of dicotyledonous weeds in sunflower, active ingredients such as linuron, flurochloridone, oxyfluorfen, pendimethalin, prosulfocarb, bifenox, aclonifen, flumioxazin, and lenacil are often used (Kilinc et al. 2011) in combination with acetamide herbicides (acetochlor, dimethenamid, pethoxamid, metolachlor, flufenacet, and propisochlor), which are intended for the control of grass weeds. Soil herbicides can influence directly the emergence and physiological process of cultivated plants and weeds. These chemicals can also change processes of nutrient transformation and indirectly effect on mineral nutrition of plants. Such herbicides as Afalon Dispersion (linuron), Galigan 240 EC (oxyfluorfen), Pledge 50 WP (flumioxazin), Proponit 720 EC (propisochlor) and Stomp 330 (pendimethalin) with the highest field suggested doses decreased

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fresh- and dry weight of four weeks age sunflower shoots in different extent, moreover influenced mineral nutrition of two hybrids unequally (Nadasy et al, 2008).

The efficacy of linuron, prosulfocarb, and pethoxamid was strongly affected by soil moisture and was insufficient under dry conditions. The majority of herbicides showed good selectivity for sunflower. Crop injury rate of 5–15% was recorded after application of flurochloridone and acetochlor. For flurochloridone, the phytotoxicity increased due to irrigation after herbicide application. The highest sunflower injury rate (27–35%) was recorded after application of oxyfluorfen. Field experiments were conducted at Agricultural Research and Development Institute from Fundulea (Romania) on a leached chernozem soil, well drained, formed on loess, with 33% clay content and 2.8% organic matter in the arable layer (Petcu Gh. and Petcu E., 2006). The developed modern crop production technologies should be improved in response to concerns about environmental impacts of agriculture towards cropping intensification reduction. In this context, choice of a good soil tillage method management is an important decision to improve grain yield and quality.

The relation between crop growing and soil tillage treatment are play important role in agricultural production (Gürsoy et al, 2014). Soils under conventional tillage (CT) generally have lower bulk density and associated higher total porosity within the plough layer than under no tillage (NT). No-till farming can reduce soil erosion, conserve soil moisture and minimize labor and fuel consumption. no differences were found between the NT and CT. There were also no significance differences in content of protein, oil and ash among six tillage methods. The highest fuel consumption was measured in conventional method (CT) whereas the lowest value was found in direct seeding method as 33.48 L ha⁻¹ and 6.6 L ha⁻¹, respectively (Halvorson et al., 1999). Soil compaction represents an important issue in the actual context of agricultural system sustainability (Aboudrare et al, 2006). Research on the various developments of root systems under tillage has been explored for many crops, whether for the biomass area or the underground, but very little concerns Sunflower (*Helianthus annuus* L.). Under compacted soil, major changes in the sunflower's root architecture occurred (55% of root length, 67% of root surface, and 42% of root diameter) and root system exploration was negatively impacted (assessed through the use of semivariogram). This resulted in a decrease of deep root exploration and in an increased lateral growth. Modifications of leaf surface, biomass, yield, and kernel components were also reported. Those modifications were the consequences of soil compaction (Mirleau-Thebaud et al, 2017; Fuentes et al., 2009). At the start of No-till sunflower planting in Argentina, weed control become a more complex topic. Herbicides for preplant chemical fallow provided effective weed control. Difficulties rose with weeds emerging after planting because two widely used pre-emergence residual herbicides (acetochlor and flurochloridona) were less effective, being dependent of rainfall and retained by stubble. As a result of research on control programs including other sunflower selective residual herbicides (sulfentrazone, diflufenican, prometryna), combined tank mixes with glyphosate, preplant plus preemergence split applications, etc. (Montoya, 2008) weed control was improved.

Crop preemergence herbicides will always be at some risk of incomplete control because rainfall must move herbicides into the soil prior to weed seed germination. While rescue treatments of escaped grasses are effective, most broadleaf weeds cannot be controlled with postemergence herbicides because the two available (acifluorfen and benazolin) are poor performing. The development of the clearfield technology (Bojanich, 2003) has been the most important progress for weed control and sunflower No-Till area increase. Trials comparing weed control programs with imazapir applied at V4-V6 were successful (Rodriguez, 2005). Furthermore, since the introduction of CL sunflower hybrids up to date, genetic progress has been important on seed yield, oil content, and high oleic CL hybrids. For crop sequences in which participate corn and sunflower, CL sunflower usually follows corn, with excellent control of voluntary glyphosate resistant corn (Montoya et al., 2008). In modern conditions, a significant part of the above-mentioned elements of oilseed production technology is not adhered to and ignored. In particular, in recent years a sunflower has been returned to its original place after 2-3 years, and even earlier, while saving on fertilizers and plant protection products. All these negative factors lead to a significant loss of the yield, decrease in its quality, and as a result to the fall of seed gross collection.

The objective of this research was to identify the most effective elements of sunflower growing technology, which ensure the maximum productivity and economic efficiency of growing oilseed crops in the Steppe of Ukraine.

MATERIAL AND METHODS

Experimental studies were carried out in 2011-2017 in the field experiments of the State enterprise of experimental farm "Dnipro" of the Institute of grain crops of National Academy of Agrarian Sciences of Ukraine according to the following schemes (Table 1-3).

Table 1. Options for application of biological active substances

Biologicals	Dose of application
Seed treatment with water before sowing (control 1)	-
Soil treatment with water before pre-sowing cultivation (control 2)	-
Seed treatment Agat-25 K	0.2 l / ha
Seed treatment EM-1	1.0 l / ha
Introduction into the soil EM-1	2.0 l / ha
Seed treatment Gumysol	400 g / t
Seed treatment Gumat potassium	400 g / t

Table 2. Effect of mechanical and chemical control for sunflower growing

Soil cultivation trials	Herbicides
Surface plowing on 25-27 cm	mechanized (control)
	mechanized (control) + manual weeding (control 2)
	without care (control 3)
	acetochlor.(harnes) -2.5 l / ha
	dual gold - 1.0 l / ha + hezagard - 2.0 l / ha
Flat-cut loosening on 14-16 cm	frontier optima - 1.4 l / ha
	mechanized (control)
	mechanized (control) + manual weeding (control 2)
	without care (control 3)
	acetochlor.(harnes) -2.5 l / ha
Disking on 10-12 cm)	dual gold - 1.0 l / ha + hezagard - 2.0 l / ha
	frontier optima - 1.4 l / ha
	mechanized (control)
	mechanized (control) + manual weeding (control 2)
	without care (control 3)
Zero cultivation (direct sowing)	acetochlor.(harnes) -2.5 l / ha
	dual gold - 1.0 l / ha + hezagard - 2.0 l / ha
	frontier optima - 1.4 l / ha
	mechanized (control)
	mechanized (control) + manual weeding (control 2)

Table 3. Options of sowing depth and seed fractions

Depth of sowing, cm	Fraction number, cm
4.0-5.0	5.0-6.0
4.0-5.0	4.0-5.0
4.0-5.0	4.0-3.0
4.0-5.0	2.5-3.0
4.0-5.0	<2.5
8.0-9.0	5.0-6.0
8.0-9.0	4.0-5.0
8.0-9.0	4.0-3.0
8.0-9.0	2.5-3.0
8/0-9/0	<2/5

The soil of the experimental plots was ordinary heavy-loamy chernozem. Topsoil is characterized with 4,2% humus, nitric nitrogen $\text{NO}_3\text{-N}$ 13.2 mg / kg, mobile forms of phosphorus and potassium 145 and 115 mg / kg respectively.

Unfavorable weather conditions for corn cultivation were in 2012. The hydrothermal coefficient (HTC) during the period of the largest water consumption of plants (the end of May - July) was equal to: 2011 – 0.8, 2012 – 0.6, 2013 – 0.7, 2014 – 0.9, 2015 – 0.8, 2016 – 0.9, 2017 – 0.8. The parameter of the HTC of less than 0.7 indicates the presence of soil and air drought, which negatively affects the formation and pouring of seeds.

RESULTS AND DISCUSSION

The external form of sunflower plants (*habitus*) play great importance in the process of yield formation. Sunflower seeds of the Middle Russian ecotype are grown in Ukraine. These plants have a height of 120-190 cm, do not branch, the seeds are shellfish. The average daily growth of the stem from germination to the formation of two pairs of real leaves is 0.8-1.0 cm, to the formation of baskets - 1.5-1.7 cm, to flowering – 3.0-4.3 cm. The upper part of the stem with a basket in most varieties is dying with an increase in the weight of baskets.

An important element in the formation of sunflower productivity is the number of leaves on a plant, which is often different even within a single hybrid. In particular, there are 24-28 units, and the mid-ripening - 28-32 units in early-ripening ones. The first pair of real leaves appears on the second or third day after appearing on the surface of the siblings, the next one - every two or three days. Death of plant leaves due to unpredictable cases (hail, drying out during a drought) usually leads to a decrease in its yield (Tkalic et al, 2018). The removal of 50% of leaves in the flowering phase reduced the seed yield by 72% in our experiments.

The formation of baskets in early-ripening hybrids starts at three to four, in medium-late - at five to eight pairs of leaves. Unfavorable conditions in this period lead to a decrease in plant productivity due to the reduction in the number of colors in the basket. Under the influence of high temperatures, drought or rain in the center of the basket seeds are often not formed, and the lack of the harvest can reach 5-78%.

The above negative factors of influence of drought and other negative factors can be partially leveled using modern adaptive and drought resistant oilseed hybrids. It is very important to choose hybrids, adapted to the growing zone with a potential yield of not less than 4.0 t / ha with a high resistance to diseases, pests and sunflower broomrape (*Orobanche cumana* Wallr.).

Such hybrids of domestic breeding as “Limit”, “Charodii”, “Basalt”, “Anthracite”, “Sonahro”, “Sibson”, “Forward”, “Rurik”, “Zorepad”, “Oreol” are highly appreciated. The performance indicators of individual registered hybrids are shown in Table 4. High drought resistance of sunflower is achieved due to the formation of a well-branched root system. So, according to our observations, individual plant roots penetrate into the soil to a depth of 3 meters, and in the horizontal direction - to 1.5-1.7 m.

Table 4. Characteristics of sunflower hybrids of Ukrainian breeding

Hybrid	Plant height, cm	Vegetation period, days	Oil content, %	Potential yield, t/ha
Time	165	98	49	4.0
Rubikon	160	98	51	3.9
Sonahro	155	99	53	4.4
Sibson	165	99	50	3.9
Yason	180	108	50	4.2
Rurik	160	101	51	4.1
Queen	175	107	49	4.4
Slavson	165	110	50	4.1
Selianin	150	112	53	4.3
Suchasnyk	170	110	50	5.0
Husliar	165	112	52	5.1
Siuzhet	160	111	51	4.2
SSD _{0,95} , t/ha				0.09

It should also be noted that hybrids that are insufficiently resistant to diseases are not suitable for sowing, because this leads in some years to a significant loss of seed yield. Hybrids that are resistant to diseases phomopsis (*Phomopsis helianthi*), sclerotinia (*Sclerotinia sclerotiorum* (Lib.)), downy mildew (*Plasmopara halstedii*), phomasum (*Phoma oleracea* var. *Helianthi* Sacc.), rust (*Puccinia helianthi* Schw.) can be returned to their original place even earlier than the recommended period, namely, in 4-5 years, but provided that after harvesting (Tsyliuryk *et al.*, 2017, 2018). It is necessary to conduct a deep surface plowing on 27-32 cm, wrapping crop residues with pathogens in the soil. If black fallow is planned after the sunflower, it is possible to carry out a surface cultivation (6-8 cm) in the spring so that pathogens die on the soil surface under the influence of unfavorable environmental factors (light, precipitation, temperature changes, etc. This is possible with good soil water availability, absence of plant diseases in the year of harvesting sunflower seeds and application of fertilizers.

In addition to the right choice of hybrids in growing sunflower, the main cultivation of the soil is also very important. As a rule, soil cultivation under the sunflower in different farms, depending on cultivation conditions, is differentiated. That is why, they perform deep surface plowing or chiseling at a depth of 25 - 27 cm, non-surface flat-cut loosening at 20-22 cm, as well as shallow plowing (disking) by 10 - 12 cm (Table 5).

The obtained scientific data show an increase in weediness and a decrease in the yield on a descending scheme: deep surface plowing - chiseling - flat-cut loosening - shallow disking - No-till system. First of all, it is caused by deterioration of the physical state of the soil, moisture and nutrition regimes, phytosanitary conditions, etc. (Tsyliuryk, 2018). Sunflower reacts well both to to macroelements

and the introduction of trace elements and bacterial agents. High increments in seed yield are provided by the treatment of seed material and the introduction into the soil of biologically active substances and bacterial agents. (Figure 1).

Table 5. Sunflower yield depending on soil cultivation and herbicides (average for 2010-2012).

Options for weed control	Yield according to treatments, t/ha			
	Surface plowing at 25-27 cm	Flat-cut loosening at 14-16 cm	Shallow disking at 10-12 cm	Direct sowing + roundup
Mechanized treatment (control)	2.39	2.19	1.91	–
Manual weeding (control 2)	3.24	2.93	2.62	2.43
Without treatment (control 3)	1.6	1.35	1.21	1.91
Harnes, 2,5 l/ha	3.05	2.30	2.33	1.78
Dual gold, 1,0 l/ha +hezagard, 2,0 l/ha	3.06	2.50	2.11	1.61
Frontier optima, 1,4 l/ha	3.04	2.60	2.19	1.77
Average	2.72	2.31	2.06	1.70
SSD _{0,95} t/ha		0.09-0.11		

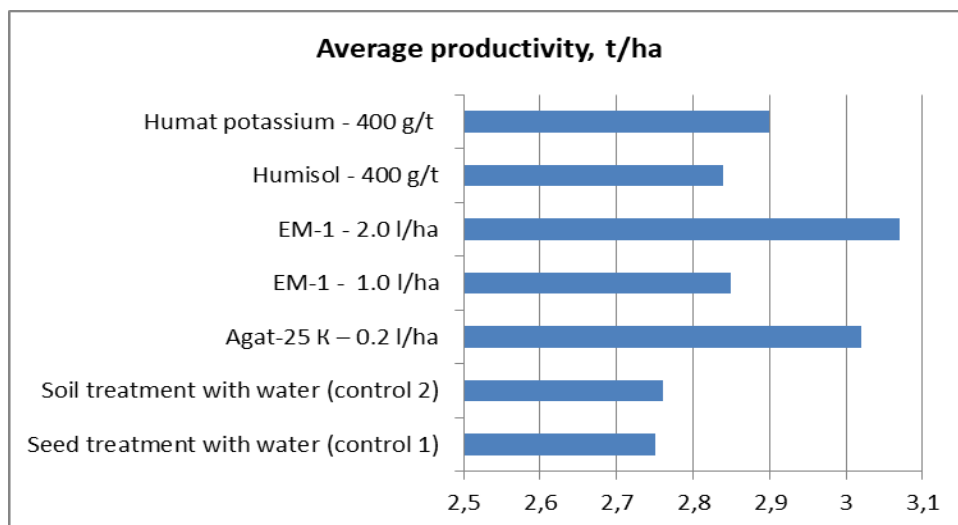


Figure 1: Effectiveness of biological active substances for sunflower yield (in average for 2013-2015).

The sunflower yield increase from their application varies within 0.09-0.32 t / ha, or 3.2-10.4%, and the maximum increase was found applying EM-1 into the soil.

An important element in the technology of sunflower growing is also the right choice of high quality seed material, which should have a similarity of at least 85%. The sunflower is adapted practically to an unlimited reduction of all organs, both vegetative and generative, including seed sizes, while preserving the reproduction function. The difference in the yield of oilseed sunflower in the next generation from seeds of different sizes is practically in focus of current tasks. The three years field experiments data to see optimum depth for different fractions of sunflower hybrid “Enei” are shown in the table 6.

Table 6. Influence of seed size on the yield of sunflower hybrid “Enei” (in average for 2008-2010).

Sunflower seeding depth, cm	Fraction №	Weight of 1000 sown seeds, g	Yield, t/ha
4-5	1	92.2	2.65
	2	53.3	2.60
	3	33.3	2.62
	4	28.6	2.55
	5	21.7	2.50
8-9	1	92.2	2.82
	2	53.3	2.68
	3	33.3	2.63
	4	28.6	2.64
	5	21.7	2.45
SSD _{0,95} , t/ha			0.07

Sowing small seeds is economically more profitable than large ones, because in order to obtain friendly germs, it requires less moisture in the soil. Better result was obtained for fraction №1. Small seeds can be sown to a depth of 8-9 cm in moist soil.

It is established that the main task of a pre-sowing tillage in the spring under the sunflower is the qualitative and optimal loosening of the sowing layer of the soil, which provides uniform seeding, contributes to the moisture preservation, the destruction of weeds and improves biological activity of the soil. In recent years, agricultural producers in Ukraine have been paying more attention to sunflower growing, due to high and attractive price of oilseed crops that grows each year (Tsyliuryk, et al., 2018). There are a lot of reserves for the further increase of the sunflower yield (Sessiz et al., 2008). One of the tasks in this direction is the elimination and leveling of the violations of the oilseed production technology, in particular, placing after the best predecessors and observance of the sunflower return policy to the original place in 5-6 years, selection of the best hybrids that are adapted to the environmental conditions,

application of optimal doses of micro and macro fertilizers, plant growth regulators, pest and disease control, compliance with all technological regulations in the care of crops, etc (Malhi et al., 2006; Heidari et al., 2008).

In conditions of late growth with a rapid increase in temperature, when the soil is leveled and not compacted, and the field is weakly overgrown with weeds, it is possible to be limited just to spring harrowing and one pre-sowing cultivation. On the untreated areas from the autumn, it is reasonable to carry out only shallow soil cultivation, using combined aggregates, disk harrows and cultivators, since spring surface tillage helps to re-dry the soil to the cultivated depth. Soil fertilization is also an important reserve for increasing the yield of sunflower seeds. The sunflower also reacts well to the result of the fertilizer introduced under the predecessor or before predecessor.

It should be noted, taking into account all mentioned reserves for increasing sunflower yield, that scientific investigations on studying their effectiveness are always relevant and should be constantly carried out due to changes in the climatic conditions of the terrain, the diversity of soils, the emergence of new technologies, hybrids, biological preparations, herbicides, fertilizers, modern machinery samples, etc. Correct selection of technological measures necessarily should be focused on energy conservation and saving material resources and time. Scientifically grounded application of technological measures for growing sunflower can significantly increase the yield of oilseed crops even by 25% or more without significant material costs and damage to the environment.

CONCLUSIONS

High productivity adaptive hybrids resistant to diseases and pests, should be used for sowing. The highest yields of sunflower hybrids are provided when plants are placed after surface plowing and chiseling at a depth of 25-27 cm. Reducing the depth of cultivation to 10-12 cm and direct sowing in untreated soils leads to a significant decline in sunflower yield by 20-30%. An important factor in increasing the crop yield is the use of fertilizers ($N_{40}P_{60}K_{30}$), which is better during the basic soil treatment in the autumn or under pre-sowing cultivation, which provides an increase in the yield of seeds at a level of 0.2-0.4 t / ha. High efficiency in the technology of sunflower growing is also provided by the use of physiologically active substances. The yield increasing from their application varies within 0.09-0.32 t / ha or 3.2-10.4%.

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FATTY ACID AND UNSAPONIFIABLE COMPOSITION OF TEN PHILIPPINE FOOD PLANT OILS FOR POSSIBLE NUTRACEUTICAL AND COSMECEUTICAL APPLICATIONS

SUMMARY

Ten readily available and underutilized plant materials in the Philippines were investigated for their oil content and composition, level and composition of unsaponifiable fraction and storage stability for possible nutraceutical and cosmeceutical applications. Based on oil extraction for each sample, the highest oil content of 25.00 ± 0.61 % was extracted from rambutan seed. Long chain fatty acids that can be used for skin-care products such as oleic, linoleic, linolenic and arachidic acids were detected in all food plant oils samples using gas chromatographic analysis. Docosahexaenoic acid (DHA), an omega-3 fatty acid that serves as the building block of human brain tissue and retina of the eye, was found in purslane oil. Multivariate discriminant analysis revealed that kernel oils of katchamita and carabao mangoes are relatively close based on their fatty acid profile. Close association was also exhibited by oils of malunggay seed and rice bran. Slow tendency to undergo hydrolytic rancidity of malunggay seed oil is indicated by its low free fatty acid value of 2.13 ± 0.13 meq/kg oil. A peroxide value of 0.95 ± 0.09 mg O₂/ kg of malunggay oil revealed that it is the most stable among the oils. These seven-month storage stability tests of the malunggay oil make it useful as ingredient in cosmetic products. Of the oils extracted, avocado kernel oil showed the highest unsaponifiable content (72.63 ± 2.91 %). The unsaponifiable fraction was subjected to TLC analysis using 5:1 v/v petroleum ether-ethyl acetate as solvent, coupled with densitometric analysis using the CP ATLAS v. 2.0 program and results showed that the highest phytosterol content of 57.59 ± 2.80 % was found in the carabao mango seed oil. Antitumor, cholesterol-lowering, antioxidant, anti-inflammatory and anti-bacterial properties are the reported bioactivities of phytosterols. This study demonstrates potential sources of nutritional lipids, which can serve as functional and innovative ingredients utilized for nutraceutical and cosmeceutical applications.

Keywords: fatty acid profile, food plant oil, unsaponifiable composition, multivariate discriminant analysis

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INTRODUCTION

Numerous indigenous and underutilized food plant materials contain high levels of bioactive components called phytochemicals, which play a vital role in disease prevention. Among these food plant materials are malunggay (*Moringa oleifera* Lamk) seed, avocado (*Persea americana* Mill.) seed, rambutan (*Nephelium lappaceum* L.) seed, chickpea (*Cicer arietinum* L.), rice (*Oryza sativa* L.) bran, purslane (*Portulaca oleracea* L.) leaves, carabao mango (*Mangifera indica* L. cv. Carabao) kernel and katchamita mango (*Mangifera indica* L. cv. Katchamita) kernel, ivy gourd (*Coccinia grandis* L.) seed and pomelo (*Citrus maxima*) seed. The oil from these food plant materials can be developed into high-value added and innovative ingredients and products promoting health and wellness. Utilizing food plant oils from plant materials (e.g., seeds) can also serve as a means to manage the environmental problems that are due to ample amount of fruit and vegetable by-products from the fruit and vegetable processing industry throughout the world (Duda-Chodak and Tarko, 2007; Ashok *et al.*, 2011).

