ISSN: 2490-3442

UNIVERSITY OF EAST SARAJEVO
FACULTY OF AGRICULTURE

Agrofor

AGROFOR International Journal

AGROFOR International Journal, Vol. 3, Issue No. 2, 2018

agrofor.ues.rs.ba

AGROFOR International Journal

PUBLISHER
University of East Sarajevo, Faculty of Agriculture
Vuka Karadzica 30, 71123 East Sarajevo, Bosnia and Herzegovina
Telephone/fax: +387 57 340 401; +387 57 342 701
Web: agrofor.ues.rs.ba; Email: agrofor@pof.ues.rs.ba

Frequency: 3 times per year
Number of copies: 300
ISSN 2490-3434 (Printed)
ISSN 2490-3442 (Online)

CONTENT

Original scientific paper
10.7251/AGRENG1802005A
UDC 632.951:595.799

IMPACT OF INSECTICIDES ON POLLINATOR POPULATIONS: ROLE OF PHYTOSANITARY PERFORMANCE INDICATORS IN TOMATO CROPS

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ABSTRACT

Agrochemicals are considered to be among the major environmental threats to pollinators, including honey bees. At the time of foraging, bees are at risk of exposure to phytosanitary treatment as a result of widespread treatment and their location, often near orchards. In Algeria, the majority of farmers systematically over-treat their orchards in order to have good quality fruits for as long as possible towards the use of an effective product. We evaluated the comparative effects of lambda-cyhalothrin and spinosad insecticide treatments on bees in tomato plots. Fluctuations of bee populations abundance were established during a 16 days period of exposure using yellow-colored plates with water placed every two days inside the experimental units. Bee populations showed very high sensitivity (0 individuals registered) to both insecticides at the homologated dose and even half dose during 10 days following application of the treatments. Depending on the estimated temporal toxicity of the respective products, differences in recovery of bee activity are presented. Consideration of indicators of the intensity of use of plant protection products is discussed.

Keywords: *Pollinators, pollutants, toxic, pesticides, agrosystems, Algeria.*

INTRODUCTION

Pesticides are a major factor affecting agrobiodiversity. They may have short-term toxic effects on organisms that are directly exposed to them, or long-term effects, causing changes in habitat and the food chain (Geiger et *al*., 2010). Broad-spectrum insecticides such as carbamates, organophosphates and pyrethroids can cause population declines of beneficial insects such as bees, spiders, or beetles. Many of these species play an important role in the food web or as natural enemies

of pest insects. Managed honey bee, *Apis mellifera* L., colonies placed in field crops are potentially exposed to carbamates pyrethroid insecticides used for broad-spectrum pest control (Pilling and Jepson, 2006). In Algeria, pesticide manufacturing was provided by autonomous pesticide management entities such as Asmidal and Moubydal. However, several companies have specialized in the importation of insecticides and various related products. Approximately 400 plant protection products are registered in Algeria, of which forty varieties are widely used by farmers (Belhadi et *al*., 2016)). Law No. 87-17 of 1st August 1987 on phytosanitary protection (J.O.R.A., 1995) introduced the mechanisms that allow the efficient use of pesticides. This law regulates aspects relating to the registration, importation, manufacture, marketing, labeling, packaging and use of pesticides (Bouziani, 2007). Numerous convergent observations show that chemical control has important effects on pollinating insects, which suffer immediate or delayed losses that affect adults or larvae (Carvalheiro et *al*., 2013)). Pyrethroids have been reported to pose repellency which alters foraging behavior with the benefit of preventing bees from encountering a lethal dose in the field (Ingram et *al*., 2015). However, sub-lethal exposure to pyrethroids may adversely impact bee behavior potentially resulting in social dysfunction or disruption of foraging (Ingram et *al.*, 2015).

This paper considers the effects and ecotoxic aspect of a pyrethroid and a bioinsecticide spinosad (Tracer) used in Algeria in vegetable field crops and orchards, on non-target fauna, particularly on functional groups of beneficial organisms.

MATERIAL AND METHODS

Experimental device and sampling

The studied tomatoe plots (variety Escudero F1 HMX 3823), spread over 5 ha area is located at 7 km north of Boufarik (Blida, sublittoral central, Mitidja region-Algeria) and belongs to a private farmer. It is bounded to the north by fallow plots, to the south and west by a road (Ben Chabane - Ben Hamdani), to the east by an apple orchard. It is surrounded to the north, to the south and to the east by cypresses windbreak hedges. No orchard maintenance was done during the study period.

The insecticide treatment solutions (L: lambda-cyhalothrin, T: spinosad,) were sprayed at the registered dose (D) and half dose (HD) with a manual sprayer at the level of 5 micro-plots or units including 30 tomato plants in each treated and control units (tm).

Lambda-cyhalothrin is a polyvalent insecticide, belonging to the synthetic pyrethroid family and acting by contact and ingestion. It is formulated as a liquid at 50 gL^{-1}, at an application rate of 60 mL HL^{-1}. Spinosad is composed of two toxins A and B, with chemical formula C41H65NO10 and C42H67NO10 respectively, formulated in concentrated suspension (SC) at 480 g L^{-1}, at a use rate of 0.2 Lha^{-1}. It acts by contact and ingestion.

The toxicity and ecotoxicity were assessed through the availability of individuals from functional communities in the treated and untreated units. We placed four yellow water traps and renewed them after each sampling every two days after application and over a period of 15 days. The captured arthropods were identified under the binocular microscope and sorted according to their taxonomic affiliation and trophic groups (phytophagous, flower dwelling, parasitoid, predatory, others with diet without interest).

Data analysis

The toxic effect of the tested insecticide was estimated by calculating the percentage of residual populations (PR) expressed by the ratio of the number of alive individuals in the treated units to the number of alive ones in the controls. The degree of toxicity of the active substance was expressed by less than 30% of PR, greater than 60% or between 30 and 60% of PR for high, neutral or average toxicity respectively. We adopted the Generalized Linear Model (GLM) using the software (SYSTAT vers 12, SPSS 2009) to evaluate the influence of exposure duration, dose and insecticide treatment on the abundance of residual populations of the captured auxiliary arthropods.

RESULTS AND DISCUSSION

As a general rule, insecticides have a negative impact, according to the families and types of molecules and adjuvants, on the majority of arthropods but also according to the life cycle of organisms (Dennis et *al.*, 1993, Hokkanen et *al.*, 1988). The impact of long-term phytosanitary treatments is likely to vary depending on the size of the plots and the presence of vegetation at the edge of fields implies the possibility of recolonization (Hole *et al*., 2005).

Obviously, when pesticides are mentioned in the causes of decline in pollinator populations, herbicides are more often referred to than insecticides (Kevan, 1999; Wilcock and Neiland, 2002).

Evaluation of studied insecticides effect on tomato trophic groups

We recorded 5 flower dwelling species, 7 species of entomophagous parasites, 22 predator species, and 26 species with varied diets (others).

Taxa respond differently when exposed to dose and half dose of lambda-cyhalothrin and spinosad respectively. This difference seems to be due to the sensitivity variation of the target species to the active substances as well as to the applied dose, the exposure duration, the insecticide activity spectrum and its persistence in the field.

The parasitic and flower dwelling species group was the most sensitive to the lambda-cyhalothrin at the homologated dose (Figure 1). These species were absent during the 10 days of the experiment ($F = 11.51$, $df = 4.199$, $p = 0.01$ and showed very low percentages of abundance (29.41% for flower dwellings and 17.39% for parasitics on the 16th day, $F = 16.54$, $df = 4.684$, $p = 0.005$). The most sensitive species include *Andrena sp* pollinators, Formicidae *Lasioglossum* sp, Halictidae, Bethylidae, *Aphidius* sp parasitoid microhymenoptera, Tachinidae and *Oxytelus*

species (Figure 3). Spinosad at homologated dose has a high toxicity on flower dwelling trophic group (Figure 1). The most sensitive species were *Andrena* sp and *Lasioglossum* sp (Figure 3). There was a period of decline during the first 10 days ($F = 11.51$, $df = 4.199$, $p = 0.01$) where relative abundances increased from 29.16% to 4% compared to control, followed by a period of increase reaching 26.08% relative to the control on the 16th day, ($F = 16.54$, $df = 4.684$, $p = 0.005$).

Population abundances were higher after application of spinosad (T) and half-dose (HD) compared with those of lambdacyhalothrin (L) at the homologated dose (Figure 1and 2). The differences in abundances for each trophic category are very highly significant from the 1st to the 2nd week after treatment ($F = 40.73$, $df = 5.183$, $p = 0.0003$).

The richness of the trophic communities of pollinators and beneficial enemies is significantly different under the effect of the two doses of lambdacyhalothrin compared to the untreated control ($p = 0.006$, $p = 0$, $p = 0.06$ respectively) throughout exposure period, while diversity is considerably low ($p = 0$).

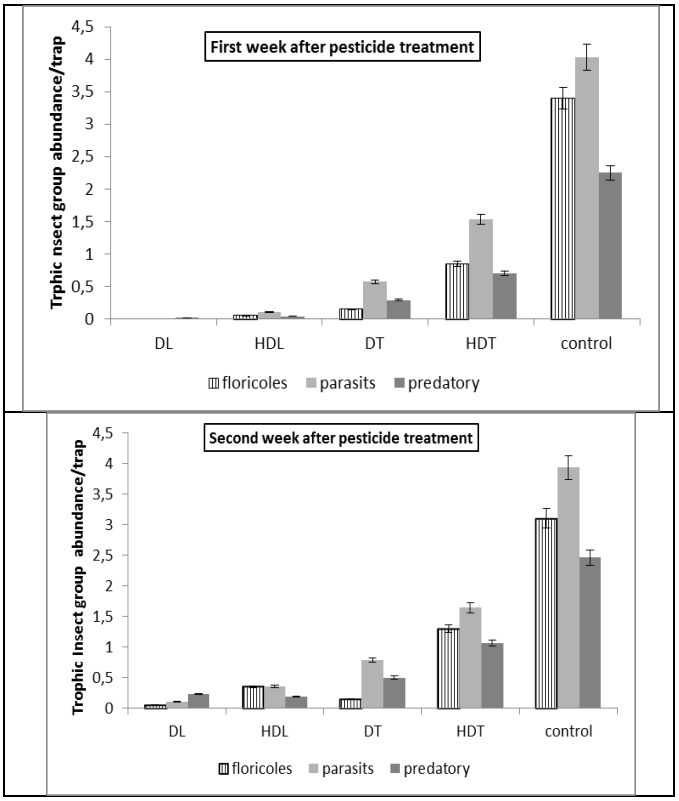

Figure 1. Variability of the abundances of main trophic groups encountered after treatment during two weeks of exposure.

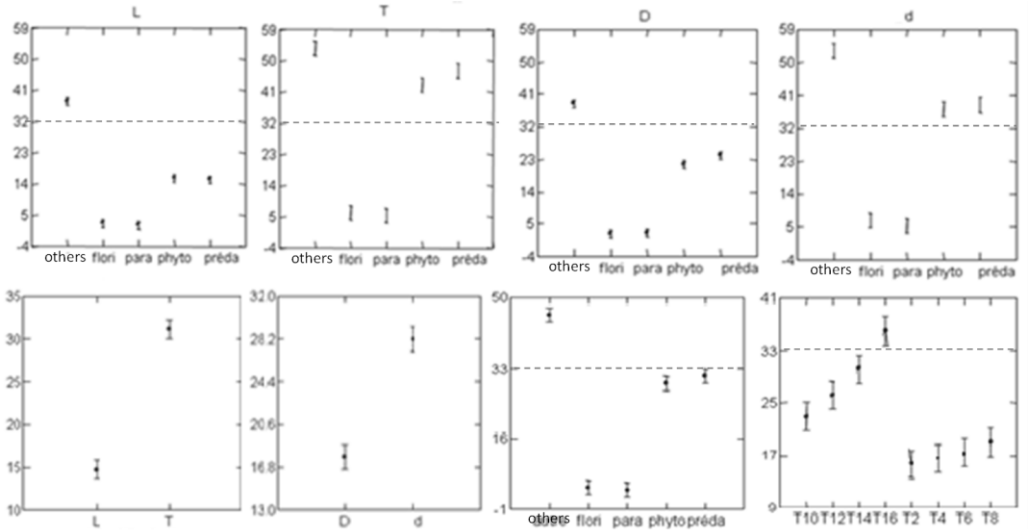

Figure 2. Influence of tested insecticides, dose, and exposure time on the abundance of trophic groups in the tomato field (L: lambdacyhalothrin, T: Spinosad, D: homologated dose, d: half dose, others, flori, phyto, pred: trophic groups, T2 to T10: time after application).

According to Cluzeau and Paternelle (2000), lambda-cyhalotrine inhibits the multiplication of Aphididae populations. Krespi (1995) also showed that lambda cyhalothrin reduces the attack of cereal aphids and infestation by their parasitoid Hymenoptera. Predators such as coccinellidae, *Empis* sp, the ant *Cataglyphis bicolor*, *Macrolophus* sp, showed a high sensitivity to lambda-cyhalotrin at registered dose, compared to spinosad. According to our observations, lambda-cyhalothrin has a toxic effect on predator populations whereas spinosad maintains this group except Chrysopidae. Half-dose lambda-cyhalothrin has destructive effect of this auxiliary group. The trophic group of parasites and flower dwelling species such as Tachinidae, Bethylidae, *Aphidius* sp, *Oxytelus* sp, *Apis mellifera*, Halictidae, *Vespula vulgaris*, Trichogrammatidae, Chalcidae, Braconidae, Ichneumonidae showed high sensitivity to lambda cyhalotrin and spinosad at registered and half dose. They are more vulnerable groups with several parasites against chemical product show sensitivity to spinosad (Rafalimanana 2003; Williams et *al.*, 2003). These two groups are more sensitive to conventional products (methidathion 400gL^{-1}l and White Oil 76 (pc) sprayed in citrus orchards in central Mitidja region ((Belhadi et *al*., 2016)).

Schneider *et al.*, (2004) reported a decrease of adult emergence and longevity endoparasitoids, *Hyposoter didymator* (Thunberg), treated with spinosad. Similarly, Tillman and Mulroney (2000) and Miles and Dutton (2000) observed spinosad toxicity on *Bracon molitor*, *Cardiochiles nigriceps* and *Cotesia marginiventris*, parasitoids on cotton.

Temporal evolution of lambda-cyhalothrin and spinosad toxicity on bees

Lambda-cyhalothrin is characterized by high toxicity on residual populations of bees during the first 15 days at registered dose, and during the first 10 days at half dose. Spinosad at registered dose has a very toxic effect only on the first 9 days. Half dose in half dose Spinosad shows a variable effect, moderately toxic from the 2nd to the 5th day, and a neutral effect from 8th day (Figure 4).

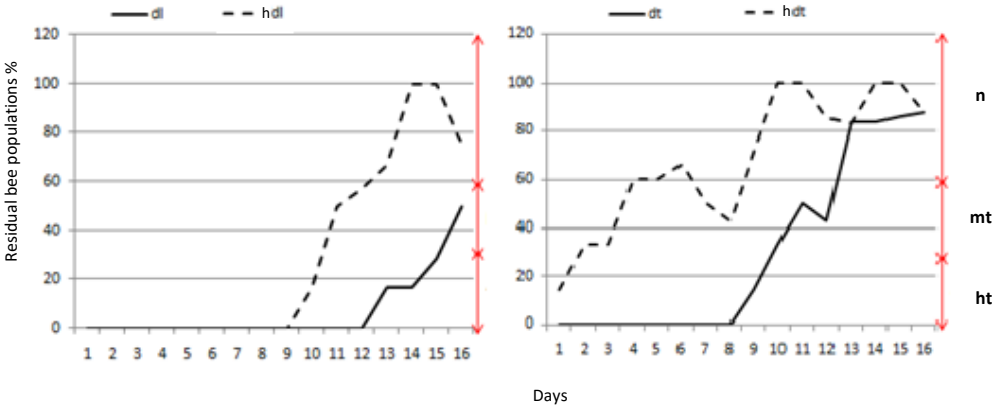

Figure 4. Evaluation of tested insecticides toxicity on bees residual populations in tomato field. (L: lambda-cyhalothrin, t: spinosad (Tracer), d: dose, hd: half-dose, n: no effect, mt: moderately toxic, ht: highly toxic).

The toxicity gradient ranges from the dose of lambda-cyhalothrin, followed by the spinosad dose, then the half-dose of lambda-cyhalothrin and finally the half-dose of spinosad which has the lowest effect. Tested insecticides toxicity on bees is due to the mode of penetration. Both act by contact and ingestion. The contact of the bee with the insecticide occurs when the foragers visit a field during or after a chemical treatment. It is when spreading in the presence of foragers that the damage is the most severe (Atkins *et al.*, 1981). Young bees will then be able to get intoxicated by consuming the contaminated pollen stores. It has been demonstrated by a tunnel assay that synthetic pyrethroids may disrupt the flight behavior of forager's bees, which took longer to return to the hive after treatment (Taylor et *al.*, 1987).

Pyrethrins are practically highly toxic to honey bees (author). However, some of the risk to pollinators is limited by their slight repellent activity and rapid breakdown. Sublethal exposure to pyrethroids impacted bee behavior over a 24-h period. Pyrethroid-treated bees traveled 30–71% less than control bees (Ingram et *al*., 2015). Esfenvalerate and permethrin decreased social interaction time by 43% and 67% (Ingram et *al*., 2015). Permethrin increased time spent in close proximity to a food source. The longevity of honey bee workers is reduced after carbaryl, diazinon and malathion treatments. Parathion also caused low losses of forager orientation due to the disruption of the information transmission system regarding the location of food resources, (Thompson, 2003). Based on laboratory dose response data, pyrethroids are considered to be either highly toxic (LD 50 of 0.1-1.0µg a.i/bee) or extremely toxic (LD 50 <0.1µg a.i/bee) to honeybees, according

to classification proposed by the International Commission for Bee Botany. An analysis of the pyrethroid data within the IOBC database shows that the synthetic pyrethroids are all classified as harmfull to non targeted arthropods, according to laboratory toxicity data. When the same pyrethroids were classified according to available IOBC semifield or field data, then classifications of moderately harmfull pyrethroids harmless were often reported for some species. This significates that the effects of the pyrethrinoids on NTAs at recommended application rates under field conditions is significantly less (Matsuo and Mori, 2012).

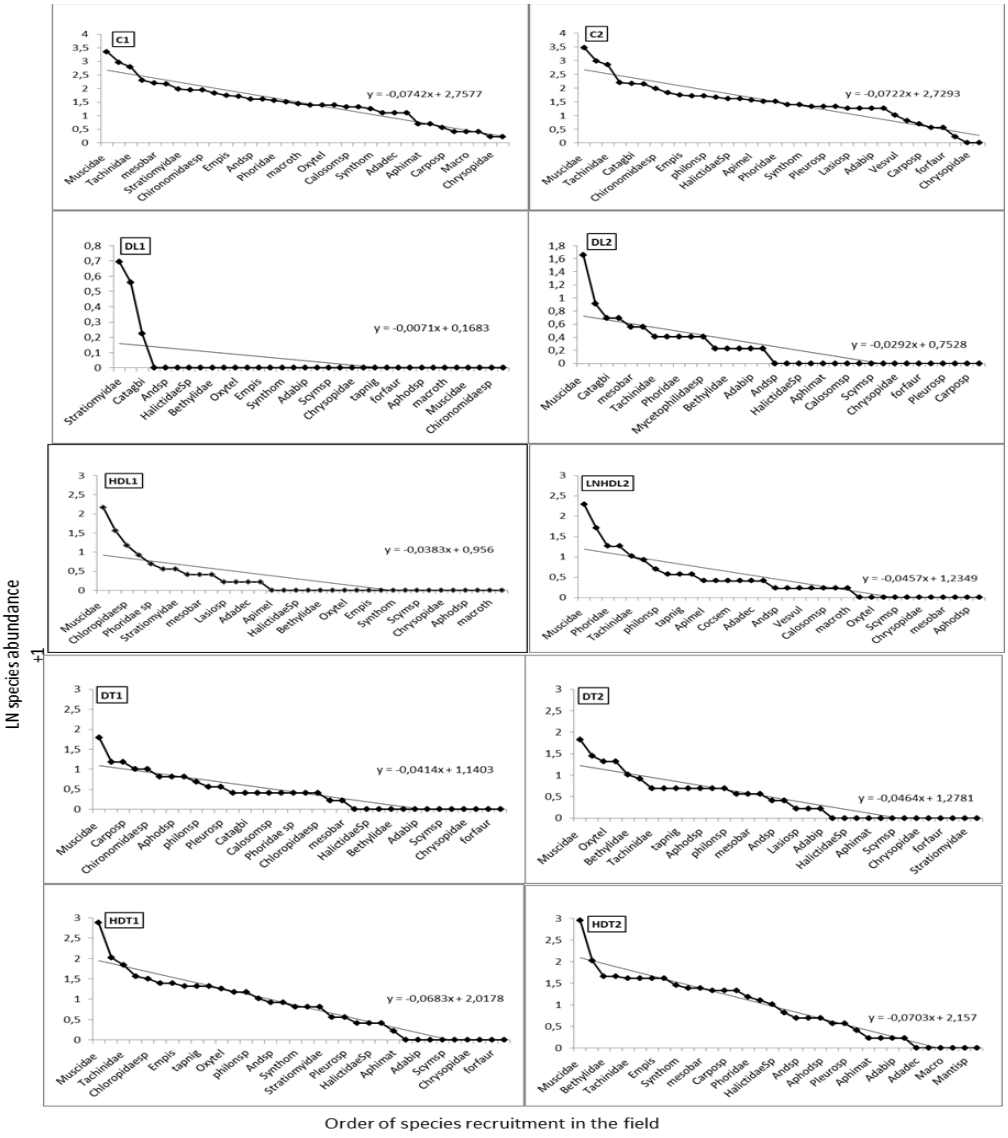

Figure 3. Recruitment order of trophic communities after treatments effect during two weeks of exposure

CONCLUSION

Like all chemical insecticides, lambda-cyhalothrin has a negative effect on non-target entomofauna, but with varying degrees depending on species and application rate. The most sensitive were parasites and flower dwelling species, followed by predators. The half-dose of this active substance showed a destructive effect on the beneficial fauna, but with low degrees compared to the homologated dose. These results lead us to predict the phytosanitary status of our crop if we use this product in an anarchic way. Thus, it is necessary to think of replacing this active substance in spite of its effectiveness on the pests and its broad spectrum of activity which minimizes the cost of protection, by other insecticides more specific on the targeted pests. For effective integrated control, spinosad has demonstrated its compatibility with most predators and its ability to regulate certain pest populations that are primarily flying insect species. The formulation with baits could be the best solution to minimize contact of parasitic and flower dwelling species with the treatment.

REFERENCES

Atkins, E.L., Kellum, D. Atkins, K.W. (1981). Reducing pesticide hazards to honey bees: mortality prediction techniques and integrated management strategies. *University of California, Leaflet*, 2883. 23p.

Belhadi, A., Mehenni, M. Reguieg, L.Yakhlef, H. (2016) Revue Agriculture. Special number 1: 09 – 16

Bouziani, M. (2007). The immoderate use of pesticides: Serious health consequences. *Journal le républicain*, 26 june 2007.

Carvalheiro, L.G., Kunin, W.E., Keil, P., Aguirre-Gutiérrez, J., Ellis, W.N., Fox, R., Groom, Q., Hennekens, S., Van Landuyt, W., Maes, D., Van de Meutter, F., Michez, D., Rasmont, P., Ode, B., Potts, S.G., Reemer, M., Roberts, S.P.M., Schaminée, J., WallisDeVries, M.F. Biesmeijer, J.C. (2013). Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. *Ecology Letters*;16 (7):870–878.

Cluzeau, S. Paternelle, M.C. (2000). Phytosanitary index. 36th edition. ACTA. 73; 78- 79, 117p.

Decourtye A, Lacassie E, Pham-Delègue MH. 2003 Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season, Pest Management Science 59(3): 269-278, 2003.

Dennis, P., Fry, G.L.A. Thomas, M.B. (1993)**.** The effects of reduced doses of insecticide on aphids and their natural enemies in oats. *Norwegian Journal of Agricultural Sciences* **7** (3/4): 311-325.

Geiger, F. et *al*, (2010). Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology* 11(2): 97-105.

Hokkanen, H., Husberg, G.B. Soderblom, M. (1988). Natural enemy conservation for the integrated control of the rape blossom beetle *Meligethes aeneus* F. *Annales Agriculturae Fennicae* 27(4): 281-194.

Hole, D.G., Perkins, J. Wilson, A.J. Alexander, D. Grice, I.H. P.V. EVANS, A.D. (2005). Does organic farming benefit biodiversity? *Biological Conservation*, 122: 113-130.

Ingram, E.M. Augustin, J. Marion, D. Blair, E. Siegfrieda, D. (2015). Evaluating sub-lethal effects of orchard-applied pyrethroids using video-tracking software to quantify honey bee behaviors. *Chemosphere* 135, September 2015, Pages 272-277.

J.O.R.A., (1995). Official Gazette, Act No. 87-17 of 1 August 1987, on plant health protection.

Kevan, P.G. (1999). Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agriculture, Ecosystems and Environment*, 74, 373-393.

Krespi, L. (1990). Study of parasitic biocenosis of cereal aphids in the Rennes basin: special case of *Aphidius uzbekistanicus* Luz. PhD thesis, University of Rennes I, pp: 80-105.

Noritada Matsuo, Tatsuya Mori (2012). Pyrethroids: From *Chrysanthemum* to Modern Industrial Insecticide. Springer Science & Business Media, 224 pages.

Miles, M. Dutton, R. (2000). Spinosada naturally derived insect control agent with potential for use in integrated pest management systems in greenhouses. In: Proceedings of the BCPC Conference—Pests and Diseases, 13–16 November 2000, Brighton, UK. British Crop Protection Council, Farnham, Surrey, UK, pp: 339–344.

Pilling, E.D. Jepson, P.C. (2006). Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (*Apis mellifera*). *Pesticide Science* 39(4): 293-297.

Rafalimanana, H.J. (2003). Evaluation of the effects of insecticides on two types of crop auxiliary Hymenoptera, domestic honeybee (*Apis mellifera* L.) and aphid parasitoids: field studies in Madagascar and laboratory in France, Thesis Doc., National Institute of Agronomy, Paris-Grignon, 205p.

Schneider M.I., Smagghe, G. Pineda, S. Viuela, E. (2004). Action of insect growth regulator insecticides and spinosad on life history parameters and absorption in third-instar larvae of the endoparasitoid *Hyposoter didymator*. *Biological Control* 31, 189–198.

Taylor, K.S. Waller, G.D. Crowder, L.A. (1987). Impairment of a classical conditioned response of the honey bee (*Apis mellifera* L.) by sublethal doses of synthetic pyrethroid insecticides. *Apidologie*, 18, 3, 243-252.

Thompson, H.M. (2003). Behavioural effects of pesticides in bees. Their potential for use in risk assessment. *Ecotoxicology*, 2003, 12, 317-330.

Tillman, P.G Mulrooney, J.E. 2000. Effect of selected insecticides on the natural enemies *Coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), and *Bracon mellitor*, *Cardiochiles nigriceps*, and *Cotesia marginiventris* (Hymenoptera: Braconidae) in cotton. *Journal of Economic Entomology* **93**: 1638-1643.

Wilcock, C. Neiland, R. (2002)**.** Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science*, 4, 270-277.

Williams, T. Valle, J. Vinuela, E. (2003). Is the naturally derived insecticide Spinosad compatible with insect natural enemies? *Biocontrol Sci. Technol.* 13, 459–475.

Original scientific paper
10.7251/AGRENG1802014K
UDC 633.11:664.236

ALLELIC COMPOSITION OF HMW-GLUTENIN PROTEIN AND THEIR RELATIONSHIP WITH QUALITY OF WHEAT

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ABSTRACT

High molecular weight glutenin subunits (HMW-GS) proteins deposited in endosperm of wheat seed which have significant impact on bread quality. The HMW-GS encoded by genes located at the long arm of chromosomes 1A, 1B, 1D. The aim of this work was study allele polymorphysms at *Glu-A1*, *Glu-B1* and *Glu-D1* locus and loaf volume, grain protein content, sedimentation volume of eight wheat genotypes (G-3130, G-35183, G-3501, G3512, G-3574, G-3027, G-3075, G-3097) harvested in two years with various weather condition. For each genotypes, flour used for extraction of glutenin which separated by method of electrophoresis on SDS gel (11.8%). Electrophoregrams used for determining *Glu-1* alleles. Technological quality parameters analyzed by standard laboratory methods. The three alleles alleles (***a, b, c***) at the *Glu-A1*, three alleles (***b, c, d***) at the *Glu-B1* and 2 alleles (***a, d***) at the *Glu-D1* were identified. The highest protein sedimentation volume had wheat genotype G-3075 in the both years (54.0ml; 58.0ml) while the lowest sedimentation volume had G-3512 (34.0ml; 36.0ml). Grain protein content (GPC) was the highest in G-3075 in both years (14.20%; 15.40%) while the lowest GPC had G-3097 (11.60%) in first and G-3512 (12.60%) in the second year. Loaf volume was the highest in G-3075 in both year (520ml; 540ml) while the lowest was in G-3512 (400ml) in both years of experiment. The estimated quality traits varied depending on genotype and year. The better quality, in average, had the wheat genotypes which carried *Glu-D1d* allele.

Keywords: *Wheat, glutenin, Glu-1 allele, quality, polymorphism.*

INTRODUCTION

Wheat grain is important source of gluten proteins (gliadin and glutenin) which determine dough quality as well end use products (Shewry et al., 2003; Li et al. 2010). Glutenin proteins comprises two groups of subunits: high-molecular weight glutenin subunits (HMWGS) and low molecular weight glutenin subunits (LMWGS). The HMW-GS are controlled by gene alleles at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arm of chromosomes 1A, 1B and 1D, respectively. The each locus consisting of two tightly linked x-type and y-type alleles. The LMW-GS are encoded by *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on the short arms of chromosomes 1A, 1B, and 1D, respectively (Payne et al. 1987). The high allele polymorphysms at each locus for storage proteins were identified ((Knežević et al., 1993; Novoselskaya-Dragovich, 2015). The composition of glutenin alleles, i.e, composition of encoded HMW-GS are in relationship with technological quality properties (Jondiko et al., 2012; 2003; Knezevic et al., 2016a), dough making quality and baking quality of wheat flour (Menkovska et al., 2002; Li et al. 2010). The previous studies showed that *Glu-D1* locus have significant influence to rheological and bread making quality. So, the HMW-GS subunit 1Ax1 and subunits pair 1Dx5+1Dy10 have the greatest relationships with flours with more suitable viscoelastic properties for bread making and that also result in bread with higher volume (Liang et al. 2010; Hernández et al. 2012; Blechl and Vensel, 2013). Also, other investigation showed that HMW-GSs such as 1Ax1, 1Ax2*, 1Bx7, and 1Bx17 + 1By18 have positive effects on dough characteristics, while 1AxNull, 1Bx20, 1Bx6 + 1By8, 1Dx2 +1Dy12 have negative effects on gluten quality and bread-making quality (Shewry et al., 2003). However, many factors are involved in variation of gluten proteins and that make dificulties for predicting bread making quality (Liu et al., 2016; Knezevic et al., 2017a; 2017b). The knowledge of diversity of wheat genotypes on the base of *Glu-1* allele composition is important, and can use in breeding program to create new new cultivars.

The present study was carried out in the aim to determine the HMW-GS composition in 8 Serbian wheat genotypes, allele polymorphysms at the *Glu-1* loci and its relationship with protein sedimentation volume, protein content and loaf volume was determined.

MATERIAL AND METHODS

The eight genetically divergent wheat genotypes ((G-3130, G-35183, G-3501, G3512, G-3574, G-3027, G-3075, G-3097) were harvested in two years of experiment. During two years of experiment, those genotypes were grown on plots $5m^2$ in five replications. At least 30 single seed were used for extratcion of glutenin proteins. Wheat flour obtained by milling of grains on Bühler laboratory mill.

The flour used for extraction glutenin proteins. The 10 mg was weighed in 1.5ml microtube and for extraction was added 400µl protein extraction SDS buffer (120 mM Tris-HCl, pH=6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and boiled for 5 min. The sample were centrifuged at 12000 rpm for 10 min. Protein resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-

PAGE) Laemmli, (1970) was performed with Bio-Rad equipment based on a previously described method He (2011) with 11.8% gel and electrophoresed at 20mA for 2h. Gels were stained by using Commassie Briliant blue dye resloved in 10% TCA and 250ml methanols. After staining, the obtained electrophoregrams are used for analysis and determining HMW-GS and identification of *Glu-1* alleles (Payne and Lawrence, 1983). Total protein content was determinated according to the Kjeldahl's method (N×5.7). Protein sedimentation volume analyzed by Zeleny method. Baking bread volume and the score was done by standard laboratory methods.

Climatic conditions in year of experiment during growing period

Experiment carried out on experimental field in two year which are characterized different regime of temperature and precipitation. Temperature values and precipitation amount measured and average values computed per months in both year of experimental investigation. Obtained values compared with average values of ten-year period (tab. 1). The average value of temperatures (8.3 ^{o}C) in the first year were similar to average of ten years' period (8.5 ^{o}C) and less than in second (11.0 ^{o}C) experimental year. The temperature and precipitation varied per months within years and were different between same period in two experimental years.

