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POLYMORPHISMS IN CANDIDATE GENES FOR BEEF QUALITY IN PINZGAU CATTLE

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ABSTRACT

The aim of present study was to identify the polymorphisms in genes encoding calpastatin (*CASTUoG*), calpain (*CAPN1*, *CAPN2*), diacylglycerol O-acyltransferase (*DGAT1*), thyroglobulin (*TG5*), and Stearoyl-CoA Desaturase (*SCD*) in order to analyse genetic structure of Pinzgau cattle. The genomic DNA for genotyping was obtained from in total 56 blood samples of Pinzgau bulls. After extraction, the concentration of DNA was controlled by the spectrophotometry measurement. The genotyping of each individual was carried out by using PCR-RFLP methods. The average value of observed (0.37±0.05) and expected heterozygosity (0.39±0.06) clearly indicated the prevalence of homozygous individuals. Observed Wright's fixation indexes showed positive values across all loci (0.03±0.06), which confirmed slight deficiency of heterozygote animals compared to the Hardy-Weinberg equilibrium expectations. The Hardy-Weinberg equilibrium was found in population, which signalizes only slight impact of factors such as selection, migration or inbreeding. The effectiveness of loci allele impact in populations has been described also by effective allele numbers (1.68±0.13) that expressed the decrease of allele activity in population. The loss of heterozygosity in analysed population was found across all of genetic markers. Each of the evaluated indicators clearly points to the need of genetic diversity monitoring. Moreover, the analyses of single nucleotide polymorphisms in genes significantly involved in control of economically important production traits are still very usable methods for identification of genetic markers that can be used in marker assisted selection of cattle.

Keywords: *cattle, genetic markers, meat quality, SNPs genotyping.*

INTRODUCTION

Improvement for carcass traits related to beef quality is the key concern in beef production. The application of traditional selection to these traits is difficult, since they are expensive and difficult to measure because it requires the slaughter of the animals. Along with traditional selection, marker assisted selection can help

improve economically important traits earlier in the breeding cycle. Knowledge of genetic variation and the search for candidate genes or genomic segments that influence production traits in the population of interest are essential for the establishment of a molecular criteria for selection (Tizioto et al., 2012; Magalhães et al., 2016).

Carcass traits related to beef quality are normally controlled or regulated by a number of genes and single nucleotide polymorphisms (SNPs) in the genes may be significant markers for improved cattle performance. Beef quality and carcass traits usually have low or moderate heritability and are often recorded post-slaughter, therefore, SNPs can be used as markers for indirectly improving beef quality traits instead of direct measurements (Liu et al., 2015). Development of molecular-genetic tools allow the identification of single nucleotide polymorphisms associated with large-effect genes that influence these traits, providing a better biological understanding of the trait and a list of candidate genes for fine mapping (Magalhães et al., 2016).

The meat tenderness is one of the most important beef traits mainly with respect to the consumer satisfaction. However, tenderness is a complex trait for breeding programs, because evaluation also depends on how animals are slaughtered. Thus, molecular marker information can be of great usefulness for identification of animals with high genetic value for tenderness and the selection process can be conducted on younger animals, even before birth (Pinto et al., 2010). Up to now, several genetic markers associated with differences in beef tenderness have been reported. These markers target two genes corresponding to the most important proteolytic system of skeletal muscle, the calcium-activated neutral protease gene (*CAPN1*) encoding the large subunit of μ-calpain and the calpastatin gene (*CAST*) encoding a specific inhibitor of the calpains (Corva et al., 2007). Moreover, the causative mutations in the *CAPN* and *CAST* genes have been shown to affect significantly not only beef tenderness but also marbling score (Casas et al., 2006; Morris et al., 2006; Lisa and Di Stasio, 2009; Pinto et al., 2010).

The key parameter of beef nutritional quality is intramuscular fat content (IMF). Nowadays people become more and more aware of what they eat and there is more interest in meat containing less fat. However, it may be at the expense of flavour and tenderness. It was shown that both flavour and tenderness scores markedly increased with increasing IMF content (Thomson, 2004). Previous studies have indicated that single nucleotide polymorphisms in the diacylglycerol-O-acyltransferase1 (*DGAT1*), thyroglobulin (*TG*) and Stearoyl-CoA desaturase (*SCD*) genes are significantly associated with intramuscular fat levels as well as marbling scores in beef (Pannier et al., 2010; Wu et al., 2012; Zhang et al., 2015).

The objective of our study was to genotype the single nucleotide polymorphisms in six genes previously reported as candidate for beef quality in order to determine the genetic structure of Pinzgau bulls population. The Pinzgau cattle is one of the most important dual-purpose cattle in Slovakia bred mainly in mountain regions of northern Slovakia due to its excellent longevity, fertility, health, and grazing ability.

MATERIALS AND METHODS

The biological samples were collected from in total of 56 Slovak Pinzgau bulls. The genomic DNA for genotyping was extracted from blood samples according to protocol of Miller et al. (1988). Subsequently, the concentration and purity of genomic DNA were tested based on the spectrophotometry measurements by the optical density at wave length of 260 nm. The single nucleotide polymorphisms in six genes encoding calpastatin (*CASTUoG*), calpain (*CAPN1*, *CAPN2*), diacylglycerol O-acyltransferase (*DGAT1*), thyroglobulin (*TG5*), and Stearoyl-CoA Desaturase (*SCD*) were analysed according to Gábor (2009) using RFLP methods. The products of PCR reaction and restriction fragments have been separated and visualised using horizontal electrophoresis in 2 % agarose gels (130 V for 50 min) and stained with day GelRed. The genetic structure of analysed population as well as population genetic indices have been analysed by using Genalex version 6.1 (Peakall and Smouse, 2012). The significance of differences between observed and expected genotype frequencies were tested by Chi-square test to assess the deviation from Hardy-Weinberg equilibrium. The genetic diversity indices derived from the frequency of alleles including observed (H_o) and expected heterozygosity (H_e), effective allele number (N_e), Shannon's information index (I) and molecular inbreeding coefficient noted as Wright's fixation index (F_{IS}) have been calculated using Genalex version 6.1 (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

In analysed population of Slovak Pinzgau bulls each of selected loci was successfully genotyped. Except SNP in *DGAT1* gene, for all loci have been identified three genotyped. Only two genotype (AA and AK) was identified in case of *DGAT1* gene, the KK genotype was not observed in analysed population. The distribution of allele frequency within each locus is shown on Figure 1. The frequency of genotypes as well as the level of genetic diversity within population derived from commonly used population genetic indices are listed in Table 1. For SNPs in *CASTUoG*, *DGAT1*, and *SCD* genes the predominance of homozygote genotypes was found, whereas for *CAPN1*, *CAPN2*, and *TG5* the prevalence of heterozygote animals was detected. The significant differences between observed and expected genotype frequencies was found only for SNP in *CAPN1* gene ($P < 0.05$). Overall, the observed Hardy-Weinberg equilibrium across loci signalize only slight impact of factors such as selection, migration or inbreeding in analysed population. But, the average value of observed (0.37±0.05) and expected heterozygosity (0.39±0.06) signalized the decrease of genetic variability due to the increase of population homozygosity. Similarly, observed Wright's fixation indexes showed in average positive value (0.03±0.06), which confirmed slight deficiency of heterozygote animals compared to the Hardy-Weinberg equilibrium expectations. The effectiveness of loci allele impact in populations has been expressed also by the effective allele numbers. Comparison of N_e showed higher effective allele numbers across populations for *CAPN2* gene and indicated good level of genetic variability in analysed population at the considered locus.

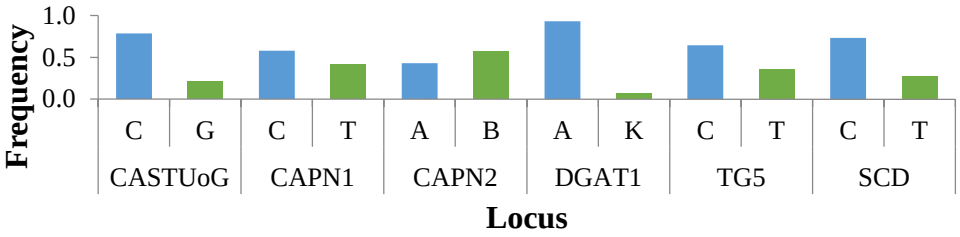

Figure 1. Frequency of alleles for each analysed locus in Pinzgau bulls population

Table 1. Summary of genetic structure and diversity indices evaluated in population

Locus	Genotypes frequency			Alleles frequency		χ^2 test	H_o	H_e	N_e	I	F_{IS}
CASTUoG	**CC** 34.571	**CG** 18.857	**GG** 2.571	**C** 0.786	**G** 0.214	ns	0.321	0.337	1.508	0.520	0.045
CAPN1	**CC** 18.862	**CT** 27.277	**TT** 9.862	**C** 0.580	**T** 0.420	*	0.339	0.487	1.950	0.680	0.303
CAPN2	**AA** 10.286	**AB** 27.429	**BB** 18.286	**A** 0.429	**B** 0.571	ns	0.500	0.490	1.960	0.683	-0.021
DGAT1	**AA** 48.286	**AK** 7.429	**KK** 0.286	**A** 0.929	**K** 0.071	ns	0.143	0.133	1.153	0.257	-0.077
TG5	**CC** 23.143	**CT** 25.714	**TT** 7.143	**C** 0.643	**T** 0.357	ns	0.500	0.459	1.849	0.652	-0.089
SCD	**CC** 30.018	**CT** 21.964	**TT** 4.018	**C** 0.732	**T** 0.268	ns	0.393	0.392	1.645	0.581	-0.002

ns – not significant; * $P < 0.05$

The obtained knowledge about genetic structure of analysed population can be used in the future for selection of animals with favourable genotypes to increase mainly economic important traits associated with muscle formation and level of intramuscular fat. The results are very perspective for breeders that use analysed bulls in mating programs. The *CAST* and *CAPN1* genes belong to the important genetic markers for beef quality. The calpastatin proteolytic axis has been identified as an important process to established meat tenderness. The μ-calpain (*CAPN1*) is a component of the calpastatin proteolytic axis (Casas and Kehrli, 2016). An association between meat tenderness and both genetic markers in the calpastatin and calpain genes has been confirmed in various studies (e.g. Casas et al., 2006; Barendse et al., 2007). The *DGAT1* gene genetic variants was primarily associated with milk production (Thaller et al., 2003), but several later studies reported the association between SNPs in *DGAT1* gene and marbling and fat thickness (Wu et al., 2012; Tait et al., 2014). Like the *DGAT1* gene, the genes encoding thyroglobulin and stearoyl-CoA desaturase are considered to be a perspective genetic marker for intramuscular fat levels as well as marbling scores in beef (Wu et al., 2012; Zhang et al., 2015; Bennett et al., 2013).

CONCLUSIONS

The loss of heterozygosity in analysed population was found across all of genetic markers. Each of the evaluated indicators clearly points to the need of genetic diversity monitoring. The analyses of single nucleotide polymorphisms in genes significantly involved in control of economically important production traits are still very usable methods for identification of genetic markers that can be used in marker assisted selection of cattle.

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EVALUATION OF DROUGHT TOLERANCE IN NEW COTTON CULTIVARS USING STRESS TOLERANCE INDICES

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ABSTRACT

Drought is a wide-spread problem seriously influencing production and quality of cotton (*Gossypium hirsutum* L.), but development of resistant cultivars is hampered by the lack of effective selection criteria. The objective of this study was to evaluate the ability of several selection indices to identify drought tolerant cultivars under different environmental conditions. Thirteen cotton cultivars were evaluated under both moisture stress (2016) and non-stress (2013) field environments using a randomized complete block design for each environment. Six drought tolerance indices including stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP) and mean harmonic productivity (HMP) were used. The significant and positive correlation of yield of genotype under non-stress condition (Yp) and MP, GMP and STI showed that these indices were more effective in identifying high yielding cultivars under different moisture conditions. The results of calculated gain from indirect selection in moisture stress environment would improve yield better than selection from non moisture stress environment. Coton breeders should, therefore, take into account the stress severity of the environment in choosing an index. The varieties Viki and Avangard-264 had the highest yields under non-stress conditions. Vega and Chirpan-539 varieties had a low yield potential and showed a high stress tolerance to drought.

Keywords: *cotton, drought tolerance index, moisture stress.*

INTRODUCTION

Cotton (*G. hirsutum L.*) is one of the most important fiber crops, which is of great economic and social importance. Despite the fact that it is a relatively drought-resistant crop and shows high tolerance to drought, insufficient soil moisture adversely affects the normal seed emergence, plant growth, development, yield and fiber quality (Hearn, 1979). The water balance deficit during the flowering-ballformation period is critical for cotton. In experiments with cotton grown under optimal and hydropower conditions, Karademir et al. (2011) found that water stress caused a decrease in fiber yield by 49.4%. The technological fiber properties were negatively affected, too.

Climatically, Bulgaria falls into the zone of unsustainable humidification (Sabeva, 1968). Cotton-producing areas are characterized by well-expressed drought in July-August, because of which the yields are below the genetic potential. Changes in the global climate, with an increase in the average air temperature and a decrease in rainfall (Aleksandrov, 2002), are also found in Bulgaria. The moisture losses from evapotranspiration has been steadily increasing and this trend will continue in the coming decades of this century (Aleksandrov, 2002), which is a prerequisite for efforts to adapt the agricultural production to the conditions of the constantly-changing climate.

The creation of high-yielding varieties to realize their yield potential, esspecially in drought conditions is an extremely difficult task for breeders (Mustatea et al., 2003; Richards et al., 2002). Susceptibility of plant to drought is often measured as a function of yield reduction in water stress (Blum, 1988), referred to yield potential values (Ramirez & Kelly, 1998). Drought indices, based on plant production losses under dry and normal conditions, are used for the screening of drought resistant genotypes (Mitra, 2001). Separate selection criteria evaluate genotypes, based on the results obtained under stress and non-stress conditions. Rosielle & Hamblin (1981) defined the stress tolerance index (TOL) as a difference in yield under irrigated and non-irrigated conditions and average productivity (MP), as the mean value of yield in stress and non-stress conditions.

Geometric mean productivity (GMP) is often used by breeders who are interested in relative productivity, as water stress varies in field conditions over the years (Fernandez, 1992). Fisher and Maurer (1978) recommend the stress susceptibility index (SSI) to measure yield stability, and this index captures changes in potential and real yield in a variable environment. The stress tolerance index (STI) is a useful tool for identifying high yielding genotypes that also have a high stress-tolerance potential (Fernandez, 1992).

MATERIAL AND METHODS

In this research 13 cotton varieties – Chirpan-539, Avangard-264, Perla, Natalia, Darmi, Colorit, Vega, Dorina, Nelina, Rumi, Helius, Boyana and Viki, created in the Field Crops Institute – Chirpan, Bulgaria were included. The trial was carried out in 2013 and 2016, in the experimental field of the Institute, on pellic vertisols (FAO), set up by randomized block design in four replications and harvesting plot of 20 m^2. Drought resistance indices were calculated using the following relationships:

Mean productivity	$MP = (Ys+Yp)/2$	(Rosielle and Hamblin, 1981)
Geometric mean productivity	$GMP = \sqrt{(Ys \times Yp)}$	(Fernandez, 1992)
Tolerance index	$TOL = Yp - Ys$	(Rosielle and Hamblin, 1981)
Stress susceptibility index	$SSI = \frac{1-\left(Ys/Yp\right)}{1-\left(\bar{Y}s/\bar{Y}p\right)}$	(Fischer and Maurer, 1978)
Stress tolerance index	$STI = \frac{Ys \times Yp}{\bar{Y}p^2}$	(Fernandez, 1992)
Harmonic mean productivity	$HMP = \frac{2(Ys)(Yp)}{(Ys+Yp)}$	(Kristin et al., 1997)

Where:
Yp – yield of genotype under non-stress condition;
Ys – yield of genotype under stress conditions;
$\bar{Y}p$ – potential yield of all genotypes in non-stress conditions;
$\bar{Y}s$ – potential yield of all genotypes in stress conditions;

RESULTS AND DISCUSSION

The years of the investigation as regards weather conditions, were characterized as follows: 2013 was considered agro-meteorologically favorable for the growth and development of cotton; in 2016 the amount of rainfall in June and July was by 77% and 92%, respectively lower than the average of many year values, while the temperature sum was higher by 9-10% (Fig. 1).

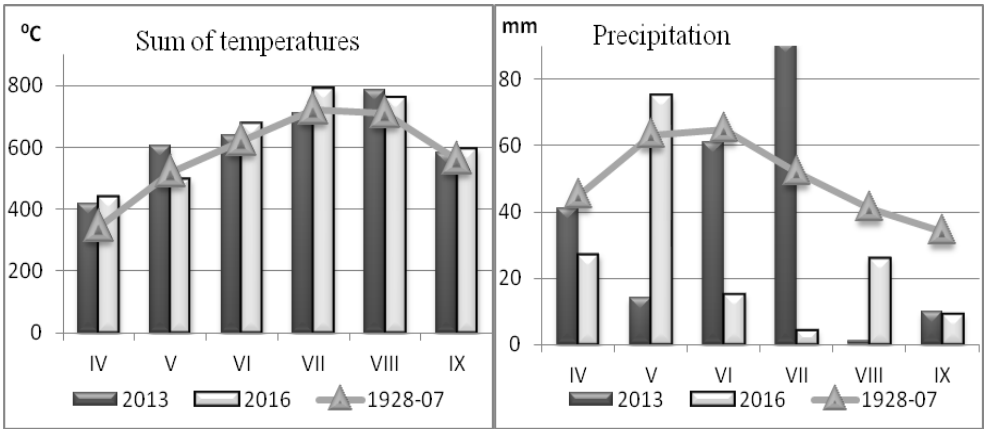

Figure 1. Meteorological conditions during the cotton growing season in 2013 and 2016 and average for the period 1928-2007

To calculate drought indexes we used the data for the total seed cotton yield obtained in 2013 and 2016 (Table 1), taking the yield reported in 2013 for potential yield - Yp, and the yield reported in 2016 marked as Ys - yield under stress conditions. The highest potential yield was recorded for Viki and Avangard-264 varieties, respectively 239.6 kg/da and 236.0 kg/da (10 da = 1 ha). The lowest potential yield was observed for Vega (176.2 kg/da) and Chirpan-539 (187.6 kg/da), which were defined as genotypes with low potential yield. In the dry 2016, the average seed cotton yield for all varieties was by 36% lower than the average potential yield. Highest yield under stress conditions (Ys) was achieved with Vega and Nelina varieties (155.0 kg/da and 148.7 kg/da). The Colorit and Dorina varieties had the lowest yields under stress conditions - 117.5 kg/da and 106.3 kg/da, respectively. The highest mean productivity (MP) values were calculated for Avangard-264 and Viki varieties. Generally, higher values of mean productivity are indicator for genotypes with high yield potential. MP shows a preference for higher yield potential and lower resistance to stress (Zangi, 2005). The lowest MP values were found for Colorit and Dorina varieties.

Geometric mean productivity (GMP) is less sensitive to larger differences between potential yield values and those of yield under stress conditions. Highest GMP values were recorded for Nelina and Avangard-264 varieties. The lowest values for GMP were found for Colorit and Dorina varieties, which again appeared to be the most sensitive to water stress. Varieties with hight HMP values were preferred under stress conditions (Farshadfar and Javadinia, 2011). The highest harmonic mean values were calculated for Nelina and Avangard-264 varieties, and the lowest - for Colorit and Dorina. Varieties having high values of the stress tolerance index (STI) possessed significant yield potential and substantial stress tolerance (Rosielle and Hamblin, 1981; Rajmani, A.1994). The highest values for STI were calculated for Nelina, Perla and Avangard-264 varieties. The variety Dorina was the most sensitive to this indicator. Stress tolerance (TOL) was calculated as a difference in yield under non-stress (Yp) and stress (Ys) conditions. Higher TOL values showed greater stress sensitivity and that's why genotypes with low values of this indicator were preferred (Zangi, 2005). The lowest TOL indexes were found for Vega and Chirpan-539 varieties. According to Zangi (2005), genotypes selected on the base of TOL will have a low yield potential and will realize high yields under stress conditions. Higher TOL values suggested greater losses under unfavorable conditions and a higher sensitivity to drought. In our investigation Viki and Dorina varieties had the highest values for this index.

Low values of the stress sensitivity index (SSI) were a prerequisite for higher stress tolerance (Zangi, 2005). According to a number of authors, when SSI values are less than 1, these varieties can be defined as drought-resistant (Ramirez and Kelly, 1998). The varieties with the lowest SSI values, i.e. having high stress tolerance were Vega and Chirpan-539. It should be noted that for Natalia, Darmi, Nelina, Rumi and Boyana varieties the SSI values were smaller than one, too. The highest values of the stress sensitivity index were recorded for Dorina and Viky varieties. By this indicator these varieties exhibited the highest sensitivity.

Table 1. Indices for assessing of drought tolerance of 13 cotton varieties on the basis of yield (2016) under stress and non-stress (2013) conditions.

	Cultivar	Yp	Ys	MP	GMP	HMP	STI	TOL	SSI
1	Chirpan-539	187,6	142,4	165,0	163,4	161,9	1,5	**45,2**	**0,662**
2	Avangard-264	**236,0**	136,2	**186,1**	**179,3**	**172,7**	**1,8**	99,8	1,161
3	Perla	231,1	137,4	184,3	178,2	172,4	**1,8**	93,7	1,113
4	Natalia	198,8	138,8	168,8	166,1	163,5	1,5	60,0	0,829
5	Darmi	206,3	132,4	169,4	165,3	161,3	1,5	73,9	0,984
6	Colorit	211,1	117,5	164,3	157,5	150,9	1,4	93,7	1,218
7	Vega	176,2	**155,0**	165,6	165,3	164,9	1,5	**21,2**	**0,330**
8	Dorina	208,5	106,3	157,4	148,8	140,8	1,2	102,3	1,347
9	Nelina	217,1	**148,7**	182,9	**179,7**	**176,5**	**1,8**	68,4	0,866
10	Rumi	213,6	144,0	178,8	175,4	172,0	1,7	69,7	0,896
11	Helius	224,8	129,8	177,3	170,8	164,6	1,6	95,0	1,161
12	Boyana	198,8	130,1	164,4	160,8	157,2	1,4	68,7	0,950
13	Viki	**239,6**	130,2	**184,9**	176,6	168,7	1,7	109,4	1,254

Table 2. Correlations between seed cotton yield and drought tolerance indices

	Yp	**Ys**	**MP**	**GMP**	**HMP**	**STI**	**TOL**	**SSI**
Yp	1,00							
Ys	-0,28	1,00						
MP	0,78***	0,39***	1,00***					
GMP	0,58***	0,62***	0,96***	1,00***				
HMP	0,37***	0,79***	0,87***	0,97***	1,00***			
STI	0,60***	0,60***	0,97***	1,00***	0,96***	1,00		
TOL	0,88***	-0,71***	0,37***	0,12***	-0,13***	0,14	1,00***	
SSI	0,78***	-0,82***	0,21***	-0,05***	-0,29***	-0,03	0,98***	1,00

* = 0.05, **= 0.01, ***= 0.001

Based on the performed correlation analysis, it was found that the relation between Yp and Ys was negative (Table 2) i.e. if the selection of genotypes is performed under optimum conditions, high yields would only be achieved under non-stress conditions. Tolerance to stress and stress sensitivity index were in a positive correlation and significant on a very high probability level.

Positive and significant correlation was found between SSI and Yp, while between SSI and Ys it was negative. This gives us reason to believe that varieties selected under this criterion will have a high stress tolerance and will produce high yields under unfavorable conditions, but under low stress conditions will have a low yield potential.

CONCLUSIONS

The varieties Viki and Avangard-264 produced the highest yields under non-stress conditions. The varieties Vega and Chirpan-539 had low potential yields and showed a high stress tolerance to drought. On the GMP, HMP, STI the varieties Nelina and Avangard-264 had the best performance, while the varieties Colorit and Dorina showed the highest sensitivity.

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OPTIMIZING WATER PRODUCTIVITY, YIELD AND QUALITY OF GRAPEFRUIT IRRIGATED BY BUBBLER AND SURFACE IRRIGATION UNDER KHARTOUM STATE SUDAN CONDITIONS

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ABSTRACT

The experiment was carried out in the private orchards at Tayba Alhasnab area of south Khartoum State, in Sudan during 2012 and 2013 to evaluate the water productivity, yield and quality of foster grapefruit irrigated by bubbler and surface irrigation system. Irrigation interval was 5 days in bubbler irrigation system and every 7 to12 days in surface irrigation system depending on the prevailing weather conditions. The results revealed that higher yield and number of fruits was obtained on bubbler irrigation system compared to surface irrigation system. Moreover, bubbler irrigation system increased the total yield of foster grapefruit by 28% and 25%, respectively as compared to surface irrigation system. Applying irrigated water under bubbler irrigation system improved the quality parameters of foster grapefruit such as fruit diameter recorded significant differences on bubbler irrigation system compared with surface irrigation system in both years, fruit weight and peel thickness recorded significant differences ($P \leq 0.001$) between bubbler irrigation system and surface irrigation system on finger weight, but on differences in peel thickness in both years, total soluble solids of foster grapefruit irrigated by bubbler irrigation system were significantly higher ($P \leq 0.001$) compared with surface irrigation system in both years.

However, bubbler irrigation system saved irrigation water by 68% and 71% and had highest water productivity (2.9 and 2.7 kg/m^3) compared to surface irrigation system (0.67 and 0.68 kg/m^3). Also highest marginal rate of return was obtained with bubbler irrigation system compared to surface irrigation.

Keywords: *grapefruit, Bubbler irrigation, surface irrigation, water productivity, crop evapotranspiration.*

INTRODUCTION

Citrus is an important cash crop in the Sudan. It is one of the major sources of human diet due to its high nutritive value, especially vitamin C (Bedri, 1984). Its cultivation is native to tropical and subtropical regions. Total area of citrus in the Sudan is estimated as 45650 ha (National Horticulture Administration, 2013).

Irrigation is one of the most important factors in the improvement of yield and quality of citrus. Poor irrigation and water stress significantly depress root elongation and reduce fruit number, size and weight (Saeed *et al*., 1990). Bubbler irrigation is a combination of surface and drip irrigation that needs a small basin because the discharge is too high, 50 to 225 liters per hour, to infiltrate. It is usually used for orchard and big trees (Ismail, 2002). Ibrahim *et al*. (2012) reported that bubbler irrigation gave the highest mean values of growth parameters on date palm, while the basin irrigation gave the lowest values. On the other hands, Amiri*et al*. (2007) investigated the response of date palm (cultivar Zahdi) under three irrigation systems: basin, bubbler and sprinkler. They found that the maximum vegetative growth was obtained on bubbler irrigation followed by basin and sprinkler irrigation. The use of modern irrigation systems became essential due to the high demand for water especially in arid and semiarid regions. Modern irrigation systems have some advantages over conventional ones with respect to improved fruit quality, lower labour costs and economic use of irrigation water (Brown, 1999). Research work on proper water managements on fruit trees is very little in the Sudan. Hence, studies on design, implementation and management of water application methods on citrus and other fruits crops are highly needed. The objective of this research work was to optimize the water productivity, yield and quality of foster grapefruit irrigated by bubbler compared to surface irrigation under Khartoum State (Sudan) conditions.

MATERIALS AND METHODS

The experiment was established in a private orchard at Tayba Alhasnab area, Khartoum, Sudan during 2012 and 2013 to evaluate performance of the bubbler irrigation system in comparison with surface irrigation on grapefruit, variety foster. The climate is semi desert with low humidity and daily mean maximum temperature of 40^{o}C in summer and 30^{o}C in winter. Summary of the meteorological data is shown in table (1).

Table 1. Monthly average climatic parameters

Month	Temperature °C		Humidity%	Wind speed Kmday^{-1}	Sunshine hrs
	Max	Min			
January	30.8	15.6	33.9	345.6	9.71
February	33	17	25	388.8	10.67
March	36.8	20.5	18.2	388.8	10.49
April	40.1	23.6	16	345.6	10.86
May	41.9	27.1	19.2	311	10.42
June	41.3	27.3	26.1	345.6	9.78
July	38.4	25.9	46.7	345.6	9.03
August	37.3	25.3	54.8	345.6	8.66
September	39.1	26	42.7	311	9.19
October	39.3	25.5	32	267.8	9.19
November	35.2	21	29.9	345.6	9.67
December	31.8	17.1	35.1	345.6	9.9
Average	**37.1**	**22.7**	**31.6**	**340.6**	**9.8**

The orchard was established in 2008 and trees were transplanted in 1.5 m^2 holes which were filled by silty loam soil with high silt content (68%) and low clay (26.7%). The plot of each irrigation method was containing 3 trees planted at spacing of 7x7 m. The plot size of surface irrigation was 21m length and 4m wide and consisted of 3 parts each one was 4x7m. Treatments were replicated 5 times. Bubblers distributors were installed in the laterals at distances of 7 m apart and one distributor per tree with discharge of 100 lha^{-1}.

The daily meteorological data were recorded during the study period to compute the daily reference evapotranspiration by REF-ET software version 2.0 Allen (2000).

The crop water requirement was calculated according to Allen *et al*. (1998) using the following formula:

$ET_c = ET_o \times K_c$…… (1)

where ET_c is crop evapotranspiration (mm/day), K_c is crop coefficient(was taken from FAO-56 documentation Table 12 (Allen, *et al*., 1998))) ET_o is reference crop evapotranspiration (mmday^{-1}).

The crop water requirement (CWR) for every 5 days in bubbler irrigation was calculated using the following equation:

$CWR = ET_c \times 5$ …… (2)

The overalllosses in discharge at the gross depth (dg) were calculated using the following equation:

$$dg = \frac{ET_c\ mm}{EU} \text{……..} \quad (3)$$

Where EU= emission uniformity (90%).

Volume of water for bubbler irrigation was applied in liter/plant using the following equation:

$V = A \times AW \times dg$…… (4)

Where V -volume of water in liter per plant, A - plant area (row spacing m × plant spacing m), Aw% - wetted area (0.3) and dg - net depth required, mm.

Time of irrigation was calculated using the following equation:

$$\text{Time of irrigation} = \frac{\text{Volume of water to be applied (liter)}}{\text{Bubbler discharge rate } (lha^{-1})} \quad \ldots\ldots\ldots\ldots \quad (5)$$

Irrigation was applied every 5 days in the bubbler irrigation system while for the surface irrigation it was applied every 7 to 12 days depending on the prevailing weather conditions. The recommended dose of fertilizer was added by fertigation in bubbler irrigation and applied manually on the surface irrigation. The special horticultural practices were carried out as recommended.

Yield per tree was recorded in tons/fed. Ten fruits were collected randomly for determination of quality such as fruit diameter (cm), fruit weight (g), peel thickness (cm) and total soluble solids (TSS %).

Flowmeter was used for measurement of total water applied in both bubbler and surface irrigation systems.

Water productivity (WP) was calculated using the following equation:

$$WP = Yield/TWA \quad \ldots\ldots\ldots\ldots \quad (6)$$

Where Yield in kg/fed and TWA is total water applied in m^3/fed.

Marginal rate of return was analyzed according to CIMMYT (1988) and used to evaluate the profitability of the bubbler irrigation system in comparison with surface irrigation based on the field information and data collected.

GraphPad statistical package (GraphPad Software, 2014) was utilized for analysis of data and t- test was used for means separation.

RESULTS AND DISCUSSION

Number of fruits per tree and total yield

There were very highly significant differences in the number of fruits per tree and total yield (t/ha) of the foster grapefruit irrigated by bubbler irrigation than those irrigated by surface irrigation in both years (Table 1). The highest yield ranged from 18.33 to 20.47 t/ha under bubbler irrigation system in the two years compared to 14.33 and 16.42 t/ha under surface irrigation. For the bubbler irrigation system, the percentage increase in total yield was equal to 22% and 19% in season one and two, respectively, as compared to the surface irrigation (Table 1). The result revealed that higher yield was produced under bubbler irrigation compared to surface irrigation. Similar results were reported by Hussien *et al*. (2013) on orange who found that trees irrigated by bubbler yielded greater amounts of fruits than those irrigated by surface for two seasons.

Table 2. Number of fruits per tree and yield (ton/ha) of foster grapefruit irrigated by bubbler and surface irrigation.

Irrigation treatments	Number of fruits/tree		Yield ton/han	
	Year 2012	Year 2013	Year 2012	Year 2013
Bubbler irrigation system	167	175	18.33	20.47
Surface irrigation system	137	148	14.33	16.42
$SE^{\pm}$	4.5	5.4	***	***
Significance level	***	***	***	***

*** Significance at P≤ 0.001.

Fruit diameter

The results on fruit diameter showed significant differences under bubbler irrigation system compared with surface irrigation in both years (Table 3). These results are in conformity with those obtained byShashidhara*et al*. (2007).

Table 3. Fruit diameter of foster grapefruit irrigated by bubbler and surface irrigation.

Irrigation treatments	Fruit diameter (cm)	
	Year 2012	Year 2013
Bubbler irrigation system	9.2	10.6
Surface irrigation system	8.9	10.1
$SE^{\pm}$	0.124	0.104
Significance level	*	**

* and ** Significance at P≤ 0.05 and P≤ 0.01, respectively.

Fruit weight and peel thickness

The results showed significant differences (P≤ 0.001) between bubbler irrigation and surface irrigation on finger weight, but on differences in peel thickness in both years (Table 4). The highest fruit weight was obtained under bubbler irrigation system. These results are in agreement with those on orange trees (Hussien *et al*., 2013).

Table 4. Fruit weight and peel thickness of foster grapefruit irrigated by bubbler and surface irrigation.

Irrigation treatments	Fruit weight (g)		Peel thickness (cm)	
	Year 2012	Year 2013	Year 2012	Year 2013
Bubbler irrigation system	540	570	0.70	0.9
Surface irrigation system	510	545	0.65	0.8
$SE^{\pm}$	0.68	2.67	0.05	0.05
Significance level	***	***	NS	NS

*** and NS significance at P≤ 0.001 and not significant, respectively.

Total soluble solids

Total soluble solids of foster grapefruit irrigated by bubbler were significantly higher ($P \leq 0.001$) compared with surface irrigation in both years (Table 5). This result is in agreement with the findings of El-Gindy*et al*. (2000) who reported that bubbler irrigation system improved the quality of mango fruits compared with gated pipe irrigation system. The best total soluble solids were obtained when muskmelon was irrigated by drip irrigation compared to furrow irrigation system as reported by Bogle and Hartz (1986).

Tab. 5. Total soluble solids of foster grapefruit irrigated by bubbler and surface irrigation

Irrigation treatments	Total soluble solids (%)	
	Year 2012	Year 2013
Bubbler irrigation system	9.0	9.4
Surface irrigation system	8.6	9.0
$SE^{\pm}$	0.09	0.1
Significance level	***	***

***: indicated significance at $P \leq 0.001$.

Total water applied

The quantity of water applied to foster grapefruit was 6321 m^3/ha and 7586 m^3/ha under bubbler irrigation compared to 21429 m^3/ha and 24000 m^3/ha for surface irrigation in season one and two, respectively (Table 6). Therefore, the percentages of applied water saving by bubbler irrigation system were 71% and 68% for season one and two, respectively, as compared to surface irrigation. Similar results of irrigation water saving by bubbler irrigation system were reported by Hussien*et al*. (2013). They found that bubbler irrigation increased water utilization efficiency (59.4 %) compared to surface irrigation.

Table 6. Total water applied (m^3/ha) on foster grapefruit irrigated by bubbler and surface irrigation.

Month	Bubbler irrigation		Surface irrigation	
	2012	2013	2012	2013
Jan	402	483	1545	1712
Feb	505	605	1743	1950
Mar	605	726	1938	2183
Apr	643	771	2010	2269
May	636	764	1998	2255
Jun	638	767	2002	2262
Jul	517	621	1767	1979
Aug	467	560	1669	1860
Sep	507	610	1748	1955
Oct	495	595	1726	1929
Nov	490	588	1714	1917
Dec	412	495	1564	1736
Total	**6321**	**7586**	**21429**	**24000**

Water productivity

The highest water productivity (2.9 and 2.7 kg/m^3) was obtained on grapefruit irrigated by bubbler irrigation system compared to surface irrigation (0.67 and 0.68 kg/m^3) in both years (Fig.1). These results are in agreement with those reported by Khalifa (2012) and Khalifa *et al*. (2013). Morever, Hussien*et al*. (2013) stated that the maximum water productivity was obtained on orange irrigated by bubbler irrigation compared to surface irrigation.

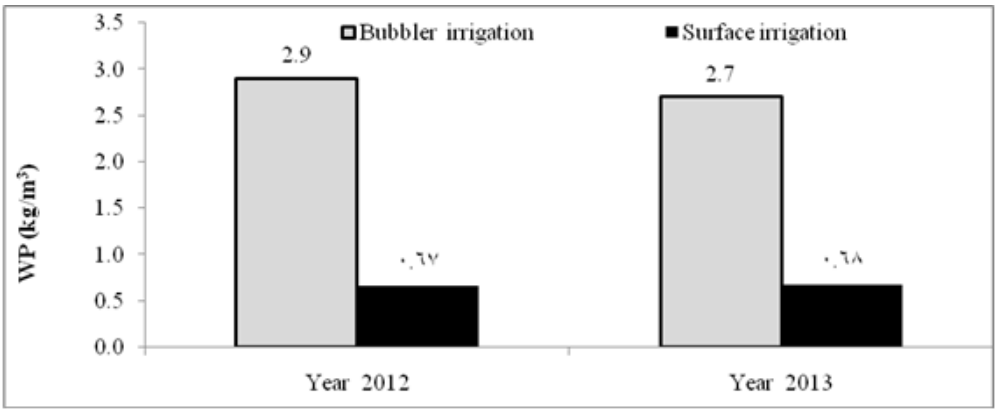

Figure 1. Water productivity (WP) (kg/m3) of foster grapefruit irrigated by bubbler and surface irrigation.