Oils belong to a heterogeneous group of hydrophobic compounds that are vital elements in healthful diets. Researches on nutrition, chiefly focused on calories and energy conversion factors of foods, led to the development of and studies on oils since they have the highest energy content compared to proteins and carbohydrates. Oils have been widely used as raw materials and inputs for industrial food and medicine. Today, fats and oils, collectively known as triacylglycerols, are fully known for their nutritional, functional, and organoleptical properties. Oils are generally regarded as emollient (products with softening and soothing properties). They also have important cosmetic applications which include use in massage oils, bath oils, oil shampoos and sunscreen formulations (Samat *et al.*, 2005; Solis-Fuentes *et al.*, 2010).

Nowadays, understanding the health-promoting benefits that can be derived from dietary fatty acids plays an essential role in evaluating the nutritional quality of oils (Rueda *et al.*, 2014). The recommended daily intake values, for each type of fatty acid (FA), in particular, saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA), have been well-reported by the Food and Agriculture Organization (FAO). Oils that have high unsaturated fatty acids (MUFA and PUFA) are now promoted to replace those that are rich in saturated fats (Aranceta *et al.*, 2013). This concern has led researchers to analyze the fatty acid composition of oils, which will help nutritionists and food manufacturers in assessing oils that are of great value due to the presence of unsaturated fatty acids (Dubois *et al.*, 2007).

Aside from fatty acid profile of food plant oils, the presence of phytochemicals with strong scientific evidence for their health promoting properties leads to potential nutraceutical and cosmeceutical applications. Nutraceuticals, products in between nutrition and pharmaceuticals, are nearly any bioactive component that delivers benefit beyond nutrition (Dover, 2008; Manela-Azulay and Bagatin, 2009). On the other hand, cosmeceuticals, an

emerging concept in 21st century personal care, are cosmetic-pharmaceutical hybrids intended to enhance beauty through ingredients that promote pharmacological action resulting in beneficial physiological effect (Moure et al., 2001). Among the segments of the skin care market, cosmeceuticals are the fastest growing and are now part of the multibillion dollar natural products industry (Sadick, 2003; Nemarundwe et al., 2008). The fusion of cosmetics and food industries results in a recent area called nutricosmetics, which is also known as “beauty pills.” Orally consumed nutraceutical supplements (nutricosmetics) and topically applied cosmeceutical are considered to work in harmony to promote physical appearance and well-being.

Recently, functional foods, a new generation of food products with added bioactive components that show a therapeutic effect, have demonstrated positive impact on human health. Among the constituents of unsaponifiable fraction are phytosterols, tocopherol, squalene & triterpenes. Phytosterols are well-recognized to have antitumor activities (Lin and Jones, 1995), cholesterol lowering properties (Hendricks et al., 1999), antioxidant, anti-inflammatory, anti-atherosclerotic, anti-oxidative, anti-ulcerative and anti-bacterial properties (Beveridge et al., 2002; Moreau et al., 2002). Phytosterols in avocado flesh oil have shown anti-angiogenic activity (Salamanes & Rodriguez 2009). It is also widely accepted that phytosterols can promote oxidative and thermal stability and shelf-life of vegetable oils (Przybylski and Eskin, 2006). Phytosterols are utilized as emulsifiers in the cosmetic industry and provide the main steroidal intermediates and precursors for the production of hormone pharmaceuticals (Grunwald, 1975). On the other hand, clinical trials have shown that tocopherol (Vitamin E) balances autonomic tone in diabetics, lowers risk for prostate and colon cancer, reduces arteriosclerosis, and retards the development of neurodegenerative disease, fibrocystic breast disease, premenstrual syndrome, osteoarthritis, and infertility (Manzella et al., 2001; Brigelius-Flohe et al., 2002; Virtamo et al., 2003).

In this study, the fatty acid profile and the unsaponifiable composition of various food plant materials were determined for possible nutraceutical and cosmeceutical applications, as part of our continuing effort on demonstrating schemes for value-adding to agriculture. Chemometric analysis of gas chromatography (GC) data based on fatty acid composition was done to determine the relationship between the type of oil (single categorical dependent variable) and the percentage (%w/w) content of the fatty acids (a set of quantitative independent variables). This paper also evaluated the storage stability of food plant oils based on free fatty acid (FFA) and peroxide value (PV).

MATERIAL AND METHODS

Sample Collection and Preparation

Chickpea, rice bran, seed samples from malunggay and fruits (avocado, rambutan, carabao and katchamita mangoes, pomelo) were bought from local

markets around Los Baños, Laguna, Philippines. Purslane samples and ivy gourd seeds were collected from areas around University of the Philippines Los Baños campus, Laguna, while rice bran samples were bought from King Erwin Rice Mill, a local milling station in Calauan, Laguna, Philippines. All seed and plant samples were initially dried under sunlight until maximum moisture was removed. Rice bran was stabilized by steaming at 100 °C for 3 minutes as described by Kuroda *et al.* (1977). Dried samples were sealed in plastic jars and were stored in a refrigerator prior to use.

Extraction of Oil

Food plant oils were extracted using modified method of Kuroda *et al.* (1977), after which the extract was salted out with saturated NaCl solution, washed with water, dried with anhydrous Na₂SO₄ and the solvent evaporated *in vacuo*.

Isolation and Determination of Relative Composition of Unaponifiable Fraction

The oil was saponified using a modified method of Gutfinger and Letan (1972) by refluxing with ethanolic KOH for 2 hours and then the unaponifiable fraction was extracted with diethyl ether. The unaponifiable fraction was obtained by evaporation of diethyl ether *in vacuo*.

The unaponifiable fraction was subjected to TLC[?] using 4:1 v/v petroleum ether-ethyl acetate as solvent. Squalene, tocopherol and phytosterol standards were also spotted on the TLC plate. The spots were visualized by spraying with 20% sulfuric acid and then charring. The mass of compound corresponding to each spot in the developed chromatogram was estimated by densitometry using the CP ATLAS v. 2.0 program.

Storage Stability of Oil

Determination of Peroxide Value (PV): The peroxide values of the extracted oils were determined spectrophotometrically using the standard method of International IDF Standards (1991). The free fatty acid values were determined using the AOAC (1960) standard procedure.

Determination of Fatty Acid Profile of Oils: The isolated oil samples were submitted to the Industrial Technology Development Institute, Department of Science and Technology, Philippines for the determination of the fatty acid profile by gas chromatography. The oils were converted to fatty acid methyl esters (FAMES) which were analyzed according to the gas chromatography AOAC method (AOAC 963.22, 1995). The fatty acid identity and percent composition were determined by comparing the retention times of the FAMES from the oil samples with authentic reference standard FAME mixture.

Statistical Analysis

All statistical analyses were carried using SPSS Version 7.5 including the determination of the average values and standard deviation. Each value is the average of three replicates. One-way ANOVA followed by Tukey test was used

to compare between means ($P < 0.05$) of the sums of fatty acids (SFA, MUFA, PUFA) and SFA/PUFA ratio of the different oils. To analyze the relationship of the type of oil (single categorical dependent variable) and the percentage (%w/w) content of the fatty acids (a set of quantitative independent variables), multivariate discriminant analysis of gas chromatography (GC) data based on fatty acid composition was used (Rueda et al., 2014).

RESULTS AND DISCUSSION

Sample Preparation and Extraction of Oils from Various Plant Samples

Photographs of some of the isolated oils are shown in Figure 1. Data on yields (dried basis) are summarized in Table 1. Highest oil content was extracted from rambutan seed ($25.00 \pm 0.61\%$), followed by malunggay seed ($19.58 \pm 0.60\%$) and ivy gourd seed ($19.20 \pm 0.57\%$).

Table 1. Oil yields and oil unsaponifiable fraction obtained from various plant samples.

Sample	Oil content, %	Oil unsaponifiable fraction, %
Malunggay seed (dried)	19.58 ± 0.60	--
Avocado kernel (dried)	3.51 ± 0.33	72.63
Rambutan seed (dried)	25.00 ± 0.61	1.16
Chickpea (dried)	6.86 ± 0.10	3.81
Carabao mango seed (dried)	13.23 ± 0.15	59.29
Rice bran	11.42 ± 2.46	4.85
Purslane leaves (edible portion, dried)	3.15 ± 0.00	40.69
Katchamita mango seed (dried)	14.44 ± 0.56	53.63
Ivy gourd (<i>Coccinia grandis</i>) seed (dried)	19.20 ± 0.57	7.30
Pomelo seed (dried)	15.59 ± 0.00	0.79

Rambutan seed oil content coincided with the value (17-39 %) reported by Solis-Fuentes et al. (2010). Extraction of malunggay seed oil is relatively lower as compared to the reported value (25.1 – 41.4 % depending on the variety) (Tsaknis et al., 1999). The avocado kernel oil yielded $3.51 \pm 0.33\%$ which was close to the reported yield (1-3 %) (Gutfinger and Letan, 1972). Extraction of carabao mango and Katchamita mango kernels yielded $13.23 \pm 0.15\%$ and $14.44 \pm 0.56\%$, respectively, which were close but little higher to the oil yield previously reported for mango oils (Stefanov et al., 1997).

In most cases, a single non-polar solvent may not isolate the polar lipids from tissues. Thus, a solvent system composed of varying proportions of polar and non-polar components were employed to ensure a complete and quantitative recovery of tissue lipids.



Figure 1. Photographs of oils and samples.

Characterization of the Unsaponifiable Fraction of Oils: Determination of Relative Composition

The unsaponifiable components of oils are those that cannot be hydrolyzed upon saponification. In general, oil samples with high unsaponifiables are regarded to have moisturizing, sunscreensing, and anti-aging properties. Among the unsaponifiable components of majority of oils are phytosterols, terpenes, tocopherols and squalene.

The highest unsaponifiable content (Table 1) was obtained from avocado kernel oil with $72.63 \pm 2.91\%$ yield, followed by oils of the kernels of carabao and Katchamita mangoes with 59.29% and 53.63% unsaponifiable content, respectively. Although the oil content of avocado kernel is quite low at $3.51 \pm 0.33\%$, it has a very high unsaponifiable content, which compensates its potential

as good source of unsaponifiable fraction in terms of over-all yield. Mango kernels are usually regarded as waste and are usually thrown away by households upon consumption of the fruit, may be used as good source of unsaponifiable lipids. Unsaponifiable components were not detected upon saponification of malunggay seed oil. The unsaponifiable fraction of pomelo oil was 0.79%, quite a low value compared to the others. Purslane leaves (edible portion, dried) contained 40.69% unsaponifiable fraction, which is double higher than record of Acedo et al. (2012).

TLC analysis of the unsaponifiable fraction (Table 2) of each oil was done using 5:1 v/v petroleum ether-ethyl acetate as solvent. Squalene, tocopherol and phytosterol standards were also spotted on the TLC plate. The spots were visualized by spraying with 20% sulfuric acid and charring. An example of TLC chromatogram of purslane unsaponifiable is shown on Figure 2. Among the oil samples, the carabao mango seed oils showed the highest phytosterol content ($57.59 \pm 2.80\%$). The avocado oils also showed high phytosterol content ($15.59 \pm 0.66\%$), and due to this property is considered as a cosmetic lipid that may be used as ingredient in massage creams, massage oils, and other cosmetic products.

Table 2. Composition of unsaponifiable fraction of analysed plant oils.

Oil	Phytosterols		Triterpenes		Tocopherol		Hydrocarbons (Squalene)	
	% comp.	mg/ 100 g oil	% comp.	mg/ 100 g oil	% comp.	mg/ 100 g oil	% comp.	mg/ 100 g oil
Avocado kernel	15.59 ± 0.66	4023.22 ± 480.75	1.37 \pm 0.05	996.94 \pm 39.70	2.53 \pm 0.21	1835 \pm 154.79	26.10 \pm 2.48	18954.01 ± 1800.46
Rambutan seed	55.04 ± 0.37	637.97 ± 4.30	8.37 ± 0.19	97.03 ± 2.17	1.33 ± 0.22	15.46 ± 2.60	21.86 ± 2.60	253.40 ± 30.18
Chickpea	19.43 ± 2.60	37.56 ± 5.03	34.16 ± 1.53	66.04 ± 2.97	-	-	36.51 ± 1.34	70.59 ± 2.58
Carabao mango seed	57.59 ± 2.80	34144.4 ± 1188.0	5.62 ± 0.03	3334.10 ± 14.90	2.31 ± 0.22	1369.60 ± 130.60	1.72 ± 0.23	1019.40 ± 133.60
Purslane	10.06 ± 2.71	16881 ± 4558	11.97 ± 0.73	20084 ± 559	7.49 ± 0.06	12561.0 ± 959.0	10.73 ± 1.71	1800.14 ± 264.43
Katchamita mango	10.57 ± 1.29	10568.0 ± 1287.8	5.33 ± 3.46	5333.3 ± 3462.3	4.37 ± 0.67	4365.9 ± 672.0	6.27 ± 0.22	6272.20 ± 223.60
Rice bran	32.12 ± 1.89	1557.31 ± 91.74	-	-	-	-	-	-
Ivy gourd seed	26.77 ± 1.04	1965.79 ± 76.14	-	-	-	-	-	-
Pomelo seed	58.71 ± 2.58	460.85 ± 20.42	21.91 ± 5.53	173.12 ± 41.35	-	-	19.38 ± 2.65	153.07 ± 20.92

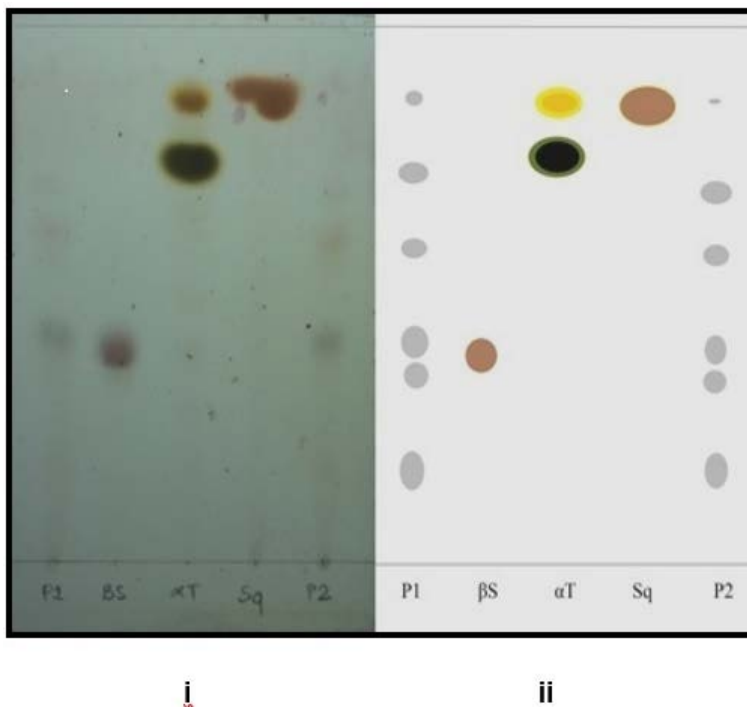


Figure 2. (i) Photograph and (ii) schematic representation of thin layer chromatogram of the purslane unsaponifiable fraction using 4:1 (v/v) petroleum ether-ethyl acetate as solvent (lane 1: purslane unsaponifiable fraction 1; lane 2: β -sitosterol standard; lane 3: α -tocopherol; lane 4: squalene; lane 5: purslane unsaponifiable fraction 2).

In some developed countries phytosterols are used as enrichment and are incorporated into various food products such as bars, vegetable oils, orange juice, mayonnaise, milk, yoghurt, soy milk, meat, green teas, among others (Berger *et al.*, 2004). The plant samples with high phytosterol content may be regarded as a good source of this bioactive compound for use in such purpose. Moreover, food plants such as purslane and avocado, shown (Acedo *et al.*, 2012; Salamanec & Rodriguez 2009) to have significantly high phytosterol content may serve as direct and quick sources of the said bioactive compound.

Data presented in Table 2 showed that chickpea had the highest triterpene (34.16 ± 1.53 %) and squalene content (36.51 ± 1.34 %). High squalene content was also observed in avocado kernel (26.10 ± 2.48 %), rambutan seed (21.86 ± 2.60 %) and pomelo seed (19.38 ± 2.65 %). Triterpenes are secondary metabolites that exhibit biological activities, such as antimicrobial, cytotoxic, antitumoral, antiviral, anti-inflammatory, hepatoprotective, anti-feedant and insecticidal activities (Alvarenga and Esteban, 2005). Squalene is a triterpene that has anti-cancer and antioxidant activities (Auffray, 2007; Charlton-Menys and Durrington, 2007; Ronco and De Stefani, 2013). Squalene can protect human

skin from lipid peroxidation caused by UV light and other sources of oxidative damage, and is also utilized as an additive in topically applied vehicles such as lipid emulsions and nanostructured lipid carriers. Squalene has the ability to help the skin to retain moisture while it provides relief, protects, nourishes, and restore harmony to dry skin (Huang et al., 2009).

Among the oils, high tocopherol (Table 2) content was obtained from purslane oil ($7.49 \pm 0.06\%$), katchamita mango kernel oil ($4.37 \pm 0.67\%$), and carabao mango kernel oil ($2.31 \pm 0.22\%$). Tocopherols were not detected in rice bran, chickpea, ivy gourd, and pomelo unsaponifiable fractions. Tocopherols, are generally associated with Vitamin E, which is known to be a lipid-soluble, chain breaking radical scavenger. The Vitamin E content of membranes often determines the susceptibility of microsomal membranes, low-density lipoproteins, hepatocytes, or whole organs to damage by peroxidizing agents such as hydroxyl radicals, alkoxy radicals, peroxy radicals, and singlet oxygen. These agents not only damage the lipids but also produce lipid hydroperoxides, which can decompose into alkoxy and organic peroxy radicals, and thus propagate chain reactions in lipid peroxidation. Tocopherols protect lipids by scavenging peroxy radicals without reacting in further chain-propagating steps (Mascio et al., 1991).

Determination of Fatty Acid Profile of Oils

Malunggay seed oil had the highest behenic acid (docosanoic acid) content ($0.49 \pm 0.01\%$) among the oils (Table 3). Behenic acid is intended for use as an oil structuring and solidifying agent (texturizer) in margarine, shortening, and foods typically requiring the use of semi-solid and solid fats, at levels of up to 8% of the oil mass of the food item. Commercially, behenic oil is used as an ingredient responsible for the smoothing properties of moisturizers and hair conditioners. The high oleic acid content ($4.91 \pm 0.01\%$) of the malunggay oil opens its potential use in cosmetic and pharmaceutical formulations as an excipient.

It is used in topical and parenteral pharmaceutical formulations. It has also been used as penetration enhancer in transdermal formulations to improve the bioavailability of poorly-water soluble drugs in tablet formulations, and as raw material for ointments and creams. Avocado kernel oil was found to contain a considerable amount of palmitic acid ($14.63 \pm 0.21\%$). Palmitic acid is a saturated fatty acid mainly used in the rubber industry. Lignoceric acid ($9.51 \pm 0.09\%$) was also detected from avocado seed oil. Lignoceric acid is a constituent of cerebrosides and sphingomyelins. The major fatty acid found in avocado kernel oil is linoleic acid ($49.10 \pm 0.70\%$). The result agrees with the finding that linoleic acid is the main fatty acid component of avocado kernel oil (Takenaga et al., 2008). Lignoceric acid ($1.15 \pm 0.05\%$) was found to be the major fatty acid component of ivy gourd seed oils.

Table 3. Fatty acid profile of the oils isolated from various plant samples*.

Plant	12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	19:0	20:0	C20:5	22:0	22:6
Malunggyz seed	ND	ND	ND	4.92 ± 0.01	0.95 ± 0.01	ND	4.91 ± 0.01	771.10 ± 0.09	0.85 ± 0.00	2.60 ± 0.01	ND	1.60 ± 0.00	0.49 ± 0.01	22.6 ± 6.53
Avocado kernel	ND	ND	14.63 ± 0.21	0.92 ± 0.00	6.54 ± 0.11	ND	5.94 ± 0.19	49.10 ± 0.70	6.28 ± 0.29	4.61 ± 0.49	1.24 ± 0.63	1.24 ± 0.06	ND	ND
Rambutan seed	ND	ND	3.56 ± 0.06	0.25 ± 0.00	ND	6.39 ± 0.02	46.20 ± 0.14	4.72 ± 0.02	13.60 ± 0.17	ND	20.40 ± 0.08	2.04 ± 0.001	4.72 ± 0.05	ND
Chickpea	ND	ND	6.29 ± 0.02	ND	ND	5.79 ± 0.01	18.80 ± 0.05	66.20 ± 0.04	1.89 ± 0.01	ND	0.57 ± 0.00	0.06 ± 0.00	0.38 ± 0.00	ND
Rice Bran	ND	0.31 ± 0.00	13.70 ± 0.12	0.07 ± 0.00	ND	1.61 ± 0.00	35.40 ± 0.22	46.90 ± 0.21	1.48 ± 0.01	ND	0.28 ± 0.00	0.16 ± 0.00	0.22 ± 0.00	ND
Purslane leaves	1.60 ± 0.19	0.48 ± 0.01	ND	ND	ND	22.56 ± 0.04	1.08 ± 1.61	4.85 ± 0.07	17.00 ± 0.33	ND	29.61 ± 1.18	ND	ND	5.08 ± 0.49
Carabao mango seed	ND	ND	6.08 ± 0.04	6.08 ± 0.04	ND	47.1 ± 0.05	37.8 ± 0.08	7.05 ± 0.01	0.86 ± 0.05	ND	1.06 ± 0.05	ND	ND	ND
Katchamita mango seed	ND	ND	5.42 ± 0.13	5.42 ± 0.13	ND	44.35 ± 0.12	39.10 ± 0.09	8.29 ± 0.02	0.96 ± 0.06	ND	1.08 ± 0.01	0.24 ± 0.00	0.43 ± 0.00	ND
Ivy gourd seeds	ND	ND	3.81 ± 0.15	3.81 ± 0.15	ND	5.17 ± 0.09	2.37 ± 0.05	29.35 ± 0.52	0.30 ± 0.00	ND	1.08 ± 0.01	ND	ND	ND
Pomelo seeds	ND	0.05 ± 0.00	19.50 ± 0.20	19.50 ± 0.20	0.09 ± 0.00	3.63 ± 0.02	15.00 ± 0.02	48.14 ± 0.09	12.20 ± 0.03	ND	58.66 ± 0.81	ND	0.04 ± 0.00	ND

*Values are expressed as means ± SEM

ND: not detected

Rambutan oil (Table 3) mainly consisted of long chain fatty acids, mostly oleic acid ($46.20 \pm 0.14\%$) and arachidic acid ($20.40 \pm 0.08\%$), and minimum amounts of behenic acid ($4.72 \pm 0.05\%$), which were shown to be similar to previous results (Solis-Fuentes et al., 2010; Harahap et al., 2012). These long chain fatty acids are good ingredients for skin-care products such as lotions and massage oils since skin irritation decreases with increasing fatty acid chain length. Fatty acid chains of at least 12 carbons are less likely to cause skin irritation (Briden and Green, 2006). Aside from this, its long chain fatty acids are also good for green-candle making since it would have higher melting point than other oils. Chickpea and rice bran oil were shown to have high linoleic ($66.20 \pm 0.04\%$ and $46.90 \pm 0.21\%$, respectively) and oleic acid content ($18.80 \pm 0.05\%$ and $35.40 \pm 0.22\%$, respectively). The fatty acid profile for chickpea was similar to the reported values (Zia-Ul-Haq et al., 2007). Rice bran oil fatty acid profile was also similar to the data provided by Riceland Foods, Inc. The predominance of oleic and linoleic acid generally adds nutritional value to these oil samples. Oleic acid was shown to have hypotensive effects (Teres et al., 2008). Linoleic acid is an essential fatty acid required for growth, physiological function, and maintenance. Ivy gourd and pomelo seed oils were also shown to have high linoleic acid content ($29.35 \pm 0.52\%$) (Table 3).