Table 1. Average values of monthly temperatures and precipitation during wheat growing period

Tem& Precpt	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
^{0}C	2005/06	11.5	5.6	3.3	-1.7	1.5	5.5	12.7	16.4	19.7	8.3	74.4
(mm)	2006/07	13.3	7.6	3.5	6.1	6.3	9.1	12.1	18.2	22.8	11.0	99.0
1990/2000		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	8.5	76.7
	2005/06	49.0	54.8	47.1	27.9	38.1	116	86.3	29.6	84.8	59.3	533.7
	2006/07	16.7	13.7	51.9	45.3	32.1	62.9	3.6	118	25.3	41.1	369.9
1990/2000		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	417.8

In the first year 2005/06 of investigation the amount of precipitation was 533.7mm and significantly higher than in second 2006/07 (369.9mm) year and the ten-year period average (417.8mm). Amounts of precipitation in the first year are was more suitable for plant grooving than in second year and without big differences between minimum and maximal values per month, as in second year (in April – 3.6mm and in May 118mm). However, in second year, dry conditions are favorable for intensive grain filling and accumulation of protein. Environmental conditions during wheat grain development can affect wheat flour quality.

RESULTS AND DISCUSSION

The identified high-molecular wight glutenin subunits were present in different frequency of analyzed wheat genotypes. The eight different *Glu-1* alleles were determined, three at *Glu-A1*, three at *Glu-B1* and two at *Glu-D1* (tab. 2). At *Glu-A1* locus three x-type subunits 1, 2* and null controlled by alleles *Glu-A1a*, *Glu-A1b* and *Glu-A1c*, respectively were identified. The subunit 2* (ecoded by ***b*** allele) was determined the most frequently in four (50.0%) genotypes, followed by subunit null (encoded by ***c*** allele) in three (37.5%) and subunit 1 (encoded by ***d*** allele) in one (12.5%) wheat genotypes. The varying in frequency of glutenin allele in wheat cultivars were found in other research (Knežević et al., 1993; Yasmeen et al., 2015; Knezevic et al., 2016a).

In three pair of eight wheat genotype the same composition of glutenin subunits were found. Among eight wheat genotypes the five different Glu-1 allele composition was identified. The glutenin subunits 2*, 7+9, 5+10 were found in G-3130 and G-3127 wheat genotype, 2*, 7+8, 5+10 in G-35183 and G-3075, while N, 7+9, 2+12 were identified in G-3501 G-3097. Wheat genotypes G-3712 and G-3574 had differnt combination of HMW glutenin subunits (tab. 2).

Protein content in seed of wheat genotypes is important quality parameter which used for estimation of selected genotype of wheat in breeding program (Knežević et al., 2016b). In this study of eight wheat genotypes identified different value of seed protein content. In the first year of investigation protein content in seed varied from 11.6% (G-3097) to 14.20% (G-3570), while in the second year, content of protein in seed was the lowest in G-3512 (12.60%) and the highest in G-3075 (15.40%) tab. 2. Mainly, in all wheat genotypes, protein content value was the higher in second year of experiment than in first zear of experiment, what indicate the more favorable condition in stage of grain filling. In second average temperature in temperature in May was 18.24^0C and June 22.8^0C, while in the first year was lower in May-16.4^0C and June-19.7^0C. Also, amount of precipitation was higher in second year (May-118mm and June-25.3mm) than in the first year (May-29.6mm and June-84.8mm). Protein content is geneticaly controlled, but affect of environmental factors (temperature, precipitation) have great on expression this trait (Godfrey et al., 2010; Knezevic et al., 2017b). Amount of gluten protein fraction will be higher when using fertilizer than without using. The high temperature influence to increasing content of gluten protein, what is the results of inhibition of starch synthesis (Hurkman et al., 2013).

Table 2. Glutenin allele encoding HMW GS composition and technological quality of winter wheat genotypes

Geno-type	*High molecular weight glutenin subunits*			*Glu-1 alleles*			*Grain protein content %*		*Protein sedimentation volume (ml)*		*Loaf volume (ml)*		*Quality score*
	1AL	1BL	1DL	***A1***	***B1***	***D1***	2005/06	2006/07	2005/06	2006/07	2005/06	2006/07	
G-3130	2*	7+9	5+10	***b***	***c***	***d***	13.60	14.60	42.0	48.0	480	480	9
G-35183	2*	7+8	5+10	***b***	***b***	***d***	13.40	15.00	52.0	54.0	500	500	10
G-3501	N	7+9	2+12	***c***	***c***	***a***	12.20	13.00	38.0	46.0	420	460	5
G-3512	N	6+8	2+12	***c***	***d***	***a***	11.80	12.60	34.0	36.0	400	400	4
G-3574	1	7+9	5+10	***a***	***c***	***d***	12.80	14.00	42.0	44.0	460	500	9
G-3027	2*	7+9	5+10	***b***	***c***	***d***	13.60	14.80	44.0	50.0	460	480	9
G-3075	2*	7+8	5+10	***b***	***b***	***d***	14.20	15.40	54.0	58.0	520	540	10
G-3097	N	7+9	2+12	***c***	***c***	***a***	11.60	13.00	40.0	46.0	440	450	5

Genotype with high protein content mainly have high protein sedimentation volume and loaf volume, in this study. Genotypes G-35183 and G-3075 withcomposition of glutenin subunits 2*, 7+8, 5+10 had the highest protein content, the highest protein sedimentation volume and the highest loaf volume in both year of experiment (tab. 2). The investigation of Brazilian wheat cultivars showed that protein content varied from 10.04 to 15.10%, while correlation between the protein content and quality parameters of the grain and flour in wheat genotypes did not find (Costa et al., 2017). However, another investigation showed that HMW-GS have connection with functional properties of wheat dough (Dvořáček et al., 2013). So, positive association of glutenin component 5+10 encoded by ***d*** allele at *Glu-D1* and component 2* encoded by ***d*** allele at *Glu-A1* with dough quality, bread volume were found (Vázquez et al., 2012; Vaiciulyte-Funk et al., 2015).

High protein concentrations often lead to high Zeleny sedimentation values. In the analyzed wheat wheat genotypes, differences were detectable on the base sedimentation values were obtained. The Zeleny sedimentation values varied in both year of experiment. The lowest value of protein sedimentation had G-3512 (34.0ml in the first year and 36.0ml in the second year) while the highest protein sedimentatio volume had G-3075 (54.0ml in the firs year and 58.0ml in the second year) tab. 2. In average for all wheat genotypes, protein sedimentation values were higher in second year of experiment. In wheat genotypes which have composition of HMW GS 2*, 7+8, 5+10 (G-3075 and G-35183) sedimentation volume was significant higher than in genotypes which had glutenin components null at 1AL, 6+8 at 1BL and 2+12 at 1DL chromosome (G-3512). Mainly, the higher sedimentation volume had wheat genotypes with 2* than null at 1AL chromosome. Also, sedimentation volume in genotypes which posses 5+10 subunits was higher than in genotypes with 2+12 subunits at the 1Dl chromosome (tab. 2). Similar resluts reported for Yugoslav wheat cultivars ((Knežević et al., 1993).

The genotype G-3075 had the highest loaf volume in the first year (520*ml*) as well in the second year (540*ml*). The lowest loaf volume had G-3512 in the first year

(400*ml*) and in second year (400*ml*). This genotypes have glutenin subunits 6+8 encoded by ***d*** allele at *Glu-B1* and subunits 2+12 encoded by ***a*** allele at *Glu-D1* (tab. 2). The previous studies showed that these subunits associated with poor dough and bread quality (Bakshi and Bhagwat, 2016).

All the HMW subunit combinations resulted in bread with good and similar appearance. The highest loaf volumes were obtained for wheat genotypes that contained subunit 2*, 7+8 and 5+10 subunits encoded by *Glu-A1c*, *Glu-B1b* and *Glu-D1d* alleles. On the base of revealed values for each HMW glutenin subunits contribution to quality (Payne, 1987) we estimate *Glu-1* quality score which was the highest (QS=10) in genotypes G-3075 and G-35183 with *Glu-A1* allele composition ***b, b, d,*** which encode 2*, 7+8 and 5+10 subunits, while the lowest (QS=4) had genotype G-3512 which is glutenin alele composition ***c,d,a***, encoding N, 6+8 and 2+12 (tab.2). The allelic variation at HMW-GS and LMW-GS and environmental conditions are important factors that influence the wheat flour quality parameters (Branlard et al., 2003; (Knežević et al., 2017b). Alleles (***a, b***) encoded at the *Glu-A1* were associated with a higher loaf volume compared to the Null subunit (***c***), and allele *Glu-D1d* encoding 5+10 associated with good baking quality (Peña et al. 2005; Vázquez et al. 2012). However *Glu-B1c* encoding 7+9 subunits are associated with low baking quality, whereas ***i*** and ***b*** allele encoding 17+18 and 7+8 subunits are associated with good baking characteristics (Menkovska et al., 2002; Liang et al. (2010).

CONCLUSION

This investigation showed allele polymorphisms at all six *Glu-1* loci and were identified 8 glutenin alleles. In this study identified five different glutenin allele formula. Among the eight genotypes for three pair of genotypes identified the same composition ofglutenin subunits encoded by same Glu-1 allele. According to values of grain protein content, sedimentation volume and loaf volume, for studied wheat genotypes were established differences between genotypes in both years which characterized diferent climatic condition. The highest protein content (14.20%; 15.40%), Zeleny sedimentation volume (54.0ml; 58.0ml) and loaf of volume (520ml; 540ml) had G-3075 wheat genotype, while G-3512 had the lowest protein content (12.60% in second year) Zeleny sedimentation volume (34.0ml; 36.0ml) and loaf volume (400ml in both year). Genotypes which carried glutenin subunits 2* encoded by *Glu-A1b*, 7+9 encoded by *Glu-B1c* and subunits 5+10 encoded by *Glu-D1d* had the highest Zeleny sedimentation volume of proteins, protein content in seed and loaf volume. The results may be used as guidelines for the breeding purposes to create wheat cultivars with better bread making quality.

ACKNOWLEDGEMENTS

This work supported financially by the Ministry of Education, Science and Technological development of Republic Serbia, Belgrade, in program of Project TR31092

REFERENCES

Bakshi, S., Bhagwat, S.G. (2016): High Molecular Weight Glutenin Subunit Variation among Indian Wheat Varieties. Greener J. of Agric. Sci., 6(11): 349-355.

Blechl, A. E., Vensel, W. H. (2013): Variant high-molecular-weight glutenin subunits arising from biolistic transformation of wheat. Journal of Cereal Science, 57:496-503.

Branlard, G., Dardevet, M., Amiour, N., Igrejas, G. (2003): Allelic diversity HMW and LMW glutenin subunits and Omegagliadins in French bread wheat (*Triticum aestivum* L.). Genetic Resources &Crop Evolution, 50:669-679.

Costa, M.S., Scholz, M.B.S., Miranda, M.Z., Franco, C.M.L. (2016): Effect of glutenin subunits on the baking quality of Brazilian wheat genotypes. Bragantia, 76.

Dvořáček, V., Bradová, J., Capouchová, I., Prohasková, A., Papoušková, L. (2013): Intra-varietal polymorphism of gliadins and glutenins within wheat varieties grown in the Czech Republic and its impact on grain quality. Czech J. Genet. Plant Breed., 49:140–148.

Godfrey, D., Hawkesford, M. J., Powers, S. J., Millar, S., Shewry, P. (2010): Effects of crop nutrition on wheat grain composition and end-use quality. J. Agric. Food Chem., 58:3012-3021.

He, F. (2011): Laemmli-SDS-PAGE. Bio101: e80. DOI: 10.21769/BioProtoc.80;

Hernández, Z. J. E., Figueroa, J. D. C., Rayas-Duarte, P., Martínez Flores, H. E., Arámbula, G. V., Luna, G. B., Peña, R. J. (2012): Influence of high and low molecular weight glutenins on stress relaxation of wheat kernels and the relation to sedimentation and rheological properties. Journal of Cereal Science, 55:344-350.

Hurkman, W. J., Tanaka, C. K., Vensel, W. H., Thilomony, R., Altenbach, S. B. (2013): Comparative proteomic analysis of the effect of temperature and fertilizer on gliadin and glutenin accumulation in developing endosperm and flour *Triticum aestivum* L. cv. Butte 86. Proteome Science. 11:8. doi:10.1186/1477-5956-11-8

Jondiko, T.O., Alviola, N.J., Hays, D.B., Ibrahim, A., Tilley, M., Awika, J.M. (2012): Effect of high-molecular-weight glutenin subunit allelic composition on wheat flour tortilla quality. Cereal Chem. 89:155-16.

Knežević, D., Šurlan-Momirović, G., Ćirić, D. (1993): Allelic variation at *Glu-1* loci in some Yugoslav wheat cultivars. Euphytica, 69(2):89-95.

Knezevic, D., Rosandic, A., Kondic, D., Radosavac, A., Rajkovic, D. (2016a): Impact of quality of grain wheat on food value. Növénytermelés, 65:99-102.

Knežević D., Maklenović, V., Kolarić, Lj., Mićanović, D., Šekularac, A., Knežević, J. (2016b): Variation and inheritance of nitrogen content in seed of wheat genotypes (*Triticum aestivum* L.). Genetika, 48(2):579-586.

Knezevic, D., Rosandic, A., Kondic, D., Radosavac, A., Rajkovic, D. (2017a): Effect of gluten formation on wheat quality. Columella – Journal of Agricultural and Environmental Sciences, 4. (1):169-174.

Knezevic, D., Zecevic, V., Micanovic, D., Menkovska, M, Glumac, S. (2017b): Effect of environment to wheat quality properties. XII Int. Conf. „Knowledge capital of the future " knowledge without borders". March 31-April 02 2017, V.

Banja, Serbia. Int. J. Inst. of knowledge Management, 16(4):609-614 (Publ.IKM) ed (R. Dimitrovski).

Li, Y., Zhou, R., Branlard, G. and Jia, J. (2010). Development of introgression lines with 18 alleles of glutenin subunits and evaluation of the effects of various alleles on quality related traits in wheat (*Triticum aestivum* L.). Journal of Cereal Science, 51:127-133.

Liang, D., Tang, J., Peña, R. J., Singh, R., He, X., Shen, X., Yao, D., Xia, X., He, Z. (2010): Characterization of CIMMYT bread wheats for high- and low-molecular weight glutenin subunits and other qualityrelated genes with SDS-PAGE, RP-HPLC and molecular markers. Euphytica, 172:235-250.

Liu, H., Wang, K., Xiao, L., Wang, S., Du, L., Cao, X., Zhang, X., Zhou, Y., Yan, Y. Ye. X. (2016). Comprehensive identification and bread-making quality evaluation of common wheat somatic variation line AS208 on glutenin composition. PLoS ONE 11:e0146933. doi: 10.1371/journal.pone.0146933

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Menkovska, M., Knežević, D., Ivanoski, M. (2002): Protein allelic composition, dough rheology, and baking characteristics of flour mill streams from wheat cultivars with known and varied baking qualities. Cereal Chemestry. 79(5):720-725.

Novoselskaya-Dragovich A.Yu. (2015): Genetics and genomics of wheat: storage proteins, ecological plasticity and immunity. Russian Journal of Genetics, 51(5):476-490.

Payne, P.I. (1987): Genetics of wheat storage proteins and the effect of allelic variation on bread–making quality. Annual Review of Plant Physiology, 38:141–152.

Peña, E., Bernardo, A., Souler, C., Jouve, N. (2005): Relationship between common wheat (*Triticum aestivum* L.) gluten proteins and dough rheological properties. Euphytica, 143:169-177.

Shewry, P.R., Halford, N.G., Tatham, A.S., Popineau, Y., Lafiandra, D., Belton, P.S. (2003): The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. Adv. Food Nutr. Res. 45:219-302.

Vaiciulyte-Funk, L., Juodeikiene, G., Bartkiene, E. (2015): The relationship between wheat baking properties, specific high molecular weight glutenin components and characteristics of varieties. Zemdirbyste-Agric., 102(2):229-238.

Vázquez, D., Berger, A. G., Cuniberti, M., Bainotti, C., Miranda, M. Z., Scheeren, P. L., Jobet, C., Zúñiga, J., Cabrera, G., Verges, R. and Peña, R. J. (2012). Influence of cultivar and environment on quality of Latin American wheats. Journal of Cereal Science, 56:196-203.

Yasmeen, F., Khurshid, H., Ghafoor, A. (2015): Genetic divergence for high-molecular weight glutenin subunits (HMW-GS) in indigenous landraces and commercial cultivars of bread wheat of Pakistan. Genetics and Molecular Research 14 (2): 4829-4839.

Zhang, P., Jondiko, T.O., Tilley, M., Awika, J.M .(2014): Effect of high molecular weight glutenin subunit composition in common wheat on dough properties and steamed bread quality. J. Sci. Food Agr. 94:2801-2806.

Original scientific paper
10.7251/AGRENG1802022K
UDC 631.459:632.125(55)

EFFICIENCY OF INTERO MODEL TO PREDICT SOIL EROSION INTENSITY AND SEDIMENT YIELD IN KHAMSAN REPRESENTATIVE WATERSHED (WEST OF IRAN)

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ABSTRACT

Application of empirical models is inevitable because of the complexity of process, features, spatial and temporal variation of soil erosion and non-existence or lack of associated data. In the present study, maximum outflow and soil erosion intensity were predicted for Khamsan representative watershed in West of Iran, using IntEro model. The results of production of erosion material in the river basin (W year), coefficient of the deposit retention (Ru) and real soil losses (G year) were then compared with the measured soil erosion, SDR and sediment yield data in Khamsan watershed. The intensity of the erosion process were medium in studied watershed. The predicted data were compared with the measured sediment yield of studied watershed and verified the acceptable results of the IntEro model in Khamsan representative watershed. The results showed that the peak flow is 27.50 m^3s^{-1} for a return period of 100 years. The value of Z coefficient of 0.876 indicates that the river basin belongs to the second destruction category out of five. The calculated net soil loss from the river basin was 12263.44 m^3 per year, specific 282.81 m^3km^{-2} per year. The strength of the erosion process is strong, and according to the erosion type, it is surface erosion.

Keywords: *IntErO Model, land use, runoff, sediment delivery ratio, soil degradation.*

INTRODUCTION

Soil erosion is one of important issues of environmental in the world, that it cause the various problems (Toy et al., 2002). The population increase and growing demand for agricultural products (Prokop and Poręba, 2012; Zhao et al., 2013) or intensive dry land (Biro et al., 2013) has generated changes in land use and resulted

in erosion and land degradation. Water erosion has several types of water erosion, including splash, sheet, interrill, rill, gully and stream bank erosion (Khaledi Darvishan et al., 2012; Khaledi Darvishan et al., 2014 and 2015, Gholami et al., 2016). Knowing or estimating the accurate quantity of soil erosion in a watershed is therefore essential and one of the basic steps of all studies to encompass lots of environmental problems and to evaluate the amount of sediment moved, transported and deposited in and out of the basin. On the other hand, direct measurements of erosion in a watershed are possible with multi-years measurement of solid transport in the closing-section (Tazioli, 2009).

The models use and modeling processes modeling, especially watersheds of without hydrometric stations, are the useful and essential tools to evaluate the amount of sediment and soil erosion (Wischmeier and Smith, 1965, 1978) for this purpose the various models have been developed (Zhang et al., 1996).

Evaluation of the applicability of soil erosion models to a watershed is not easy, as it is difficult to accurately measure soil erosion in the field (Conoscenti et al. 2008, Rawat et al. 2011). In contrast, sediment yield models are easier to apply, because the data for these models can be measured at the watershed outlet (Kinnell and Riss 1998; Erskine et al. 2002; Kinnell, 2010).

Among several models, Erosion Potential Method – EPM, originally developed for Yugoslavia by Gavrilovic (1972), was in recent times repeatedly applied in the watersheds of Apennine and in the Balkan Peninsula (Blinkov and Kostadinov, 2010; Kostadinov et al., 2006, 2014; Lenaerts, 2014; Milevski et al., 2008; Ristic et al., 2012; Sekularac, 2000, 2013; Spalevic et al. 2012a, 2012b, 2013a, 2013b, 2013c, 2013d, 2013e, 2013f, 2014a, 2014b, 2014c; Stefanovic, 2004; Tazioli, 2009, Zorn and Komac, 2008), but also in the other regions in the world, for example in arid and semi-arid areas of the south-western USA (Gavrilovic Z., 1988), Saudi Arabia (Aburas Al-Ghamdi, 2010). The method is based on the factors affecting erosion in a catchment; its parameters dependent on the temperature, the mean annual rainfall, the soil use, the geological properties and some other features of the catchment.

The Intensity of Erosion and Outflow - IntErO program package (Spalevic, 2011), developed to predict the intensity of soil erosion and the runoff peak discharge in a watershed, is a computer-graphic method based on the Erosion Potential Method - EPM, which is embedded in its algorithm.

This present study, the IntErO model was verified and tested in Khamsan watershed in Kurdistan province and west of Iran.

MATERIAL AND METHODS

Study area

The Khamsan watershed with the area 43.37 km^2 has two sub-watersheds with exclosure treatment and under grazing, respectively. The main river length, total river length, average elevation, maximum elevation, minimum elevation, average annual temperature and average annual rainfall are 5.18 km, 198.85 km, 1936.27

m, 2378 m, 1580 m, 12.86 °C and 308.04 mm, respectively. Figure 1 shows the location of Khamsan Representative and treated and control sub-watershed

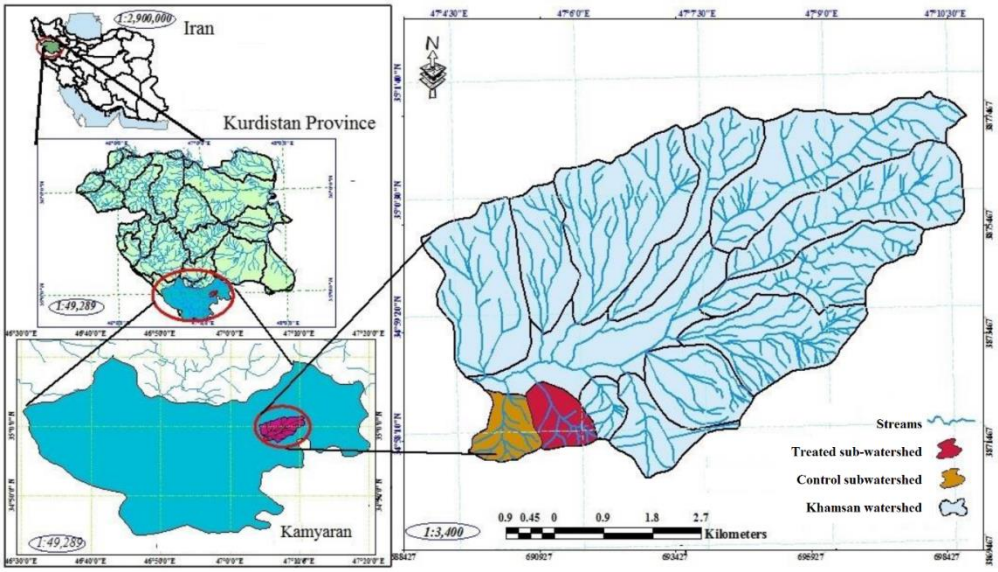

Fig. 1. Location of Khamsan Representative and treated and control sub-watersheds in Iran

IntERO model application

The Intensity of Erosion and Outflow - IntErO program package (Spalevic, 2011) was used to estimate maximum runoff discharge from the basin and the intensity of soil erosion, with the Erosion Potential Method – EPM (Gavrilovic, 1972) embedded in the algorithm of this computer-graphic method.

The above methodology was used in Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia and Slovenia (Kostadinov et al., 2014). In Iran, the IntERO have been successfully used previously in the Regions of Chamgardalan; Kasilian (Amiri, 2010; Zia Abadi & Ahmadi, 2011; Yousefi et al., 2014) and some other sub-catchments (Behzadfar et al., 2014 and 2015; Barovic et al., 2015).

RESULTS AND DISCUSSION

Climatic characteristics

The average annual temperature is 12.86 °C, the average annual air temperature (t0) and the average annual precipitation (Hyear) are 12.5 °C and 428 mm, respectively, based on the data from the Khamsan meteorological station. The temperature coefficient of the region (T) was calculated equal to 1.16.

The geological structure and soil characteristics of the area

To calculate some inputs of IntERO, the geological data was extracting from the geological map of Iran (Bolourchi et al., 1987). The geological data showed that the structure of the river basin, according to bedrock permeability, is the following: poor water permeability rocks (f0), medium permeable rocks (fpp) and very permeable products from rocks (fp) were 6%, 51% and 35%, respectively. The coefficient of the region's permeability, S1, was calculated about 0.61 (source: original).

Vegetation and land use

According to the analysis, the main portion of the river basin is totally under grass, meadows, pastures and orchards (60%). The coefficient of the river basin planning (Xa) and the coefficient of the vegetation cover (S2) were calculated about 0.73 and 0.88, respectively.

Soil erosion and runoff characteristics

According to the results, surface erosion has taken place in all the soils on the slopes as the dominant erosion form in the studied area which is the most pronounced on the steep slopes with scarce vegetation cover.

The coefficient of the river basin form, A, calculated as 0.55 using IntErO software. Coefficient of the watershed development, m, was 0.47 and the average river basin width, B, was 4.23 km. (A)symmetry of the river basin, a, which indicates that there is a possibility for large flood waves to appear in the river basin, was calculated as 0.17.

Drainage density, G, was calculated as 3.53 km km^{-2} which corresponds to high density of the hydrographic network. The height of the local erosion base of the river basin, Hleb, was 700 m and also the coefficient of the erosion energy of the river basin's relief, Er, was calculated as 86.63.

The value of Z coefficient as 0.876 indicates that the river basin belongs to II destruction category. The strength of the erosion process is high, and according to the erosion type, it is surface erosion, the second destruction category out of five.

For the current state of land use, calculated peak flow is 27.50 m^3s^{-1} for a return period of 100 years.

The production of sediments in the basin, Wyear, is calculated as 55552.22 m^3 $year^{-1}$; and the Coefficient of the intra-basin deposition, Ru, at 0.221 which indicates that 21% of the eroded materials will deposit and remain in the watershed.

Sediment yield at catchment outlet (Gyear) was calculated as 12263.44 m^3year^{-1}; and specific sediment yield at 282.81 m^3 km^{-2} $year^{-1}$.

Part of the detailed report for the Khamsan watershed is shown in Table 1.

Table.1. Part of the IntErO report (inputs and outputs of the model) for Khamsan watershed

Inputs	Amount and Unit
River basin areas (F)	43.36 km^2
The length of the watershed (O)	31.28 km
The area of the bigger river basin part (Fv)	23.57 km^2
The area of the smaller river basin part (Fm)	19.79 km^2
Natural length of the main watercourse (Lv)	11.08 km
Length of the contours / isohypses (liz)	223.57 km
Areas / surfaces between neighbouring contours / isohyets (fiz)	43.36 km^2
Altitude of the first contour line	1690 m
Incidence (Up)	100 years
Equidistance (Δh)	100 m
The lowest river basin elevation	1687 m
The highest river basin ele`vation	2387 m
A part of the river basin consisted of a very permeable products from rocks(fp)	35 %
A part of the river basin area consisted of medium permeable rocks (fpp)	59 %
A part of the river basin consisted of poor water permeability rocks (f0)	6 %
A part of the river basin under forests (fs)	0 %
A part of the river basin under grass, meadows, pastures and orchards (ft)	60 %
A part of the river basin under bare land, plough-land and ground without grass vegetation (fg)	40 %
The total length of the main watercourse with tributaries of I and II class	152.86 km
The shortest distance between the fountainhead and mouth(Lm)	1.34 km
The volume of the torrent rain (hb)	44.4 mm
Average annual air temperature (t0)	12.5 C
Average annual precipitation (H year)	428 mm
Types of soil products and related types (Y)	1
River basin planning, coefficient of the river basin planning (Xa)	0.73
Numeral equivalents of visible and clearly exposed erosion process(φ)	0.49
Outputs	
Coefficient of the river basin form (A)	0.55
Coefficient of the watershed development (m)	0.47
Average river basin width (B)	4.23 km
(A)symmetry of the river basin (a)	0.17
Coefficient of the river basin tortuousness (K)	8.25
Average river basin altitude (Hsr)	1860.04 m
Average elevation difference of the river basin (D)	173.04 m
Average river basin decline (Isr)	51.56%
The height of the local erosion base of the river basin (Hleb)	700 m
Density of the river network of the basin (G)	3.53
Coefficient of the erosion energy of the river basin's relief (Er)	86.83

Coefficient of the region's permeability (S1)	0.61
Coefficient of the vegetation cover (S2)	0.88
Analytical presentation of the water retention in inflow (W)	0.24
Energetic potential of water flow during torrent rains (2gDF^½)	383.69 m km s^{-1}
Maximal outflow from the river basin (Qmax)	27.50 m^3 s^{-1}
Temperature coefficient of the region (T)	1.16
Coefficient of the river basin erosion (Z)	0.876
Production of erosion material in the river basin (Wyr)	55552.22 m^3 $year^{-1}$
Coefficient of the deposit retention (Ru)	0.221
Real soil losses (Gyr)	12263.44 m^3 $year^{-1}$
Real soil losses per km^2 (Gyr/km^2)	282.81 m^3 km^{-2} $year^{-1}$

CONCLUSION

The study was conducted in the Khamsan watershed of Kurdistan province in west of Iran. The soil erosion intensity and runoff were calculated using the IntErO model. According to the findings, it can be concluded that there is a possibility for large flood waves to appear in the studied Khamsan river basin.

Calculated peak flow is 27.50 m^3s^{-1} for a return period of 100 years. The value of Z coefficient of 0.876 indicates that the river basin belongs to the second destruction category out of five. The calculated net soil loss from the river basin was 12263.44 m^3 per year, specific 282.81 m^3km^{-2} per year. The strength of the erosion process is strong, and according to the erosion type, it is surface erosion.

This study further confirmed the findings of Amiri (2010), Zia Abadi & Ahmadi, (2011), Yousefi et al. (2014), Behzadfar et al. (2014 and 2015) as well as Gholami et al. (2016) in successful implementation of the Erosion Potential Method – EPM and/or IntERO model in Iran, what leads to the conclusion that the IntErO model may be a useful tool for researchers in calculation of runoff and sediment yield at the level of the river basins.

REFERENCES

Aburas Al-Ghamdi, S. (2010). Application of Gavrilovic' Model for water erosion hazards in wadinaman basin through remote sensing and GIS techniques. p43, www.dr-aburas.com.

Amiri, F. (2010). Estimate of erosion and sedimentation in semi-arid basin using empirical models of erosion potential within a geographic information system. Air Soil and Water Research, 3, 37-44.

Barovic, G., Leandro Naves Silva, M., Veloso Gomes Batista, P., Vujacic, D., Soares Souza, W., Cesar Avanzi, J., Behzadfar M. & Spalevic, V. (2015). Estimation of sediment yield using the IntErO model in the S1-5 Watershed of the Shirindareh River Basin, Iran. Agriculture and Forestry, 61(3), 233-243

Behzadfar, M. Tazioli, A., Vukelic-Shutoska, M., Simunic, I. & Spalevic, V. (2014). Calculation of sediment yield in the S1-1 Watershed, Shirindareh Watershed, Iran. Agriculture and Forestry, 60(4), 207-216.

Behzadfar, M., Curovic, M., Simunic, I., Tanaskovik, V. & Spalevic, V. (2015). Calculation of soil erosion intensity in the S5-2 Watershed of the Shirindareh River Basin, Iran. International Conference on Soil, Tirana, Albania; 4-7 May,

Biro K, Pradhan B, Buchroithner M. & Makeschin F. (2013). Land use/land cover change analysis an its impact on soil properties in the Northern part of Gadarif region, Sudan. Land Degradation and Development, 24, 90- 102.

Blinkov, I. & Kostadinov, S., (2010). Applicability of various erosion risk assessment methods for engineering purposes, BALWOIS 2010 Conference - Ohrid, Republic of Macedonia. 25 - 29 May 2010.

Bolourchi, M., Mehr Parto, M. &Afsharharb, A. (1987). Geological quadrangle map of Iran no. J5 (Bojnurd sheet), scale 1:250,000, Geological Survey of Iran.

Conoscenti, C., Di Maggio, C. & Rotigliano, E. (2008). Soil erosion susceptibility assessment and validation using a geostatistical multivariate approach: a test in Southern Sicily. Natural Hazards, 46(3), 287-305.

Erskine, W.D., Mahmoudzadeh, A. & Myers, C. (2002). Land use effects on sediment yields and soil loss rates in small basins of Triassic Sandstone Near Sydney, NSW, Australia. CATENA, 49, 271–287.

Gavrilovic, S. (1972). Inzenjering o bujicnim tokovima i eroziji. Izgradnja. Beograd.

Gavrilović, Z. (1988). The use of empirical method (erosion potential method) for calculating sediment production and transportation in unstudied or torrential streams. In: White, W. R. (ed.), International Conference on River Regime; 411–422. Chichester.

Gholami, L., Batista, P.V.G., Behzadfar, A., Khaledi Darvishan, A. & Behzadfar, M. (2016). Application of Intero Model for Soil Loss Estimation Case Study: S7-1 Watershed of Shirindareh River Basin, Iran. Agrosym 2016, 2169-2177.