Economic analysis

Economic analysis showed that the bubbler irrigation had the highest net return of (38663.8 SDG/ha) and marginal rate of return 18%, which indicates that every monetary unit (SDG) invested in bubbler irrigation system would be returned back plus additional amount of 0.18 SDG (Table 7). These results are in agreement with those reported by Khalifa (2012) and Khalifa*et al*. (2013). Moreover, Khalifa*et al*. (2014) who found that the highest net returns and benefit cost ratio were obtained in the drip irrigation and the lowest were obtained in the surface irrigation.

Table 7. Marginal analysis of foster grapefruit irrigated by bubbler and surface irrigation.

Treatments	Variable cost (SDG/ha	Marginal cost (SDG/fed)	Net return (SDG/fed)	Marginal net return (SDG/fed)	Marginal rate of return (%)
Surface irrigation	2845.0		29405.0		
Bubbler irrigation	2336.5	-508.5	38663.5	9258.5	18.2

CONCLUSION

- The highest yield and yield components of foster grapefruit were obtained by bubbler irrigation system.
- Bubbler irrigation system increased the total yield of foster grape fruit by 19% and 22%, and saved irrigation water by 68% and 71%, respectively, as compared to surface irrigation.
- The highest irrigation water productivity (2.9 and 2.7 kg/m^3) was obtained on bubbler irrigation and the lowest (0.67 and 0.68 kg/m^3) on surface irrigation.
- The highest marginal rate of return was obtained on bubbler irrigation system.

RECOMMENDATION

Based on the above findings, were commend the use of bubbler irrigation for foster grapefruit production under Khartoum state conditions.

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APPLICATION OF MULTI CRITERIA DECISION TECHNIQUE TO DETERMINE THE BEST CHICKPEA CULTIVARS WITH HIGH ANTIOXIDANT POTENTIAL

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ABSTRACT

In this study, technique for order preference by similarity to ideal solution (TOPSIS) analysis, which is multi criteria decision making method, was firstly applied to rank the most suitable cultivars among 12 registered chickpeas for high antioxidant potentials. Registered chickpea cultivars were grown in trial fields of state research institute in 2015, Adana, Turkey. The cultivars were analyzed for the criteria such as their water-soluble protein content (WSPC), total phenolic content (TPC), free radical scavenging activity (FRSA) and iron chelating activity (ICA) which were related to their antioxidant potentials. However, depending on each criterion, the ranking of the cultivars was completely different so that TOPSIS analysis was applied to the obtained data in six steps. Firstly, the decision matrix was constructed and then each criterion was weighted as respectively 0.40, 0.30, 0.20, 0.10 for FRSA, TPC, ICA, and WSPC by the researchers. After the weighted normalized decision matrix was constructed, the positive ideal and negative ideal solutions were determined. Then the separation measures for each alternative were calculated (Si* and Si- for the separation from positive and negative ideal alternative, respectively). Finally, the relative closeness to ideal solution was calculated (Ci*). The cultivar Seçkin with the highest Ci* value (0.776) was the first rank and followed by Aydın, Azkan, and Çakır. This study showed the usefulness of TOPSIS analysis in the multi criteria decision making process when the presence of different parameters related to same property of sample set such as antioxidant potential of chickpea cultivars.

Keywords: *multi criteria decision technique, TOPSIS, chickpea, antioxidant potential.*

INTRODUCTION

Chickpea is an ancient crop which was domesticated in southeastern Turkey and spread to other parts of the world dominantly in tropical, subtropical, and temperate regions about 45 countries (Atalay and Babaoglu, 2012; Ozkilinc et al., 2011; Özer et al., 2010). The major producer of chickpea is India with 9.9 M tones production

and followed by Australia, Myanmar, Ethiopia, and Turkey (FAO, 2014). It is the third most important pulse crop after dry bean and pea and consumed as flour, canned, roasted, boiled, fermented, fried steamed, or eaten as snack food (Bibi et al., 2007; Coşkuner and Karababa, 2004; Çelik et al., 2016; Özer et al., 2010). Chickpea is good source of protein (15-29 %), carbohydrate (41-47 %), dietary fiber (14-18 %), minerals and vitamins (Bibi et al., 2007; Çelik et al., 2016; Mafakheri et al., 2011; Özer et al., 2010; Torutaeva et al., 2014). However, chickpea is cultivated mostly in arid or semiarid Mediterranean environment of West Asia and North Africa, it has been adopted in North America, western Canada, Australia, New Zealand, and Central Europe due to its high nitrogen utilization efficiency and high protein yield under drought conditions (Neugschwandtner et al., 2015; Oweis et al., 2004; Sadras and Dreccer, 2015; Siddique et al., 2012). Biotic factor such as ascochyta blights and abiotic factors such as cold, drought, salinity, and micronutrient deficiencies lower the chickpea yield (Atalay and Babaoglu, 2012; Mafakheri et al., 2011; Siddique et al., 2012). Due to its low genetic polymorphism and limited genetic variation, it is relatively difficult to develop resistant varieties through classical breeding methods to mentioned stress factors (Atalay and Babaoglu, 2012). The studies showed that winter sown chickpea is more tolerant to biotic and abiotic stresses than spring or summer chickpea (Oweis et al., 2004).

Chickpea seeds have many bioactive and functional properties that are important for health-related products and other processed products. Chickpea proteins, hydrolysates, and peptides had considerable phenolic content with important antioxidant activities based on free radical scavenging activities and metal chelating abilities (Kou et al., 2013; Torres-Fuentes et al., 2015; Zhao et al., 2014). Chickpea proteins had also the potential to be used as functional plant based protein source because their functional properties were comparable with or superior than those of soy and animal origin proteins (Aydemir and Yemenicioglu, 2013). Many researchers were also reported the studies related to good functional properties of chickpea flours or proteins (Ghribi et al., 2015; Withana-Gamage et al., 2011; Xu et al., 2017). Processed chickpea products are also suitable as replacement of wheat flour or value-added ingredient for functional food productions such as mayonnaise, muffin batter, gluten-free spaghetti, salad dressing, etc (Alamri et al., 2013; Alu'datt et al., 2017; Alvarez et al., 2017; Demi et al., 2010; Flores-Silva et al., 2014; Ma et al., 2016). In addition to tolerance to stress factors and yield, the bioactive and functional properties of chickpea seed should be considered if value added products formation is aimed. In this study, a comprehensive evaluation of the antioxidant potentials of 12 registered chickpea cultivars, which were developed by breeding methods and grown in 2015, Adana, Turkey, was performed by using Technique for Order Performance by Similarity to Ideal Solution (TOPSIS) method which was one of the best and widely used multiple criteria decision making technique to choose the best alternative that had the shortest geometric distance from the positive ideal solution and the longest geometric distance from the negative ideal solution (Kou et al., 2015).

MATERIALS AND METHODS

Materials: 12 registered chickpea cultivars were kindly donated by Dr. Dürdane Mart from Eastern Mediterranean Agricultural Research Institute. *Preparation of chickpea extracts*: Chickpea seeds were firstly grounded to obtain chickpea flour and extracted in deionized water as 500 mg chickpea flour in 5 ml deionized water stirring overnight. Then the suspensions were centrifuged (15000×rcf, 25˚C, 30 min) and supernatant were collected and named as chickpea extract. Total moisture content of chickpea flours was measured with moisture analyzer (Ohaus MB 45, Switzerland).

Antioxidant potentials of chickpea extracts: Total phenolic content (TPC) of extracts were spectrophotometrically determined by using Folin-Ciocalteu's reagent. The absorbances were measured at 765 nm as 3 replicates and results were expressed as mg gallic acid/g dry weight basis (dwb). Free radical scavenging activity (FRSA) of extracts were determined spectrophotometrically at 734 nm. The inhibition of ABTS free radical solution by antioxidants in cickpea extract was measured as three replicates and the results were expressed as μmol Trolox/g dwb. Iron chelating activity (ICA) of extracts were spectrophotometrically determined at 562 nm by measuring the binding of Fe^{2+} (formed by reaction of $FeCl_2.4H_2O$ and ferrozine) with the groups in chickpea extract as three replicate sand the results were expressed as μmol EDTA/g dwb (Aydemir et al., 2014). Water soluble protein content (WSPC) of chickpea extracts were determined by Lowry method as three replicates and the results were expressed as mg casein / g dwb.

TOPSIS comprehensive evaluation method: TOPSIS method was developed to overcome rank problems in multiple criteria decision making (Hwang and Yoon, 1981). The details of the steps of TOPSIS method were expressed as follow: In step 1, decision matrix was established. In step 2, normalized decision matrix was calculated. In step 3, the weighted normalized decision matrix was calculated. In step 4, positive and negative ideal solutions were determined. In step 5, the distance of each alternative from positive and negative ideal solution was determined. In step 6, the closeness coefficient of each alternative was determined (Kou et al., 2015).

RESULTS AND DISCUSSIONS

*Antioxidant potential of chickpea extracts***:** Phenolic compounds were related to antioxidant activity of plant extracts due to their free radical scavenging activities and metal chelating activities. In this study, water extracts of registered chickpea flours had significantly varied TPC between 1471 - 2152 μg gallic acid / g (Table 1). While their FRSA changed from 1544 to 2514 μmol Trolox / g, ICA values were between 1.1 and 17.7 μmol EDTA / g. Their WSPC was similar to each other and varied from 48 to 77 mg casein / g.

Table 1. Antioxidant parameters of registered chickpea cultivars (dry weight basis)

Registered Cultivar	FRSA (µmol Trolox/g)	ICA (µmol EDTA/g)	TPC (µg GA/g)	WSPC (mg casein/g)
Aksu	15.44±0.8	4.5±0.7	1474±73	57±1.4
Arda	23.08±0.4	10.8±1.0	2060±14	48±2.8
Aydin	21.18±0.1	15.9±2.0	1782±71	68±5.0
Azkan	18.97±0.9	17.7±0.3	1559±521	52±6.4
Çakir	25.14±1.7	14.4±0.7	2152±20	62±4.8
Diyar	20.96±1.9	4.8±2.5	1995±114	65±7.1
Gülümser	21.04±1.7	1.1±0.3	2080±72	55±4.6
Hasanbey	21.83±0.9	8.7±0.4	1993±136	51±2.9
Ilgaz	19.43±0.7	8.2±1.1	1785±161	68±5.9
İnci	20.45±1.5	3.1±1.4	1983±128	61±4.2
İzmir	18.45±0.5	10.3±0.6	1655±49	66±4.7
Seçkin	23.15±1.5	14.3±0.8	1984±78	77±5.5

TOPSIS method: When all the measured parameters were evaluated it is hard to decide which cultivar is the best in terms of antioxidant potential. Because in each criterion, the ranking is different. Therefore, in order to rank the cultivars that fits more to the desired purpose, TOPSIS was applied. In TOPSIS method, the researchers joined the study assigned different weights to the measured parameters subjectively. The attributed weights were 0.40 for FRSA, 0.30 for TPC, 0.20 for ICA, and 0.10 for WSPC. After the normalized decision matrix was established, the weighted normalized matrices were established (Table 2).

The positive and negative ideal solutions of antioxidant parameters were determined by taking the maximum and minimum values for each criterion:

The positive ideal solutions = {0.133, 0.095, 0.099, 0.036}

The negative ideal solutions = {0.088, 0.006, 0.068, 0.023}

Then the distances from positive and negative ideal solution were calculated and the relative closeness of each value was calculated. The final rankings according to distances and closeness coefficient of each sample was shown in Table 3.

Table 2. The weighted normalized matrices for registered chickpea cultivars

Registered Cultivar	FRSA	ICA	TPC	WSPC
Aksu	0.0883	0.0243	0.0676	0.0267
Arda	0.1326	0.0576	0.0945	0.0227
Aydin	0.1085	0.0850	0.0818	0.0320
Azkan	0.1138	0.0950	0.0716	0.0245
Çakir	0.1071	0.0769	0.0988	0.0292
Diyar	0.1031	0.0258	0.0916	0.0305
Gülümser	0.1114	0.0060	0.0955	0.0258
Hasanbey	0.1244	0.0466	0.0915	0.0241
Ilgaz	0.1145	0.0437	0.0819	0.0320
İnci	0.1284	0.0164	0.0910	0.0285
İzmir	0.1261	0.0550	0.0760	0.0309
Seçkin	0.1200	0.0765	0.0911	0.0363

Table 3. The ranking of registered chickpea cultivars evaluated by the TOPSIS method.

Registered Cultivar	S*	S^-	C*	Rank
Aksu	0.0895	0.0188	0.1732	12
Arda	0.0401	0.0731	0.6458	5
Aydin	0.0314	0.0833	0.7260	2
Azkan	0.0351	0.0927	0.7253	3
Çakir	0.0320	0.0800	0.7141	4
Diyar	0.0758	0.0353	0.3174	10
Gülümser	0.0922	0.0363	0.2826	11
Hasanbey	0.0511	0.0593	0.5371	7
Ilgaz	0.0570	0.0490	0.4622	8
İnci	0.0795	0.0479	0.3759	9
İzmir	0.0468	0.0630	0.5734	6
Seçkin	0.0236	0.0820	0.7765	1

S^*: The distance of each alternative from positive ideal solution, **S^- :** The distance of each alternative from negative ideal solution, **C*:** The closeness coefficient of each alternative

According to the analysis, Seçkin cultivar had the 1st rank as having the highest antioxidant potential and followed by Aydın, Azkan, and Çakır. This study was the first study that TOPSIS method was applied to cultivar selection based on defined property. In the literature of food science and agriculture, 21 studies were reported

comprising TOPSIS evaluation to choose the best alternative according to Web of Science.

CONCLUSIONS

Chickpea is one of the most important crop in the world due to being cheap and having high nutrition quality especially good protein content. However, it is mostly cultivated in arid and semi-arid area, the adaptation studies have been conducted for Central Europe, North America and Canada. Chickpea products such as flours, proteins, hydrolysates are suitable for functional food additive in processed foods with their good technological properties and considerable bioactive properties. This study screened the antioxidant potentials of registered chickpea cultivars based on different property measurements and the problem about the choosing the best cultivars was tackled by TOPSIS method for the first time in the literature. It was also seen that TOPSIS method has the potential to choose the best cultivar in multi criteria decision problems in this area.

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RAPD ANALYSIS OF GENETIC VARIATION IN NATURAL POPULATIONS OF AEGILOPS SP. FROM SOUTH ADRIATIC

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ABSTRACT

New challenges that food production is facing, requires novel approach in agricultural strategy. The scissors of growing demand for food and the limits of the Earth's resources are forcing plant breeders to run for the new borders, utilizing all the available genetic variation in order to create fruitful and economically sound cultivars. *Aegilops* sp. (*Poaceae*) is a potential source of genetic variation for wheat improvement. RAPD marker analysis was used in order to distinguish and evaluate different genotypes of *Aegilops* sp. population samples from the collection gathered during few years' expeditions in South Adriatic, along the coastal, littoral and the inland parts of Montenegro. Ten randomly amplified polymorphic DNA markers (RAPDs) were tested: OPA-05, OPA-08, OPB-06, OPA-02, OPA-07, OPA-25, OPB-07, OPB-18, OPC-06, OPC-10 to examine genetic structuring on 18 samples of 6 populations of different *Aegilops* sp. According to global AMOVA, 75% of total gene diversity was attributable mostly to diversity within population (Φ_{PT} =0.205 p=0.001), indicating that the groups of studied goat grass populations were seemingly to differing genetically. In contrast, 25% of the variation came from variation among populations. According to PCoA, the distribution of 18 goat grass accessions by Principal Coordinate Analysis shows 3 distinct groups. PCo axis 1, PCo axis 2, and PCo axis 3 account for 20.8%, 18.2% and 14.1% of the variation, respectively. The results showed that RAPD markers could be a convenient tool for investigating genetic variation and for detecting genetic structuring of populations. Genetic variability formed under natural selection was entrenched.

Keywords: *Aegilops, goat grass, RAPD, population, genetic diversity.*

INTRODUCTION

Humanity is facing the challenges of overpopulation, environmental erosion, and climate changes. Answering these challenges will be crucial to affect the very survival of the human species on Earth. As well as miners, we do not have the right to a mistake. The clock is ticking. The exact number of people on Earth on 6th of July, 2017, at 14:35 hours was 7,516.477.305, Land lost due to the erosion this year

up to this moment was 3,578.485 hectares. Ten minutes later human population rose to 7,516.478.889, and land erosion grew to 3,578.619 ha (worldometers, 2017). Humane species entered 20th Century with 1.7 billion souls, and left it with 6.1 billion. By the 2050, that number is going be 9.1 billion. Moreover, the ongoing processes are urbanization, environmental erosion including arable land degradation and climate change (Bongaarts, 2009; Myers *et al*., 2017). The previous century is going to be remembered by Green Revolution, the second agricultural revolution after the Neolithic one. Green Revolution enhanced average yield of the most important cultivars 3 to 5 times, however it has made primary food production highly dependent on fossil fuel, chemical plant protection, monoculture or nearly monoculture production organization. Consequences of agricultural industrialization are soil degradation, salinization mostly, environmental pollution, biodiversity loss, human diet narrowing, monoculture in food production. Furthermore, narrowing of genetic variability, genetic erosion, and species loss means the erosion of knowledge, as well. Loss of biological resources implies the loss of specific knowledge about them (Mooney, 2001). Agriculture has to meet the challenge in producing more food, adapt to climate change and adopt more sustainable and efficient mode of production. To achieve these goals, a novel genetic variability from wild relatives, local populations, old instinctively selected populations and varieties and other available genetic resources has to be utilized. Available agrobiodiversity is to be screened for usable genes, in order to broaden genetic variability suitable to meeting the sustainable food production requirements. Wheat (*Triticum* sp.) has been used as a food for about 10,000 years right from the "Neolithic Agricultural Revolution". With the rise of civilization the wheat growing, as well as, breeding had been improving. In our times, wheat is counted among the 'big three' cereal crops, with over 600 million tons being harvested annually. However, in order to meet new selection criteria in modern breeding programs for realization of wheat ideotype suitable for contemporary and future agricultural production requirements, genetic variability is to be broadened. Since, goat grass (*Aegilops* sp.) played an important role in wheat evolution, this genus possess a number of desirable genes to broadening wheat genetic variability, to withstand biotic and abiotic stress growth conditions and to satisfy growing demands for food (Shewry, 2009). Collecting genetic resources, gene bank establishing, genetic variability studying, by phenotyping and genotyping, using protein and molecular markers, are required in order to recognize and isolate desirable gene variation (Petrović and Dimitrijević, 2005; Dimitrijević *et al.*, 2011). The aim of this article is to investigate genetic variation of *Aegilops* sp. samples collected in Montenegro.

MATERIAL AND METHODS

Samples of goat grass (*Aegilops* sp.) have been taken for genetic variability examination from an *ex situ* conservation gene bank consisting of 200 entries of landraces and wheat wild relatives (*Triticum* sp., *Hordeum* sp., *Aegilops* sp.), that had been established after six years of collecting expeditions, mainly in

Montenegro (Dimitrijević *et al*., 2011). *Aegilops* species classification is given after Kimber and Feldman (1987).

Table 1. Ten RAPD primers used for screening *Aegilops* sp. genotypes.

Primers	Sequence	Usability
OPA-02	5′-TGCCGAGCTG-3′	+
OPA-05	5′-AGGGGTCTTG-3′	-
OPA-07	5′-GAAACGGGTG-3′	+
OPA-08	5′-GTGACGTAGG-3′	-
OPA-25	5′-GACAGACAGA-3′	+
OPB-06	5′-TGCTCTGCCC-3′	-
OPB-07	5′-GGTGACGCAG-3′	+
OPB-18	5′-CCACAGCAGT-3′	+
OPC-06	5′-GAACGGACTC-3′	+
OPC-10	5′-TGTCTGGGTG-3′	+

A discontinuous genetic variation of *Aegilops* sp., was examined using ten Random Amplified Polymorphic DNA (RAPD) primers, where three did not yield any product (tab. 1). DNA extraction has been done using the method of Somma (2004). In order to test amplification profiles for polymorphism, readability and reproducibility, six decamer (10 nucleotides length) primers from ROTH®GmbH kits were tested. PCR was carried out in a 25-μL reaction volume containing 2.5 μL buffer; 0.2 mM of each dNTP; 0.5 μM of primer; 2 units of Taq polymerase (Fermentas) and 30 ng of DNA. Reactions were performed in Tpersonal PCR (Biometra) and Mastercycler ep gradient S (Eppendorf) thermocyclers with amplification profile: denaturation at 94°C for 4 min, followed by 40 cycles with 94°C for 2 min, 36°C for 1 min and 72°C for 2 min, with final elongation on 72°C for 10 min. PCR products were separated on 1.2% or 1.7% agarose gels containing 0.005% ethidium bromide and visualized under UV light. Each fragment amplified using RAPD primers was treated as binary unit character and scored "0" for absence and "1" for presence. Estimation of genetic variation was carried out by using the POPGENE and GenAlEx (Yeh and Boyle, 1997; Peakall and Smouse 2006, 2012, respectively).

RESULTS AND DISCUSSION

Variation of six goat grass (*Aegilops* sp.) populations was examined by RAPD molecular markers. Samples of these populations had been collected at three sites along the Adriatic coast, and, inland area of Montenegro around the town of Podgorica, during previous decade. All the tested samples of *Aegilops* sp., have a common U genome, and different ploidy level, and most of them, all except *Ae. kotschy* (SU), have genome M (tab. 2).

Table 2. *Aegilops* species, accession number, geographical location, elevation, ploidy level, genome constitution of goat grass samples examined in the experiment.

Accession label	Population	Genome	Ploidy level	Latitude (N)	Longitude (E)	Altitude (m)
L-16/03	*Aegilops biuncialis*	UM	4x	42.463322	18.505762	84.9
L-28A/02	*Aegilops kotschy*	SU	4x	42.389494	18.664502	102.3
L-44/01	*Aegilops columnaris*	UM	4x	42.229633	18.909559	95.6
L-8/01	*Aegilops triaristata*[*]	UM(X)	4x/6x	42.444066	19.242864	41.6
L-17/01	*Aegilops ovata*[**]	MU	4x	42.411666	19.339257	78.8
L-10/01	*Aegilops umbellulata*	U	2x	42.382146	19.316676	57.0

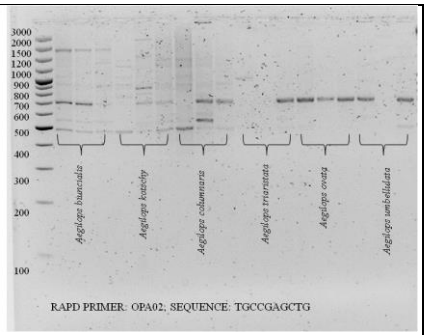
Remarks:
* A map of Montenegro shows exact collection site locations
[*] *Aegilops triaristata* (old name *Ae. recta*) apply to the hexaploid but they are indistinguishable from tetraploids of *Ae. neglecta* in the field. *Aegilops triaristata* is an illegitimate name. Currently used name is *Aegilops neglecta*.
[**] *Aegilops ovata*, which is an old name, is known by its current name *Aegilops geniculata*.
Source: http://herbarium.usu.edu/Triticeae/genomesaegilops.htm

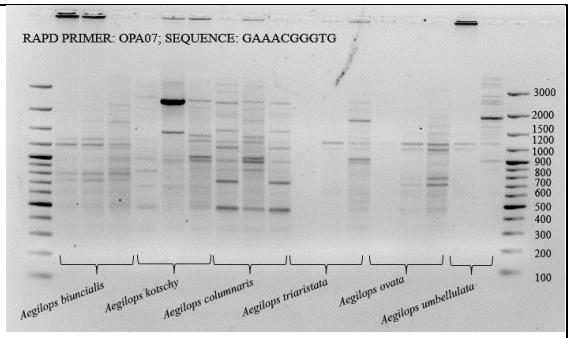
*Source: Google map.

Ae. umbellulata is considered as the main genetic source of genome U (Kihara and Yanashita, 1956; Kadosumi *et al*., 2005). According to the Kadosumi *et al*. (2005), U genome has at least three distinct sequences, and that could be the reason of different patterns obtained with the same RAPD primer for *Ae. umbellulata*. *Ae. comosa* (2x) is donor of genome M. A number of tetraploid *Aegilops* sp. obtained their genomes out of spontaneous hybridization of the diploids *Ae. comosa* (2n = 26= 14, MM) and *Ae. umbellulata* (2n = 26= 14, UU). However, genome M appeared to be even more polymorphic than U genome (Kihara and Yamashita, 1956; Molnár *et al*., 2013). Hence, a considerable variation that was denoted in products of RAPD markers in this study, even within products of the same primer, is in accordance to previously reported results. RAPD primers OPA02, OPA25, and particularly OPA07 that gave products in *Ae. umbellulata* are usable to mark

the presence of U genome in genetic constitution. A total of 61 bands were generated, ranging from 500 to 3000 bp. The high overall level of polymorphism was denoted. The highest number of polymorphic bands was obtained using primers OPA07, OPC 10, and OPA 02 (10), followed by OPB07 (9), and OPC06 (8), tab. 3.

Table 3. Random amplified polymorphic DNA profiles for six goatgrass species using RAPD primer OPA02 (on the left) and OPA07 (on the right). Below, number of RAPD markers generated by each one of the oligonucleotide primers that revealed polymorphism among examined goat grass samples. 1- unique band; 2- shared by two; 3- shared by three; 4- shared by four; 5- shared by five; 6- shared by six.





Species	*Aegilops biuncialis*							*Aegilops kotschy*							*Aegilops columnaris*							*Aegilops triaristata*							*Aegilops ovata*							*Aegilops umbellulata*						
Primers	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18
Molecular Size (bp)																																										
500	4							4			2			2	4		1	2	3	2	2					3	2						3			4						
600	3							2		2	2				3		2		3	2						3	2	2					2	3		2						
700	6			3			5	6					2	5	6		1	3	3		5	6				3		5	6				3	3		5	6					
800		4		3							3					4		3	3							3				4			3				4					
900		1						1		4	4						4	4	3					4	4	3						4	4	3								
1000				5			5	1	5	1	5		3	5		5		5	3	3	5	1	5		5	3	3	5		5			3		5		5		5			
1200				6					2	2	6						2	6	3				2		6	3						6	3					6			
1500	3		2				5	1			5			5	3	2		5	3	2			2		5	3	2	5			2	5	3		5				5			5
2000	1							3	2	3	5		3	4			3	5	2	3	4		2	3	5		3	4				5	2		4				5			
3000							4	1		1			3	4						3						2	3	4		1			2		4							

Number and percentage of polymorphic loci, effective number of alleles, expected heterozygosity and Shannon's information index were used to estimate genetic variation. *Ae. columnaris*, *Ae. triaristata*, *and Ae. kotschy* had the highest values for exhibiting the highest level of variation, whereas varieties *Ae. biuncialis* and *Ae. umbellulata* exhibited the lowest. *Ae. columnaris* had the highest effective number of alleles (1.368), as well as, expected heterozygosity (0.213), and Shannon's information index (0.316). The lowest values of all three parameters, leading to the low diversity had *Ae. biuncialis* (1.080, 0.050, and 0.077, respectively) and *Ae. umbellulata* (1.087, 0.055, and 0.085, respectively), tab. 4.

Six *Aegilops* sp. population under study were grouped in two clusters. The first consisting of *Ae. columnaris*, solely, and the second consisted of all the others. This clustering partly correlates with the different estimates of genetic variation obtained for the samples, since *Ae. columnaris* expressed the highest divergence in this study. The same goes for *Ae. kotschy*, at the next level of grouping (fig. 1).

Table 4. Genetic variation out of 61 bands in *Aegilops* sp. using RAPD markers in experiment

Species	P (No.)	P (%)	Ne±σ	He±σ	I±σ
Aegilops biuncialis	9	14.75	1.080±0.212	0.050±0.126	0.077±0.190
Aegilops kotschy	30	49.18	1.305±0.360	0.181±0.197	0.271±0.287
Aegilops columnaris	34	55.74	1.368±0.384	0.213±0.204	0.316±0.294
Aegilops triaristata	33	54.10	1.240±0.233	0.165±0.155	0.261±0.244
Aegilops ovata	24	39.34	1.315±0.422	0.170±0.220	0.245±0.312
Aegilops umbellulata	10	16.39	1.087±0.217	0.055±0.130	0.085±0.196

P (No.): number of polymorphic loci; P (%): the percentage of polymorphic loci; Ne: effective number of alleles; He: expected heterozygosity; I: Shannon's information index; σ:standard deviation

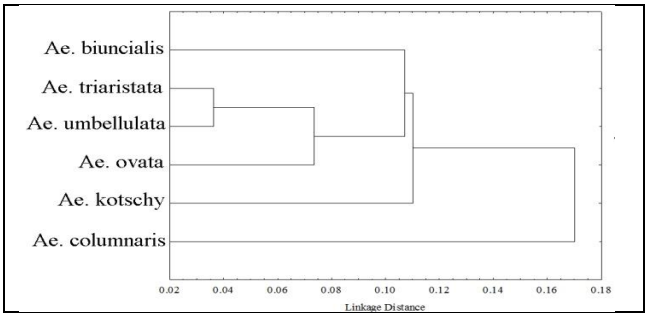
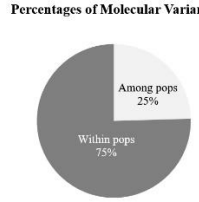


Figure 1. Dendrogram of genetic relationships among 6 populations of *Aegilops sp.* based on RAPD primers

AMOVA partitioned 75% of the total variation within samples of goat grass populations in study, and 25% among samples. This corresponds to previously reported results since all the populations in study share the genome U, and most of them the genome M, as well. However, RAPD primers that were used, gave different DNA patterns within populations, resulting in high level of polymorphism, that contributed greatly by its AMOVA sum of squares to total variation that appeared in the experiment (tab. 5).

Table 5. Analysis of molecular variance (AMOVA) of 6 *Aegilops sp.*, based on RAPD primers

Source of varition	Degree of freedom	Sum of squares	Mean squares	Est. var.	%
Among populations	5	76.833	15.367	2.530	25%
Within populations	12	93.333	7.778	7.778	75%
Total	17	170.167		10.307	100%



CONCLUSIONS

The RAPD marker analysis could be of use in getting the general insight in genetic variation of samples of *Aegilops* sp. RAPD primers used in this experiment gave a considerable variation, not only among, but also within the examined goat grass population of six different species. The nature of this variation is complex consisting on variation in RAPD primers that were utilized, and on genetic variation of the natural populations sampled in their natural habitats in Montenegro. This genetic variation appears as a consequence of inter- and intra-species hybridization, as well as, diversity in genetic constitution of U and M genomes. The complex nature of variation illustrates Principal Coordinates Analysis (PCoA), which had to be omitted due to six pages limit, which carried out three significant axes of variation that contributed 21% (PCoA1), 18% (PCoA2) and 14% (PCoA3) to total denoted variation in the experiment. Though, some RAPD markers could be used in following the presence of particular genomes, more detailed and more expensive investigations are needed to get further information.

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PLANT GENETIC RESOURCES AND GASTRONOMIC TOURISM

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ABSTRACT

Plant genetic resources for food and agriculture (PGRFA) came into the world attention due to their genetic erosion upon the adoption of the International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty) in 2001. Among these, landraces are recognized for their value when are maintained in the same agro-ecosystem for more than 50 years. However, food security as a complex and sensitive subject, is acting between national and local levels and depends on socio-economic attributes of rural societies deeply embedded in the history of the place grounding the traditional knowledge (TK) related to local communities' lifestyle. In the past 25 years Romania lost more than 75% of its own plant genetic resources based on recorded official data, even its economy depends on the activity of more than 69% small landowners (i.e. over 800,000.00). From economic point of view, such type of agriculture is not productive. However, 32% of the today Romania's territory is declared as protected areas and most of these arable lands are in buffering zones or inside protected areas. Studying the village Ațel, from Sibiu county, Valea Târnavelor (i.e. in the buffering zones of protected areas), reveals that rural areas are rich pools of landraces that have been preserved and cultivated by at least 50% of local householders for more than 50 years (i.e. wheat, corn, rye, barley, oats, peas, cabbage, beans, onions, lettuce, spinach, celery, parsley, garlic, carrot, dills). The poorness of these villages in the today economic terms is counteracted by the richness of biodiversity, TK, PGRFA and local gastronomy. The scope of this article is to envisage original approaches, for connecting local TK to economy based on gastronomic tourism that may provide these villages the chance to become part of it.

Keywords: *landraces, food security, rural development, gastronomic tourism, traditional knowledge.*

INTRODUCTION

Complying commitments to multilateral environmental agreements (MEAs) become more and more difficult for signatory Parties in terms of financial mechanisms implementation (Horn and Mavroidis, 2014). It is also the case of biodiversity and heritage conservation that are claimed by different MEAs. On the

other hand, exists lots of opportunities that may be accessed for developing financial innovative mechanisms that may also act for biodiversity or heritage conservation and securing genetic resources for food and agriculture. Among these very appreciated are those related to business and especially connected to tourism (Gelcich and Donlan, 2015). However, a key role in the sustainability of environmental policy implementation belongs to regional and local stakeholders, rather than to national level and bottom-up and top-down approaches should be developed based on peculiarities of regions, especially by tackling synergies among all identified activities (Sala *et al.*, 2015). Moreover, developing rural economy is a challenge in protected areas and it becomes more relevant when climate change and food security are at the edge (Smith *et al.*, 2016). Food security is highly depending on the conservation of biodiversity and securing all genetic resources for food and agriculture will become essential for future strategies development (Khoury et al., 2015). Furthermore, a complete analysis of all above mentioned drivers, will pave the way for developing more resilient rural communities (Lipper *et al.*, 2014). According to these authors, for rural areas, climate-smart agriculture should be relevant and they are grounding their theory on the results regarding the estimation of climate change effects on world agriculture, presented into the report of the Intergovernmental Panel on Climate Change or IPCC (IPCC, 2014). Furthermore, it is predicted for southern Europe, the decline in rainfall and meltwater from snow, that would increase the costs of production and living (Falloon and Betts, 2010; Spinoni *et al*., 2015; Antofie *et al*., 2015). On the other hand, the Carpathian Region, is recognized as a mosaic of crop fields, grasslands, forests as well as a host of a rich biosphere (Loos *et al*., 2014) due to a diverse relief and peculiarities of geographical position (Mráz and Ronikier, 2016). However, rural communities in the region, rich in biodiversity, are highly depending on their own production that is not reflected yet in coherent policies for sustainable development based on applied holistic approaches (Spânu and Nicula, 2016). Traditional knowledge (TK) related to the region (TK related to resources management and practices) is poorly studied and less used in developing appropriate conservation measures (Hanspach *et al.*, 2014). In 2016, Sibiu County was awarded as a *European Region of Gastronomy for 2019* based on a white paper entitled "Sibiu richness and legendary tastes" and analysed by an independent jury of the International Institute for Gastronomy, Culture, Arts and Tourism from Barcelona, Spain according to a specific procedure (Richards, 2014). The dossier is a regional political commitment to support the development of gastronomic tourism from cities to villages inside the borders of Sibiu county. The third objective of this white paper focused to ensure long-term food security, the development of adaptation and mitigation measures related to climate change for safeguarding the resilience of local communities. This study has the aim of analysing new opportunities for connecting TK related to local resources to culinary tourism for Aţel commune into Sibiu county. Based on this approach it might be possible to increase connectivity between cities and villages, secure genetic resources for food and agriculture, that may further support local

communities' resilience towards climate change and ensure food security for the future.

MATERIAL AND METHODS

The paper is based on data surveys collected between 2009 and 2016 from Aţel householders and producers as well as based on provided data from the Division for soil analysis of the County Direction for Sustainable Agriculture, Sibiu. The survey focused on crops and agricultural practices and it was applied on 62 householders originating from Aţel (10.95%). A survey regarding the origin of producers in the agri-food market from Sibiu city was applied. All collected data have been analysed against the white paper, published in 2016 and entitled: "Sibiu richness and legendary tastes", as a Programme for the award *Sibiu European Region of Gastronomy 2019*.

Description of the administrative territory unit (ATU) Aţel. Aţel ATU is located in Sibiu County at the confluence of the Plateau Târnavelor, Hârtibaciului Plateau and Târnava Mare Valley (Lat: 46.15; Long: 24.47). This ATU is occupied today by two villages: Aţel and Dupuş. The history of the place goes before XIII century and the microregion is recognized as a multicultural place influenced by different ethnical groups (e.g. Romanian, Germans, Hungarians, Gypsies and others (Corsale and Iorio, 2014). From historical point of view Aţel was assessed for its agricultural potential and landscape values since XIII century. It was presented as being located near a wetland and having a medium fertile soil especially for spring time crops. However, in 1750 it was stated that maize was cultivated in better conditions compared to wheat that was often attacked by wheat leaf rust (*Puccinia recondita* f.sp. *tritici*). Vineyards and pastures are among other types of agricultural occupations of the time. The ratio between forests, agricultural land and grasslands is almost equal and according to the historical evidences this equilibrium supports local community to rely on their own resources for more than two centuries (Gyémánt *at el*., 2009). Agricultural land encompasses a surface of 2700 ha (98,8% of the total agricultural territory) of the total of 3900 (Acelenescu et al., 2007). A surface of 1205 ha is arable lands, 972 ha are pastures, 439 ha are meadows (a total of 1411 ha grasslands) and 191 ha are vineyards (over 90% of the vineyards have been lost during communist times). 1470 ha of 3902 ha are public properties and the rest are private. 37 of the total 568 houses are public property and Aţel has no public sewerage. There live 1601 people which means 46 people/km^2. The major occupation is agriculture and more than 60% are retired people. The today annual average temperature is 8,6 °C and the annual average rainfall is 627 mm. Dupuş is part of Natura2000 European ecological network of protected areas SCI 200706 Sighişoara - Târnava Mare.