Purslane is generally known for its high omega-3 fatty acid content. Docosahexaenoic acid (DHA), an omega-3 long chain polyunsaturated fatty acid, was detected in purslane oil, more than two-fold ($18.3 \text{ mg}/100\text{g FW}$ or $5.08 \pm 0.49\%$; Table 3) higher than the previously reported value ($0.8 \text{ mg}/100\text{g FW}$) (Omara-Alwala et al., 1991). DHA is the building block of human brain tissue. It is the primary structural fatty acid in the gray matter of the brain and retina of the eye. Brain tissue is about 60% lipid (structural fat, not adipose fat) and about 25% of that is DHA. Eicosapentanoic acid (EPA) was shown to be present in malunggay ($1.60 \pm 0.00\%$), avocado kernel ($1.24 \pm 0.06\%$), rambutan ($2.04 \pm 0.00\%$), rice bran ($0.28 \pm 0.00\%$), chickpea ($0.06 \pm 0.00\%$) and katchamita mango ($0.24 \pm 0.00\%$) kernel oils. EPA was not observed from the purslane oil sample due to the lack of EPA reference standard during the time of its analysis. EPA is the precursor to prostaglandin-3 (PGE3) and also provides a natural approach to lower blood cholesterol and triglycerides. PGE3 is directly responsible for making blood platelets less sticky, thus leading to an easier flow of blood throughout our bodies. This natural antithrombotic effect of EPA has been well researched. This means that EPA is intimately involved in bodily processes that inhibit blood clots, particularly in the small capillaries of the heart (Stefanov et al., 1997).

Oils from carabao and katchamita mangoes were mainly composed of oleic ($37.80 \pm 0.08\%$ and $39.10 \pm 0.09\%$, respectively) and stearic acids ($47.10 \pm 0.05\%$ and $44.35 \pm 0.12\%$, respectively) which are considered to be the main fatty acids required in the cosmetic industry. The results are similar to the reported values (Nzikou et al., 2010). Mango oils may be useful as ingredients in vanishing and shaving creams, lotions, deodorants, and shampoos. However,

other specifications for use as cosmeceutical products of these oils must also be considered such as good color and odor, crystalline structure and low iodine number.

Statistical and Multivariate Discriminant Analyses of Fatty Acid Profile

Table 4 presents the related sum (Σ SFA, MUFA and PUFA) and ratio of Σ SFA/ Σ PUFA for each food plant oil. One-way ANOVA followed by Tukey's test ($P < 0.05$) showed significant difference among the fatty acid constituents of each sample. Pomelo seed oil had the highest Σ SFA (83.12), malunggay seed oil had the highest Σ MUFA (78.10), and chickpea oil had the highest Σ PUFA (68.13). Oils of katchamita and carabao mangoes and purslane showed the high ratios of Σ SFA/ Σ PUFA (6.86, 5.42 and 5.71, respectively).

Table 4. Sum of fatty acids in food plant oils.*

	Σ SFA	Σ MUFA	Σ PUFA	Σ SFA/ Σ PUFA
Malunggay seeds	17.96 ^a	78.10 ^a	3.94 ^a	4.55 ^a
Avocado kernel	35.53 ^b	7.33 ^b	56.61 ^b	0.65 ^b
Rambutan seeds	35.06 ^c	46.40 ^c	18.51 ^c	1.89 ^c
Chickpea	13.04 ^d	18.83 ^d	68.13 ^d	0.19 ^d
Rice Bran	16.09 ^e	35.49 ^e	48.52 ^e	0.33 ^e
Purslane leaves	54.25 ^f	1.08 ^f	26.93 ^f	5.71 ^f
Carabao mango seeds	54.26 ^f	37.83 ^g	7.91 ^g	6.86 ^g
Katchamita mango seeds	51.40 ^g	39.10 ^h	9.49 ^h	5.42 ^h
Ivy gourd seeds	68.72 ^h	6.18 ⁱ	29.65 ⁱ	2.32 ⁱ
Pomelo seeds	83.12 ⁱ	34.50 ^j	60.34 ^j	1.38 ^j
SEM	0.0527	0.1477	0.0875	0.0160
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*Values are expressed as means \pm SEM.

^{a-j}Different superscripts indicate significant differences between oils (one-way ANOVA followed by Tukey's test, $P < 0.05$)

Σ SFA (saturated fatty acid) = sum of lauric, myristic, palmitic, heptadecanoic, stearic, arachidic, behenic and lignoceric acids;

Σ MUFA (monounsaturated fatty acid) = sum of palmitoleic and oleic acids;

Σ PUFA (polyunsaturated fatty acid) = sum of linoleic, linolenic and docoheptaenoic acids

Multivariate discriminant analysis, which graphically describes the similarities and differences among oils based on categorical variables, is used to study the interdependence among the oils by providing discriminating functions related to the type of the oils and the fatty acid components. Figure 3 depicts a graphical chart showing the similarity of the ten oils based on three dimensions

or discriminating functions, which were selected according to eigen values. Higher eigen values signify better function that can distinguish the groups (Rueda et al., 2014). The measure of distance corresponding to the type of data determines similarity or dissimilarity of objects in a multidimensional scaling. The characteristics of the oil is influenced by major and minor fatty acid content; hence there could be variation in two oils which have close percentage of major fatty acid but differ in the percentage of minor fatty acid constituents (Rezanka and Rezankova, 1999). Multivariate discriminant analysis showed that oils of katchamita and carabao mangoes are relatively close (Figure 3), similarity between oils of malunggay seed and rice bran and a considerable relationship among pomelo seed and avocado kernel oils. A close relationship also exists among malunggay seed, rice bran, chickpea and ivy gourd seed oils (Figure 3).

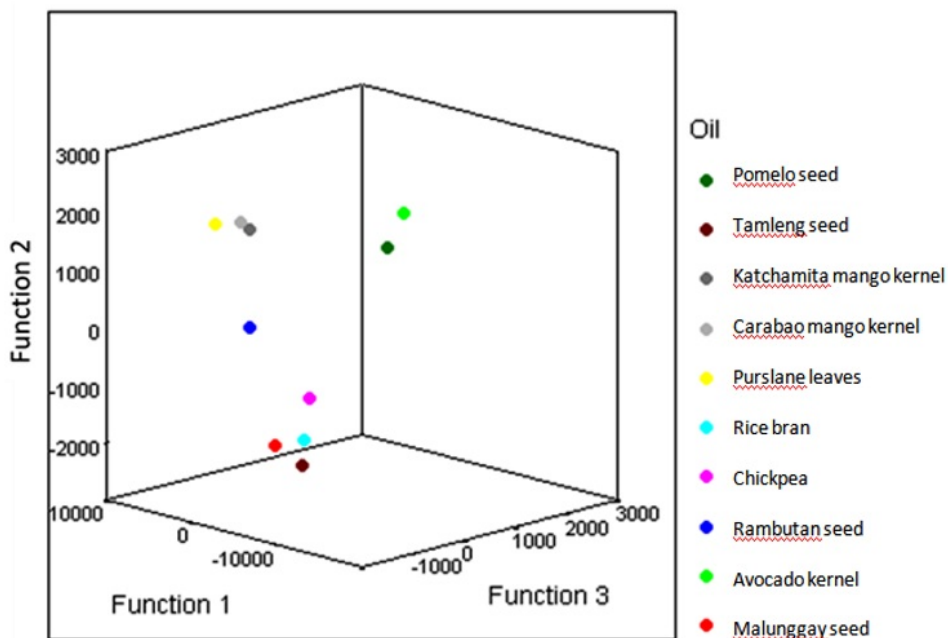


Figure 3. Similarity in fatty acid composition of the food plant oils based on the three discriminant functions of the multivariate discriminant analysis.

Determination of Storage Stability of the Oils

The storage stability of the isolated oils was evaluated by determining their peroxide value (PV) and free fatty acid value (FFA) for a seven-month duration. The effect of mode of storage was also investigated by comparing the PV and FFA of the oil samples at room temperature and upon storing by refrigeration.

Free Fatty Acid Values of the Oils

The FFA values of the oils stored at room temperature (25°C) and upon refrigeration (4°C) were determined to measure their degree of hydrolytic rancidity through time (Figure 4; Table 5).

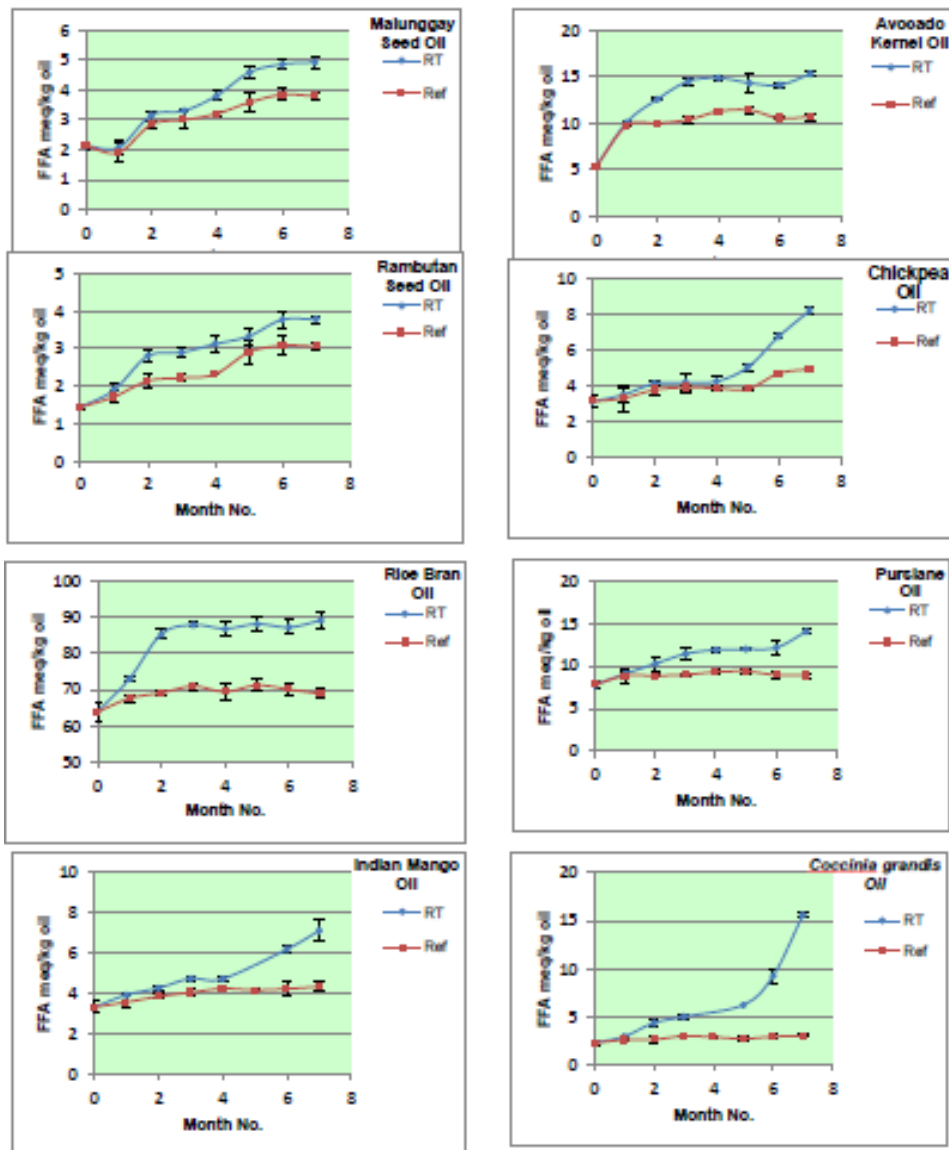


Figure 4. Free fatty acid values of malunggay seed, avocado kernel, rambutan seed, chickpea, rice bran, purslane, katchamita mango kernel, and ivy gourd seed oils upon 7-month storage. RT-at room temperature; Ref-at 0°C.

Rambutan oil showed the lowest FFA value (1.44 ± 0.08 meq/ kg oil) at the end of the storage period which suggests its slow tendency to undergo hydrolytic rancidity, and may be useful as ingredient in cosmetic products given its relative stability.

Table 5. Peroxide and free fatty acid values of the various plant oils.

	Peroxide Value (mg O ₂ /kg oil) on Extraction	Free Fatty Acid (meq/kg oil) on Extraction
Malunggay seeds	0.95 ± 0.09	2.13 ± 0.13
Rambutan seeds	0.98 ± 0.08	1.44 ± 0.08
Chickpea	5.60 ± 0.56	3.15 ± 0.34
Rice bran	0.83 ± 0.11	63.85 ± 2.89
Purslane leaves	59.76 ± 7.91	7.82 ± 0.40
Katchamita mango kernel	3.99 ± 0.05	3.33 ± 0.29
<i>Coccinia grandis</i> seeds (ivy gourd)	9.80 ± 0.36	2.28 ± 0.18
Pomelo seeds	2.66 ± 0.10	0.96 ± 0.17

The initial free fatty acid content of the isolated malunggay seed oil was found to be 2.13 ± 0.13 meq/kg oil which is close to the FFA value reported by Abdulkarim et al. (2005) and higher compared to the results of Tsaknis et al., 1999; Lalas and Tsaknis, 1999. Among the oil samples, malunggay oil still showed a low free fatty acid value (2.13 ± 0.13 meq/ kg oil) after 7 months which suggests that like rambutan oil, it is also a good cosmetic ingredient. Rice bran oil showed the highest free fatty acid value (63.85 ± 2.89 meq/kg oil), which continued to elevate during the 7-month storage period. The rest of the oil samples showed a moderate increase in FFA value throughout the duration of 7-month storage. Generally, storage at lower temperature (refrigeration) minimized the tendency of the oils to undergo hydrolytic rancidity. Although there is no reported threshold value for FFA, fresh oil samples that have undergone purification.

Peroxide Values of the Oils

The standard IDF spectrophotometric method (IDF 1991) was adapted in determining the peroxide values (PVs) of the oil samples. This is highly sensitive in detecting PV of samples even as low as 0.03 g. It is based on the oxidation of Fe²⁺ to Fe³⁺ by the peroxide. The Fe³⁺ ions that formed are then quantified by complexation with thiocyanate thereby giving a colored complex whose concentrations can be readily determined using spectrophotometry.

The actual state of oil oxidation is indicated by the amount of peroxides formed during oxidative processes (Giuffre et al. 2016). The more saturated fatty acid contained in oil, the less susceptible it is to oxidative rancidity. On the other hand, the greater the amount of unsaturated fatty acid in an oil, the more likely it is to become rancid via peroxidation. Since the supposed healthiest plant oils are all highly unsaturated, they are especially susceptible to rancidity. During the process of oxidative rancidity, oxygen molecules interact with the structure of the oil and damage its natural structure in a way that can change its odor, its taste, and its safety for consumption. In this process the long-chain fatty acids are degraded and short-chain compounds are formed.

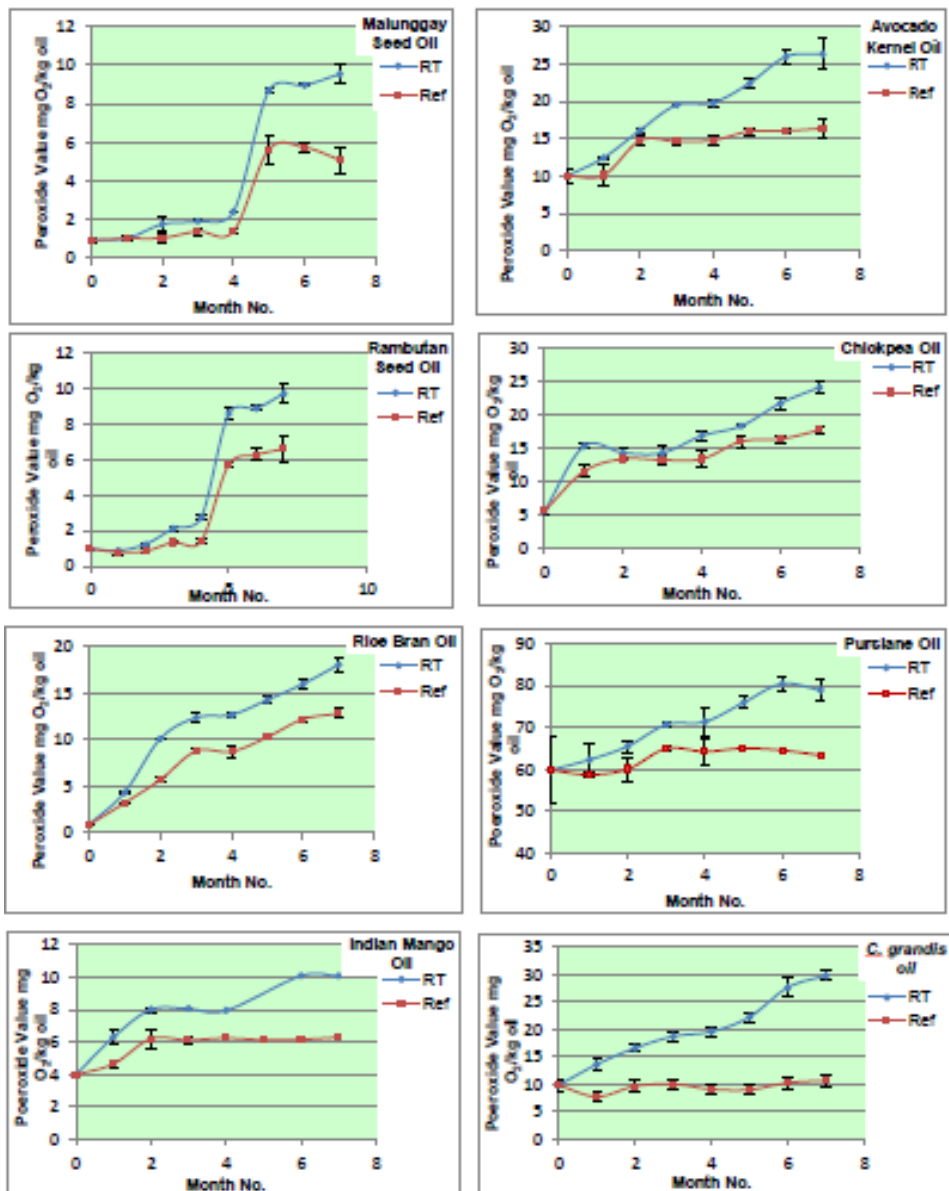


Figure 5. Peroxide values of malunggay seed, avocado kernel, rambutan seed, chickpea, rice bran, purslane, katchamita mango kernel, and ivy gourd seed oils upon 7-month storage. RT-at room temperature; Ref-at 0°C.

The threshold value for oxidative rancidity is 20 mg O₂/ kg oil. Typically, initial peroxide values of crude oil range from about 2-3 mg O₂/kg oil while purified oil PV is below 1. Among the samples, malunggay oil was the most

stable in terms of peroxide value (0.95 ± 0.09 mg O₂/ kg oil; Table 6). Several other factors affect the oxidative degradation of oils such as the presence of antioxidants, e.g. tocopherol. Antioxidants lower the rate of oxidative degradation of samples depending on its amount in it (Martinez-Alvarez et al., 2005). On the other hand, the presence of chlorophyll results in higher tendency for oxidative degradation (Werman et al., 1990). Thus, the high peroxide value of purslane oil (59.76 ± 7.91 mg O₂/ kg oil) could be due to their high chlorophyll content as indicated by the dark green color of the oil extracts.

Generally, the rates of increase in the peroxide values of the oil samples kept at room temperature were higher than those of the refrigerated samples (Figure 5). Therefore, oxidative degradation of the oil may be slowed down by storing at lower temperature. Oxidative rancidity proceeds with a low activation energy and is highly accelerated by sunlight, temperature increase and polyvalent ions. Oxidative rancidity happens when the sample reacts with oxygen which results in the degradation of fat and hyperperoxide formation.

CONCLUSIONS

As part of our continuing effort to maximize readily available and underutilized plants in agriculture and seeds, the fatty acid and unsaponifiable composition of various food plant materials were investigated. Results showed that avocado kernel oil contains the highest unsaponifiable fraction (72.63 %), followed by kernels of carabao mango (59.29 %) and katchamita mango (53.63 %). Upon analysis of the composition of unsaponifiable fraction, the mango seed oil had the highest phytosterol content. Phytosterols have been shown to possess anti-tumor, cholesterol-lowering, antioxidant, anti-inflammatory and anti-bacterial properties. Oil from purslane leaves and kernels of carabao and katchamita mangoes had high tocopherol content.