Khaledi Darvishan, A., Banasik K., Sadeghi S.H.R., Gholami, L. & Hejduk L. (2015). Effects of rain intensity and initial soil moisture on hydrological responsesin laboratory conditions. International Agrophisics, 29,165-173.

Khaledi Darvishan, A., Sadeghi, S.H.R., Homaee, M. & Arabkhedri, M. (2012). Potential use of synthetic color-contrast aggregates and a digital image processing technique in soil splash measurements, IAHS Publication, 356, 364-368.

Khaledi Darvishan, A., Sadeghi, S.H.R., Homaee, M. & Arabkhedri, M. (2014). Measuring sheet erosion using synthetic color-contrast aggregates. Hydrological Processes, 28(15), 4463-4471.

Kinnell, P.I.A. & Riss, L.M. (1998). USLE-M: empirical modeling rainfall erosion through runoff and sediment concentration. Soil Science Society of America Journal, 62, 1662-1672.

Kinnell, P.I.A. (2010). Event soil loss, runoff and the Universal Soil Loss Equation family of models: a review. Journal of Hydrology, 385, 384–397.

Kostadinov, S., Zlatic, M., Dragicevic, S., Novkovic, I., Kosanin, O., Borisavljevic, A., Lakicevic, M. & Mladjan, D. (2014). Anthropogenic influence on erosion intensity changes in the Rasina river watershed - Central Serbia.

Kostadinov, S., Zlatic, M., Dragovic, N. & Gavrilovic, Z. (2006). Soil erosion in Serbia and Montenegro. In Boardman, J., Poesen, J. (eds), Soil Erosion in Europe. John Wiley & Sons, Ltd; London, 271-277.

Lenaerts, T. (2014). Geomorphological mapping of Montenegro: Landform genesis and present processes. Master thesis, University of Gent, Faculty of Geography, p128.

Milevski, I., Blinkov, I. & Trendafilov, A. (2008). Soil erosion processes and modeling in the upper Bregalnica catchment. XXIVth Conference of the Danubian Countries. On the hydrological forecasting and hydrological bases of water management, 2-4 June 2008, Bled, Slovenia. p190.

Prokop P. & Poręba G.J. (2012). Soil erosion associated with an upland farming system under population pressure in Northeast India. Land Degradation and Development 23, 310- 321.

Rawat, P.K., Tiwari, P.C., Pant, C.C., Sharama, A.K. & Pant, P.D. (2011). Modelling of stream run-off and sediment output for erosion hazard assessment in Lesser Himalaya: need for sustainable land use plan using remote sensing and GIS: a case study. Natural Hazards, 59, 1277–1297.

Ristic, R. Kostadinov, S., Abolmasov, B., Dragicevic, S., Trivan, G., Radic, B., Trifunovic, M. & Radosavljevic, Z. (2012). Torrential floods and town and country planning in Serbia. Nat. Hazards Earth System Science, 12, 23–35.

Sekularac G. (2000). Odnos intenziteta erozije i stepena erodiranosti zemljišta sliva reke Kamenice. Doktorska disertacija. Agronomski fakultet, Čačak, Srbija.

Sekularac, G., Jelic, M., Kulina, M., Jakisic, T. & Jugovic, M. (2013). Soil erosion of the Cuverak River Basin (West Serbia). IV International Symposium „Agrosym 2013“, p. 807-810.

Spalevic, V. (2011). Impact of land use on runoff and soil erosion in Polimlje. Doctoral thesis, Faculty of Agriculture of the University of Belgrade, Serbia, p 1-260.

Spalevic, V., Curovic, M. Tanaskovik, V., Oljaca, M. & Djurovic, N. (2013b). The impact of land use on soil erosion and run-off in the Krivaja river basin in Montenegro. The First International Symposium on Agricultural Engineering, 4th - 6th October 2013, Belgrade–Zemun, Serbia, VI: 1-14.

Spalevic, V., Curovic, M., Andjelkovic, A., Djekovic, V. & Ilci, S. (2014a). Calculation of soil erosion intensity in the Nedakusi Watershed of the Polimlje Region, Montenegro. International Scientific conference: Challenges in modern agricultural production, December 11, 2014, Skopje, Macedonia.

Spalevic, V., Čurović, M., Borota, D. & Fuštić, B. (2012). Soil erosion in the river basin Zeljeznica, area of Bar, Montenegro. Agriculture and Forestry, 54(1-4), 5-24.

Spalevic, V., Curovic, M., Simunic, I., Behzadfar, M. & Tanaskovic, V. (2014). Land degradation, land use and soil erosion: the case of Montenegro. CIHEAM, The Watch Letter: Land Issues in the Mediterranean Countries, 28(1), 58-62.

Spalevic, V., Curovic, M., Tanaskovik, V., Pivic, R. & Djurovic, N. (2013e). Estimation of soil erosion intensity and runoff in the river basin of Bijeli Potok, Northeast of Montenegro. In proceeding of the 1st International Congress on Soil Science, XIII Congress of Serbian Soil Science Society "SOIL – WATER – PLANT", October, 2013. Belgrade, Serbia.

Spalevic, V., Curovic, M., Uzen, N., Simunic, I. & Vukelic-Shutoska, M. (2013d). Calculation of soil erosion intensity and runoff in the river basin of Ljesnica, Northeast of Montenegro. In proceeding of the 24th International Scientific-Expert Conference on Agriculture and Food Industry, Sarajevo, Bosnia and Herzegovina.

Spalevic, V., Djurovic, N., Mijovic, S., Vukelic-Sutoska, M &, Curovic, M. (2013a). Soil erosion intensity and runoff on the Djuricka River basin (North of Montenegro). Malaysian Journal of Soil Science,17, p.49-68.

Spalevic, V., Grbovic, K., Gligorevic, K., Curovic, M. & Billi, P. (2013). Calculation of runoff and soil erosion on the Tifran watershed, Polimlje, North-East of Montenegro. Agriculture and Forestry, 59(4), 5-17.

Spalevic, V., Hübl, J. Hasenauer, H. & Curovic, M. (2014c). Calculation of soil erosion intensity in the Bosnjak Watershed, Polimlje River Basin, Montenegro. The 5th International Symposium "Agrosym 2014", Jahorina, 23-26 October 2014, Bosnia and Herzegovina, p 730-738.

Spalevic, V., Mahoney, W., Djurovic, N., Üzen, N. & Curovic, M. (2012). Calculation of soil erosion intensity and maximum outflow from the Rovacki river basin, Montenegro. Agriculture and Forestry, 58(3), 7-21

Spalevic, V., Nyssen, J., Curovic, M., Lenaerts, T., Kerckhof, A., Annys, K. Van Den Branden, J. & Frankl, A. (2013c). The impact of land use on soil erosion in the river basin Boljanska Rijeka in Montenegro. In proceeding of the 4th International Symposium "Agrosym 2013" (3-6 October, 2013, Jahorina, Bosnia). Key note speakers, p. 54-63.

Spalevic, V., Nyssen, J., Curovic, M., Lenaerts, T., Kerckhof, A., Annys, K. Van Den Branden, J. & Frankl, A. (2013). The impact of land use on soil erosion in the river basin Boljanskarijeka in Montenegro. Proceeding "Agrosym 2013". Key note papers, p. 54-63.

Spalevic, V., Radanovic, D., Behzadfar, M, Djekovic, V., Andjelkovic, A. & Milosevic, N. (2014). Calculation of the sediment yield of the Trebacka Rijeka, Polimlje, Montenegro.Agriculture and Forestry, 60(1), 259-272.

Spalevic, V., Railic, B., Djekovic, V., Andjelkovic, A. & Curovic, M. (2014). Calculation of the Soil Erosion Intensity and Runoff of the Lapnjak watershed, Polimlje, Montenegro.Agriculture and Forestry, 60(2), 261- 271.

Spalevic, V., Simunic, I., Vukelic-Sutoska, M., Uzen, N. & Curovic, M. (2013). Prediction of the soil erosion intensity from the river basin Navotinski, Polimlje (Northeast Montenegro). Agriculture and Forestry, 59 (2), 9-20.

Spalevic, V., Tazioli, A. Djekovic, V., Andjelkovic, A. & Djurovic, N. (2014b). Assessment of soil erosion in the Lipnica Watershed, Polimlje, Montenegro. The 5th International Symposium "Agrosym 2014", Jahorina, 23-26 October 2014, Bosnia and Herzegovina, p 723-729.

Stefanovic, M., Gavrilovic, Z. & M. Milojevic, M. (2004). Erosion potential method and erosion risk zoning in mountainous regions. In Internatioales Symposion Iterprevent-RIVA\TRIENT.

Tazioli, A. (2009). Evaluation of erosion in equipped basins: preliminary results of a comparison between the Gavrilovic model and direct measurements of sediment transport. Environmental Geology, 56(5), 825-831.

Toy, T. J., Foster, G.R., & Renard, K.G. (2002). Soil erosion: processes, prediction, measurement, and control. John Wiley & Sons.

Wischmeier, W.H. & Smith, D.D. (1965). Predicting rainfall-erosion losses from cropland east of the Rocky Mountains. Agr. Handbook No. 282, US Department of Agriculture, Washington, DC.

Wischmeier, W.H. & Smith, D.D. (1978). Predicting rainfall erosion losses. Agriculture Handbook, n. 537.Agriculture Research Service, US Department of Agriculture, Washington DC.

Yousefi, S., Kivarz, N., Ramezani, B., Rasoolzadeh, N., Naderi, N. & Mirzaee, S. (2014). An Estimation of sediment by using erosion potential method and geographic information systems in Chamgardalan watershed: A Case Study of Ilam Province, Iran. GRIB, Vol. 2 (2): XXXIV to XLI

Zia Abadi, L. & Ahmadi, H. (2011). Comparison of EPM and geomorphology methods for erosion and sediment yield assessment in Kasilian Watershed, Mazandaran Province, Iran. Desert, 16, 103-109.

Zhang, X. C., Nearing, M. A., Risse, L. M. & McGregor, K. C. 1996. Evaluation of WEPP runoff and soil loss predictions using natural runoff plot data. Transactions of the ASAE, 39(3), 855-863.

Zhao G, Mu X, Wen Z, Wang F. & Gao P. (2013). Soil erosion, conservation and Eco-environment changes in the Loess Plateau of China. Land Degradation and Development, 24, 499- 510.

Zorn, M. & Komac, B. (2008). Response of soil erosion to land use change with particular reference to the last 200 years (Julian Alps, Western Slovenia). XXIVth Conference of the Danubian Countries. Hydrological forecasting & hydrological bases of water management, June 2008, Bled, Slovenia. p205.

Professional paper
10.7251/AGRENG1802032M
UDC 631.147(497.6)

ORGANIC AGRICULTURE AT HIGH ALTITUDES: EXPERIMENTAL ORGANIC GARDEN IN ILOVICE, BOSNIA AND HERZEGOVINA

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ABSTRACT

Agriculture at high altitudes is a challenge, especially in terms of organic vegetable production. In mountainous regions, most of the arable land is at high altitude, which significantly affects agricultural production, limiting the number of crops. This research was carried out in order to verify the possibility of growing a wide range of vegetables and herbs in these regions. The experimental plot (4500 m^2) was formed in the village Ilovice (municipality Trnovo, Bosnia and Herzegovina), at 950 m above sea level. After the analysis of the soil fertility, the organic production of vegetables, some spices and medicinal herbs was designed in the open field, without additional fertilization. Some typical Mediterranean species, such as artichoke and kale, were also planted. Some of the most important principles of organic agriculture - beneficial organisms, companion crops and intercropping - were fully met in the garden. Finally, although the production on the experimental plot took place at high altitudes, the health of plants, the appearance of fruits and the yield, confirmed the possibility of successful organic vegetable production in such regions. In addition, the experimental organic garden in Ilovice is a place for practical education of those interested in organic agriculture. This is of great importance for conducting applied research, acquiring new knowledge and helping producers to promote organic agricultural production, especially in less developed regions. To the best of our knowledge, this is the first study that carried out in Bosnia and Herzegovina, where the possibility of growing a wide range of plant species, at such high altitudes, has been practically verified.

Keywords: *Organic agriculture, high altitude, environmental education, mountain farming.*

INTRODUCTION

The most intensive agricultural production is carried out in the plain up to 200 m above sea level, as well as on terrain up to 600 m. Climatic conditions enable diverse agricultural production, while hilly and mountainous areas are suitable for fruit production. Above this height, there is a decrease in the number of crops that

can be grown, there are less cultivable areas and yields are lower. At altitudes above 1000 m (hilly-mountainous areas), the configuration of the terrain is even more unfavorable for the development of farming. This represents a transition zone between the fruit and grass belt (Lazić et al., 2014). At higher altitudes, light intensity is greater, the utilization of solar radiation is higher and spectral light composition is altered. Also, with an increase in altitude, temperatures decrease, and the amount and frequency of precipitation increase. Another important factor at high altitudes is the thinness of the atmosphere, and as a consequence, water evaporates faster from the ground.

In Bosnia and Herzegovina, mountainous areas account for 35% of agricultural land (Volk, 2010). These areas have short summers, with temperature up to 35 °C, and long harsh winters. Dominant in those regions are sheep farming, potatoes production, cereals, fodder plants, medicinal herbs, mushrooms and forest fruits (Lazić et al., 2014). Agriculture, and especially organic vegetable production, under these conditions represents a real challenge. However, unpolluted land and water are the most important potential of these regions for organic agriculture. As these are often the least developed regions, the additional value represents a possibility of economic development and reduction in the unemployment rate. Hence, organic farming can play an important role for socio-economic development and to make villages self-sustainable. Degradation of environmental quality and food safety concerns due to excess use of fertilizers and pesticides promoted organic farming in recent decades (Mishra et al., 2015).

In Bosnia and Herzegovina, only 3.9% of arable land is under vegetable production, including potato as a dominant crop (Kaba, 2017); in Sarajevo region it is 1.41% (Kaba 2014). According to the available literature, number of crops and vegetables that can be grown at higher altitudes is significantly limited, mainly to cereals (Plećević, 1985). In order to provide better conditions, vegetable growing is recommended in greenhouses or other indoor places. Also, vegetables that require a growth stage before producing seeds and fruits (tomatoes, zucchini, peppers, green beans) require more time and therefore are more risky.

This research was carried out in order to evaluate the possibility for organic production of a wide range of vegetables and herbs at high altitudes.

MATERIAL AND METHODS

The experimental field was formed on an area of 4500 m^2, in the village of Ilovice, municipality of Trnovo (Bosnia and Herzegovina), on the southern slopes of Jahorina mountain, at 950 m above sea level (Figure 1). In this garden, agricultural production has not been implemented for the last 50 years, and pesticides and fertilizers have not been applied. In order to start organic production on a particular land, it is necessary to adequately clear it and prepare it for healthy, organic production. By clearing and plowing, the completely grounded land has been turned into a field with crushed land suitable for agricultural production (Figure 2). A reduced soil tillage was applied, which did not deteriorate the soil characteristics but contributed to the improvement of fertility.

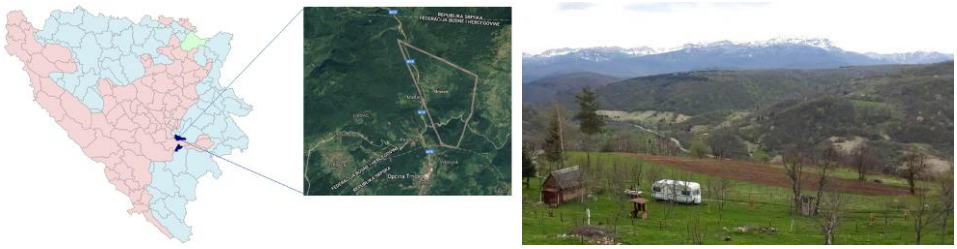

Figure 1. Geographical position of the experimental field
Source: *GoogleMaps;* photo*: D. Močević*

Before the agricultural production, the soil fertility and chemical properties of the surface soil layer from a depth of 0-30 cm were determined. The pH value (ISO 10390: 2005), $CaCO_3$ content (ISO 10693: 2005), total N (ISO 11261: 1995), humus content, P_2O_5 and K_2O content were determined. For the analysis, the standard methods were applied. At the location of the experimental field, weather conditions were monitored; during the growing season (May – October) the average temperature was 16.5 °C, while the precipitation ranged from 80 to 120 mm (Federal Hydrometeorological Institute, B&H).

On the experimental garden in Ilovice, the organic production of vegetables (cherry tomato, pepper, potato, eggplant, onion, garlic, parsley, carrots, white radish, asparagus, green beans, cucumbers, red beet, celery, pumpkin varieties hokkaido and stambolka, zucchini), as well the cultivation of spices and medicinal herbs (basil, dill, fennel, lemon balm) was planned. Some typical Mediterranean species, such as artichoke and kale, were sown/planted, as well. Only certified organic seeds were used, while vegetable and herb seedlings were obtained from the certified organic producer "PPI Ahmići", Vitez.

The geographical position, climate and altitude in such areas led to the postponement of the beginning of the agricultural season. Due to late frosts, sowing/planting was carried out in the period 15 - 30 May.

Agricultural production started in the beds (200 × 100 cm) and in the open field (Figure 2), without additional fertilization. For irrigation, only spring water was used, and in the beds and in the field there was a drip irrigation system.

Figure 2. The experimental field 'before and after'
(photo: D. Močević)

RESULTS AND DISCUSSION

Immediately before agricultural production, the soil fertility was determined (Table 1). Soil fertility is fundamental in determining the productivity of all farming systems, and it is most commonly defined in terms of the ability of a soil to supply nutrients to crops (Watson et al., 2002). The soil on the experimental field is of a poorly acidic reaction, as such it is suitable for growing vegetables. Humus content of 5.27% indicates good soil quality, while the amount of total nitrogen shows that content of this element in soil is sufficient. The soil on the field on which the organic production of vegetables was started is poorly phosphorous, but with a very significant amount of potassium.

Table 1. Chemical properties of soil on experimental plot

pH	6.67
$CaCO_3$	0%
Humus content	5.27%
Total N	0.33%
Available P	4.28 mg/100 g
Available K	41.55 mg/100 g

When weather conditions allowed, in the second half of May, sowing, i.e. planting of the vegetables and herbs, started (Figure 3). As organic production always implies growing of more crops at the same time, the appropriate choice of plants species and varieties, adapted to agroecological conditions, must be made (Filipović et al., 2010). Although, referring to the time of sowing/planting, vegetation was delayed, as early as the beginning of August the first fruits began to ripen. At the end of August and during September, the garden was rich in fruits (Figure 4 and 5). All plant species formed healthy fruits, and the yield was well above the expected. The production of vegetables such as zucchini, peppers, cherry tomatoes, green beans at high altitudes is risky however, in the garden in Ilovice these plants kept giving fruits until the appearance of stronger frosts.

The most suitable species for these agroclimatic conditions is definitely potato; high yields and healthy fruits have been achieved with the complete absence of its most significant pests, colorado potato beetle (*Leptinotarsa decemlineata* Say.).

Figure 3. The experimental organic garden in June and August (photo: D. Močević)

During August, the first fruits of tomatoes, peppers, hot peppers, potatoes and onions were picked. Expectedly, red beet had also a very good yield, as it is easily grown and tolerant to cold weather. However, pumpkins and zucchini, that require higher temperatures, also provided an extraordinary yield in the organic garden in Ilovice. Green beans, a plant of warmer areas, gave a particularly good yield. It is also known that at temperatures above 35°C and less than 6°C green beans lose flowers (Lazić, 2008). However, by selecting the appropriate varieties, at an altitude of almost 1000 m, a good yield and healthy fruits were obtained (Figure 5). The eggplant is also a species of warm climates; on the experimental plot, growing in the beds, healthy fruits were formed at the end of September (Figure 4).

Figure 4. Healthy fruits grown in organic garden in Ilovice (photo: D. Močević)

As a special part of this research, artichoke was planted. *Cynara scolymus* L. (artichoke) is a plant originating from the Mediterranean (Reolon-Costa et al., 2015), and requires specific growing conditions. It is a perennial plant, but in colder regions it is grown as an annual plant. Artichoke was planted at the end of May in the open field, on a sloping terrain, with a distance of 1 m between rows and among plants, and a drip irrigation system was applied (Figure 4). When it comes to cultivating artichokes, irrigation was paid extraordinary attention, as due to the terrain configuration, the plants are often suffocated. At the end of September, in the experimental garden in Ilovice, flower developed on healthy plants of artichoke (Figure 4).

Figure 5. The experimental field in September (photo: D. Močević)

The typical brassicae of the coastal and island areas, kale (*Brassica oleracea* L. *var. acephala* DC) was grown at almost 1000 m above sea level, in the organic garden in Ilovice. A slightly forgotten vegetable, recently kale has increased popularity, as a result of its high nutritional properties (Batelja et al., 2009, cit. Dumičić et al., 2014). Among all the brassicas, it is the richest in carotene, provitamin A and Ca, especially its older leaves (Lefsrud et al., 2007). In kale cultivars, the content and amount of secondary metabolites vary significantly depending on endogenous developmental factors such as genetic expression and protein modification, as well as environmental conditions (Jeon et al., 2018). The cultivation of kale in colder climates increases their quality; anthocyanin accumulation in purple kale is strongly induced by cold, the total anthocyanin content of purple kale exposed to cold was approximately 50-fold higher than those of plants grown in a greenhouse (Zhang et al., 2012).

In Ilovice, Jerusalem artichoke (*Helianthus tuberosus*) was also planted in the open field. This species, also known as wild potato, represents a healthy alternative to potatoes, given the high content of inulin polysaccharides. Tubers contain inulin instead of starch and sucrose found in most other tubers. In addition to the high levels of inulin in the tubers, Jerusalem artichoke is also regarded as a good source of soluble and insoluble fiber (Terzić and Atlagić, 2009; Saengkanuk et al., 2011). Above-ground parts of the plant can be used as animal feed. Jerusalem arthichoke was planted from tubers in May, while the first broods were harvested in October (Figure 5).

During the second half of May, the cultivation of spices and medicinal herbs (lemon balm (*Melissa officinalis* L.), fennel (*Foeniculum vulgare* Mill.) and sweet basil (*Ocimum basilicum*)) was started (Figure 5). Lemon balm and sweet basil were planted in the field, and fennel in the beds. The first picking of lemon balm was in mid-August, and after drying, this plant has been used for tea production.

Figure 6. Jerusalem artichoke, kale and lemon balm in the organic garden in Ilovice (photo: D. Močević)

Production at the organic garden in Ilovice was conducted respecting very important principles e.g. beneficial organisms, companion crops, intercropping. In order to attract useful insects that contribute to plant production by pollination (bumblebees, bees and axes) and suppression of harmful insects (ladybirds, spiders, mantises), on the field were grown sunflower, fennel, marigold, dill, basil and

lemon balm. On the farm, i.e. on the field next to the garden, buckwheat was also grown in organic production, which additionally attracted useful insects.

Plant species were cultivated in line with the system of compatible species, which by mutual action support growth and development, play a role of protection from the causal agents of diseases and pests (Lazić, 2008). In the garden, according to this principle, parsley and white radishes, blue eggplant and marigold, and sunflower near cucumber, were cultivated.

Since it is a natural repellent, basil was considerably present in the garden. It was sown close to tomatoes and peppers, because these species shade basil, which postpones flowering, prolongs the picking and increases yield, while on the other hand, basil repels lice that lay eggs in the fruit of tomatoes and peppers. Basil was also useful in protecting cucumber and tomatoes from the attack of powdery mildew, and as a repellent of trips, flies and mosquitoes.

When it comes to plant protection, on the experimental garden in Ilovice, with the exception of mechanical weed removal, other measures, including bio-pesticides, were not applied. This was achieved using healthy plant material and appropriate resistant varieties, crop rotation, time of sowing/planting, but also by the fact that the production took place at 1000 m. At this altitude, the pressure of harmful agents is significantly reduced, and given the long and cold winters, there is no possibility of survival of their forms in the soil or old plant parts.

This study has shown that, even at high altitudes, there is a potential for the successful organic agricultural production and growing of many different vegetable varieties. In order to achieve this, one of the most important factors is the geographical position and slope of the garden. Due to the generally small fields in mountain regions, organic vegetable production is an appropriate option for the development of family farms. Often, in such regions, agriculture represents the only possibility for the economic development and unemployment reduction.

The additional and very important value of this experimental garden is the practical education of those interested in organic agriculture. Under education, it is generally assumed the acquisition of theoretical knowledge, but when it comes to agricultural production, practical advisory work is required (Filipović and Ugrenović, 2009). As organic production requires a high level of knowledge from different fields, and education plays a crucial role, organic farm in Ilovice serves as a trial field for practical training (Figure 1).

Furthermore, UNDP, UNICEF, and UNESCO, in partnership with the Presidency of Bosnia and Herzegovina, financed the "Organic Peace Building" program, within the project "Dialogue for the Future". This project aimed to link and educate young and unemployed people through education and promotion of organic production of healthy food in underdeveloped areas at high altitudes, such as the municipality of Trnovo. Within the project, in the course of several months, "Small School of Organic Agricultural Production" was realized at the experimental organic garden in Ilovice. Through a theoretical and practical part of the teaching, training provided knowledge on the basics of organic agricultural production for fifty participants, primarily young unemployed persons (Figure 6).

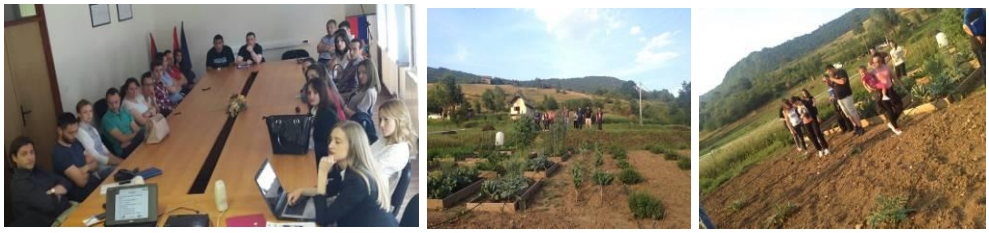

Figure 7. "Small School of Organic Agricultural Production" at the experimental organic garden (photo: D. Močević)

CONCLUSION

In the garden in Ilovice, the basic principles of organic agricultural production were fully met i.e. principles of health, ecology, fairness and care (IFOAM). Although the production on the experimental plot took place at altitude of almost 1000 m, results confirmed the thesis about the possibility of successful realization of organic vegetable production, including spice and medicinal herbs, in such regions. In addition, such experimental gardens are of great importance for conducting applied research, acquiring new knowledge and giving recommendations to producers, to promote of organic agricultural production. Above all, this research had the aim to point out to the possibility for successful organic farming, even in the mountain regions of Bosnia and Herzegovina. To the best of our knowledge, this is the first study that carried out in Bosnia and Herzegovina, where, unlike the assessment of the potential for organic agricultural production based on statistics and available literature data, the possibility of growing a wide range of plant species, at such high altitudes, has been practically verified.

ACKNOWLEDGEMENT

The authors would like to express their appreciation to the owner of the garden.

REFERENCES

Batelja, K., Goreta Ban, S., Žanić, K., Miloš, B., Dumičić, G., Matotan, Z. (2009). Autochthonous kale populations (*Brassica oleracea* L. *var. acephala*) in Croatian coastal region. Agriculture 2: 1-7.

Dumičić, G., Díaz-Pérez, J.C., Singh Sidhu, H., Urlić, B., Goreta Ban, S., MacLean, D., Workman, S. (2014). Kale (*Brassica oleracea* L. *var. acephala* DC) leaf water loss as affected by genotype and bagging. 49th Croatian & 9th International Symposium on Agriculture, February 16–21, 2014, Dubrovnik, Croatia, 305-309.

Kaba, Z. (2017). Federation of Bosnia and Herzegovina in figures. Institute for Statistics of FBiH, Sarajevo.

Kaba, Z. (2014). Federation of Bosnia and Herzegovina in figures. Institute for Statistics of FBiH, Sarajevo.

Filipović, V., Ugrenović, V., Ugrinović, M., Jaćimović, G., Glamočlija, Đ., Radivojević, S. (2010). Organska proizvodnja u funkciji zaštite i unapređenja biodiverziteta /Organic production in the function of protecting and improving biodiversity/. XV Međunarodno naučno – stručno savjetovanje agronoma Republike Srpske ″Poljoprivreda i hrana – izazovi XXI veka″, Trebinje, Republika Srpska, 171.

IFOAM, https://www.ifoam.bio/sites/default/files/poa_english_web.pdf

Jeon, J., Kim, J.K., Kim, H., Kim, Y.J., Park, Y.J., Kim, S.J., Kim, C., Park, S.U. (2018). Transcriptome analysis and metabolic profiling of green and red kale (*Brassica oleracea var. acephala*) seedlings. Food Chem. 241:7-13.

Lefsrud, M., Kopsell, D., Wenzel, A., Sheehan, J. (2007). Changes in kale carotenoid pigment concentration during leaf ontogeny. Sci. Hort. 112:136–141.

Lazić, B. (2008). Bašta zelena cele godine. Novi Sad, 2008.

Lazić, B., Mentov, A., Vladisavljević, A., Tanović, N., Salčinović, A.(2014). Organska proizvodnja u prekograničnom području Republike Srbije i Bosne i Hercegovine /Organic production in the cross-border area of the Republic of Serbia and Bosnia and Herzegovina/. Belgrade.

Mishra, J., Tewari, S., Singh, S., Arora, N. K. (2015). Biopesticides: Where We Stand? In Plant Microbes Symbiosis: Applied Facets, (Arora, N.K., ed.), Springer India.

Plećević, M. (1985). Tehnološke osnove poljoprivredne proizvodnje, I deo / Technological basics of agricultural production, I part/. Zavod za izdavanje udžbenika, Novi Sad.

Reolon-Costa, A., Grando, M.F., Cravero, V.P.Y., Almeida, A. (2015). Selection of artichoke plants and analysis of correlation between quantitative traits for fresh consumption. Agriscientia 32: 67-76.

Saengkanuk, A., Nuchadomrong, S., Jogloy, S., Patanothai, A., Srijaranai, S. (2011). A simplified spectrophotometric method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Eur Food Res Technol. 233: 609-616.

Terzić, S., Atlagić, J. (2009). Nitrogen and sugar content variability in tubers of Jerusalem artichoke (*Helianthus tuberosus*). Genetika 41: 3,289 -295.

Zhang, B., Hu, Z., Zhang, Y., Li, Y., Zhou, S., Chen, G. (2012). A putative functional MYB transcription factor induced by low temperature regulates anthocyanin biosynthesis in purple kale (*Brassica Oleracea var. acephala f. tricolor*). Plant Cell Rep. 31(2):281-9.

Volk, T. (ed.) (2010). Agriculture in the Western Balkan Countries. Leibniz Institute of Agricultural Development in Central and Eastern Europe. IAMO, Volume 57.

Watson, C.A., Atkinson, D., Gosling, P., Jackson, L.R., Rayns F.W. (2002). Managing soil fertility in organic farming systems. Soil Use and Management 18: 239-247.

Original scientific paper
10.7251/AGRENG1802041K
UDC 577.2

GENETIC MARKERS AND BIOSTATISTICAL METHODS AS APPROPRIATE TOOLS TO PRESERVE GENETIC RESOURCES

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ABSTRACT

The aim of presented study was to assess the most suitable way how to distinguish different breeds based on molecular markers. One of the most difficult aspects of quality assurance schemes is their reliability. The verification of fraud needs great efforts in control strategies. The use of DNA markers has been shown to be a useful tool for individual identification. It is necessary to use modern statistical method based on data mining and supervised learning. Supervised pattern recognition techniques use the information about the class membership of the samples to a certain group (class or category) in order to classify new unknown samples in one of the known classes on the basis of its pattern of measurements. Large scale of supervised learning oriented method was used for traceability and identification on individual level. A result of provided study shows the possibility to classify unknown samples according to genetic data. Model is also useful for classification on many logical levels as brand, region and many others. If we take in the account only Slovak and Austrian Pinzgau cattle, based on SNP chip data, it is not possible to separate them using Bayesian approach. Once we considered with the admixture of breeds involved in the historical development as well as inbreeding, selection signatures and migration, we were able to separate even genetically similar breeds. It is possible distinguish between closely related populations based on different markers. We just need to select the appropriate type of analysis.

Keywords: *cattle, markers, supervised learning, structure assessment.*

INTRODUCTION

Research of cattle breeding is a complex contemporary issue of interdisciplinary scientific interest, including research of agricultural landscapes and resilient urban food systems (Tóth et al, 2016). Considering that wild cattle no longer exist and that all surviving genetic diversity is now present in domestic animals, a better understanding of cattle genetics could help us to reduce some of these undesirable

effects (Canavez et al., 2012). Molecular markers have been comprehensively exploited to access genetic variability as they contribute information on every region of the genome, regardless of the level of gene expression. Employment of microsatellite markers is one of the most powerful means for studying the genetic diversity, calculation of genetic distances, detection of bottlenecks and admixture because of high degree of polymorphism, random distribution across the genome, codominance and neutrality with respect to selection (Putman and Carbone, 2014).