RESULTS AND DISCUSSION

The award *Sibiu European Region of Gastronomy for 2019* implies among others the sustainable development of agriculture in Sibiu county, covered by protected areas for more than 50% of county' surface. A series of regional developing

programmes were committed before and after accessing the European Union, for supporting the sustainable development of Central Region of Romania where resides Sibiu county, especially those related to structural funds (Mazilu *et al.*, 2011). In addition, Sibiu was awarded in 2007 with the title of *European Capital of Culture* that boost the economy of the city (Richards and Rotariu, 2007; Mitrut and Constantin, 2009). However, the current target for Sibiu city, from tourism perspective, is that tourists need to spend more than one night in hotels in Sibiu or region (Nicula et al., 2013) that is hard to be reached after 2008 (Bodosca *et al.*, 2014). However, accessing new political commitments such as the *European Region of Gastronomy* award, may develop regional economy based on culinary tourism extended in rural areas (Sasu and Epuran, 2016). Based on these assumptions in 2016 it was released a regional political commitment, as a white paper, entitled "Sibiu richness and legendary tastes". According to this white paper in Sibiu exists 175 restaurants, 3 certified producers with 11 certified products and a sole product with geographical indication (i.e. Sibiu salami).

Conservation and sustainable use of PGRFA. According to the second specific objective of the white paper entitled *Cultural diversity*, in Sibiu County will be further supported *research, inventory and conservation, valorisation and promotion of local gastronomic heritage and its official recognition as intangible immaterial heritage*. It is a very first step in connecting cities with villages and furthermore with the scope of securing genetic resources for food and agriculture as a basis for local gastronomy. Based on this white paper it is possible to further connect TK to local gastronomy as an intangible heritage of the regional culinary tourism. However, traditional gastronomy needs to be surveyed and recognized up to the level of hotels and restaurants from cities (i.e. Sibiu, Mediaș), as a future possible connection for tourism development. The second objective of the white paper supports the *interconnecting between urban and rural areas* as well as the interconnection of rural producers and cities markets. We consider that this white paper is grounding the opportunity for raising awareness about traditional values in countryside and use their values for community in a regional context. But, the real economic support of rural communities may become relevant only based on their commitment to this programme and universities may play a crucial role in collecting scientific data and supporting the implementation of an encouraging and maintaining participatory system (Middendorf and Busch, 1997). Only based on local communities' commitment in a regional context, it will be possible to fulfil the objectives of the current white paper. Thus, it can represent an opportunity for further foster the proper implementation of international MEAs that are relevant for the country in synergy with culinary tourism. It is also the case of the Plant Treaty, that should be implemented down to the local level considering the provisions of Art. 5, regarding the compulsory need for surveying all PGRFA. Moreover, by engaging local communities in the regional context based on securing PGRFA the white paper will further support the increasing of food security for *long term*. The Plant Treaty, since its adoption in 2001, is highly supporting food security through the PGRFA conservation and sustainable use, free access to genetic resources for

signatory Parties to the Treaty as well as rights of framers (Padulosi et al., 2012). The white paper will further support through its objectives the increase of food security in the region based on developing interconnectivity between cities and rural communities. This objective is also related to the objective related to *Cultural diversity* that supports the inventory of local gastronomic heritage including all genetic resources for food and agriculture.

Genetic resources for food analysis. The results of our survey reveal, for Ațel ATU that wheat occupies 30 ha followed by triticale with 2 ha and six row barleys, with 5 ha in the past 10 years. It was recorded an old wheat variety: 'Arieșan' registered into the Official Catalogue for varieties in 1985, removed after 2004 and reintroduced after 2014. Local people remains attached to this cultivar due to the decrease attack from wild herbivores (i.e. ears due to long palea development) and as well as due to the resistance to wheat leaf rust. For barley, it was possible to identify an old variety 'Precoce', first officially recorded in 1986 and for triticale it is a commercial variety. Maize is still cultivated at the gardening level and not in the arable land of the ATU due to the presence of wild herbivores in the region (i.e. boars and dears). However, the cultivated surface reach more than 20 ha spread on small plots between 0.1 and 5 ha. In this ATU were identified different maize landraces (yellow and red with 10 and 12 and 14 rows on the cob and one or two cobs on the plant) with low productivity (i.e. about 3500 kg/ha). The maize is cultivated for local use only but it can be used as a valuable resource for gastronomy at the region level. Considering the history of the place, local communities are following the long history traditions in cultivating crops as in 1750 (Gyémánt *et al.,* 2009). Even during the communism times for more than 50 years local communities were imposed to cultivate for high production it seems that TK related to the land use, genetic resources choosing is prevalent. Cabbage is a very common vegetable present all the time in the menu of the local's cuisine, traditions and legends. All investigated householders (over 10% of the total) are cultivating peas, cabbage, beans, onion, tomatoes, lettuce, spinach, celery, parsley, garlic, carrot, dills and different spices. However at least two householders are applying complete traditional technologies for onion, garlic, lettuce, spinach, celery, parsley and tomatoes, pepper and eggplant. These two producers (i.e. Families Bunea and Mija) are not officially registered and act only locally for all year duration. Moreover, one producer has the potential to develop their own affairs and wish to be included in the regional market place. The family Mija provides vegetables seedlings for almost half of community during the spring time and have experience for selling agricultural products all year. In this regard, our analysis in the agri-food market from Sibiu revealed that no one from this ATU is recorded as a producer especially due to the lack of connectivity measures. Moreover, based on our survey only 16% of the commodities trade on this market are originating from Sibiu region. Over 45% of registered persons in the market are only traders, the access of producers being limited in Sibiu market. This result should determine local political factors to find solutions for increasing the access of local producers from rural communities to agri-food markets in the cities. As a

general remark, it can be said that in Aţel exists valuable genetic resources that are unique as well as potential producers that can become part of the future European Region of Gastronomy 2019.

Traditional knowledge analysis. Local communities apply, TK related to the use and access of genetic resources for food and agriculture (35.68% of respondents are applying traditional practices in agriculture, storage and use), landscape management (15.23% of respondents do not change land use as historically established by their ancestors), agricultural practices (68.23% of respondents are applying agricultural practices for crops gardening and livestock), social organization and management in the community level (82.23% of respondents are supportive for each other in neighbourhood organized at the street level). Thus, TK is still part of the social life of community. These data resulted after the survey of more than 45% of householders. In case of genetic resources for food and agriculture, TK includes wild plant genetic resources and edible mushrooms, local people knows places and time for collecting from the wild of berries, mushrooms and medicinal plants. TK is associated to the grasslands flora composition and agricultural practices related to. The most relevant case it is related to plant associations between *Juncus effuses* and *Molinia coerulea* (inferior pastoral value) present on gleysoils and associations *Phragmites australis* and *Gliceria maxima* present on gleysoils and land with ponds and swamps. According to inhabitants these habitats should be avoided when are traditionally mowed due to the presence of wild bird's eggs (i.e. *Crex crex*). Usually this is related to the period of May and June.

Climate change commitments taken under the United Nations Framework Convention on Climate Change are engaged under the third general objective of the Programme. It is already proved that food security is highly depending on the management of resources and negatively influenced by climate change effects (Cheeseman, 2016). Therefore, adaptive management tools need to be applied especially in marginal areas inhabited by highly vulnerable population for ensure food security framework (Kahane *et al*., 2013). It can be considered that for rural communities, climate change effects are of outmost importance considering the need for improving their resilience (Mc. Evoy *et al.*, 2013).

Landscape analysis For the Central part of Romania, the historical province South-Easy Transylvania, we proposed to be taken into consideration the administrative territorial unit or ATU at the commune level (this includes couples of villages separated through hills landforms) because they usually share similar agricultural landscape type or types. However, these villages are similar for all Târnava Valley that is a historical larger administrative unit established some over 800 years ago (Hanspach *et al*., 2014). In Aţel case, the ATU includes Dupuş village and both villages share similar landscape features: arable land (i.e. it occupies 30% of the total surface) separated by hills landforms (covered 50 years ago with vineyards and occupying 30% of the total surface) forests (i.e. it occupies 30% of the total surface) and constructed area (10%). The constructed area of these villages is following the crick course and is placed in the bottom of the valley. Each locality is

protected during the winter season of strong cold winds and during the summer by hot weather. Such a resilient landscape was set during 1260 according to historical evidences (Gyémánt *et al*., 2009). Even each village is a very well defined both local communities still share the same administration. Inside this landscape unit it can be defined hilly pastures, arable land and two folds terraces covered in the top of the hills with forests. The crick valley is towered by old white willow on the whole course as an indicator species for high humidity of the region (*Salix alba*). First dramatical change in landscape is recorded during the communist times when vineyards where transferred from the top of the two folded terraces to the bottom of these hills. Vines do not survive during in the valley due to the extended periods of fog in spring and autumn that is a peculiarity of the region. Therefore, the entire vineyard surface of the commune was lost and only vines planted in the yards survived up today. Thus, today these landscapes sub-units are composed of two folded terraces covered with pastures. After the communism times, the landscape suffered again due to the invasion of some exotic species starting with 2000. *Solidago canadensis* and *S. gigantea* were established in the arable land as well as in ruderal areas of ATU Aţel. In the ruderal areas of Dupuş, plantains (*Plantago media* and *P. lanceolata*), knotweed (*Polygonum aviculare*), white clover (*Trifolium repens*) have been replaced completely by these invasive alien species. A second issue of present days is the spreading of invasive alien species in arable and ruderal areas by removing native species and changing the habitats structure. The official recorded land abandonment is 3% for more than 10 years and it is especially due to the deficit of agricultural policy for small landowner protection as well as due to property' disputes. However, if land abandonment should become beneficial for native species and habitats conservation (Queiroz *et al*., 2014) however, in this case the land will be populated by invasive alien species (Zimmermann *et al.,* 2015). Dupuş Village that is inside protected area of European importance Natura2000 SCI 200706 Sighişoara - Târnava Mare is invaded by *S. canadensis and S. gigantea* in the arable land, gardens and ruderal areas. Based on the European regulatory framework for nature protection, all invasive alien species need to be part of a controlling or eradication programme for invasive alien species (Vicente *et al.*,2013). Moreover, some of the Common Agricultural Policies measures imposed to local inhabitants that are claimed to support biodiversity conservation some time needs adjustment according to some authors (Mikulcak *et al.*, 2013), and in this case, are in favour of spreading invasive alien species as the mowing is forbidden up to August. Thus, forbidding traditional mowing during late May up to July it will support these invasive alien species to better install in this landscape. Based on this observation for Aţel it will be beneficial to restore vineyards on the two folded hills and apply traditional mowing in late May up to August for removing invasive alien species. A positive aspect observed during these field missions is related to the attachment of the owner to the land as their property. They know better all peculiarities of the arable land and making him or her the very first witness of any possible unpredictable change that may become relevant for climate change and biodiversity conservation. The proper

information and awareness of local communities may gain the best supporter in this programme implementation. The landscape is unique in terms of aesthetic for Transylvania and therefore may be used as a potential destination for the future regional culinary tourism network.

Culinary tourism potential of Aţel In Aţel ATU the rural economy is low as only two producers are officially recorded at the county level: one is for beekeeper and another for traditional products based on wheat (i.e. traditional bread, bread for church ceremony and traditional cakes). However, these products or processes are not certified even they follow traditional procedures and are accessing traditional resources. Tourism is not developed in the region as no accommodation unit is yet developed. However, at least 5 householders express their interest in developing a rural tourism based on availability of houses. Thus, the community existence is due to several key economic drivers (i.e. agriculture, forestry, water, education, transport, energy sector) that must synergic work together for supporting food security on *long term*.

CONCLUSIONS

Regional political commitments are keys factors that may work for the proper implementation up to local level of national political commitments taken for food security and biodiversity conservation. They are relevant in understanding all local peculiarities and developing more suitable business. Local gastronomy may be treated as an ecosystem service that may be integrated into policies supporting culinary tourism in the context of the award Sibiu European Region of Gastronomy 2019. Connecting ecosystem services and regional business may provide an appropriate framework for securing the conservation of biodiversity and plant genetic resources for food and agriculture. Promoting unique culinary tourism in Sibiu region may further support the conservation of all genetic resources for food and agriculture and will provide an appropriate framework for ensuring food security in the region.

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IN VITRO EFFECTS OF *ACINETOBACTER BAUMANNII* AND SELECTED NATURAL BIOMOLECULES ON RABBIT SPERMATOZOA MOTILITY

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ABSTRACT

The aim of this study was to assess the potential efficiency of selected biologically active substances on the motility behaviour of rabbit spermatozoa subjected to *in vitro* induced *A. baumannii* contamination. The semen samples used for *A. baumannii* detection were collected from 10 New Zealand white male rabbits and the presence of the bacterium was confirmed using MALDI-TOF Mass Spectrometry. For the *in vitro* experiments rabbit spermatozoa were re-suspended in PBS, containing mineral supplements, BSA and glucose in the presence of $3x10^5$ CFU *A. baumannii* and diverse concentrations of selected biomolecules (resveratrol - RES, quercetin - QUE, curcumin - CUR, epicatechin - EPI, isoquercitrin - ISO). The sperm motility was assessed using the computer-aided sperm analysis at 0h, 2h, 4h and 6h. *A. baumannii* significantly decreased the sperm motility ($P<0.001$) at Time 2h and maintained this negative impact throughout the *in vitro* culture. Meanwhile, the motility at Time 2h was significantly higher in the samples subjected to *A. baumannii* together with 10 µmol/L RES ($P<0.01$); 5, 10 and 50 µmol/L QUE ($P<0.001$); 1 µmol/L CUR ($P<0.05$); 10, 50 and 100 µmol/L EPI ($P<0.01$) as well as 50 µmol/L ($P<0.05$) and 100 µmol/L ISO ($P<0.001$) in comparison to the control exposed to the bacterium exclusively. After 4h, the motility remained significantly higher in the groups co-treated with the inoculum and 10 µmol/L RES ($P<0.05$), 50 µmol/L QUE ($P<0.05$) as well as 50 µmol/L EPI ($P<0.05$) when compared to the positive control. Nevertheless, none of the biomolecules was effective against the rapid decline of sperm motility caused by *A. baumannii* during later stages of the experiment (Time 6h). Based on these results, one can conclude that RES, QUE and EPI exhibit antibacterial properties providing a selective advantage to spermatozoa in the presence of *A. baumannii*, particularly during short-term rabbit semen handling.

Keywords: *Acinetobacter baumannii, spermatozoa, biomolecules, contamination, motility.*

INTRODUCTION

A variety of studies have reported that the loss of sperm motility, morphological alterations, acrosome dysfunction, disruption of membrane integrity and oxidative damage may be linked to bacterial contamination of semen under both *in vivo* and *in vitro* conditions (Villegas *et al*., 2005; Fraczek and Kurpisz, 2007; Fraczek *et al.*, 2012). Most data connected to bacteriospermia are related to well-known causative agents of urogenital tract infections, such as *Escherichia coli*, *Staphylococcus aureus*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Chlamydia trachomatis*. Nevertheless, some authors have emphasized that other bacteria, responsible for the colonization of the male urogenital tract, rather than the actual infection, could also contribute to a decreased sperm quality (Fraczek *et al*., 2012).

The genus *Acinetobacter* comprises Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive and oxidase-negative bacteria that are typically short, almost round and rod-shaped. Acinetobacters have been shown to colonize the skin, respiratory and oropharynx secretions of infected individuals (Howard *et al.*, 2012). *A. baumannii* can be an opportunistic pathogen in mammals, affecting individuals with compromised immune systems, and is becoming increasingly important as a nosocomial infection agent. In recent years, the bacterium has been designated as a "red alert" pathogen, generating alarms among the scientific society, arising largely from its extensive antibiotic resistance spectrum (Peleg *et al*., 2008).

Most ejaculates collected from otherwise healthy animals may be contaminated to a certain degree, as semen collection is not a completely sterile process (Bielanski, 2007). As such, antibiotics are commonly added to semen extenders used for artificial insemination to control possible microbial contamination of semen during collection and processing. Since antibiotics themselves may be toxic to spermatozoa, and because of an alarmingly increasing bacterial resistance, there is an urgent need to find alternatives to conventional antibiotics to be used in animal reproduction biotechnologies (Morrell and Wallgren, 2014).

Recent studies have emphasized on the rebirth of naturally occurring compounds with a variety of beneficial properties, rich diversity, complexity and availability, lack of significant toxic effects and intrinsic biologic activity. A broad array of flavonoid and polyphenolic compounds has been shown to possess beneficial properties which could potentially provide a selective advantage to spermatozoa under stress conditions.

This study focused to assess the effects of five natural biologically active compounds (resveratrol, quercetin, curcumin, epicatechin, isoquercitrin) in comparison with three traditional antibiotics (penicillin, gentamycin, kanamycin) on the motion of rabbit spermatozoa subjected to *in vitro* induced *Acinetobacter baumannii* contamination.

MATERIAL AND METHODS

Sample collection

Ten male rabbits (New Zealand white broiler line) were used in the experiment. The animals were 4 months old, with a weight of 4.0±0.2 kg and kept at an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic. The rabbits were housed in a partially air-conditioned rabbit house under a photoperiod of 16L:8D (a minimum light intensity of 80 lux), kept in individual cages and fed with a commercial diet. Water was provided *ad libitum*. The air temperature of 20-24 °C and relative humidity of 65% were maintained in the rabbit house. Institutional and national guidelines on the care and use of animals were followed, and all the experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic (no. 3398/11-221/3) and Ethics Committee. One ejaculate was collected from each rabbit using an artificial vagina. Immediately upon collection, the samples were transferred to the laboratory.

Cultivation and identification of microorganisms

100 µl of each semen sample were transferred into the MacConkey agar (Biomark, Pune) and MRS agar (Biolife, Italy). The cultures were maintained at 37°C during 24h for microorganisms which grew on the MacConkey agar and 37°C during 48-72h for microorganisms which grew on the MRS agar. Purification of all microorganisms was done by four ways streak plate method after the first cultivation. The Chromogenic coliform agar (Oxoid, England) and the URI Select IV (Biolife, Italy) were subsequently used to purify those microorganisms which contaminated the MacConkey agar. Microorganisms which contaminated the MRS agar were repeatedly purified in the MRS agar. All steps of recultivation were done at the same conditions (Hleba *et al.*, 2017).

Matrix-assisted laser desorption/ionization time-of-light (MALDI TOF MS) (Brucker Daltonics, Germany) was used for bacterial identification in the semen samples. Cells from a single colony of fresh overnight culture were used for each isolate to prepare samples according to the manufacturer's recommendations for microorganism profiling using the ethanol-formic acid extraction procedure. Each sample spot was overlaid with 2 µl of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile with 2.5% trifluoroacetic acid) and again air-dried for 15 min. To identify the microorganisms, raw spectra obtained for each isolate were imported into the Biotyper software, version 2.0 (Brucker Daltonics), and analyzed without any user intervention (Hleba *et al.*, 2017). *A. baumannii* was identified in seven out of ten semen samples.

The isolated *A. baumannii* was aseptically transferred to the culture medium selected for the *in vitro* experiments and cultured at 36°C for 24 to 48h. Following culture, *A. baumannii* concentration was adjusted to 0.3 McF using a densitometer (DEN^{-1} McFarland Densitometer, Grant-bio, UK). Such inoculum was suitable for the simulation of an *in vivo* environment under *in vitro* conditions taking into consideration an ideal environment for the sperm cells as well as the bacterium.

In vitro experiments

One ejaculate was collected from 10 male rabbits used for previous *in vivo* experiments on a regular collection schedule (twice a week for two consecutive weeks) using an artificial vagina. Only samples with a minimum motility of 60% were used in the experiments.

The resulting semen sample was centrifuged (300 x g) at 25°C for 5 min, seminal plasma was removed and the sperm pellet was washed twice with PBS (Dulbecco's phosphate-buffered saline without calcium chloride and magnesium chloride; Sigma-Aldrich, St. Louis, MO, USA), resuspended in a culture medium consisting of PBS, mineral supplements for semen cultures (Minitube, Tiefenbach, Germany), 5% glucose (Centralchem, Bratislava, Slovak Republic) and 4% BSA (bovine serum albumin, Sigma-Aldrich) using a dilution ratio of 1:20. Two controls were established – the Negative Control was resuspended in the culture medium exclusively, while the Positive Control contained the culture medium with 0.3 McF *A. baumannii*. Each experimental group was exposed to the bacterium and different concentrations of chosen antibiotics or biomolecules as follows: 300 μg/mL penicillin (PEN; Sigma-Aldrich); 1 mg/mL gentamycin (GEN; Sigma-Aldrich); 80 μg/mL kanamycin (KAN; Sigma-Aldrich); 50, 10 and 5 μmol/L^{-1} resveratrol (RES; Sigma-Aldrich); 50, 10 and 5 μmol/L^{-1} quercetin (QUE; Sigma-Aldrich); 10, 5 and 1 μmol/L^{-1} curcumin (CUR; Sigma-Aldrich); 100, 50 and 10 μmol/L^{-1} epicatechin (EPI; Sigma-Aldrich); 100, 50 and 10 μmol/L^{-1} isoquercitrin (ISO; provided by the Center of Biocatalysis and Biotransformation, Czech Academy of Sciences).

At culture times of 0h, 2h, 4h and 6h the spermatozoa motility (percentage of motile spermatozoa; motility > 5 μm/s; %; MOT) was assessed using the computer-aided sperm analysis (CASA; Version 14.0 TOX IVOS II.; Hamilton-Thorne Biosciences, Beverly, MA, USA). The samples were stained using the IDENT stain, a DNA-specific dye based on Hoechst bisbenzimide (Hamilton-Thorne Biosciences). The IDENT dye provided in Eppendorf tube was diluted with 1 ml of the culture medium and mixed with the sample using a ratio of 1:1. Following 10 min of incubation in the dark, the sample was analyzed under fluorescent illumination. Ten microscopic fields were subjected to each analysis in order to include at least 300 cells.

Statistical analysis

All the data were subjected to statistical analysis using the GraphPad Prism program (3.02 version for Windows, GraphPad Software incorporated, San Diego, California, USA, http://www.graphpad.com/). The results are quoted as the arithmetic mean ± standard error of mean (SEM). The comparative analysis was carried out by a one-way ANOVA with the Dunnett's post test. The level of significance for the analysis was set at * $P<0.05$; ** $P<0.01$; *** $P<0.001$. The comparative analysis was performed as follows: Positive Control (PC) was compared to the Negative Control (NC), while experimental fractions exposed to *A. baumannii* and antibiotics or biomolecules were compared to both Controls.

RESULTS AND DISCUSSION

The initial MOT was lower in the Positive Control when compared to the Negative Control. Moreover, MOT was insignificantly lower in groups exposed to *A. baumannii* together with PEN, GEN, KAN, 50 and 5 µmol/L RES, 10 and 5 µmol/L CUR, 10 µmol/L EPI and 10 µmol/L ISO (Figure 1).

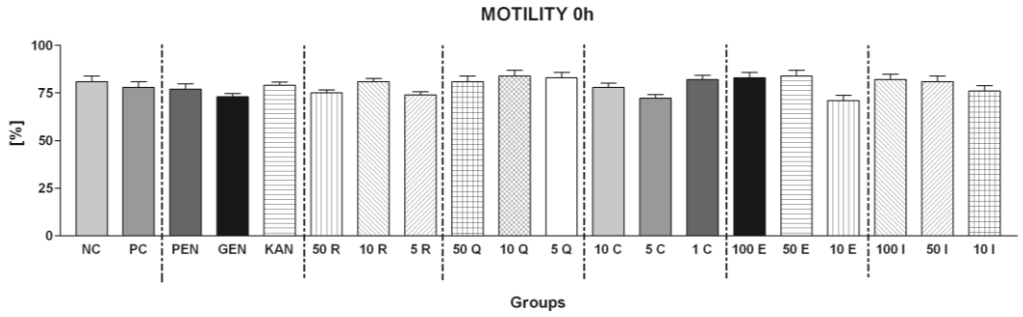

Figure 1. Immediate effects (Time 0h) of *A. baumannii*, selected antibiotics and biomolecules on rabbit spermatozoa motility [%]. Mean±SEM. * P<0.05; ** P<0.01; *** P<0.001. NC – Negative Control; PC – Positive Control; PEN – Penicillin; GEN – Gentamycin; KAN – Kanamycin; R – Resveratrol; Q – Quercetin; C – Curcumin; E – Epicatechin; I – Isoquercitrin.

After 2h the presence of *A. baumannii* significantly decreased MOT (P<0.001) when compared to the untreated Control. Meanwhile, the MOT was significantly higher in the samples subjected to *A. baumannii* with GEN (P<0.01), KAN (P<0.01), 10 µmol/L RES (P<0.01), all QUE doses (P<0.001), 1 µmol/L CUR (P<0.05), all EPI concentrations (P<0.01), 100 µmol/L (P<0.001) and 50 µmol/L (P<0.05) ISO. On the other hand, MOT was significantly decreased in samples exposed to *A. baumannii* with PEN (P<0.01), 50 µmol/L RES (P<0.01), 10 µmol/L and 5 µmol/L CUR (P<0.001) as well as 10 µmol/L ISO (P<0.01).

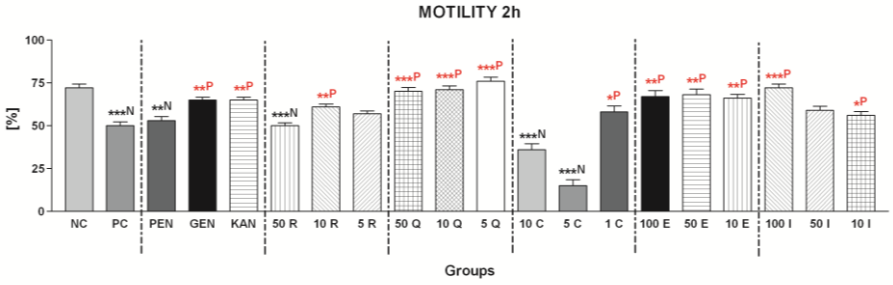

Figure 2. The effects of *A. baumannii*, selected antibiotics and biomolecules on rabbit spermatozoa motility following 2 hours of in vitro culture [%]. Mean±SEM. * P<0.05; ** P<0.01; *** P<0.001. NC – Negative Control; PC – Positive Control; PEN – Penicillin; GEN – Gentamycin; KAN – Kanamycin; R – Resveratrol; Q – Quercetin; C – Curcumin; E – Epicatechin; I – Isoquercitrin. N – vs. Negative (untreated) Control. P – vs. Positive Control (exposed to *A. baumannii* exclusively).

After 4h, the highest MOT was detected in the Negative Control, while the MOT in the Positive Control was significantly lower (P<0.001). While the MOT in all experimental groups was lower when compared to the Negative Control, tit remained significantly higher in the groups co-treated with the inoculum together with PEN (P<0.01), GEN (P<0.001), KAN (P<0.01), 10 µmol/L RES (P<0.05), 50 µmol/L QUE (P<0.05) and 50 µmol/L EPI (P<0.05). In the rest of the experimental groups, the MOT was significantly decreased when compared to the Negative Control (P<0.001).

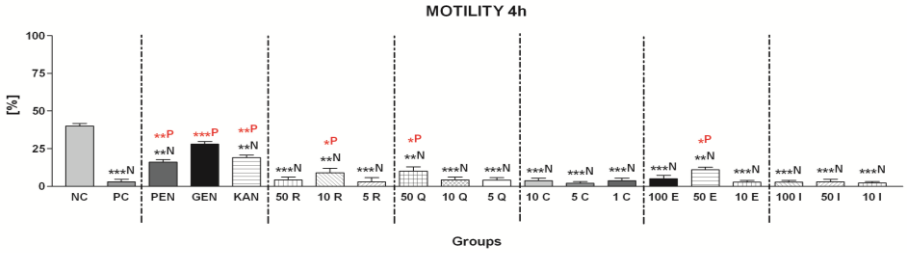

Figure 3. The effects of *A. baumannii*, selected antibiotics and biomolecules on rabbit spermatozoa motility following 4 hours of in vitro culture [%]. Mean±SEM. * P<0.05; ** P<0.01; *** P<0.001. NC – Negative Control; PC – Positive Control; PEN – Penicillin; GEN – Gentamycin; KAN – Kanamycin; R – Resveratrol; Q – Quercetin; C – Curcumin; E – Epicatechin; I – Isoquercitrin. ^{N} – vs. Negative (untreated) Control. ^{P} – vs. Positive Control (exposed to *A. baumannii* exclusively).

The final (Time 6h) assessment revealed that the only group containing actively moving spermatozoa was the Negative Control. Only few moving spermatozoa were detected in the Positive Control (P<0.001). Sperm MOT was preserved to a certain degree by the presence of PEN (P<0.05) and GEN (P<0.01). None of the biomolecules applied was able to maintain the sperm MOT comparable to the Negative Control, and none was effective against the rapid decline of sperm motility caused by the presence of *A. baumannii*.

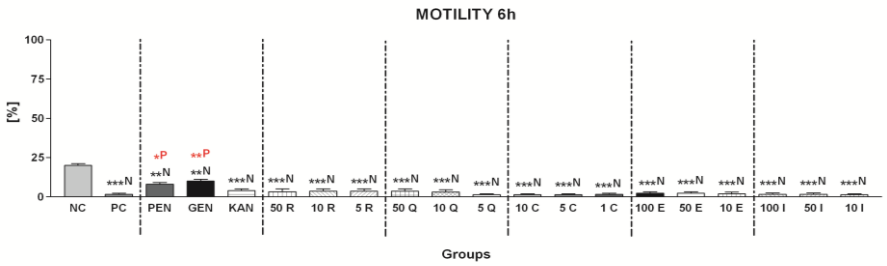

Figure 4. The effects of *A. baumannii*, selected antibiotics and biomolecules on rabbit spermatozoa motility following 4 hours of in vitro culture [%]. Mean±SEM. * P<0.05; ** P<0.01; *** P<0.001. NC – Negative Control; PC – Positive Control; PEN – Penicillin; GEN – Gentamycin; KAN – Kanamycin; R – Resveratrol; Q – Quercetin; C – Curcumin; E – Epicatechin; I – Isoquercitrin. ^{N} – vs. Negative (untreated) Control. ^{P} – vs. Positive Control (exposed to *A. baumannii* exclusively).

A. baumannii has recently emerged as a critical bacterial contaminant of ejaculates. Similarly to our MALDI TOF data, *A. baumannii* was identified in semen by other investigators (Kiessling *et al*., 2008; Sarah *et al.*, 2015) in individuals suffering from oligozoospermia, teratozoospermia and chronic prostatitis. Furthermore, Kiessling *et al.* (2008) performed PCR amplification of bacterial rDNA on 34 semen samples, and identified *Acinetobacter* as one of the largest groups in different specimens. The authors conclude that the abundance of bacteria in semen may influence fertility, and may reflect an inadequate cellular immune response. Moreover Sarah *et al.* (2015) conclude that the presence of *Acinetobacter* in semen samples makes *in vitro* fertilization procedures not viable. Semen extenders with single or combination of antibiotics such as kanamycin, ampicillin, gentamicin, linomycin, penicillin-streptomycin, tylosin and lincospectin have been tried with different success rates in eliminating microbes in animal studies (Hasan *et al.*, 2001). Nevertheless, a large number of antibiotics have been shown to exhibit toxic effects on the sperm motility, viability and DNA integrity. Furthermore, long term use of the same antibiotic may lead to antibiotic resistant varieties. As such, there is an urgent need to search for alternative substances which could provide a selective advantage to male reproductive cells against the stress resulting from microbial contamination (Morrell and Wallgren, 2014). RES has been recently discovered to possess a wide range of cardiovascular, anticancer, anti-inflammatory and protective effects. Our data are contradictory to Collodel *et al.* (2010) who reported that human spermatozoa were more sensitive to the potentially toxic effect of RES with a LD50 between 30 and 50 μmol/L. Moreover, Tvrdá *et al.* (2015b) reported a significant decrease of bull sperm MOT following exposure to 100 or 200 μmol/L RES during a 24h *in vitro* culture. On the contrary, protective effects of lower RES concentrations on spermatozoa are in agreement with Tvrdá *et al.* (2015) who emphasize on the beneficial effects of 5-50 μmol/L RES on the motion behaviour, mitochondrial activity and superoxide production by bovine sperm. Our data are in agreement with Mojica-Villegas *et al.* (2014) who reported that pre-treatment with 15 μmol/L RES prior to incubation with ferrous ascorbate showed an 8.0-fold increase in murine spermatozoa MOT. QUE is a common dietary flavonoid, reported to exhibit a broad variety of favourable biological effects. Protective effects of QUE on rabbit spermatozoa in our study disagrees with earlier reports, according to which QUE compromised human sperm motility and viability (Khanduja *et al.*, 2001). Talking in favour of our results, Tvrdá *et al.* (2016b) emphasized on the protective effects of QUE on bovine sperm motion activity when incubated without the presence of seminal plasma (Tvrdá *et al.*, 2016b), although we must take into consideration that QUE may act dose dependently as either a stimulant at low concentrations or as an inhibitor at high doses. Previous reports on the impact of CUR on male fertility are controversial. Naz (2011) reported that exposure of human and mouse sperm to CUR caused a concentration-dependent decrease of sperm MOT, capacitation and acrosome reaction. Furthermore, our results disagree with Tvrdá *et al.* (2016a) suggesting stimulating and protective effects of CUR on spermatozoa MOT and antioxidant status.

Motility parameters recorded by our IDENT CASA technique partially complement previous findings by Bucak *et al.* (2010) demonstrating a significant motion improvement of ram spermatozoa supplemented with CUR. Interestingly, their later study on CUR administration to bovine semen revealed non-significant differences in the sperm motion (Bucak *et al.*, 2012).

EPI is a flavonoid and antioxidant commonly found in green tea and cocoa. Jamalan *et al.* (2016) evaluated the effects of different flavonoids including EPI on the recovery of sperm MOT and prevention of membrane damage from aluminium, cadmium and lead. Contrary to our study, the report revealed that EPI did not protect spermatozoa from heavy metal-mediated damage, rather, it showed inhibitory effects on the sperm motion associated with co-incubation with selected heavy metals. Inversely Purdy *et al.* (2004) revealed that EPI may aid in maintaining the MOT of cooled goat sperm in a dose dependent manner.

ISO is found in a variety of medicinal plants, likely contributing to their pharmacological qualities (Appleton, 2010). According to our data, although being structurally similar to QUE, the molecule did not exhibit beneficial effects on the sperm MOT. On the other hand, numerous animal studies emphasize on potential ameliorative effects of plant extracts containing ISO on the testicular structure and function, as well as sperm concentration, motility and morphology (Awoniyi *et al.*, 2011). As such, we may suggest that more specific experiments on the roles of ISO are to be designed in order to elucidate its beneficial or harmful roles in male reproduction.

CONCLUSIONS

Based on our preliminary CASA results it can be concluded that resveratrol, quercetin and epicatechin exhibit antibacterial properties providing a selective advantage to the male gametes in the presence of *Acinetobacter baumannii*, particularly during short-term rabbit semen handling. On the other hand, curcumin and isoquercitrin did not prove to possess significant protective or beneficial effects on the *in vitro* survival of rabbit spermatozoa in the presence of uropathogenic bacteria. Last but not least, more experiments will be necessary to unravel specific molecular mechanisms of action of *A. baumannii*, antibiotics and/or natural biomolecules on the structure and function of male reproductive cells.

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IMPACT OF LAND USE ON SOIL WATER CONTENT IN A HILLY RAINFED AGROSYSTEM: A CASE STUDY IN THE CAP BON PENINSULA IN TUNISIA

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ABSTRACT

The relation of soil moisture, evapotranspiration and vegetation is fundamental for improving management of water and soil resources on rainfed agrosystems. The present study focused on the soil moisture (SM) and the evapotranspiration (ETa) patterns in a hilly rainfed agrosystem. We analyzed five datasets from measurement at 15 sites during various crop growth cycles under the common cereals/legumes/pasture cropping systems within the Kamech catchment in Tunisia. Results indicated a strong seasonality in the precipitation and evapotranspiration dynamics that influences soil moisture patterns, with a reduction in the availability during summer (average SM = 20 mm) as compared with winter (average SM = 40 mm). This reduction was tied to higher evapotranspiration ratio in late winter and in spring (average ETa = 5 mm/day) compared with summer (average ETa = 2.5 mm/day). The data of two consecutive years showed that the spatial variation, expressed through the coefficient of variation, between the various land uses was at its highest point during this period of the year. The averaged soil moisture of the two years yielded a reasonable significant linear relation (R^2=0.67**) indicating temporal stability of spatial pattern. ETa estimated using water balance was accurate compared with energy balance during the growth cycles.