Analysis of fatty acid profile showed that oleic acid was the major fatty acid of malunggay and rambutan oils; while linolenic acid was the major fatty acid of avocado kernel, chickpea, rice barn and pomelo oils. Omega-3-fatty acids (DHA and ALA), known to reduce risk of cardiovascular diseases and to possess anti-inflammatory properties, were found in purslane oil. Purslane had high omega-3-fatty acid (DHA and ALA) and ivy gourd seed contained lignoceric acid as the major fatty acid. All oils contained ALA; purslane having the highest amount. EPA was found in malunggay, avocado kernel, rambutan, chickpea, rice bran and Katchamita mango oils. Multivariant discriminant analysis of oil revealed similarities and differences between food plant oils based on fatty acid profile; oils of Katchamita and carabao mangoes were relatively close. On monitoring the storage stability of the isolated oils over a seven-month period, rambutan and malunggay seed oils were found to be the most stable in terms of free fatty acid (FFA) and peroxide value (PV). This study is novel for the analysis of the unsaponifiable and fatty acid compositions of ten food plant oils in the Philippines and the comparison of the oils using multidiscriminant analysis. The results indicate that the ten plant materials can be sources of

functional and innovative ingredients that can be used for nutraceutical and cosmeceutical applications.

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CHEMICAL COMPOSITION AND TOXICITY OF THE ESSENTIAL OILS OF *LIPPIA CITRIODORA* FROM TWO DIFFERENT LOCATIONS AGAINST *RHYZOPERTHA DOMINICA* AND *TRIBOLIUM CASTANEUM*

SUMMARY

The lesser grain borer, *Rhyzopertha dominica* (F.), and the red flour beetle, *Tribolium castaneum* (Herbst), are among broad-based damaging insect pests of agricultural stored products. Because of several side-effects of conventional chemical insecticides to the human health, non-target organisms, and environment, searching for and introducing healthy, inexpensive, accessible and efficient agents is necessary. In the present study, chemical profiles and fumigant toxicity of the essential oils isolated from *L. citriodora* against *R. dominica* and *T. castaneum* were evaluated. Chemical compositions of *L. citriodora* essential oils from two different Iranian regions including Germi and Parsabad was evaluated by gas chromatography–mass spectrometry (GC-MS) and it was found that the terpenic compounds such as caryophyllene oxide, citral, limonene, and neral has the high amount in both oil. Some components such as sabinene, sulcatone, and β -ocimene were only identified in the essential oil from Germi region while some others such as verbenone, camphor, borneol with considerable amount in Parsabad region had not any trace in Germi' origin essential oil. The results of bioassays showed that the essential oils of *L. citriodora* from both regions had significant fumigant toxicity against the adults of *R. dominica* and *T. castaneum*. In general, essential oils' concentrations and exposure times had direct positive effects on the observed mortality. According to the results of present study, the terpene rich *L. citriodora* essential oils from two Iranian regions (Germi and Parsabad) have a high potential for the management of *R. dominica* and *T. castaneum*.

Keywords: chemical composition, essential oil, insect pest, lemon verbena, toxicity

INTRODUCTION

Lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is between the damaging group of whole grain pests. This cosmopolitan insect pest feeds and tunnels through cereals grain which leads to small pieces of kernels and powdery residues. Along with cereals, *Rhyzopertha dominica* can

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damage to other grains such as legumes and even to stored pharmaceuticals, leather, and packaging materials made from wood and paper (Edde, 2012). Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is also well-known as a cosmopolitan and serious worldwide insect pest of stored products containing broken cereals and legumes, dried fruits, and spices but flours are favourite diets. In addition to direct feeding damage, product quality reduction also occurs due to the residues of insect bodies and skin, and the release of quinones with their unpleasant smell (Villaverde et al., 2007, Johnson, 2013).

Although chemical pesticides have an important role in worldwide plant protection strategies, but their indiscriminate utilization lead to several negative side-effects such as threat to the human health (Zhang et al., 2018), non-target organisms including fish, birds, bees, (Mitra et al., 2011, Köhler et al., 2013) and even biological control agents (Cruz et al., 2017), the soil and water pollution (Goulson, 2013, Zheng et al., 2016), and spread of pest resistance (Sudo et al., 2018). In this circumstance, the use of healthy and effective compounds to alternate harmful chemical pesticides is necessary.

Plant-derived essential oils as secondary metabolites are constructed in different organs of aromatic plants, including leaves, roots, stems, flowers and seeds. These volatiles are the mixtures of several compounds, which are divided mainly in two groups of terpenes (by the coupling of isoprene units) and aromatics (derived from phenylpropane) constituents (Bakkali et al., 2008, Bajpai & Baek, 2016). Along with widely applications of essential oils in the pharmaceutical and perfume industries (Che & Zhang, 2019), their potential in the management of major agricultural pests has also been demonstrated in the recent years (Nikolic et al., 2018, Singh & Pandey, 2018, Gaire et al., 2019).

Lemon verbena [*Lippia citriodora* Kunth (Verbenaceae)], is one of the most cultivated medicinal plants, which used in the preparation of herbal tea for its lemon-like aroma (Argyropoulou et al., 2007, Kizil et al., 2018). Along with diuretic, antipyretic, antispasmodic, and sedative properties, the antimicrobial and antioxidant effects of this GRAS (Generally Regarded As Safe) listed plant was also demonstrated (Gomes et al., 2006, Farahmandfar et al., 2018, Fitsiou et al., 2018). Therefore, the aim of present study was to describe the chemical profile of two Iranian *L. citriodora* essential oils and assess their fumigant toxicity against *R. dominica* and *T. castaneum*.

MATERIALS AND METHODS

Plant samples and essential oils

The leaves of *L. citriodora* were collected from the 10 cm end of young stems during April and May 2019 from the Germe and Parsabad regions, Ardebil province, Iran. The plant samples were grinded by using an electric grinder and dried at room temperature. Fifty grams of powders were poured into a 1000 ml balloon of a Clevenger apparatus and the essential oil was extracted within 3 h. Extracted essential oils were stored in a refrigerator at 4 °C.

Chemical profile of the essential oils

Chemical analysis of the essential oils was performed by using an Agilent gas chromatographic system model 7890B equipped with the Agilent 5977A mass spectrometer detector system. Chromatographic separation was performed on the HP-5MS (5% phenyl-methyl-polysiloxane) capillary column with 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness. This detector was equipped with an electron ionization system with 70 eV ionization energy. The injected volume was 1.0 μl with the injector temperature of 280 $^{\circ}\text{C}$. The temperature program of the column was adjusted from 50 to 350 $^{\circ}\text{C}$. Helium (99.999%) was used as a carrier gas at 1 ml/minute. Component identification was done by comparison of their mass spectra with those from Wiley's MS library (7th edition) and NIST (National Institute of Standards Technology) found in the device library (Ebadollahi et al., 2017a).

Tested insects

The adult insects of *T. castaneum* were collected from contaminated wheat grains in the warehouses of Parsabad city, Ardabil province, Iran. In the breeding container, 50 pairs of adult insects were separately released on wheat grains and then removed from the diet 48 h later. Infected grains with insects' eggs were kept separately in an incubator at $25 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ relative humidity in the dark (Arnaud et al., 2005). One to seven old-days adults were selected for fumigant bio-assays.

Fumigant toxicity

Twenty adult insects of *R. dominica* and *T. castaneum* were separately placed into 340 ml containers as fumigant chambers. The tested concentrations of Germi origin's *L. citriodora* essential oil, based on the preliminary experiments, were from 8.18 to 47.06 $\mu\text{l/l}$ and from 20.59 to 52.94 $\mu\text{l/l}$ for *R. dominica* and *T. castaneum*, respectively. These values for essential oil from Parsabad region were from 5.88 to 55.88 $\mu\text{l/l}$ and from 23.53 to 61.76 $\mu\text{l/l}$, respectively. The concentrations were poured on 2×2 cm filter papers, treated filter papers were sealed to the inside of the lids of the containers and the lids were impenetrably to the air closed using parafilm. Insects' mortality were documented after 24, 48 and 72 h exposure times. All experiments were carried out for control groups without adding any essential oil concentrations and each treatment was repeated four times (Ebadollahi, 2018). Calculation of LC_{50} values (lethal concentrations to kill 50% of tested insects) and linear regression analysis were conducted using SPSS software version 24 (SPSS 24, IBM, Chicago, IL). Heterogeneity of the data was confirmed by a Chi-squared test.

RESULTS AND DISCUSSION

The results of chemical analysis of the *L. citriodora* essential oils from two Iranian regions are presented in Table 1. From 99.15% total identified compounds, verbenone (15.62%), camphor (11.03%), α -pinene (9.70%), borneol (8.26%), 1.8-cineol (7.33%), methyl oleate (6.87%), and bornyl acetate (4.93%) were identified as the main components from Germi region.

Table 1. Chemical composition of the essential oils of *Lippia citriodora* from Germi and Parsabad regions.

Components	Formula	RT	Percentage	
			Germi	Parsabad
α -Pinene	C ₁₀ H ₁₆	5.24	0.35	9.70
Camphene	C ₁₀ H ₁₆	5.52	-	2.91
Verbenene	C ₁₀ H ₁₄	5.62	-	0.28
Sabinene	C ₁₀ H ₁₆	5.98	0.76	-
β -Pinene	C ₁₀ H ₁₆	6.04	-	0.76
3-Octanone	C ₈ H ₁₆ O	6.22	-	1.42
Sulcatone	C ₈ H ₁₄ O	6.22	0.88	-
β -Myrcene	C ₁₀ H ₁₆	6.29	-	0.88
β -Cymene	C ₁₀ H ₁₄	6.95	0.14	0.89
Limonene	C ₁₀ H ₁₆	7.04	5.57	1.69
1,8-Cineole	C ₁₀ H ₁₈ O	7.11	2.33	7.03
β -Ocimene	C ₁₀ H ₁₆	7.38	0.26	-
β -Terpineol	C ₁₀ H ₁₈ O	7.80	0.32	-
p-Cymen-7-ol	C ₁₂ H ₁₈ O	8.41	0.13	-
Linalool	C ₁₀ H ₁₈ O	8.49	0.33	2.48
Filifolone	C ₁₀ H ₁₄ O	8.61	-	0.32
4-Methyl benzenemethanol	C ₈ H ₁₀ O	9.13	-	0.34
Camphor	C ₁₀ H ₁₆ O	9.67	-	11.03
Chrysanthemal	C ₁₀ H ₁₆ O	9.73	0.11	-
Isopinocampone	C ₁₀ H ₁₆ O	10.05	-	0.48
1-Tert-butyl-3,3-dimethylcyclopropene	C ₉ H ₁₆	10.09	0.23	-
Borneol	C ₁₀ H ₁₈ O	10.24	-	8.26
Rosefuran epoxide	C ₁₀ H ₁₄ O ₂	10.40	0.37	-
(E)-Pinocampone	C ₁₀ H ₁₆ O	10.46	-	1.39
Terpinene-4-ol	C ₁₀ H ₁₈ O	10.52	-	1.39
Vinylcyclohexane	C ₈ H ₁₄	10.60	0.39	-
α -Terpineol	C ₁₀ H ₁₈ O	10.91	1.13	2.54
Verbenone	C ₁₀ H ₁₄ O	11.58	-	15.62
Neral	C ₁₀ H ₁₆ O	12.51	9.97	2.24
1-Methylene-2-vinylcyclopentane	C ₈ H ₁₂	12.61	-	0.26
Carvol	C ₁₀ H ₁₄ O	12.63	0.25	-
Citral	C ₁₀ H ₁₆ O	13.60	13.26	3.38
Bornyl acetate	C ₁₂ H ₂₀ O ₂	14.23	-	4.93
α -Copaene	C ₁₅ H ₂₄	17.66	0.68	-
Geranyl acetate	C ₁₂ H ₂₀ O ₂	17.97	1.34	-
Methyl eugenol	C ₁₁ H ₁₄ O ₂	18.69	0.25	-
α -Cedrene	C ₁₅ H ₂₄	18.88	0.71	-

β -Caryophyllene	C ₁₅ H ₂₄	19.14	2.38	1.02
α -Caryophyllene	C ₁₅ H ₂₄	20.20	0.29	-
Aromandendrene	C ₁₅ H ₂₄	20.43	0.79	-
γ -curcumene	C ₁₅ H ₂₂	20.75	0.25	-
α -Amorphene	C ₁₅ H ₂₄	20.91	0.45	-
Germacrene D	C ₁₅ H ₂₄	21.05	0.62	-
Curcumene	C ₁₅ H ₂₂	21.14	11.48	1.55
Germacrene B	C ₁₅ H ₂₄	21.52	0.90	-
Cedrene	C ₁₅ H ₂₄	21.96	0.44	-
γ -Cadinene	C ₁₅ H ₂₄	22.01	0.30	-
δ -Cadinene	C ₁₅ H ₂₄	22.67	1.41	0.55
Nerolidol	C ₁₅ H ₂₄ O	23.46	2.44	-
β -Gurjunene	C ₁₅ H ₂₄	23.65	0.46	-
Spathulenol tricyclic sesquiterpenoid	C ₁₅ H ₂₄ O	23.80	16.51	1.43
Caryophyllene oxide	C ₁₅ H ₂₄ O	23.95	8.96	3.90
2-Phenyl-1-pentene	C ₁₁ H ₁₄	24.15	1.55	-
2-Quinazolinamine	C ₈ H ₇ N ₃	24.28	0.89	-
Z- α -Bisabolene epoxide	C ₁₅ H ₂₄ O	24.58	1.08	0.40
8-Isopropyl-5-methyl-2-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene	C ₁₅ H ₂₄	25.38	3.72	-
Widdrol	C ₁₅ H ₂₆ O	25.70	0.62	-
α -Elemene	C ₁₅ H ₂₄	25.84	0.31	-
1,4-1,7-trans-Acorenone	C ₁₅ H ₂₄ O	26.23	1.11	-
Fitone	C ₁₈ H ₃₆ O	29.80	0.23	-
2,4-Dimethyl-2,4-Heptadiene	C ₉ H ₁₆	30.66	0.10	-
Methyl palmitic acid	C ₁₇ H ₃₄ O ₂	30.71	0.09	0.64
8-bromo-Neoisolongifolene	C ₁₅ H ₂₃ Br	31.28	0.64	
Methyl lineoleate	C ₁₉ H ₃₄ O ₂	31.87	0.07	1.29
Methyl oleate	C ₁₉ H ₃₆ O ₂	31.91	-	6.87
Zingiberenol	C ₁₅ H ₂₆ O	31.96	0.13	-
Neric acid	C ₁₀ H ₁₆ O ₂	32.15	0.22	-
Hexafluoroacetylacetone	C ₅ H ₂ F ₆ O ₂	32.19	0.18	-
Ethyl Oleate	C ₂₀ H ₃₈ O ₂	32.23	-	0.98
Neryl acetone	C ₁₃ H ₂₂ O	32.44	0.21	-
1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane	C ₁₆ H ₅₀ O ₇ Si ₈	32.81	-	0.24
Methyl eicosenate	C ₂₁ H ₄₂ O ₂	32.90	-	0.08
Isoheneicosane	C ₂₁ H ₄₄	34.76	0.17	-
Icosane	C ₂₀ H ₄₂	36.31	0.39	-
Total			99.15	99.17

RT: Retention Time

In total, 99.17% of components were identified in the essential oil of Parsabad region, in which spathulenol (16.51%), citral (13.26%), curcumene (11.48%), neral (9.97%), caryophyllene oxide (8.66%), and limonene (57.5%) had the highest amount.

According to the study of Amini et al. (2016), geranial (22.52%), citral (15.88%) germacrene D (7.42%), caryophyllene (7.11%), benzenamine (6.30%), spathulenol (6.00%), and curcumene (5.44%) were the main components in the essential oil of *L. citriodora* from Iran. In the other study from Iran, limonene (18.41%), nerol (16.1%), geranial (13.02%), neral (6.94%), and β -caryophyllene (4.78%) were the main ingredients (Farahmandfar et al., 2018). Geranial, nerol and benzenamine were not detected in the present study but the others with different values were recognized. Fitsiou et al. (2018) revealed that geranial (26.4%), neral (17.2%), nerol (8.0%), geraniol (5.7%), spathulenol (3.3%), 1,8-cineol (3.2%) and limonene (2.2%) had high amount in the essential oil of *L. citriodora* from Greece. Geranial, geraniol and nerol were not found in the essential oil of present study while neral, spathulenol and 1,8-cineol with different percentages were detected in both tested oils. There are obvious differences in the chemical composition of the essential oil of *L. citriodora* from two inspected regions: some components such as sabinene, sulcatone, β -ocimene, β -terpineol, p-cymen-7-ol, chrysanthemal, geranyl acetate, and nerolidol were only identified in the essential oil from Germi region. In contrary, verbenone (15.64%), camphor (11.03%), borneol (8.26%), methyl oleate (6.87%), bornyl acetate (4.39%) and terpinene-4-ol (1.39%) with considerable amount in Parsabad region had not any trace in Germi' origin essential oil. In general, plant essential oils are affected by several exogenous and endogenous factors such as geographical habitation, seasonal variation, harvesting time, nutritional conditions, and genetic makeup (Jaimand & Rezaee, 2001, Barra, 2009, Ligan, 2018). Regarding *L. citriodora* essential oil, it was found that phenological stages (Shahhoseini et al., 2013), nutritional and fertilization conditions (Amini et al., 2016), and harvesting periods (Kizil et al., 2018) have direct effect on the chemical profile. Accordingly, mentioned differences between chemical profiles of the essential oils of *L. citriodora* from Germi and Parsabad regions in the present study and even from other studies may be due to such factors.

The results of fumigant toxicity showed that the adults of *R. dominica* and *T. castaneum* had a significant sensitivity to the essential oils of *L. citriodora* from both Germi and Parsabad regions. The amount of 50% lethal concentrations (LC₅₀ values) for *R. dominica* affected by the essential oil from the two regions of Germi and Parsabad after 24 h exposure time were 22.762 (15.656 - 37.801) μ l/l and 20.680 (17.471 - 24.937) μ l/l, respectively. These values for *T. castaneum* were 37.349 (34.644 - 40.745) μ l/l and 43.835 (41.588 - 47.978) μ l/l (Table 2). The high R^2 values (from 0.896 to 0.963) showed a positive relationship between different concentrations of essential oils from both regions and the pests' mortality (Table 2).

Table 2. The results of probit analysis for fumigant toxicity of essential oils isolated from *Lippia citriodora* form Germi and Parsabad regions.

Insect	Essential oil origin	Time (h)	LC ₅₀ (95% confidence limits) (µl/l)	χ ² (df = 3)	Slope ± SE	P value*	R ²
<i>R. dominica</i>	Germi	24	22.762 (15.656 – 37.801)	7.160	1.925 ± 0.227	0.067	0.913
		48	17.307 (10.758 – 25.348)	6.850	1.895 ± 0.228	0.077	0.912
		72	14.237 (12.162 – 15.251)	5.149	2.081 ± 0.235	0.161	0.938
	Parsabad	24	20.680 (17.471 – 24.937)	4.752	1.593 ± 0.175	0.191	0.948
		48	17.514 (14.611 – 21.139)	6.849	1.482 ± 0.173	0.077	0.896
		72	12.141 (7.423 – 16.549)	6.058	1.743 ± 0.185	0.109	0.903
<i>T. castaneum</i>	Germi	24	37.349 (34.644 – 40.745)	2.833	3.562 ± 0.422	0.418	0.963
		48	34.679 (27.969 – 45.136)	7.114	3.480 ± 0.419	0.068	0.910
		72	30.245 (25.003 – 34.314)	5.478	3.966 ± 0.432	0.140	0.941
	Parsabad	24	43.835 (41.588 – 47.978)	3.801	3.499 ± 0.414	0.284	0.951
		48	40.516 (32.722 – 53.129)	6.617	3.336 ± 0.409	0.081	0.912
		72	34.262 (27.909 – 39.228)	6.411	4.162 ± 0.431	0.093	0.937

*Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits. The number of insects for each exposure time, essential oil's origin and coleopteran species are 360, 1080 and 2160 insects, respectively. LC₅₀ = median lethal concentration necessary to kill 50% of tested insects.

The LC₅₀ values for insects decreased with increasing exposure times from 24 to 72 h in Germi and Parsabad origin essential oils. In the case of *R. dominica* and under the influence of essential oil from Germi, the LC₅₀ value was significantly decreased from 22.762 (15.656 - 37.801) µl/l after 24 h to 14.237 (12.162 - 15.251) µl/l after 72-h exposure time. This value for essential oil from the Parsabad region was 20.680 (17.471 - 24.937) µl/l during 24 h which was significantly decreased to 12.141 (7.423 - 16.549) after 72 h (Table 2).

In the case of *T. castaneum* and under the influence of essential oil from Germi, the LC₅₀ value was significantly decreased from 37.349 (34.644 - 40.745) µl/l at 24-h exposure time to 30.245 (25.003 - 34.314) µl/l after 72 h. This value in the essential oil extracted from the Parsabad region was calculated as 43.835 (41.588 - 47.978) µl/l after 24 h which significantly decreased to 34.262 (27.909 - 39.228) µl/l after 72 h (Table 2).

Although the pesticidal effects of plant-derived essential oils have been evaluated in several scientific works (Regnault-Roger et al., 2012, Isman & Grieneisen, 2014, Ebadollahi & Jalali-Sendi, 2015), there are a few studies related to the insecticidal effects of *L. citriodora* essential oil; the insecticidal effects of two Argentinian *Lippia* essential oils, including *L. citriodora* with 83.5% carvone and 16.5% limonene and *L. polystachya* with 51.3% citronellal and 22.9% sabinene, were verified against the soybean pest (*Nezara viridula*). The LC₅₀ values of *L. citriodora* and *L. polystachya* essential oils against *N.*

viridula were 29.9 and 13.5 mg/l 24 h after treatment, respectively (González et al., 2010). In the same work, *L. citriodora* and *L. polystachya* essential oils also exhibited significant fumigant toxicity against *T. confusum* with LC₅₀ values of 5.53 and 5.92 mg/l but there was not significant toxicity on the adults of *T. castaneum* (Benzi et al., 2014). In the other study, Khani et al. (2012) revealed that *L. citriodora* essential oil, with citral (11.3%), limonene (10.6%), and neral (7.9%) as main components, had significant fumigant toxicity on the adults of cowpea weevil (*Callosobruchus maculatus* (F.)) and the confused flour beetle (*Tribolium confusum* Jacquelin du Val). In this research, *C. maculatus* with LC₅₀ value of 10.2 µl/l was susceptible than *T. confusum* (LC₅₀ = 497.8 µl/l). Results of the mentioned above studies are in line with the results of the present study in view of the fumigant toxicity of the essential oil of *L. citriodora* on some of the common insect-pests. The presence of different chemical components in the studied essential oils and the differences in the tested insect species can be expressed as the main reasons for the differences in the amount of reported LC₅₀ values. However, the toxicity of *L. citriodora* essential oil has been demonstrated for the first time on *T. castaneum* and *R. dominica* in the present study.