Machine learning (ML) is the science of building systems that automatically learn from data (Swan et al, 2013). The ML represents a set of topics dealing with the creation and evaluation of algorithms that facilitate pattern recognition, classification, and prediction, based on models derived from existing data. The data can present identification patterns which are used to classify into groups. The result of the analysis is the pattern which can be used for identification of data set without the need to obtain input data used for creation of this pattern. An important requirement in this process is careful data preparation validation of model used and its suitable interpretation (Židek et al., 2014). Tarca et al. (2007) described supervised as well as unsupervised learning methods in their study. In supervised learning, objects in a given collection are classified using a set of attributes, or features. The result of the classification process is a set of rules that prescribe assignments of objects to classes based solely on values of features. Supervised learning algorithms induce models from these training data and these models can be used to classify other unlabelled data (Židek et al., 2014). During the last twenty years, supervised learning has been a tool of choice to analyse the always increasing and complexifying data generated in the context of molecular biology, with successful applications in genome annotation, function prediction, or biomarker discovery (Guerts et al., 2009).

However, recent advances in genome sequencing and high-throughput DNA techniques has led to the development of single nucleotide polymorphism (SNP) genotyping arrays as a new molecular tool. Single nucleotide polymorphism arrays provide information on a large number of markers distributed over the whole genome at an affordable price. Consequently, this improvement enables a more realistic estimation of genetic diversity, population structure and admixture level (Kukučková et al., 2017). High-throughput technologies have already been used in many areas, as a genomic inbreeding measure (Ferenčaković et al., 2013), genetic and population structure (Mastrangelo et al., 2014).

The aim of this study was to classify the observed animals into Slovak and Austrian cattle using supervised and unsupervised learning models based on different molecular data.

MATERIALS AND METHODS

DNA of 412 selected Slovak (346) and Austrian (66) Pinzgau cows was isolated from hair roots and amplified in one multiplex PCR with 8 microsatellites (TGLA227, SPS115, ETH3, BM1824, CSRM60, CSSM66, TGLA122 and INRA23) localized on 8 chromosomes (18, 15, 19, 1, 10, 14, 21 and 3,

respectively). The polymorphism of microsatellite sequences was determined by fluorescent fragmentation analysis using capillary electrophoresis and the sizes of alleles were evaluated. All observed animals were divided into 2 logical groups based on country of origin. The classification models for identity verification of animals was developed. Statistical analysis was performed using Tanagra software (Rakotomalala, 2005).

Data mining statistical approaches using supervised classification were used in the learning phase. In total, 20 different methods of supervised machine learning and their ability to classify examined data were analysed. The basic output of supervised learning methods was "confusion matrix" representing the number of classified individuals using statistical method to some logical group. Bootstrapping and cross validation have been applied to minimize the model error. For construction of the algorithm in the using phase 75% of the data were used and remaining 25% were presented to algorithm as unknown classification.

The software Tanagra 1.4 was used for analysis of relatedness and principal component analysis (PCA) of microsatellite data (Rakotomalala, 2005). PCA is used to characterize how different multiple populations are, often using only the two first principal components (Albrechtsen et al., 2010). Mixture partition based on unsupervised clustering using Bayesian Analysis of Population Structure (BAPS v. 6.0) software was executed, further described in Cheng et al. (2013). The interpretation of the optimal number of clusters is directly inferred by the implemented algorithm in BAPS. The maximum number of clusters was set to 1, 2 or 4, repeated ten times. Each run has led to the same results.

Slovak Pinzgau cattle were genotyped by the Illumina BovineSNP50 v2 BeadChip (Illumina Inc., San Diego, CA). Ten active breeding bulls of Pinzgau cattle from Slovakia used in breed management were analysed. Genotyping information (BovineSNP50 v1 BeadChip) for 33 Austrian Pinzgau sires described in Ferenčaković et al. (2013) was used. The consensus map with the same number of autosomal SNPs for both breeds considered in the further analysis was firstly created. The population structure and the admixture level were inferred by the program BAPS version 6.0 (Corander et al. 2004) where the interpretation of the optimal number of clusters is directly inferred by the implemented algorithm. The maximum number of clusters was set to 5, because this number it is recommended to be higher than expected number of populations (Corander et al. 2004). An admixture analysis conditional on the optimal genetic mixture estimated from the individual level analysis was performed. Results were based on 5000 simulations from the posterior allele frequencies. The number of clusters containing more than 10 individuals as a point estimate of K was used, since the lowest population size was 10. Furthermore, to assess the significance of the admixture estimates, 200 individuals were generated from each identified ancestral source to provide an approximation to the distribution of the estimates under the hypothesis of no admixture. Ten iterations for the reference individuals were run.

RESULTS AND DISCUSSION

A model for animal identity verification was developed using microsatellite panel and machine learning methods. The reliability of individual methods was observed by application of all available models of supervised learning for data set preparation. Three of 20 tested methods have been selected with highest value of reliability (Table 1). The method with the lowest algorithm error in direct classification was Rnd Tree, applying decision trees techniques. Methods C4.5 and CS-MC4 appeared as preferred due to memorization phenomenon of Rnd Tree method. Although both methods recorded the higher value of the algorithm error in the phase of direct learning, after verifying the reliability using bootstrapping and cross validation lower error rate was recorded. Using CS-MC4 (C4.5) method 99.6% (98.6%) of animals can be correctly assigned to Slovak population and excluded from Austrian population only with 3.6% (7.9%) error rate.

Modern biology can benefit from the advancements made in the area of machine learning. Caution should be taken when judging the superiority of some machine learning approaches over other categories of methods. Of special concern with supervised applications is that all steps involved in the classifier design (selection of input variables, model training, etc.) should be cross-validated to obtain an unbiased estimate for classifier accuracy. For instance, selecting the features using all available data and subsequently cross-validating the classifier training will produce an optimistically biased error estimate (Tarca et al., 2007).

Table 1 Reliability of learning process, validation reliability (bootstrapping and cross validation) and reliability of using process expressed as a percentage

Method		Recall	Precision	Algorithm error	Bootstrap. 632+	CV error	Recall	Precision	Method error
C4.5	A	87.9	92.1	3.16	**7.4**	8.1	84.2	88.9	**4.8**
	S	98.6	97.7				97.6	96.5	
CS-MC4	A	57.5	96.4	6.8	8.1	**7.8**	79.0	93.7	**4.8**
	S	99.6	92.9				98.8	95.4	
Rnd Tree	A	100	100	**0.0**	9.0	12.0	73.7	70.0	10.7
	S	100	100				92.9	94.0	

The test set (25% of individuals) is used for the generalization error assessment of the final chosen models (Table 1). The methods C4.5 and CS-MC4 have been confirmed as the most reliable for classification of animals by country origin ($p < 0.05$). Algorithm is able to mark animals with specific pattern typical only for Slovak population. Precision of assessment is 94-96.5%. Similarly is possible to mark animals which do not belong to pure Slovak Pinzgau with recall probability 92.9-98.8%. The correct classification rate obtained with the reliability validation of the model were sufficient for identification of animals. Evaluation of classification models is essential to determine their ability and accuracy; ideally this would be performed by producing the model on a training set and testing it on

an independent test set (Swan et al., 2013). Although in learning process appeared the Rnd tree method as the most appropriate, after verification were all three observed models very balanced. In using process have proven methods C4.5 and CS-MC4 as the most accurate and therefore most suitable for this type of analysis.

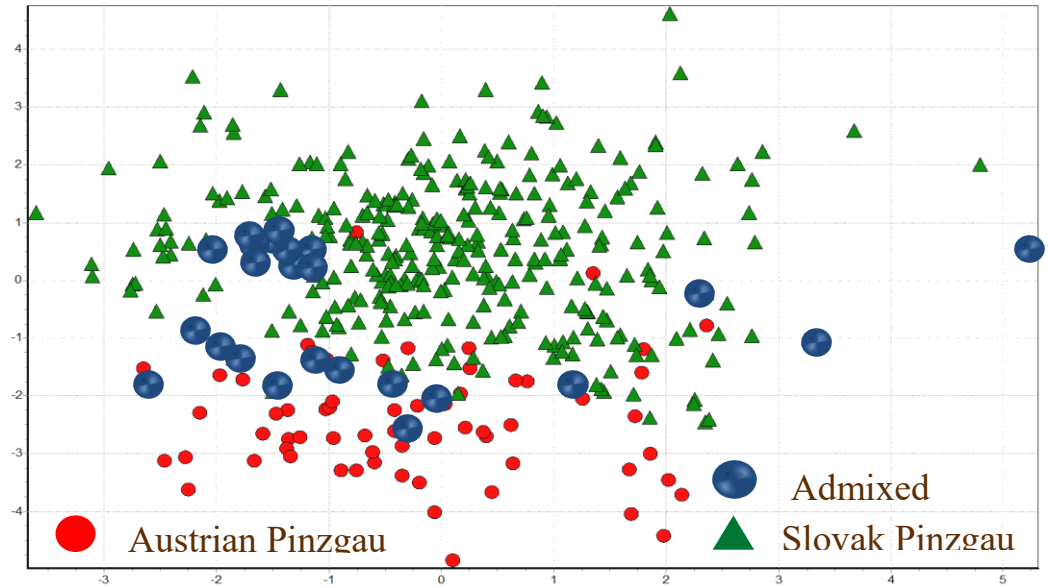

Figure 1. Animals of Slovak Pinzgau admixed with Austrian Pinzgau

The PCA and ancestry model were used to cluster animals, to explore the relationships among and within breeds, and to place the Slovak Pinzgau in a context with closely related Austrian Pinzgau. The PCA of 3 Pinzgau groups is visualised in figure 1. Slovak and Austrian Pinzgau created separate clusters although populations are very close. Admixed animals were between those 2 groups regardless of whether it was the Slovak and Austrian individual. Mixture partition based on unsupervised clustering using Bayesian approach clearly distinguished even genetically similar breeds (Figure 2). The approach used for populations' structure assessment is characterized as unsupervised learning methods with specific computation algorithm. It is possible to use or do not use a priori information about population unlike supervised learning.

Figure 2 Stacked bar plot of the cluster membership suggested by the BAPS algorithm ("unsupervised") presenting Slovak cattle in green and Austrian cattle in red

In comparison to microsatellite analysis the high-throughput genotyping data was used in subsequent analysis. Using genomic information estimated from 43 animals and 41,135 SNPs, the population structure of 2 cattle breeds was evaluated. A detailed analysis of genetic structure at both the individual and population level was performed based on the Bayesian clustering method adopted in BAPS. Comparing only Slovak and Pinzgau population based on SNP chip data it is not possible to separate them. Since both populations of Pinzgau cattle have the same origin and thus they are genetically similar, the Bayesian approach considered both populations as one cluster. But finally we were able to separate even these closely related breeds (S and A) since they incorporated the admixture with breeds involved in the historical development as well as inbreeding, selection signatures and migration. Each of the 15 clusters presented one exact population from the metapopulation of 15 European breeds (Figure 3). It is possible distinguish between closely related populations based on different markers. We just need to select the appropriate type of analysis.

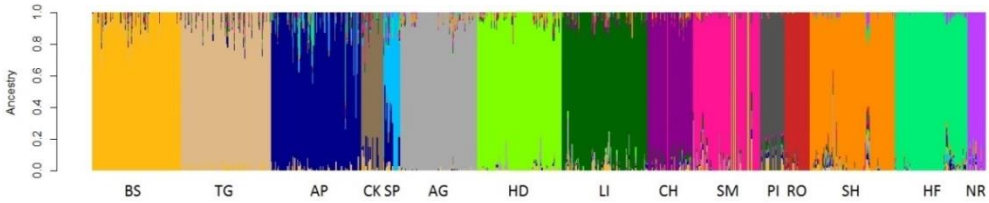

Figure 3. Posterior admixture analysis for 15 European cattle breeds based on the optimal genetic mixture estimate with 15 clusters using the BAPS uniform prior clustering model for individuals. Brown Swiss (BS), Tyrol Grey (TG), Austrian Pinzgau (AP), Cika (CK), Slovak Pinzgau (SP), Angus (AG), Hereford (HD, Limousine (LI), Charolais (CH), Simmental (SM), Piedmontese (PI), Romagnola (RO), Shorthorn (SH), Holstein (HF), Norwegian Red (NR).

The intensive selection of Slovak and Austrian Pinzgau cattle due to mass artificial insemination could increase the similarity among animals. The change in breeding goals to preserve the dual-purpose character of the Slovak Pinzgau was proposed for the long time, and consequently, a positive impact on population structure is expected. According to Jemma et al. (2015), the presence of purebred local individuals has become rare and thus highlights the need to implement a national conservation strategy. There is clearly a race between the characterization of genetic resources and their loss. In the same way, the development of genomic tools will allow to optimize the breeding strategies for ensuring the improvement of performance together with the preservation of genetic diversity. For breeders, it is important to know the origin of animals from the point of the genetic diversity. In case of missing pedigree information, other methods can be used for traceability of animal´s origin. Genetic diversity written in genetic data is holding relatively useful information to identify animals originated from individual countries (Židek et al., 2014).

CONCLUSION

Many of the local farm animal breeds substituted by more efficient breeds in the past are now endangered and preserved in situ as small populations in some regions. The possible extinction of these breeds would also mean irrecoverable loss of the genetic variability and so the damage of unique gene and allele combinations that would be very useful in the future for the generation of new farm animal genotypes. The global breeding program including very close populations will be more efficient providing higher genetic progress and diversity. Classification of individuals on the level of DNA is a valuable tool for origin traceability. The use of supervised learning allowed apparent distinction of closely related animals with Austrian and Slovak origin based on microsatellite markers. We can conclude the correct classification rate obtained with the reliability validation of the model were sufficient for identifying of animals. Datamining techniques based on genetic data are applicable in protection of Pinzgau cattle, breeding management and herdbook core conservation. Using high-throughput molecular information based on the method with linked markers, including inbreeding, gene flow, mutation, and thus introgression of other breeds, the more accurate view on the genetic structure of the observed breed was successfully performed. Presented methodology for differentiation of genealogically close breeds (Slovak and Austrian Pinzgau) based on various molecular markers can be proposed as general, how to distinguish among all highly related breeds.

ACKNOWLEDGEMENT

This study was supported by the Slovak Research and Development Agency under the Contract no. APVV-14-0054.

REFERENCES

Albrechtsen A., Nielsen F.C., Nielsen, R. (2010). Ascertainment Biases in SNP Chips Affect Measures of Population Divergence. Molecular Biology and Evolution, 27(11), 2534-47.

Canavez F.C., Luche D.D., Stothard P., Leite K.R.M., Sousa-Canavez J.M., Plastow G., Meidanis J., Souza M.A., Feijao P., Moore S.S., Camara-Lopes L.H. (2012). Genome Sequence and Assembly of Bos indicus. Journal of Heredity, 103(3), 342-348.

Corander J., Waldmann P., Marttinen P., Sillanpä M.J. (2004). BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics, 20, 2363-2369.

Cheng L., Connor T.R., Sirén J., Aanensen D.M., Corander J. (2013). Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. Molecular Biology and Evolution, 30(5), 1224-1228.

Ferenčaković M., Solkner J., Curik, I. (2013). Estimating autozygosity from high-throughput information: effects of SNP density and genotyping errors. Genetics Selection Evolution, 45(1), 42.

Guerts P., Irrthum A., Wehenkel L. (2009). Supervised learning with decision tree-based methods in computational and systems biology. Molecular BioSystems. 5(12), 1593-1605.

Kasarda R., Mészáros G., Kadlečík O., Hazuchová E., Šidlová V., Pavlík I. (2014). Influence of mating systems and selection intensity on the extent of inbreeding and genetic gain in the Slovak Pinzgau cattle. Czech Journal of Animal Science, 59(5), 219-226.

Kukučková V., Moravčíková N., Ferenčaković M., Simčič M., Mészáros G., Sölkner J., Trakovická A., Kadlečík O., Curik I., Kasarda R. (2017). Genomic characterization of Pinzgau cattle: genetic conservation and breeding perspectives. Conservations Genetics.

Jemma S.M., Boussaha M., Mehdi M.B., Lee J.H., Lee S.-H. (2015). Genome-wide insights into population structure and genetic history of tunisian local cattle using the illumina bovinesnp50 beadchip. BMC Genomics, 16, 677.

Mastrangelo S., Saura M., Tolone M., Salces-Ortiz J., Di Gerlando R., Bertolini F., Fontanesi L., Sardina M.T., Serrano M., Portolano B. (2014). The genome-wide structure of two economically important indigenous Sicilian cattle breeds. Journal of Animal Science, 92, 4833-4842.

Putman A.I., Carbone I. (2014). Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecology and Evolution, 4(22), 4399-4428.

Swan A.L., Mobasheri A., Allaway D., Liddell S., Bacardit J. (2013). Application of Machine Learning to Proteomics Data: Classification and Biomarker Identification in Postgenomics Biology, OMICS, 17(12), 595-610.

Tarca A.L., Carey V.J., Chen X.W., Romero R., Drăghici S. (2007). Machine Learning and Its Applications to Biology. Plos Computational Biology, 3(6), e116.

Tóth A., Rendall S., Reitsma F. (2016). Resilient food systems: A qualitative tool for measuring food resilience. Urban Ecosystems, 19(1), 19-43.

Židek R., Šidlová V., Kasarda R, Fuerst-Waltl B. (2014). Methods for Distinction of Cattle Using Supervised Learning. International Journal of Biological, Veterinary, Agricultural and Food Engineering, 8(5), 500-502.

Original scientific paper
10.7251/AGRENG1802049P
UDC 634.22:631.563

ANALYTICAL INDICATORS OF PLUM FRUIT SENSORY CHARACTERISTIC CHANGES AFTER SEVEN-DAY STORAGE AT ROOM TEMPERATURE

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ABSTRACT

Consumer acceptance of plum is based, primarily, on the corresponding sensory characteristics of the fruits: appearance, colour, firmness, taste and aroma. If not consumed immediately after harvesting, the aforementioned characteristics of plum will change. Still, those changes are less expressed when fruits are kept in cold storage, at low temperatures. However, during the transport and storage of plum at room temperature, which is often the case in local market plum sale, intense changes of certain characteristics of the fruit happens as well as a significant shortening of shelf life. The most expressive is firmness change where fruits become completely soft after seven days. Appearance and colour of the fruit are less susceptible to change. Since plum taste and odour depend on sugar and acid content, i.e., sugar-acid ratio and the content of certain volatile aromatic components, changes of their contents have been examined in some important plum cultivars in Serbia after seven-day storage at the temperature 20±1 °C. Sugar-acid ratio that determines sweetness, i.e., fruit acidity, has not significantly changed during the storage period. Among 10 examined aromatic components, the most significant changes have been observed in 2-E-hexenal, which gives a distinctive green note aroma and nonanal, characteristic plum-like scent. During the seven-day storage of picked plums, the content of 2-E-hexenal decreased while the content of nonanal increased.

Keywords: *Plum, shelf life, sugar/acid ratio, volatile aromatic components, odour activity values.*

INTRODUCTION

The quality of fresh fruits is prescribed by different official standards. It lays down the commercial value of fruits (which is usually based on a subjective evaluation of its external characteristics - colour, size, firmness, freshness) and guarantees uniformity of appearance. Such approach ignores sensory, nutritional, health and

other aspects that in a more comprehensive way define fruit quality. Increasing interest in the sensory quality of fruits reflects in the fact that 38% of consumers in the EU consider taste as a crucial criterion for purchasing fruits (Huyskens-Keil and Schreiner, 2004). When it comes to plums, even 75% of consumers consider taste and 71% of them, flavour, as very important characteristics of fruit, while the size and skin colour are considered to be very important characteristics only by 6% and 8% of consumers, and firmness of 14% of them (Hoehn *et al.*, 2005).

Like other fruits, the quality of plums for fresh consumption is, apart from the cultivar, crucially influenced by ripening stage at harvesting (Crisosto, 1994; Kader, 1999; Hoehn *et al.*, 2005; Kader, 2008; Usenik *et al.*, 2008), as well as by temperature and storage period if not consumed immediately after the harvest (Paunović and Grković, 1956; Guerra and Casquero, 2008; Casquero and Guerra, 2009; Guerra *et al.*, 2009). For plum sale on distant markets, the fruits have to be picked far before full ripe stage (regardless of not having a distinctive varietal flavour and aroma) and usually transported and stored at temperatures between 0 and 5 °C. It is considered much more important for the fruits to be in the wholesale market before they reach full ripe stage, in order to maintain attractive appearance and reach adequate ripening stage in retail, without major decrease in fruit firmness (Childers, 1949). For fresh consumption on local markets, plums are usually picked shortly before full ripe, that is, at their ripening stage when the fruits are still firm enough and have an established varietal flavour and aroma. In this ripening stage plums also have a very short shelf life considering that post-harvest handling, including transport and storage, is usually carried out at about room temperatures. It is often the case that, for local markets as well, plums are picked far before their full ripe stage in order to, on one hand, achieve higher price at the beginning of season when supply is low and provide sufficient fruit firmness and higher storage potential at about room temperature (Kader, 2008).

Although during storage period, numerous changes of physiological and physicochemical, as well as some sensory (colour and firmness) plum fruit characteristics have been established, there are almost no studies dealing with changes of taste and odour and their association with changes in content of the corresponding components of the fruit. Guerra *et al.* (2009) have attempted, for the sake of objective assessment of changes in taste, odour and aroma (determined by sensory analysis) during plum storage, to establish a correlation between their variations with changes in fruit characteristics (colour, firmness, total soluble solids/acid ratio) that can be determined by quick and simple instrumental analytical methods. This resulted in a partial success, since taste, odour and aroma of the fruit does not directly depend on its characteristics, but on the content of sugars, acids and volatile aromatic components, for which determination both time consuming methods and expensive analytical instruments need to be used. Sugar/acid ratio determines the sweetness i.e. acidity of the fruits, and concentration of the certain volatile aromatic components and odour threshold ratios (so called Odour activity values – OAV) determine the impact of each component on the overall volatile aroma, that is on aroma profile of the fruit.

The aim of this study was to determine the changes in the content of individual constituents of the fruit that decisively influence the taste and odour of the plum fruits Čačanska Rodna and Stanley, cultivars widely cultivated in Serbia and used, among other things, for fresh consumption.

MATERIAL AND METHODS

For a two-year study (2008 and 2009), fruits of the plum cultivars Čačanska Rodna and Stanley from a commercial orchard, planted in 1999 near Čačak (Site Premeća), at an altitude of 440 meter were used. Myrobalan was used as a rootstock and the spacing was 5 × 4 m, with standard agro-technical measures applied in the orchard.

Harvesting was performed on the basis of visual assessment, i.e. at the moment when characteristic fruit colour of the cultivar is developed and with maximum up to 10% of the skin area with green colour observed, which is 3 weeks (in case of the cultivar Čačanska Rodna), that is, 4 weeks (the cultivar Stanley) before reaching full ripe stage. At this ripeness stage fruits are firm, which makes them very suitable for transport and post-harvest handling; it is common that the fruits of such characteristics are picked for transport to distant markets, and sometimes for local markets, for fresh consumption. Picked fruits are stored for 7 days at a temperature of 20 ± 1 °C, a period during which the plums are usually sold in local market groceries, which is approximately their shelf life, at this temperature. After that, in addition to the change of certain sensory characteristics, the fruits soften a lot and there is an intensive development of mould and fruit rot (results not shown).

For the analysis of sugar and acid content in freshly-picked fruits and fruits after 7 days of storage, 20 fruits were used each. Standard methods of analysis were used (Trajković *et al.*, 1983): total sugars content (Luff-Schoorl method), total acids content (by neutralisation with 0.1 M NaOH, phenolphthalein added). Based on the results obtained, sugar/acid ratio was calculated. In order to isolate the volatile aromatic components from 250 g of plum fruits with stones, the Lickens-Nickerson method of simultaneous distillation/extraction was used. The GC/MS (Gas Chromatography/Mass Spectrometry) analysis was performed using the gas chromatograph Agilent 6890 (Agilent Technologies, Inc., USA), column Agilent 19091S-433 HP-5MS (30 m x 0.25 mm x 0.25μm) connected with the detector Agilent 5973 MSD. The identification of components was performed by comparing the GC/MS analysis results with the spectres of NIST (National Institute of Standards and Technology) and Wiley and Adams libraries. The quantitative (GC/FID) analysis was performed using the gas chromatograph HP 5890 II with FID (Flame Ionization Detector) and column HP-5 MS (30 m x 0.25 mm x 0.25μm), with the internal standard (menthol solution in dichloromethane). Contribution of certain volatile components to the overall fruit aroma was given on the basis of Odour activity values (OAV) representing the quotient of the concentration of certain volatile components in a fruit and its odour threshold in water (Leffingwell and Associates, 2016).

RESULTS AND DISCUSSION

Compared to freshly picked plums, after 7 days of fruit storage at room temperature, in most cases, there are no significant changes in the content of total sugars and total acids, so there are no significant changes in the value of sugar/acid ratio (Table 1). Similar results were found by other authors (Paunović and Grković, 1956; Guerra and Casquero, 2008; Casquero and Guerra, 2009) during the storage of different cultivars of European plum (*Prunus domestica* L.), at about room temperature.

Table 1. Contents of total sugars and acids (%) and sugar-acid ratio in plum fruits immediately after harvesting and after 7 day storage at a temperature of 20±1 °C

Characteristics	Year	Čačanska Rodna		Stanley	
		At harvest	After a 7-day storage	At harvest	After a 7-day storage
Total sugars	2008	8.20	7.95	8.70	8.95
	2009	9.70	9.95	9.70	11.95
Total acids	2008	0.98	0.94	0.78	0.78
	2009	1.20	1.12	0.75	0.69
Sugar/acid ratio	2008	8.37	8.45	11.15	11.47
	2009	8.08	8.88	12.93	17.32

Since the fruits of both tested cultivars are picked at very early stage, at the beginning of ripening on tree, they belong, due to the very low value of sugar/acid ratio (<16), according to Mitrović *et al.*, (2016), to a group of plums with sour taste. Since there is no change in value of ratio of sugar/acid, they remain acidic even after 7-day storage. The only exception were fruits of the cultivar Stanley in 2009, because their somewhat more remarkable change of sugar and acid content led to the value sugar/acid ratio to be greater than 16 (17.32), so these fruits could be classified into a group of fruits with sour-sweet taste (with the range of this ratio from 16 to 20) (Mitrović *et al.*, 2016). According to these authors, fruits of the cultivars Čačanska Rodna and Stanley, picked up for drying at full ripe stage have the value of sugar/acid ratio 18.67 (fruits of sour-sweet taste) and 20.34 (fruits of sweet-sour, harmonized taste, as the ratio sugar/acid is within the limits of 20-25). If we take into account that fruits for drying are picked at full ripe stage, it can be seen that early picked fruits (3-4 weeks before full ripe) cannot, even after 7-day ripening after harvest, in terms of room temperature, get the taste of the fruits at full ripe stage, with fully developed varietal sensory characteristics.

Table 2. Content of volatile compounds (μg/kg) in fruits of the plums picked immediately after harvesting and after 7-day storage at a temperature of 20±1 °C (ND - not detected)

Compounds	Year	Čačanska Rodna		Stanley	
		At harvest	After a 7-day storage	At harvest	After a 7-day storage
2-E-Hexenal	2008 2009	7318.81 6419.99	5408.16 4368.57	5348.65 6665.57	5843.67 2221.71
2-E-Hexenol	2008 2009	ND ND	ND 69.11	ND 179.67	ND 74.05
Hexanol	2008 2009	204.98 195.69	280.38 406.82	319.69 457.56	365.19 521.81
Heptanal	2008 2009	43.30 43.48	67.49 82.84	ND 97.75	63.33 134.28
Octanal	2008 2009	ND 62.10	57.28 45.10	57.57 49.74	84.08 57.03
Nonanal	2008 2009	352.74 356.69	733.96 715.95	277.49 454.19	597.54 1005.97
Benzaldehyde	2008 2009	ND 31.41	ND ND	ND ND	33.37 136.63
Benzeneacetaldehyde	2008 2009	33.58 85.85	ND ND	ND ND	399.05 ND
Linalool	2008 2009	ND 140.50	ND 48.89	ND ND	ND 57.66
α-Terpineol	2008 2009	56.02 61.58	275.41 179.10	49.75 70.61	26.27 277.21
Sum of C6 compounds	2008 2009	7682.90 6755.18	5842.58 4966.71	5795.00 7478.18	6360.91 2867.73
Sum of volatile compounds	2008 2009	8168.54 7536.79	6976.71 6038.58	6179.81 8150.46	7564.54 4536.78

Among 10 identified and quantified volatiles in freshly picked plums and the plums after 7-day storage (Table 2), the most were C6 aldehydes and alcohols, and nonanal, which is in accordance with the results of other authors (Herrmann, 1991; Chai *et al.*, 2012). The contribution of each individual volatile compound to the fruit odour, based on their OAV (Odour activity values) was shown in Table 3. Since they have OAV>1, the fruit aroma is significantly affected by nonanal, 2-E-hexenal, octanal, heptanal, linalool and benzeneacetaldehyde. On the other hand, because of OAV<1, α-terpineol, hexanol, 2-E-hexenol and benzaldehyde, do not have significant influence on the fruit odour. Having in mind the odour quality and changes of OAV in individual components, after 7 day storage of both studied cultivar fruits, at room temperature, a decrease in green note (due to a decrease of

OAV for 2-E-hexenal) and an increase of fruit aroma (due to an increase of OAV for nonanal, heptanal and octanal) could be observed. OAVs for 2-E-hexenal accounted for a total OAV of freshly picked plums from 41.2% (Stanley, 2009) to 53.4% (Č. Rodna 2008), whereas in 7 day stored plums, from 10.3% (Stanley 2009) to 29.1% (Stanley 2008). On the other hand, involvement of OAVs for nonanal in a total OAV of freshly picked plums was from 40.4% (Č. Rodna 2009) to 47.8% (Stanley 2009), and in 7 day stored plums at room temperature, from 50.5% (Stanley 2008) to 78.9% (Stanley 2009).

Table 3. Odour threshold (OT) and Odour activity values (OAVs) of aroma compounds in plum fruits

Compounds	Odour quality	OT (µg/kg)	Year	OAV			
				Čačanska Rodna		Stanley	
				At harvest	After a 7-day storage	At harvest	After a 7-day storage
Nonanal	fruity, woody, canned plum	1	2008	352.7	734.0	277.5	597.5
			2009	356.7	716.0	454.2	1006.0
2-E-Hexenal	green	17	2008	430.5	318.1	314.6	343.7
			2009	377.6	257.0	392.0	130.7
Octanal	citrus-like, fruity	0.7	2008	-	81.8	82.2	120.1
			2009	88.7	64.4	71.1	81.9
Heptanal	citrus-like, fresh	3	2008	14.4	22.5	-	21.1
			2009	14.5	27.6	32.6	44.8
Linalool	flowery, citrus, fruity	6	2008	-	-	-	-
			2009	23.4	8.2	-	9.6
Benzeneacetaldehyde	pungent, green	4	2008	8.4	-	-	99.8
			2009	21.5	-	-	-
α-Terpineol	peach-like, lilac-like	330	2008	0.2	0.8	0.2	0.1
			2009	0.2	0.5	0.2	0.8
Hexanol	green, fresh, fruity	2500	2008	0.1	0.1	0.1	0.1
			2009	0.1	0.2	0.2	0.2
2-E-Hexenol	leafy, green, fruity, fresh	400	2008	-	-	-	-
			2009	-	0.2	0.4	0.2
Benzaldehyde	bitter almond-like	350	2008	-	-	-	0.1
			2009	0.1	-	-	0.4

CONCLUSION

After 7 day storage of the cultivars Čačanska Rodna and Stanley at room temperature there are no significant changes in sugar/acid ratio and, consequently no change in the fruit flavour. On the other hand, due to changes in concentration of certain volatile components (2-E-hexenal, nonanal, octanal and heptanal) odour of fruit loses its green note and receives a fruity character.

ACKNOWLEDGEMENTS

This paper was financially supported by the Ministry of Education, Science and Technology of the Republic of Serbia (Projects TR 20013A i TR 31093).

REFERENCES

Casquero P. A., Guerra M. (2009). Harvest parameters to optimise storage life of European plum „Oullins Gage“. International Journal of Food Science and Technology, 44, 2049-2054.

Chai Q., Wu B., Liu W., Wang L., Yang C., Wang Y., Fang J., Liu Y., Li S. (2012). Volatiles of plums evaluated by HS-SPME with GC–MS at the germplasm level. Food Chemistry, 130, 432-440.

Childers N. F. (1949). Fruit science – Orchard and small fruit management. J. B. Lippincott Company, Chicago, USA.

Crisosto C. H. (1994). Stone fruit maturity indices: a descriptive review. Postharvest News and Information, 5, 65-69.

Guerra M., Casquero P. A. (2008). Effect of harvest date on cold storage and postharvest quality of plum cv. Green Gage. Postharvest Biology and Technology, 47, 325-332.

Guerra M., Sanz M. A., Casquero P. A. (2009): Influence of harvest dates on quality, storage capacity and sensory attributes of European plum cv. Green Gage. Food Science and Technology International, 15 (6), 527-534.

Herrmann K. (1991). Die aromastoffe des obstes. Teil IV: Steinobst. Erwerbsobstbau, 33, 174-180.

Hoehn E., Gasser F., Naepflin B., Ladner J. (2005). Consumer expectations and soluble solids, acidity and firmness of plums (*Prunus domestica* ‘Cacaks Beauty’). Acta Horticulturae, 682, 665-672.