Keywords: *soil water availability, crop water use, crop sequences, agricultural hilly catchment, Tunisia.*

INTRODUCTION

Rainfed agrosystems have significantly contributed to economic output and agricultural production. At the global scale in 2000, approximately 75% of the harvested agricultural area and 66% of cereal production were associated with rainfed agrosystems (Portmann et al., 2010). Hilly rainfed agrosystems have

experienced agricultural intensification, since their topographical features allow rainwater-harvesting techniques. Meanwhile, they are threatened by climate change, and they are subjected to human-induced pressures (Niu et al., 2015). Therefore, particular attention should be given to the management of upstream agrosystems, so that land degradation is reduced, crop rainwater consumption is more efficient, and downstream water availability and quality are improved. Among the various drivers to be considered when addressing management of upstream agrosystems, an important factor is soil water storage, so-called green water resource (Rockström et al., 2010). Soil water content plays a key role in hydro-agricultural processes, including partitioning of precipitation into runoff and infiltration, percolation towards aquifers, soil evaporation and plant transpiration, crop functioning and related biomass production, as well as transport of chemical and biological soil constituents (Endale et al., 2006; Hébrard et al., 2006; Loague, 1992; Mekki et al., 2006; Pan et al., 2008; Vivoni et al., 2010, Zucco et al., 2014). Therefore, reliable information is needed on soil water content in relation to agricultural practices, so that it is possible to assess the impact of management strategies (Chen et al. 2008). Among the various action means to be considered when addressing management of upstream agrosystems, land use has several implications for agricultural practices and watershed hydrology. Further, land use significantly affects soil water content through changes in hydrodynamic properties and in evapotranspiration rate (Famiglietti et al., 1998; Hu et al., 2010; Wang et al., 2013; Zhu et al., 2012). Thus, several studies have shown that inappropriate land uses have negative impact including deforestation, overgrazing, and deficient agricultural practices, leading to soil erosion, salinization and vegetation degradation, as a consequence of drastic changes in the water balance (Pla, 2006). The effects of land use on soil water variations have been investigated via statistical analysis or simulation of physical-based models combined with measurements (Chen et al., 2008; Hu et al., 2009; Li et al., 2009; Romano, 2014). In a specific context of the dry land vineyards of Catalonia (Spain), Pla (2006), showed that the farming cropping practices resulted in drastic changes in the soil moisture regime. Agricultural practices such as soil tillage, chemical weed control or grass covering affect strongly soil profile water recharge at the plot level and water balance at the catchment level (Gomez-Plaza et al., 2001; Mekki et al., 2006; Hebrard et al., 2006; Zucco et al., 2014). Beyond existing studies, accurate field measurements of soil moisture depletion are scarce and there are not many experiments that observed the influence of land use over whole growing seasons and crop sequences. Therefore, campaigns to collect more field measurements should be carried out to lead to a better understanding of the influence of land use choices on water availability and requirements. The available studies analyzing the relationship between land use and water balance and then soil water content rarely deal with the context of in site-specific cropped field in rainfed Mediterranean hilly watersheds. In this context, the main purpose of this paper was to study the spatio-temporal patterns of soil moisture and actual evapotranspiration within a hilly rainfed semi-arid Mediterranean agrosystem. A specific objective of the study was

to determine the respective influence of weather conditions, land use on the variations of soil moisture, in relation to soil type and topography. We attempt to deepen our understating of land use impact on soil moisture, by analyzing runoff and evapotranspiration.

MATERIALS AND METHODS

Study site and experimental design description

The study area is the Kamech watershed situated in the central part of the Cap Bon Peninsula, northeastern Tunisia. It is an agricultural hilly watershed, 2.6 km² in size, with elevations ranging from 100 to 200 m. It is an area of ongoing research efforts and part of the long-term collaborative environmental research observatory labeled ORE OMERE (http://www.umr-lisah.fr/omere). The mean annual precipitation of the study area ranges from 400 to 650 mm, with a marked winter dominance which accounts for 75% of the total annual rainfall and occurs between winter and spring (October and April). Average annual potential evapotranspiration is 1250 mm (Zitouna Chebbi, 2009). Four major soil types mostly used for annual crops were distinguished and mapped according to the FAO classification (FAO, 1998). The predominant soils are cambisols, and cover about 46% of the watershed. Luvisols and Vertisols cover about 26% and 10%, respectively. Regosols, which are thin and commonly associated with pasture and shrubs, cover about 18%. The watershed is characterized by a diversity of land uses, which are associated with a large diversity of agricultural operations and overland flow fluxes (Mekki et al. 2006). Agricultural land use includes rainfed cropping systems (cereals, legumes, vineyards and fallow), and pasture systems (pastures-annual and pastures-shrubs). The most common crops are winter cereals (wheat, triticale, oat and barley), and legumes (fababean, chickpeas, peas, fenugreek).

Data collection included five different sets of measured soil moisture profiles and eddy covariance crop evapotranspiration. The 2 year period (Y1 2000-01 and Y2 2001-02) consists of a succession of winter arable crops (cereals, legumes) and pastures (annuals and shrubs) and vigneyard. For the Y3 (April to June 2004), Y4 (January to June 2005) and Y5 (April to July 2006) growing seasons, land use plots include winter arable crops (cereals, legumes) and pastures (annuals and shrubs). The studied fields were defined by 5 classes of texture and 3 classes of soil depth (Table 1).

Table 1. Classes of texture and soil depth.

Soil texture					Soil depth		
S1	S2	S3	S4	S5	d1	d2	d3
Clay	Clay loam	Sandy clay loam	Silty clay	Sandy loam	<1m	>1 m <1.5	> 1.5m

Measurements

Climatic data were obtained from an automatic weather station located at the experimental site, in operation at the watershed outlet since 1994. The sensors and the experimental datasets are detailed in Zitouna-Chebbi (2009), Zitouna-Chebbi et

al. (2014). Potential evapotranspiration (ET0) is computed from the micrometeorological observations. Due to failures of the weather station during Y1 and Y2, potentiel evapotranspiration was computed from evaporation data of a Colorado-ORSTOM type evaporation pan. Vegetation height measurements were acquired approximately every 15 days throughout the five crop growth cycles. During the Y1 and Y2 growing seasons, soil moisture profiles were collected using a neutron probe (Solo 25, Nardeux, St-Avertin, France) from a steel access tube installed in the center of each plot. Volumetric water content was calculated from gravimetric water content with a calibration curve, determined by using bulk density measured with a gamma-density probe (Solo 40, Nardeux, St-Avertin, France) and gravimetric measurements taken nearby the access tube. The experimental plots locations were distributed according to the watershed land uses, providing a good representation of the distribution of vegetation types and successions in the basin, with a dominance of annual crops. The field experimental and the resulting datasets are detailed in Mekki et al. (2006); Mekki et al. (2014). For the Y3, Y4 and Y5 growing seasons, measurements of soil moisture were conducted on five different types of land use plots. The latter were selected according to soil type and topographic position. Gravimetric sampling was performed at different moisture states. An eddy covariance station (EC) was used to characterize radiation, energy and water fluxes. Sensors included a three-dimensional sonic anemometer (CSAT3, Campbell Sci.) or Young-81000V (R.M. Young, USA) a pyranometer (NR-lite, Kipp & Zonen, NL), a krypton hygrometer (KH20, Campbell Sci.), an hygrothermometer (HMP45C, Vaisala, Fl), three soil heat flux plates at 2 and 10 cm depth in bare soil and vegetated areas (HFP01, Hukseflux, NL). Intruments were installed to take measurements at 2 m above the soil surface. Covariances of vertical wind speed, temperature and water vapor concentration were recorded at 10 Hz and processed to obtain sensible (H) and latent heat (LE) flux at 30 min intervals. with ECPACK library version (van-Dijk et al., 2004). The field experimental and the resulting datasets are detailed in Zitouna Chebbi (2009) and Zitouna Chebbi et al. (2012). The water and energy balances in the measurements plots within the watershed were computed at daily, monthly and over all the periods of measurements. Crop evapotranspiration (ETa) was estimated as a residual of the water balance (ETa (WB) =P+ΔS -R) where R is the runoff amount, P is the the precipitation and ΔS is the variation of the soil water storage in the 0-100 cm soil depth layer between the two dates. Daily ETa was calculated by dividing total amount by the number of days of the considered period. When using energy residual energy balance, ETa (EC) is estimated (ETa (EC) =LE= Rn-G-H) where LE is the latent heat flux; Rn is the net radiation, G is the soil heat flux, and H is the sensible heat flux.

RESULTS AND DISCUSSION

Climatic and vegetation conditions

The analysis of the climatic conditions for the 5 years (Y) experiments are presented on the Figure 1a and Figure 1b. In these figures we consider the sub-period from February to June that can be representative of the major crop growth cycle at this time of the year. In the 5 experiments, the driest year was Y2, and monthly rainfall varied from 2 mm in June to 120 mm in February during the studied periods. We observed a large interannual variability of the accumulated seasonal precipitation. The precipitations mainly occur during the winter months; soils are wet, and evaporative demand is low. ET0 shows the same monthly dynamic between years and varied between < 2 mm/day in February and > 7 mm/day in June.

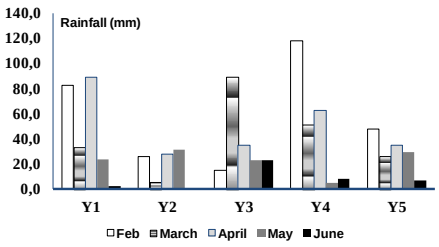

Fig. 1a: Monthly rainfall and ET0 at different years (Y) of data acquisition

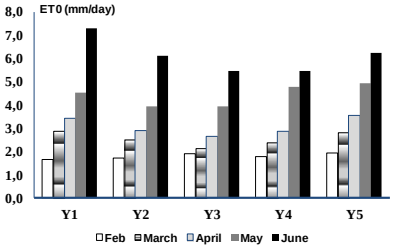

Figure 1b: Monthly ET0 at different years (Y) of data acquisition

Monthly dynamics of vegetation heights were computed for the same periods. The values of the vegetation height (Hv) for the three most common land cover (cereals, legumes and pastures) types in the area throughout the growth cycles show that: the Hv evolution is typically characterized by little development until February, followed by a period of exponential growth, and reaches its maximum level between March and May. It appears that the height did not reach 1 m for all cases. For the cereals their height could be also important between April and May. The maximum heights are found in the clay loam soil with depth > 1.5 m even in the driest year (Y2). Indeed crops exhibit better development in the clay loam soil, which is probably due to the larger soil moisture supply of this soil during its most active growing period. The triticale growth was slightly faster than that of wheat and oat, which is likely due to the difference in characteristics of these species. The vegetation heights of pastures, pea and chickpeas is smaller and the vegetation covered the soil surface for a shorter period than cereals.

Soil moisture and crop evapotranspiration (ETa) dynamics

The impact of the succession of crop periods and bare soil inter-crop periods on soil moisture dynamics is presented during Y1 and Y2 monitoring periods. The temporal variations of averaged soil moisture profile from 0 to 100 cm depth for the period January 2001 to August 2001 and January 2002 to May 2002, in the

different land uses are shown on Figure 2a and Figure 2b. As can be seen on Figure 2, the profile-mean soil moisture exhibits very similar temporal patterns at the different sites. There is a very significant and fast replenishment of the soil water store after the rainfalls whereas the decrease in soil moisture after rainfall is gradual and evidently maximum at the end of the dry season. The profiles refill in January of Y2 after a significant accumulation of rain water within the soil during the August-December period in Y1. The mean soil moisture dynamics varies according to the development stages of the crop. The soil moisture in the vineyard plot remains at a value of 40% during the dormant period and only starts to decrease by end of May with the development of the vegetation and of root extraction during the growing period. In contrast, the other land uses, like pastures and annual crops whose vegetation has already developed in January, exhibit a decrease in soil moisture starting in February. The site on pasture-shrubs has the lowest mean soil moisture that should be related to the influence of the root depth parameter. We observed large differences in soil water dynamics among the fababean, wheat, oat, and triticale. This could be caused by (i) differences in the growth periods of the different crops and the larger vegetation cover of cereals compared to fababean, (ii) the varying evapotranspiration of the different plants and (iii) management effects (e.g., planting date, tillage and fertilization). We noted that the development of winter wheat is completed by the end of June or beginning of July, whereas fababean and oat start their growing cycle later in the year and finish their development with their harvest in May. The lower mean soil moisture in oat may also be attributed to the sandy soil texture with a lower water retention capacity. The low soil moisture at the start of the inter-crop periods directly results from the plant water uptake during the previous crop cycle.

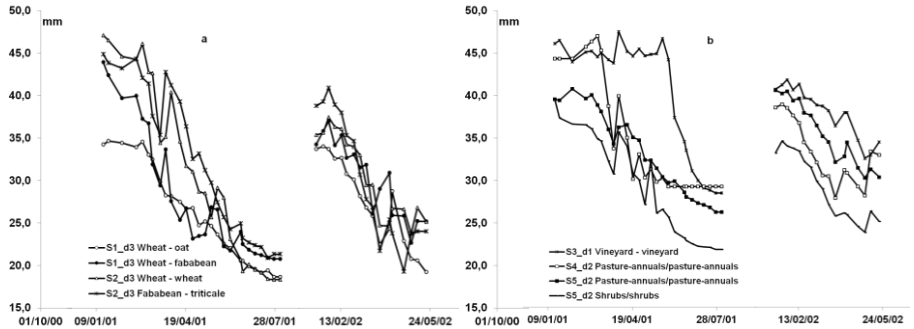

Fig. 2. Temporal variations of mean soil moisture under different land uses for two seasons (Y1 and Y2): (a) the plots with annual crops sequences and (b) the plots with pastures and vineyards

The mean soil moisture ranges from 9 to 32%, with an average value of 18.5%, in the first growing season and it ranges from 11.5 to 25.5%, with an average value of 18.5%, in the second growing season. At the watershed scale, the data of two consecutive growth cycles show that the spatial variation of soil moisture, expressed through the coefficient of variation was at its highest point, reaching almost a CV of 0.25, from march to end of may (Figure 3a, b). This period of large

variation also corresponds to the period over which root water extraction is the highest and can vary largely between the crops dues to differences in canopy extension but also rooting patterns. During the dry summer season and during the winter period, the spatial variation of soil moisture is much smaller. Indeed during these periods, the water consumptions by most crops is low because either the evapotranspiration demand is low or the crop is no longer active. Consequently crops do not appear to influence soil moisture dynamics very much from a quantitative point of view, differences between plots are then restricted and are more likely driven by soil texture. When comparing the average soil water storage at each point between both growing seasons, it may be observed that the points rank similarly as can be seen on Figure 4: the driest and wettest plots remain the same during the two years. Accordingly there is strong linear correlation between the plot soil water storages of the two growing seasons (Figure 4).

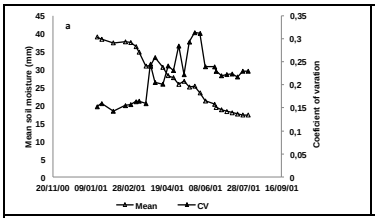

Fig. 3 . Mean soil moisture and coefficient of variation at the watershed scale: (a) the Y1 and (b) the Y2.

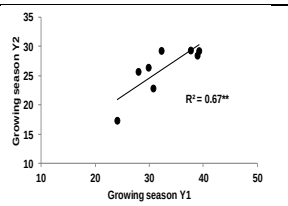

Fig. 4. Regression analysis of average water storage (mm), in the soil layer 0-1 m, for the Y1 and Y2.

Variation of mean soil water content profile for different land uses

The variation of mean SWC profile within 0-100 cm is shown in Figure 5, for the period [January 2001 - August 2001], the period [January 2002 - May 2002], and the period [April 2006 - July 2006], and for different land uses. The mean SWC profile significantly changes with field location within the hill slope, and changes with soil thickness, as previously observed by Qui et al. (2001). Inspection of the 2001-2002 data shows that the pasture sites have the lowest mean SWC profiles and that the largest mean SWC profiles are found on chickpea and pea. We observe increasing and waving trends of soil moisture changes with depth, which is consistent with previous results from Fu et al. (2003). This is ascribed to the high evapotranspiration as compared to the rainfall amount, to the differences in soil physical properties and in root vertical distributions. The increasing type corresponds to the annual crops. The waving type is observed for pastures (mainly shrubs) and vineyard. For pasture on vertisols (field P11), soil moisture varies greatly and is strongly affected by flows of rainfall water within shrinkage cracks in the root zone, where such shrinkage cracks are observed on swelling soils during drying periods. For the [2005-2006] season, we observe that the wheat crop on plot H located on the rim bottom near the watercourse bed, with deep soils depicts larger mean SWC profiles than those observed on the plot M that is located on the

rim top with medium soil thickness. The fababean is located on plot L, a plot that depicted the same characteristics than plot M. However, the fababean shows relatively large mean SWC profile. This difference is ascribed to a larger wheat fraction cover and consequently to a larger evapotranspiration rate. The mean SWC observed on pasture is low.

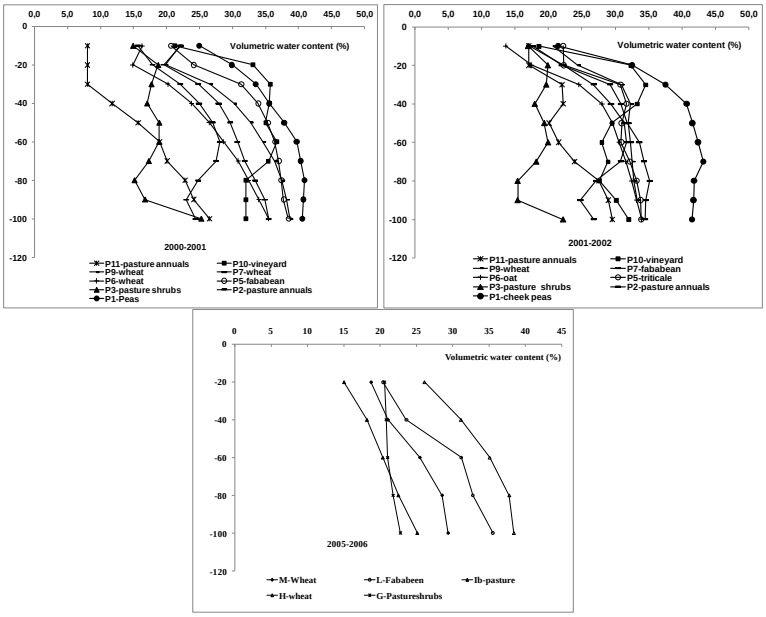

Fig. 5. Variations of mean soil moisture profile in different seasons and land uses.

The monthly ETa values over the period of February to June were estimated either by a soil water balance approach or by eddy covariance according to the available observed data. The period analyzed here can be considered representative of crop growth conditions at this time of year. The cropping periods are marked by increases in ETa, which is consistent with the observed general decrease in soil moisture and canopy growth. Mean daily ETa keeps value lower than 3 mm/day mainly during all the winter-spring seasons. During April, the average ETa shows a higher rate in year Y1 than the ETa of Y2. When soil water is available, during months with high precipitation, the mean ETa can exceed 4 mm/day for wheat and legume fields. The maximum mean ETa rate (5 mm/day) occurred during the maturity stage of wheat with a large spatial variation (3 mm/day). The natural vegetation shows a high ETa during May. After harvest of legumes crops in late spring, the evapotranspiration amounts were significant on bare soils in rows, where weed development is considerable and soil moisture is available.

Impact of land use on water balance partition

The point scale water balance over the 1 m depth layer was obtained by integrating the soil moisture profiles. A large between-plot variation of precipitation can be

observed. There was no runoff for most of the sites; the observed amounts corresponded to less than 10% of the annual rainfall. The observed amount of available water at a given date is also variable, which can be explained by the spatial variation in soil retention capacity, infiltrated rainfall amounts and evpotranspiration fluxes. The amount of available water ranges from 160 mm to 290 mm following the observation time and the location. The amount of available soil moisture at the end of summer ranges from 0 mm to 55 mm during Y1. A larger variability is found in the soil water storage changes ΔS calculated from the observed soil moisture. The available soil moisture varies in relation to land use and crop succession. The observed average potential evapotranspiration for the whole experimental period is equal to 4.4 mm/day in Y1, to 2.2 mm/day in Y2, to 1.6 mm/day in Y5, and about 2.9 mm/day in Y3 and 3.9 mm/day in Y4. This is consistent with previous findings that during summer months, evaporative demand is high and tends to be relatively small in winter season. Despite drought conditions during summer, bare soils following annual pasture and legumes provide larger amounts of soil water as compared to soils with annual crops. During the wheat growing periods, the soil with medium or low soil thickness remains dry in Y1, Y2 and Y5 due to water use by wheat crop. The results show that annual rainfall is mainly converted into evapotranspiration (ETa) during the growing cycle for different land uses. Therefore, ETa is the predominant factor that influences the soil moisture dynamics. The average ETa for the whole experimental period varies from 1.6 to 2.4 mm/day in Y1, from 1.2 to 2 mm/day in Y2, from 0.6 to 1.6 mm/day in Y5, and about 1.3 mm/day in Y3 and 2.1 mm/day in Y4. We observed differences up to 60% in Y1 and Y2 seasons and of 40% in Y5 season. The agreement between the methods measuring ETa (EC, soil water loss) was found to be satisfactory on a cropping cycle periods basis but large differences were observed for daily and monthly timescales. This is due to the inherent difficulty in closing the monthly energy and water balances using independent and spatially-distributed sensors, each characterized by measurement uncertainty as well as issues of representativeness of the contributing area's conditions.

In the site studied, which has a semi-arid climate, significant seasonal variations occurred, in the precipitation and evapotranspiration dynamics that influence soil moisture temporal patterns. With dry soils in summer and wet soils in winter, the variation of the soil moisture content is low, while the spring season corresponds to high rates and large variability of crop water consumption. Accordingly, the spatial variation of soil moisture between the various land uses of the catchment was also at its highest point during this short period, as previously observed by, Hebrard et al. (2006). Land use and agricultural practices are the main factors affecting soil surface conditions and controlling soil moisture. These controlling factors can be more easily identified when different locations within a catchment are compared, especially in areas where soil and vegetation spatial heterogeneities are important, inducing marked differences in soil moisture dynamics (Williams et al., 2004). Here in a semi arid environment, the spatial variation of soil moisture is mainly

locally controlled. This was also shown earlier by Hebrard et al. (2006) in a vineyard catchment located in the south of France. The results show that annual rainfall is mainly converted into evapotranspiration during the growing cycle for different land uses. Therefore, evapotranspiration is the predominant factor influencing soil moisture dynamics. The evapotranspiration ratios differ significantly in relation to land use, soil properties and climate influences. This finding confirms results of previous work. Korres et al. (2015), found a homogenizing effect of the uniform vegetation at the forest test site, contrary to the cropped areas, where the shifted periods of maximum water uptake of the different crops on different fields and the agricultural management generated an increase of spatial variability in the soil moisture patterns. In the current study, it appears that the soil properties affect the interseasonal water storage below the root zone. We observed differences in ETa between seasons up to 60% in 2001 and 2002 seasons and of 40% in 2006 season. As shown by Korres et al. (2015) for a catchment with heterogeneous agricultural use, autocorrelation lengths are :i) short within the growing period of different crops and they are caused by land use patterns, i.e. varying transpiration rates of different crops, and ii) long outside of the growing period and were mainly caused by large scale patterns of soil properties. This experiment shows the impact of the succession of crop periods and bare soil inter-crop periods on the water balance of the field over some period of time.

However, we have just begun to investigate how changes in land use might affect soil moisture profile distribution and evapotranspiration patterns in semi-arid rainfed agrosystems. At the wider scale, the effect of land use and on the water balance is very complex and difficult to determine which, if any, other environmental factor was dominant on both spatial and temporal variability.

CONCLUSION

Despite this limitation, the coupling of soil moisture and evapotranspiration measurements clearly complement each other, contribute to the understanding of the variability and suggest that land use exercised a dominant influence on the spatiotemporal patterns, therefore, on the continuity of hydrological pathways at the catchment scale. The spatial and temporal variability was clearly evident in this experiment and stress the large influence of land use. This has two main implications in water management in rainfed agrosystems, which can lead to positive environmental impacts on surrounding ecosystems. One is the possibility to control in part runoff and downstream water yield by choosing appropriate agricultural practices. It must, however, be stressed that the variation in land use patterns should always be taken into account when estimating and predicting the potential production of water harvesting systems. Second, is the possibility to control the amount of green water. It is also driven and drive the uses of green and blue water since land use and related management practices influence the overall water needs for plant production through the choice of crop types, cropping periods, sowing densities, fertilization levels. Therefore, the following actions are recommended. Ideally, in rainfed agrosystems land use should be optimized to an

efficient use of rainwater. Considerable differences occur with different crop sequences. During initial development of plants, soil water storage directly results from the plant's water uptake during the previous crop cycle. The high soil moisture at the start of the inter-crop periods is accessible to the successive crop. Crop management should be compatible with soil water availability and take into account the beneficial effect of crop sequences as a management strategy. Finally, crop transpiration generates high ETa over short time periods, thus accounting for crop type to accurately estimate ETa amount and ETa temporal dynamic, which are both critical to properly represent land-surface atmosphere interactions.

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COMMUNAL LANDS AND RURAL DEVELOPMENT IN THE NORTHWESTERN IBERIAN PENINSULA

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ABSTRACT

Communal lands occupy about one million hectares in the northwest Iberian Peninsula with high average areas (500 hectares in Portugal and 200 in Galicia). The region is among the poorest in the European Union with a notably lower gross domestic product in comparison with the most developed regions of Europe. Over centuries, *'Baldios'* in Portugal and *'Montes Veciñais en Man Común'* (MVMC) in Galicia played an essential role in the rural economy of their owner's communities. They were mainly used in forestry, but several reasons resulted in a current sub-utilization of them. This role was lost during the twentieth century due to great reforestation and a decline in agriculture prominence. The restoration of democratic regimes returned Baldios and MVMC to their owners, now declining, aging and disorganized. Taking into account the extension of these lands and their average size, this paper looks into the main historical determinants of the commons existence and tries to illustrate their present-day with reference to the collective action problem; features related to the commoners' (*'veciños'* and *'compartes'*) characteristics and to the way they use their lands are analysed. Both Galician and Portuguese realities exhibit similarities and complementary benefits requiring social innovation to make better use of rural resilience. Communal lands and small-scale business initiatives could support the network of the local produce markets with attractive values, and also the conservation of the biodiversity. These data are discussed focusing at the human and natural resources.

Key words: *Common lands, northwest Iberian Peninsula, local development*

INTRODUCTION

For centuries the communal lands of northwest Iberian Peninsula (*'Baldios'* in Portugal and *'Montes Veciñais en Man Común'* −MVMC− in Galicia played a crucial role in the rural economy of their owner communities (Baptista *et al*., 2002). This function was lost during the 20th century due to the massive forestation and the decline of agriculture. The restitution of democratic regimes in both countries returned these commons to their owner communities, now declining, aging, disrupted and disorganized (Magariños, 1999). As the rural world they

belong to, they are now facing a number of threats and challenges. Communal lands occupy approximately 1 million hectares, 400,000 in north Portugal and 600,000 in Galicia, with high average areas (around 500 ha in Portugal and 200 ha in Galicia). They are owned by approximately 2900 communities in Galicia and 1000 in north Portugal. The use of these lands is primarily forestry, but several reasons resulted in a current sub-utilization of their potential (Sineiro, 1998).

The MVMC and the Baldios are currently faced with challenges that are common to the rural world, as well as with other challenges arising from the peculiar nature of their form of property (Baptista *et al*., 2002).The living strength and the reason for their survival lies in the fact that they are an inseparable reality from the life of the local populations, strongly marked by a communal way of living. According to Saco (1998), *'this is a form of communalism that expresses itself not only in the organization of commons uses but also in the joint usufruct of certain facilities (such as clothes washbasins, hearths, wind- and water-mills) as well as the collective management of water supplies for irrigation, festivities, pathway maintenance and repair and for performing a number of farming tasks'*.

Our aim is to lay the ground for a debate on to what extent the commons may feasibly subsist, given their unique form of property and the widely democratic nature of their organizational aspects, and surrounded as they are by a different reality that appears to be less and less indifferent to their existence.

MATERIAL AND METHODS

Resorting mostly to Galician and Portuguese literature, the paper seeks to describe the more determining factors of their historic evolution, from a time when communal lands set a essential balance with the traditional agrarian system, through periods of intense privatizations, alienations and administrative reforms that coincided with liberalism, to the systematic afforestation imposed in the 20th century by both Franco's and Salazar's regimes (Baptista, 2001). These common areas were the mainstay of the traditional agricultural system up until modern times and they have played a unique role in preserving the relationship between landowners, land users, and land. Stockbreeding and agriculture were, according to Balboa (2000), the two activities making the most use of the MVMC, largely on account of the use of furze as livestock bedding material, small-ruminant feed for its shoots, household fuel and organic fertilizer allowing for the development of other crops. Estêvão (1983) points to a similar role played by the Baldios: besides firewood, charcoal, timber, honey, etc. They provided the necessary pasture for livestock feeding while the brushwood and manure combined provided the traditional fertilizer used by the peasantry. So within the context of traditional agriculture, the use of MVMC and Baldios was a practical and appropriate way of ensuring a balanced agrarian system (Pereira, 1999).

RESULTS AND DISCUSSION

The demographic growth from the 18th century onwards (and the resulting need to expand croplands) was partly responsible for disrupting this balance. The

ideological arguments (namely, with 19th century liberalism, the full support for private property ownership rights) and the developmental theories would soon make themselves heard. Bica (2004a) referring to Portugal, and Pereira (1999), referring to Galicia, use the same expression, *'disentailment frenzy'*, to describe the type of liberal intervention that was directed at the commons. Pereira (1999) points to an estimated one million hectares having been withdrawn from communal ownership in Galicia between 1860 and 1930. As for Portugal, Estêvão (1983) estimates that approximately 2.5 million hectares were turned into cultivated land between 1874 and 1902 and another 300 thousand followed suit in the period between 1903 and 1933, adding that *'the commons played a vital role in the increase of arable lands in the last quarter of the 19th century'* (…) and that *'in the first thirty years of this century (1903-33), such role was played by privately-owned holdings in the South'*.

The move towards disentailment raised complaints, protests and resistance of all sorts (Rodrigues, 1987; Magariños, 1999; Pereira, 1999; Bica, 2004a). New roadways for social development were being sought, but the foundations of the traditional agrarian and stock farming system and its centuries-old balance were being put at stake. The transition period into the 20th century witnessed a decisive commitment on part of the state, both in Galicia and in Portugal, towards afforestation of wildlands, communal lands and hillside areas. On top of the consequences (identical in Galicia and in Portugal) of this foretold intervention, there were the pressures for the shared allocation of the MVMC and Baldios. Ribeiro (1970) referring to the region where the largest private pinewood-planted area is located in Portugal, says that *'in the beginning of the century, on the initiative of the peasants and before the state would intervene and confiscate their commons, pinewoods came to cover these wildlands, up until then known to goat keepers and coalmen alone'*. Likewise, in this disentailment period, rural communities in Galicia divided the commons among the veciños on several occasions, *'anticipating what they presumed would most certainly be an expropriation, given the legal uncertainty surrounding community-owned properties'* (Pereira, 1999).

The individualization of the commons was, after all, a means of securing the resources they had always provided to the peasants. With individualization the road was open towards the progressive loss of the functions of the MVMC. One of the main reasons for this *'defunctionalisation'*, as Balboa López (1995) calls it, is the disappearance of joint disciplines and the new dependency of the commons on individual (and often diverging) strategies and decisions. The author adds that *'such defunctionalisation'* has a social dimension to it as well, for the community looses much of its cohesion capacity. The productive balance between commons and cultivated lands is lost, and so is the social balance between the household and the community, a situation most apparent during Franco's regime' (Balboa López, 1995).

The emergence of authoritarian regimes in Portugal and Spain meant that the state carried through the expropriation of those communal lands that had survived these

processes of shared allocation, alienation and individualization. In both cases, there were notorious coincidences of financial and industrial interests in the assignment of vast areas of land for afforestation. Among other examples, with regard to Galicia, Rodríguez (1999) refers to the interests of paper monopolies, and Magariños (1999) denounces the systematic use of eucalyptus in the reforestation activities. Estêvão (1983) draws a connection between this trend and the industrial expansion in Portugal. For one thing, in the aftermath of the cereal campaigns in the south of the country, the fertilizer industry gained new clients, in fact due to afforestation, farmers (north of the Tajo River) saw themselves deprived of their basic source of organic matter, i.e., brush lands and animal manure. More significantly, *'since it would be unlikely that the lesser layers of the peasantry would buy large quantities of chemical fertilizers, in the end it was the expulsion of this social stratum of the peasantry from their place of residence that would lead to the influx of abundant, cheap and unskilled labour into the urban centres, while forcing those that remained to take up a modern, intensive form of agriculture, based on the use of fertilizers, machinery and reduced labour'* (Estêvão, 1983).

In Galicia, communal land property was forbidden by law as from 1940 and the related traditional uses were restricted or even prohibited (Magariños, 1999). In Portugal, the great plantation of hillside areas, better said, of the commons, was announced in 1938 with the promulgation of the Afforestation Law. This law became the main regulation concerning communal lands and, as opposed to what applied until then, determined that the commons would become the property of the state as and when they became afforested. Pereira (1999) work *'O monte comunal na Galicia contemporánea: Unha historia de resistencia'* (The commons in contemporary Galicia: A story of resistance) contains many descriptions of the struggles and protests raised in defence of the commons. Such resistance is also signalled in Portugal by a number of authors, e.g.: Rodrigues (1987) and Gralheiro (1990). Quoting Estêvão (1983) again, *'in some instances, instead of starting afforestation in areas where the rural communities would be less affected, in both their actual farming and others activities, like sheep grazing, wood logging, etc., tree planting would begin precisely in the commons closest to the villages'*. Pereira (1999) also denounces the forced recruitment by the Civil Guards to fight forest fires. Bica (2004b) notes that the villagers deprived of their commons *'would find no other work apart from the humiliation of having to take up a job in planting their own commons on behalf of the Forestry Services'*.

Nevertheless, the disregard for or the failure to safeguard the rights of the people were not sufficient to erase completely an age-old social reality: the exclusive right held by the villagers of certain places or *'parroquias'* to use certain MVMC and Baldios. Besides, the migration flows, on one hand, and the new industrial policies linked to the emergence of different markets, on the other, weakened some aspects of the afforestation approach. The advance of the forest would go only as far as it proved relevant for industrial development and could actually risk affecting agricultural crops that had evidenced sufficient economic strength. The lack of interest for massive reforestation, the inefficiency of the state authorities in

maintaining the wealth thus created, the rural communities' protests against the occupation of their lands, the artificial, not to say parasitic, nature of the management carried out by many local authorities, all this gave way, with the advent of political changes towards democratic practices, to a number of legislative initiatives seeking to hand back the MVMC and Baldios to the local communities.

In 1976 is promulgated in Portugal the law returning the *'use, the income and the administration of the Baldios to their respective compartes'*. This law allows two administration procedures for the Baldios –fully by the compartes or by an association between the compartes and the state–. In Spain, there were several initiatives to legislate the *'veciñal'* property between 1957 and 1968. In 1980 is published a new law highlighting the non-public attribute of the MVMC, and in 1989 (when the MVMC are already under the juridical competence of the Government of Galicia) is promulgated the law establishing that the MVMC belong to the *'agrupacións vecinãis na calidade de grupos sociais'*, communities, and the communities may collectively manage these or may delegate their management to the Forestry Administration by way of a contract (López, 1995; Díaz, 1999).

In 2001, twenty five years after the promulgation of the legislation which returned the Baldios to the local communities, the performance of the state regarding the application of the law was strongly disapproved by the communities (Carvalho, 2001). The main criticisms regarded the lack of state investments in the commons and the neglected accompanying of its own projects, and the delayed responses regarding for instance the permission to sale the goods produced in the Baldios. In addition, complaints were also made regarding the lack of use of the potential European Union financial supports.

In Galicia, the commons were also the object of strange behaviours on part of the state structures. Sineiro (1998) denounces *'the notorious lack of government support to the real autonomy of the comunidades veciñais'* and *'the non fulfilment of its legal duties, such as the preparation of the Rexistro de Montes Veciñais (commons registration) containing an update on their situation, their use and their boundaries'*. Garcia (1998) also points out *'the government's neglect towards the monte comunal'*, evidenced in particular by *'the lack of technical and economic support, the non-existence of a fire prevention policy, the scarce interest in considering the commons as a distinct reality that must be preserved under the same conditions as any other aspect of the national heritage'*. From another viewpoint, Escariz (1998) stated that *'in the current legal framework there is no clear rule on the tax regime of the Comunidades de Montes Veciñais en Man Común, or communally held properties'*, which he adds, *'besides being a violation of the principle of legal security, stands against the constitutional principles of legality and equality'*. In its turn, referring to the ways in which the communities are organized, a Galician survey underlines the long distance between reality and what is provided by law –in half of the communities, either there is no Xunta Rectora (Ruling Board) or if it exists it is only a name, i.e. it doesn't operate–. The same survey further points out that the organized communities, while being less

numerous than the non-organized, hold altogether a larger commons area and predominate in regions with a higher economic development and a more dynamic demographic behaviour, being more abundant when the MVMC include a forest component (Fernández *et al*., 2006).

Recent work, support by extensive field surveys and experimental research, helped to expand the theory of common property. According to Poteete (2004) *'the clarification of concepts allowed scholars to recognize the possibilities for sustainable management of resources under common property as opposed to open access, and to raise questions about the supposed superiority of private property rights for the management of common-pool resources'*.

A lot of the motivation to perform early case studies and to develop theoretical work was based on an effort to try to bring forward evidences to refute Hardin's theories and the policies of privatization and state takeover of resources. However, to further develop the Common Pool Resources (CPR) theory, it is still necessary to go beyond simple descriptions (almost always successful ones) and designing principles (Edwards and Steins, 1997).