The chemical compounds of plant essential oils, especially terpenes, have a direct effect on their pesticidal properties. The toxicity of some of the terpenic compounds identified in the present essential oil of *L. citriodora* has been investigated and recorded in some recent studies; the considerable toxicity of 1,8-cineole, borneol, bornyl acetate, camphene, camphor, carvacrol, geraniol acetate, limonene, linalool, myrcene, nerol, terpinen-4-ol, thymol, α -pinene, β -pinene, β -ocimene and γ -terpinene has been shown on the adults of granary weevil (*Sitophilus granarius* (L.)), Colorado potato beetle (*Leptinotarsa decemlineata* Say), and even the larvae and pupae of mosquito (*Culex quinquefasciatus* Say) (Kordali et al., 2007, Kordali et al., 2017, Andrade-Ochoa et al., 2018). Therefore, the insecticidal properties of *L. citriodora* essential oil can be attributed to the presence of such compounds in it. Moreover, compounds such as sabinene, β -ocimene, β -terpineol, nerolidol, and geranyl acetate, which insecticidal potency are well-established (Kordali et al., 2007, Wang et al., 2011, do Nascimento et al., 2018), were identified only in the essential oil obtained from Germi region but not in the Parsabad origin's essential oil. Therefore, the higher insecticidal potential of the essential oil from the Germi regions, especially on the *T. castaneum*, can be due to the presence of such compounds.

CONCLUSIONS

The possibility to use plant essential oils as an effective and eco-friendly tools has been considered by many researchers in recent years. Also, the multiple modes of action of these natural agents on the insect pests increase their potential and reduce resistance to the pests (McAllister & Adams, 2010, El-Wakeil, 2013). Although the antifungal, antibacterial and antiviral properties of *L. citriodora* have been considered in the previous researches (Tatsadjieu et al., 2009, Ocazonez et al., 2010), results of present study showed that the essential oil extracted from the

aerial parts of *L. citriodora* has significant toxicity on the adults of *R. dominica* and *T. castaneum* as two cosmopolitan and detrimental pests of stored product grains. In general, plant essential oils have very low durability and have a half-life of about 24 to 48 h (Turek & Stintzing, 2013).

Therefore, it is recommended that the *L. citriodora* essential oil stability is enhanced using applicable methods such as micro-and nano-encapsulation in the base of controlled-release techniques (Asbahani et al., 2015, Ebadollahi et al., 2017b) to increase its practical application in the pest management.

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INFLUENCE OF TEMPERATURE AND LIGHT CONDITIONS TO COLONY GROWTH OF BROWN ROT FUNGI OF STONE FRUITS IN MONTENEGRO

SUMMARY

This research was performed to determine the influence of temperature and light conditions to colony growth of *Monilinia* isolates collected on stone fruits in Montenegro. The fungi cause brown rot on stone fruits and have a great economic impact in Montenegrin stone fruit production.

Effects of temperature (at 15°, 22°, 27° and 30°C) and light (light 16h /darkness 8h and constant darkness) were studied on *Monilinia laxa* (Aderh. & Ruhland) Honey and *Monilinia fructicola* (G. Wint.) Honey isolates.

Optimal temperature for both *M. laxa* and *M. fructicola* was 22 °C and at this temperature *M. fructicola* isolates grew faster than *M. laxa*. Temperatures higher than optimal favoured *M. fructicola*, while lower temperature favoured *M. laxa*. Isolates of both species developed better in darkness while sporulation was instigated by light/dark alteration.

Keywords: *Monilinia fructicola*, *Monilinia laxa*, brown rot, micelial/colony growth, temperature, light regime, Montenegro

INTRODUCTION

Brown rot is a fungal disease caused by one or more of three closely related fungi – *Monilinia fructigena* Honey, *Monilinia laxa* (Aderh. & Ruhland) Honey and *Monilinia fructicola* (G. Winter) Honey (Lane, 2002). These species are economically very important pathogens of stone and pome fruits. *M. laxa* and *M. fructicola* are mainly spread on stone fruits while *M. fructigena* is widely present on pome fruits (Duduk et al., 2017).

Monilinia species cause similar symptoms on stone fruits – flower and twig blight and brown rot of fruits. *M. fructicola* is common in North and South America, Australia, New Caledonia and New Zealand, while the other two species occur in Europe. Hence, *M. fructicola* is called „American brown rot fungus”, while *M. laxa* and *M. fructigena* are designated as „European brown rot fungi” (Ivić and Novak, 2012). Since *M. fructicola* is considered the most destructive, in Europe it was regulated firstly as quarantine pest from the A1 pest

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list of the EPP0 region and after its appearance in several European countries it was moved to the A2 pest list (Carstens *et al.*, 2010).

Detailed study of *Monilinia* species distribution on stone fruits in Montenegro was done by Vučinić (1994). In the study performed from 1980 to 1983 and later in 1988 it was concluded that *M. laxa* and *M. fructigena* were present on stone fruits in Montenegro while *M. fructicola* has not been recorded. However, in 2016 Latinović *et al.* (2017) reported the presence of *M. fructicola* in Montenegro for the first time. It was detected in a commercial orchard of nectarine in the region of Podgorica.

Since the disease is widespread in Montenegro causing blossom, branch and twig blight and fruit brown rot on stone fruits, and especially because the new fungal species (*M. fructicola*) was recently discovered, the aim of our study was investigation of ecological features (temperature and light conditions) of selected *Monilinia* isolates obtained from different regions and hosts in Montenegro. Isolates were compared, identified and examined in different conditions of the abiotic factors for better understanding of their influences on fungal development and reproduction. The results can be useful in prediction of the two species during the season and control of brown rot fungi on stone fruits.

MATERIAL AND METHODS

In order to explore the presence of *Monilinia* species on stone fruits in Montenegro, field survey was conducted from March to October 2018. Orchards of plum, cherry, peach and nectarine at localities: Pljevlja, Žabljak, Bijelo Polje, Nikšić, Podgorica surroundings Zeta, Mataguži, Gornja Gorica, Lješkopolje, Vranj, Čemovsko polje and Tuzi were checked. In case of apparent disease symptoms, samples were collected and transferred to the laboratory.

The research was performed at the Plant Pathology Laboratory of the Biotechnical Faculty in Podgorica, Montenegro. A total of 18 *Monilinia* spp. isolates were isolated from diseased fruits or flowers. Isolation was performed by placing small pieces of decayed tissue on potato dextrose agar (PDA, Biolife) in Petri dishes (90 mm in diameter) and incubated at 22°C for a week. Four representative isolates have been chosen in order to study influence of different temperatures and light conditions to fungal colony growth.

Determination of the isolates was made according to the synoptic key established by Lane (2002).

Influence of temperature to isolates colony growth was examined at 15°, 22°, 27° and 30°C on PDA nutrient medium in dark. Effect of light was studied on isolates' colonies grown on PDA at 22°C in two treatments: in darkness and in natural light/dark rotation (16 h light / 8 h dark long-day photoperiod).

Colony growth of different isolates was evaluated by measurement of diameter of the colonies on the third and the seventh day. Average daily growth of the colonies was calculated according the formula:

$$\frac{D_2 - D_1}{T_2 - T_1}$$

where D_1 and D_2 represent colony diameters in mm after the first and the last measurement while T_1 and T_2 corresponds to the days at which the measurements were made (Brasier and Webber, 1987), in this case at 3 and 7 days after medium inoculation, respectively.

The experiment was set up in four repetitions.

RESULTS AND DISCUSSION

Among 18 obtained isolates, four of them were selected as representative in relation to the fungal species and the host, in order to study influence of different temperatures and light conditions to fungal colony growth. The isolates were determined as *Monilinia laxa* (isolates MT1 and Mšpv-r from cherry and plum, respectively) and *Monilinia fructicola* (isolates MN₁ and MBP from nectarine and peach, respectively). These isolates are presented in Table 1.

Table 1. List of *Monilinia* isolates used in the study

Isolate	Species	Host	Variety	Locality
MT1	<i>Monilinia laxa</i>	Cherry	Burlat	Tuzi
Mšpv-r	<i>Monilinia laxa</i>	Plum	Zerdelija	Pljevlja
MN1	<i>Monilinia fructicola</i>	Nectarine	Unknown	Vranj
MBP	<i>Monilinia fructicola</i>	Peach	Early May Crest	Ćemovsko polje

Effect of different temperatures on mycelial growth. Influence of different temperatures on colony growth of the studied isolates is presented in Fig. 1. Development of examined isolates of *M. laxa* and *M. fructicola* colonies at different temperatures is shown in Fig. 2.

Among different temperatures studied (15°, 22°, 27° and 30°C), colony growth of the tested isolates was the best at 22°C. *M. laxa* developed better at 15° then at 27° and 30°C while it was the opposite for *M. fructicola*. In general, growth rate of *M. fructicola* isolates were faster than those of *M. laxa*. Those results could be important for the disease epidemiology especially related to aggressiveness of *M. fructicola* which can be considered more certain in warmer climate of the country. On the other hand, *M. laxa* can be expected more at lower temperatures which are common for the northern parts of Montenegro.

Those results are in accordance with findings of the other authors. Vučinić (1994) studied the effects of temperatures on growth of *M. laxa* and *M. fructigena* on stone fruits in Montenegro and revealed that both fungi developed in a broad range of different temperatures with the optimum at 24 °C, well growth at 21° and 18 °C while the temperature at 33 °C completely stopped the fungi development.

As cited in Bernat et al. (2017), *M. laxa* cannot develop at 33 °C, but it is even able to grow below 0 °C *in vitro*. *M. fructicola* mycelia can develop at 33 °C however, no mycelia were observed at 0 °C. The authors stated that *M. fructicola*

is better adapted to high temperatures, whereas *M. laxa* is better adapted to low temperatures.

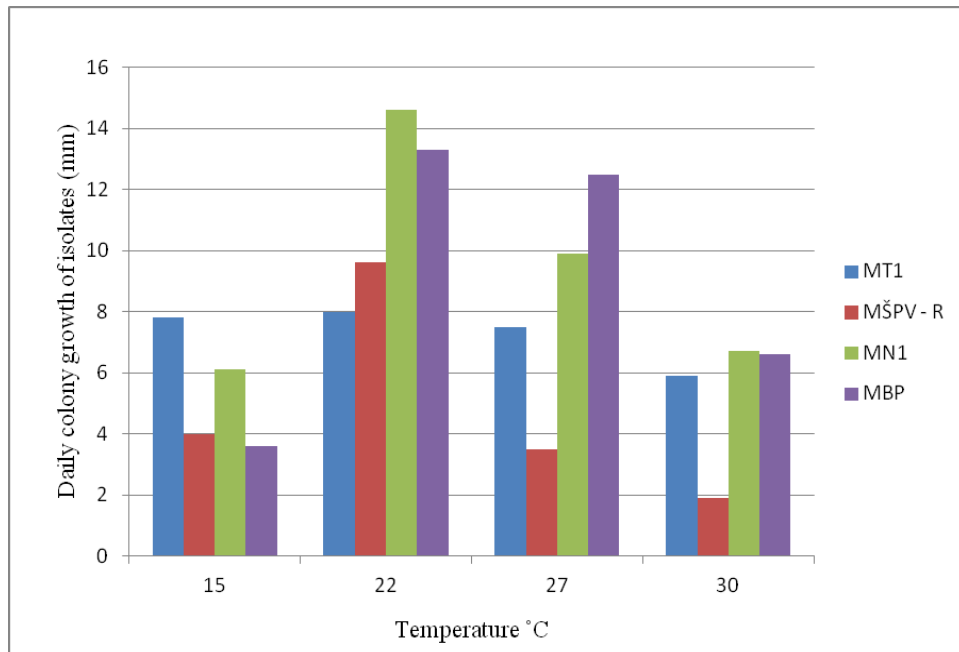


Figure 1. Average daily growth (mm) of the *Monilinia laxa* and *Monilinia fructicola* isolates at different temperatures.

Batra (1979) stated that *M. laxa* can be discriminated from *M. fructicola* by slower growth rate. De Cal and Melgarejo (1999) have also reported that growth rates of *M. fructicola* isolates were faster than those of *M. laxa*. Similar, Lane (2002) founded that in general, colony diameter for *M. fructicola* was greater than for *M. laxa*.

All these findings are important for understanding the disease epidemiology. Based on ecological parameters it can be considered when and in what extent the disease can be expected. According to Hrustić *et al.* (2012), heavy rains in the period of flowering, with temperatures ranging from 20 to 25°C during the day are ideal conditions for the disease development.

Angeli *et al.* (2017) cited that there are differences in ecological requirements in areas where these two species co-exist. In these areas, *M. fructicola* is mostly reported on fruit, whereas *M. laxa* is mostly prevalent on flowers and twigs (EFSA, 2011). The reason for these differences could be in weather conditions during flowering and fruit ripening, since flowering occurs during spring at lower temperatures, while ripening of the fruit occurs in summer when temperatures are significantly higher. The results obtained in our study are in accordance with this opinion since isolates of *M. fructicola* developed better at higher temperatures than *M. laxa* isolates. Detailed studies done by Angeli *et al.*

(2017) indicate that *M. fructicola* is favoured by warmer weather than *M. laxa*. These authors reported that in Brazil the optimum temperature for development of brown rot caused by *M. fructicola* was 24.5°C and for *M. laxa* was 19.8°C. In their experiments *M. laxa* lesions produced more conidia than *M. fructicola* at 10°C, while at 30°C *M. fructicola* lesions produced more conidia than *M. laxa*. The temperature which influenced on lesion development was also higher for *M. fructicola* than for *M. laxa*.

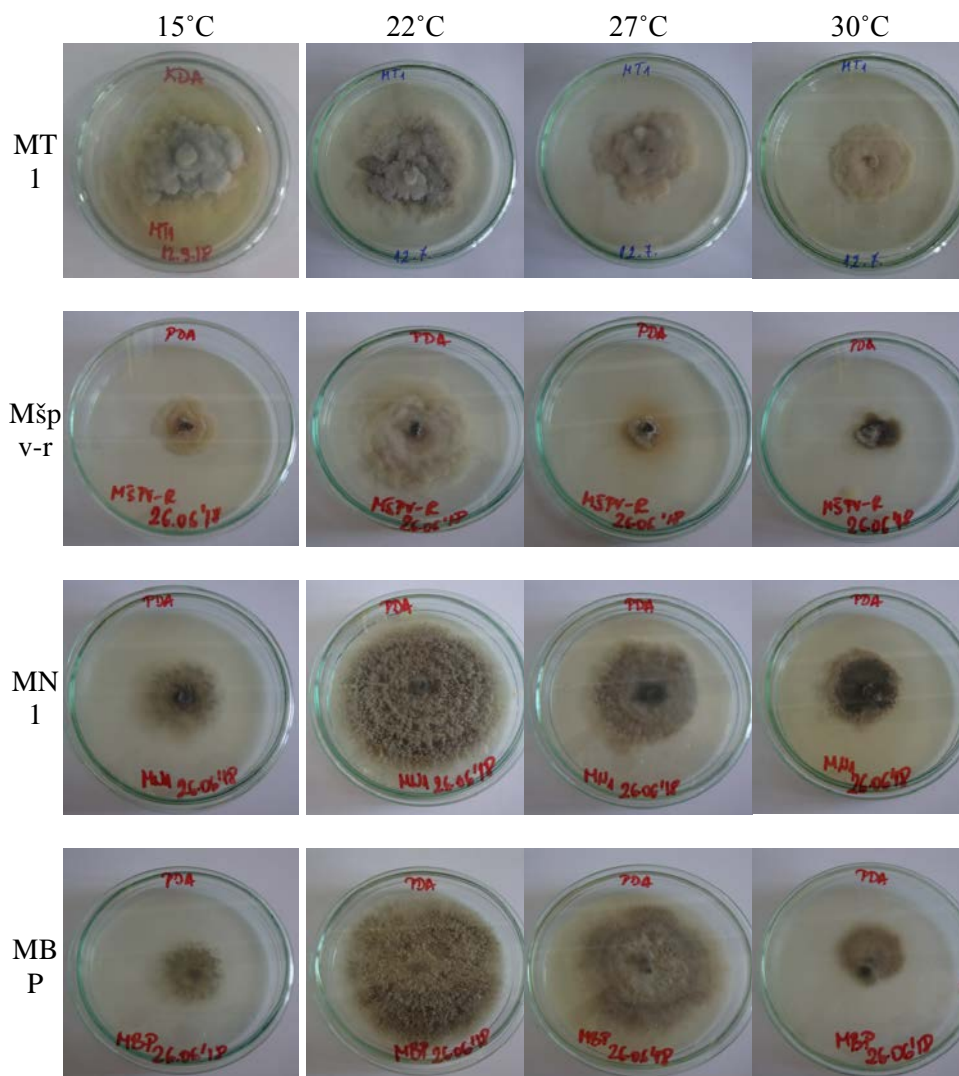


Figure 2. Colony growth of *Monilinia laxa* (MT1, Mšpv-r) and *Monilinia fructicola* (MN₁, MBP) isolates on PDA at different temperatures

Effect of different light conditions on mycelial growth. Influence of different light conditions on colony growth of the studied isolates is presented in Fig. 3. Development of examined isolates of *M. laxa* and *M. fructicola* colonies at different light conditions is shown in Fig. 4.

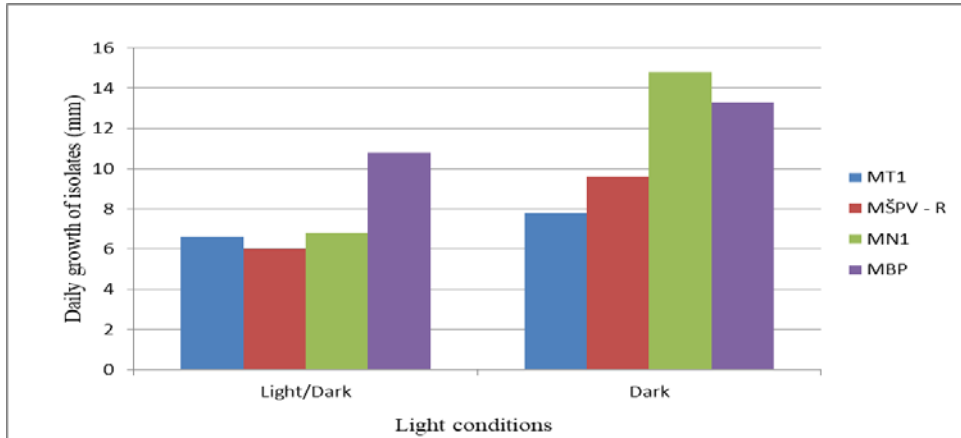


Figure 3. Average daily growth (mm) of the *Monilinia laxa* and *Monilinia fructicola* isolates at different light conditions (16 h light / 8 h dark and darkness)

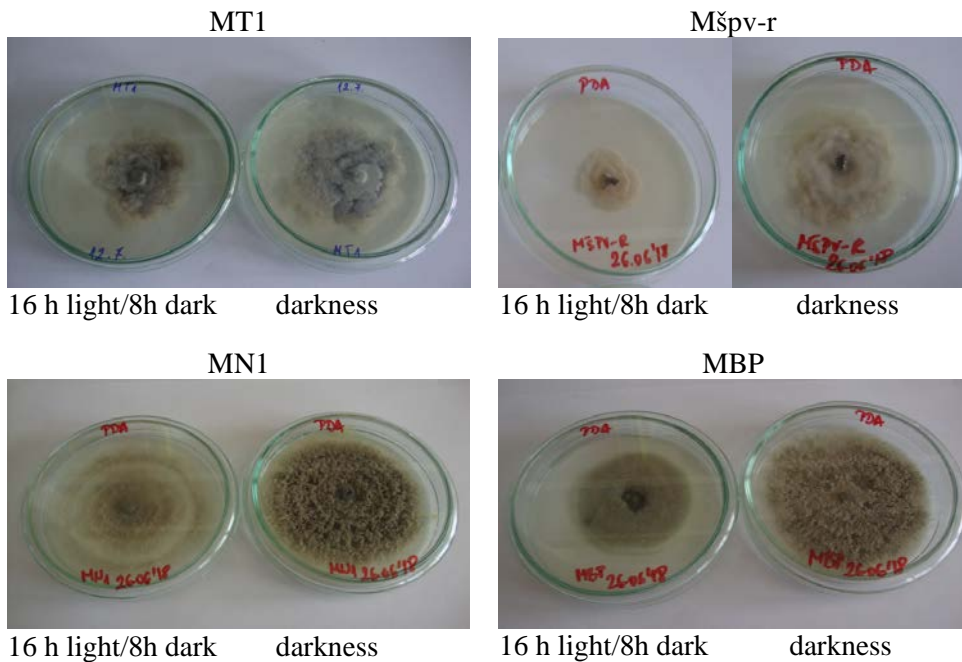


Figure 4. Colony growth of *Monilinia laxa* (MT1, Mšpv-r – upper row) and *Monilinia fructicola* (MN1, MBP – bottom row) isolates on PDA at different light conditions

The colonies of the all studied isolates grew faster in conditions of total darkness. Also, isolates of *M. fructicola* exhibited faster colony growth than isolates of *M. laxa* both in conditions of total darkness, as well as in the conditions of light/dark rotation. Differences in sporulation were noted between the isolates of *M. fructicola* and *M. laxa*. *M. fructicola* isolates sporulated in abundance in both light regimes but more abundant in condition of light and darkness alternation. However, isolates of *M. laxa* sparsely sporulated in darkness but the sporulation was induced with the presence of light.