Huyskens-Keil S., Schreiner M. (2004). Quality dinamics and quality assurance of fresh fruits and vegetables in pre- and postharvest. In: Production practices and quality assessment of food crops, Vol. 3, „Quality handling and evaluation“, Dris R. and Jain S. M. (eds.), Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 401-449.

Kader A. A. (1999). Fruit maturity, ripening, and quality relationships. Acta Horticulturae, 485, 203-208.

Kader A. A. (2008). Flavor quality of fruits and vegetables. Journal of the Science of Food and Agriculture, 88, 1863-1868.

Leffingwell and Associates (2016): Odor and flavor detection thresholds in water. http://www.leffingwell.com/odorthre.htm (Access date 22.05.2017)

Mitrović O., Zlatković B., Popović B., Kandić M., Miletić N. (2016). Total sugars and total acids content in plum fruit as affected by drying. Journal of Pomology, 50 (193/194), 47-54, (in Serbian).

Paunović S., Grković Lj. (1956). Investigating the keeping qualities of the Požegača plums in storage. Journal for Scientific Agricultural Research, 9 (24), 81-89, (in Serbian).

Trajković J., Baras J., Mirić M., Šiler S. (1983). Analize životnih namirnica /Analyzes of foodstuffs/. TMF, Beograd.

Usenik V., Kastelec D., Veberič R., Štampar F. (2008). Quality changes during ripening of plums (*Prunus domestica* L.). Food Chemistry, 111, 830-836.

Original scientific paper
10.7251/AGRENG1802056W
UDC 631.1:631.147(438)

ORGANIZATIONAL FORMS OF ORGANIC FARMS IN POLAND

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ABSTRACT

Organic farms form a perspective segment of the Polish agriculture, which have been developing very dynamically since Poland accession to the European Union (from 2004). Changes in the population of these agricultural holdings` group are the result of the growing demand for organic agricultural products, the growing environmental awareness of society, as well as current agricultural policy focused on agrienvironmental practices, for which farmers are remunerated in the form of subsidies. Organic production methods include good soil condition maintenance, plant nutritious needs and animal welfare requirements. Organic farms may be differently organised. There are three basic forms of organic farms organization, namely: farms that are in transition to organic production system (are implementing environmental practices in stages); farms that combine organic and conventional production system; farms organized solely according to the organic principles. Legal requirements strictly specify what features an organic farm should have, in each of the identified organisational forms. The aim of the article is to compare organizational forms of organic farms in Poland in terms of production and economic efficiency. There were analyzed organic farms in comparison to conventional farms included in Farm Accountancy Data Network 2015. The research were conducted on the basis of indicators analysis, considering calculation reflected costs, production, economics and subsidy value connected with the Common Agricultural Policy directed to farms.

Keywords: *organic farms, organizational forms, economic analysis, Farm Accountancy Data Network, Poland.*

INTRODUCTION

Organic production is a system of farm management and food production combines best environmental practices, a high level of biodiversity, preservation of natural resources and application of high animal welfare standards (EC, 2007). This production is based on the use of the substance and natural processes that occur in nature, ensuring its quality. The use of organic method of agricultural production in accordance with the requirements of the soil, plants, animals and consumers` preferences characterizes this farms` group. Therefore, organic farms realize the concept of sustainable development of agriculture (Wrzaszcz and Zegar, 2015). For this reason, organic farms are an important, albeit niche group.

Organic farms are the demanded and the fast-growing form of environmental-friendly agriculture. Since Poland accession to the EU, there is observed dynamic growth in the number of organic farms and agricultural land. During the period of 2004-2014 the number of organic farms in Poland has increased around 7 times, from 3.7 thousand to 24.8 thousand. The surface of organic plants increased from 83.7 thousand ha to 657.9 thousand ha, nearly 8 times. Organic sector development is also reflected in the number of food-processing plants and the range of organic products. The number of organic food-processing plants increased from 50 in 2004 to 484 in 2014, which is almost 10 times (AFQI, 2015; MARD, 2017). Accelerating organic production growth was especially observed after 2005. In Poland since the end of 2004, a wide-ranging support directed to organic producers has been implemented, mainly within rural development programmes. Farmers also have received support through existing advisory structure. Institutional environment certainly has been essential, perhaps the most important, factor determining the development of this agricultural production system in Poland in the past dozen years.

The systematic increase in organic farms` potential in Poland should be regarded as a positive and desirable direction of agriculture development, and this is due to the many environmental, economic and social benefits, that they provide (Runowski, 2012), as well as correspond with the future model of agriculture, based on renewable resources and environmental-friendly practices (Zegar, 2012).

The aim of the article is to compare organizational forms of organic farms in Poland in terms of production and economic efficiency.

MATERIALS AND METHODS

The analysis considered about 12 thousand individual farms covered by the Farm Accountancy Data Network in Poland in 2015[1]. There were analysed conventional (C), organic (ORG; organized according to organic system requirements; with legal certificate), and mixed farms, with parallel production, that combine two production system, namely: organic (certificated) and conventional (ORG&C) farms[2]. There were presented organizational and environmental issue, such as farm specialization and soil management[3]. The article focuses on production and economic results, including their effectiveness[4].

RESULTS AND DISCUSSION

The FADN farm population was dominated by conventional farms (96.7%, Table 1). Farms managed only according to the organic production principles accounted for only 2.5% of the analysed population (those farms held organic production

[1] See: http://fadn.pl/

[2] Due to the insufficient number of organic farms in the reorganization process (only 12 farms), their results could not be published. According to the FADN methodology, analysed group must cover at least 15 farms.

[3] See method details of soil organic matter calculation in (Wrzaszcz, 2017).

[4] See method details of farms` effectiveness method in (Wrzaszcz and Zegar, 2015).

certificates). Some farms combined both production systems, i.e. conventional and organic, but they were the least numerous group (0.7%). In accordance with the law, combining both systems of production in the farm is possible, as far as environmental practices are observed in a specific part of the farm. Thanks to the separation of both production systems, farms may diversify their agricultural activity and thus obtain various sources of income.

The average farm keeping agricultural accounting had an area of 36 ha of agricultural land and generated income at the level of EUR 18 thousand. The value of crop and livestock production generated in those farms was comparable. The similar production potential, and the results obtained were characteristic of conventional farms. They will constitute a point of reference in the assessment of the situation of organic and mixed farms – combining both production systems.

Organic farms significantly differed *in minus* from conventional farms. Their production potential was lower (both the area and quality of land used, as well as the animal population). The stocking density in those farms was by almost 1/3 lower than that in conventional farms. This result indicated the relatively smaller scale of livestock production in organic farms. The value of livestock production slightly exceeded that generated from crop activities, although, of significant importance here was the structure of crop production. In organic farms, as much as 30% of the utilised agricultural area were permanent grassland, in conventional farms, it was 13%. Such land use resulted directly from the lower quality of soils in organic farms, and then livestock production adapted to these. The structure of the animal population was dominated by cattle (61%, in conventional farms it was 51%), while the pig population was only 12% (respectively, in conventional farms it was 44%). Interesting is the fact that organic farms developed the activity in the field of poultry production (here, poultry accounted for as many as 19% of the animal population, while in conventional farms this percentage was 5%). Organic farms also held more orchards (5% of the utilised agricultural area, while in conventional farms it was slightly more than 1%). The labour intensity of organic production was slightly higher, which was determined by both the legal requirements enabling only the incidental use of chemical plant protection products, as well as the more frequent specialisation in rearing grazing animals and fruit-growing production (Chart 1).

Table 1. Farms` characteristic and outcomes (average/farm)[1]

No.	Specification	C	ORG	ORG&C	ORG/C %	ORG&C/C %
1	Farms` number	11 701	304	88	2.5	0.7
2	Agricultural land, AL (ha)	35.84	27.44	40.91	77	114
3	Soil quality	0.83	0.60	0.65	72	79
4	Labour (AWU)	1.96	1.87	2.01	95	102
5	Livestock (LU)	27.85	15.09	15.05	54	54
6	Livestock/AL (LU/ha)	0.78	0.55	0.37	71	47
7	Assets (mln €)	0.34	0.21	0.27	61	77
8	Standard output (thous. €)	47.6	27.3	41.5	57	97
9	Standard gross margin (thous. €)	28.0	12.3	25.0	44	89
10	Total output (thous. €)	59.5	23.9	36.5	40	61
11	- Crop production (%)	51.1	44.5	66.4	#	#
12	- Livestock production (%)	48.0	53.7	32.4	#	#
13	- Other production (%)	0.9	2.9	1.3	#	#
14	Gross farm income (thous. €)	30.2	21.9	29.6	73	98
15	Family farm income (thous. €)	18.2	14.6	18.1	80	99
16	Gross investment (thous. €)	10.5	3.6	5.1	35	49
17	Net investment (thous. €)	1.2	-1.6	-2.9	#	#

[1] *Soil quality – site index for soil classification; 1 Annual Work Unit is equivalent to full-time own and paid labour, i.e. 2,120 hours of work/year; 1 Family Work Unit is the equivalent of a full-time labour of a farming family member; 1 Livestock Unit is a conventional unit of farm animals with a mass of 500 kg; Standard output – the sum of standard value of all agricultural activities on the farm; Standard gross margins – the difference between output and specific (direct) costs of all activities occurring on the farm; Total output is the sum of crop and livestock production value and other activities; Gross Farm Income is the result of difference of total output and total intermediate consumption, adjusted for the outcome of the balance of current subsidies and taxes; Family Farm Income – net value added adjusted for the cost of total external factors and the balance of subsidies and taxes on investments (Bocian and Malanowska, 2014; Goraj, 2009; www.fadn.pl). The used exchange rate, 1EUR = 4PLN was used. Source: Prepared on the basis of FADN 2015 data.*

Organic farms were also characterised by different organisation of crop production when compared to that in conventional farms. In case of organic farms, soil degrading plants had a lower share, for the benefit of soil structure forming plants. For example, cereals, industrial crops and root crops accounted for, respectively, 41%, 1% and 1.5%, while in case of conventional farms – 59%, 13% and 5%. They were characterised by a significant share of legumes (16%) and papilionaceous plants and field grasses (39%), which in conventional farms accounted for a symbolic part of sowings (respectively, 6% and 5%). This structure of sowing was certainly determined by limitations associated with the compliance with the prohibitions of applying mineral fertilisation and plant protection products, as well as the frequent concentration on cattle rearing, which resulted in internal balancing of fodder and fertiliser needs of organic farms. Thanks to the reproduction of soil organic matter as a result of growing soil structure forming plants and using natural fertilisers, the adequate restoration of soil fertility was provided in those farms (Table 2).The decisive disparity of organic farms became visible in case of production and economic results as well as investment activity

(Table 1). The greatest differences *in minus* were visible in the production value. In contrast, received support in a form of subsidies to the operational activity reduced by nearly half their production distance in relation to conventional farms. Finally, income of organic farms was lower by about 1/5 than the result of conventional farms. Those values translated into assets of organic farms and a possibility to reproduce them. Certainly, the investment needs of organic and conventional farms are different due to the different specificity of agricultural production. However, in the longer term, the lack of the sufficient investment activity will be gaining importance due to the further consumption of assets and a need to introduce adequate organisational changes in the farm, which will translate into the account of the agricultural producer.

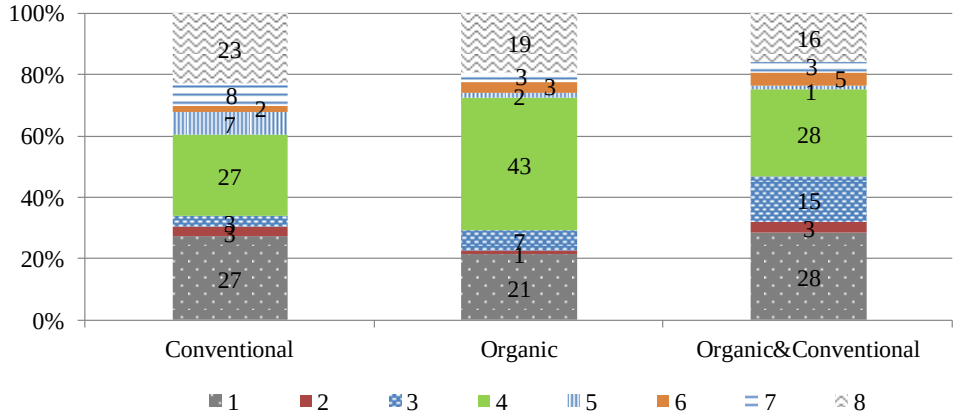

Chart 1. Farming type structure

Types: specialised in field crops (type I), horticulture (II), permanent crops (III), rearing grazing livestock (IV) and granivores (V), nonspecialised with mixed crops (VI), mixed livestock (VII) and mixed crops and livestock (VIII). Source: Prepared on the basis of FADN 2015 data.

Table 2. Elements of soil organic matter balance (SOMB in t/ha)

No.	Specification	C	ORG	ORG&C
1	Crop degradation	-0.60	-0.26	-0.34
2	Crop reproduction	0.12	0.74	0.69
3	Crop balance [1+2]	-0.48	0.48	0.34
4	Natural fertilizers	0.68	0.48	0.32
5	Organic fertilizers	0.44	0.09	0.21
6	**SOMB [3+4+5]**	**0.64**	**1.05**	**0.88**

**Source: Prepared on the basis of FADN 2015 data.*

Mixed farms, some part of which are managed according to environmental principles and some according to the conventional system, were characterised by the definitely bigger utilised agricultural area and the lower stocking density when

compared to conventional farms (by about 15% and 50%). Those farms were characterised by a high share of orchards (6.5%). As shown in Chart 1, they were relatively often specialised in fruit-growing production. The structure of field crops in mixed farms should be considered to be more beneficial in terms of the reproduction of soil organic matter when compared to conventional farms, although mixed farms were inferior to organic farms in this regard (Table 2). They had a 47% share of cereals, as well as a 36% share of legumes and field grasses (in conventional farms, it was, respectively 59 and 4.5%). In case of livestock production, it was dominated by cattle (63%) and pigs (28%).The production result of mixed farms was lower than that achieved by conventional farms (lower by about 40%), although it was more beneficial when compared to certified organic farms. The production value in mixed farms was, to a greater extent, determined by crop production. Income categories (both gross value added and income) corresponded to the values achieved by conventional farms. The differences between the analysed groups of farms with regard to the production value were reduced through support in a form of subsidies as well as cost advantages.

The efficiency of management is determined by the productivity and profitability of the production factors. The components of this account are illustrated in Table 3. The average land productivity in analysed farms amounted to EUR 1.6 thousand. In case of organic and mixed farms, this result was lower by nearly half. Those differences indicated a limited ability to produce the volume of organic production from a unit of the agricultural area, and then, the supply of organic products. Organic farms are bound by significant restrictions on the use of mineral fertilisers and plant protection products, which significantly determines the level of their productivity. In these farms, average costs spent for this purpose accounted only for a few percent of expenses incurred by conventional farms. Mixed farms, due to combining the organic and conventional management system, incurred higher costs of production means when compared to organic farms but still those costs were definitely lower than in conventional farms (lower by about 60%).

Subsidies received by organic farms definitely exceeded the value of transfers addressed to conventional farms, in particular those obtained with respect to the implementation of the agri-environmental programme. Support related to this programme substantially reduced the economic distance between organic and mixed farms and conventional farms. The stream of subsidies to the operational activity in case of organic farms corresponded to the half of the value of total output, which indicates their important role in shaping the economic situation. In case of mixed farms, this indicator amounted to more than 40%. For comparison, in conventional farms this value was only 16%. Subsidies to the organic system largely applied to the fruit-growing activities. In agricultural holdings taking up organic production (certified and mixed), also the depreciation value, derived from assets and investment activity, remained below the value corresponding to conventional farms. In addition, lower costs of external factors were in favour of organic farms. It should also be pointed out that differences in the level of costs associated with the operation of organic, mixed and conventional farms were not

nearly so high as in case of the production value. Finally, both lower costs of the operation of organic farms, along with significant support in a form of subsidies, equalised income of organic and conventional farms per area unit. The economic situation of mixed farms was less beneficial. In case of the productivity and profitability of labour inputs, organic farms were left behind conventional farms, while mixed farms developed a significant advantage. According to the FADN methodology, subsidies are included in the farm`s income account. However, excluding this element, relationships of the highlighted groups of farms in terms of the profitability of the production factors would be different – definitely, to the detriment of organic farms. With this calculation, the land profitability in organic and mixed farms, when compared to the result of conventional farms, would be lower by 58% and 73%, while in case of the labour profitability the result would be lower by 64% and 66%, respectively. In case of certified organic farms, income was mainly determined by external support in a form of subsidies, where more than 80% of income came from this source of financing. Mixed farms were dependent on those funds even more (85%). For comparison, in the average farm, about half of income came from received subsidies. These indicators show the importance of government programmes supporting this form of agricultural activity in the operation of environment-friendly farms.

Table 3. The main economic calculation categories

No.	Specification	C	ORG	ORG&C	ORG/C %	ORG&C/C %
1	**Total output (€/ha AL)**	**1 661**	**871**	**892**	**52**	**54**
2	*Total output (thous. €/AWU)*	*30.4*	*12.8*	*18.2*	42	60
3	Total intermediate consumption (€/ha AL)	1 053	481	520	46	49
4	- total specific costs (€/ha AL)	3 118	1 070	1 335	34	43
5	-- fertilisers (€/ha AL)	190	15	72	8	38
6	-- crop protection (€/ha AL)	73	2	26	2	36
7	Balance on current subsidies & taxes (€/ha AL)	234	409	351	175	150
8	- agri-environmental subsidies (€/ha AL)	14	148	103	1 086	753
9	-- organic subsidies (€/ha AL)	1	126	92	25 200	18 419
10	**Gross farm income (€/ha AL)**	**843**	**799**	**723**	**95**	**86**
11	*Gross Farm Income (thous. €/AWU)*	*15.4*	*11.7*	*14.7*	76	96
12	Depreciation (€/ha AL)	259	190	196	73	76
13	**Farm net value added (€/ha AL)**	**584**	**610**	**527**	**104**	**90**
14	*Farm net value added (thous. €/AWU)*	*10.7*	*9.0*	*10.7*	84	101
15	Total external factors	95	79	98	83	103
16	Balance on investment subsidies & taxes (€/ha AL)	-24	-12	-17	#	#
17	**Family farm income (€/ha AL)**	509	533	443	105	87
18	*Family farm income (thous. €/FWU)*	10.7	9.4	11.9	88	112
19	**Current subsiedies/Total output (%)**	**16**	**49**	**42**	#	#
20	**Current subsiedies/ Farm income (%)**	**52**	**81**	**85**	#	#

**Source: Prepared on the basis of 2015 FADN data.*

CONCLUSIONS

Organic farms have various organisational form. They include certified organic farms (fully adapted to the requirements of the system, implementing the practices throughout the farm), farms shifting to organic production (partly adapted, in the reorganisation process), as well as mixed, combining both organic and conventional production systems, whereby agricultural activities carried out under these systems are separated.

So far, organic farms are a niche fraction of farms in Poland, although they are of particular importance in production due to the growing demand for organic products and environmental services, as they fit in the sustainable model of agriculture.

Organic and mixed farms differ from conventional farm in terms of the production potential, production organisation, production and economic results and investment activity. In case of organic and mixed farms, the production potential is smaller than expected, production organisation is more environment-friendly, and the outcomes are definitely lower.

Organic and mixed farms are predominantly specialised entities with the production specificity differing from that in conventional farms. The former are more often specialised in cattle rearing and fruit growing which is dictated by, *inter alia*, production conditions (lower quality of soils, high share of permanent grasslands).

The productivity of the production factors (land and labour) in organic and mixed farms differs significantly *in minus* from that in conventional farms.

The profitability of the production factors (land and work) is comparable among the analysed groups of farms, which results from reducing the differences in the productivity thanks to lower costs of the operation of farms with organic production, and generally higher transfers in a form of subsidies to the operational activity. In the situation, where subsidies are not included in the income account, an important economic distance characterises farms with the organic production comparing them with conventional farms.

The efficiency of organic and mixed farms to a much greater extent is dependent on government support (subsidies) when compared to conventional farms. These farms could not operate without these subsidies.

REFERENCES

Agricultural and Food Quality Inspection (2015). Condition of organic farming in Poland. The report 2013–2014, IJHARS, Warsaw.

Bocian M., Malanowska B. (2014). Wyniki Standardowe 2012 uzyskane przez indywidualne gospodarstwa rolne uczestniczące w Polskim FADN. Część I. Wyniki Standardowe, IERiGŻ-PIB, Warszawa [Standard results 2012 of individual farms in Polish FADN. Part I. Standard results, IAFE-NRI, Warsaw].

EC, Council Regulation No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91,Official Journal of the European Union, L. 189/1, 20.7.2007.

Farm Accountancy Data Network (2017). www.fadn.pl, access: 10.06.2017.

Goraj L. (2009). Rachunkowość i analiza ekonomiczna w indywidualnym gospodarstwie rolnym, Difin, Warszawa [Accountancy and economic analysis of individual farms, Difin, Warsaw].

Ministry of Agriculture and Rural Development (2017)., www.minrol.gov.pl/Jakosc-zywnosci/Rolnictwo-ekologiczne/Rolnictwo-ekologiczne-w-Polsce, access: 10.05.2017.

Runowski H. (2012). *Rolnictwo ekologiczne w Polsce*, [w:] *Z badań nad rolnictwem społecznie zrównoważonym,* ed. J. St. Zegar, No. 15, IAFE-NRI, Warsaw [Organic farming in Poland, in: research on Sustainable agriculture].

Wrzaszcz W., Zegar J. St. (2015). Economic challenges of organic farms in Poland, Book of proceedings, Sixth International Scientific Agricultural Symposium, "Agrosym 2015", Jahorina, October 15 - 18, 2015, 1132-1139.

Zegar J. St. (2012). Description of organic holdings in Poland, in: Sustainable agriculture – selected papers, IAFE-NRI, Warsaw, 139-155.

Original scientific paper
10.7251/AGRENG1802065M
UDC 633.11:757.2

VARIABILITY, AMMI AND CLUSTER ANALYSIS FOR QUALITY COMPONENTS OF DIFFERENT WHEAT GENOTYPES

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ABSTRACT

The present study was carried out to investigate the variability and heritability of wheat quality components and to evaluate the stability of ten wheat genotypes in different environmental conditions. The experiment was conducted in the Center for Small Grains in Kragujevac, Serbia during two growing seasons (2010 and 2011). Thousand grain weight had the highest value of GCV and PCV (7.13 and 7.7%), while test weight had the lowest PCV (3.24%) and protein content had the lowest GCV (1.82%). The highest heritability was observed for thousand grain weight (H^2=85.37%), while the lowest one was found for protein content (H^2=19.56%). The AMMI analysis showed significant effect of the G×E interaction, where first main component was significant for all components. Genotypes KG-56, Arsenal and Osječanka are close to the average values for all components and expressed the highest stability. Genotypes with the highest or lowest average values for analyzed traits, such as Norin 10, Mironovskaya 808, Gruža and Spartanka, showed moderate to high instability. Cluster analysis categorized the genotypes into four groups. The genotype Norin 10 showed the highest distance from other genotypes, whereas the stable genotypes grouped together.

Key words: *Heritability, AMMI analysis, stability, cluster.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most commonly planted and used crop in the world. Therefore, development of high yielding varieties with good end-use quality is a challenging objective for many wheat breeding programs (Abdipour *et al.*, 2016). Wheat grain yield and protein content are the dominant determinants in the economic value of the harvest product (Shewry, 2007). Test weight, thousand grain weight and protein content are the important traits for wheat breeding programs (Aydin *et al.*, 2010). Grain protein content and thousand grain weight are the most important indirect quality traits and can be used for the purpose of quality

predictions at the early generations of breeding programs (Mut *et al.*, 2010). Heritability is a parameter which is widely used in establishment of breeding programs and formation of selection indexes (Falconer, 1985). Heritability is low for the traits with agronomical importance because these traits are influenced by a large number of genes. Many authors observed low heritability for protein content (Zečević *et al.*, 2007 and Taghouti *et al.*, 2010) and moderate to high heritability for thousand grain weight and test weight (Aydin *et al.*, 2010). The G×E interaction complicates breeding of superior genotypes (Hintsa and Fetien, 2013). Therefore, assessing any genotype without including its interaction with environment is incomplete (Crossa, 1990). The additive main effect and multiplicative interaction (AMMI) analysis, using principal component axis (PCA), can be used to determine stability of genotypes across environments and, therefore, it has been efficient tool in determining stable and high yielding genotypes (Zobel and Gauch, 1988).

The goal of this study was to determine variability and heritability of wheat quality components and to evaluate the stability of different wheat genotypes in various environments.

MATERIAL AND METHODS

Ten wheat genotypes (Arsenal, KG-56, Gruza, Mironovskaya 808, Norin 10, Rana Niska, Spartanka, Sterna, Osjecanka, and Szegedi 765) were tested in two different vegetation seasons (2010 and 2011) at the Center for Small Grains in Kragujevac, Serbia. Growing season 2010 was determined by higher total sum of precipitation in April, May and June (293.2 mm) in relation to 2011 growing season (119.1 mm) in region of Kragujevac (Republic Hydro-meteorological Service of Serbia). The trial was conducted in randomized complete block design with three replications. It is performed rare sowing where seeds were sown in 1 m long rows, with 20 cm space between the rows and 10 cm distance between each seed in a row. Rare sowing was applied to allow that genotypes express their maximal genetic potential for tillering and other spike characteristics. Three quality components are analyzed: thousand grain weight, test weight and protein content. Analysis of variance and cluster analysis based on squared Euclidean distance and Linkages between groups method were calculated using the IBM SPSS Statistics Trial Version 22.0 (https://www.ibm.com/). The generated components of the variance were used to estimate the genotypic and phenotypic variance and broad-sense heritability. Genotype × environment (G×E) interaction was calculated through AMMI and biplot analysis using GenStat Trial Version **18.1.0.17005 (**https://www.vsni.co.uk/**).**

RESULTS AND DISCUSSION

For all analyzed traits, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV), which indicates that environment has significant effect on present variation. This is in agreement to results obtained by Tyagi *et al.* (2016). Thousand grain weight had the highest values of GCV and PCV (7.13 and 7.70%, respectively), while the test weight had the lowest PCV (3.24%) and grain protein content had the lowest PCV (1.82%). Similar results for GCV and PCV of thousand grain weight were reported by

Başçıftçi *et al.* (2013). Heritability estimates in broad sense were high for thousand grain weight (85.37%), while moderate for test weight (61.67%), which indicated a high response to selection in these traits. The low heritability (19.56%) was observed for grain protein content, which indicated low inheritance of this trait and high effect of environment. This is in accordance with results obtained by Zecevic *et al.* (2007) (Table 1).

Table 1. The mean value, variance and heritability of analyzed yield components

Traits[1]	Mean values	Estimates of variance components[2]				CV (%)	GCV (%)	PCV (%)	H^2 (%)
		σ^2_g	σ^2_{ph}	$\sigma^2_{g\times y}$	σ^2_E				
PC	16.47	0.09	0.46	1.01	0.36	5.03	1.82	4.11	19.56
TGW	37.02	6.97	8.13	2.68	1.16	6.39	7.13	7.70	85.37
TW	76.80	3.83	6.21	6.93	2.37	2.27	2.54	3.24	61.67

[1]PC – protein content, TGW – thousand grain weight, TW – test weight; [2]σ2g, σ^2_{ph}, σ^2_{g*y} and σ^2_E indicate the genetic, phenotypic, genotype and phenotype interaction and environment variance, respectively

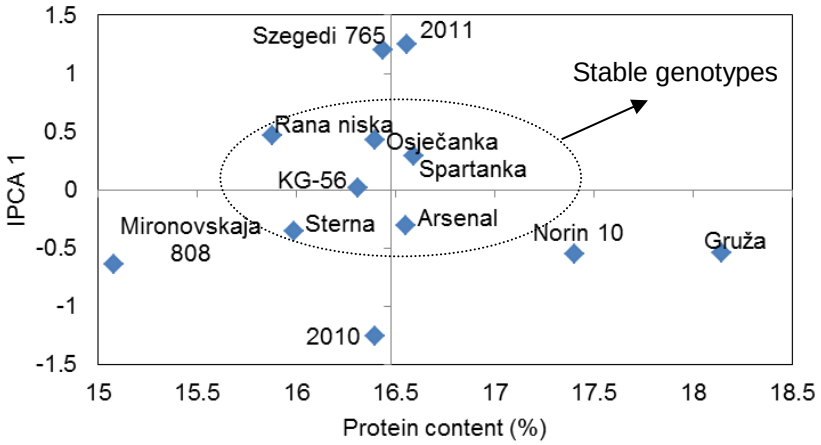
AMMI analysis shows highly significant influence of genotype and G×E interaction for all analyzed traits. The significance of G×E interaction effects demonstrated that genotypes responded differently to various environmental conditions. Main effects had the highest share in total variation for all traits, where the largest share belonged to genotype effect. In relation to protein content, Taghouti *et al.* (2010) observed larger variances associated with genetic effects than the variances associated with G×E interaction. Higher genotype effects than environment effect is found by Altinabas *et al.* (2004) for thousand grain weight. The total contribution in G×E interaction gave the first principal component (IPCA1), which confirmed that the additive effects were more important than multivariate effects in all of analyzed traits (Table 2). Mladenov *et al.* (2012) also reported that protein content, thousand grain weight and test weight are important to IPCA1.

Table 2. AMMI ANOVA for protein content, thousand grain weight and test weight of ten wheat genotypes in two growing seasons

Source of variation	df	Protein content			Thousand grain weight			Test weight		
		SS	MS	F-value	SS	MS	F	SS	MS	F-value
Total	59	77.09	-	-	966.8	-	-	720.7	-	-
Genotypes	9	37.29	4.14	17.05**	658.9	73.2	30.3**	503.0	55.9	99.1**
Environments	1	0.40	0.40	1.65	117.1	117.1	48.4**	2.8	2.77	4.92
Interactions	9	29.69	3.29	13.57**	94.2	10.4	4.33**	192.4	21.4	37.9**
IPCA1	9	29.69	3.29	13.57**	94.2	10.4	4.33**	192.4	21.4	37.9**
IPCA2	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Error	40	9.72	0.24	-	96.7	2.42	-	22.6	0.56	-

** $p<0.01$; * $p<0.05$

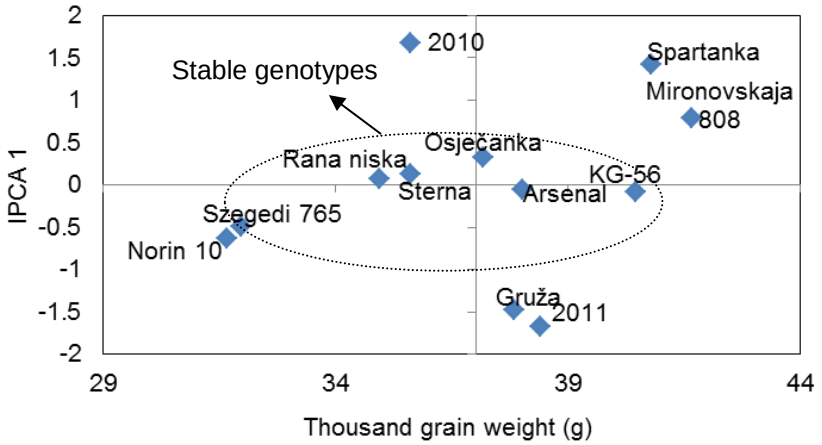
AMMI 1 biplot (Graph 1) shows that genotypes KG-56, Spartanka, Osječanka, Arsenal, Rana Niska and Sterna are found near to origin and considered to be stable.



Graph 1. AMMI 1 biplot for the protein content of 10 wheat genotypes in two growing seasons

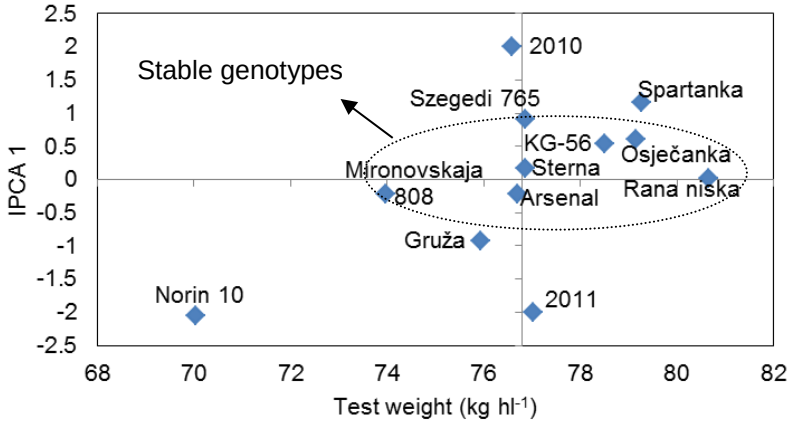
Genotypes Norin 10 and Gruža, with protein content higher than grand mean (17.4 and 18.14%, respectively), were middle stable genotypes. This indicates that it is harder to achieve stability in genotypes with high value of certain trait. Genotype Mironovskaya 808 with the lowest protein content (15.08%) had low adaptability, also. Genotype Szegedi 765 achieved the strongest interaction with the agroecological conditions of the second growing season, which is distinguished by lower amount of precipitation (Graph 1). Mut *et al.* (2010) also observed that grain protein content was higher in low rainfall environments.