CONCLUSIONS

The heterogeneity of existing situations and the complexity of the involved institutions (including property) exclude only one *'scientific truth'*. Common property, private property, state property, all have shown to be able and unable in providing sustainability, organization, and investment capacity in the management of the natural resources.

As a global conclusion of our work, the communities owning the communal lands currently seem to have the conditions to successfully manage their commons if the commoners are able to mobilize and organize their communities. For that purpose, they should be able to valorise: i) their cultural and heritage patrimony; ii) the natural resources and the biodiversity conservation potential; iii) the productive potentials; iv) the new uses presented by their lands.

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THE ROLE OF INNOVATION ON COMMERCIAL FARMS IN POLAND

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ABSTRACT

Changes in relations between production factors necessitate surveys into their effect on the total value of production. This paper aims to determine the extent to which the involvement of production factors in the production process, in particular in connection with progress, that is, broadly interpreted innovation, had an influence on the value of production of specific size farms determined based on cropland area. The surveys were based on figures recorded for individual commercial farms registered in the database of the Polish Farm Accountancy Data Network (FADN). It ensured the methodological uniformity of data used in this paper. The analysis of the production process on farms was carried out by means of the Cobb-Douglas (C-D) production function method. The study makes it possible to evaluate changes in the productivity of production factors on commercial farms in years covered by the surveys. The flexibility of relations between total production in PLN (Polish Zloty) and production factors, i.e. labour output in man-hours and total costs in PLN, was analyzed. Changes in the management effectiveness of commercial farms which occurred when Poland joined the European Union were evaluated. According to the survey, the level of technical and organisational progress was the highest on farms with the largest cropland area, i.e. 30 =< 50 ha and more than 50 ha, as well as on farms with the smallest cropland area, i.e. less than 5 ha. The size of the farm sufficient to satisfy the requirement of farming products is 10=<20 ha of cropland and 20=<30 ha of cropland.

Keywords: *agriculture, commercial farms, Poland, production factors, innovation.*

INTRODUCTION

With regard to the necessity to secure the food requirements, despite its small share in the Gross Domestic Product (GDP) agriculture supports permanent economic development. The fundamental task of agriculture is producing easily accessible food at a relatively lower cost. In compliance with the theory of economics, full rationality in agriculture leads to the effective utilization of resources and achievement of the economic optimum; however, neglecting external effects.

Previous studies indicate that agriculture based exclusively on the criteria of microeconomic rationality leads to discrepancies between the microeconomic minimum and the social optimum, which causes the loss of social parts and social environmental benefits (Zegar, 2010). The development of Polish economy based on innovations makes it possible to include Poland in the group of highly developed countries (Chyłek et al., 2016). Innovation is inseparably linked with change. The term 'innovation' was introduced into economic studies by Schumpeter who understood it broadly as the production of new products or improvement of the existing ones, the introduction of a new or improved method of production, the opening of a new market, the use of the new selling or purchasing method, the use of new raw materials or semi-finished products, and the introduction of the new organisation of production (Schumpeter, 1960). In turn, the definition of innovation according to the OECD is the implementation of a new or significantly improved product, service or process in business practices, including the implementation of a new marketing or a new organisational method redefining the method of work or relations between the business and the environment (OECD, 2005). The knowledge capital, meaning R&D-related activities, plays a key role in enhancing competitiveness and accelerating economic growth and transformation, both in the domestic economy and in relation to particular sectors, including agriculture (Kijek et al., 2016). In Poland the rate of innovative progress in agriculture is much lower than in other sectors of economy and the implementation of the 2020 strategy in Polish agriculture is carried out using funds provided under the Rural Development Programme (Nosecka and Zaremba, 2016). Improvement of the economic results of all farms is one of the main objectives which can be achieved thanks to increasing the profitability of farms and their competitiveness. Poland has noted down quite a rapid growth in agricultural production, export of agricultural and food products, an increase in income and life standard in rural areas with a relatively low level of productivity of agriculture, overemployment, and poor agrarian structure (Wilkin and Nurzyńska, 2016).

Changes in relations between production factors necessitate surveys into their effect on the total value of production. This paper aims to determine the extent to which the involvement of production factors in the production process, in particular in connection with progress, that is, broadly interpreted innovation, had an influence on the value of production of specific size farms determined based on cropland area. It also attempts to evaluate the effectiveness of production of the analysed factors during the term of Poland's membership in the European Union.

MATERIALS AND METHODS

The surveys were based on figures recorded for individual commercial farms registered in the database of the Polish Farm Accountancy Data Network FADN (Goraj and Mańko, 2009). In the European Union commercial farms have a cropland area of at least 1 hectare, and if they are smaller than 1 hectare – they supply a major part of their products to the market or their production exceeds the standard production volume (Goraj and Olewnik, 2011). The analysis made use of

the classification of commercial farms according to cropland area. Table 1 presents the number of farms in a sample covered by the survey grouped according to farm area.

Table 1. The sample of farms covered by the survey according to classes of cropland area in 2004, 2009 and 2012.

Specification	Number of farms in the sample					
	<5 ha	5=<10 ha	10=<20 ha	20=<30 ha	30=<50 ha	>=50 ha
2004	574	1571	3484	2130	1834	1399
2009	354	1185	3012	2156	2134	2096
2012	203	928	2671	1923	2035	2171

Source: Own calculations based on unit empirical data from the monitoring of the Polish FADN

The evaluation of factors shaping the economic situation of agriculture in the period of accession to the European Union is of particular importance with regard to high expectations but also with regard to fears (Zegar, 2009). Years covered by the survey: 2004, 2009, 2012. For the purposes of the study the relationships describing the process of production on farms were quantified. The production function is used for mapping relationships between the outcome of this process and factors involved in this process (Welfe and Welfe, 1996). The production process on commercial farms covered by the survey was analysed by means of the Cobb-Douglas (C-D) production function, which facilitated the analysis of substitution of production factors, the analysis of the productivity of production factors as well as horizontal and vertical comparisons.

The studies made use of the production function taking labour and capital into account (Tomczak, 1983; Bezat and Rembisz, 2011). Land was not included in the equation, among other reasons, with regard to significant limitation of the scope of variability of this characteristic in comparison to others by identifying farm groups according to their area. The objective of the studies was accomplished by means of the Cobb-Douglas (C-D) production function method because it constitutes the theoretical basis for explaining most regularities concerning effectiveness in the economics of agriculture (Bezat and Rembisz, 2011). The function is as follows:

$Y = aX_1^{\alpha} X_2^{\beta} d$, where:

a – constant describing the specific level of technical and organisational progress,
Y – total value of production in PLN (according to Polish FADN: SE131),
X_1 – total labour input in man-hours (SE011),
X_2 – stream of capital determined by the costs of production (SE270) in PLN,
α, β – regression coefficients specific to respective factors,
d – random factor.

The measurement of business production effectiveness has been a developing concept in recent decades. Production effectiveness surveys are a standard procedure allowing rational allocation of resources. High operating effectiveness of a commercial farm is a reason for continuing investment, whereas low effectiveness is a warning that the activity should be limited or discontinued (Adamkiewicz-Drwiłło, 2002).

RESULTS AND DISCUSSION

The symbols of characteristics are used consistently throughout the paper and the content and symbolic of the variables comply with the methodology used in the Polish FADN. The initial value of the production potential contributes to multiplying but does not ultimately determine this potential (Góral, 2016). The cross-functional surveys assume that production factors are continuously substituted and that every combination of production factors matches a clearly defined level of production (Niezgoda, 1986). The production function expressing the relationship between total production in PLN (SE131) as a dependent variable and human labour in man-hours (SE011) and the total cost in PLN (SE270) as independent variables was illustrated by the following equations:

I. Cropland area group <5 ha

2004: $SE131' = 1.2091\ SE011^{0,0682}\ SE270^{0,9538}$

2009: the analysis of the production function was abandoned since the constant (a) and the human labour in man-hours (SE011) turned out to be statistically insignificant.

2012: $SE131' = 1.6101\ SE011^{0,1141}\ SE270^{0,9012}$

II. Cropland area group 5=<10 ha

2004: $SE131' = 0.6280\ SE011^{0,1251}\ SE270^{0,9698}$

2009: $SE131' = 0.3109\ SE011^{0,0774}\ SE270^{1,0610}$

2012: $SE131' = 0.4944\ SE011^{0,1067}\ SE270^{1,0079}$

III. Cropland area group 10=<20 ha

2004: $SE131' = 0.9143\ SE011^{0,0903}\ SE270^{0,9648}$

2009: $SE131' = 0.1670\ SE011^{0,1178}\ SE270^{1,0827}$

2012: $SE131' = 0.4941\ SE011^{0,1210}\ SE270^{0,9944}$

IV. Cropland area group 20=<30 ha

2004: $SE131' = 1.3508\ SE011^{0,1039}\ SE270^{0,9254}$

2009: $SE131' = 0.1794\ SE011^{0,1008}\ SE270^{1,0858}$

2012: the analysis of the production function was abandoned since the constant (a) turned out to be statistically insignificant

V. Cropland area group 30=<50 ha

2004: $SE131' = 1.5202\ SE011^{0,1080}\ SE270^{0,9162}$

2009: $SE131' = 0.2641\ SE011^{0,0657}\ SE270^{1,0752}$

2012: $SE131' = 1.7748\ SE011^{0,0462}\ SE270^{0,9430}$

VI. Cropland area group >=50 ha

2004: $SE131' = 2.0156\ SE011^{0,0604}\ SE270^{0,9268}$

2009: the analysis of the production function was abandoned since the human labour in man-hours (SE011) turned out to be statistically insignificant

2012: $SE131' = 2.4493\ SE011^{-0,0307}\ SE270^{0,9728}$

The analysis was limited to statistical elements of functions that are significant at the adopted level of probability. Statistical verification of regression coefficients in the equations was performed by means of t-Student test assuming the level of significance $\alpha = 0.01$. A statistically significant level of multiple correlation coefficients indicates that this function model matches the coordinates of the analysed characteristics. The presented equations are characterised by a high degree of probability for regression coefficients in the groups of farms and years covered by the survey.

The power function is a function with a constant (irrespective of the value of respective variables) elasticity of the dependent variable, and the elasticity of respective variables equals the evaluations of parameters describing such variables (Czekaj, 2006). The coefficients of production elasticity for respective production factors indicate that the increase in the value of production was to the highest extent shaped by the stream of capital. Such trends in 2004-2007 were also identified in the studies by Niezgoda (2010). The optimum utilization of production factors on a farm using the mechanism of substituting capital for human labour considerably determines the resulting level of production.

Table 3 presents the level of the coefficient of total elasticity of production factors in relation to the production value and the parameter "a" describing a specific level of technical and organisational progress.

Table 3. The level of total production elasticity coefficient (SE131) in relation to workload (SE011) and the stream of capital (SE270) and the parameter "a" describing a specific level of technical and organisational progress for farms covered by the survey grouped according to cropland area in 2004, 2009 and 2012.

Specification	<5 ha	5=<10 ha	10=<20 ha	20=<30 ha	30=<50 ha	>=50 ha
	Total production elasticity coefficient for the analysed factors					
2004	1.0220	1.0950	1.0552	1.0294	1.0241	0.9872
2009	-	1.1384	1.2005	1.1866	1.1409	-
2012	1.0153	1.1146	1.1154	-	0.9892	0.9421
Specification	Parameter "a" – describing a specific level of technical and organisational progress					
2004	1.2091	0.6280	0.9143	1.3508	1.5202	2.0156
2009	-	0.3109	0.1670	0.1794	0.2641	-
2012	1.6101	0.4944	0.4941	-	1.7748	2.4493

Source: Own calculations based on unit empirical data from the monitoring of the Polish FADN.

The total elasticity coefficient is a measure of the impact of changes in production factors on the scale of production (Santeramo, 2014). The power function is characterised by the constant elasticity of production in relation to the factors irrespective of the level of their utilization and the resulting production volume. Regression coefficients are also coefficients of production elasticity in relation to factors (Doszyń, 2012). The analysis indicates that a simultaneous increase in the involvement of every factor by 10% maintaining proportions between factors

resulted in an increase in total production in the analysed years for very small farms by 10.22% in 2004 and by 10.15% in 2012, whereas for small farms by 10.95% in 2004, 11.38% in 2009, and 11.15% in 2012, which means the benefits of scale (Milewski and Kwiatkowski, 2013). Similarly for quite small farms, the increase in the involvement of factors by 10% contributed to an increase in total production by: 10.55%, 12.01%, and 11.15% respectively and for quite large farms by 10.29% in 2004 and by 11.87% in 2009. In turn, for large farms the increase in the involvement of every factor by 10% contributed to an increase in total production by: 10.24%, 11.41%, and 9.89% respectively and for very large farms by 9.87% in 2004 and by 9.42% in 2012. It means that in 2004 the increase in the involvement of factors by 10% contributed to a less than proportional increase in total production only for very large farms, and in 2012 also for large and very large farms, whereas for very small, small and quite small farms the increase was more than proportional. Those were the effects of less intensive organisation of production on small farms compared to farms with a large area. In 2009 the increase in production was more than proportional for all the analysed farms grouped according to area, while the level of parameter "a" describing technical and organisational progress was low. This suggests that it is difficult to increase the already high level of technical and organisational progress (innovation) for farms with a large area, whereas increasing the level of innovation for farms with a small area where the level of innovation is lower brings better effects. Due to the deficiency of capital some farmsteads cannot increase innovation in the production potential, e.g. change production techniques to more rationally accommodate their resources in products e.g. introducing new varieties or ecological production (Jóźwiak at al., 2012).

CONCLUSION

The basis for determining the competitive advantage of farms is product, process and marketing innovations. According to the survey, the level of technical and organisational progress was the highest on farms with the largest cropland area, i.e. 30=<50 ha and more than 50 ha, as well as on farms with the smallest cropland area, i.e. less than 5 ha. In turn, the differentiated level of production elasticity coefficient confirms the advisability of separating area groups from the whole collective of farms. It also means that the size of the farm sufficient to satisfy the requirement of farming products is 10=<20ha of cropland and 20=<30ha of cropland. Those were the effects of less intensive organisation of production and probably of substituting human labour with capital. It was economically justified in connection with EU grants. The surveys aim to improve the agrarian structure.

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INVESTIGATING THE EFFECT OF ARBUSCULAR MYCORRHIZA *GLOMUS* Sp. AS A BIOFERTILIZER ON LETTUCE PRODUCTION

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ABSTRACT

Arbuscular mycorrhiza used recently as biopesticide has shown beneficial effects on plant growth. An experiment was conducted in West Bekaa in 2016 in order to investigate the effect of a commercial biostimulant (MYCOSAT) containing 5 *Glomus* species on the production of two lettuce varieties: Romaine and Iceberg. Plant growth and nutritional quality were compared between mycorrhizal plants (mycorrhizal Romaine: MR and mycorrhizal Iceberg: MI) and non-mycorrhizal plants (non-mycorrhizal Romaine: NMR and non-mycorrhizal Iceberg: NMI). Measurements were done on root and leaf parameters and results showed a significant positive effect of mycorrhizal inoculation on plant growth. Best results were obtained for root parameters as well as leaf area and leaf weight in mycorrhizal plants of both varieties compared to non-mycorrhizal plants. An improvement was found in root length, root diameter, number of secondary roots and root weight by 81%, 81%, 61% and 60% for MR plants and of 80%, 88%, 84% and 94% in MR and MI in comparison to NMR and NMI. Leaf number was only enhanced in MI plants. The improved crop performance was associated with an ameliorated nutritional status with higher percentages of N, P, and K in leaves and was correlated to a stronger root development in mycorrhizal plants due to the action of arbuscular mycorrhiza. Finally, the application of the biostimulant MYCOSAT could provide a biological tool for improvement of growth and quality of lettuce grown in clay soils of West-Bekaa.

Keywords: *Bekaa, Lactuca sativa, Arbuscular mycorrhiza, Root growth, Plant Growth*

INTRODUCTION

Sustainable agricultural production is based on enhancing natural biological processes above and underground. Symbiotic associations are underground

biological processes considered as important approach for a profitable sustainable agriculture (Smith *et al*., 1968). Arbuscular mycorrhiza is one biological strategy (Cekic *et al.,* 2012) that could be suitable in Mediterranean regions including semi-arid conditions where soil is poor in organic matter and soil particles inhibit root development and spread (Coons *et al.,* 1990). Arbuscular mycorrhizal colonization is reported to promote plant growth (Evelin *et al.,* 2011); In fact, the association of AMF with plant roots creates an intimate link between plant roots (Johnson et *al.*, 1997) and improves plant adsorption of water and nutrients, consequently plants are better fed, watered and are more tolerant to biotic and abiotic stress factors. Therefore, this strategy induces a reduction in the use of chemical fertilizers and pesticides resulting in higher crop sustainability (Candido *et al.,* 2013).

AMF (Arbuscular Mycorrhizal Fungi) is associated with the vast majority of higher plants. It is the most common endomycorrhiza (Brundrett *et al.,* 1996) and it stimulated the hormones regulating plant growth and accelerates the rate of photosynthesis (Al-Karaki, 2006). Glomus is one very common genus of the AM (Torrey, 1992). The potential of mycorrhization differs among species (Ma *et al*., 2001). For instance, the beneficial effect of AMF on plant growth has been proven in the traditional cultivation of lettuce (Kowalska *et al.,* 2015). This shallow-rooted crop is sensitive to any soil deficit and has the ability for a beneficial symbiotic mycorrhizal fungi association (Ma *et al.,* 2001). Lettuce (*Lactuca sativa* L.) is widely grown under greenhouse and open-field conditions in the Mediterranean region, particularly in Lebanon where it is a valuable crop, and its high quality yield is an essential prerequisite for its economic success. In 2014, harvested area reached 1417 ha with a total production of 37709 tonnes (FAOSTAT, 2017). Bekaa plain is one the main areas of lettuce cultivation in Lebanon that is characterized by heavy clay soils. Lettuce production in this region employs a heavy chemical fertilization to promote plant growth. Therefore, the study investigated the effect of applying a commercial biostimulant of symbiotic endomycorrhizal fungi (MYCOSAT TAB, VALLE D'AOSTA, CCS) with 5 *Glomus* sp. (*Glomus coronatum, Glomus Caledonium, Glomus intraradices, Glomus mosseae, Glomus viscosum*) on the performance of two common lettuce varieties: Romaine and Iceberg as an environmentally safe method that could alternate heavy chemical fertilization in Bekaa.

MATERIALS AND METHODS

The experiment was carried out in open field, under summer conditions at an open-field situated in West Bekaa where soil was a silt-clay soil, rich in calcium, poor in organic matter and containing acceptable values of nitrogen, potassium and phosphorus. Seedlings were transplanted at the stage of 2-3 leaves on 20 May 2013 into previously prepared seedbeds.

Seedlings of Romaine and Iceberg varieties were subjected or not to mycorrhizal inoculation. The experimental design was a randomized complete block design and investigated treatments were four: MR: Mycorrhizal Roman, NMR: Non-Mycorrhizal Roman, MI: Mycorrhizal Iceberg, and NMI: Non-Mycorrhizal Iceberg

with 200 plants per treatment. The commercial biostimulant was added at the same day of transplantation. It was dissolved with water to prepare a solution of a concentration 5g/l, thus of 5ml of the solution were provided to each plant. Data was recorded twice: 25 DAT (days after transplanting): date 1 and 50 DAT: date 2 on a sample of 15 plants from each treatment. Diameter and length of the principal roots were measured using a sliding caliper. Then, plants were cut at the junction level to measure roots and leaves weight separately. Leaf area was calculated by multiplying the length and width of leaves. Finally, the macronutrients composition was analyzed on the mill of oven-dried leaves; nitrogen content was determined using Kjeldhal digestion procedure, phosphorus content was measured using Vanadate-Molybdate-Yellow method and potassium content was evaluated through flame photometer. The effects of the factors (variety and mycorrhizal application v.s. mycorrhizal absence) and their interactions on the averages of the measurements were analyzed using Factorial ANOVA. Correlations between aboveground and underground parameters were also studied.

RESULTS AND DISCUSSIONS

Results of the factorial analysis (Table 1) showed that the non-interactive effect of the experimental factors was significant regarding all parameters. In addition, for the first (line 4 to line 6) and second (line7) orders, interactive effects of the different investigated factors were mostly significant with some exceptions:

For the first order interactive effects: the combination effect of time and variety on number of leaves, the combination effect of time and mycorrhizal application on the number of leaves and the combination effect of variety and mycorrhizal application on root diameter and root length. For the second order interactive effects: the combination effect of time, variety and mycorrhizal application on root length and leaf area.

Table 1. ANOVA null hypothesis rejection probability ($P_{value} \leq 0.05$) for the effects of the experimental factors and their interactions on the different measurements averages.

Effect	N.S.R	R.D	R.L	R.W	L.N	L.W	L.A
Date (d)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Variety (v)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mycorrhiza (m)	0.0000	0.0000	0.0000	0.0000	0.0312	0.0000	0.0000
d*v	0.0000	0.0000	0.0000	0.0000	0.0955	0.0000	0.0000
d*m	0.0000	0.0000	0.0000	0.0000	0.4542	0.0000	0.0000
v*m	0.0000	0.1046	0.1503	0.0000	0.0102	0.0000	0.0029
d*v*m	0.0000	0.0000	0.0720	0.0000	0.0218	0.0000	0.7660

N.S.R.=Number of Secondary Roots, R.D=Root Diameter (cm), R.L=Root Length (cm), R.W= Root Weight (g), L.N=Leaf Number, L.W=Leaf Weight (g), L.A=Leaf Area(cm^2).

Results (Figure 1) reflected a positive effect of mycorrhizal inoculation on the majority of parameters and this effect was mainly evident at the second date of sampling. It improved the diameter of the root neck and its effect differed among both varieties according the date of sampling. At date 1, root neck diameter did not differ significantly between mycorrhizal and non-mycorrhizal plants of each variety and between treated plants of both varieties, however at date 2 it differed significantly for both cases with superiority for mycorrhizal plants of both varieties (MR: 31.52 cm v.s NMR: 25.46 cm and MI: 24.81 cm v.s NMI: 21.85 cm). The same tendency was observed for the variation of the average values of the following parameters: root length, number of secondary roots and weight of leaves where superiority was maintained for mycorrhized plants of both varieties at date 2. Also, root weight was obviously not affected by mycorrhizal application at date 1 since there was no significant difference between average values related to MR and MI compared to NMR and NMI. However, at date 2, differences in root weight were observed only for the Romaine variety where MR recorded a significantly higher average compared to NMR (35.23 g and 21.03 g respectively). In addition, at date 2, arbuscular mycorrhiza enhanced the average number of leaves of Iceberg lettuce (MI: 35.0 v.s NMI: 31.6) while it did not create any significant difference of leaf number of Romaine lettuce during the whole vegetative cycle. Finally, at date 1 average leaf area was almost similar in Romaine and Iceberg plants with or without mycorrhizal application while at date 2 it was significantly higher in treated plants of both varieties (MR: 125.03 cm^2 v.s NMR: 75.94 cm^2 and MI:164.55 cm^2 v.s NMI: 106.34 cm^2).

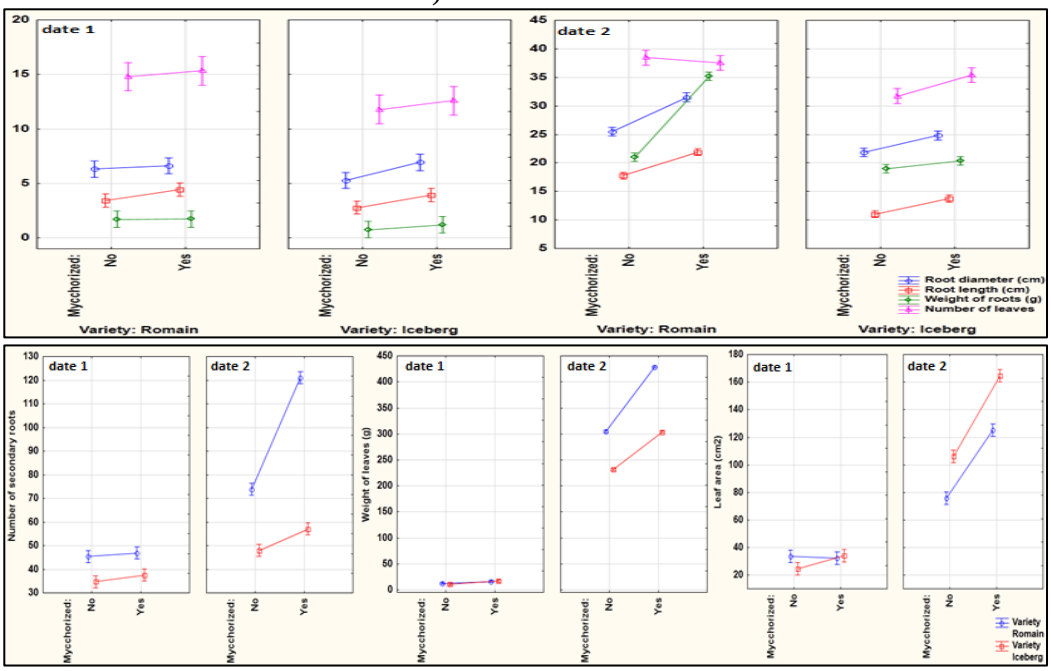

Figure 1. Variation of the average measurements under the effect of variety at date 1 (25 DAT) and date 2 (50 DAT)

All investigated plant parameters were positively correlated (Table 2). This positive correlation was strong between measurements of the root system. For instance, the weight of roots was strongly correlated to the number of secondary roots (R=0.85), length of main root (R=0.86), and diameter of root neck (0.78). Moreover, all measurements of root development were positively correlated with those of leaf development with more or less different strength of correlations. Leaf area and leaf number were less positively correlated to the number of secondary roots than root diameter, root length and root weight.

Table 2. Correlations between plant parameters

	N.S.R	R.D	R.L	R.W	L.N	L.W	L.A
N.S.R	1.000000	0.785703	0.866678	0.858275	0.695628	0.824803	0.519864
R.D		1.000000	0.954215	0.972208	0.956144	0.987667	0.878394
R.L			1.000000	0.956239	0.921144	0.970094	0.757169
R.W				1.000000	0.909891	0.984640	0.851637
L.N					1.000000	0.947818	0.824247
L.W						1.000000	0.863898
L.A							1.000000

N.S.R.=Number of Secondary Roots, R.D=Root Diameter (cm), R.L=Root Length (cm), R.W= Root Weight (g), L.N=Leaf Number, L.W=Leaf Weight (g), L.A=Leaf Area(cm^2).

Extraradical hyphae of *Glomus* sp. that formed the main components of the product MYCOSAT used in various treatments can metabolize both organic and inorganic sources of nitrogen by glutamate synthetase activity (Hawkins *et al.,* 2000). Also, plants cannot readily utilize P in an organic or complex inorganic form due to its low solubility and mobility, thus arbuscular fungi intervene to hydrolyze any available sources of P through the spread with the aid of secreted enzymes like phosphatase (Carlite *et al.,* 2001). Mycorrhizal symbiosis can facilitate the absorption of various other minerals like potassium. Therefore, the percentage of nitrogen, phosphorus and potassium was higher in leaves of MR and MI compared to NMR and NMI leaves (Table 3).

Table 3. Macronutrient content in lettuce leaves of various treatments

	MR	NMR	MI	NMI
N (%)	3.2	2.2	2.5	2.0
K (%)	7.5	5.4	8.0	7.5
P (%)	1.0	0.6	0.9	0.5

Arbuscular mycorrhiza symbiosis improved nutrient uptake by improving soil exploration (Beltrano *et al.*, 2013) by allowing plants to explore larger volumes of soils. It induced amelioration in root length, root diameter, number of secondary roots and root weight of 81%, 81%, 61% and 60% for MR plants and of 80%, 88%, 84% and 94% in MR and MI in comparison to NMR and NMI. It helped roots of mycorrhizal plants to grow deeper and ramify more and consequently to absorb more water and immobile mineral elements like phosphorus and to improve water-use efficiency (Beltrano *et al.*, 2003). Improvement in nutrition of mycorrhizal plants was coupled with an increase in leaf number of the Iceberg variety as well as an increase in leaf area and total leaf biomass in Romaine and Iceberg with an improved nutritional quality which confirmed the findings confirmed of Baslam *et al*. (2011).

CONCLUSIONS

Arbuscular mycorrhiza application can improve crop conditions of Romaine and Iceberg lettuce and contribute to reduction or even to prevent the fertilizers usage to this type of Lebanese horticulture. The application of the biostimulant MYCOSAT could provide a key to a safe improvement of lettuce production and to a biologically-based sustainable farming in Lebanon. However, it could be better to apply it twice during the vegetative cycle in order to promote a higher benefit on root development and consequently on head formation.

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AZOXIMERI BROMIDUM - PROTECTIVE ACTION OF IMMUNOSTIMULATOR DRUG IN EXPERIMENTAL TRICHINOSIS OF MICE

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ABSTRACT

Trichinosis is a parasitic disease caused by roundworms of the Trichinella type. Geographic distribution - worldwide. Nematodes of the genus Trichinella are one of the most widespread zoonotic pathogens on the world, and they can still cause major public health problems in many parts of the world. In our study we evaluated the protective effect of azoximeri bromidum in experimental trichinosis of mice. The azoximeri bromidum, is a polymer, a combined innovative product with immunomodulating, detoxifying, and antioxidative action, it is part of the Russian influenza vaccine. An assay was carried out on 20 white outbred mice weighting 16-18 g, divided into 2 groups of 10 animals in each. The first group was injected with azoximeri bromidum; the second group was injected with 0.9% NaCl. The drug was injected twice with an interval of 48 hours intramuscularly in a dose of 0,004 mg/mouse. After 48 hours the mice were infected by Trichinella spiralis larvae in a dose of 80±5 larvae/mouse. Analysis of the data indicates that in the experiment the application of this drug had significant protective effect. The number of T. spiralis larvae detected in animals was 142.5±11.1 larvae/mouse, respectively. This was 31.5 times less than in the mice of control group (4485 ± 430.6 larvae/mouse). Based on this, we consider it is expedient to continue the study of this immunostimulator drug in the complex immunoprophylaxis of trichinosis.

Keywords: *immunostimulator drugs, Trihinella spiralis, immunoprophylaxis, immunomodulators, mice.*

INTRODUCTION

Trichinosis is a parasitic disease caused by roundworms of the Trichinella type. Geographic distribution - worldwide. Most common in parts of Europe and the United States. Light infections may be asymptomatic. Intestinal invasion can be accompanied by gastrointestinal symptoms (diarrhea, abdominal pain, vomiting). Larval migration into muscle tissues (one week after infection) can cause

periorbital and facial edema, conjunctivitis, fever, myalgias, splinter hemorrhages, rashes, and peripheral eosinophilia. Occasional life-threatening manifestations include myocarditis, central nervous system involvement, and pneumonitis. Larval encystment in the muscles causes myalgia and weakness, followed by subsidence of symptoms (https://www.cdc.gov, 2017).

The therapy and immunoprophylaxis of this disease represents an important problem. Scientific research for effective means of protection against this deadly disease is conducted throughout the world.

The results of the study suggest that the parasite Trichinella spiralis made an effort to reduce the effectivity of the host immune response in order to ensure its own survival (Piekarska et al., 2010). Studies established that interleukin-25 (IL-25) promotes efficient protective immunity against Trichinella spiralis infection by enhancing the antigen-specific IL-9 response (Angkasekwinai et al., 2013). In this way the using immunostimulators drugs in recent years are reasonably. There is a lot of references to research about strengthening the body's resistance to helminth infection by the application of non-specific immune stimulating (Berezhko. et all., 2004, Rudneva et all., 2014, Smolencev, 2011). Thus, in the model of experimental alveolar echinococcosis, the protective effect of the immunostimulating drug ribotan was established (Rudneva et all.,2016). Immunomodulator Roncoleukin has a protective effect (within 40%) with echinococcosis of dogs (Berezhko. et all., 2004). The protective effect of the immunostimulating antiparasitic agent on the basis of the seed *Artemisia vulgaris* was also revealed in experimental trichinosis (Rudneva et al., 2016). Combined use of antiparasitic drugs and immunomodulators, according to Ismagilov AM (2010), contributes to the faster recovery of the organism in the case of melophogosis. The use of immunomodulator Roncoleukin in experimental trichinosis provides protection against invasion by 80.6% (Napisanova et al., 2016).

Given these data, it is certain that the effectiveness of the protective action of a specific drug improves with the use of an immunostimulating agent. Its use makes it possible to increase the antiparasitic resistance of the organism.

In our study, we focused on the study of immunoprophylaxis activity of azoximeri bromidum – is an N-oxidized polyethylene-piperazine derivative, a water-soluble high-molecular synthetic immunomodulator. Polyoxidonium can be used as adjuvant in combined treatment of acute and chronic infections of any etiology, in the treatment of first and secondary immunodeficiencies simultaneously with basic drugs (Pinegin et al., 2003). It is part of the russian influenza vaccine (https://www.drugs.com, 2017). Also we evaluated the protective effect immunostimulant azoximeri bromidum in experimental trichinosis of albino outbred mice.

MATERIALS AND METHOD

We used in our study 20 albino mice weighing 16-18 g, divided into 2 groups of 10 animals in each. The first group was injected with azoximeri bromidum; the second group control group was injected with 0.9% NaCl. The azoximeri bromidum,

polymer, a combined innovative product with immunomodulating, detoxifying, and antioxidative action. The drug was injected twice with an interval of 48 hours intramuscularly in a dose of 0,004 mg/mouse in 0,2 ml 0.9% NaCl. After 48 hours the mice were infected by T. spiralis larvae in the dose of 80±5 larvae/mouse. Study of the protective effect of immunostimulatory drug was carried out 45 days after invasion. The mice of each group include controls that are subjected to human euthanasia and digested in the artificial gastric juice separately (Figure 1).

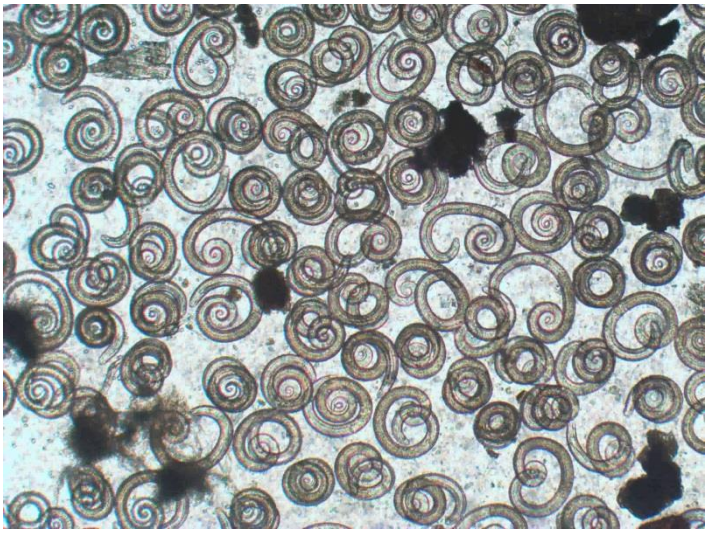

Figure 1. The larvae of Trihinella spiralis in the fluid after digested mice's in the artificial gastric juice (control group).

We then washed of T.spiralis larvae in the sedimentary fluid. The larvae were counted for each mouse separately by a binocular microscope. We have defined the arithmetic mean of the number of larvae in each group of experimental animals to evaluate. The average arithmetic number of larvae for each group of animals was determined.

RESULTS AND DISCUSSION

The number of T. spiralis larvae detected in animals was 142.5±11.1 larvae/mouse respectively (figure 2). The degree of protection of mice injected with azoximeri bromidum. Even so, this was 31.5 times less than in the mice of control group (4485 ± 430.6 larvae/mouse).

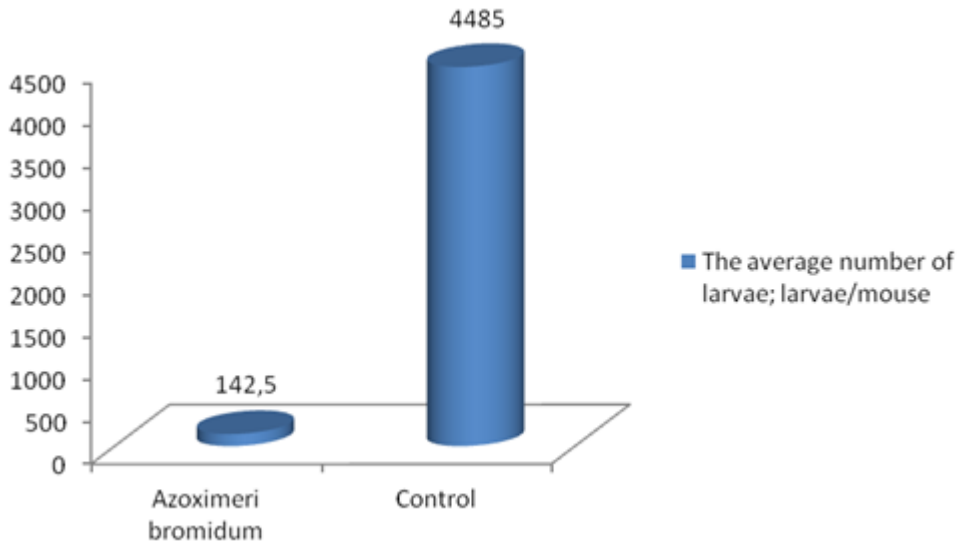

Figure 2. The number of detected *Trihinella spiralis* larvae in the experiment on the evaluation of the protective properties of the immunostimulator in experimental trichinosis

In this case, the use of this drug activates the immune response and the injection of this is immunomodulator significantly reduces subsecuently infected of parasits animals. Thus, the smallest number of larvae was observed in the experimental group mice what evidenced about a high biological activity immunostimulatory azoximeri bromidum in experimental trichinosis.