Effect of light on *Monilinia* species (*M. laxa* and *M. fructigena*) was studied by Vučinić (1994). There were no significant differences between the *Monilinia* isolates grown in total dark or in alternation of light and darkness. However, in total darkness zones in the colony were less pronounced than in a presence of light and in *M. laxa* sporulation was sparse especially in darkness. The influence of light to *Monilinia* species is indubitable, but it is different concerning growth rate, sporulation and zonal colony distribution. Willetts (1969) concluded that total darkness stops conidia formation so when sporulation is inhibited the energy is directed to vegetative growth; Harada (1975) similarly reported that mycelial growth is reduced at light in comparison to darkness while sporulation is more pronounced in the presence of light (as cited in Vučinić, 1994).

Van Leeuwen and van Kesteren (1998) determined growth rate and sporulation intensity under two light regimes (darkness, 12 h light : 12 h dark) among a wide collection of isolates of *M. fructicola* and *M. laxa* and their results showed that increase in colony diameter and sporulation intensity was remarkable higher in *M. fructicola*. According to these authors, sporulation was more abundant in the light : dark regime. In darkness sporulation was the highest in *M. fructicola*, but in the light : dark regime sporulation intensity of some of the isolates of *M. fructicola* overlapped with some of the profusely sporulating isolates of *M. laxa*.

As stated by De Cal and Melgarejo (1999), differences in mycelial growth under long-wave UV may be a useful tool to identify *Monilinia* spp. They investigated the effect of long-wave UV/dark period on mycelial growth of *Monilinia* isolates. Growth in the dark was faster than growth under long-wave UV/darkness. Growth rates of *M. fructicola* were faster than those of *M. laxa* under both test conditions.

CONCLUSIONS

The results showed that ecological factors had significant effects on *Monilinia fructicola* and *Monilinia laxa* isolates from stone fruit in Montenegro.

Growth rates of examined isolates were the highest at 22°C, however, at this temperature *M. fructicola* isolates grow faster than isolates of *M. laxa*. The temperature below optimum (15°C) is more favourable for the growth of *M. laxa* isolates, while the temperatures above optimum (27° and 30°C) suit more for *M. fructicola* isolates.

The studied isolates developed more intensively under condition of total darkness compared to the condition of natural light and dark alteration. On the contrary, sporulation in both species was more abundant in the light:dark regime than in darkness but this was much more expressed in *M. laxa* isolates. Sporulation of *M. fructicola* isolates was abundant also in darkness while in isolates of *M. laxa* it was sparse.

The effects of climate change, especially changes in temperature can affect the distribution of *Monilinia* species on different stone fruit hosts which should be considered for the future in the relevant risk analysis and strategies for disease management.

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THE IMPACT OF NUTRITION OPTIMIZATION ON CROP YIELD OF WINTER WHEAT VARIETIES (*TRITICUM AESTIVUM* L.) AND MODELING OF REGULARITIES OF ITS DEPENDENCE ON STRUCTURE INDICATORS

SUMMARY

The article presents the results of studies conducted in 2011 -2016 years on the southern chernozem in the southern Steppe of Ukraine, studied the efficiency of processing winter wheat crops modern growth-regulating drugs in the main periods of vegetation of the crop on the background of mineral fertilizers application. It was determined that the introduction of pre-sowing cultivation of winter wheat fertilizer in a dose of N₃₀P₃₀ (background) and the use of foliar fertilizing of crops at the beginning of the resumption of spring vegetation and the beginning of stooling complex organic fertilizers Organic D2 and Escort-bio created favorable conditions for the formation of optimal indicators of the structure of the crop and, accordingly, a high level of grain yield of the studied varieties. In the variants of fertilizer Organic D2 and Escort-bio plants of winter wheat variety Kolchuga formed 4,42-4,48 t / ha of grain, and plants of Zamozhnist formed 4,96 – 4,99 t/ha, which exceeded the control by 52,9 – 55,0 and 62,6-63,6%, respectively. From the studied varieties of winter wheat on a set of indicators, it was determined Zamozhnist as the best variety.

Key words: winter wheat, variety, plant nutrition, crop structure, grain yield, modeling of regularities.

INTRODUCTION

Today, grain production has a special place in the structure of the agro-industrial complex. It is the grain and products of its processing which are vital products that ensure the food security of the state, play an important role in the socio-economic development of the national economy, form the basis of agricultural exports and determine the degree of its participation in international cooperation. Favorable natural and climatic conditions and fertile lands of Ukraine allow to grow all crops and to receive high-quality food products in sufficient volumes both for domestic needs and for the formation of export potential (Kushniruk and Tolmach, 2016). Over the past five years, Ukraine strengthened its position in the international agricultural market and it is confidently in the world's top ten grain producers. Wheat is the top food crop in

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Ukraine as well as in the whole world and the biggest part of grain is obtained primarily from winter wheat. The total area for winter wheat cultivation in Ukraine covers 6.8 mln. ha with actual productivity of 24 mln. tons and average capacity of 2.8 t/ha. Due to its favorable natural and climatic conditions the South of Ukraine is considered one of the leading regions for the production of soft winter wheat of high quality (Korchova et al., 2018; Nazarenko and Kharytonov, 2016).

Improving the technology of winter wheat growing is an extremely urgent task, because in the current economic conditions, reducing the cost of grain production and increasing its profitability it is possible only in the case of the introduction of new agricultural techniques which do not involve large costs. Modern intensification of crop production in the conditions of acute shortage of organic fertilizers and too high prices for mineral fertilizers provides for the development of alternative measures for the technology of growing crops. In this context, it is becoming increasingly important to study the effect of high-performance polymer chelated fertilizers, biological products, growth-regulating drugs, etc. in combination with other agrotechnical elements on the formation of biometric indicators of plants, yield and product quality (Rozhkov and Gutyansky, 2017). There is a need to develop and implement resource-saving elements of plant nutrition technology, which consist in the introduction of low doses of mineral fertilizers and on their background the use of foliar fertilizing with modern drugs in the main periods of their vegetation (Panfilova and Gamayunova, 2018).

Research of grain yield is the main objective of modern agricultural science (Macholdt and Honermeier, 2017). It primarily depends on such structural elements as the number of productive stems per unit area, the number of grains in the ear and its mass, the 1000 grains weight, etc. Larger grain has better indicators of quality and germination, which contributes to the formation of higher crop yields (Campbell et al., 1999; Dholakia et al., 2008; Bresseghele and Sorrells, 2006; Sun et al., 2008; Chamurliyski et al., 2015). It should be noted that the indicators of the yield structure depend on the varietal characteristics of culture and agrotechnology of its cultivation (Cooper et al. 2001), therefore, the purpose of our research was to study the effect of optimizing the nutrition of winter wheat plants on crop productivity and modeling the laws of dependence of its yield on the structure indicators.

MATERIALS AND METHODS

Experimental studies were conducted during 2011-2016 years on the experimental field of the Mykolaiv National Agrarian University (Mykolayiv district, Mykolaiv region, Ukraine). The object of research was winter wheat varieties such as Kolchuga and Zamozhnist. The variety Kolchuga (variety-lutescen) refers to the early maturing varieties, its vegetation period is 275-278 days. The height of the plant is medium-sized (96 cm). In the field conditions during the testing years winter hardiness is 8.8 points, resistance to lodging is 8.7

points, resistance to shedding is 8.9 points, drought resistance is 8.1 points. It is the variety of intensive type, universal use. The variety Zamozhnist (variety – erythrosperrum) is mid-ripening. The vegetation period is 282-287 days. Plant height is 94-104 cm, the variety is highly resistant to lodging and shedding, frost and winter hardiness above average, it is characterized by high drought and heat resistance. It is the variety of high-intensity type and universal use on different agricultural backgrounds.

The technology of growing winter wheat in the experiment, with the exception of the studied factors, was generally accepted to the existing zonal recommendations for the southern steppe of Ukraine. The territory of the farm is located in the third agro-climatic region and it belongs to the subzone of the southern Steppe of Ukraine. The climate is temperate continental, warm, dry, with unstable snow cover. Weather conditions for hydrothermal indicators in the years of research varied, which made possible to obtain objective results, but in general, they were typical for the location of the farm.

The soil of experimental sites was represented by the southern, resiliently weakly sunny, heavy-sooty black soil on the loesses. The reaction of the soil solution was neutral (pH 6.8 - 7.2). The content of humus in the 0 - 30 cm layer was 123 - 125 g kg⁻¹. The arable layer of soil contained moving forms of nutrients on average: nitrates (by Grandval Liagou - this method is based on interactions between nitrates and disulpho-phenolic acid from which trinitrophenol (picric acid) is formed. In alkaline environment it gives us yellow coloring due to formation of potassium trinitrophenolate (or sodium, depending from alkali used) in quantity equivalent to nitrates content) as 15 - 25 mg kg⁻¹, mobile phosphorus (by Machigin - this method is based on extraction of mobile phosphorus and potassium compounds from the soils with 1% ammonium carbonate solution, pH 9.0, at 25 ± 2⁰C) as 41 - 46 mg kg⁻¹, exchangeable potassium (on a flame photometer) as 389 - 425 mg kg⁻¹ of soil.

The experiment scheme included the following variants:

Factor A – variety: 1. Kolchuga; 2. Zamozhnist.

Factor B – nutrition: 1. Control (without fertilizers); 2. N₃₀P₃₀-for pre-sowing cultivation-background; 3. Background + urea K1 (1 l/ha); 4. Background + Urea K2 (1 l/ha); 5. Background + Escort-bio (0,5 l/ha); 6. Background + urea K1 + Urea K2 (0.5 l/ha); 7. Background + Organic D2 (1 l/ha).

The norm of the working solution was 200 l/ha. Fertilizing of crops with modern growth regulating drugs was carried out at the beginning of the resumption of spring vegetation and at the beginning of the winter wheat stooling. Plants of the control variant were sprayed with tap water in the specified phases of growth and development.

Preparations used for foliar nutrition of winter wheat crops are included in the list of pesticides and agrochemicals allowed for use in Ukraine. Preparations Urea K1 and Urea K2 are registered as fertilizers which contain respectively N as 11-13%, P₂O₅ as 0,1-0,3%, K₂O as 0,05-0,15%, trace elements as 0,1%, succinic acid as 0,1% and N as 9 -11%, P₂O₅ as 0,5 - 0,7%, K₂O as 0,05 - 0,15%,

sodium humate as 3 g/l, potassium humate as 1 g/l, trace elements as 1 g/l. Organic D2 is a organo-mineral fertilizer, which contains N as 2,0 – 3,0%, P₂O₅ as 1,7 – 2,8%, K₂O as 1,3 – 2,0%, calcium total as 2,0 – 6,0%, organic substances as 65 – 70% (in recalculation on carbon). Escort-bio is a natural microbial complex which contains strains of microorganisms of the genera Azotobacter, Pseudomonas, Rhizobium, Lactobacillus, Bacillus and biologically active substances (BAS) produced by them.

In the process of research the method it was used the state variety testing of agricultural crops (Volkodav et al., 2001). The structure of the crop was analyzed by sheaves, which were selected before harvesting from sites of 1 m². The yield structure was determined by the method of continuous harvesting of each accounting area (grain harvester "Sampo-130").

Statistical dependence of winter wheat yield was studied on the basis of correlation and regression analysis, the task of which was to establish the form of dependence between the indicators, evaluation of the regression function and prediction of the values of the dependent variable. In general, the yield of winter wheat is characterized by a nonlinear dependence. Since, in this form of connection, the increasing in the factor characteristic leads to an uneven increasing (or decreasing) in the effective characteristic, or the growth of its value varies in descending order, and the decrease in increasing, then at a certain small interval the yield is estimated by the equation of linear multivariate regression of the form:

$$\hat{y}_x = a_0 + a_1x_1 + a_2x_2 + K + a_nx_n, (1)$$

where \hat{y}_x - dependent variable, x_1, x_2, \dots, x_m - independent factors,

$a_0, a_1, a_2, \dots, a_n$ - parameters of the model.

RESULTS AND DISCUSSION

The crop yield structure is a quantitative expression of the result of the life of the plant organism, which determines the value of the crop and reflects the interaction of plants and the environment at certain stages of growth and development. Important components of the crop yield structure of the winter wheat are the length of the ear, the number of grains in the ear, the weight of the grain per ear and the 1000 grains weight (Table 1).

On average, over the years of research and on nutrition factor, the winter wheat variety Zamozhnist had a slightly longer ear length by 6.5%. It should be noted that the longest ear was characterized by plants of both studied varieties of winter wheat on the nutrition variants Background + Organic D2 and Background + Escort-bio. So, on average, over the years of research, the length of the ear of plants of winter wheat variety Kolchuga on these variants of the experiment was 9.0-9.2 cm, the length of the ear of variety Zamozhnist was 9.8-10.1 cm, which exceeded the control version, respectively, by 15.4-17.9 and 19.5-23.2%.

On average, over the years of research, nutritional variants to some extent influenced on the number of grains in the ear of the studied varieties of winter wheat. Thus, if no application of fertilizers the ear of plants variety Kolchuga consisted of 24.9 PCs, and the ear of plants variety Zamozhnist consisted 27,5 PCs, use of seed and application of only mineral fertilizers ensured the increasing of this indicator in the context of the undertaken study on the varieties 6.8 – 7.8 % and for the background of fertilizers foliar nutrition it increased by 11.1 – 16.4% for the cultivation of variety Kolchuga and it increased by 8.9 - 13.2% for variety Zamozhnist.

Table 1. The structure of the yield of winter wheat varieties depending on the diet (average for 2012-2016 years)

Variety	Nutrition variant	Structure indicators			
		Length of ear, cm	The number of grains in the ear, PCs	The weight of grain per the ear, g	The 1000 grains weight, g
Kolchuga	Control	7,8	24,9	0,88	35,0
	N ₃₀ P ₃₀ (background)	8,4	27,0	1,00	36,8
	Background +Urea K1	8,5	28,0	1,08	38,3
	Background + Urea K2	8,6	28,2	1,11	39,2
	Background + Urea K1 + Urea K2	8,9	28,7	1,15	31,1
	Background + Escort-bio	9,2	29,8	1,25	41,9
	Background + Organic D2	9,0	29,3	1,19	40,5
Zamozhnist	Control	8,2	27,5	1,02	36,9
	N ₃₀ P ₃₀ (background)	8,7	29,5	1,15	38,9
	Background +Urea K1	9,2	30,2	1,23	40,7
	Background + Urea K2	9,1	30,5	1,26	41,3
	Background + Urea K1 + Urea K2	9,6	31,2	1,31	42,1
	Background + Escort-bio	10,1	31,7	1,35	42,7
	Background + Organic D2	9,8	31,3	1,32	42,1

A slightly larger number of grains in the ear during all the years of research was formed by plants of variety Zamozhnist. So, on average for years of researches on the nutrition factor, they were formed 30.3 PCs, which exceeded the variety Kolchuga by 2.3 PCs or by 7.6%.

We found that, on average, over the years of research, varieties and nutrition variants were reflected in the weight of grain per ear. So, for the introduction of the background recommended dose of mineral fertilizer for winter wheat variety Kolchuga the weight of grain from the ear compared to the inconvenient control increased by 12.0%, and it increased for the variety of Zamozhnist by 11.3%. Carrying out foliar fertilizing on the background of mineral fertilizers increased this indicator of the crop structure by 18.5 – 29.6 and 17.1 – 24.4%, respectively, to the control.

It was noted that the 1000 grains weight of winter wheat depended primarily on the varietal characteristics of the crop. So, on average, over the years of research on the nutrition factor, the 1000 grains weight of the Kolchuga variety was 37.5 g, which was less than the indicators for the variety of Zamozhnist by 3.2 g or 7.9%.

It should be noted that the introduction of mineral fertilizers in a moderate dose for pre-sowing cultivation contributed to the growth of this indicator, depending on the variety, by 4.9-5.1%. Carrying out foliar fertilizing of plants during the growing season with modern growth-regulating drugs contributed to increasing in the 1000 grains weight of the Kolchuga variety by 3.3 – 6.9 g or by 8.6 – 16.5%, and it increased for variety Zamozhnist by 3.8 – 5.8 g or 9.3-13.6% respectively. Thus, irrespective of the studied varieties of winter wheat the highest 1000 grains weight was observed on applying of the drug Escort-bio background $N_{30}P_{30}$ as 41.9 – 42.7 g.

In the effective use of fertilizers, an important role belongs to the variety (Fig. 1).

Thus, on average, over the years of research and nutrition factor, the grain yield of winter wheat variety Zamozhnist compared with the yield of variety of Kolchuga formed higher by 0.41 t/ha or 10.2%.

In our studies, the increasing in grain yield of winter wheat variety Zamozhnist for the introduction of $N_{30}P_{30}$ in the control was 0.53 t/ha or 17.4%. The use of growth-regulating drugs on the background of application of $N_{30}P_{30}$ provided the increasing in grain yield of winter wheat 1.59-1.94 t/ha or 52.1 – 63.6% depending on the drug.

Foliar nutrition of winter wheat variety Kolchuga also had a positive impact on grain yield. So, on average for years of researches specified agronomic techniques contributed to the increasing in the yield by from 1.34 up to 1.59 t/ha or by 46.4 – 55.0 % in comparison to the control.

The use of plant growth-regulating drugs on winter wheat and other crops is a common practice (Panfilova and Gamayunova, 2018; Yeremenko et al., 2018). Thus, in the UK, the use of plant growth-regulating drugs in the spring vegetation period contributed to the growth of grain yield by 4 t / ha, compared

with the natural background (Griffin and Hollis, 2017). Nutrition is an important factor in the cultivation of wheat, as it affects the growth, yield and quality of grain. Studies by Yuxue Zhang et al. conducted in the greenhouse at the Ottawa Research and Development Centre (ORDC) showed that growth-regulating drugs contributed to an increase in plant height, but the grain yield decreased.

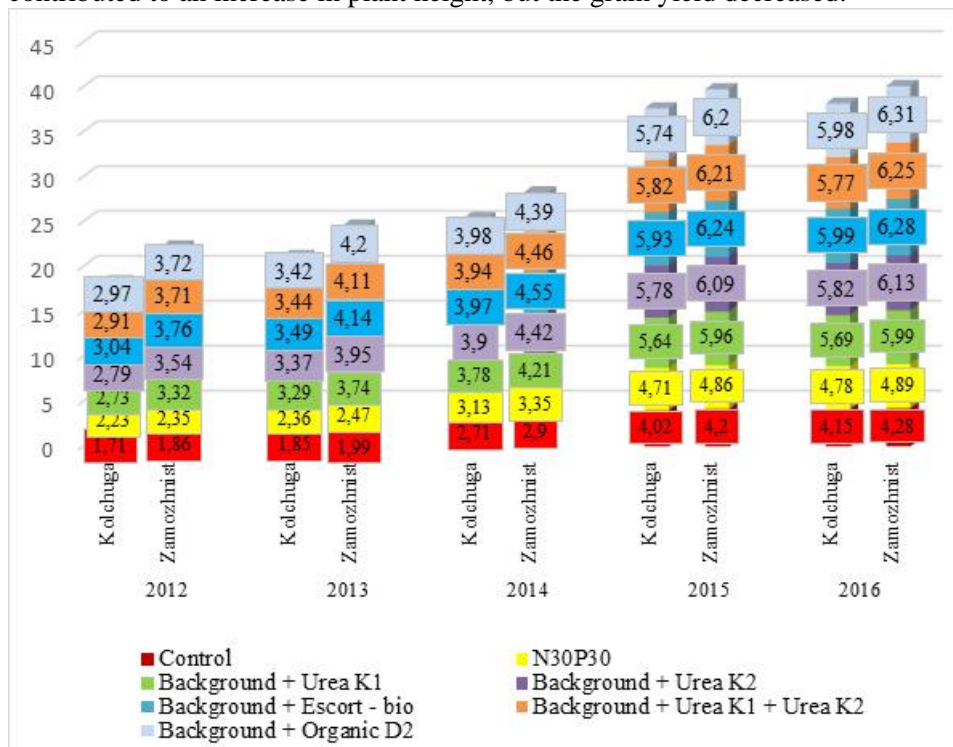


Figure 1. The crop yield of winter wheat on the different nutrition variants, t/ha

Higher crop yields are likely to be contributed by resource-saving technologies which increase the yield of crops up to 10-15% (Markov et al., 2011; Dwyer et al., 1995). The combined use of organic and mineral fertilizers in combination with humic substances in studies by Indian scientists increased the yield of wheat by 27% and it had a positive effect on the nutrient content and organic carbon in the soil (Bharali et al., 2017).

Yield of many crops rarely meets its maximum potential for production (Smith et al., 2018). In our research the yield of winter wheat greatly depended on the variety features. Currently, the variety acts as an independent factor in increasing of grain yield (Panfilova and Gamayunova, 2018; Nazarenko et al., 2019). According to Ayranci et. al., (2014) and Ahmadi et. al. (2016), on the contrary, the grain yield was more dependent on environmental conditions during the growing season than on the genotype effect.

We would determine the statistical dependence of winter wheat yield in our studies on the structural elements on the basis of correlation and regression analysis, as it could give an idea of the change in the effective feature in the variation of factor variables. Also, the results of regression analysis of the data of long-term multifactor field experiments make it possible to derive mathematical models of the relationship of crop yields with the factors that determine them.

The yield of winter wheat depends on the level of manifestation of the structure elements, such as the length of the ear, the number of grains in the ear, the mass of grain from the ear and so on, which vary significantly under the influence of agrotechnical factors, in particular due to the nutrition method.

For identifying the dependence of the yield on the structure indicators we construct a correlation matrix, such as a matrix of paired correlation coefficients. Let's determine the variables of the econometric model: let Y – winter wheat yield, t/ha; X_1 – the ear length, cm; X_2 – the number of grains in the ear, PCs.; X_3 – the mass of grain from the ear, g; X_4 – the 1000 grains weight, g (Table 2).

Table 2. The paired correlation coefficients of relationship of yield and structure indicators (average for 2012 – 2016 years)

Nutrition variant	Variety			
	X_1	X_2	X_3	X_4
Variety Kolchuga				
Control	0,775867	0,949413	0,964352	0,953291
N ₃₀ P ₃₀ (background)	0,934191	0,520464	0,98125	0,949085
Background + Escort-bio	0,860109	0,804483	0,979362	0,927868
Variety Zamozhnist				
Control	0,824169	0,904006	0,989757	0,988779
N ₃₀ P ₃₀ (background)	0,87356	0,965198	0,977377	0,975628
Background + Escort-bio	0,95136	0,939118	0,980013	0,983574

To determine the closeness of the connection of independent indicators on the factor characteristic, we find the pair correlation coefficients, as the higher the coefficient value is, as the greater the influence of the factor is on the yield. According to the results of our calculations, in the control version of the experiment the number of grains in the ear, the mass of grain from the ear and the 1000 grains weight had the greatest impact on the yield of winter wheat variety Kolchuga. When applying mineral fertilizers for pre-sowing cultivation in a moderate dose of N₃₀P₃₀ and the background + Escort-bio nutrition variant, a strong relationship was observed between the yield and the length of the ear, the weight of the grain from the ear and the 1000 grains weight.