Genotypes Rana niska, Sterna, Arsenal, KG-56, Osječanka and Szegedi 765 lie near the origin of biplot and hence shows high stability in thousand grain weight. Genotypes Mironovskaya 808 and Spartanka, as non stable genotypes, had the highest value of thousand grain weight (41.66 g and 40.81 g, respectively). The lowest value of this trait is recorded in genotype Norin 10 (31.66 g), which is characterized by middle stability. Genotype Gruža showed high instability and strong interaction with environmental condition of second year which was characterized by optimal amount of precipitation. Analyzed genotypes achieved higher thousand grain weight in 2011 year than in 2010 year which was distinguished by higher amount of precipitation that led to the appearance of wheat diseases (Graph 2).



Graph 2. AMMI 1 biplot for the thousand grain weight of 10 wheat genotypes in two growing seasons

Genotypes Mironovskaya 808, Arsenal, Sterna, KG-56, Osječanka and Rana niska had high stability and the lowest values of IPCA1 for test weight (Graph 3).

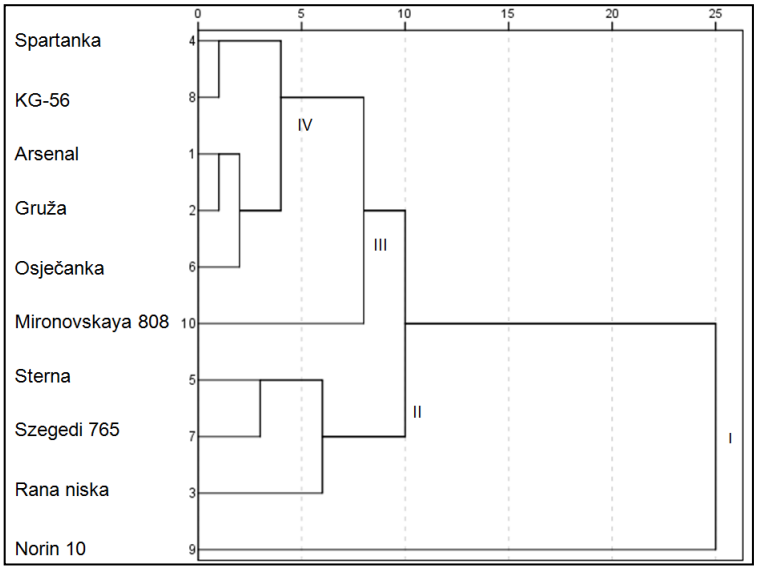


Graph 3. AMMI 1 biplot for the test weight of 10 wheat genotypes in two growing seasons

Genotype Rana niska, with the highest value of test weight (80.67 kg hl^{-1}) showed the highest stability. The lowest test weight was observed in genotype Norin 10 (70.04 kg hl^{-1}), which had the strongest G×E interaction. Genotypes Szegedi 765 and Gruža were characterized by middle stability and test weight around the grand mean, while genotype Spartanka had middle stability and test weight above the grand mean. In both growing seasons genotypes achieved test weight near the grand mean (Graph 3).

Cluster analysis based on squared Euclidean distance divided analyzed genotypes into four major groups. Cluster 1 including only genotype Norin 10 which showed the highest distance from others genotypes, and it is observed in AMMI 1 biplot,

also. This genotype had the lowest values of test weight and thousand grain weight and it is characterized by middle to low stability. Cluster II including genotypes Sterna, Szegedi and Rana niska, which showed the high stability in thousand grain weight. Also, Mutwaly *et al.* (2016) reported that stable and well-associated genotypes with all end-use quality attributes grouped together in same cluster. Genotype Mironovskaya 808 is allocated in Cluster III and it is distinguished by highest value of thousand grain weight and lowest value of protein content. Genotypes which are grouped in Cluster 4, Spartanka, KG-56, Arsenal, Gruža and Osječanka, had the highest average stability in all analyzed genotypes (Graph 4). Grouping of genotypes by the cluster analysis highly corresponds with grouping genotypes in the AMMI 1 biplot.



Graph 4. Dendrogram of cluster analysis for ten wheat genotypes in two years for analyzed quality components

CONCLUSION

In relation to our results, it can be concluded that environment has significant effect on present variation in all quality traits. The significance of G×E interaction effect indicated that genotypes responded differently to various environment conditions. Thus, testing genotypes under different environment conditions, and using analysis such as AMMI, is important for evaluating stability of genotypes. Genotypes Norin 10, Mironovskaya 808, Gruža and Spartanka, showed moderate to high instability, where genotype Norin 10 had the lowest average values of analyzed traits. Genotypes Arsenal, Osječanka and KG-56 have the highest stability and average values of analyzed traits near or above grand mean. Therefore, these genotypes can be used as a good breeding material for future breeding programs in terms of quality components.

ACKNOWLEDGEMENTS

This investigation was supported by Ministry of Education, Science and Technology Development of Republic of Serbia, Project TR 31092

REFERENCES

Abdipour, M., Ebrahimi, M., Izadi-Darbandi, A., Mastrangelo, A.M., Najafian, G., Arshad, Y., Mirniyam, G. (2016). Association between grain size and shape and quality traits, and path analysis of thousand grain weight in Iranian bread wheat landraces from different geographic regions, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(1), 228-236.

Altinbas, M., Tosun, M., Yuce, S., Konak, C., Kose, E., Can, R.A. (2004). Effects of genotypes and location on the grain yield and some quality traits in bread wheat (Triticum aestivum L.), Journal of Agricultural Faculty Edge University, 41, 65-74.

Aydin, N., Mut, Z., Ozcan, H. (2010). Estimation of broad-sense heritability for grain yield and some agronomic and quality traits of bread wheat (Triticum aestivum L.), Journal of Food, Agriculture and Environment, 8(2), 419-421.

Başçıftçi, Z.B., Kinaci, G., Kinaci, E. (2013). Genetic variability and heritability association with spike yield and related characters in durum wheat (Triticum durum (L.) Desf.), Journal of Food, Agriculture and Environment, 11(3,4), 781-784.

Crossa, J. (1990). Statistical analysis of multilocation trials. Advances in Agronomy, 44, 55-85.

Falconer, D.S. (1985). Introductions to Quantitative Genetics. Longman, London.

Hintsa, G., Fetien, A. (2013). AMMI and GGE biplot analysis of bread wheat genotypes in the Northern part of Ethiopia, Journal of Plant Breeding and Genetics, 1, 12-18.

Mladenov, V., Banjac, B., Krishna, A., Milošević, M. (2012). Relation of grain protein content and some agronomic traits in European cultivars of winter wheat, Cereal Research Communications, 40(4), 532-541.

Mut, Z., Aydin, N., Bayramoglu, O., Ozcan, H. (2010). Stability of some quality traits in bread wheat (Triticum aestivum) genotypes, Journal of Environmental Biology, 31, 489-495.

Mutwali, N.I.A., Mustafa, A.I., Gorafi, Y.S.A., Mohamed Ahmed, I.A. (2016). Effect of environment and genotypes on the physicochemical quality of the grains of newly developed wheat inbred lines, Food Science and Nutrition, 4(4), 508-520.

Shewry, P.R. (2007). Improving the protein content and composition of cereal grain. Journal of Cereal Science, 46, 239-250.

Taghouti, M., Gaboun, F., Nsarellah, N., Rhrib, R., El.Haila, M., Kamar, M., Abbad-Andaloussi, F., Udupa, S.M. (2010). Genotype x Environment interaction for quality traits in durum wheat cultivars adapted to different environments, African Journal of Biotechnology, 9(21), 3054-3062.

Tyagi, B.S., Sing, M.K., Singh, G., Kumar, R., Verma, A., Sharma, I. (2016). Genetic Variability and AMMI Bi-Plot analysis in bread wheat based on multilocation trials conducted under drought conditions across agro-climatic cones of India, Triticae Genomics and Genetics, 7(1), 1-13.

Zecevic, V., Knezevic, D., Micanovic, D. (2007). Variability of technological components in winter wheat, Genetika, 39(3), 365-374.

Zobel, R.W., Wright, M.J., Gauch, H.G. (1988). Statistical analysis of a yield trial, Agronomy Journal, 80, 388–39.

https://www.ibm.com/ Accessed on the 15th of February, 2017.

https://www.vsni.co.uk/ Accessed on the 20th of February, 2017.

Original scientific paper
10.7251/AGRENG1802073K
UDC 581.162.3:633.15

EFFECTS OF POLLEN CONTAMINATION AND KERNEL WEIGHT ON KERNEL STRUCTURE OF MAIZE IN OPEN AND SELF POLLINATION TREATMENTS

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ABSTRACT

In small plot experiments conducted in maize, the mostly used pollination methods are open and self-pollination treatments. Comparative studies using these treatments are abundant in scientific literature; however studies on the effect of cross pollination and kernel weight on kernel biochemical properties in different treatments are limited. In this study, we conducted a comparative experiment to investigate the effect of pollen contamination and mean kernel weight on kernel biochemical composition of ten different maize genotypes by using two different pollination methods.Open and self-pollination treatments were applied as pollination methods. Eight different traits; kernel weight, cross pollination rate, protein, oil, carbohydrate, oleic acid linoleic acid and carotenoid content were measured. Regression analyses were performed to understand the effects of cross pollination and mean kernel weight on biochemical constituents of maize kernel in different treatments. Results showed that the major biochemical traits, such as protein, oil and carbohydrate content were significantly affected by pollen contamination but minor traits were not. When data were combined (n=60) the effect of pollen contamination in different treatments was not clearly understood. When data (n=30) of each treatment were separately analyzed, it was found that cross pollination rate had significant effect on the most of biochemical constituents in open pollination. Overall, results suggested that pollen contamination had an effect on major biochemical traits in maize and hand pollination could be used for preventing of unwanted effect of pollen contamination in small plot experiments. However, it should be considered that the effects of hand pollination on kernel weight affect the some biochemical traits in maize.

Keywords: *Xenia, quality traits, Zea mays, pollen contamination.*

INTRODUCTION

Maize (*Zea mays* L.) is used in both human and animal nutrition and it has important place in the World's cereal production. Normal maize contains 8-11% protein, 3-18% oil, and 72-73% carbohydrates (Khan et al., 2104). These are major components that directly affect grain quality in maize kernel. In addition to major components, minor constituents such as amino acids, secondary metabolites and fatty acids are available and they are present in relatively small proportions within the kernel but which have significant grain quality effects,.

In recent years, the development of kernel quality has become an important breeding goal in maize. Maize breeding experiments are carried out in breeding nurseries where a large number of materials are grown together in the same area. Due to maize is a cross pollination species, different methods of pollination are used to prevent pollen transmission among different breeding materials in this species (Kahriman, 2016). In the scientific literature, the mostly used pollination treatments are self- pollination and open pollination techniques. The effect of the pollination methods on major kernel quality components has been the subject of different studies. It was reported that the biochemical structure of the kernel has been changed by pollen transmission (Letchworth and Lambert, 1998; Kahriman *et al*., 2015a). Another factor affecting kernel structure is the size and weight of the kernel. The single kernel weight is also an important variable affecting final yield (Prado *et al.*, 2014). In different studies, the relationship between major kernel quality traits and grain weight characteristics has been discussed (Aliu *et al*., 2012; Chuckwu *et al.*, 2013; Scrob *et al.*, 2014). It has been shown that open and self-pollination have an effect on kernel structure (Sulewska *et al.*, 2014) and on calculated parameters in breeding studies (Kahriman *et al.*, 2015b). However, no comparative study subjected to the relationship between kernel composition and pollen transmission and mean kernel weight had been conducted in open or self-pollination treatments. A detailed study in this context was thought to give beneficial results in the understanding of this relationship. From this point, this study was conducted to investigate the relationships between kernel quality features and cross pollination rate and mean kernel weight in open and self-pollinated samples of the half-diallel set consisting of 4 parents and 6 hybrids.

MATERIAL AND METHODS

In this study, six hybrids (Q2xIHO, Q2xIHP, Q2xPR, IHOxIHP, IHOxPR, IHPxPR) and their 4 parents (IHO, IHP, Q2 and PR) were used as plant material. IHO has high oil content, IHP has high protein content, PR high anthocyanin content and Q2 opaque genotypes.

The field experiment was carried out at the Dardanos Research and Application Center of Çanakkale Onsekiz Mart University, Faculty of Agriculture, during the summer growing season of 2016. The experiment was conducted according to the split plot design and genotypes were located in main plots whereas pollination methods were in sub-plots. Drip irrigation was applied according to the state of the plants. Fertilization was applied by taking into consideration the results of soil

analysis with account of 18 kg / da pure N. Self-pollination treatment was performed the method proposed by Kahriman *et al* (2015a).

In the harvest, six ear samples were taken open and self-pollinated plants. These samples were shelled and kernel number of each sample was determined. Afterwards, kernel weights of the samples were determined by a laboratory scale. Cross pollination rate was calculated by the ratio of foreign kernel number to total kernel number in each sample. The mean kernel weight (g) was determined by dividing the value for total kernel weight to value for total kernel number in each sample. Kernel samples were grounded using a laboratory mill (Fritsch pulverisette 14, Germany) then subjected to laboratory analyses. Protein, oil and carbohydrate ratios and oleic and linoleic acid contents were determined using NIR spectroscopy apparatus (Spectrastar 2400D, Unity Scientific, USA) with local calibration models (Egesel and Kahrıman, 2012; Egesel *et al.*, 2016). Carotenoid content was determined according to the method recommended by Rodriguez-Amaya and Kimura (2005).

The obtained data were analyzed in the R 3.0.3 package program (R Development Core Team, 2012) using the *stargazer* package. In the regression analysis, each quality trait was assigned as a dependent variable and mean kernel weight (MKW) and cross pollination rate (CPR) were assigned as estimators. In order to compare the results of three different models, intercept, slope and R^2 values of the model were used.

RESULTS AND DISCUSSION

The mean values for the investigated traits in the study are shown in Table 1. As clearly seen in this table, cross pollination rate is higher in the open-pollination than self-pollination treatment. The mean percentage of protein content, oil content, oleic acid content, and carotenoid content were found to be numerically higher in the self-pollination treatment than in the open-pollination (Table 1).

Table 1. Mean values for measured traits in open-pollinated samples (n=30), self-pollinated samples (n=30) and combined data (n=60)

	n	MKW (g)	CPR (%)	Protein (%)	Oil (%)	Carb (%)	Oleic (%)	Linoleic (%)	Carotenoid (µg/g)
Open	30	0.21	17.71	12.12	5.78	74.2	30.96	49.67	7.80
Self	30	0.22	0.14	13.04	6.39	72.9	31.85	48.78	8.27
Combined	60	0.21	8.92	12.53	6.05	73.5	31.32	49.38	8.03

The R^2 values of the models formed from the open pollinated samples varied between 0.001 and 0.309, between 0.018 and 0.216 in the self-pollinated samples, and between 0 and 0.162 in the combined dataset. It has been understood that the R^2 values vary considerably in different pollination treatments. For all features, it was determined that the regression coefficient for CPR was higher than the other sets for CPR in open pollination. R^2 values were dropped with the effect of self-pollination and they were similar in combined dataset. It has been understood that the R^2 values of the models was high in which the CPR and MKW variables were

included, but this increase did not available for all measured traits. It is noteworthy that this increase was particularly pronounced in open-pollination treatment. In the models where CPR and MKW variables coexist as predictors in the samples obtained from open pollination treatment had higher R^2 values compared to the others. Indeed, it was determined that the 15.4% of total variation for protein content, 30.9% for oil content, 25.9% for carbohydrate content, 23.8% for oleic acid content and 25.6% for linoleic acid content can be explained when two variables was used as predictors together (Table 2).

Table 2. The R^2 values of the regression models for predicting grain quality characteristics based on the CPR and MKW.

		Dependent Variables					
	Predictors	Protein	Oil	Carbohydrate	Oleic	Linoleic	Carotenoid
Open Pollination (n=30)	CPR	0.138	0.180	0.252	0.049	0.049	0.078
	MKW	0.001	0.209	0.027	0.228	0.246	0.027
	CPR+MKW	0.154	0.309	0.253	0.238	0.256	0.087
Self Pollination (n=30)	CPR	0.110	0.054	0.174	0.0001	0.032	0.020
	MKW	0.079	0.062	0.018	0.091	0.100	0.025
	CPR+MKW	0.216	0.104	0.209	0.092	0.120	0.040
Combined Data (n=60)	CPR	0.007	0.026	0.016	0.011	0.009	0.033
	MKW	0.025	0.123	0.000	0.152	0.162	0.027
	CPR+MKW	0.039	0.132	0.016	0.153	0.163	0.050

For all three models, the regression constant for protein to oil ratio was found to be lower in open pollination than the combined dataset and self-pollination. On the contrary, the exact opposite case was observed for carbohydrate content (Figure 1).The effect of cross pollination rate on protein and oil ratio was negative while its effect on carbohydrate ratio was positive. Considering the effect of mean kernel weight alone, it was observed that one unit increase in the weight of kernel resulted in a decrease in oil ratio and an increase in protein ratio. This effect was similar in open and self-pollination treatments. It has been reported that there was a negative correlation between oil content and kernel weight in some studies, whereas this relationship was found to be positive in some others. Therefore, it can be stated that the relationship between kernel weight and oil ratio may change depending on the material used.

The effect of mean kernel weight on carbohydrate ratio was significantly different from that of open pollination and self-pollination (Figure 1). This effect was found to be negative in the open pollination while it was negative in the self-pollination treatment. This can be attributed to the fact that the number of kernels in the ears was higher in the open pollination treatment. This is because the increase in the number of kernels may have reduced the mean kernel weight, thus causing the carbohydrate content to be low in the open pollination treatment. Indeed, this conclusion was confirmed by previous studies where there is a positive relationship between the kernel weight and the carbohydrate content.

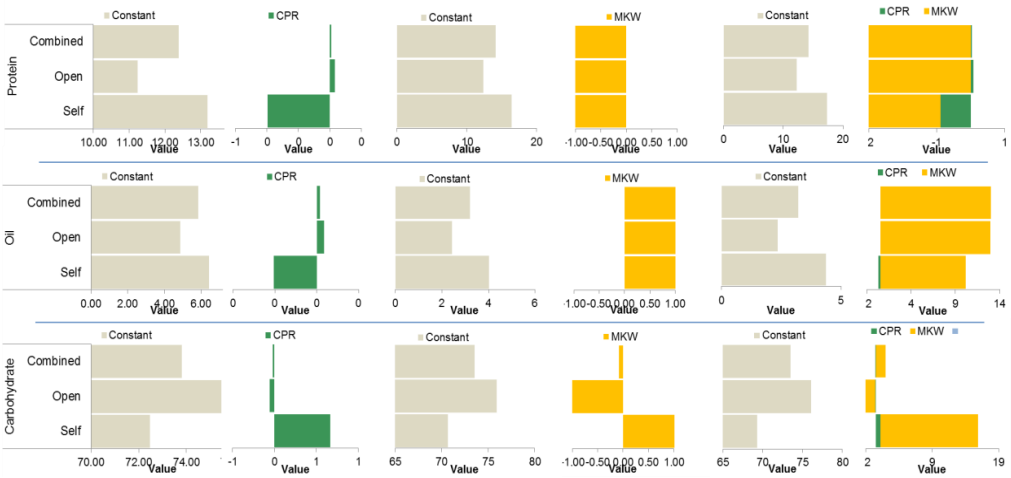

Figure 1. Graphical representation of constant and intercept values of regression models for protein, oil and carbohydrate ratio.

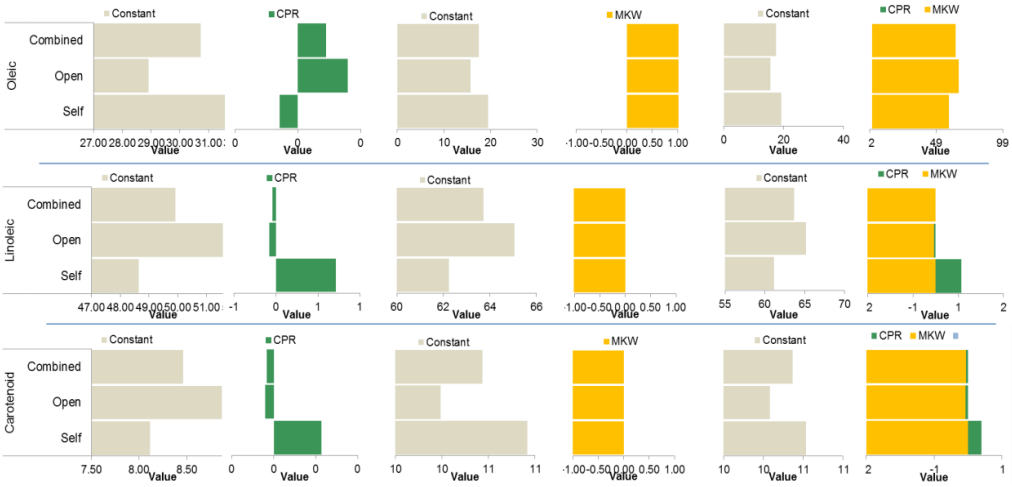

Figure 2. Graphical representation of constant and intercept values of regression models for oleic acid ratio, linoleic acid ratio and carotenoid content.

For oleic acid, the effect of cross pollination rate was positive in the open pollination and negative in the self-pollination. The effect of mean kernel weight on this trait was found to be positive in all data sets. In contrast to these findings, the effect of cross pollination rate on linoleic acid and carotenoid contents was positive in self-pollination treatment. Mean kernel weight had an negative effect on both tratis. It was understood that the effect of mean kernel weight and cross pollination rate were also effective in changing of linoleic acid and carotenoid content. In this study, the effect of mean kernel weight and cross pollination rate on the oleic and linoleic acid contents differed according to the pollination treatments.

This result can be attributed to the synthesis mechanism of the respective components. Because linoleic acid is a fatty acid synthesized from oleic acid (Huang *et al.,* 2016), and for this reason, the proportional increase of one of these components causes the decrease in other. It is not possible to discuss the findings of the study in a comprehensive way, since the relationships between major components and mean weight are generally considered in the scientific literature and minor compenents are not. However, it is understood that oleic and linoleic acid were highly affected by changes in mean kernel weight change and cross pollination rare, while carotenoid content was not affected much by these variables (Figure 2). This result is attributable to the fact that carotenoid content is a property controlled by maternal effects (Egesel *et al.,* 2003).

CONCLUSION

The results of this study showed that major and minor components of kernel quality characteristics changed with the effect of pollen transmission and mean kernel weight. This variation was affected by open and self-pollination treatments. Cross pollination rate and mean kernel weight can explain the variation in the quality traits examined. According to the results of the regression analysis, it was seen that a significant part (30.9%) of the variation in oil content was explained by mean kernel weight and cross pollination rate in open pollination treatment. Except the carotenoid content, there is a need to be careful in selecting pollination treatments that have the potential to affect pollen transmission risk and weight of the kernel. In this study, pollen contamination was examined depending on kernel color. In the future studies, more detailed results may be possible to obtain using different methods in determining of pollen contamination (such as molecular markers) and sensitive reference analyzes for kernel quality traits.

ACKNOWLEDGEMENT

This work was supported by Research Fund of the Çanakkale Onsekiz Mart University. Project Number: FHD-2016-768.

REFERENCES

Aliu S., Rusinovci I., Fetahu S., Simenoovska E. (2012). Genetic diversity and correlation estimates for grain yield and quality traits in Kosovo local maize (*Zea mays* L.) populations, Acta Agriculturae Slovenica, 99(2), 121–128.

Chukwu S.C., Ekwu L.G., Onyishi G.C., Okporie E.O., Obi I.U. (2013). Correlation between agronomic and chemical characteristics of maize (*Zea mays* L.) genotypes after two years of mass selection. International Journal of Science and Research, 4(8), 1708-1712.

Egesel C.Ö., Kahriman F., Ekinci N., Kavdir İ., Büyükcan M.B. (2016). Analysis of fatty acids in kernel, flour and oil samples of maize by NIR spectroscopy using conventional regression methods. Cereal Chemisty, 93, 487-492.

Egesel C.Ö., Wong J.C., Lambert R.J., Rocheford T.R. (2003). Gene dosage effects on carotenoid concentration in maize grain. Maydica, 48, 183-190.

Huang J., Zhang T., Zhang Q., Chen M., Wang Z., Zheng B., Xia G., Yang X., Huang C., Huang Y. (2016). The mechanism of high contents of oil and oleic acid revealed by transcriptomic and lipidomic analysis during embryogenesis in Carya cathayensis Sarg. BMC Genomics 17:113 10.1186/s12864-016-2434-7.

Kahriman F., Egesel C.Ö., Aydın T., Subaşı S. (2015a). The role of artificial pollination and pollen effect on ear development and kernel structure of different maize genotypes. Journal of Pollination Ecology, 15, 6-14.

Kahriman F., Egesel C.Ö., Zorlu E. 2015b. Effects of open- and self-pollination treatments on genetic estimations in maize diallel experiment. Spanish Journal of Agricultural Research, vol.13, no.e0704, pp.1-11.

Kahriman F. (2016). Mısırda polen etkisi ve bu etkinin kontrolünde uygulanan yöntemler, Lambert Academic Publishing, Saarbrükhen.

Khan A.H., Minhas N.M., Asad M.J., Iqbal A., Ilyas M., Mahmood R.T. (2014). Estimation of protein, carbohydrate, starch and oil contents of indigenous maize (*Zea mays* L.) germplasm. European Academic Research, 2(4), 5230-5240.

Letchworth M.B., Lamber R.J. (1998). Pollen parent effects on oil, protein, and starch concentration in maize kernels. Crop Science, 38, 363–367.

Prado S.A., López C.G., Senior M.L., Borrás L. (2014). The genetic architecture of maize (*Zea mays* L.) kernel weight determination, G3, 4: 1611-1621.

R Development Core Team, (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org

Rodriguez-Amaya D.B., Kimura M. (2004). HarvestPlus handbook for carotenoid analysis. HarvestPlus Technical Monograph Series 2. IFPRI, Washington, D.C., and CIAT, Cali.

Scrob S., Muste S., Has I., Muresan C., Socaci S., Fărcas A. (2014). The biochemical composition and correlation estimates for grain quality in maize. Journal of Agroalimentary Processes and Technologies, 20(2), 150-155

Sulewska H., Adamczyk J., Gygert H., Rogacki J., Szymanska G., Smiatacz K., Panasiewicz K., Tomaszyk K. (2014). A comparison of controlled self-pollination and open pollination results based on maize grain quality. Spanish Journal of Agricultural Research, 12(2), 492-500.

Original scientific paper
10.7251/AGRENG1802080L
UDC 630*26(497.11)

CARBON STORAGE IN SHELTERBELTS IN THE AGROFORESTRY SYSTEMS OF THE BAČKA PALANKA AREA (SERBIA)

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ABSTRACT

Field shelterbelts as agroforestry practice provides numerous ecosystem services. Carbon capture and storage potential in biomass and soil is among regulating services shelterbelts provide. Designing shelterbelts to address the various demands and provide services, requests special attention to choosing structural and spatial characteristics of shelterbelts, and species selection for shelterbelts. This paper presents the research results of C storage in 20-years old shelterbelts established on Gleyic Phaeozem in the area of Bačka Palanka (Serbia). Investigated shelterbelts were consisted of the most commonly used species for shelterbelt establishment in Serbia: Siberian elm (*Ulmus pumila* L.), poplar (*Populus x euramericana* (Dode) Guin. cv. „Serotina“) and black locust (*Robinia pseudoacacia* L.). The diameter at breast height (d) and the height (h) of all trees in studied shelterbelts were measured. Carbon stock in biomass was estimated according to IPCC (2003) methodology. Soil profiles were opened in studied shelterbelts with soil sampling carried out at fixed depths of 0-10 cm, 10-20 cm and 20-40 cm. Assessment of carbon storage in soil was performed according to IPCC (2003). According to the research results, living biomass C stock in 20-years old Siberian elm and poplar shelterbelts per tree is almost the same 0.333 t per tree and 0.300 t per tree, respectively. In black locust shelterbelt carbon stock is considerable less 0.111 t per tree. However, in species selection for shelterbelts some characteristics should take into account such as adaptability and suitability to the environmental conditions, longevity and their impact on crops that are grown in the sheltered fields, as well as the natural potential vegetation communities of the area. The results of this study indicate that the poplar is preferred species than the Siberian elm in given environmental conditions. The average carbon stock in the soil of studied shelterbelts in a layer 0-40 cm is 9.33 kg m^{-2} C.

Keywords: *agroforestry, field shelterbelts, carbon storage, species selection*.

INTRODUCTION

Field shelterbelts are among the most commonly used practices in temperate agroforestry systems. Field shelterbelts have the potential to provide numerous ecological and environmental services, starting from wind erosion control and protection of soil and crops, biodiversity maintenance and protection, the mitigation of greenhouse gases (GHGs) by carbon (C) sequestration (Possu *et al.*, 2016). The characteristics of shelterbelts (biological, structural and spatial) should be compatible with the other components of the agroforestry system, in order to meet various requirements. The selection of species for shelterbelts is an important task particularly in designing the field shelterbelt network in agricultural lands and in the absence of natural vegetation communities. There are different criteria in the selection of species for shelterbelts, ranging from the need for the provision of the protective function of shelterbelts as soon as possible by choosing fast-growing species, through the intention to provide some ecological services such as biodiversity conservation by providing habitats for birds and wildlife and unimpeded pollination, providing connectivity by creating corridors between habitat remnants etc. (Jose, 2009), by choosing tree species of natural vegetation communities. The tree species for shelterbelts could, also be chosen in accordance with the intentions to provide various socio-economic services such as the improvement of the touristic potential of the area by creating shelterbelt networks at local, national and regional levels for recreation and cultural heritage preservation by choosing autochthonous tree species of the area. Field shelterbelts provide an important ecosystem regulating service of atmospheric CO_2 capture through photosynthesis and its storage in biomass (Kirby and Potvin, 2007; Shoeneberger, 2009). Forest ecosystems can be C sources or sinks, depending on the dominant biological and physical factors. Considering that forests accumulate the highest amount of C among terrestrial ecosystems, the concept of planting trees, as a strategy for using trees to mitigate global climate change, is widely accepted (Marland and Marland, 1992). According to Oelbermann *et al.* (2004), agroforestry systems have the potential to sequester atmospheric C in trees and soil, while maintaining sustainable productivity.

The aim of this research was set according to the multifunctional role of the field shelterbelts and primarily has ecological character. The research was aimed at determining the efficiency of tree species in C storage in the shelterbelts of agricultural areas, highlighting the importance of species selection for shelterbelts in terms of C accumulation and pointing out the role of shelterbelts as agroforestry practice in climate change mitigation.

MATERIAL AND METHODS

The investigated shelterbelts located in the area of Bačka Palanka were established by planting 2-year old seedlings in 1995 (Dožić et al., 1995) (Fig. 1). The Siberian elm shelterbelt (*Ulmus pumila* L.) (SE) with total length of 935 m and average width 4 m, was mowed every year since established, and in 3rd and 7th year, side branches were pruned and removed from the shelterbelt. The poplar shelterbelt

(*Populus x euramericana* (Dode) Guin. cv. „Serotina“) (P) with total length 800 m and average width 3,5 m was mowed occasionally. Regular care and maintenance were not applied in the black locust (*Robinia pseudoacacia* L.) shelterbelt (BL) (total length 750 m, average width 2 m) so there were a number of forked trees of smaller dimensions. According to the General project of field shelterbelts network the proposed shares of species in total number of seedlings in the whole area of Bačka Palanka (Dožić et al., 2001) was as follows: Siberian elm 9.41%, poplar 11.49% and black locust 23.67%.

In order to estimate C stock in the biomass of shelterbelts the diameters at breast height (dbh) and heights (h) of all trees in the shelterbelts were measured. Soil investigations were performed to estimate the C stock in the soil of the shelterbelts. The assessment of C stock was obtained according to the recommendations of IPCC (2003) methodology.

The diameter at breast height (dbh) and height (h) of all trees in the shelterbelts were measured including those with a dbh less than 10 cm. On the basis of this data, the structural and production elements per ha of Siberian elm, poplar and black locust shelterbelts were obtained, using the following parameters: the number of trees (N) and their distribution by diameter classes, basal area (G), height curves and volume (V) (Banković and Pantić, 2006). A total of two soil profiles were opened in each shelterbelt with soil sampling carried out at fixed depths of 0-10 cm, 10-20 cm and 20-40 cm. Basic physical and chemical properties of air-dry soil were determined using the following methods: the pipette method was used for particle size analysis (ISO-11277:1998), bulk density was calculated according to Adams (1973), soil pH was determined in distilled water with solid-liquid, hydrolytic acidity (Y_1) and the sum of exchangeable basis (S) was determined using the Kappen's method (1929), the total capacity of cation adsorption (T) and the degree of base saturation (V%) were calculated (Hissink, 1925), CaCO3 was determined using the Scheibler's calcimeter. Humus content was measured using the Turin method (Nelson and Somers, 1996), total N using the Kjeldahl method (ISO 11261:1995) and C/N was calculated. The available P and K were determined by the Egner-Riehm method (Čakmak et

Figure 1. Study area with marked shelterbelts

al., 2010). In this paper are presented the results of C stock assessment in shelterbelts which are an integral part of the agroecosystems of the area of Bačka Palanka. Although shelterbelts are a part of agroforestry systems, C stock evaluation was carried out according to the methodology proposed for forests, because the Law on Forests (Official Gazette of RS 30/10) recognized shelterbelts as forests. General C pools for forest ecosystems are: living biomass, dead organic matter and soil.