Our data are comparable with the data of other researchers working in this field. So, according to Klenova, the protective action of the drug ribotan is in alveolar echinococcosis (Klenova, 1999). Cycloferon has a protective effect (within 95.75%) with experimental trichinosis (Rudneva, 2016). Podophyllum Theta, Cina 30 and Santoninum 30 reduced the larval population in the studied mice by 68.14%, 84.10% and 81.20%, respectively, as compared to the untreated control group (Sukul et all., 2005). Numerous studies on experimental models have shown that the combined use of specific and nonspecific drugs leads to successful immunization against helminthiases, especially in tissue invasion (Dalton et all., 2013, Rudneva, 2006).

In this way immunostimulating drugs significantly increase the protective reaction of the organism and are able to prevent subsequent infection by different helminths.

CONCLUSION

Thus, in the experiment it was clearly demonstrated high power effect azoximeri bromidum to achieve the best protective effect. Our data are actually not inconsistent with the results of other researchers which have used various

immunomodulating and adjuvant agents. That enhance the immunogenicity of antigens as a means of increase potentiation animal body defense mechanisms against helminthiasis.

Analysis of the data indicates that this substance in the experiment showed hight protect effect and can be considered as a medicament for increasing the effect of specific antigens in parasitic diseases. We consider it necessary to continue research in this direction, but now in combination with specific antigenic drugs in the complex immunoprophylaxis against trichinosis.

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THE EFFECT OF FOLIAR FERTILIZING ON THE CHEMICAL COMPOSITION OF PEPPERS GROWN IN PROTECTED SPACES IN THE STRUMICA AREA IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

The influence of foliar fertilizing on the chemical composition of peppers grown in protected spaces in the Strumica area was examined. The experiment was set in four variants and three repetitions. The variants used in the experiment were, as follows: Control (untreated variant); NPK+Ever green (55% organic matter, 2%w/w Mg, 2%w/w Fe, 2%w/w Zn, 2% w/w Mn, 0.5 %w/w Cu, 0.5 %w/w B); NPK+Biolinfa (34% organic matter, 3% N, 5.80 % K_2O); NPK+Oligomix (1.20 % B, 0.10 % Cu, 4 % Fe, 1.50 % Mn, 0.10 % Mo, 2 % Zn). The experiment was set in 18 rows, and each variant and repetition comprised 62 plants. During the vegetation period, 7 foliar treatments were made with listed fertilizers at a concentration of 0.4%. Before setting up the experiment, an agrochemical analysis of the soil was performed enabling the researchers to determine good soil fertility with available nitrogen and potassium, and average fertility with available phosphorus. Foliar fertilizing had a positive influence on the chemical composition of the peppers. In the variants treated with different organic fertilizers the researchers recorded a higher content of the analyzed parameters than in the control, untreated variant. The highest average content of dry matter (14.80%), the highest average content of ash (0.90%) and the highest average vitamin C content (120 mg/100g) were determined in the pepper in Variant 2. The highest average content of nitrogen (1.37%), phosphorus (0.53%), potassium (2.25%) and calcium (1.42%) was also determined in the pepper in Variant 2. The highest average magnesium (0.38%), iron (0.0067%) and manganese (0.0017 %) content was determined in the pepper in Variant 3.

Key words: *foliar fertilizing, peppers, protected spaces.*

INTRODUCTION

Plant nutrition is one of the most important agrotechnical measures in the agricultural production. Quality and well-balanced nutrition is one of the basic conditions for achieving high, stable and high-quality crop yields (Domagalski et al., 2008; Kannan 2010). Determination of doses and types of fertilizers, timing and method of use are based on the consumed nutrients from the soil (Datnoff et al., 2007). Intake of nutrients through the soil often does not give the expected results due to their unavailability for plants (due to drought, unfavorable soil properties, underdeveloped root system). Hence, foliar nutrition is of great importance for the successful cultivation of agricultural crops (Dzamić and Stevanović, 2000). According to numerous authors that plants can give the maximum of their genetic potential, a foliar nutrition is necessary (Jekić and Brković, 1986, Saciragić and Jekić, 1988). The advantage of foliar fertilizing compared to soil fertilizing is that the utilization of nutrients does not depend on the soil moisture content, the pH of the soil and other chemical and physical properties (Kostadinov and Kostadinova, 2014). The effects of foliar nutrition are rapid. After several days of using foliar fertilizers, the plants receive intense green color, their habitus is rapidly increasing, the formation of organic matter accelerates (Kerin and Berova, 2003). The development of the root system speed up, thus allowing better utilization of nutrients from the soil. In this way, plants become more resistant to adverse weather conditions, diseases and pests.

For the normal growth and development of agricultural crops, many macro and micro biogenic elements are of great importance (Sarić et al., 1989; Taiz and Zeiger, 2006). Each nutrient has its specific influence on the individual parts of the plant. Plant nutrition affects numerous physiological and biochemical processes as growth, development and fruit formation (Vukadinović and Loncarić, 1997, El-Bassiony et al., 2010). Plants that are timely and properly nourished produce fruits with characteristic shape, color and size, with typical organoleptic properties (Fageria, 2007; Fageria et al., 2009). The use of foliar fertilizer in the diet of garden crops is of great importance for obtaining higher yields but also products that are characterized with better quality (Epstein and Bloom, 2005, Fewzy et al., 2012).

The pepper (*Capsicum annuum L*) originates from South America. The Spaniards brought it to Europe in the 15th century, from where it spread to Turkey, and today it is mostly cultivated in Hungary.

In the Republic of Macedonia, pepper is one of the most common vegetable. It is a one-year culture of great economic significance. The fruits of the pepper vary in shape, color, but also in smell and taste. The fruits are characterized by high nutritional value. They are rich in many vitamins, organic and mineral substances (Kolota and Osinska, 2001). They also have great technological value.

The pepper contains about 89% water. It contains sugars from the group of monosaccharides and disaccharides. Of the monosaccharides, 90-98% contains glucose, the rest is fructose and sucrose. Of vitamins, the pepper contains mostly vitamin C. In the pepper there are also significant amounts of vitamin B, especially

B_1 and B_2. It contains vitamin E, pantothenic acid, and in the form of provitamin contains vitamin A, which is present as beta-carotene and cryptoxanthin (Karakurt et al. 2009, Nassar et al. 2001). Of minerals, pepper is the richest with potassium, phosphorus and iron (Youssef et al., 1996; Fawzy et al., 2005). The fruits are consumed both in fresh and processed form.

The aim of this research was to determine the impact of foliar fertilizing with various organic fertilizers on the chemical composition of fruit peppers grown in protected spaces in the Strumica region.

MATERIALS AND METHODS

In the Strumica region in the area of the village Kuklish, field crop experiment was set in the protected spaces of 300 m^2 during the 2013 and 2014.

The experiment was set in 18 rows. Four variants and three repetitions were included.

The material for the work was the pepper's variety *bela dolga*. The seedling was planted in rows with row by row distance of 60 cm, and between plants, 40 cm. The experiment was set in conditions of irrigation. During the vegetation period of peppers, basic agro-technical measures were applied. Before the planting took place, soil fertilization with mineral fertilizer NPK 6-10-30 + 2% MgO in the amount of 12 kg in the hall with an area of 300 m^2 was applied.

The variants in the experiment were:

1. Control (untreated);
2. NPK+Ever green (55% organic matter, 2%w/w Mg, 2%w/w Fe, 2%w/w Zn, 2% w/w Mn, 0.5 %w/w Cu, 0.5 %w/w B);
3. NPK+Biolinfa (34% organic matter, 3 %N, 5.80 % K_2O);
4. NPK+Oligomix (1.20 % B, 0.10 %Cu, 4 % Fe, 1.50 % Mn, 0.10 % Mo, 2 % Zn).

In each variant and repetitions, 62 plants were involved, and for the entire experiment 1116 plants were involved.

Each variant was treated with tasted foliar fertilizer in concentration of 0.4% solution. The application of fertilizers was done with hand sprayer, by spraying the leaves. During the vegetation seven foliar treatments were conducted, starting from the stage of growth of the first fruits. The harvest was done when the peppers were 18 cm long, separated in variants and repetitions. During the vegetation five harvests were done. The first harvest was done on the 23th of May, and the last one on the 12th of July.

During the last harvest, fruits were taken separately by variants and following parameters were performed:

- The content of hygroscopic water content was determined by drying the material in dryer on temperature of 105°C matters is deducted;
- The content of total dry matters was determined by calculation when from 100%, the percentage of hygroscopic water;
- The content of organic matter was determined by calculation when from 100% the percentage of total ash will be deducted.

- The content of total ash was examined by removing moisture from the prepared material, drier on temperature of 105°C. Then the rest was burned in electric oven by gradually increasing the temperature to 550°C. The burning was done until ashes became grey or white;
- The content of vitamin C was determined by method of Thilmans, which is based on the redox reaction between L-ascorbic acid and organic colour 2.6-dichlorophenolindophenol;
- The content of phosphorus (P_2O_5) was determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1986);
- The content of potassium (K_2O) was determined by incineration of the material with concentrated H_2SO_4 and flame-photometer (Sarić et al., 1986);
- The content of calcium (SAT) was determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1986);
- The content of magnesium (Mg) was determined by applying atomic; emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1986);
- The content of iron (Fe) was determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1986);
- The content of manganese (Mn) was determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1986);
- The content of zinc (Zn) was determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Sarić et al., 1986).

Before setting up the experiment, soil samples were taken for agrochemical analyses performed on the following parameters:

- pH value determined with pH meter (Bogdanović et al., 1966);
- Content of easy available nitrogen – chosen by method of Tjurin and Kononova (Bogdanović et al., 1966);
- Content of easy available phosphorus – chosen by AL method and reading of spectrophotometer (Bogdanović et al., 1966);
- Content easy available potassium – chosen by AL method and reading of spectrophotometer (Bogdanović et al., 1966);
- Content of carbonates – chosen with Schaiblerov Calcimetar (Bogdanović et al., 1966).

RESULTS AND DISCUSSION

For the achievement of high and quality yields, the pepper requires favorable soil and climatic conditions. Pepper that is grown in protected spaces has a greater need for nutrients, and in particular requires a greater amount of potassium (Lazić et al.,

2001; Salama and Zake, 2000). In a short time, the pepper creates a massive vegetative mass, but there is a less developed root system. Therefore, it is necessary to grow on good fertile soils (Shafeek et al., 2014).
The best yields are obtained if the pepper is grown on deep and friable soils rich in easily accessible nutrients. The optimum soil reaction for the pepper is slightly acidic with a pH of 5.5 to 6.0.

Table 1. Agrochemical soil analysis

Order No.	Plot	Depth cm	pH		Available form (mg/100 g soil)			$CaCO_3$ %
			H_2O	KCl	N	P_2O_5	K_2O	
1	Pepper 1st part	0-20	7.35	6.65	9.55	18.30	21.20	/
2		20-40	7.40	6.64	10.20	14.20	17.00	/
Average			**7.37**	**6.64**	**9.87**	**16.25**	**19.10**	**/**
3	Pepper 2nd part	0-20	7.43	6.70	8.90	15.25	23.10	/
4		20-40	7.40	6.60	9.70	17.00	20.50	/
Average			**7.41**	**6.65**	**9.30**	**16.12**	**21.80**	**/**

From the data in Table 1, it can be concluded that the soil on which the experiment was set, has a neutral pH, good fertility with nitrogen and potassium, and medium fertility with available phosphorus. There is no presence of carbonates.

Table 2. Chemical content of pepper fruit in % of dry matter, average 2013/2014

Variant	Hygroscopic water %	Dry matter %	Organic matter %	Ash %	Vitamin C mg/100g
1	90.30	9.70	99.40	0.60	103.50
2	85.20	14.80	99.10	0.90	120.00
3	87.00	13.00	99.37	0.63	105.10
4	90.20	9.80	99.25	0.75	115.00

Table 3. Chemical content of pepper fruit in % of dry matter, average 2013/2014

Variant	N	P_2O_5	K_2O	Ca	Mg	Fe	Mn
1	**1.22**	0.43	1.95	1.25	0.29	0.0053	0.0011
2	**1.37**	0.53	2.25	1.42	0.30	0.0062	0.0015
3	**1.28**	0.49	2.07	1.33	0.38	0.0067	0.0017
4	**1.32**	0.47	2.15	1.32	0.32	0.0058	0.0012

LSD 0.05=0,062 LSD 0.05=0.065 LSD 0.05=0.103 LSD 0.05=0.103 LSD 0.05=0.101 LSD 0.05=0.00086 LSD 0.05=0.0004

LSD 0.01=0,090 LSD 0.01=0.091 LSD 0.01=0.145 LSD 0.01=0.145 LSD 0.01=0.142 LSD 0.01=0.00120 LSD 0.01=0.00060

From the data in Table 2 and Table 3 it can be concluded that foliar fertilization had a positive influence on the content of the examined parameters in pepper fruits. In all variants, the analyzed parameters gave better results compared to the untreated control variant.

The highest average content of dry matter (14.80 %), the highest average content of ash (0.90%) and the highest average vitamin C content (120 mg/100g) were determined in the pepper fruits in the variant 2.

The content of hygroscopic water is in correlation with the dry matter content and it is the highest in the control variant (90.30 %). The content of organic matter (99.40 %) was highest in the control variant. The highest average content of nitrogen (1.37), phosphorus (0.53 %), potassium (2.25 %) and calcium (1.42 %) was determined in the pepper fruits in the variant 2. The highest average magnesium (0.38 %), iron (0.0067 %) and manganese (0.0017 %) content is determined in pepper fruits in variant 3. The positive foliar effect of the used organic fertilizers on the yield of peppers is the result of their chemical composition. The organic matter in the fertilizer is of great importance for the intensification of all the processes taking place in the individual organs of the plant. It participates in many biochemical and oxidative processes. It affects the migration and redistribution of elements in plants, too. Through these processes it affects the general growth, development and the increase both in yield quantity and quality. The presence of micro elements in the composition of the analyzed fertilizers is of great importance for the correct growth, development and fruit formation of peppers. These elements influence numerous physiological and biochemical processes that are vital in the vegetative cycle of culture. Statistically significant differences compared to the control variant were obtained for the nitrogen content of variants 2 and 4 at both examined levels. There is no statistically significant difference in phosphorus content. For potassium content, there are statistically significant differences in variants 2, 3, and 4 at the two levels. For the content of calcium, statistically significant differences in the two levels are found in variant 2. For the content of manganese, the statistically significant difference is found in variant 3 at the LSD level of 0.05. For iron content statistically significant difference at LSD level 0.05 is found in variants 2 and 3, and at LSD level 0.01 in variant 3.

CONCLUSIONS

Based on the obtained results for the influence of foliar fertilizing on the chemical composition of pepper fruits grown in protected spaces, can be concluded that in all variants treated with foliar fertilizers, higher content of the tested elements has been determined compared to control variant. The highest average content of nitrogen (1.37), phosphorus (0.53 %), potassium (2.25 %) and calcium (1.42 %) was determined in the pepper fruits in the variant 2. The highest average magnesium (0.38 %), iron (0.0067 %) and manganese (0.0017 %) content is determined in pepper fruits in variant 3. The highest average content of dry matter (14.80 %), the highest average content of ash (0.90%) and the highest average vitamin C content (120 mg/100g) were determined in the pepper fruits in the variant 2. For future used, variant 2 is recommended (NPK+Ever green (55% organic matter, 2%w/w Mg, 2%w/w Fe, 2%w/w Zn, 2% w/w Mn, 0.5 %w/w Cu, 0.5 %w/w B);

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WHAT ORCHARDISTS EXPECT FROM FARMER FIELD SCHOOLS ON INTEGRATED PEST MANAGEMENT: A CASE OF IRAN

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ABSTRACT

Since 2002, the implementation of integrated pest management (IPM) program through farmers' field schools (FFS) approach has gained some priority on the agenda of Iran's extension services. Different nature of agricultural activities and the specific context of farming in each area of the country have raised some questions regarding the suitability of these training programs to meet the needs of participants. In line with this, a need assessment survey in the summer of 2015was conducted to extract the educational needs of orchardists living in west part of Iran. The study population consisted of all the grape and pomegranate growers that participated in the IPM/FFS programs (N= 420). A sample of 201 individuals was selected through a simple random sampling manner. To collect data, a questionnaire based on Borich model was administered. Results indicated that the most important need was "to be skillful in tree pruning" and the least important was "to train participants based on lecturing and formal classes", scoring 4.30 and 3.24 out of 5, respectively. Moreover, "the ability to recognize the best time to spray pest-herbicides", "practical identifying of the symptoms of plant diseases and direct observations", "pre-assessment of participants' information before launching the programs" and "acquisition of the know-how knowledge to use bio-fertilizers and manures" were the first four priorities of respondents' needs among a total of 49. The study concluded with the idea that most of orchardists were aware of the need to conserve the environment and produce safe crops but IPM/FFS training programs could not provide them with adequate skills to perform the sound method and practice in their professional activities.

Key words: *IPM/FFS programs, orchardists, Borich need assessment model, Iran.*

INTRODUCTION

Integrated Pest Management (IPM) was born in the late 1950s primarily out of a crisis in agriculture from the overuse of synthetic pesticides and an increasing concern over the ecological consequences of these pest-control tools (Brunner, 2014). The main objective of IPM is to reduce pesticide use to minimize/reduce risks to human health and environment (Sharma *et al*., 2015). However, chemical pesticide is a two edge sword. While the rising level of pesticide use certainly has helped farmers to reduce crop yield loss, the high level of pesticide use may have had a number of adverse consequences (Chen *et al*., 2013). Excessive use of pesticides can threaten food and user safety, the environment, and increasingly, the export of agricultural products to global markets (Hashemi *et al*., 2008). Nonetheless, in many cases, farm workers engaged in pesticide application are the most affected by pesticides, as they do not wear any protective clothing while applying pesticides (Sharma *et al*., 2015).

IPM is an approach that applies the principles of ecology, especially population biology, to the management of pests in agricultural, forest, or urban environments (Brunner, 2014). IPM requires farmers to integrate different pest control methods including varietal resistances, cultivation, mechanical, biological and chemical control according to their specific field conditions (Yang *et al*., 2008). An IPM program is built on a foundation that includes the knowledge of pest and natural enemy biology, their correct identification, and ecology. Intervention against a pest should be based on the risk of crop injury and is only taken if the pest's population exceeds a level where the cost of preventing injury exceeds the cost of the intervention. Control actions are not implemented unless the pest's population exceeds a specified level, a treatment threshold. Multiple tactics to mitigate the negative impacts of pests are used in an integrated approach with the goal of limiting or relegating the use of tools that disrupt natural controls to options of last resort (Brunner, 2014). There are many factors affecting farmers' pesticide use activities. These factors include farmers' characteristics, environmental factors as well as the level of pest infestation (Chen *et al.*, 2013). Thus farmers need skills in pest monitoring and knowledge of pest ecology (Yang *et al*., 2008). Farmers are facilitated to conduct their own research, diagnose and test problems, and come up with solutions (Davis *et al*., 2012). Recent farmer field school (FFS) training on integrated pest management has involved adult, non-formal, education using the learning-discovery approach (Yang *et al*., 2008).

To overcome the negative consequences of pesticide use in Iran agricultural sector, the Ministry of Agriculture, promoted the Integrated Pest Management (IPM) as a strategy for plant protection. Since 2002, the implementation of integrated pest management (IPM) program through farmers' field schools (FFS) approach has gained some priority on the agenda of Iran's extension services. During recent years, FFS approach has been implemented by extension workers for orchardists especially grape and pomegranate growers in west part of Iran, Ilam Province. This region produces 6094.68 tons of grape and 1742.09 tons of pomegranate annually from an area of 1101.731 and 274.44 hectares, respectively. The average

productivity of these two crops is 6237.01 and 8958.59 kg per hectares, respectively. Local orchardists annually lose a huge part of their production due to invasion of insect pest. However, the extent of crop loss due to insects varies with the crop type, crop location, damage potential of the insect pests involved, and the cropping season (Sharma *et al*., 2015). The main insect pest of pomegranate is the *Ectomyelois ceratoniae* zeller (Lep.: Pyralidae) which annually inflict crop losses of 40% in pomegranate production. In addition, the extent of annual crop loss due to *Polychrosis* (=Lobesia botrana), as the main insect pest of grape farms, is about 20% of the total crop production.

There seems to be an essential need to monitor and evaluate the efficacy of such IPM/FFS program. To make decisions about training program priorities, a need assessment is a useful tool. Training programs can apply Borich model by defining what is as the measured behaviors, skill, and competencies of the trainee and what should be as the goals of the training program. The distance between these two poles can then be used as an index of the training program's effectiveness. Discrepancies can be ranked for priority by a panel of trainers or by statistical techniques that weight the relative importance of each goal statement from values assigned to them by the respondents. Discrepancies ranked in descending order of priority provide the framework for deciding what parts of the program to modify or revise (Borich, 1980). The overall objective of this study was to identify performance requirements and the gap between what performances are required and what presently exists. Put another words, the question of interest was to investigate how much the changes brought about by IPM-FFS program at orchardists could meet their training needs? What training needs were addressed by IPM-FFS programs compared to conventional training courses? And what are the opportunities for upgrading IPM training and to direct future IPM programs?

MATERIAL AND METHODS

A need assessment survey was conducted to extract the training competencies of orchardists living in west part of Iran, Ilam Province. The study population consisted of all the grape and pomegranate growers that participated in the IPM/FFS programs (N= 420) during three recent years. A sample of 201 individuals was selected through a two-stage simple random sampling manner. First, the districts where the integrated pest management courses had been implemented identified, and then, at the second stage, 50 percent of participants in the IPM courses of each district randomly selected. All participants were male. To collect data, two questionnaires based on Borich (1980) needs assessment model was developed for assessing the orchardists' perceived level of importance and perceived level of performance regarding 49 competencies. These two forms of questionnaire were administered for conventional and IPM training courses. To develop the questionnaires an in-depth literature review following with several interviews with local extension workers and field observations were used and then, a list of competencies for fruit production (e.g. grape and pomegranate) as well as for holding a training course was provided. All competencies should be checked

against program activities and materials to ensure that they actually represent program objectives (Borich, 1980). To find these competencies/training needs, seven categories based on the main components needed for holding a training course i.e. content (knowledge-awareness, attitude, and skill), goal and objective, teaching-learning method, instructional media, time, place, and evaluation of outcomes were taken into account. For each one, three measurements containing competency importance, present performance (knowledge) and satisfaction of courses in addressing the needs were launched. Finally two questionnaires consisting of seven multi-item constructs, that were measured on a five-point Likert type scale (1 = very low, to 5= very high), were developed. A response of one indicated the competency was not important and a five indicated the competency was very important to production process. Survey data were collected, via face-to-face interviews with randomly selected participants. The instrument's content and face validity was approved by a panel of agricultural experts and its reliability, also, was confirmed by calculating the Cronbach's alpha coefficient through a pilot study. All scales indicated an acceptable reliability coefficient. After collecting data, a *discrepancy score* for each individual on each competency was calculated by taking the importance rating minus the ability (competence) rating. A *weighted discrepancy score* was then calculated for each individual on each of the professional competencies by multiplying the discrepancy score by the mean importance rating (Garton and Chung, 1997). To analyze data SPSS software was used.

RESULTS AND DISCUSSION

Descriptive analysis of the data revealed that the age of the participants ranged from 19 to 88 with a mean value of 48.21 years (standard deviation=15.20). Most of them aged over 50. While the majority of participants (27.4%) had high school level, the minority of them (9.25) had academic educational level. The average of the garden ownership was 1.43, with the minimum and maximum of 0.1 and 4 hectares, respectively. Most of the orchardists (47.5%) had less than 1 hectare grape and pomegranate garden.

Analysis of the participants' needs, using the Borich model for IPM/FFS programs, indicated that 15 of the 49 competencies were in greater need for grape and pomegranate growers (Table 1). The 15 highest rated competencies had mean weighted discrepancy scores greater than 3.70. Table 1 also shows the perceived importance of the first 15 competencies. The mean score of all the 49 needs was obtained as 3.6 out of 5. The most important need was "to be skillful in tree pruning" and the least important was "to train participants based on lecturing and formal classes", scoring 4.30 and 3.24 out of 5, respectively. Moreover, Weighted Discrepancy Score shows that "the ability to recognize the best time to spray pest-herbicides", "practical identifying of the symptoms of plant diseases and direct observations", "pre-assessment of participants' information before launching the programs" and "acquisition of the know-how knowledge to use bio fertilizers and manures" were the first four priorities of respondents' needs among a total of 49.

Table 1. The needs of orchardists using the Borich Needs Assessment Model for IPM/FFS program (N=201).

Rank	Needs	Imp. level	Present Perf.	WDS
1	The ability to recognize the best time to spray pest-herbicides	3.92	2.30	5.83
2	Practical identifying of the symptoms of plant diseases and direct observations	3.87	2.40	5.29
3	Pre-assessment of participants' information before launching the programs	3.72	2.25	5.29
4	Acquisition of the know-how knowledge to use bio-fertilizers and manures	3.83	2.51	4.75
5	To match the content with orchardists' profession	3.64	2.34	4.68
6	To be skillful in tree pruning	4.30	3.02	4.61
7	Awareness of cultural (agronomic) control (irrigation time management; collecting infected fruits; variety selection; etc.)	3.67	2.41	4.54
8	Formative evaluation and modifying the deficiencies (if any)	3.59	2.33	4.54
9	Favorable educational place based on ventilation	3.78	2.55	4.43
10	Using instructional media (poster, leaflet, etc.) to motivate more participation in discussion	3.60	2.38	4.39
11	Long-time and several-days instructional courses along with issuing certification	3.51	2.30	4.36
12	Using instructional media in accordance with the class content	3.22	2.02	4.32
13	Seeking ideas regarding the satisfaction of extension classes	3.68	2.49	4.28
14	One-day instruction	3.27	2.21	3.81
15	To train participants based on lecturing and formal classes	3.24	2.20	3.74

Imp. Level: Importance level; present perf.: present performance; WDS: Weighted Discrepancy Score

- To avoid prolonging the table, only 15 first needs have been reported.

These 15 competencies with regard to conventional courses have been rated differently from the participants' viewpoint (Table 2). With the same score on importance, present performance of participants based on conventional courses was different. With the mean score of 3.6, the weighted discrepancy score for each competency was calculated. Surprisingly, the outcomes on the same competencies for conventional courses were much higher than those for IPM/FFS program. This shows that our participants feel more need to learn competencies in the conventional courses. A precise examination of findings indicated in Table 2 revealed that the first three competencies are practical skills which have gained the least performance score in conventional courses. This finding also can be

confirmed by the result of the measuring of participants' satisfaction indicated in Table 3. The results showed that the mean score of participants' satisfaction on the seven categories of main components needed for holding an IPM/FFS course (Mean= 2.35) was higher than conventional courses (Mean=2.11). As expected, the conventional courses were perceived weak in transferring need skills from the orchardists' point of view. In total, the mean of orchardists' satisfaction of both programs were lower than average.

Table 2. The needs of orchardists using the Borich Needs Assessment Model for conventional courses (N=201).

Rank	Needs	Imp. level	Present Perf.	WDS
1	To be skillful in tree pruning	4.30	1.57	9.83
2	The ability to recognize the best time to spray pest-herbicides	3.92	1.35	9.25
3	Practical identifying of the symptoms of plant diseases and direct observations	3.87	1.39	8.93
4	Favorable educational place based on ventilation	3.78	1.55	8.03
5	Pre-assessment of participants' information before launching the programs	3.72	1.53	7.88
6	Acquisition of the know-how knowledge to use bio-fertilizers and manures	3.83	1.90	6.95
7	Using instructional media (poster, leaflet, etc.) to motivate more participation in discussion	3.60	1.73	6.73
8	Seeking ideas regarding the satisfaction of extension classes	3.68	1.99	6.08
9	Long-time and several-days instructional courses along with issuing certification	3.51	1.85	5.98
10	Awareness of cultural (agronomic) control (irrigation time management; collecting infected fruits; variety selection; etc.)	3.67	2.15	5.47
11	To match the content with orchardists' profession	3.64	2.13	5.44
12	Formative evaluation and modifying the deficiencies (if any)	3.59	2.24	4.86
13	Using instructional media in accordance with the class content	3.22	1.93	4.64
14	One-day instruction	3.27	2.11	4.18
15	To train participants based on lecturing and formal classes	3.24	2.47	2.77

Imp. Level: Importance level; Present Perf.: Present Performance; WDS: Weighted Discrepancy Score

Table 3. Participants' satisfaction of IPM/FFS and conventional courses

Item		Awareness	Skill	Attitude	Aim/objectives	Teaching-learning method	Instructional Media	Time	Place	Evaluation	Mean (out of 5)
No. of Statement		14	6	2	2	8	3	4	4	6	-
Mean (out of 5)	IPM/FFS	2.39	2.38	2.30	2.29	2.37	2.40	2.32	2.32	2.38	2.35
	Conventional Courses	2.02	1.81	2.11	2.22	2.14	2.16	2.21	2.21	2.14	2.11

CONCLUSIONS

The substantial feature of IPM program is to utilize all appropriate pest management techniques to keep pest populations below economically injurious levels. Each technique must be environmentally sound and compatible with producer purposes. While most of the orchardists were aware of the need to conserve the environment and produce safe crops, the current implemented IPM/FFS training programs in west part of Iran, could not provide them with adequate skills to perform the sound method and practice in their professional activities. Nonetheless, the orchardists are more satisfied by IPM/FFS programs compared to the conventional courses implemented by the agricultural extension and education services centers. With regard to this point that for leaf vegetable a similar project has been promoted through April 2015, the extension workers must emphasize more on practical aspects.

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EFFECT OF ORGANIC SUBSTRATES ON GINGER GROWTH, YIELD AND [6]-GINGEROL CONTENT CULTIVATED USING SOILLESS CULTURE SYSTEM

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ABSTRACT

Ginger (*Zingiber officinale* Rosc.) belongs to a tropical and sub-tropical *Zingiberaceae* family, which originated from Southeast Asia. Ginger is one of the most widely used herbs contains several interesting bioactive constituents including [6]-gingerol that has potent antioxidant activity and health promoting properties. Substrates plays an important role for plants to growth in the soilless culture system. Given the nature of the substrate may play a crucial role in determining water and nutrient availability for the plant and hence may affect the metabolic pathways involved in the synthesis of specific biochemical compounds, this study was conducted to determine the effects of organic soilless substrates such as coir dust and burnt paddy husks on ginger growth, yield and [6]-gingerol content using soilless culture system. The treatments were arranged in a randomized complete block design (RCBD) with five levels of treatment with three replicates. The treatments include, T1 = 100% coir dust; T2 = 100% burnt paddy husks; T3 = 70% coir dust and 30% burnt paddy husks; T4 = 30% coir dust and 70% burnt paddy husks; and T5 = 50% coir dust and 50% burnt paddy husks. Results showed that plant in T1 gave rise to highest rhizomes yield (5480 ± 325 gm) compare to other treatments. Media containing high amount of coir dust (70 – 100%) showed good growth and increased the rhizome yield up to 36% compared to those containing high amount of burnt paddy husks. There was no significant difference between all treatments in term of [6]-gingerol content in the fresh and dry ginger rhizomes. The studies suggested that the secondary metabolites like [6]-gingerol content and accumulation were not affected by the substrates. It can be concluded that 100% coir dust are the best substrates for growing ginger in soilless culture system.

Keywords: *ginger, substrates, [6]-gingerol, soilless culture system.*

INTRODUCTION

Soilless culture system is the most intensive production method in Malaysia today's agriculture industry, which can result in higher yields even in limited and adverse growing conditions. Yields of chillies, rock melons and tomatoes cultivated in soilless system increased 3 – 5 times compared to those using conventional method (De Rijck and Schrevens 1998). In soilless production system, many types of growing media or substrates such as rockwool, perlite, vermiculite and peat have been used to grow many kinds of crops (Komada et al. 1997). Media such as rockwool, perlite and vermiculite are expensive because they have to be imported. Hence, alternative substrates that are cheaper and locally available such as coconut husks and burnt paddy husks should be used as alternative media (Ortega et al. 1996). Substrates plays an important role for plants to growth in the soilless culture system.

Ginger is one of the most widely used herbs that contains several interesting bioactive constituents and possesses health promoting properties. [6]-gingerol, a major pungent ingredient of ginger, also has potent antioxidant activity (He et al., 1998). Several studies have assessed and modified component of soilless culture production system including irrigation techniques and nutrient elements to increase the plant yields and quality (Hargreaves et al., 2008, 2009). Limited studies have yet investigated the role of substrates may have on the plant productivity and final product such as fruit quality and composition. Given the nature of the substrate may play a crucial role in determining water and nutrient availability for the play and hence may affect the metabolic pathways involved in the synthesis of specific biochemical compounds, this study was conducted to determine the effects of organic soilless substrates such as coir dust and burnt paddy husks on ginger growth, yield and [6]-gingerol content using soilless culture system.

MATERIALS AND METHODS

Bentong ginger was selected and used in this study. Each of the rhizomes was cut into smaller pieces of about 4 cm long and 40 g in weight. Each of the seed rhizomes contained 2 – 3 point buds. The seed rhizomes were treated with previcur-N prior to planting. A side-netted rain shelter of 30 m long x 10 m wide x 4.5 m high located in MARDI Station, Kluang, Johor was used in the study. The treatments were arranged in a randomized complete block design (RCBD) with five levels of treatment with three replicates and 30 plants per treatment. The coir dust and burnt paddy husks were weighed in accordance to the quantity required for each treatment. There were five coir dusts and burnt paddy husks mixtures used as treatments in this study. These treatments were as follows: T1 = 100% coir dust; T2 = 100% burnt paddy husks; T3 = 70% coir dust and 30% burnt paddy husks; T4 = 30% coir dust and 70% burnt paddy husks; and T5 = 50% coir dust and 50% burnt paddy husks. Each mixture was thoroughly mixed in a 10-litre pail before filled into 60 cm x 60 cm black polyethylene bags. The seed rhizomes were sown into the substrate according to the treatments. Each polyethylene bag was

placed randomly on four irrigation lines under the side-netted rain shelter and individually irrigated with nutrient solution via a dripper on the surface of the medium. The irrigation system, which was built in the side-netted rain shelter, consisted of a 1,500-litre tank, 1.5 Hp water pump, water liter, pressure meter and four lateral lines (28 m each) which looped to each other. Each of the lateral lines was equipped with 100 drippers that were placed into 100 polyethylene bags, side by side. The distance between each line was 1.5 m and the distance between each dripper point in the lateral line was 0.3 m. The nutrient solutions were supplied through 0.3 m micro tubes and arrow drippers.

The fertilizer was formulated by MARDI based on the needs of the plant rhizomes (Yaseer Suhaimi et al. 2009). All the fertiliser components were water soluble. The fertiliser stocks were prepared according to Yaseer Suhaimi et al. (2011).

The irrigation solutions were prepared in a 1,500-litre tank. Stock A and stock B were added into the tank at 1:1 ratio until the needed electricity conductivity (EC) was achieved. The EC of the fertigation solution was between 1.8 uS and 2.3 uS. The irrigation scheduling was automatically implemented by a digital timer, three times per day in the first 3 months (0800 h, 1200 h and 1600 h), six times per day in the $4^{th} - 7^{th}$ months (0700 h, 0800 h, 1000 h, 1200 h, 1400 h and 1600 h), and once per day in the last 2 months (1000 h).

The duration of irrigation was 3 min and an identical amount of fertilizer solution was applied to all polyethylene bags. The daily irrigation volumes per plant were 675 ml in the first 3 months, 1,350 ml in the $4^{th} - 7^{th}$ months, and 75 ml in the last 2 months. Routine horticultural practices for pest, disease and weed control were followed. Insecticide (*Malathion*) and fungicide (*Benlate*) were applied once every 2 weeks.

The growth of the ginger plants was measured monthly by measuring the height and weight of leaves/shoot and rhizomes. The ginger plants were randomly selected and the rhizomes were harvested after 3 – 9 months of sowing to determine the yield and growth of rhizomes. The weight was measured immediately after harvest to prevent desiccation and water loss from the rhizomes.

Fresh ginger rhizomes were graded, washed thoroughly with tap water, peeled and cut into cross-sections of 2 ± 1 mm thickness. Cut ginger samples were dried in an air dryer at 50 ± 2 $^{\circ}$C to achieve 10-12 % moisture content for 3 hours then the samples were taken for the analyses of [6]-gingerol content for dried ginger. 2 g of dry ginger were put in a 250 ml conical flask contain 150 ml of methanol then were shaking about 7 hours on the orbital shaker at 250 rpm. The methanol extract was filtered using 0.20 µm Nylon membrane filter (Whatman, England). 10g of cut fresh ginger were blended with 50 ml methanol (HPLC grade) by electrical blender for 1min and centrifuged at 5,000 rpm for 5 min. The supernatant was subsequently filtered through a 0.20 µm Nylon membrane filter (Whatman, England). A 20 µl ginger extract from both dry and fresh ginger extract were then subjected to HPLC

for the [6]-gingerol analysis. The analysis method of 6-gingerol content was done according to Sharizan et al. (2014) with modification.