Analyzing the calculations on the variety of wheat Zamozhnist, it could be concluded that on the control option and the option of making N₃₀P₃₀ the number of grains in their ear, the mass of grain from the ear and the 1000 grains weight

had the greatest impact on yield. Foliar nutrition of winter wheat crops in the main periods of vegetation with the drug Escort – bio on the background of application of $N_{30}P_{30}$ contributed to the creation of a strong relationship between the yield and the length of the ear, the weight of the grain from the ear and the 1000 grains weight.

Let's construct a linear multivariate regression of the form (1), taking into account those exogenous variables which have the strongest relationship with the endogenous variable (Table 3).

Let's provide the correlation analysis of regression equation for winter wheat variety Kolchuga on application of moderate doses of mineral fertilizers ($N_{30}P_{30}$):

$$Y = 0,007 + 0,083X_1 + 16,99X_3 - 0,39X_4$$

From the regression equation, we could see that for increasing in the length of the ear X_1 (cm) by 1%, the yield of winter wheat would increase by 0.083 t/ha, and for increasing in X_3 (the weight of grain from the ear, g) it would increase by 16.99 t/ha. It should be noted for increasing in X_4 (the 1000 grains weight, g) by 1%, the yield of winter wheat would decrease by 0.39 t/ha.

Table 3. Mathematical modeling of winter wheat crop structure (average for 2012 – 2016 years)

Nutrition variant	Regression equation	Coefficient of determination	Multiple correlation coefficient
Variety Kolchuga			
Control	$Y = 155,86 - 6,56X_2 + 175,61X_3 - 4,09X_4$	0,969103328	0,984430459
$N_{30}P_{30}$ (background)	$Y = 0,007 + 0,083X_1 + 16,99X_3 - 0,39X_4$	0,994792455	0,997392829
Background + Escort-bio	$Y = -20,49 + 0,97X_1 - 3,21X_3 + 0,48X_4$	0,987441678	0,993701
Variety Zamozhnist			
Control	$Y = -24,36 + 0,62X_2 - 9,64X_3 + 0,54X_4$	0,988868	0,994419
$N_{30}P_{30}$ (background)	$Y = 176,77 - 6,25X_2 + 169,35X_3 - 4,72X_4$	0,989904559	0,994939475
Background + Escort-bio	$Y = -44,47 - 0,23X_1 + 10,63X_3 + 0,87X_4$	0,987511136	0,993735949

The Constructed table of variance analysis (ANOVA – table), has the following form:

Table 4. ANOVA – table (variance analysis table)

Source of variables	Degrees of freedom	Sum of squares	Dispersions (average squares)
Regressions	$k_1 = m - 1 =$ $= 4 - 1 = 3$	$SSR = 6,103131293$	$MSR = 2,034377098$
Residues	$k_2 = n - m =$ $= 5 - 4 = 1$	$SSE = 0,0319487$	$MSE = 0,031948707$
of total variable	$n - 1 =$ $= 5 - 1 = 4$	$SST = 6,13508$	$MST = 1,53377$

The coefficient of determination without taking into account the number of degrees of freedom:

$R^2 = 0,99479$ testifies that the variation of the yield by 99,48% determines by the variation of the ear length, the weight of grain from the ear and the 1000 grains weight.

Multiple correlation coefficient: $R = \sqrt{R^2} = \sqrt{0,99479} = 0,993701$ is a measure function of linear connection of dependent variable Y with independent variables X_1, X_3, X_4 . Its value 0,993701 shows the close connection between corresponding indicators.

Using the ANOVA table we could define:

– the variance of the regression: $\hat{\sigma}_p^2 = MSR = 2,034377098$;

– unbiased estimate of the variance of the residues:

$$\hat{\sigma}_u^2 = MSE = 0,031948707;$$

– standardized error of residues: $\hat{\sigma}_u = \sqrt{\hat{\sigma}_u^2} = \sqrt{0,031948707} =$;

– variance of the dependent variable (total variance) $\hat{\sigma}_Y^2 = MST = 1,53377$.

Partial elasticity coefficients:

$$E_{\frac{Y}{X_1}} = \frac{\frac{\partial \hat{Y}}{\partial X_1}}{\frac{\bar{Y}}{\bar{X}_1}} = \hat{a}_1 \cdot \frac{\bar{X}_1}{\bar{Y}} = 0,200949;$$

$$E_{\frac{Y}{X_2}} = \frac{\frac{\partial \hat{Y}}{\partial X_2}}{\frac{\bar{Y}}{\bar{X}_2}} = \hat{a}_2 \cdot \frac{\bar{X}_2}{\bar{Y}} = 4,938201;$$

$$E \frac{Y}{X_3} = \frac{\frac{\partial Y}{\partial X_3}}{\frac{Y}{\bar{Y}}} = \hat{a}_3 \cdot \frac{\bar{X}_3}{\bar{Y}} = -4,14113.$$

The calculated partial elasticity coefficients show when if the ear length increases by 1%, the yield of winter wheat would increase by 0.200949, provided that the remaining factors are stable, if the mass of grain from the ear increases by 1%, the grain yield would increase by 0.443943, provided that the remaining factors are stable, and if the 1000 grains weight increases by 1%, the yield would decrease by 4.14113, provided that the remaining factors are stable.

The total elasticity

$$A = \sum_{j=1}^n E \frac{Y}{X_j} = 0,200949 + 4,938201 - 4,14113 = 0,998016.$$

The total elasticity shows when if all the factors taken into account increase simultaneously by 1%, the yield of winter wheat would increase by 0.998016.

Check the statistical significance of the estimates of the model parameters t – Student's statistics. Let's determine the actual values t – statistics:

$$t_{\hat{a}_0} = 0,00196129; \quad t_{\hat{a}_1} = 0,113124165; \quad t_{\hat{a}_3} = 4,244929172; \quad t_{\hat{a}_4} = -2,283886522.$$

The table value t – criterion for the degree of freedom $k = k_2 = 5$ and the significance level $\alpha = 0,05$ is equal $t_{ma\bar{o}l} = t_{0,05;5} = 2,57$.

As $t_{\hat{a}_3} > t_{ma\bar{o}l}$, as the parameter a_3 of the model is statistically significant, therefore the parameter x_3 significantly impacts on the dependent variable Y . As $t_{\hat{a}_1} < t_{ma\bar{o}l}$, $t_{\hat{a}_4} < t_{ma\bar{o}l}$ and $t_{\hat{a}_0} < t_{ma\bar{o}l}$, so corresponding parameters of the model are statistically insignificant. When if to decrease the confidence level, for example $\alpha = 0,1$, so $t_{ma\bar{o}l} = 2,015$. And it would prove that the 50% confidence level validates the significance of the parameter a_4 .

Let's check by F – criterion of Fischer the adequacy of the econometric model to actual data, such as the hypothesis of significance of connection between independent variables and the dependent variable $F_{\phi akm} = 63,61635$ The table value for the specified confidence level $\alpha = 0,05$ and the number of degrees of freedom $k_1 = 2$ i $k_2 = 3$: $F_{ma\bar{o}l} = F_{0,05;2;3} = 9,552$.

As $F_{\phi akm} > F_{ma\bar{o}l}$, so we decline the null hypothesis and with the specified probability $p = 0,95$ the econometric model would be consider to be adequate to actual data, therefore the hypothesis of significance of connection between independent variables and the dependent variable should be proved.

Checking the accuracy of the econometric model by the average relative approximation error $\bar{\varepsilon} = \frac{1}{n} \sum_{i=1}^n \left| \frac{y_i - \hat{y}_i}{y_i} \right| \cdot 100\% = 1,53 < 10\%$, so it would testify of the high quality of the model.

That would be illustrated by Figure 2 as the insignificant deviation of y_i and \hat{y}_i could be observed.

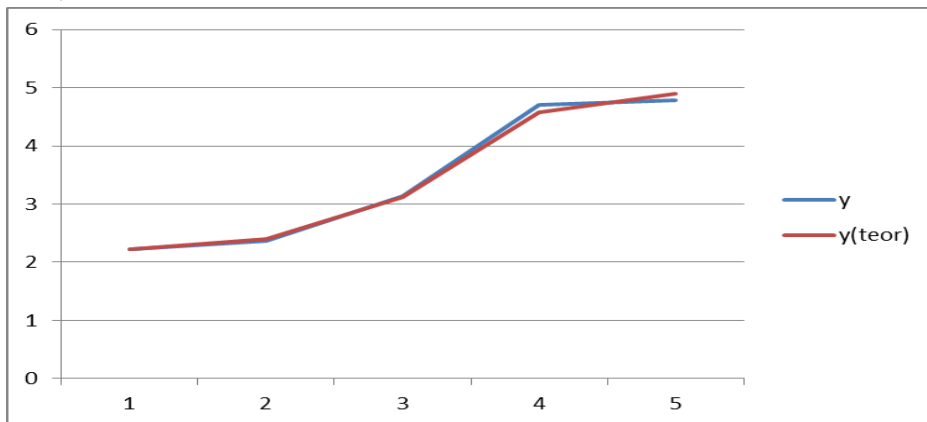


Figure 2. Diagrams of actual data and line of regression

So, the model is built with precision $\alpha=0,05$. For further research possible directions include developing a better model structure with less computation time and complexity, using some hybrid algorithms to build the embedded model, and using parallel algorithms.

In the process of research there is a need on the basis of available information about the yield to establish a statistical pattern for this set of observations. At the same time, the main point is to determine the factors affecting its level, in particular the influence of soil and climatic conditions, plant nutrition, economic indicators of crop cultivation. To that end, the researchers use various methods of economic analysis, in particular the method of analytical groupings, correlation and regression analysis, index method, etc. (Oprah, 2011; Kiani and Agahi, 2016).

Crop selection is influenced by many factors like the weather, soil type, market, etc. Weather and soil type are the major factors which affect on the crop yield. Crop yield prediction helps the farmers in the selection of the crop for plantation. Crop yield can be accurately predicted by considering the parameters like the soil type, amount of rain, crop characteristics, etc. Results show that the C-ANN model performs better with a higher R2 statistic and a lower percentage prediction error than the MLR and D-ANN models on the test dataset. Prediction of crop yield is very important in the agriculture community. In this study wheat yield was predicted by considering its different parameters. Better wheat yield was predicted by using C-ANN model (Aditya Shastry et al., 2016).

Using an experiment on integrated nutrient management in the rice-wheat processing system, a regression analysis of the biological yield and yield index of rice and wheat was studied to assess the contribution of various vegetative and reproductive traits (Gupta and Sharma, 2013). Two-year data on both crops showed that plant height, the number of cultivators during harvesting, the length of the stem and grain on fibrin were crucial for biological yield and yield index.

CONCLUSION

Under the conditions of southern Steppe of Ukraine, the application of mineral fertilizers at a dose of $N_{30}P_{30}$ for pre-sowing cultivation and foliar fertilizing of winter wheat crops at the beginning of the resumption of spring vegetation and the stooling with Escort – bio and Organic D2 drugs ensures the formation of the highest rates of crop structure. According to the results of mathematical calculations, in the control version of the experiment, the number of grains in the ear, the mass of grain from the ear and the 1000 grains weight have the greatest impact on the yield of the winter wheat variety Kolchuga. When applying mineral fertilizers for pre-sowing cultivation in a moderate dose of $N_{30}P_{30}$ and fertilizing during the growing season with the drug Escort-bio, there is a strong correlation between the yield and the length of the ear, the weight of the grain from the ear and the 1000 grains weight. The same dependence is observed in the variety Zamozhnist. For further research, possible directions include developing a better model structure with less computation time and complexity, using some hybrid algorithms to build the embedded model, and using parallel algorithms.

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**CITIES EXPANSION AND LAND USE CHANGES OF AGRICULTURAL
AND GARDEN LANDS IN PERI-URBAN VILLAGES
(CASE STUDY: BOJNURD)**

SUMMARY

Sustainable development looking to reduce threats and harmful effects of economic, social and followed proper development patterns and preservation from lands in periphery cities. Urbanization in developing countries in comparison with developed countries accelerating the process has been increasingly and this problem caused to the physical expansion without any program in cities, leads to impose the harmful effects on the natural environment and agricultural lands in periphery cities. In Iran that share urban and rural population between 1941 to 2016 is contrary from 31.5 per cent to 74 percent have physical changes such as expansion of cities and merged or dissolve villages in cities space and swallowing agricultural lands and gardens in periphery cities. Bojnurd city in North Khorasan province with 2,584 hectares moved toward dissipated growth pattern with modernism paradigm and with accepting urban sprawl pattern swelled of peripheral lands and this issue increased in recent years. Four villages include Halghehsang, Malkesh, Yengeghalee and Bagherkhan 3 were added to the city services margin from 2009. Results shows decreasing in gross urban density from 153.6 to 65.3 per cent in the period from 1941 to 2016. This issue caused that sprawl growth pattern in this city swallowing lands. These lands mainly are agricultural lands and gardens. Reviews on city creep phenomenon in Bojnurd with urban sprawl development pattern shows that this pattern will continue due to promotion of Bojnurd city to the capital of North Khorasan province and acceptance of new superior role and capacity of new regional investment and job creation in the upcoming decades. The other villages like Yngeghalee, Bagherkhan3 will swallow the peri-city lands and the productive role of these villages will changed to services. Therefore, sustainable development and preservation of lands surrounding the city should change sprawl urban pattern in Bojnurd city and replaced by a pattern of smart growth that forms the ideal city is a compact city

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that will search development at within the city. This pattern emphasis on preserving agricultural lands within and outside the city. The fact is 24 percent of city space belongs public lands and gross city density is 71.5 person. Therefore, it is not recommended any increasing in city limits because more than 50 per cent of the surrounded lands include agricultural lands and gardens are in the immediate vicinity of the city.

Keywords: Agricultural lands, Garden, land use change, Peri-urban villages, Sprawl development, Bojnurd

INTRODUCTION

By emerging industrial revolution in 18th and 19th century, Cities and surrounded villages experienced vast evolutions. After the second world war, the main scheme of cities growth was the machinery and dissipated urban development. These types of developments led to degrading and emitting agricultural lands especially clear cutting gardens and also some problems to support facilities and environmental crisis (Rahnam and Abbaszadeh, 2008). As a result, policy makers are trying to preserve environment and settle environmentally friend cities based on sustainable development thought and eco-friendly cities as a world awakening (Mahdizadeh, 2011).

Urbanization and agriculture are two different cases which are completely related together as all researchers believe in the necessity of existence of surplus agricultural products in appearing of cities in history (Harvey, 1992). But today the relationship of these two has reached a specific level. With the fast increase of urbanization and the continuous raise in population, we could not only see that big cities are appearing but also with this expansion toward existing biological the spaces, it would cause irreversible damages in agricultural lands and gardens and even these incorrect plans will occasion that the suburb villages turn into informal settlements in transition phase of rural nature into urban nature (Daneshpour,2006).

The sharp rise in urban population and subsequent irrepressible physical development of cities in recent decades are some of the threats that are occupying habitations and sustainable urban development is a case looking for proper patterns of development and suburb lands preservation in order to reduce these threats and the harmful economic social impacts of them.

Urbanization in developing countries in comparison with developed countries has been accelerating and this has caused by unplanned physical expansion results in more harmful undesirable effects on natural environments and the agricultural lands around and the trouble which is happening a serious treatment of urban-rural prospective.

In I.R.Iran where the rural and urban population rate was reversed between 1941 and 2016 and urbanization grew from 31.5 to 68.5 percent, some changes such as physical expansion and merging the suburb villages and swallowing the agricultural lands and gardens appeared.

Peri-urban environments are some spaces where are strongly under effect of daily growth of urban population and experience constitutional changes. Firstly, their land use which turns from agricultural and husbandry into residential use and secondly they merge with urban fabric that the main reason of this phase are comparative low life expenses especially the costs of land and house in these villages and emigrants' tendency toward these areas.

The purpose and subject of this research is analyzing and presenting the general and comprehensive image of growth model of Bojnurd and land use changes consequent by this growth in the villages around the city which on one hand the physical growth process of the city and on the other hand the physical growth and expansion process of the villages will be studied and the main question in this research is that "is there any relationship between the physical growth model of cities and land use changes of the villages around them?" And to answer to this question, a hypothesis is given that the model of sprawl growth and expansion of Bojnurd is the rational of land use change if agricultural lands and gardens of the suburb villages.

Records and revision of previous researches

Based on several researches accomplished about different cities, physical development and expansion of cities was the main factor of changes in peri-city residential function and also in land use which will be mentioned below.

A research about development and expansion of Tehran has been accomplished showing that along city growth, the villages in its influence domain and around it face lots of changes in their functions which sometimes led to appearing of new villages (Rahnamayi,1990,24-53) and also in this process some villages around Tehran such as Hesarak, Vanak, Darabad, Darband, Saadatabad, Velenjak, etc were swollen which sometimes caused to appearance of newly born informal settlements for example Abkouh Castle in Mashhad (Saeedi Rezvani,2006).

Analysis of Sanandaj space development in last 3 decades shows that 8 villages including all their agricultural lands and gardens and farms were merged with city fabric (Zia Tavana and Ghadmarzi, 2005) that the most important characteristics of these villages were lack of urban installations, facilities and services (Farhoudi and Mohamadi,2005).

The analysis of physical development of Tabriz in last 4 decades shows that irrepressible expansion of this metropolis had a wide range of changes specifically through changing land use of agricultural land and gardens and turning them into residential land use in influence domain villages especially along the communication paths of this city (Zaheri,2008).

The study which has been done in valley settlements of Skochai, Azarbayejan shows that in a 13-year period (1989-2002), the population raised almost 12 per cent meanwhile approximately 77 per cent was added to the construction space in the area of study and in this process there is a dramatic decline in the space of gardens and agricultural lands for the interest of

residential land use and none agricultural land use (Sadr Mosavi & Ghorbani, 2007).

Another research has been done to analyze the construction of the new industrial city of Alborz and the influences of its change on Alvand villages that the results indicate constitutional direct effects of city development on economic, social and spatial, somatic and environmental changes of the influence domain villages which are resulted by expansion of industrial units (Soleymani,2002).

Physical Expansion process of our cities is growing sharply at the moment as one study shows that the fast sprawl development of Yazd between 1983 to 2006 and the continuance of its center-suburb movement are accelerating and in spite of spongy fabric and existence of the major number of idle lands in it, the lands around the city are being swallowed by this physical expansion (Azizpour,2009) and this matter is admitted by the comparison 4 percent growth and 8 percent space growth and this problem is the reason why 21 percent of city space is turned into idle land use (Taghvayi and Sarabi, 2004).

There is a study related to the subject matter done in village area about analysis of rural infrastructure development plans and the process of construction in Chenaran. Results shows that the construction in agricultural lands instead of specified lands in the direction of predicted expansion plans is still in progress which has caused to an increase in urban problems (Yasouri,2009)

Research theoretical foundation

Urbanization is defined by some experts based on its communication and in this case David Harvey believes that every city includes environs and no centre could be imagined without the environs and these two help each other to be defined (Harvey,2003). Based on Hamilton and Anas (2008) decentralized development of cities leads to activity and population replacement. So, travel time and costs will increase and cause to pollution and urban problems (Bestani & Klein, 2006). The main issue is decentralized development of cities being to the main problem of developing countries especially in Asia and Africa (Musallam, 2012).

Somatic and physical growth of a city is a progress which takes place in 2 general structures of internal and external and the international searches show that compact city approach is nearer to the aims of sustainable development (Masnavi,2003). External city expansion is appeared like city limit increase or sprawl and internal expansion like compact city growth pattern which is connected to urban smart growth.

City smart growth is a method which mentions expansion principles and set piece related with land use pattern and transportation. Smart growth finally ends in vertical expansion pattern and compactness in cities which occupies less land space and with use of different types of transportation makes a decrease in traffic and journeys.

Some advantages of compact cities are decreasing the distance of facilities and job opportunities and nearing free time to each other and finally saving fuel and efficient use of urban lands and protecting agricultural and rural lands around

city (Jurgis,2007), overhauling old buildings and abandoned lands, increasing mobilization resulted by transportation increase and creating social spirit by merging land uses (Zenonas,2006).

In developed countries a lots of methods and patterns related to strategies of protecting agricultural lands, gardens and green lands are used which are divided in to groups: suburban and intercity. Suburban strategies such as green rings and bows are accomplished to save suburb agricultural lands and limit irrepressible growth of cities. Intercity strategies such as method of economic revenue operation of agricultural lands and gardens with the purpose of providing some needs of city habitants.

The method of transferable development law which is used to prevent agricultural lands and gardens land use change into residential land use is provided for owners and farmers and subsequently land use change of agricultural lands and gardens would be forbidden and method of ecological benefits and tax support which is resulted by the desired effect of these lands on weather modification and protecting urban landscapes help these owners to be included in these supports and different tax discounts. (Sadr Mosavi & Ghorbani, 2006).

MATERIALS AND METHODS

In this research the method of descriptive-analytical method is taken to analyze and explain connections and communications of Bojnurd and the villages around it and documentary method is taken for analyzing its changes and transitions and because the final aim is operational kind, these two methods are merged with desk and survey methods.

In order to analyze process of somatic expansion of the city and progress of swallowing the villages located in peri-city domain, first level of the research was spent on desk research and analysis of aerial pictures and maps of different periods and after collecting information and data and categorizing them, by using direct observation method, second level was spent on completing information and finally by merging and analyzing the existing information, assessment and analysis of existing growth model and pattern of the city was accomplished to find the best physical expansion pattern for the city coincided with sustainable urban expansion on one side and on the other side with sustainable rural expansion. The area of study in this research is urban area of Bojnurd and pericity domain villages which are merged with urban fabric and immediate villages.

RESULTS AND DISCUSSIONS

There is a wide fertile valley located on the north of Khorasan from West to east and is surrounded by Kopedagh and Hezarmasjed mountains in the north and Aladagh-Binaloud in the South.