According to the IPCC (2003) a total annual change in C stock in forests is the sum of changes in C stock in living biomass (aboveground and belowground), dead organic matter (dead wood and litter) and forest soil, expressed in t C yr^{-1}.

In this research, C stock estimation included only the living biomass pool and soil pool. Dead organic matter (dead wood includes wood lying on the surface, dead roots or stumps) was excluded from the estimation, because dead wood was removed from the shelterbelts, and also litter (includes litter, fumic, humic layers and fine roots), because shelterbelts were regularly mowed.

Carbon stock in aboveground living biomass (*B*t) was obtained using the following equation (IPCC, 2003):

$$B_t = V_t \times D_w \times BEF_2 \times CF$$

where V_t is the total bole volume (m^3 ha^{-1}), *Dw* is the wood density (Mg dry mass m^{-3} green; 0.57 for Siberian elm (Martin et al., 2009), 0.42 for poplar and 0.74 for black locust (Šoškić and Popović, 2002), BEF_2 is the biomass expansion factor (dimensionless), which is the conversion factor to expand under-bark bole biomass to include non-merchantable biomass such as bark and branches (1.4 – IPCC, 2003) and *CF* is the conversion factor from dry biomass to carbon (Mg C [Mg dry mass]-1; 0.5 – IPCC, 2003).

Belowground biomass was calculated using an allometric equation which related aboveground biomass to root biomass (IPCC, 2003):

$$Y = [-1.0587 + 0.8836 \times ln(B_t) + 0.2840]$$

C stock in belowground biomass was obtained by multiplying root biomass (Y) and the C fraction of dry matter (0.5; IPCC, 2003).

Carbon stock in soil (*SCD*) was calculated using the following equation (Stolbovoy et al., 2003):

$$SOCD = \sum_{i=1}^{n} \left[SOC_i \times BD_i \times T_i \times \left(1 - \frac{C_i}{100}\right) \right]$$

where *SCD* is the soil carbon density for the *j*-th layers (*l*) of the sampling site (Mg C ha^{-2}), SOC_i is the soil organic carbon content for a single sampled depth (g kg^{-1}), BD_i is the soil mass of the undisturbed volume of a single sampled depth (g cm^{-3}), T_i is the thickness of the sampled layer (cm), C_i is the volume of the coarse fragments in the single sampled depth (%).

RESULTS AND DISCUSSION

In the Siberian elm shelterbelt there were 1304 trees per hectare, and timber volume was 865.6 m^3 ha^{-1}. The poplar shelterbelt had a lower planting density and 980 trees per hectare with a timber volume 785.9 m^3 ha^{-1}. The highest number of trees was found in the black locust shelterbelt and it amounted to 2115 trees per hectare, and timber volume was 355.3 m^3 ha^{-1}. The achieved timber volume in 20-years-old black locust shelterbelt was a consequence of the high number of trees in the shelterbelt, and indicates low productivity on the one hand and the absence of silvicultural measures on the other.

The average volume of a single tree in the Siberian elm, poplar and black locust shelterbelts were 0.664 m^3, 0.802 m^3 and 0.168 m^3, respectively. Since all three species in 20-years-old shelterbelts were far from their ecological optimum, poplar had the highest productivity in specific conditions. The absence of care and maintenance in the black locust shelterbelts was probably one of the causes of its low productivity. Siberian elm is between poplar and black locust in terms of productivity. However, the estimation of production characteristics is only one (and less significant) element in the assessment of the suitability of species for shelterbelts. Primarily, the selected species should be wind-tolerant and adapted to environmental conditions in terms of soil, vegetation, climate, etc.

The living biomass of the 20-years-old Siberian elm shelterbelt was 867.6 t ha^{-1}, where aboveground biomass was 690.8 t ha^{-1} (79.6%), and belowground biomass amounted to 176.8 t ha^{-1} (20.4%). The living biomass of the poplar shelterbelt amounted 588.4 t ha^{-1}, where aboveground biomass was 462.1 t ha^{-1} (78.5%), and belowground biomass accounted for 126.3 t ha^{-1} (21.5%). In the black locust shelterbelt, 471.2 t ha^{-1} of living biomass was distributed in the aboveground biomass 368.1 t ha^{-1} (78.1%) and belowground 103.1 t ha^{-1} (21.9%). The ratio of aboveground and belowground biomass was not significantly different among the investigated species and it was about 0.8:0.2 for each species.

According to the results (Table 1) the largest portion of C stock per hectare was accumulated in the living biomass of the Siberian elm shelterbelt (433.8 t ha^{-1}), where the aboveground C stock was 345.4 t ha^{-1}, and the belowground C stock 88.4 t ha^{-1}. The total C stock in the poplar shelterbelt was 294.2 t ha^{-1} of which the aboveground C stock was 231.1 t ha^{-1}, and 63.1 t ha^{-1} was the belowground C stock. In the black locust shelterbelt total C stock was 235.6 t ha^{-1}, while C stock in the aboveground biomass was 184.1 t ha^{-1}, and belowground C stock was 51.5 t ha^{-1}. Average C stock per tree in the Siberian elm shelterbelt was 0.333 t ha^{-1}, in the poplar shelterbelt it amounted to 0.300 t ha^{-1} and 0.111 t ha^{-1} in the black locust shelterbelt.

Table 1. Carbon stock in 20-years-old shelterbelts in the area of Bačka Palanka

Diameter class	Carbon stock n living biomass								
	Aboveground biomass			Belowground biomass			Total		
	SE	P	BL	SE	P	BL	SL	P	BL
	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}
2.5									
7.5			0.56			0.25			0.81
12.5	0.16		18.37	0.09		5.56	0.25		23.93
17.5	2.04	1.49	41.77	0.80	0.61	11.50	2.84	2.10	53.27
22.5	17.05	5.88	69.72	5.21	2.03	18.08	22.27	7.91	87.81
27.5	65.27	17.55	29.01	17.06	5.34	8.33	82.33	22.89	37.35
32.5	132.91	62.19	10.43	31.98	16.35	3.37	164.89	78.54	13.80
37.5	100.64	50.74	14.17	25.01	13.66	4.43	125.66	64.39	18.60
42.5	24.03	62.22		7.06	16.35		31.09	78.58	
47.5	3.27	30.98		1.21	8.83		4.48	39.81	
Total	345.39	231.05	184.04	88.42	63.17	51.54	433.80	294.22	235.57

According to Bura (1962), plantations of Euramerican poplar hybrids established in agricultural systems have always had a higher productivity than plantations of the same hybrids outside agricultural production. A significant amount of C stock and nutrients were accumulated in biomass of hybrid poplars in systems of riparian buffer strips (Fortier et al., 2010/b). Fast growth of poplar clones and cultivars defines the production cycle (rotation) of poplar plantations. If the production targets were set as secondary, as in shelterbelts, then the life cycle of poplar plantations could be significantly longer. In addition, poplar (*Populus x euramericana* (Dode) Guin. cv. „Serotina“) reaches its peak productivity later in life cycle compared to Siberian elm, and unlike Siberian elm still has high productivity in the investigated shelterbelts. Siberian elm is a fast growing species and at the age of 10 years in favourable environmental conditions it could reach the height of 13 m and a diameter of 9.3 cm (Jovanović, 1982). In its ecological optimum Siberian elm can reach the age of 100-150 years. However in the conditions of continental climate prevailing in the study area, Siberian elm is a short-lived species that hardly reaches more than 60 years of age (Grbić, 2014). It was widely applied in shelterbelts' establishment in steppe conditions (Jovanović, 1982), but also in Serbia (Grbić, 2014). Siberian elm tolerates a variety of conditions such as poor soil and low soil and air moisture and it is fairly wind-tolerant, which makes it suitable for shelterbelts, but shows the tendency to be invasive (Grbić, 2014). The extract from the leaf litter of Siberian elm inhibits the radical growth of herbaceous plant native species, such as *Dactylis glomerata* L., *Trifolium repens* L. and germination of *Chenopodium album* L. which reduces the growth of understory species (Perez-Corona et al. 2013). The allelopathic effect of Siberian elm that may limit the establishment of the most abundant species of natural vegetation communities may lead to clear effects on understory vegetation structure and function. Although every monoculture has adverse effects on soil properties and function and could cause soil sickness (Pavlović et al., 2011).

Considering previously mentioned facts, poplar is a preferred species for shelterbelts establishment in the area studied. Poplar is well adapted to environmental conditions and has beneficial effects on crops in protected fields

(Bura, 1962). It also does not have the allelopathic potential to suppress native species. In multifunctional agroforestry systems, poplar shelterbelts represent an appropriate way for the production of high amounts of biomass and wood in short rotation, and at the same time contributing to the mitigation of ecological problems (such as agricultural non-point source pollution) which had occurred as a consequence of intensive agricultural production (Fortier et al., 2010/a). Carbon stock in the biomass of black locust of the same age expressed per tree is significantly lower and amounts to 30% of the C stock accumulated in the Siberian elm and poplar. Black locust in shelterbelts in the area of Bačka Palanka is the least efficient species, not only in C accumulation but in its adaptation potential to the environmental conditions. In the given site conditions, black locust has a low increment and low trunk quality which partly is a consequence of the complete absence of care and maintenance, but also its unconformity with the environmental conditions.

The observed soil type was Gleyic Phaeozem (WRB, 2006). It belongs to the textural class of clay and contains no particles sized 2.00 mm. Soil reaction (pH in water) ranged from 7.82 to 8.14. Concerning nutrient content in the studied soil, the following values were recorded: the humus content ranged from 2.04 to 6.16%, indicating moderate to high humus level, the total N ranged from 0.26 to 0.40%, indicating a soil well supplied with N, while the average content of available P was 4.8 mg/100g soil, and the average content of available K was 24.13 mg/100g soil. C:N ratio ranked from 4.08 to 9.67. Narrow C:N ratio indicating the formation of a better quality humus (mull humus) (Knežević and Košanin, 2007).

The average C stock in the studied soil in the 0-20 cm layer was 9.33 kg m^{-2}. The largest amount of C stock was accumulated in the soil layer up to 10 cm and amounts to 3.24 kg m^{-2} on average, while decreasing with depth, consequently amounting to 1.84 kg m^{-2} and 1.42 kg m^{-2} in the 10-20 cm and 20-40 cm layers, respectively.

Compared to the C stock in the 0-20 cm layer in soil of the same type in Srem, which ranged from 2.72 to 5.05 kg m^{-2}, and averagely 3.56±0.94 kg m^{-2}, there was a larger amount of C stock accumulated in the studied soil in these shelterbelts, that amounted 5.07±0.16 kg m^{-2} on average in the 0-20 cm layer. The soil organic matter content in arable land on Chernozems of Vojvodina is constantly decreasing, and in the period 1990-2004 by up to 0.05-0.2% (Ličina et al., 2011). According to Vidojević et al. (2013), C stock was decreased in agricultural lands due to the increase in agricultural production and land use changes. By the implementation of agroforestry systems and reintroduction of trees in agricultural lands by shelterbelt networks establishment, rapid decrease in soil organic matter could be mitigated in agricultural soils.

The interactions between trees and soil are very complex. In the same soil condition investigated tree species shown different average timber volume as a result of different species requirements and allelopathic effects. The soil properties such as pH and availability of nutritive macro and microelements, organic matter quality, structure, texture and soil moisture are some of the most important factors

for biomass production and timber volume increment. Also, the backward effect of trees on soil should not be neglected. Leaf litter and dead wood influences the organic matter quality and turnover which is beneficial in treeless agricultural land. These beneficial interactions between trees and soil are utilized in agroforestry systems (Jose, 2009).

The total C stock in biomass and soil (0-40 cm) of the studied shelterbelts was presented in Table 2. The largest amount of C stock was accumulated in the Siberian elm shelterbelt 527.1 t ha^{-1}, and the lowest one was accumulated in the black locust shelterbelt 328.9 t ha^{-1}. The share of accumulated C by C pools in the total C stock differed among the studied tree species. According to the results, aboveground C stock was the largest in the Siberian elm shelterbelt (65.5%), and the smallest one was in the black locust shelterbelt (56.0%). The share of belowground C stock was slightly different in the Siberian elm, poplar and black locust shelterbelts (16.8%, 16.3% and 15.6%, respectively), while the share of C stock in soil was the largest in the black locust shelterbelt (28.4%), and in the Siberian elm shelterbelt it amounted to the lowest value of 17.7%.

Table 2. Carbon stock in biomass and soil in the studied shelterbelts

	Siberian elm		Poplar		Black locust	
	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
Total C stock	527.09	100.0	387.51	100.0	328.86	100.0
Aboveground C stock	345.39	65.5	231.05	59.6	184.04	56.0
Belowground C stock	88.42	16.8	63.17	16.3	51.54	15.6
Soil C stock	93.29	17.7	93.29	24.1	93.29	28.4

The C stock per tree was slightly different in the 20-years-old Siberian elm and poplar shelterbelts in the given site conditions, with that being the total C stock differently distributed in the aboveground, belowground and soil C pools. Siberian elm accumulated more C in the aboveground biomass at the expense of C accumulation in the soil, unlike poplar which accumulated less C in the aboveground biomass in favour of the C accumulation in soil. These ratios indicate the beneficial effects of poplar on the content and quality of soil organic matter. When selecting species to establish field shelterbelts in the area studied, a number of relevant ecological and economic factors, and above all, interactions of the tree species and specific site conditions should be considered.

CONCLUSION

Designing shelterbelts to address the various demands and provide services, requests special attention paid to the choice of structural and spatial characteristics of shelterbelts and the selection of species for shelterbelts. An appropriate selection of species provides the multifunctionality of these shelterbelts. The C stock in 20-year old shelterbelts in the area of Bačka Palanka consisted of Siberian elm and poplar, expressed per tree, is almost the same 0.333 t and 0.300 t, respectively, and in the black locust shelterbelt it is considerably lower amounting to 0.111 t per tree.

Although it has a lower C stock in biomass (20-years old shelterbelts), poplar is a preferred species in comparison to Siberian elm in the given environmental conditions, due to its adaptability and suitability to the environmental conditions, longevity and the impact on crops that are grown in the sheltered fields. Namely, in contrast to Siberian elm, poplar still reaches high productivity in the examined shelterbelt. The distribution of C stock in living biomass and soil indicates that poplar litter gives a better quality organic matter and affects more C accumulation in soil than Siberian elm. Siberian elm, also expresses a tendency to be invasive, especially in monocultures, thus having a negative impact on crops in sheltered fields.

Poor conditions of the black locust shelterbelt, its volume and C stock indicate the importance of regular care and maintenance, which were not applied in the examined shelterbelt.

AKNOWLEDGEMENTS

The part of this investigation was conducted through the project: "Climate Change and Its Impact on the Environment – Monitoring, Adaptation and Mitigation", reference no. 043007 financially supported by the Ministry of Education and Science of the Republic of Serbia.

REFERENCES

Adams WA (1973). The effects of organic matter on the bulk and true densities of some uncultivated podzolic soils. Journal of Soil Science 24: 10-17

Banković S, Pantić D (2006). Dendrometrija [Dendrometry]. Univerzitet u Beogradu – Šumarski fakultet, Belgrade, Serbia, pp. 556. [in Serbian]

Bura D (1962). Plantaže topola sa poljoprivrednim kulturama. Šumarski list 9-10:297-316

Čakmak D, Saljnikov E, Mrvić V, Jakovljević M, Marjanović Z, Sikirić B, Maksimović S (2010). Soil properties and trace elements contents following 40 years of phosphate fertilization. J. Environ. Qual. 39 (2): 541-547

Dožić et al. (1995). Projekat šumskog zaštitnog pojasa pored puta Čelarevo – Gajdobra. [Technical documentation: The Project of Forest Shelterbelt along the Road Čelarevo Gajdobra]. DTM Podunavlje, Pokret gorana Srbije, Beograd. [in Serbian]

Dožić et al. (2001). Glavni projekat podizanja poljezaštitnih pojaseva na području Bačke Palanke. [Technical documentation: The Main Project of Field Shelterbelts in the Area of Bačka Palanka Municipality, Vojvodina, Serbia]. Pokret gorana Srbije - Komisija za ozelenjavanje opštine Bačka Palanka – Univerzitet u Beogradu - Šumarski fakultet [in Serbian]

Fortier J, Gagnon D, Truax B, Lambert F (2010a). Biomass and volume yield after 6 years in multiclonal hybrid poplar riparian buffer strips. Biomass Bioenergy 34: 1028-1040.

Fortier J, Gagnon D, Truax B, Lambert F (2010b). Nutrient accumulation and carbon sequestration in 6-year-old hybrid poplars in multiclonal agricultural riparian buffer strips. Agric Ecosyst Environ 137: 276-287.

Grbić (2014). Sibirski brest - za i protiv. [Siberian Elm – pro and contra]. Zbornik radova 11. seminar Pejzažna hortikultura, Beograd 6-21 [in Serbian]

IPCC (2003). Good practice guidance for land use, land-use change and forestry (Penman J, Gytarsky M, Hiraishi T, Krug T, Kruger D, Pipatti R, Buendia L, Miwa K, Ngara T, Tanabe K, Wagner F eds). IPCC, Institute for Global Environmental Strategies (IGES), Kanagawa, Japan, pp.593

ISO-11261 (1995). Soil quality - Determination of total nitrogen, modified Kjeldahl method. International organization for standardization, Geneva, Switzerland, pp. 4.

ISO-11277 (1998). Soil quality - Determination of particle size distribution in mineral soil material. Method by sieving and sedimentation. International organization for standardization, Geneva, Switzerland, pp. 34.

Hissink DJ (1925). Base exchange in soils. Transactions of the Faraday Society 20: 551-556

Jose S. (2009). Agroforestry for ecosystem services and environmental benefits: an overview. Agroforest Syst 76:1-10

Jovanović B (1982). Dendrologija. [Dendrology]. Univerzitet u Beogradu - Šumarski fakultet, Belgrade, Serbia, pp. 713. [in Serbian]

Kappen H (1929). Die Bodenaziditat. Springer Verlag, Berlin, Germany, pp. 363. [in German]

Kirby KR, Potvin C (2007). Variation in carbon storage among tree species: Implications for the management of a small-scale carbon sink project. For. Ecol. Manage. 246:208-221

Knežević M. Košanin O. (2007). Praktikum iz pedologije. [Pedology Practicum]. Univerzitet u Beogradu Šumarski fakultet, pp. 153. [in Serbian]

Ličina V, Nešić Lj, Belić M, Hadžić V, Sekulić P, Vasin J, Ninkov J (2011): The Soils of Serbia and Their Degradation, Ratar. Povrt. / Field Veg. Crop Res. 48: 285-290

Marland G, Marland S (1992). Should we store carbon in trees? Water Air Soil Pollut 64: 181-195

Martin JA, Solla A, Esteban LG, de Palacios P, Gil L. (2009). Bordered pit and ray morphology involvement in elm resistance to *Ophiostoma novo-ulmi*. Can J For Res 39: 420–429

Nelson DW, Sommers LE (1996). Total carbon, organic carbon and organic matter. In: "Methods of soil analysis. Part 3 - Chemical Methods" (Sparks DL ed). SSSA Book Series No. 5, SSSA and ASA, Madison, WI, USA, pp. 961-1010.

Oelbermann M, Voroney RP, Gordon AM (2004). Carbon sequestration in tropical and temperate agroforestry systems: a review with examples from Costa Rica and southern Canada. Agric Ecosyst Environ 104: 359–377

Official Gazette of Republic of Serbia (2010). Law on Forests - Official Gazette of Republic of Serbia 30/10. 07/05/2010

Pavlović P, Djurdjević L, Mitrović M (2011). Soil sickness in Forestry. In: Narwal SS, Politycka B, Wu F, Sampietro DA (Eds.), Reseach Methods in Plant Science, Vol. 3: Soil Sickness. Studim Press LLC, Houston, pp. 241-272.

Pérez-Corona ME, de las Heras P, Vázquez de Aldana BR (2013). Allelopathic potential of invasive *Ulmus pumila* on understory plant species. Allelopathy J 32:101-112

Possu WB, Brandle JR, Domke GM, Schoeneberger M, Blankenship E (2016). Estimating carbon storage in windbreak trees on US agricultural lands. Agrofor Syst 90:889-904

Schoeneberger MM (2009). Agroforestry: working trees for sequestering carbon on agricultural lands. Agrofor Syst 75:23-27

Šoškić B, Popović Z (2002). Svojstva drveta. [The Properties of Wood]. Univerzitet u Beogradu - Šumarski fakultet, Belgrade, Serbia, pp. 97-108. [in Serbian]

Stolbovoy V, Montanarella L, Filippi N, Selvaradjou S Gallego J (2003). Soil Sampling Protocol to Certify the Changes of Organic Carbon Stock in Mineral Soils of European Union. EUR 21576 EN, 19 pp. Office for Official Publications of the European Communities, Luxembourg

Vidojević D, Dimić B, Baćanović N (2013). Praćenje stanja zemljišta - zakonski osnov, ciljevi i indikatori, [Monitoring of the soil condition – law framework, goals and indicators] (ed. Vidojević D), Ministarstvo energetike, razvoja i zaštite životne sredine R Srbije - Agencija za zaštitu životne sredine, str. 30 [in Serbian]

WRB (2006). World reference base for soil resources 2006 - a framework for international classification correlation and communication. IUSS International Working Group, World Soil Resources Reports 103, FAO, Rome, Italy, pp. 128.

Review paper
10.7251/AGRENG1802091S
UDC 639.2:616.99

BOTHRIOCEPHALUS SPP. INFECTION OF CYPRINIDAE: EPIZOOTOLOGY, CLINICAL FEATURES AND PATHOGENESIS, DIAGNOSTICS, THERAPEUTIC AND PROPHYLACTIC MEASURES

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ABSTRACT

Bothriocephalosis of fish is a disease caused by tape worms Bothriocephalus opsariichthydis and Bothriocephalus acheilognathi parasitizing in the anterior part of intestine. Bothriocephalus spp. infection is widely spread among fish at pond farms, cage fish farms in cooling ponds of thermal power and nuclear power stations and in natural reservoirs. One have revealed Bothriocephalus in 26 species of fish attributed to Cyprinidae family, in salmons (Arctic salmon) and in some predatory fish (catfish, pikeperch). Different species of cyclopes serve as the intermediate hosts necessary for development of helminths. Fish fry and fingerlings are the most susceptible ones to infection. Fish of older age groups are less susceptible to this parasite. The data on biology, epizootology, clinical features and pathogenesis, diagnostics, therapeutic and prophylactic measures against this infection are represented. The results of the field trials with microsal against Bothriocephalosis in carps and grass carps carried out in different regions of the Russian Federation as well as monitoring of the safe use of microsal for the study period (2007 to 2016) are described. The daily dose of medicated feed with 2% of microsal corresponds to the daily feeding for fish. The therapeutic feeding is carried out during one day without preliminary starvation period according to the current technology of fish feeding with granulated feed. The dose level according to the active substance depends on water temperature and average fish weight and ranges 12 to 40 mg/kg. Prophylactic treatment is carried out twice a year: in late April – early May and in late August – early September (at pond farms) and in late September – early October (at cage fish farms) when the water temperature is not higher 150 C. In general the data obtained for 10 year period evidence about microsal's safety for fish and at it's current application (according to the instructions) the reasonable benefit/risk ratio is maintained in all cases.

Keywords: *Bothriocephalus opsariichthydis, Bothriocephalus acheilognathi, fish farms, microsal.*

EPIZOOTOLOGY

Carps, common carps, silver and gold carps and grass carps are mainly susceptible to Bothriocephalus spp. infection. Fish fry and fingerlings are especially sensitive to this infection. Fish of older age groups are less susceptible to this parasite. Infected fish of different age groups as well as infected Cyclops function as the source of infection. At cage fish farms transmission of infection occurs with excrements containing parasite eggs and carried by water flow. First infection of young fish occurs in trays (or fry ponds) at feeding of infected zooplankton. After transfer of fish into cages the infection level increases reaching the maximum value to the end of summer and then decreases. Due to siltation of wire gauze each cage becomes a separate biotope and Bothriocephalus infection microfocus with independent circulation of the pathogen. Two-year old fish become infected with Bothriocephalus in spring and summer, but the rate of their infection usually is not high. Infection of fish fry at pond farms occurs at feeding of zooplankton and consumption of infected Cyclops. The peak of infection extensity and intensity is recorded in July-August at rich development of zooplankton and intensive feeding of fish. The infection extensity value achieves 80-100% with the intensity of infection - from several specimens to one hundred helminths per one fish. In autumn when the grown juveniles transfer to eating of forage and the number of copcpods becomes fewer the rate of infection reduces. Increase of Bothriocephalus infection level among fish depends on the temperature conditions. Temperature lowering retards the development of infection. Helminths develop from eggs to adult stages at the water temperature of 16-19°C for 33-34 days as while at 20-25°C for 19-25 days. If fish becomes infected by Bothriocephalus in autumn parasites reach mature stage only to April of the following year (post 200-240 days).

CLINICAL SIGNS AND PATHOGENESIS

Pathoanatomical changes in fish infected with Bothriocephalus depend on the infection intensity and terms of parasitizing. One can observe the most manifested changes in the anterior and middle parts of the intestine. In places of contact of helminth strobila with the intestine thinning of its wall usually is observed as a result of destruction of the mucous, muscular and partially serous membranes. Helminth parasitizing in the intestine of fish leads to deterioration of digestion processes. In infected carp fingerlings and yearlings hemoglobin level in blood reduces by 25- 30% as while polymorphonuclear leukocyte and neutrophil counts increase. Carp tiny infected by Bothriocephalus demonstrate growth and development decrease. Exhaustion, anemic gills, movement reduction are recorded in infected one-year- old fish. They cannot survive winter and die in the middle of March or early April. Chronic inflammation of the intestinal mucosa is observed in infected two-year-old carps. They have a growth slowing, poor digestion of feed and anemic condition. Intestinal obstruction and reduced growth rate take place at the infection intensity more than 12 helminths per one fish. Death of carp

fingerlings caused by Bothriocephalus occurs at parasitizing of more than 50 helminths in one fish.

DIAGNOSTICS

Bothriocephalosis is diagnosed on the basis of epizootologic data, clinical signs of disease and the results of helminthological examination of fish. The structure of parasite scolex is the most reliable diagnostic sign of Bothriocephalus species. B. opsariichthydis has a heart-shaped scolex with muscular pariental disk and deep, open bothridia as while B. acheilognathi - a spherical scolex with deep, half closed bothridia. For investigations one use not less than 25 fry, fingerlings and yearlings as well as 10-15 two-year-old carps from each pond (at pond farms); 40-50 fingerlings and 10-15 two-year-old fish from each pontoon line (at cage fish farms). Breeding herd and rearing fish are examined using coprologic methods. To reveal the latent parasite carriage two-week fry (the most susceptible ones to Bothriocephalus infection) are transferred to adult fish suspected in infection. 2-3 weeks later fry is examined and diagnosed for latent Bothriocephalus infection. Autopsy of infected fish is carried out as follows: one cut abdominal wall of fish with scissors, extract intestine with tweezers, put it into Petri dish, separate intestine from other organs, cut it along or squeeze out the contents from the intestines adding some water and count parasites according of the number of scolexes. Then the pathogen species is identified. Coprologic examinations for presence of helminth eggs include collection of fish excrements and recovery of parasite eggs. Fish excrements are collected from cage walls by net made of gauze № 50 during lifting. Samples of excrements from 3(or fewer) cages from each pontoon line are combined into one 3-4 g sample and examined by one of the following methods.

Method 1. One take a sample of 0.5 g from each combined excrement sample and examine by the method of native smear.

Method 2. 0.5 g from each combined excrement sample is added into a 100 ml glass beaker and 30 ml of water is added. The sample is stirred thoroughly using a glass rod and the obtained suspension filtered through strainer. Water is added. The volume of filtered suspension is adjusted to 60 ml. The contents is transfused into Petri dish in small portions of 10-15 ml and examined under microscope (MBS; magnification 7X8) for detection of Bothriocephalus eggs. The size of eggs - 0,05 - 0,054 ><0,03 - 0,038.

THERAPEUTIC AND PROPHYLACTIC MEASURES

Bothriocephalus infection in Cyprinidae is included in the list of quarantine and especially dangerous diseases of fish according to The Order №173 of September 29, 2005 of the Ministry of Agriculture of Russia. If Bothriocephalus infection is diagnosed in fish then the pond farm is announced as unsafe for this infection and restrictions are imposed. It is forbidden to transfer the fish stocking material to non-infectcd waters, pond and cage fish farms.

At pond farms: infected ponds are drained and subjected to disinfection using caustic lime (25 cwt/ha) or by bleaching powder (6 cwt/ha). In winter the drained ponds are deep freezed, in spring arc dried that leads to death of helminth eggs and infected intermediate hosts - crustaceans and Cyclops (Muzykovsky A.M., Skachkov D.P., et all., 1987; Skachkov D.P., Muzykovsky A.M., et all., 1990).
At cage fish farms with high level of Bothriocephalus infection: cages for juvenile fish with a mesh up to 10 mm is changed weekly and with larger mesh size – according to contamination. Cages from infected fish are cleaned from dirt, washed with water and dried at the temperature 20°C at least for 24 hours or kept in 2% formalin solution for 2 hours and then washed with water. Warm water for incubation shop, sites for rearing of juvenile fish and keeping of producers as well as lor try ponds is taken from the channel or cooling reservoir upstream to cage lines or from some other water source stationary safe for Bothriocephalus infection. Cages with juvenile fish are placed at the distance of 15-20 meters from the shore in sections of cooling reservoirs with a depth not less than 5 meters, forming sanitary zone between cage lines in range of 50-60 m. Herewith the cages with fry and fingerlings on pontoon lines should be located upstream in relation to cages with fish of older age (Skachkov D.P., Gorokhov V.V., et all., 1995).
Prophylactic treatment by anthelmintics is carried out twice a year: at the end of April-beginning of May and at the end of August- beginning of September (at pond farms) and at the end of September-beginning of October (at cage fish farms) at the water temperature not higher 15°C. Medical treatment by anthelmintics is performed at any time of the year if diagnostic indications are present. Microsal is used for such treatment of fish. The daily dose of medical feed with 2% of Microsal corresponds to the daily norm of fish feeding by mash. The therapeutic feeding is carried out for one day without preliminary starvation period using the current technology of fish feeding with granulated feed. The dose level according to the active substance depends on water temperature and average fish weight and ranges 12 to 40 mg/kg (Skachkov D.P., 2008).

Table 1.The daily dose of 2% medicated feed with Microsal, the % of fish body weight

Water temperature, °C	Fish body (weight), g				
	20-50	50-100	100-250	250-500	More than 500
12	2,0	1,6	1,3	1,0	0,8
15	3,0	2,0	1,6	1,2	1,0
18	4,0	3,0	2,0	1,6	1,3
21	5,0	4,0	3,0	2,0	1,6
24	6,0	5,0	4,0	3,0	2,0
27	7,0	6,0	5,0	4,0	2,2
30	8,0	7.0	6,5	4,5	2,5

Before general treatment every lot of the drug as a part of medical feed is tested on tish in one pond. If no complications are observed within 3 days treatment of all fish in every pond is carried out.
25 fish recovered from each pond are subjected to helminthological autopsy before and 4-5 days after medical feeding to determine the treatment effectiveness. If necessary the medical feeding can be repeated 10-20 days after the first treatment (Skachkov D.P., Arkhipov I.A., 2009).
During the period of 2007-2016 one produced and sold 37225 kg of the Microsal to fish farms of 23 Territories and Regions (Altai, Krasnodar and Stavropol I erritories; Astrakhan, Bryansk, Vladimir, Volgograd, Voronezh, Kaliningrad, Kaluga, Kursk, Lipetsk, Moscow, Orenburg, Orel, Perm, Rostov, Ryazan, Samara, Saratov, Sverdlovsk, Tambov, Tula, Chelyabinsk Regions). 1098150 kg of fish was treated [4-6]. The therapeutic effectiveness of the agent for carps appeared to be 100% as while for white Amur - 87.5% (Skachkov D.P., Pavlovich G.M., 2012).

Table 2. The data on used agent amounts and quantity of treated fish

The country	Years	Amount of the drug	Average dose according to the active substace per 1 kg of fishmass (ichthyomass)	Treatment course (days)	Treated fish (kg)
Russia	2007	3450			69000
	2008	1720			34400
	2009	2460			49200
	2010	3770			75400
	2011	3555	40 mg/kg	1	71100
	2012	6050			121000
	2013	3980			79600
	2014	6080			121600
	2015	4300			86000
	2016	1860			37200

In general all data obtained for 10 year period evidence about Microsal safety for fish and at it's correct application (according to the instructions) the reasonable benefit/risk ratio is maintained.
Microsal is packed by 20 kg in paper bags which are put in polyethylene bags. This agent amount is sufficient for preparation of one ton of medicated feed.
According to the results of our work The Federal Service for Veterinary and Phytosanitary Supervision issued the State Registration Certificate on Microsal as an agent for application against cestodoses of Cyprinidae in ponds (Skachkov D.P., 2013, 2015).