Data obtained were subjected to statistical analysis using analysis of variance (ANOVA) procedures to test the significant effect of all the variables investigated using SAS version 9.1. Means were separated using Duncan Multiple Range Test (DMRT) as the test of significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

There were significant differences in plant height between treatments (*Table 2*). The tallest plants were produced by ginger cultivated in 100% coir dust with an average height of 123 ± 23 cm and the lowest were those cultivated in mixture of 30% coir dust and 70% burnt paddy husks (average height 105 ± 8 cm). Treatment containing 100% coir dust produced the tallest plants compared to burnt paddy husks and mixtures of both substrates. This could be due to the higher porosity of coir dust compared to the other treatments. This higher porosity property drained out the excess fertiliser solution between the irrigation schedules more quickly. Raviv et al. (2001) found that the number of rose flowers was 19 per cent higher in coir dusk than in others substrates. The obtained results were agreed with Neamati et al. (2010) who observed the highest stem and root length tomato cultivated in the coco peat media. The mixtures between coir dust and burnt paddy husks could have increased the water holding capacity and subsequently decreased the dissolved oxygen availability in the growing medium. Plant height grown in mixture of 30% coir dust and 70% burnt paddy husks was significantly affected. The higher content of burnt paddy husks in the medium added more moisture content that lowered dissolved oxygen in the media, which consequently reduced height of the ginger plant compared to 100% coir dust. Similar studies also showed that high water holding capacity reduces the growth and yield of cucumber (Peyvast et al. 2010). The shoots were cut and trimmed 2 weeks before harvesting. This allowed the rhizomes to harden in the media (Paul et al. 2004). There were significant differences in shoot fresh weight between treatments. The highest shoot fresh weight was recorded from plants cultivated in 100% coir dust with an average weight of 1,340 ± 235 g (*Table 2*), while the lowest weight was obtained from plants cultivated in mixture of 30% coir dust and 70% burnt paddy husks. The shoot fresh weights were higher with higher content of coir dust in the growing media. However, there was no significant difference between 100% burnt paddy husks, mixture of 70% coir dust and 30% burnt paddy husks, and mixture of 50% coir dust and 50% burnt paddy husks at $p \leq 0.05$. A study by Fukuda and Anami (2002) also revealed an increase in melon biomass when grown in coir dust.

Table 2. Plant growth and rhizomes yield after 9 months of cultivation periods

Treatment	Plant height (cm)	Shoot fresh weight (g)	Average rhizome yield per plant (g)	Rhizome to shoot ratio
100% CD	123 ± 23a	1,340 ± 235a	5,480 ± 325a	4.09a
100% BPH	114 ± 15b	1,210 ± 223b	3,480 ± 150d	2.87d
70% CD + 30% BPH	112 ± 12c	1,130 ± 127b	4,580 ± 170b	4.05b
30% CD + 70% BPH	105 ± 8d	1,090 ± 115c	2,570 ± 135e	2.33e
50% CD + 50% BPH	115 ± 16b	1,120 ± 120b	4,400 ± 180c	4.03c

Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$
CD = Coir dust; BPH = Burnt paddy husks

For commercial purposes, ginger rhizomes are harvested 7 – 9 months after sowing (Wilson and Ovid 1993). In this study, the rhizomes were harvested after 3 – 9 months and the fresh weight of the rhizomes were measured. The interior fresh and epidermis were lighter in colour than the mother seed piece. There was also fibre development in the interior fresh. The rhizomes also produced a pungent odour with a distinctive ginger flavour. They were marketable as fresh young ginger between 3 and 6 months of cultivation and mature ginger between 8 and 9 months. There were significant differences in rhizome yield between treatments after 9 months of cultivation. The highest average fresh rhizome yield was obtained from plants cultivated in 100% coir dust, followed by mixtures of 70% coir dust and 30% burnt paddy husks, 50% coir dust and 50% burnt paddy husks, 100% burnt paddy husks, and 30% coir dust and 70% burnt paddy husks. These results showed that ginger cultivated in higher amount of coir dust media increased the rhizome yield up to 36% compared to those grown in media containing higher amount of burnt paddy husks. High oxygen availability in the coir dust media supported the underground rhizomes requirement for high oxygen for growth. Tomatoes grown very well in coir dusk compared to others substrates, even under temperature stress (Islam et al., 2002). It has been shown that a number of potted plants, nursery, leafy and fruity vegetables performed better in coir dusk (de Kreij and van Leeuwen, 2001). For crops grown in containers, it is important to consider the tendency of most root systems to grow gravitropically to form a dense layer at the bottom of the containers (Raviv et al. 2001). Coir dust has a strong capillarity that provides more uniform moisture conditions for roots. These conditions are able to increase aeration in the base mix and reduce drying of the surface by lifting the moisture higher up in the polyethylene bags. This increases the volume of the mixture that is suitable for root development and improve access to moisture and fertilizer. This redistribution of moisture is perhaps one of the reasons for plants grown in pure coir dust to have higher rhizome yield. Aeration in the growing medium is positively related to AFP and negatively to water content (Raviv and Lieth 2008).

The coir dust is less acidic with a pH suitable to facilitate ginger to grow and consequently allows the plant roots to absorb nutrient efficiently.

In the early cultivation period between 1 and 3 months, the growth of rhizomes between treatments was similar. The exponential growth of the rhizomes began in the fifth month and the rhizomes in 100% coir dust treatment showed the highest growth compared to other treatments (*Figure 1*). Media with higher content of burnt paddy husks gave lower rhizome yield throughout the cultivation period with a mixture of 30% coir dust and 70% burnt paddy husks exhibited the lowest rhizome yield.

These results were similar with the study conducted by Kratky (1998), who found that ginger rhizome yield increased significantly when grown using soilless system under rain shelter. Previous study done by Hayden et al. (2004) found that the growth of rhizomes is dependent on the type of medium. The growing medium acts as heat insulator and provides heat that enhances the growth of rhizomes.

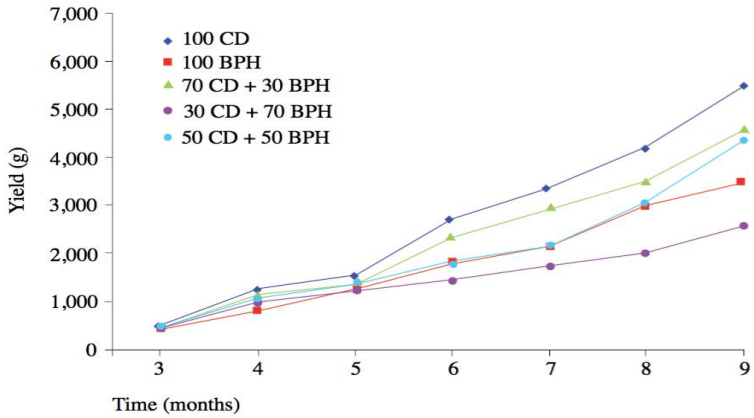

Figure 1. Growth of ginger rhizomes between third and ninth months of cultivation

Overall biomass of ginger plants can be divided into two parts: above ground biomass consisting of leaves and stem (shoots), and underground biomass consisting of rhizomes and roots. In this study, there were significant differences between treatments in rhizomes to shoot ratio. The ratio of underground biomass to aboveground biomass was highest in plants cultivated in 100% coir dust with a ratio of 4.09 (*Table 3*). There was higher underground biomass compared to aboveground biomass in plant grown in the 100% coir dust. The higher ratio of underground biomass to aboveground biomass reflects that the roots were well able to supply the top of the plant with water, nutrient, stored carbohydrates and certain growth regulators (Harris 1992). The rhizome to shoot ratio in plants cultivated in high coir dust media was 4 to 1, while that in plants cultivated in high burnt paddy husks was 2 to 1. Long and quicker root development of tomatoes and strawberry were observed in the coir dusk growth substrate (Lopez- Medina et al., 2004).

Table 3. The dry weight of shoot and rhizomes biomass after 9 months of cultivation periods

Treatment	Shoot dry weight (g)	Rhizome dry weight (g)
100% CD	804	3288
100% BPH	726	2088
70% CD + 30% BPH	678	2748
30% CD + 70% BPH	654	1542
50% CD + 50% BPH	672	2640

[6]-gingerol was the major ginger oleoresin. The amounts of [6]-gingerol content from fresh and dried ginger are shown in Table 1. β-hydroxyl keto functional group is main molecular structure of gingerol which was thermally labile. [6]-gingerols tends to degrade into shogaols and aliphatic aldehydes due to heat or thermal process during occurrence of drying process (Bhattarai et al., 2001). As a result, the dried ginger had a smaller amount of [6]-gingerol compared to the fresh product. The HPLC chromatograms of [6]-gingerol standard are shown in Figure 2. The retention time of [6]-gingerol standard as shown in the chromatograms at 11.5 minutes.

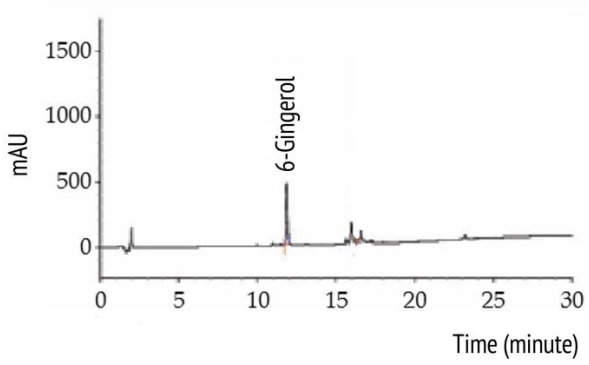

Figure 2: The retention time of [6]-gingerol standard injected into HPLC instrument

There were no significant different between all treatments in term of [6]-gingerol content in the fresh and dry ginger rhizomes. However, plant cultivated in the media contained coir dust showed slightly higher [6]-gingerol content in the fresh weight of ginger (Table 4). Similar pattern also was observed in [6]-gingerol content in the dry weight of ginger (Table 5). The accumulation of [6]-gingerol in both fresh and dry ginger samples were higher from month three to month six and started to decrease at month seven towards the end of the cultivation period. The [6]-gingerol content was higher in the month six and lowest at months nine of cultivation periods. Both fresh and dry ginger samples showed the similar pattern. Studies by Sharizan et al. (2014) revealed that accumulation of [6]-gingerol compound were higher in 6-month ginger rhizomes and lower in 9 and 12-month ginger rhizomes. No differences in [6]-gingerol were observed according to the

type growth media. Investigation by Rodriguez et al. (2006) on the effect of different substrates for hydroponic production of 'Galia' muskmelons (Cucumis melo L.) revealed that fruit quality and composition were not affected by substrate. Others studies also had showed that rising concentration of electric conductivity (EC) of nutrient solution and nutrient solution composition can caused increase in total soluble solids, organic acids and plant secondary metabolites for tomatoes (Petersen et al., 1998), sweet pepper and cucumber (Trajkova et al., 2006). Overall, the nature of the soilless media substrate did not affect overall rhizomes quality and chemical compounds. Appropriate root zone aeration is effective for higher fruit yield and bioactivity of the fruit in perlite culture, but excessive aeration inhibited root respiration, nutrients, bioactivity, and water uptake, and it resulted in the reduction of plant growth and fruit yield (Jong et al., 2014)

Table 4. Accumulation of [6]-Gingerol in the fresh weight of rhizomes from three to nine months if cultivation periods (mg/g fresh weight)

Treatment/ months / mg/g fresh weight	**3**	**4**	**5**	**6**	**7**	**8**	**9**
100% CD	1.879	2.045	2.389	2.546	1.767	1.542	1.183
100% BPH	1.521	1.756	2.023	2.298	1.437	1.247	1.094
70% CD + 30% BPH	1.732	1.906	2.274	2.456	1.612	1.434	1.013
30% CD + 70% BPH	1.597	1.845	2.154	2.318	1.556	1.228	0.754
50% CD + 50% BPH	1.621	1.889	2.171	2.308	1.678	1.390	0.806

Table 5. Accumulation of [6]-Gingerol in the dry weight of rhizomes from three to nine months if cultivation periods (mg/g fresh weight)

Treatment/ months / mg/g dry weight	**3**	**4**	**5**	**6**	**7**	**8**	**9**
100% CD	1.474	1.045	0.643	0.434	0.367	0.236	0.122
100% BPH	1.125	0.906	0.512	0.302	0.290	0.104	0.093
70% CD + 30% BPH	1.305	1.006	0.734	0.404	0.313	0.209	0.114
30% CD + 70% BPH	1.138	0.943	0.705	0.398	0.285	0.201	0.105
50% CD + 50% BPH	1.224	0.912	0.684	0.351	0.255	0.112	0.100

CONCLUSIONS

The yield and quality of the products reflects to the different cultivation guidelines that applied to each substrate. The mixture of coir dust and burnt paddy husks significantly affected plant height, shoot biomass, rhizome yield, and rhizome to shoot ratio. Media containing high amount of coir dust (70 – 100%) showed good growth and increased the rhizome yield up to 36% compared to those containing high amount of burnt paddy husks. The [6]-gingerol content were higher in 100% coir dust throughout the cultivation periods. However, there were not significant difference in the [6]-gingerol content between each treatment. The studies suggested that the secondary metabolites like [6]-gingerol content and accumulation did not effected by the substrates or substrates mixtures alone. It can be concluded that 100% coir dust or any combinations with high amount of coir dust are the best substrates for growing ginger in soilless culture system.

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EFFECTS OF CULTIVAR AND MULCHING ON THE POTATO YIELD

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ABSTRACT

Plant growing technology involves usage of complex agrotechnical operations aimed at creating favorable conditions for plant growth and development, that is, for better exploitation of cultivar genetic potential. Effects of mulching with white and black polyethylene foil, and organic mulch (straw) were studied in three, drip-irrigated potato cultivars: Carrera (early), Laura (medium early) and Agria (medium late). Treatments were arranged in a randomized complete block design with four replications at the site of Zemun Polje, Serbia (44°88'N, 20°35'E, 79 m a.s.l.) in three consecutive years (2011–2013). Results obtained on the variants with different mulch materials were compared with results attained on the plot with bare soil (control). The highest average number of tubers per plant was determined in cv. Laura's control variant (12.0), while the lowest average number of tubers was determined in the cv. Agria with white mulch (8.2). The highest average tuber mass (146.3 g) and total tuber yield (59.6 t ha^{-1}) was determined in cv. Carrera subjected to the straw mulch treatment. The lowest average tuber mass and tuber yield was found in the cv. Agria on the variant with black polyethylene foil (83 g and 27.8 t ha^{-1}). The results obtained in our study indicate positive effect of the combination of straw mulch and irrigation on the productivity of potato. Modern growing technology, which includes the regulation of temperature in the surface soil layer by combination of soil mulching, drip irrigation system, and the use of adequate genotypes, can result in high potato yield.

Key words: *mulching, potato, potato yield, tuber mass.*

INTRODUCTION

The average area under potatoes in Serbia is 73.000 ha with a small average yield of 14.5 t ha^{-1} (Statistical Yearbook of Serbia, 2016). Wider application of intensive agrotechnology is lacking in potato production in Serbia, especially considering small and economically poor agricultural holdings (Broćić and Stefanović 2012).

Plant growing technology involves usage of complex agrotechnical operations aimed at creating favorable conditions for plant growth and development, that is, for better exploitation of the genetic potential of cultivars. Term agronomic variety relates to variety specific requirements regarding particular agrotechnical measures. Detailed monitoring of important morphological characteristics, such as fertility, yield quantity and quality, disease resistance and abiotic stress tolerance, must be a basic prerequisite for assessing the suitability of a particular breeding variety to specific agroecological conditions (Momirović et al., 2000). Agrotechnical procedures applied in modern potato production are selection of plots, basic and additional soil cultivation, fertilization of crops, selection of assortments and preparation for planting, crop protection measures (hoeing, sheltering, protection from diseases and pests, feeding, mellowing of compacted soils, irrigation), extraction of potato tubers, tuber storage and preparation for the market.

Mulching is an agrotechnical technique that directly determines the microclimate of plants in several ways. It reduces evaporation (Gao and Li, 2005; Zhao et al., 2012), warms the surface soil layer after sowing (Wang et al., 2003; Zhao et al., 2012), increases the microbiological activity (Yang et al., 2003; Agüero et al., 2008) and inhibits the development of most one-year and perennial weeds (Jodaugiene et al., 2006a, b). Soil covering with mulch also reduces the destructive action of rain drops, prevents formation of crust and maintains favorable air regime of the land. Through greater heat accumulation, as well as the photo-physiological effect of reflected diffused light, soil mulching significantly affects potato yield quantity and quality, the bulk density and marketability of the tubers, as well as the greater content of protective, colorful compounds (Momirović et al., 2011). Since the application of a plastic mulch technique most often involves irrigation with the "drop by drop" system, the loss of nutrients is minimal. Nutritional substances can be introduced into the irrigation system and thus, if necessary, precisely directed into the soil root zone.

For soil mulching, differently colored polychloropenic foils and organic mulch are used. White/black foil is characterized by extremely high reflection which allows cultivation of crops in the warmer part of the vegetation season. Black mulch foils are used for vegetable cultivation in general; the advantages are mainly related to water savings (up to 50% in drip-irrigation system), successful weed control, better phytosanitary conditions and directed carbon dioxide emissions from soil to photosynthetic area (chimney effect). Dark foil can be considered as absolutely non-toxic herbicide, harmless to plants, land and man (Kovačević and Momirović, 2008). Straw is the most commonly used material for ground covering in the crop and vegetable production because of its good thermal insulation properties. The temperature of the soil under the straw can be 5-8 ^{o}C lower than the temperature of bare soil which is especially important in the summer. The aim of the present paper was to evaluate effects of different mulch materials (organic and plastic mulch) and cultivar/genotype on productive characteristics of potato grown in the lowland region of Serbia.

MATERIALS AND METHODS

Effects of mulching with white and black polyethylene foil, and organic mulch (straw) on productive characteristics of three drip-irrigated potato cultivars: Carrera (early), Laura (medium early) and Agria (medium late) were investigated. Treatments were arranged in a randomized complete block design with four replications at the site of Zemun Polje, Belgrade (44°88'N, 20°35'E, 79 m a.s.l.) in the period 2011–2013. Experimental field was divided into four blocks with unit size of 7 m^2 (cultivar x mulch combination). Standard tillage treatment for potato crop was used. Irrigation tapes were placed in the middle of a shallow bank and incorporated into the soil (sub-irrigation) and soil moisture maintained at about 75% of FWC.

The variants with different mulch materials were established, as well as control plot with bare soil. In plots with plastic mulch, ridges were formed firstly and then covered by the polyethylene film. Tubers were hand-planted in previously prepared holes in the plastic mulch. In the variant with organic mulch, straw was layered to 25 mm thickness directly after planting and the formation of ridges.

In the years of study the planting of tubers (planting density: 80 x 30 cm) was carried out in the first decade of April. Potato harvesting was carried out at the stage of full maturity in the second ten days of September and number of tubers per plant, tuber mass and total yield were determined for all cultivar/treatment variants and averaged.

The climate conditions during potato growing season are presented in the Table 1. The average air temperatures during potato vegetation period in 2011, 2012 and 2013 were significantly higher (for 2.1-3.6 ^oC) than 1961-1990 multi-year average (18.4 ^oC); this was mostly result of average temperature increase in June, July and August. Temperature increase in June and July were especially significant, considering that this period is important for tuber bulking. Precipitations were below limits of multiple-year average with an uneven distribution during the growing season in all three years of investigation.

Climate conditions during potato growing season

Table 1. The average monthly air temperatures and monthly precipitation sums at Belgrade, Serbia during the growing seasons 2011, 2012 and 2013 and multi-year average

Month	2011		2012		2013		1961-1990	
	^oC	mm	^oC	mm	^oC	mm	^oC	mm
April	14.7	14.1	14.6	66.9	15.2	21.3	12.4	58.8
May	18.3	66.8	18.0	127.9	19.3	104.4	17.2	70.7
June	21.9	41.1	24.6	16.0	21.1	50.1	20.1	90.4
July	24.3	95.0	27.0	39.0	24.1	2.9	21.8	66.5
August	24.6	14.0	26.0	4.5	25.5	44.3	21.4	51.2
September	23.0	47.7	22.0	30.7	17.6	58.7	17.7	51.4
Average/Sum	**21.1**	**278.7**	**22.0**	**285.0**	**20.5**	**281.7**	**18.4**	**389.0**

Statistical analysis was performed using STATISTICA 10 (StatSoft, Inc. 1984-2011, USA). The data concerning tuber number, tuber mass and total yield were subjected to two-factor analysis of variance (ANOVA) with Cultivar and Mulch-Type as factors, followed by Fisher's-LSD test at significance levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

The results of investigation given in Table 2. show that the highest average number of tubers per plant (all three cultivars/genotypes) was recorded in the variant with white plastic mulch and control (10.0), somewhat smaller was determined on variants with black mulch (9.9), while the lowest number of tubers per plant (9.5) was recorded on variant with straw.

Considering cultivar/genotype factor, cv. Laura was forming the highest average number of tubers per plant (10.8), followed by Carrera (10.2), while the lowest number of tubers per plant was recorded for cv. Agria (8.6).

Considering both investigated factors (Table 2.), Mulch and Cultivar, cv. Carrera grown on white foil mulch has shown significantly higher number of tubers per plant (11.2) compared to straw mulch (10.1), black foil mulch (10.0) and control (9.4) variants. Cv. Laura formed the highest number of tubers on bare soil (12.0), followed by variants with black and white foil mulch, and significantly the lowest number on variant with straw mulch (9.1).

Tuber size and mass are traits primarily determined by potato genotype. Nevertheless, these traits depend also on ecological factors, agrotechnical practices, size of seed tuber, number of primary shoots, number of tubers per plant, etc. (Poštić et al., 2015; Momirović et al 2016). Significant difference was determined between tuber mass on organic, straw mulch and other investigated mulches (Table 2.). The highest average tuber mass was recorded on organic mulch (134 g), followed by control (126 g), variant with black plastic mulch (102 g), while the lowest tuber mass was determined on variant with white plastic mulch (95 g).

Comparison between cultivars/genotypes has revealed that cv. Carrera forms significantly heavier tubers (130 g) compared to cvs. Agria (111 g) and Laura (101 g).

Considering both investigated factors (Table 2.), Mulch and Cultivar, cv. Agria grown on straw mulch and bare soil formed significantly heavier tubers (134 g and 129 g, respectively) compared with white and black foil mulch (98 g and 83 g, respectively). Tuber mass of two other cultivars, Carrera and Laura, did not significantly differ between investigated mulch variants.

Table 2. Impacts of mulch and cultivar on potato status

Mulch (polyethylene foil A1=white, A2=black; organic mulch A3=straw; bare soil A4), cultivar (B1= Carrera, B2=Laura, B3=Agria) and potato status (3-year averages 2011-2013)

	Tuber number per plant				**Tuber mass** (g)				**Tuber yield** (t ha^{-1})			
	B1	B2	B3	**xA**	B1	B2	B3	**xA**	B1	B2	B3	**xA**
A1	11.2	10.7	8.2	**10.0**	103	83	98	**95**	48.0	34.7	33.1	**38.6**
A2	10.0	11.5	8.2	**9.9**	127	96	83	**102**	53.9	45.6	27.8	**42.5**
A3	10.1	9.1	9.3	**9.5**	146	122	134	**134**	59.6	44.5	48.2	**50.7**
A4	9.4	12.0	8.5	**10.0**	142	105	129	**126**	55.1	51.8	45.1	**50.7**
x B	**10.2**	**10.8**	**8.6**	**x B**	**130**	**101**	**111**	**x B**	**54.2**	**44.1**	**38.6**	
LSD	A	B	AxB		A	B	AxB		A	B	AxB	
0.05	0.57	0.38	1.50		34.5	29.9	45.8		12.1	10.8	19.1	
0.01	0.97	0.79	2.57		41.5	30.6	55.5		16.2	14.0	25.0	

The highest average yield of three cultivars (Table 2.) was determined in variant with organic mulch (50.7 t ha-1), followed by yield of potato plants treated with black plastic mulch (42.5 t ha-1), while the lowest yield was determined in variant white plastic mulch (38.6 t ha-1). Similar results were obtained in research on cvs. Marabel (medium early), Desiree (medium late) and Jelly (medium late) where significant difference in tuber yield was determined between organic mulch and two plastic mulch treatments (Oljača et al., 2016a). Beneficial effect of plastic foil mulches on potato yield is usually observed in cold climate regions (Singh and Ahmed 2008), while in Serbia plastic foil mulching can be useful in production of new potato during spring (Oljača 2016. b).

Considering cultivar/genotype factor, cv. Carrera has shown the highest average yield (54.2 t ha^{-1}), followed by Laura (44.1 t ha^{-1}), while the lowest tuber yield was recorded for cv. Agria (38.6 t ha^{-1}). However, significant difference in yield was detected only between Carrera and two other cultivars, Laura and Agria.

Considering both investigated factors, Mulch and Cultivar, cv. Agria has shown significantly higher tuber yield on straw mulch (48.2 t ha^{-1}) and bare soil (45.1 t ha^{-1}) compared with white (33.1 t ha^{-1}) or black (27.8 t ha^{-1}) plastic foil mulch. Similarly to tuber mass parameter, Carrera and Laura tuber yield on different mulch variants did not significantly differ. Also, no significant difference was observed between mulch treatments and control (bare soil).

CONCLUSIONS

The results obtained in our study indicate positive effect of the combination of straw mulch and irrigation on the productivity of potato. Considering production potential of particular cultivars/genotypes, the highest average tuber mass all three varieties were achieved in treatments with organic mulch or bare soil. Cultivars Carrera and Agria was showing the highest yield on variant with organic mulch,

while the Laura variety achieved the highest yield on bare soil. Conversely, the lowest yield of each particular cultivar was determined in treatment with white and black plastic mulch indicating that this mulch type is not appropriate for potato growing under agroecological conditions of southern Srem, Serbia. Modern growing technology, which includes the regulation of temperature in the surface soil layer by combination of straw mulching, drip irrigation system, and the use of adequate genotypes, can result in high potato yield.

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EFFECT OF BIOFERTILIZER AND COMPOST ON NITRATE STATUS, YIELD AND QUALITY OF POTATO TUBER UNDER NEWLY RECLAIMED SANDY SOIL

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ABSTRACT

This investigation was carried out during the two summer seasons of 2015 and 2016 in sandy soil on potato culitvar "Sante" to study the effect of using 100% compost (15 t/fed.) and 50% compost + nitrogen fixing bacteria (*Azotobacter*, and *Pseudomonas* alone or together) on potato yield and quality as compared to the conventional mineral fertilization (120-75-150 kg/fed. NPK + 5 ton compost/fed.(control)). No significant differences in tubers yield/fed. were detected between mineral fertilization (control) and using 100% compost (15 t/fed).However, control treatment significantly produced a high yield per feddan, more than using 50% compost + any biofertilizer treatment.Using compost treatment at 15 t/fed.exceed all biofertilizer treatments in marketable yield in both seasons, but without significant differences as compared with mineral fertilization (control).No significant differences in tuber dray matter and content of starch in tuber were found between using compost treatment at 15 ton/fed. and mineral fertilization treatment (control)in both seasons. Nevertheless, application of 50% compost+ 4 applications of Azotobacter and Pseudomonas had the highest tuber concentrations of starch and nitrogen with significant differences as compared with the mineral fertilization.Using50% compost + 4 applications of Azotobacter or Pseudomonas or both (Azotobacter + Pseudomonas)and application of 100% compost caused producing potato tubers with the lowest concentration of nitrate with significant differences as compared with the mineral fertilization. No significant differences were detected between mineral and organic fertilizers concerning P and K concentrations in tubers.

Keywords: *potato, compost, Azotobacter, Pseudomonas, biofertilizer.*

INTRODUCTION

Organic farming/products are becoming very necessary in today's world to control ecosystem health and to impart related human health benefits, world over there is growing demand for organic produce. Therefore, renewed interest in organic

farming has resulted in a need for research in sustainable farming practices this interest is in response to environmental and health concerns. In addition, there is a perception that organic farming will help alleviate problems associated with food safety, environmental quality and impact, market concentration, and the survival of rural communities. Over fertilization in agriculture has led to surface water and ground water contamination. Nitrogen fertilizer pollution is responsible for eutrophication, hypoxia, and algal blooms in rivers, marshes, ground water, and runoff, and may be a public health risk (Kramer *et al*., 2006). Organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. These goals are met, where possible, through the use of cultural, biological, and mechanical methods, as opposed to using synthetic materials to fulfill specific functions within the system (USDA National Organic Standards Board (NOSB) definition, 2001).Egypt has an extended growing season along with an abundance of insect, disease and weed species, both beneficial and pest, making it an ideal region for the extensive study of organic farming. One aspect of organic production that is in need of study is fertilizers and their application rates in organic systems. One of the main problems of organic production is that organic fertilizers are often bulky or are necessary in large quantities. Potato (Solarium tuberosum) It is economically important both in Egypt and worldwide, and it is a world staple crop, able to grow in an array of environments. Up to 85% of potato plant biomass is edible as compared to about 50% of cereals. Potato consumption has increased in the developing world, and over the last decade world, potato production has increased at an annual average rate of 4.5 percent (FAO, 2007). Among other crops, potato is one of the most highly demanded products on the market for organic produce.

The present work aimed to study the effect of using 100% compost and 50% compost + nitrogen fixing bacteria on potato growth and yield as compared to the conventional mineral fertilization.

MATERIALS AND METHODS

This investigation was carried out in Sandy soil farm at Alexandria Desert Rod, Egypt, during the two summer seasons of 2015 and 2016. Investigation material used was potato cultivar Sante, Treatments were arranged in Complete Randomize bloke design with four replicates for each treatment.Potato seeds were cut (approximately 35 g pieces), All potato tuber seeds were treated with the biofungicideBiohealth (containg*Trichoderma sp.* + *Bacillussubtilus*) at a rate of 150 ml/ 100 l. water to avoid infection with soil borne diseases.Potato tubers were mechanically planted by using a four-rows-planter. Each plot was 180 m^2 and included 4 rows.

Treatments

1- T_1: Recommended control (Mineral NPK at rate of 120-75 -150 kg + 5 ton compost/fed.)

2- T_2: 15 t/ Compost fed. (100%) + Feldspar
3- T_3: 50% Compost + Feldspar + Rock Phosphate +Azotobacter (Applied 0, 6 weeks after planting).
4- T_4: 50% Compost + Feldspar + Rock Phosphate + Azotobacter (Applied 0, 3, 6 and 9 weeks after planting).
5- T_5: 50% Compost + Feldspar + Rock Phosphate + Pseudomonus (Applied 0, 6 weeks after planting).
6- T_6: 50% Compost + Feldspar + Rock Phosphate + Pseudomonus (Applied 0, 3, 6 and 9 weeks after planting).
7- T_7: 50% compost + Feldspar + Rock Phosphate + Azotobacter (0, 6 weeks) + Pseudomonus (0, 6 weeks)
8- T_8: 50% compost + Feldspar + Rock Phosphate + Azotobacter (0, 6 weeks)+ Pseudomonus (0, 3, 6, 9 weeks)
9- T_9: 50% compost + Feldspar +Rock Phosphate+ Azotobacter (0, 3, 6, 9 weeks) + Pseudomonus (0, 6 weeks)
10- T_{10}: 50% compost + Feldspar + Rock Phosphate + Azotobacter (0, 3, 6, 9 weeks) + Pseudomonus (0, 3, 6, 9 weeks)
11-

Data recorded

The experiment was harvested after 105 day after planting, using digger and the following yield data were recorded:1. Total yield (ton/fed).2. Marketable yield (ton/fed).

At harvest, a sample of 100 gram fresh weight of tubers was taken from each plot to determine the percentage of dry matter, nitrates, starch, N, P and K in tubers.

Data of the present study were statistically analyzed using M Stat and the differences between the means of the treatments were considered significantly, when they were more than least significant differences (LSD) at the confidence level of 5%.Sndecor and Cokran, (1967).

RESULTS AND DISCUSSION

Yield and its components: Total Yield

Using mineral fertilization (control treatment) exceeded all biofertilizer treatments in total yield in both seasons (Table1). Meanwhile, there were no significant differences between using compost treatment at 15 ton/fed. and using mineral fertilization (control treatment). No significant differences between application of *Azotobacter* at T3 and *Azotobacter*application atT4 in both season. Applied of *Pseudomonas* T5 and T6 had no significant differences in first season, in contrary in second season T6 recorded higher value of total yield than T5. All combinations between *Azotobacter* and *Pseudomonas* had no significant differences except treatment no. 10, which exceeded all bio fertilizer treatments. This was true in both seasons.

Table 1.Effect of different fertilization, treatments on tuber total yield(ton/fed).

Treatments		Total yield (ton/fed.)		Combined data
		First season	Second season	
1	Mineral NPK(Control)	12.667	15.11	13.89
2	100% Compost(15 ton/fed.)	11.91	13.00	12.46
3	50% Compost + *Azotobacter* 0, 6	5.983	5.750	5.867
4	50% Compost + *Azotobacter* 0, 3, 6, 9	6.350	5.917	6.133
5	50% Compost + *Pseudomonas* 0, 6	8.600	9.517	9.058
6	50% Compost + *Pseudomonas* 0,3, 6, 9	6.533	6.550	6.542
7	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 6.	8.050	8.383	8.217
8	50% Compost + *Azotobacter*0, 6 + *Pseudomonas* 0, 3, 6, 9.	6.917	7.667	7.292
9	50% Compost + *Azotobacter*0, 3, 6, 9 + *Pseudomonas* 0, 6.	8.183	7.667	7.925
10	50% Compost + *Azotobacter*0, 3, 6, 9 + *Pseudomonas* 0, 3, 6, 9.	10.08	10.03	10.058
Mean		8.528	8.959	
LSD $_{0.05}$ for: Season (S) 1.084 Treatments (T) 3.428 S X T 2.424				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks after planting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P_2O_5 and 195 K_2O kg/fed).

Marketable yield

Using compost treatment at 15 ton/fed. exceeded all biofertilizer treatments in marketable yield in both seasons, without no significant differences compared with mineral fertilization (control treatment). Treatment no.4 had the lowest marketable yield value in both seasons. Applied of *Pseudomonas*T5 and T6 had no significant differences in both season. Different combinations between *Azotobacter* and *Pseudomonas* (treatments no. 7, 8, 9 an10) showed no significant differences among them. Treatment no. 7 recorded the highest value of marketable yield in first season, while treatment no. 10 recorded the highest value of marketable yield in the second season. All biofertilizer treatments had no significant differences except treatments no. 7 and 10 at the first season. (Table2).

Tuber quality: Starch concentration

Data in Table 3 revealed that there were no significant differences between using compost at 15 ton/fed. and mineral fertilization in starch concentration in potato tuber. However, compost application showed higher concentration than the mineral application in both seasons. Treatment no.10 surpassed mineral fertilization treatment and had the highest value of tuber starch concentration. The lowest value of tuber starch content was recorded with T9.

Nitrate concentration

Application of compost treatment as well as T4, combination T10,T6 and T8 had the lowest value of NO_3 concentration in tuber. Compost application had significantly lower nitrate concentration in tuber than mineral fertilization. There were no significant differences between using mineral fertilization and T3, T5 and T7 in NO_3 concentration in tuber (Table 4).

Dry matter concentration

No significant differences in tuber dray matter were found between using compost treatment at 15 ton/fed. and mineral fertilization treatment (control). Using T3 had the lowest value with significant differences than all the other biofertilizer treatments except T6, which had no significant differences than T3(Table5). Using both of mineral fertilization and compost treatment had no significant differences with all biofertilizer treatments except T3 and T6 which had the lowest value with significant differences.

Table 2.Effect of different fertilization, treatments on marketable yield(ton/fed).

Treatments		Marketable yield (ton/fed)		Combined data
		First season	Second season	
1	Mineral NPK(Control)	8.45	11.196	12.33
2	100% Compost(15 ton/fed.)	11.231	10.19	13.83
3	50% Compost + *Azotobacter* 0, 6	4.333	4.048	5.167
4	50% Compost + *Azotobacter* 0, 3, 6, 9	2.667	4.508	4.333
5	50% Compost + *Pseudomonas* 0, 6	5.667	6.667	6.167
6	50% Compost + *Pseudomonas* 0, 3, 6, 9	4.333	5.338	5.667
7	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 6.	8.269	6.667	8.000
8	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 3, 6, 9.	5.333	6.632	6.667
9	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 6.	3.667	6.260	6.000
10	50% Compost + *Azotobacter*0, 3, 6, 9 + *Pseudomonas* 0, 3, 6, 9.	8.000	9.000	8 .000
Mean		6.195	7.050	
LSD $_{0.05}$ for:Season 1.084 Treatments (T) 3.428 S X T 4.424				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks afterplanting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P2O5 and 195 K2O kg/fed).

Table 3. Effect of different fertilization, treatments on Starch concentration in potato tuber.

Treatments		Starch %		Combined data
		First season	Second season	
1	Mineral NPK (Control)	75.50	72.54	74.02
2	100% Compost (15 ton/fed.)	78.99	75.61	77.30
3	50% Compost + *Azotobacter* 0, 6	74.77	72.97	73.87
4	50% Compost + *Azotobacter* 0, 3, 6, 9	73.88	79.96	76.92
5	50% Compost + *Pseudomonas* 0, 6	75.14	74.93	75.04
6	50% Compost + *Pseudomonas* 0,3, 6, 9	74.72	79.45	67.94
7	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 6.	74.27	73.42	73.84
8	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 3, 6, 9.	78.13	76.01	77.07
9	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 6.	72.40	72.93	72.67
10	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 3, 6, 9.	75.96	79.21	77.58
Mean		72.54	75.71	
LSD $_{0.05}$ for: Season (S) 1.575 Treatments (T) 4.981 S X T 3.522				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks afterplanting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P2O5 and 195 K2O kg/fed).

Table 4. Effect of different fertilization, treatments on Nitrate concentration.