Alongside this building hole, there are some small and medium sized plains where sediments accumulation has made appropriate agricultural domains in and has prepared the base of city creation and expansion of some cities including Bojnurd. Bojnurd is located in 57° 20' latitude and 37° 29' longitude

and is 1070 meters higher than sea level and is limited by faults in North and South West and by dry rivers in North and East (Fajre tose'eh,2007).

Population: according to the census data collected in 2016, the population of Bojnurd is 228931 (Statistical Centre of Iran, 2016) that is calculated 186297 by urban comprehensive plan of Bojnurd with ratification of its city proper and attaching 3 villages to the city in 1388 (Naghshe Jahane Pars, 2009).

Urbanization ranking of this city didn't fluctuate a lot in urban order system from 1941 to 2016 and went up from ranking 54th in 1941 to 42th in 2016 as this city is in the population class of 100,000 to 499,000 people and is known as a city with an average population or middle city.

Occupation: In 2016, Classification of occupants based on main activities showed from 58977 people (10 and over 10 years old) who lived in the city were 9 per cent work in agriculture part, 26 percent in industrial part, 63 in service part and 2 percent in unknown part.

Assessment of economic status of the city expresses a transition in economic structure of the city which has changed from economic status dependent on agriculture into industry and service expansion and specifying a major space of town to industrial and service occupations has caused some changes in somatic structure and land use pattern.

Somatic structure: assessment of population increase process and alongside it expansion of city space between the years 1941 and 2009 indicates on acceleration of city space increase in comparison with population increase as gross urban density has had a decline from 153.6 people per hectare to 65.3 people per hectare (table 1) and this problem is obvious in comprehensive plans of 1976, 1995 and 2009 as gross urban density is 104.8, 78.9 and 65.3 respectively. On the other hand, population grew 368 percent during these 40 years which equals an increase of 4.6 times while city space grew 611 percent (equal to an increase of 7.1 times).

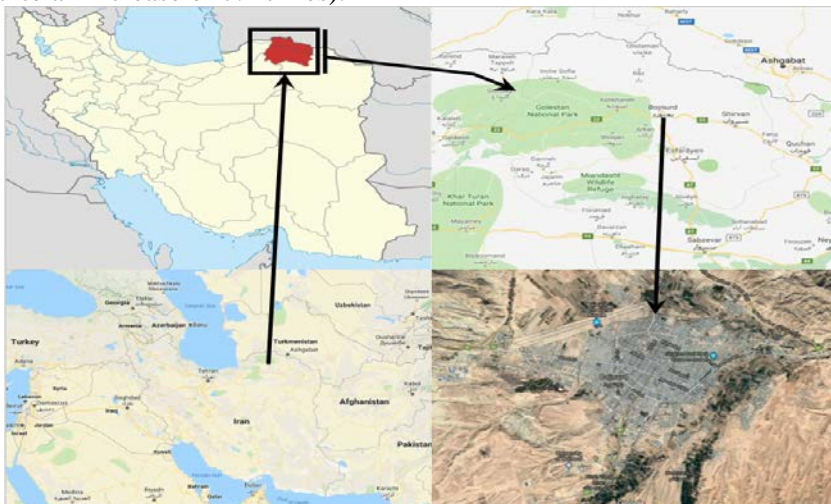


Figure1. Location map of Bojnurd

Table1: gross urban density of Bojnurd (1941-2016)

Year	City space (hectare)	Population	Gross density (person per hectare)
1941	19.5	15293	153.6
1956	142	19253	135.5
1966	270	31248	115.7
1976	450	48850	108.6
1986	770	93392	121.2
1996	1653	134835	82.46
2006	2854	186297	65.27
2011	3050	199791	65.5
2016	3200	228931	71.5

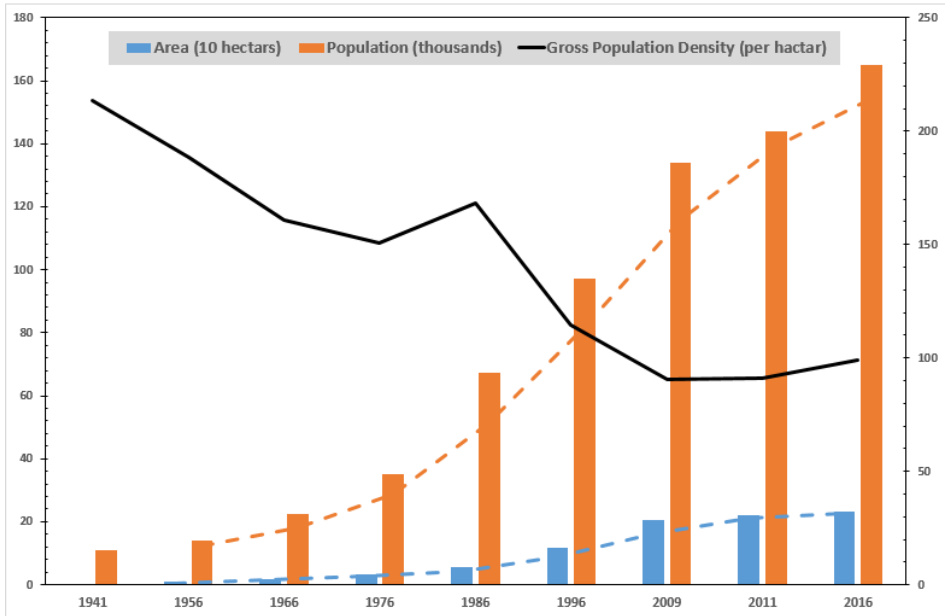


Figure 2. Time series and comparison of city area, population and gross population density of Bojnurd

By analyzing somatic expansion of Bojnurd, we can separate 3 different fabrics which are categorized as Inner, Middle and Outer. Each of these fabrics has different situation according to their creation circumstances and time. In external fabric which mostly has been formed after land reformation period and has grown after Islamic revolution, not following the plans, principles and rules of construction, lack of desired land use in residential areas, sprawl construction and undesirable status of quality and quantity parameters are obvious that explains serious need of control and leading.

Analysis of idle lands in this fabric which is 33.4 percent of the whole

space indicates the significant sprawl expansion of city during recent decades whereas the least space is belonged to urban service. Table 2 shows a comparison of different land use categories to comprehend somatic features of these lands.

Table 2: Portion of different land use categories from the space of existing fabrics of Bojnurd

Land use	Portion of land uses from fabrics		
	Outer	Middle	Inner
Residential	17.4	40.5	53.3
Service	9.4	11.3	7.5
Idle lands	33.4	6.8	7.9
Passage way	16.3	26	24
Others	28	15.4	7.3

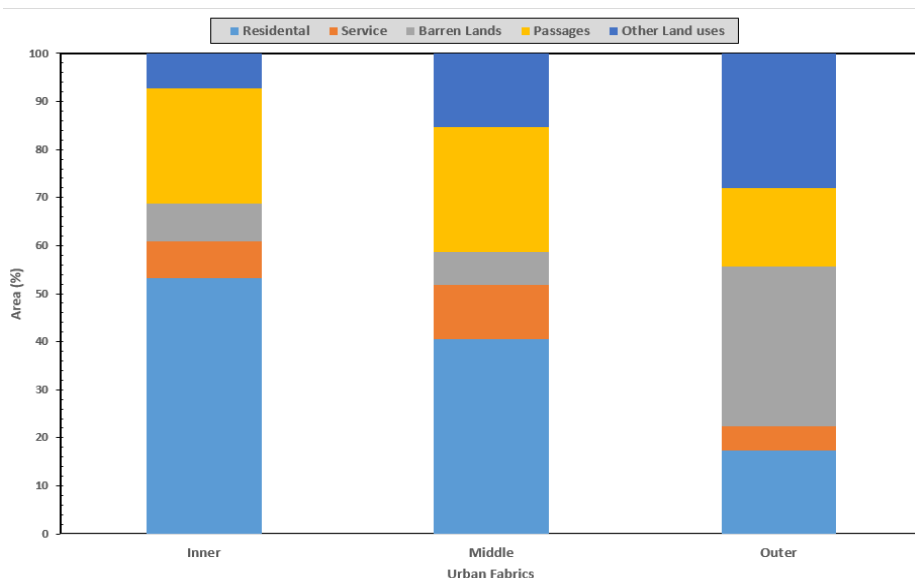


Figure 3. Portion of different land use categories

Geographical location villages of the study and process of their somatic expansion:

In this research, the villages of the study are categorized in two groups. First category includes some villages that are merged in somatic body of Bojnurd in its somatic expansion and Malkesh and Halghesang are the villages which are the cases of study in this research and were attached to city fabric in comprehensive plan of Bojnurd in 1388 and are a part of urban service now. And second group include those villages which are located around city and in peri-city domain and is under attack of being swallowed by somatic expansion of Bojnurd in its physical development and the case of study are Yengeh Ghaleh and

Bagherkhan 3. Malkesh and Halghesang are in the south of Bojnurd in the road side of Bojnurd- Esfarayen. Assessment of population growth of these 2 villages during a 30-year period (1976-2016) shows a sharp growth of population (table 3) as their population growth overtakes Bojnurd population growth which expresses that these villages were immigrant status of extra population of Bojnurd because of their cheaper lands and settlements.

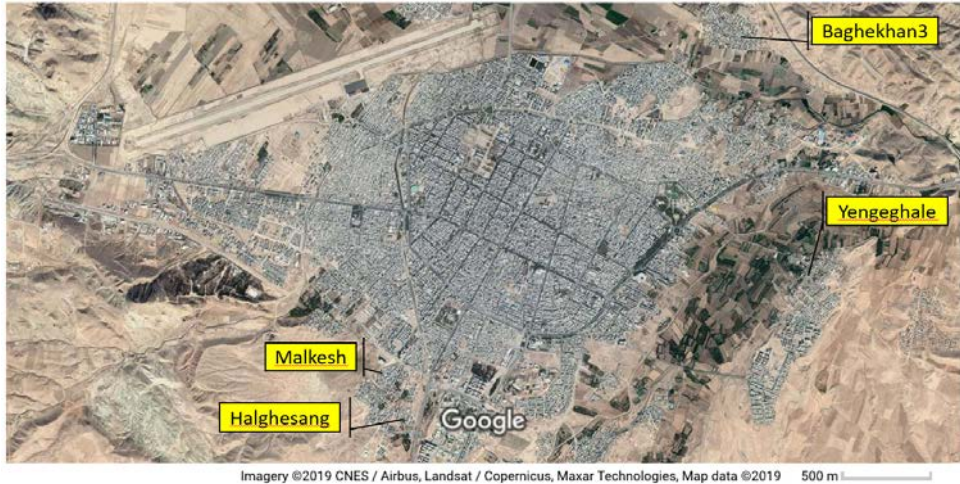


Figure 4. Location of Villages as case study

Table 3: Population Growth rates of Malkesh, Halghesang and MohammadAbad from 1976 to 2016

Village	1976-1986	1986-1996	1996-2006	2006-2011	1976-2016
Malkesh	3.5	8	7.5	6.3	6.7
Halghesang	1.6	18.3	12.4	10.5	11.2
MohammadAbad	5.2	9	3.4	6.5	5.9

Yengehaleh is located in the east of Bojnurd in the vicinity of Valiear suburb and Bagherkhan 3 is located in the distance of 500 meters of north of city on the route of new cemetery of Bojnurd and analysis of their population growth process indicates the immigrant status of them in recent 3 decades (table 4). Although their population rate is lower than the previous villages but it's still more than Bojnurd population growth. Cheaper land and houses could be some rationales of immigrant status of these 2 villages.

Table 4: Population Growth rates of Yengehaleh and Bagherkhan 3 between 1355 and 1385

Village	1976-1986	1986-1996	1996-2006	2006-2011	1976-2016
Yengehaleh	6	1.7	3.5	5.67	4.22
Bagherkhan 3	2.8	6.8	5.8	12.5	6.9

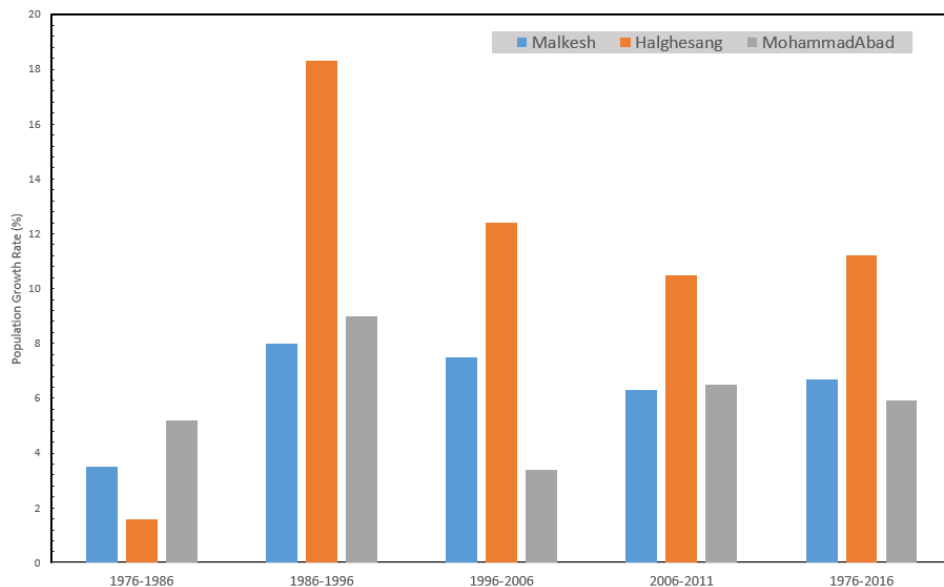


Figure 5. Population Growth rates of Malkesh, Halghesang and MohammadAbad from 1976 to 2016

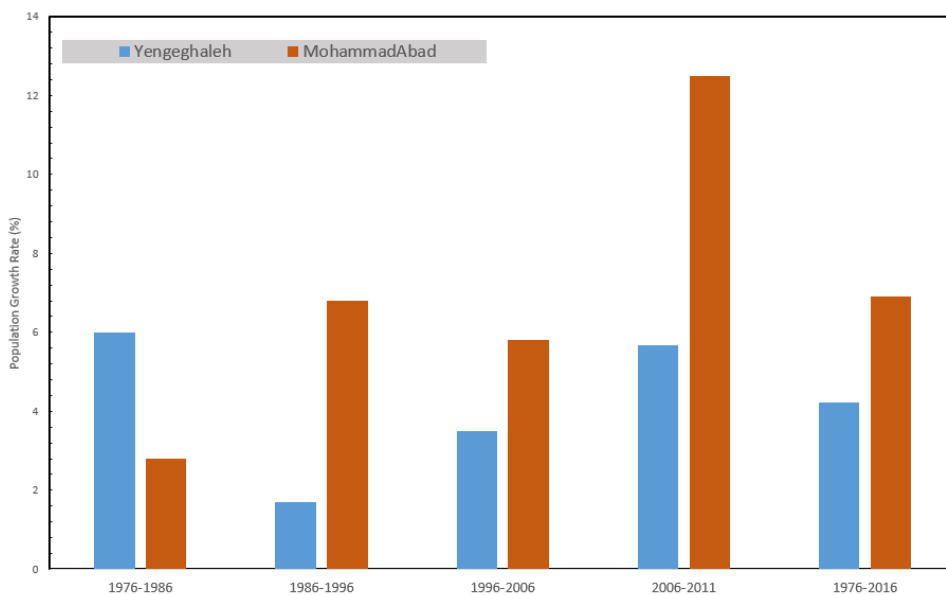


Figure 6. Population Growth rates of Yengeghaleh and Bagherkhan 3 and 1976 to 2016

But this population growth which is under effect of Bojnurd vicinity has caused major transitions in occupation structure and body of these villages that has direct or indirect influences on land use change of agricultural lands and

gardens. Based on economic features, the results of studies on rural infrastructural development plans show that closer villages to physical domain of the city have less occupants in agriculture (table 5) and this is the main reason of increase of occupants in industry and service parts in Bojnurd.

Table 5: Income portion of the villages occupants in 3 categories

Village	Agriculture	Industry	Service
Malkesh	52.6	24.6	22.8
Halghesang	2.1	91	6.8
Yengeghaleh	85.4	4.3	10.4
Bagherkhan 3	66	16	18

In terms of service and installation and land use, population growth has also caused a special structure in these villages as residential portion has resulted a decline in service portion which is shown in table 6.

Table 6: Major existing land use in the villages of study

Land use	Malkesh	Halghesang	Yengeghaleh	Bagherkhan 3
Residential	41.4	42.4	43.9	69.6
Service	2.7	3.2	7.8	0.9
Passage way	23.7	30.9	20.3	12.1
Agricultural lands and gardens	1.6	18.2	4.5	1.6
Idle lands	30.6	5.3	18	15.8
Cemetery	0	0	5.5	0
Total	100	100	100	100

CONCLUSION

Bojnurd has experienced growth in 2 general forms during the time. Organic model that was dominant till Pahlavi and before 1960s land reformations grew smoothly, continuously and slowly, and non-organic model that took place in recent 50 years and passed domination of irregular sprawl expansion which caused creation of external fabrics in different forms of suburbanites (growth of informal settlements), discontinuous development and swallowing the villages around and major problems of this city is also resulted by this growth model which caused loss of integration and spongy development, increasing idle and useless lands and destroying in demand agricultural lands and gardens. In accelerated process of scattered expansion of the city, vast areas are situated in the suburbs as rural fabrics that most agricultural lands are illegally partitioned and came into market and made environmental problems and informal settlements.

The results of the assessments on urban creep phenomenon of Bojnurd with scattered expansion pattern indicates progress swallowing suburb villages and changing land use from agricultural and gardens in to residential that if this process continues, other villages will also be in danger if this merger (map 1).

The results of these studies show that while Bojnurd population is increasing, the body of this city tends to expand sprawl more quickly and to encroach the lands around which confirms the decline of gross urban density and occupation of idle lands. One of the consequences of this encroachment is swallowing suburb villages that with merging these villages, on one side, agricultural land and gardens disappear and we witness this threat nowadays and in the other side, the balance of land use was harmful to service land use so the accomplished analysis confirms the given theory named "sprawl growth and expansion of Bojnurd has given a rise to land use change of rural agricultural and gardens around city"

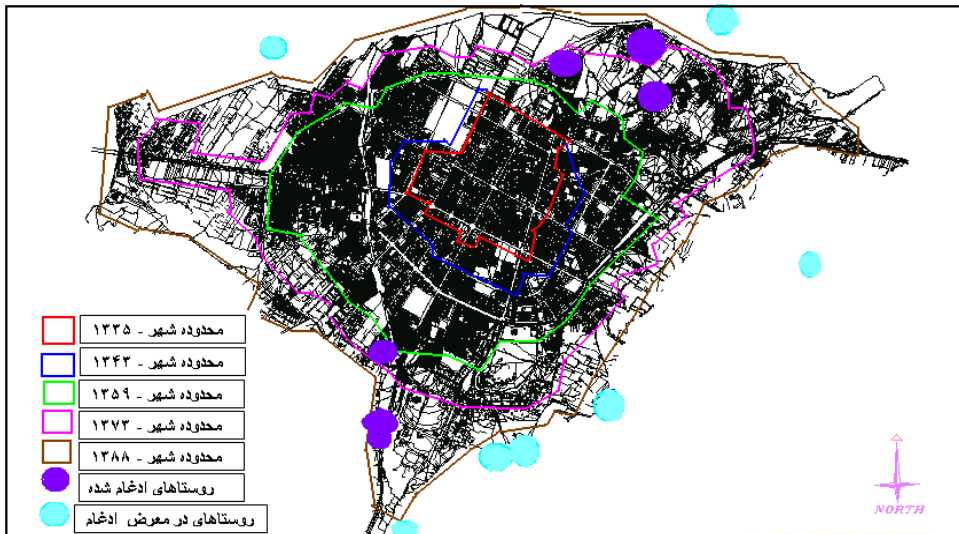


Figure 7. Process of somatic expansion of Bojnurd Status of peri-city villages



Figure 8. Some Sharp Land Use Changes (Clear Cutting of Gardens) in the South of Bojnurd Between 10/25/2005 (Left) to 7/23/2019 (Right)



Figure 9. Sharp Land Use Change (Rain Fed Agricultural Lands to Residential Area) in the South West of Bojnurd Between 10/20/2005 (Left) to 7/23/2019 (Right)



Figure 10. Urban Creep Between 10/20/2005 (Left) to 7/23/2019 (Right) in the East of Bojnurd

RECOMMENDATIONS

What is deduced from population and somatic assessment of Bojnurd and the villages around is that Bojnurd because of being promoted to be center of the province and accepting better and new zonal roles and new investment and job creation capacities in following decades will be an immigrant city that some emigrants will live in the villages around and this problem will make the agricultural lands and gardens in danger of disappearing more than before so regarding to urban sustainable development and also rural sustainable development, there must be some plans to have these high grade agricultural lands with their desirable landscapes as an opportunity not a threat for sustainable development.

If the settlements located in peri-city areas get led and correct plans get accomplished for protecting their agricultural and gardens landscapes there will be the opportunity for moving toward urban sustainable development and rural sustainable development and if we don't make correct decisions in these areas, they will find a residential approach in form of informal settlements and it will be a threat die sustainable development. Therefore, in the first step we must recognize suburb villages as a part of an integrated system with metropolis that have constitutional impacts on system so then we must see them through

collision location of city-village-nature in the form of a zonal planning and a combined rural and urban planning and management and regarding to this some recommendations are given as below:

In order to protect agricultural land and gardens of peri-city villages and to prevent them from land use change, we must replace pattern of compressed city instead of scattered city pattern and according to the existence of idle and nonagricultural lands in urban fabric which occupied 24 percent of city space, prevent all kinds of limit rise and control all types of irregular construction out of existing limit seriously and construction of green rings roads and green bows must be included in urban managers' working schedule to prevent sprawl expansion of city and invasion of the city to agricultural lands and gardens especially those between attached city fabric and separate cores of Golestan and Valieasr Suburbs which are talented to get into market.

Precise observation is needed for adjacent villages which are in danger if getting merged with somatic fabric of the city that in this case training village assistants would have a major influence.

Infrastructure development plans of these villages must be up to date and revision off them must be included in planning schedule of housing foundation and providing them must be with advisement of the most eligible and experienced consultants according to social and economic affairs and partnership of people and village managers including village assistant, council and local trustees and finally prevent all kinds of change in land use of agricultural lands and gardens of villages and around them and subjoining must be with the permission of land use change commission.

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