Treatments		Nitrate conc.		Combined data
		First season	Second season	
1	Mineral NPK(Control)	0.212	0.290	0.251
2	100% Compost	0.112	0.099	0.105
3	50% Compost + Azotobacter 0, 6	0.282	0.137	0.210
4	50% Compost + Azotobacter 0, 3, 6, 9	0.109	0.087	0.098
5	50% Compost + Pseudomonas 0, 6	0.220	0.232	0.226
6	50% Compost + Pseudomonas 0,3, 6, 9	0.117	0.125	0.121
7	50% Compost + Azotobacter 0, 6 + Pseudomonas 0, 6.	0.237	0.177	0.207
8	50% Compost + Azotobacter 0, 6 + Pseudomonas 0, 3, 6, 9.	0.111	0.124	0.118
9	50% Compost + Azotobacter 0, 3, 6, 9 + Pseudomonas 0, 6.	0.190	0.132	0.161
10	50% Compost + Azotobacter 0, 3, 6, 9 + Pseudomonas 0, 3, 6, 9.	0.084	0.084	0.084
Mean		0.167	0.149	
LSD $_{0.05}$ for: Season 0.08966 Treatments (T) 0.06340 S X T 0.06340				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks after planting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P2O5 and 195 K2O kg/fed).

Table 5. Effect of different fertilization, treatments on dry matter concentration in potato tuber.

Treatments		Dry matter %		Combined data
		First season	Second season	
1	Mineral NPK(Control)	19.76	22.44	0.2512
2	100% Compost(15 ton/fed.)	21.78	22.18	0.1059
3	50% Compost + *Azotobacter* 0, 6	17.26	18.40	0.2100
4	50% Compost + *Azotobacter* 0, 3, 6, 9	19.34	21.90	0.09825
5	50% Compost + *Pseudomonas* 0, 6	22.16	22.08	0.2262
6	50% Compost + *Pseudomonas* 0,3, 6, 9	17.76	19.02	0.1213
7	50% Compost + *Azotobacter* 0, 6 +*Pseudomonas* 0, 6.	21.10	22.90	0.2075
8	50% Compost + *Azotobacter* 0, 6 + Pseudomonas 0, 3, 6, 9.	21.18	20.26	0.1180
9	50% Compost + *Azotobacter*0, 3, 6, 9 + Pseudomonas 0, 6.	21.96	23.30	0.1612
10	50% Compost + *Azotobacter*0, 3, 6, 9 +*Pseudomonas* 0, 3, 6, 9.	22.96	21.72	0.0843
Mean		20.52	21.42	
LSD $_{0.05}$ for: Season (S) 0.973 Treatments (T) 3.076 S X T 2.176				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks afterplanting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P2O5 and 195 K2O kg/fed) N-P-K concentration in tuber

Nitrogen concentration in tuber

Data in table 6 indicated that there were no significant differences between using compost at 15 ton/fed. and mineral fertilization in nitrogen concentration in tuber. This was true in both seasons. T8, T9 and T10 had significantly higher nitrogen concentration in tuber than mineral fertilization treatment and compost.

Phosphorus concentration in tuber

Data presented in Table 7 indicated that there were no significant differences between using compost at 15 ton/fed. and mineral fertilization regarding phosphorus concentration in potato tubers in both seasons. Tubers obtained from treatment No. 4 had lower of phosphorus concentration than those got from compost treatment. The same Table indicates no significant differences between mineral fertilization treatment and all biofertlization treatments regarding phosphorus concentration in potato tubers. There were no significant differences between both seasons.

Potassium concentration in tuber

Data in Table 8 showed that there were no significant differences between using compost at 15 ton/fed. and mineral fertilization as well as between these two treatments and all biofertilzation treatments in potassium concentration in tuber. There were no significant differences between both seasons.

Table 6. Effect of different fertilization treatments on Nitrogen concentration in tuber.

Treatments		N % in tuber		Combined data
		First season	Second season	
1	Mineral NPK(Control)	1.640	2.661	2.151
2	100% Compost (15 ton/fed.)	2.176	2.576	2.376
3	50% Compost + *Azotobacter* 0, 6	1.527	2.721	2.124
4	50% Compost + *Azotobacter* 0, 3, 6, 9	1.960	2.796	2.378
5	50% Compost + *Pseudomonas* 0, 6	1.654	2.642	2.148
6	50% Compost + *Pseudomonas* 0, 3, 6, 9	1.622	2.444	2.033
7	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 6.	1.699	3.285	2.492
8	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 3, 6, 9.	3.466	5.617	4.542
9	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 6.	3.344	5.518	4.431
10	50% Compost + *Azotobacter*0, 3, 6, 9 + *Pseudomonas* 0, 3, 6, 9.	3.862	5.400	4.631
Mean		2.295	3.566	
$LSD_{0.05}$ for:Season (S) 0.2381 Treatments (T) 0.7528 S X T 0.5323				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks after planting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P_2O_5 and 195 K_2O kg/fed).

Table 7. Effect of different fertilization treatments on Phosphorus concentration in tuber.

Treatments		P % in tuber		Combined data
		First season	Second season	
1	Mineral NPK(Control)	0.939	1.072	1.005
2	100% Compost (15 ton/fed.)	1.140	1.264	1.202
3	50% Compost + *Azotobacter* 0, 6	1.009	1.416	1.213
4	50% Compost + *Azotobacter* 0, 3, 6, 9	0.859	0.809	0.834
5	50% Compost + *Pseudomonas* 0, 6	1.014	0.905	0.959
6	50% Compost + *Pseudomonas* 0, 3, 6, 9	1.011	1.283	1.147
7	50% Compost + *Azotobacter*0, 6 + *Pseudomonas* 0, 6.	0.982	0.900	0.941
8	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 3, 6, 9.	1.326	0.989	1.158
9	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 6.	1.316	1.015	1.166
10	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 3, 6, 9.	1.016	1.113	1.064
Mean		1.061	1.077	
$LSD_{0.05}$ for: Season (S) 0.1292 Treatments (T) 0.4084 S X T 0.2888				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks after planting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P2O5 and 195 K2O kg/fed).

Table 8. Effect of different fertilization treatments on Potassium concentration in tuber.

Treatments		K % in tuber		Combined data
		First season	Second season	
1	Mineral NPK(Control)	1.608	1.590	1.599
2	100% Compost (15 ton/fed.)	1.396	2.349	1.873
3	50% Compost + *Azotobacter* 0, 6	1.479	2.377	1.928
4	50% Compost + *Azotobacter* 0, 3, 6, 9	1.682	1.537	1.609
5	50% Compost + *Pseudomonas* 0, 6	1.847	1.973	1.910
6	50% Compost + *Pseudomonas* 0, 3, 6, 9	1.848	1.716	1.782
7	50% Compost + *Azotobacter*0, 6 + *Pseudomonas* 0, 6.	2.343	1.421	1.882
8	50% Compost + *Azotobacter* 0, 6 + *Pseudomonas* 0, 3, 6, 9.	2.454	1.387	1.921
9	50% Compost + *Azotobacter*0, 3, 6, 9 + *Pseudomonas* 0, 6.	1.849	1.355	1.602
10	50% Compost + *Azotobacter* 0, 3, 6, 9 + *Pseudomonas* 0, 3, 6, 9.	1.735	1.307	1.521
Mean		1.824	1.701	
$LSD_{0.05}$ for: Season(S) 0.16 Treatments (T) 0.53 S X T 0.37				

0, 3, 6, and 9 =Azotobacter + Pseudomonas were applied after 0, 3, 6 and 9 weeks after planting. Rock phosphate and feldspar were added to the treatments no. 2 to no.10 to have same levels of P and K added to the control treatment (130 kg/fed P2O5 and 195 K2O kg/fed).

The present study revealed that mineral fertilizer exceeded the compost at 15 ton/fed regarding total tuber and marketable yield. However, the differences between the two treatments were significant only regarding total tuber yield. These results reflected the effect of these treatments on plant productivity. Although several investigations indicated that the yields between conventional and organic are comparable (Delate *et al*., 2003; Lang, 2005;Powon*et al*., 2005; Abou-Zeid and Bakry 2011 andMandic*et al*., 2011), the present results revealed the superiority of conventional fertilization over the organic ones. Since the soil, where the present experiment was virgin, it is logic to get lower yield from the organic fertilization. These results are in line with others proved that organic production is generally lower than the conventional one in the first years of organic production. An increase in potato tuber yield due to using conventional fertilization was also recorded by various previous researchers. Also, the effect of organic fertilizer on yield depended also on potato cultivar. Regarding effect of different fertilization treatments on the concentrations of N, P and K in potato tubers, the present study revealed that there were no significant differences between using compost at 15 ton/fed. and mineral fertilization regarding the concentrations of these three elements in potato tubers in both seasons.Similar results were recorded by some researchers under similar conditions. In the same connection, Mourão *et al*., (2008) found that The differences between the composted organic pig manure at rates of 0, 15, 30 and 45 ton ha^{-1} and conventional mineral N fertilizer (120 kg N ha^{-1}) regarding the concentrations of N, K, Ca and Mgtubers were not significant.The present study clearly indicated that using a combination of nitrogen fixing bacteria (as in the T 8, 9 and 10) led to a significant increase in nitrogen concentration in

potato tubers. Also, it was clear that potassium and phosphorus release micro-organisms that used in the present study were similar in their effect on P and K concentrations in tubers to the mineral fertilization. The most previous research revealed increase in the concentrations of N, P and K in potato tubers due to biofertilization, as compared to mineral fertilization.Conventional fertilization significantly contained higher nitrate than all other treatments, except T3, T5 and T7 which had similar effect to the conventional fertilization. The previous studies also revealed the effectively of biofertilizers in increasing potato quality by reducing nitrate content. El-Banna and Tolba, 2000, usingNitrobien (a biofertilzer contains *Azospirillum* sp., *Azotobacter* sp. and phosphate dissolving bacteria, namely, *Bacillus* sp.) in plots received 75% of recommended N and P, Abdel-Salam and Shams 2012, adding a Biofertilizer consisting of a combined mixture of N-free fixing bacteria (*Azotobacter*and *Azospirillium*) + P-dissolving bacteria (*Bacillus megaterium*) + silicate dissolving bacteria (SDB) (*Bacillus circulans)* to feldspar . Respecting effect of different treatments on dry matter and starch percentages in tubers, it was clear that compost fertilization caused production of higher percentages of starch and dry matter than conventional one, but without significant differences between them. The previous studies revealed either increase in starch percentage in potato tuber due to using organic fertilizers (Merzlaya*et al*., 2008; Baniuniene and Zekaite, 2008; Järvan and Edesi, 2009 and Abou -Zeid and Bakry, 2011). Concerning effect of biofertlization, T10 significantly exceeded mineral fertilization in starch percentage in tubers, whereas T3 and 6 had lower dry matter % than the mineral fertilization. No significant differences were detected between mineral and biofertlization regarding these two traits. Meanwhile compost at 15 ton/fed resulted in significant increase in starch percentage than T9 and significant increase in the percentage of tuber dry matter than the T3, and T6. Abdel-Salam and Shams (2012) found under clay soil conditions, that adding a biofertilizer consisting of a combined mixture of N-free fixing bacteria (*Azotobacter*and *Azospirillium*) + P-dissolving bacteria (*Bacillus megaterium*) + silicate dissolving bacteria (SDB) (*Bacillus circulans)* to feldspar had no effect on starch concentration in tubers as compared to using feldspar alone without inoculation.

CONCLUSION

The present study proved that we can produce organic potato in sandy soil by using100% compost without any significant differences in tubers yield per fed. as comparing to the conventional fertilization. Using compost treatment at 15 ton/fed. exceeded all biofertilizer treatments in marketable yield in both seasons, without significant differences compared with mineral fertilization. There were no significant differences between using compost at 15 ton/fed. and mineral fertilization in nitrogen, phosphorus, Potassium, starch and dray matter concentration in tuber. Compost application had significantly lower nitrate concentration in tuber than mineral fertilization

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DEFINING OPTIMAL LOCATIONS OF ESTABLISHMENTS AND TRANSPORTATION ROUTES FOR TREATMENT AND STORAGE OF ANIMAL WASTE

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ABSTRACT

In order to ensure a high level of public health and animal health protection in Bosnia and Herzegovina, it is necessary to improve the existing and/or provide quality management of animal by-products, i.e. to establish an infrastructure for quality and efficient treatment/disposal of animal by-products and waste of animal origin. This implies a wide range of activities in this field, such as measures to improve the legal and institutional framework, better data system management, establishment of by-product management model including transport solutions and technologies and provision of an adequate financial framework and sources of funding. At this point, the issue of management of animal by-products and animal waste in Bosnia and Herzegovina (BiH) has not been adequately addressed and poses a threat to both human and animal health. In this regard, establishment of a sustainable management system for animal by-products and animal waste is of utmost importance for further development of BiH agriculture. Inadequate management of animal by-products and animal waste poses a huge threat to the environment, endangering natural resources, watercourses, sources of drinking water, soil and atmosphere. This paper presents some of the activities related to establishment of this infrastructure, relating to the methodology of selection of locations for central plant and intermediate establishments for treatment and collection of animal waste and the definition of optimal transport routes and transport capacities.

Keywords: *animal by-products, animal waste, multi-criterial optimization, location analysis, categorization of animal waste.*

INTRODUCTION

One of the biggest challenges of the EU's standards adoption in the field of agriculture and the environment is to address the problem of harmless removal of

animal by-products and animal waste. Due to the increasing amounts and environmental impacts, waste, including animal by-products and animal waste (ABP / AW), is considered to be one of the most significant ecological problems of the contemporary world. Regulation EC 1069/2009 prescribes the health conditions that must be met when ABP / AW is manipulated on EU territory, or countries claiming to become EU members. It regulates the conditions under which the ABP / AW may be safely removed in order to exclude the risks to human and animal health, and prescribes the conditions under which ABP / AW may be used for animal nutrition, manufacture of cosmetic products, medical products or used for technical purposes. Also, Regulation 142/2011 regulates ways of implementing health conditions and determines the way of handling or managing ABP / AW.

According to the definition given in Regulation EC 1069/2009, animal by-products are parts of the animal body or whole carcasses of animals, products of animal origin, or other products derived from animals not intended for human consumption (Anonymous, 2009). These products defined in Regulation EC 1069/2009 include food waste obtained in the process of preparing food, used edible oil, foodstuff debris from restaurants and catering, animal waste produced from butchers and slaughterhouses, animal blood, feathers, wool, hooves, popcorn, skin, died of domestic animals, pets carcasses, bodies of dead animals originating from zoos and circuses, hunting trophies, manure, eggs, embryos and animal semen which are not intended for reproduction of animals. Anonymous, 2009).

Animal by-products and animal waste in BiH are mainly generated during slaughtering of animals for human consumption, during the production of products of animal origin in dairy factories, in animal husbandry during animal production e.g. technological mortality, manure and during the eradication of diseases as consequence of implementation of disease control measures. The main generators are farms, slaughterhouses or meat cuttings, processors and meat products producers, small scale farms, backyard farms and small rural holdings (Gagić, 2012). Regardless of their source and quantities, they pose a potential risk to public and animal health and threatens the environment, in particular with regard to transmissible spongiform encephalopathy (TSEs), pollution by dioxins and various exotic diseases (Gagić, 2012; Pearson and Dutson, 1992).

Animal by-products are an organic matter that, in the external environment, under the influence of atmospheric factors, is degraded by the formation of gases of unpleasant odors and other decomposition products (Feiner, 2006). These ABP / AW degradation products directly or indirectly pollute the environment. Such places become the habitats of stray dogs, rodents, scavengers, birds and insects, which in search for food become vectors of transmitting infectious diseases. At the same time, these substances penetrate the soil and become the risk of long-term pollution of the ecosystem. Inadequate handling of manure and other animal waste in rural areas, in addition to the risk of the occurrence of destructive epidemics of contagious animal diseases, can also lead to the pollution of rural wells, drinking water sources or contamination of agricultural crops in the fields, fruits and

vegetables with the agents of infectious animal diseases from which people can get sick (Sannik et al., 2015).

Animal waste according to EC Regulation 1069/2009 is classified into one of three categories (according to the level of risk), where its treatment and use is regulated for each of the categories (Anonymous, 2009). Technologies of ABP/AW safe destruction, disposal and processing can be based on incineration, rendering, production of motor fuel, production of biogas, composting, alkaline hydrolysis and other approaches (Sannik et al., 2015; Oreopoulou and Russ, 2007). Categorization of animal waste should be carried out at the place of its generation, using appropriate clearly marked containers, in the manner regulated by applicable regulations (Sannik et al., 2015). Animal waste producer (generator) should be responsible for its separation and categorization in the manner stipulated by regulations. In general, category 1 consists of brains of ruminants, parts their intestine (ileum), spinal cord and similar tissue of ruminants. The dead animals are classified in category 2a and may be Category 1 if it is found that they have died from the disease such as Bovine Spongiform Encephalopathy (BSE), TSE in sheep, or from diseases that can cause people to become ill (zoonosis). The content of the digestive tract and manure fall into category 2, while category 3 consists of low-risk materials and can be treated in a rendering machine, composted, and used for the production of biogas, pet food production and in another acceptable way.

The infrastructure for management of animal waste in the narrow sense consists of all facilities, equipment and means for its collection, transport, storage, disposal, destruction or processing, whether it is a space or collection containers, intermediate facility for temporary collection or other means of care.

Central treatment facilities are spaces and facilities for the final treatment of animal waste and disposal in such a way that it becomes harmless to health and the environment. Intermediate facilities are places and temporary storage areas, from which regular discharge should be provided to the central treatment facility, or to the place of final disposal.

Identification and selection of locations for animal waste management infrastructure facilities means conducting detailed geospatial (location) analysis. The result of this analysis is the optimal solution for the central treatment facility and intermediate establishments for the collection of animal waste. Based on these, it further defines and analyses the transport routes, identify transport solutions and calculates the investment and operational costs of the animal waste management infrastructure. Location optimization is carried out in two phases: the first one is definition solutions at the level of smaller administrative spatial units (local administration), and in the second one is selection of micro-locations of objects according to defined criteria (Huisman, 2009). When developing an analytical model, particular attention should be paid to sources and data sets to be used for computing and presentation of the quantity of generated animal waste, which are further used, along with the criteria, to select sites for animal waste management facilities (Ponjavic, 2011). The next paper section describes the methodology and criteria for selecting the location of such objects.

MATERIAL AND METHODS

The analytical process of selecting optimal solutions can be implemented through five steps: problem definition, analysis planning, data collection, location analysis, and conclusion (de Smith, 2015). Data collection is the most sensitive step in this process and has a direct impact on all the steps in the analytical process. Location analysis includes application of appropriate analytical methods, tools and models. At the end of the process, the results are analyzed and conclusions are made. Based on the solutions offered, the final decision on location selection is made by the representatives of the competent institutions.

The location analysis is based on data on calculated amounts of generated animal waste at the level of local governments with projections for the year in which the animal waste management infrastructure would be started in full capacity. For the purpose of designing these capacities in Bosnia and Herzegovina it is necessary to use all available official data relating to: animal import and export, generator register per municipality (slaughterhouses and farms register), number of animals and slaughtered animals by municipalities, number of registered companies for meat production and other records.

Quantities of certain categories of animal waste are calculated on the basis of statistics on livestock, livestock balance and number of slaughtered animals obtained from competent institutions for statistics (Sannik et al., 2015; Sannik et al., 2016). In order to properly assess the needs and projected capacities of storage facilities and treatment / disposal of animal by-products and their location, it is necessary to undertake a study to estimate the expected quantities of animal waste and by-products of animal origin arising in all stages of primary agricultural production of domestic animals (Anonymous, 2009). This study must include an estimate of the expected quantities generated by all generators of the animal by-products, regardless of their capacity, precisely because the livestock production in BiH is fragmented and is mainly based on a significant number of small rural farms, which, in mass, give significant quantities of animal by-products or waste. It is also necessary to estimate the quantities that occur in all plants for the production of food of animal origin. It is necessary to determine the value of all categories of by-products, precisely because of the fact that for each category special infrastructure is provided: separation and categorization in special collection courts, separate transport of different categories of waste, special processing plants, etc. The analysis should be based on precise data on the number of cattle, structured at the level of animal species and animal categories and distributed to the level of the municipality. Data on the number of slaughtered cattle must also be available, or data on the quantities generated by slaughterhouses and processing plants for meat, dairies, etc. The above information must be kept continuously for several years (min. 3 years) in order to correctly determine the trends and to perform forecast of future quantities of ABP/AW based on trends from the past. However, these data are often unavailable or data that can be obtained carries a certain degree of error, so it is necessary to check them well before making final conclusions and also adjust them if they are not correct with theoretical data such

as expected number of offspring per cow, piglets per sow, etc. (Kučević, 2015; Gutić sa saradnicima., 2016). This adjustment is also useful whenever central database on animal registration and identification is not present or operational, system of public statistics is not optimal, etc., as is the case in BiH. The total quantities of different categories of animal by-products are assessed by calculating the conditional heads, LSU (livestock units), using the standard methodology and multiplying the obtained values with the literature data on the expected technological mortality of animals during primary production (Uremović, 2004; Gagić, 2012), (Eurostat Statistics Explained, Glossary: Livestock unite (LSU), 2013);.

Data on the theoretical values of waste resulting from the slaughter of different animal species and categories have been taken from the literature and used to calculate the expected amount of waste that occurs when slaughtering different animal species and categories of livestock (Rede and Petrović, 1997; Vuković, 1998).

The criteria used for site selection for treatment and collection of ABP / AW are:

- road distance from the site to generated quantities aggregated at the level of municipality
- quantities of ABP/AW for treatment (collection) generated during slaughter of livestock in households (for private purposes)
- quantities of ABP/AW generated during slaughter of livestock in slaughterhouses
- quantities of ABP/AW generated by animal deaths

The aim of optimization is minimization of the transport costs ie equal accessibility of the site to all generators. Transport costs are minimal when the total length of all routes, pondered with generated quantities of ABP/AW, to the central location is minimal. This may be expressed by the formula:

$$\text{SUM } d_i(q_{HHi}+q_{SHi}+q_{Ci}+q_{IMEi}) = \text{min. } (i=1\ldots n)$$

where:

- n – total number of municipalities
- d_i – length (duration) of transport from central location to *i* municipality
- q_{HHi} – quantity of ABP/AW generated by slaughter of livestock at households in *i* municipality (HH – *household*)
- q_{SHi} – quantity of ABP/AW generated by slaughter of livestock in slaughterhouses in *i* municipality (SH – *slaughterhouse*)
- q_{IMEi} – quantity of ABP/AW collected at location of intermediate establishment (IME - *intermediate establishment*)
- q_{Ci} – quantity of ABP/AW generated by animal deaths in *i* municipality (C – *cracass*)

RESULTS AND DISCUSSION

The following table summarizes the aggregate quantities of animal by-products / animal waste (ABP / AW) for Bosnia and Herzegovina calculated on the basis of available statistical data with a prediction in 2020:

Table 1. Amounts of ABP / AW for BiH

Enitity/district/state Category of ABP/AW	Federation of BiH (t/year)	Republika Srpska (t/year)	Brcko District (t/year)	BiH (t/year)
ABP/AW category 1	2,864	3,743	45	6,652
ABP/AW category 2a	11,022	10,381	361	21,764
ABP/AW category 3	37,159	34,238	200	71,597
Total 1 + 2a + 3	51,045	48,362	606	100,014

Figure 1 (left) shows a thematic map of ABP / AW calculated per category for all municipalities. In order to determine the location of intermediate establishments, it is necessary to define service areas in BiH, which will be serviced by individual facilities. These areas can be formed by grouping adjacent municipalities, depending on the criteria set, i.e. the higher administrative and economic-functional organizations (eg. cantons or regions). Intermediate locations are defined for these areas according to formula (1), and then the location of the central treatment facility for the 1st, 2nd and 3rd ABP / AW treatment (Figure 1, right) is also determined.

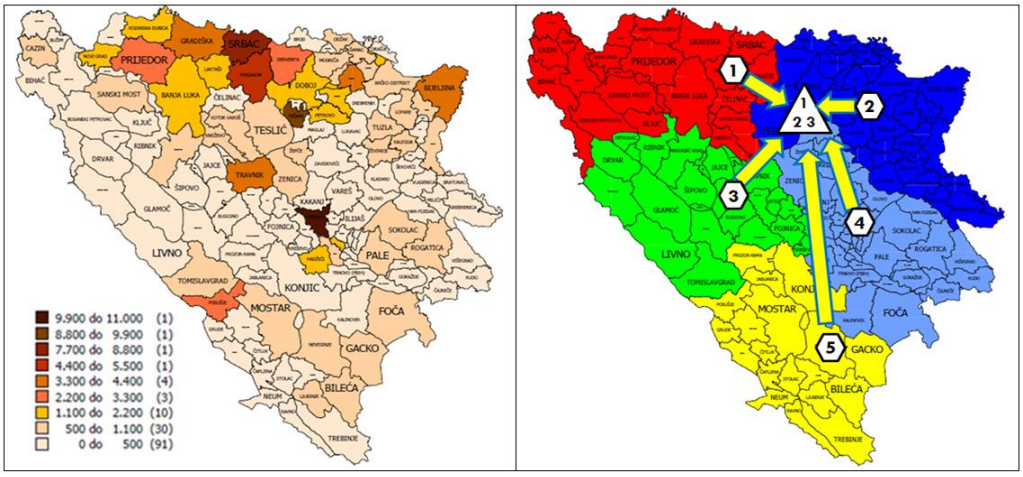

Figure 1. Total quantity of ABP/AW 1st, 2nd and 3rd category prediction in tones for 2020 (left), and service areas with locations of intermediate establishments and central plant (right).

Based on route network analysis within individual service areas, it is possible to identify routes for transporting animal waste to intermediates and central facilities, and using other statistical data (length of road, animal waste quantity, etc.) to

define transport solutions and calculate investments and operational transport costs of animal waste management.

CONCLUSIONS

Quality control of by-products and waste of animal origin implies a wide range of activities such as the assessment and implementation of measures to improve the legal and institutional framework, better database management, the establishment of an appropriate management model including transport solutions and technologies, and the provision of an adequate financial framework and sources of funding. One of the most important tasks within the framework of the construction and development of animal waste management infrastructure is the selection of optimal locations for the facilities. This task is carried out by applying the appropriate spatial and non-spatial criteria. The most important criteria are the road distance and the amount of generated waste for the transport between generators, intermediate establishments and central plant. When optimal locations are found, transport routes and capacities can be analysed, and investment and operating costs calculated. After determining the location in the wider area follows the selection of micro location for objects by the techniques of multi-criteria analysis and cooperation with the local administration to carry out public debate and make the final decision.

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INFLUENCE OF MANGANESE FERTILIZER ON GRAPES EFFICIENCY ON SANDY SOILS OF THE SOUTH RUSSIA

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ABSTRACT

As a result of the studies, there has been obtained a new information about manganese influence on productivity of grape plantations on sandy soils of the South Russia. Manganese fertilizing of 4 kg active ingredient per 1 ha on the background addition of nitrogen 90 kg ha^{-1}, phosphorus 90 kg ha^{-1} and potassium 90 kg ha^{-1} into a phase of grape sap flow contributes to higher yields and increase the sugar content of the berries with a significant decrease in juice acidity as compared with other variants. On determining the average weight of the fruit mass there has been found that manganese stimulates growth of berries significantly increasing their weight. The greatest effect of manganese fertilizer insertion is achieved on introducing it into the phase of sap flow at a dose of 4 kg ha^{-1} on the background of N90, K90, P90. In order to expedite the recovery of vineyards damaged by frosts to enhance the development of reproductive organs 4 kg ha^{-1} manganese into the phase of sap flow must be introduced on sandy soils.

Keywords: *grapes, soil, mineral nutrition, manganese, productivity.*

INTRODUCTION

The role of manganese in the metabolism of plants is similar with magnesium and iron functions. This compound activates the numerous ferments, especially during the process of phosphorylation. The manganese participates in various redox chemical reactions, being a part of redox ferments involved in the processes of respiration, photosynthesis, and carbohydrate and nitrogen metabolism of plants, due to the ability to transfer electrons by changing the valence (Benito et al., 2013; Pinskii et al., 2014). The average manganese uptake by the grapes harvest is nearly 1.2-1.5 kg ha^{-1}. The manganese deficiency affects many metabolic processes, in particular for the synthesis of hydrocarbons and proteins, since activates ferments in the plant (Romic et al., 2012). Signs of manganese deficiency in plants is mostly observed in carbonate soil, in strongly limed and some peaty and sandy soils at pH above 6.5 (Bell et al., 2013). Manganese deficiency becomes noticeable firstly on young leaves by a lighter green color or discoloration (chlorosis). Results of patent

searches and generalization of published data show the studies were conducted in the are of manganese effect on grape plants growth in sandy soils (Saleh et al., 2013). Such studies are not only of great practical but also of the theoretical value. The aim of the research is to determine the content of manganese in soils of Tersky sands and to identify the physiological reaction to manganese fertilizer of Platovsky grapes.

MATERIALS AND METHODS

The studies were carried out on Terek-Kumskiy sands of vine producing farm "Burun" of Shelkovskoy District in the Chechen Republic. Soil and plant samples were collected simultaneously for the determination of macro and microelements by atomic absorption method. Sampling was carried out according state standard methods (GOST, 2008); general requirements for conducting soil analyzes. Sugar content of the berries and titratable acidity were determined according to according state standard methods. Statistics of results were determined by Statistica 11.0. The purpose of field experiment is investigation of different doses and timing effects of manganese fertilizer on growth, development and productivity of plantations. The scheme of the field experiment was as follows: control (kg ha^{-1}: $N90+P_2O_590$ +Mn 0 kg ha^{-1}), the Mn spikes level to soil were 2, 4, 6 and 8 kg Mn per ha^{-1}. The field experiment was carried out in 2011, 2012 and 2013 years. In the work were used different fertilizers: sulphate manganese, ammonium silitra, super phosphate, potassium salt. Fertilizers were added into the soil during the phases of sap flow, or into the flowering phase, or the phase growth and the beginning of the ripening berries by the hydro drills method at the distance of 80 cm from the bush, to a depth of 30 cm per year. The Platovsky type grapes were planting upon to 3 x 1.0 m scheme. Variants of experience were laid in four variants four plants per every replication. Forming of vineyards is long sleeved, unsheltered.

RESULTS AND DISCUSSION

The content of humus in the 0-20 cm soil layer of studied sandy soils is 0.67%, in the 20-40 cm soil layer - 0.66%, and in the 60-150 cm soil layer - 0.95%. pH ranges from 8.5 up to 8.8. The phosphorus content in the 0-20 cm soil layer was 14.3 mg kg^{-1}, in the 20-40 cm soil layer - 10 mg kg^{-1}, and in the 60-150 cm soil layer – 13.0 mg kg^{-1}. The average of potassium total amount at all soil profile varied from 121 to 143 mg kg^{-1}. The total carbonates content in studied soils is 2.1-2.3% (Batukaev et al., 2014). The content of nitrogen in the sandy loam soil was observed in a very small amount 0.02-0.04%. The average content of total manganese in studied soils varied in the test area from 8.5 to 24.3 mg kg^{-1} (Table 1, Table 2).

Table 1. Nutrients content at different depths of sandy soil farm Burunny of Shelkovskoy District in the Chechen Republic (status 2012)

Soil depth (cm)	pH	Humus %	Nutrients in dry soil (kg ha^{-1})		Content of microelements (mg kg^{-1})				
			P_2O_5	K_2O	Zn	Cu	Mn	B	
0-20	8.8	0.7	14.3	143	0.8	1.9	16.8	0.4	0.53
20-40	8.8	0.7	10.0	121	0.7	1.3	14.4	0.5	
40-60	8.8	0.7	12.0	143	0.7	0.8	24.3	0.4	0.12
60-150	8.8	1.0	13.0	132	0.5	0.3	8.5	0.8	0.12

The share of firmly bound compounds more than 90% accounts for the major part of the total manganese content in the soil. Results of geobotanical research of field experiment in 2011, 2012 and 2013 showed that adding of manganese fertilizer had positive effect on plants growth (Table 2). The average length of shoots on the control variant without fertilizer was 154.6±36.4 cm in 2011. In the variant with the adding of manganese into the phase of sap flow at the dose of 4 kg ha^{-1} the average length of shoots was 180±37.4 cm. It has been considerably reduced the growth on insertion of manganese into the flowering phase, 164.4±26.4 cm, respectively, and even less growth was in the growth phase and in the beginning of berries ripening - 149.4±22.1 cm.

Table 2. Time and dose effect of manganese fertilizer adding on growth, and productivity of Platovsky grapes plants

Impact of manganese fertilization (kg Mn ha^{-1}: 0, 2,4, 6 and 8) added by control (kg ha^{-1}: N90 + P_2O_5 90 + K_2O 90) on status of Platovsky grape plants: length of shoots (LS), diameter of shoots (DS), yield (Y: t ha^{-1}) and sugar contents of the berres (SCB: g/dm^3)												
	The 2011 growing season				**The 2012** growing season				**The 2013** growing season			
Mn	LS	DS	Y	SCB	LS	DS	Y	SCB	LS	DS	Y	SCB
	cm	mm			cm	mm			cm	mm		
	Manganese (kg Mn ha^{-1}) adding into the phase of sap flow											
0	154.6	5.6	69.9	174.0	133.8	5.1	68.7	182.0	146.2	5.1	19.9	176.4
2	172.4	5.8	71.6	176.2	146.9	5.4	72.4	183.1	156.8	5.3	21.9	185.4
4	180.0	6.0	74.6	185.6	167.9	5.8	75.7	190.3	171.8	5.9	24.5	198.4
6	171.4	5.9	73.3	183.8	150.0	5.6	74.3	186.4	162.4	5.5	23.3	184.5
8	160.5	5.9	70.5	176.4	145.6	5.8	70.7	184.5	143.6	5.4	20.7	179.1
	Manganese (kg Mn ha^{-1}) adding into the flowering stage											
0	153.9	5.4	69.9	174.0	127.8	5.1	68.7	181.4	133.7	5.4	19.6	175.6
2	160.3	5.5	71.0	174.3	141.7	5.7	72.1	181.0	142.1	5.7	21.7	181.5
4	164.4	5.7	73.0	176.8	150.0	5.7	75.8	186.6	156.9	5.8	24.8	186.0
6	161.5	5.6	72.3	175.5	142.8	5.6	74.7	183.3	147.7	5.4	23.9	183.1
8	161.0	5.5	70.0	173.8	134.9	5.6	70.7	182.7	135.6	5.5	20.9	182.5
	Manganese (kg Mn ha^{-1}) adding in growth and beginning of grapes ripening											
0	140.0	5.1	69.9	174.0	125.8	5.1	68.7	182.2	132.8	5.2	19.7	177.2
2	141.6	5.1	70.1	171.0	129.9	5.2	72.1	182.6	130.8	5.3	21.6	182.0
4	149.4	5.2	71.6	173.5	145.9	5.5	73.6	186.8	149.5	5.6	22.6	186.2
6	140.6	5.1	71.0	172.0	130.0	5.2	71.5	185.9	138.6	5.2	21.1	185.1
8	139.4	5.0	71.0	172.0	127.0	5.2	70.3	184.1	135.4	5.2	20.0	184.8

The maximum value of shoots length in the variant 4 kg ha^{-1} Mn added into the phase of sap flow. Using the low and high doses of Mn reduced its effectiveness on grapes morphometric characteristics (Table 3). The rate of accumulation of dry biomass aboveground organs of plants is the criteria of photosynthesis efficiency.

Table 3. Influence of manganese fertilizer on root development of Platovsky grapes in stage of sap flow

Impact of manganese fertilization (kg Mn ha^{-1}: 0, 2,4, 6 and 8) added by control (kg ha^{-1}: N90 + P_2O_5 90 + K_2O 90) on status of Platovsky grape plants in stage of sap flow: air-dry weight of root and number of skeletal roots (NSR)						
Mn	**The 2011** growing season		**The 2012** growing season		**The 2013** growing season	
	Root weight (kg 10^{-3})	NSR	Root weight (kg 10^{-3})	NSR	Root weight (kg 10^{-3})	NSR
0	385	400	379	387	368	397
2	398	422	386	421	385	435
4	426	462	432	479	435	462
6	419	451	417	452	429	451
8	416	453	434	437	427	453

The study of the nature of the leaf surface Platovsky grapes showed that the number of leaves on the bush, the area of the leaf blade, as well as the total area of leaves on one bush and 1 ha, changed depending on the dose and timing of manganese fertilizing. The increase in sugar content of berries was up to 1.8 g/dm^3. The highest rates in growth and productivity of grapes were obtained by manganese insertion into the phase of sap flow in the amount of 4 kg ha^{-1}. The most intensive restoration of Platovsky grapes roots after Mn addition in the phase of sap flow at a dose of 4 kg ha^{-1} (Table 3). The addition of Mn into the phase of sap flow increased the average length of shoots on the control compare to control up to 167.9±35.4 cm in 2012. Adding the higher amount of Mn (6 kg $ha^{-1}$and 8 kg ha^{-1}) increased the growth of shoots.

CONCLUSIONS

Manganese content in the soil profile studied soils varies greatly, ranging from 8 to 24.3 mg or 15 times less than in the alluvial soils of the Chechen Republic. Manganese fertilizer is effective farming techniques promoting the growth of development, hardiness and productivity of grape plants in Shelkovskoy District of Chechen Republic. The most effectiveness цфы manganese fertilizфешщт by adding active manganese into the phase of sap flow at a dose of 4 kg ha^{-1} on the background of N90, K90, P90.

ACKNOWLEDGEMENTS

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The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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