At cage fish farms to prevent reinfection with Bothriocephalus spp. fish should be transferred to other cages in a day after treatment at water temperature higher 16°C and on day 3-5 at the water temperature 14°C and lowcr. At farms with high incidence of Bothriocephalus infection a complex of other measures according to the corresponding regulation documents can be carried out along with treatment by Microsal. Restrictions are cancelled and farms are considered to be safe for Bothriocephalus inlection if no infected fish are revealed during parasitological examinations throughout a year.

CONCLUSION

It can be concluded that the obtained results of our ten-year work evidence microsal's safety for fish and at it's current application (according to the instructions) the reasonable benefit/risk ratio is maintained in all cases.

REFERENCES

Muzykovsky A.M., Skachkov D.P., Zhukov N.I., Parpalak E.S. // Cyprinocestin-2 at cestodoses of carps. J. Veterinariya . – M., 1987. – N 10. – pp. 34-36.

Skachkov D.P., Muzykovsky A.M., Zabudsky S.A., Zhukov N.I., Aksenova I.N., Sadovoj A.V. // Cyprinocestin-2 at bothriocephalosis in carps. J. Veterinariya. – M., - 1990. – pp. 42-44.

Skachkov D.P., Gorokhov V.V., Borisova M.N., Samarin N.I., Altkseeva N.B. // Usage (application) of microsal at bothriocephalosis in carps at termal cage fish farms. J. Veterinariya. – M., 1995. – N 9. – pp. 38-40.

Skachkov D.P. // The experience of application of microsal at cestodoses of carps at the pond farms. Materials of reports of scientific conference: Theory and practice of control of parasitic diseases. M., 2008. – issue 9. – pp. 442-444.

Skachkov D.P, Arkhipov I.A. // Control of cestodoses in pond carp fish. J. Fish farming. – M., 2009. – N. 1. – pp. 46-48.

Skachkov D.P., Pavlovich G.M. // Microsal at cestodoses in pond carp fish. J. Fish farming. – M., 2012. – N. 2. – pp. 40-41.

Skachkov D.P. // Results of monitoring of safety in application of microsal at cestodoses of fish. Materials of reports of scientific conference: Theory and practice of control of parasitic diseases. M., 2013. – issue 14 . – pp. 369-371.

Skachkov D.P // Fenasal formulations for control of cestodoses in pond carp fish. J. Veterinariya. – M., 2015. – N 4. – pp. 40-43.

Original scientific paper
10.7251/AGRENG1802097Q
UDC 631.115.11(436)

FAMILY FARMS IN AUSTRIA, ITALY AND POLAND

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ABSTRACT

Recently there has been a surge of interest in family farms – in particular because 2014 was declared by the United Nations as the International Year of Family Farming. This focus on family farms is mainly a reaction to several trends, such as economic pressures, large-scale land grabbing or the restructuring of agrifood chains, etc. Yet it has to be acknowledged that the changes taking place in agriculture and agrifood systems put into question the role of family farms. This paper responds to the need for a review of family farms by examining the situation in Austria, Italy and Poland. It uses the legal form 'sole holder' as the criteria to identify the family farm. It analyses the status of family farms in terms of (I) numbers, area cultivated, livestock and labour force, (II) their contribution to nutrition and food security, and (III) their consideration within the relevant agricultural policies. The situations in Austria, Italy and Poland are outlined using official agricultural census data, 2013. The results show that family farms are by far the prevailing form of agriculture in these three countries. Furthermore, we explore the country specific characteristics within the policy environment given in which family farms operate and how this policy supports them. Finally, this form of farm poses significant challenges for food production and systematic policy design. We conclude by giving some suggestions on future perspectives and the areas for further research.

Keywords: *Austria, family farms, Italy, Poland, policy aspects*

INTRODUCTION

The family farm is an icon of Austria, Italy and Poland. It embodies the disparate character of traditional agriculture. Moreover, the family farm embodies firstly the perceived independence, autonomy and resilience of a form of farming, secondly the intricate meshing of work and family life and finally a location for production (Gasson and Errington, 1993; Hill, 1993). Yet, because of the changes taking place in agriculture and agrifood systems worldwide family farms are now receiving global attention. There is a renewed interest in family farms – especially from the political point of view. The United Nations International Year of Family Farming 2014 was created (De la Campos and Garner, 2014). In this context, family farms

have been widely analysed and discussed by both research and political bodies (*e.g.* Davidova, Thomson, 2013, 2014; European Parliament, 2014a,b; FAO, 2010, 2015, 2018; Graeub *et al.*, 2016; Parlinska and Parlinska, 2015; van der Ploeg, 2016). Therefore this paper reviews the role of family farms with regard to land cultivation, food production and food security as well as their place in the agrarian policy. For the purposes of working with available statistical data we use the legal form, *i.e.* sole holder, to identify family farms (De la Campos and Garner, 2014; FAO, 2018). In this respect family farms comprise farms belonging to a single natural person (sole holder) and functioning as full-time and part-time farms (Statistics Austria, 2018, 35). This paper uses best available estimates to date, firstly, of the percentage of family farms and further structural characteristics and secondly the calories produced and distribution channels used by family farms in the countries sampled. We attained results quite similar to those obtained by Eurostat (2018) and Graeub *et al.* (2016). And thirdly, the situation raises the question of whether there exist or whether there is a need for context-specific policies for family farms. Thus, to illustrate the complex challenges facing family farms, we profile the policy environment in the countries sampled. The focus and importance of the role of family farms in the national policy and agrarian model depends on the respective policy orientation of the time. Finally, we propose a number of issues related to family farms to be examined in more depth. They appear necessary in order to meet the challenges of food security and policy advice more effectively in terms of a sustainable, smart and resilient European Model of Agriculture.

MATERIAL AND METHODS

This paper builds on the prior analysis by Davidova and Thomson (2013, 2014), Graeub *et al.* (2016), Parlinska and Parlinska (2015), van der Ploeg (2016). The terms family farms, family farmer and family holding can be used interchangeably. Family farms do not constitute a statistical category. In response to practical constraints given we identify family farms for Austria, Italy and Poland by drawing on the best available data in the agricultural statistics. In the Farm Structure Survey, Eurostat differentiates three types of legal form: sole holder, group holding (partnership) and legal entity (Davidova and Thomson, 2014, 16). Normally, the family farmer is a sole holder, often (but not always) registered for statistical and policy purposes as a farmer but not constituting a legal business entity (Davidova and Thomson, 2013, 13). We distinguish between family farms defined by a sole natural person and others such as group holding and legal persons. This is one definition for family farms (*c.f.* Graeub *et al.* 2016) that is also used by FAO (De la Campos and Garner, 2014; FAO, 2018) and Statistics Austria (2018, 35). While not a perfect measure for family farms the sole holder criteria identifies family farms in such a precise way that further statistical evaluations are well possible for EU Member States. Austria, Italy and Poland were chosen because of the time of their accession to the EU, the high proportion of family farms and their specific family policy. The data used for Austria, Italy and Poland has been derived from the

agricultural statistics 2013, *e.g.* EU's Farm Structure Survey (Eurostat, undated). The parameters analysed are the number of farms, the utilized agricultural area (UAA), livestock units (LSU) and labour force (total and family in annual working units, AWU), household consumption and rural development support. The empirically based estimates for the potential contribution of family farms to food production are based on the calculation of the country's Average Dietary Energy Requirements (ADER) by Graeub *et al.* (2016). This calculation estimates how many calories were produced by family farms in the countries sampled. To learn more about country specific needs we look at the policy environment in Austria, Italy and Poland. In composing these policy profiles, we follow research done on family farming and the policy environment derived from existing literature (Bélières *et al.*, 2015; Graeub *et al*., 2016, Davidova and Thomson, 2013), alongside our own extensive experience and research in each country.

RESULTS AND DISCUSSION

Structural profile

Family farms are central to the agriculture in Austria, Italy and Poland. In 2013, as shown in Table 1, family farms by the natural person sole holder account for 94.0% of 140,430 farms in Austria, 98.6% of 1,010,330 farms in Italy and 99.7% of 1,429,010 farms in Poland. These farms operate in Austria on 87.4% of 2,726,890 ha UAA, in Italy on 89.6% of 12,098,890 ha and 90.9% of 14,409,870 ha in Poland. This broadly confirms the findings of Eurostat (2018), Graeub *et al.* (2016) and Lowder *et al.* (2014). Furthermore, the labour force in agriculture is about 111,160 AWU in Austria, 816,920 AWU in Italy and 1,918,550 AWU in Poland. Considering all of the farms in Austria, family farms employ 91.9% of the national agricultural labour force. 83.6% of this is made up of family members. In Italy these figures are 95.3% and 75.5% and in Poland 98.3% and93.8% respectively. In Austria 2,439,090 LSU are kept on farms, in Italy 9,374,270 and in Poland 9,164,570. Family farms rear 95.1% of all livestock (in terms of LSU) in Austria, 90.2% of the LSU in Italy and about 91.0% of the LSU in Poland. (Table 1)

Within Austria, Italy and Poland as in the European Union (Eurostat, 2018) family farms are the dominant, although not the exclusive, form of farming. Family farms still dominate the structure of agriculture in Austria, Italy and Poland in terms of their numbers, their UAA, their contribution to agricultural employment and the keeping of animals. This strong presence of the family farming business can be explained. Family farms have grown over centuries and stand for tradition. There are a number of different areas of development expected for family farms in the future (Davidova, Thomson, 2014; European Parliament, 2014a,b; FAO, 2010). As the development of the past shows, a certain percentage will have to give up agriculture (Eurostat undated). The surviving full-time and part-time farms will have different focuses due to, for instance, liability or social security, work organisation, orientation of production and services as well as food security.

Table 1. Share (%) of family farms by select structural data, Austria, Italy and Poland 2013.

County	Farms	UAA	LSU	AWU total	AWU family
Austria	94.0%	87.4%	95.1%	91.9%	98.6%
Italy	98.6%	89.6%	90.2%	95.3%	100.0%
Poland	99.7%	90.9%	91.0%	98.3%	100.0%

*Source: Eurostat (undated).

Nutrition and food security

The provision of nutrition and food security, as a condition related to the ongoing availability of food, is an important function of family farms (FAO, 2014, IX). Family farms are the main producers of foodstuff that feeds billions of people (Graeub *et al*., 2016). Furthermore, family farms have been acknowledged as a key link in the effort to build nutritious food systems that allow people to lead healthy, productive lives, as well as being a cornerstone in the global fight against poverty (FAO, 2014).

The importance of family farms in Austria, Italy and Poland is emphasised by the fact that most agricultural products are produced by such farms. Family farms have traditionally accounted for a very large share of total agricultural produces for food (Graeub *et al.*, 2016). Using country-level averages of sufficiency by family farms, Poland scores the highest (174.2%) — even when limiting maximum sufficiency levels to 100% of the country's Average Dietary Energy Requirements (ADER), followed by Austria (133.1%) and Italy (65.9%). Poland and Austria stand out with comparatively high levels of sufficiency while Italy reaches 65.9% sufficiency levels with family farms. Climate, cultural, regional and economic conditions explain Italy's ADER sufficiency levels (Ciani, 2018). This follows a historical trend and is confirmed by Graeub *et al.* (2016). To further substantiate our empirically based estimates for the potential contributions of family farms to food production, we looked at household consumption. As reported by Eurostat (undated) 37.7% of family farms in Poland and 13.1% in Italy also produce for their own household consumption; in Austria this data is not collected. Although Poland still has a very high percentage producing for the family's sustenance this is no longer the family farmer production strategy. In developing countries there is an explicit reference here to agricultural production for on farm consumption (Bélières *et al.*, 2015, 73). Beside the household consumption 75.6% of family farms in Poland[5] (GUS, 2017), 15.6% in Austria (Statistics Austria, 2018, 100) and 11.0% of Italian family farms (Italian Institute of Statistics, 2018) sell their products directly to the consumer. In more detail, according to Eurostat (undated) 4.1% of the family farms in Austria, 9.1% in Italy and 17.5% in Poland sell more than half of their production directly to the consumer. Direct sales are practised by the majority of family farms and play a very minor role for non family farms.

[5] Over 1 ha for the data on any size of direct sale.

Family farms embody a strategic pillar that contributes to nutritional value and food security in the countries sampled. In Italy and Poland they still provide food for their own households. In Poland the contribution to the revitalisation of the local market (direct sales) is very high. Having direct connection to people, the farmer becomes more sensitive to the needs of consumers. In Austria and Italy direct sales are relatively small and in these countries consumers are mainly reached using just a few agents along the way. In this context family farms are currently considered a guarantee of nutrition and food security.

Table 2. Contribution (%) of family farms to nutrition and food security, Austria, Italy and Poland 2013.

County	% adequacy of ADER produced by	% adequacy of ADER	Direct sales – more than 50% of production	Household consumption
Austria	133.1%	100.0%	4.1%	0.0%
Italy	65.9%	66.0%	9.1%	13.1%
Poland	174.2%	100.0%	14.9%	37.7%

*Source: Eurostat (undated).

Policy aspects

Family farms are a key element of the European Model of Agriculture (Davidova and Thomson, 2014). In this context supporting the family farm has been at the centre of the Common Agricultural Policy (CAP) since its foundation (European Parliament, 2014a, 9).

CAP Pillar I or direct payments policies have been designed to support the economic viability of farms (European Parliament, 2014a, 17f). CAP Pillar I schemes, mainly decoupled direct payments, have transferred significant funds to family farms. The decoupled direct payments also act as an income stabilisation tool by reducing the exposure to market and production risk and have an influence on credit issues. (European Parliament, 2014a, 18)

Unlike Pillar I, Pillar II programmes are more targeted to supporting specific policy objectives. They provide support aimed at improving the competitiveness of farming and forestry, protecting the rural environment and maintaining sensitive farming activity, diversifying the rural economy and promoting quality of life for rural inhabitants (European Commission, 2017). As programmes and payments are not typically linked to farm size, Pillar II payments do not suffer from the same scale effects as Pillar I payments (Davidova and Thomson, 2013). In 2013, 83.1% of Austrian family farms with 96.0% of the UAA cultivated benefitted from the Rural Development Programme. In contrast, to a lesser degree, 13.3% of Italian family farms that cultivate 34.8% of UAA and 11.4% of family farms in Poland farming 34.2% of the total UAA participated in the Rural Development Programme. (Eurostat, undated)

Country specifics

The GAP provides a common framework and agriculture is a "State affair". The public policies implemented do not necessarily and solely depend on the general characteristics of the family farms in question, or their specific economic, environmental, social and cultural contributions. Across the various Member States there are certain constellations within the national policies that enhance family farms. This is discussed in further detail for Austria, Italy and Poland below.

In Austria the agricultural policy is geared to farms held by a natural person as a sole holder. In this paper family farms are defined by this criterion. These farms are at the centre of rural areas and the agricultural policy (Agrar-Europe, 2018). Wherever possible Austrian agricultural policy takes into account the specificities of this type of farm. Some Austrian specificities are (I) flat-rate system (the profit of non-bookkeeping farmers up to a taxable value of 65,500 euros has to be determined by average rates.), (II) support for diversification (*e.g.* direct sales, social farming) and food quality activities and (III) support for young farmers taking over a farm or business start-up in agriculture.

In Italy, article 41 of the Constitution and article 2135 of the Civil Code promote and guarantee agricultural activity. According to the Stresa Agreement of 1958 the pillar of the European agriculture and, of course of Italy, is the Family Agriculture Entrepreneurship. In the last decades the multifunctional and integrated farms with part time management have been growing. In accordance with the CAP regulations on rural development, Italy established a national law supporting young farmers aiming to ensure the sustainability of family farms.

The position of family farms in Poland is guaranteed constitutionally. Article 23 of the Constitution of the Republic of Poland says that "*The basis of the state's agricultural system is the family farm* ..." (Konstytucja Rzeczypospolitej Polski, 1997). The Operational Program, which provides the widest support to this kind of farm, is the Rural Development Program for 2014 - 2020 (PROW 2014 - 2020). Among others, the program provides support for starting a business activity, support for modernisation of farms and a special bonus for non-agricultural activities to diversify sources of income, a bonus for young farmers and others (Ministry of Agriculture and Rural Development, 2017). Another way to support is the farmers' rights, 1 January 2017, to direct sales allowing farmers to sell not only unprocessed but also processed goods. The farmers deciding for direct sell are exempt from income tax to the sales value of 20000 PLN (~4,611 euros). Exceeding this amount, they are obliged to pay a 2% flat-rate tax. Furthermore, a Strategy for Responsible Development 2020 (with a prospect until 2030) (2017), a main middle term strategy document published by the Polish Council of Ministers defines small and medium farms as the basis for Polish agriculture. The document announces a loan line to support investments that are not eligible for support from public funds, it also indicates high quality food production as a one of the strategic goals, what cannot be achieved without the engagement of family farms.

Austria, Italy and Poland provide country-specific strategies targeted to family farms. This is done with policy approaches that aim to address the corresponding challenges of addressing economic development, food insecurity and improving rural livelihood.

CONCLUSION

Family farms have a very long tradition in Austria, Italy and Poland and remain by far the most common operational model for farming. The renewed interest in and the continuing existence of family farms as a main actor in the field of land management, food security and agricultural policy is certainly not solely due to the high proportion of family farms over the total number of total farms or the International Year of Family Farming 2014. It can also be assumed that agricultural politicians use this term to define a target group whose services they recognise and whom they want to encourage to continue. In addition, politicians expect a higher contribution to a sustainable, smart and resilient European Model of Agriculture from family farms than from other forms of production organisation, *e.g.* capitalist firms. Broadening the spectrum and potential of policies to support family farms first of all entails affirming their recognition as a form of production. Looking at the situation in three European countries, this paper has conclusively shown the importance of family farming and emphasises the need for policy to support the latter if it is to continue to provide for a food security within Europe.

The findings show that today's family farms conforming to the European model of agriculture are important and can greatly complement larger scale commercial agribusinesses. In order to achieve this, targeted agricultural and rural development policies still need to be further developed. Targeted agricultural policies should help semi-commercial and commercial family farmers upgrade their knowledge and skills, practice environmentally sound agricultural practices, become more integrated in value chains, and thus enhance their productivity and competitiveness with regard to consumers' needs.

The paper has allowed an initial review, but has also raised new questions worth subsequent development. The field of research is vast and would allow a renewed approach to family farms and sustainable agricultural as well as rural development. Much empirical and systemic research remains to be done to (I) document the contribution of family farms to inclusive sustainable development and food security in terms of quality and the diversity of the food produced, (II) analyse the impacts on sustainable development of the different forms of organisation of agricultural production, (III) understand the precise limits and contextual dependencies of family farms in terms of innovation in food products and technology, (IV) build on the different approaches used in designing and implementing policies on family farms, and (V) identify successful principles that could be shared in the policies of different countries.

REFERENCES

Agrar-Europe (2018). EU-Agrarpolitik muss bäuerliche Familienbetriebe stärken. EU-Nachrichten 22/18, 28. Mai 2018. [EU agricultural policy need to strengthen family farms]

Bélières, J.-F. et al (2015). Family Farming Around the World. Definitions, contributions and public policies. https://agritrop.cirad.fr/576161/1/Belieres_Bonnal_Bosc_Losch_Marzin_ Sourisseau-Family_farming-AFD-2015.pdf.

Ciani, A. (2018). Own research. Perugia.

Davidova, S., Thomson, K. (2014). Family Farming in Europe: Challenges and Prospects. Brussels. http://www.europarl.europa.eu/RegData/etudes/note/join/2014/529047/IPOL-AGRI_NT%282014%29529047_EN.pdf.

Davidova, S., Thomson, K. (2013). Family Farming: a Europe and Central Asia Perspective. Background Report for Regional Dialogue on Family Farming: Working towards a strategic approach to promote food security and nutrition [Draft]. Brussels. http://www.fao.org/3/a-i6536e.pdf.

De la Campos, A.P., Garner, E. (2014). Identifying the "family farm" An informal discussion of the concepts and definitions. http://www.fao.org/3/a-i4306e.pdf.

European Commission (2017). Previous rural development programming periods. https://ec.europa.eu/agriculture/rural-development-previous/2007-2013_en.

European Parliament (2014a). CAP 2014-2020 Tools to enhance family farming: opportunities and limits. http://www.europarl.europa.eu/RegData/etudes/note/join/2014/ 529051/IPOL-AGRI_NT(2014)529051_EN.pdf.

European Parliament (2014b). Farming in Europe: challenges and prospects. http://www.europarl.europa.eu/RegData/etudes/note/join/2014/529047/IPOL-AGRI_NT(2014)529047_EN.pdf.

Eurostat (2018). Agriculture statistics – family farming in the EU. http://ec.europa.eu/eurostat/statistics-explained/index.php/Agriculture_statistics_-_family_farming_in_the_EU.

Eurostat (undated). Agricultural statistics. http://ec.europa.eu/eurostat/web/agriculture/overview.

FAO (2018). Family Farming Knowledge Platform. Countries & Regions. http://www.fao.org/family-farming/countries/en/.

FAO (2015). Innovation in family farming in Europe and Central Asia. http://www.fao.org/3/a-mo296e.pdf.

FAO (2014). The state of food and agriculture 2014: Innovation in family farming. Rome: FAO. http://www.fao.org/3/a-i4040e.pdf.

FAO (2010). Challenges for succession in family farming – Perspective and research question.

http://www.nordlandsforskning.no/getfile.php/Dokumenter/Arbeidsnotater/2010/Notat_1007_2010.pdf.

Gasson, R., Errington, A. (1993). The Family Farm Business. Wallingford, UK: CAB International.

Graeub, B.E., Chappell, M.J., Wittmann, H., Ledermann, S., Bezner Kerr, R., Gemmill-Herren, B. (2016). The State of Family Farms in the World. World Development Vol. 87: 1-15. http://dx.doi.org/10.1016/j.worlddev.2015.05.012.

Gray, J. (1998). Family farms in the Scottish Borders: a practical definition by hill sheep farmers. Journal of Rural Studies, Vol. 14(1): 341-356.

Hill, B. (1993). The 'Myth' of the Family Farm: Defining the family farm and assessing its importance in the European Community. Journal of Rural Studies, Vol. 9(4): 359-370.

Italian Institute of Statistics (2018). Household consumption 2013. Oral communication.

Lowder, S.K., Skoet, J., & Singh, S. (2014). What do we really know about the number and distribution of farms and family farms in the world? Background paper for The State of Food and Agriculture 2014. ESA Working Paper No. 14-02. Rome: FAO. http://www.fao.org/docrep/019/i3729e/i3729e.pdf.

Parlinska, M., Parlinska, A. (2015). Characteristic of family farming in the European Union. http://web.a.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=16913078&AN=102910747&h=hC5dPNXGcXNQOXtCGatXgFOd1vhojbbwhuK0NO94ifx0gvBn1QY%2fOHHIJnQGjDvP1xNeEQVVBQJ7BC49R6ByCQ%3d%3d&crl=f&resultNs=AdminWebAuth&resultLocal=ErrCrlNotAuth&crlhashurl=login.aspx%3fdirect%3dtrue%26profile%3dehost%26scope%3dsite%26authtype%3dcrawler%26jrnl%3d16913078%26AN%3d102910747.

Statistics Austria (2018). Agrarstrukturerhebung 2016. Wien. [Farm Structure Survey]

van der Ploeg, J.D. (2016). Family farming in Europe and Central Asia: history, characteristics, threats and potentials. Working paper No. 153. http://www.fao.org/3/a-i6536e.pdf.

GUS, Central Statistical Office (2017). Charakterystyka gospodarstw rolnych w 2016. Informacje i opracowania statystyczne, Warszawa. [Characteristics of the Farms 2016].

Konstytucja Rzeczypospolitej Polski (1997), [The Constitution of the Polish Republic].

Ministry of Agriculture and Rural Development (2017), Program Rozwju Obszarów Wiejskich na lata 2014-2020, Ministerstwo Rolnictwa i Rozwoju Obszarów Wiejskich, Warszawa.

Strategia na Rzecz Odpowiedzialnego Rozwoju do 2020 (z perspektywą do 2030), (2017) [A Strategy for Responsible Development 2020 (with a prospect until 2030)] Polish Council of Ministers, Warszawa.

Original scientific paper
10.7251/AGRENG1802106F
UDC 63:556.18(55)

BARRIERS TO FARMERS' PARTICIPATION IN ESTABLISHING WATER USER ASSOCIATIONS: THE CASE OF IRRIGATION AND DRAINAGE NETWORK OF NORTHEAST AHWAZ, IRAN

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ABSTRACT

Concern about water resources in semi-arid areas of the world has led to the introduction of a participatory management system of water, which potentially challenges farmers' willingness to involvement. Establishing water user associations has altered the water management system in irrigation and drainage networks. Undoubtedly, promoting these changes in rural areas, where the new social changes are slowly accepted, is encountered with various obstacles. As such, this study was conducted to recognize the impediments of establishing water user associations through the eyes of those working in an irrigation and drainage network. Data were collected through a questionnaire which consisted of questions regarding social, financial, cultural, organizational, management and attitude barriers. Analysis of data revealed that management barriers were ranked at the first place followed by the cultural, attitude and social ones. However, lack of motives to stimulate users into participatory system of water management, farmers' inabilities to combat with those who illegally extract water, inability to equal and justice-based allocation of water to different users, farmers' preferences to instant individual advantages instead of future common advantages, negative attitude of farmers toward efficacy of local associations, lack of informative opportunities for making farmers aware of the WUAs' benefits were recognized as the strongest barriers, respectively. The results also showed that there was significantly positive correlation among four categories of barriers including social, cultural, management and attitude. This means that they were interrelated and any intervention to change one could affect the others. Hence, to initiate involvement of the local people into decentralized systems of water resources management, salient attempts are needed to empower farmers for removing the barriers, mainly management and social.

Keywords: *Water user association, participation, barriers, Iran.*

INTRODUCTION

Water shortage mainly due to reduced rain, mismanagement or poor management of users has made principal concerns for governments to solve supply problems. Eventually, concern about agricultural water resources, especially in arid and semi-arid areas of the world, has led to the introduction of a participatory management system of water, which potentially challenges farmers' willingness to involvement. Many countries have adopted reform policies such as transferring rights and responsibilities of irrigation systems from government agencies to farmers' associations and other private institutions (Qiao *et al*., 2009). Irrigation management transfer and the creation of Water User Associations (WUAs) seemed to be a promising solution to reduce conflicts, to make water management more efficient and to keep up the irrigation infrastructure (Wegerich, 2008). Lopez-Gunn (2003), in this respect, pointed out that WUAs can play an important role in facilitating factors that encourage collective action. In addition, they can increase the political capital among small land holders, as well.

Generally, a water user association (WUA) is empowered to maintain and manage the irrigation and drainage system and to collect fees to cover its expenses (Qiao *et al*., 2009). Mustafa *et al*. (2016) insisted that farmers nevertheless prefer WUAs because they are helpful in gaining access to patronage and water. Establishing such associations has altered the water management system in irrigation and drainage networks. It is widely believed that direct participation in irrigation management by farmers is an effective way of improving farmers' knowledge of irrigation and efficiency of water use (Qiao *et al*., 2009). Undoubtedly, promoting these changes in rural areas, where the new social changes are slowly accepted, is encountered with various obstacles. Taking this into account, it is questionable what factors would be kept in mind as the inhibitors of establishing WUAs. Gholamrezaei *et al*. (2014) stated that five factors including inattention of government authorities, unsound water allocation mechanism, lack of negotiation with farmers as the users, impartial rules and lack of commitment among authorities are the most important political-administrative impediments of farmers' tendency to establish WUA. Ataei and Izadi (2014) also in their study on comparison of WUAs' adopter and non-adopter farmers recognized that farmers who were agree with involvement in WUAs showed more positive attitude and higher information level about WUAs, more amount of trust, solidarity and social participation, less experiences of conflict over water with peer, and less satisfaction of governmental authorities' function regarding water allocation. Moreover, other studies (Khanal, 2003; Lopez-Gunn, 2003; Omid *et al*., 2012; Wegerich, 2008) indicated that government central role in water allocation, negative attitude of authorities for transferring the power to local community, infrastructural inefficacy of irrigation and drainage networks, inequality in water distribution, lack of trust to association's management committee, low financial supports either by government or farmers, and top-down hierarchical structures which refer to management instead of governance, hamper the establishing of WUAs. Hence, one strand of the literature suggests that the major of studies done to identify the inhibitors or drivers

of WUAs' creation have been focused on the water users' perspectives, while another strand highlights the contribution of various factors to prevent establishing WUAs. As such this study was conducted to recognize the impediments of establishing WUAs through the eyes of those working in an irrigation and drainage network. To do this, the impediments were classified into six major factors including social, financial, cultural, management, organizational and also attitude, as a separate category.

In Iran WUAs dominantly deal with surface water distribution. Khuzestan province, with an area equal to 64,057 square kilometers in southwest of Iran, has third of total surface water resources of the country. While, five main rivers of Karoon, Dez, Karkheh, Maroon and Zohreh-Jarahi and fertile lands are the most important natural features in this province, high levels of land salinity and water table are the limiting factors for agricultural activities which are privileged in four seasons of the year. Development of agriculture in this region with saline soils, ground water and climatic conditions requires design, implementation, operation and maintenance of the irrigation and drainage networks (Golabi *et al*., 2017). Irrigation and drainage network of Northeast Ahwaz in Khuzestan provides irrigation water for 19510 ha of farmlands through canals which have been built since 1998. No water user association or at least a local entity has been established up to now, for managing and monitoring the water distribution as well as safekeeping the canals and other infrastructures. All of these tasks routinely are performed by the staff of irrigation and drainage network agency.

MATERIALS AND METHODS

Data was collected in November 2017 using a questionnaire. All the staff working in the irrigation and drainage network of Northeast Ahwaz in Khuzestan Province, Iran, was surveyed leading to a final number of 30 persons. The view of respondents was measured on six factors which may hamper the establishing of WUAs in the region. Our questionnaire was classified into two parts: personal characteristics of respondents and barriers. In total, to measure the barriers which composed of social, financial, cultural, organizational, management and attitude aspects, 29 items were used. A likert scale rating from 1 to 5, reflecting very low to very high respondents' agreement, was applied. A number of socio-demographic questions were asked covering age, education, work experience and residency in rural areas. All of the respondents were male. Data were analyzed using SPSS software.

RESULTS AND DISCUSSION

While 80 % of respondents had academic degree, about 67 % of them was younger than 40. Almost most of them lived in urban areas and only near 17 % were inhabitant of rural. All of the respondents worked in the irrigation and drainage network agency for more than 5 years. About 67 % of our respondents have experienced working in the irrigation and drainage network agency for more than 10 years.

In the following sections, the respondents' view regarding each category of barriers have been analyzed and explained in detail.

Social barriers heavily depend on the context in which farmers live and relate with each other. Our findings showed that the most important barriers to establish a WUA in the study area, respectively, are "lack of motives to stimulate users into participatory system of water management ($\bar{X}$= 4.47)", "farmers' reluctance to financial participation in protecting of canals and infrastructures ($\bar{X}$= 3.92)", and "illegally water extracting from river and canals ($\bar{X}$= 3.85)" (Table 1). In fact, lack of policies that give farmers incentives to involve in participatory water management was highlighted by our respondents. As Wegerich (2008) insisted transferring rights and responsibilities of irrigation systems from government agencies to farmers' associations should not imply rapid and complete withdrawal of the state. In line with this, government bodies must pay more attempts on preparing an enabling environment which contain providing incentive and motives for participation. Unexpectedly, "farmers' non-commitment to pay water charge ($\bar{X}$= 2.85)" ranked at the last place (Table 1), showing that if farmers are supplied with a fair share of the water in a timely manner, they will incline to pay for it. Because they already have to pay for water are supplied for them during the planting season from the irrigation and drainage agency which administered totally by the state.

As demonstrated in Table 1, our respondents highlighted if farmers are motivated to establish a WUA, the most important financial barriers which hamper them are "farmers low income ($\bar{X}$= 3.66)" following by "no need to establish an entity to distribute water due to enough access to available water ($\bar{X}$= 3.28)". As pointed out by Lopez-Gunn (2003), while solutions like subsidies and payments can help mitigate aquifer overuse, these are not a long-term or sustainable option. Therefore, financial support by farmers is crucial. On the other hand, clearly stated by our respondents that as long as water is readily available for farmers and they have to pay just the water charge, they will be reluctant to establish an entity which enforced them into excessive costs. Moreover, water charge, in practice, is estimated rather than calculated by cubic meter. From our respondents' view, in compare to other items, "diversity of current water resources which obviate need to establish a WUA ($\bar{X}$= 2.85)" could not be a substantial obstacle, because there were no diverse water resources in the region.

Table 1 also shows a descriptive statistics of the items used to measure cultural barriers. Findings indicated that although "lack of informative opportunities for making farmers aware of the WUAs' benefits ($\bar{X}$= 3.90)" ranked at the first place, other items also gained a nearly similar mean scores. This reflects that all the items which mainly focused on lack of awareness about benefits, function and task of WUAs due to absence of educational opportunities and information sources are moderately important as the cultural barriers in the study area.

Considering barriers listed in Table 1, "non-autonomy of WUAs in water management due to government interference in affairs ($\bar{X}$= 3.04)" was regarded as the most important factor which hamper establishing a WUA from an

organizational point of view. As noted by Lopez-Gunn (2003), only sound institutional design of WUAs can favor self-governance and management by farmers. When farmers or WUAs are not involved "in decision making on water allocation, hence farmers are supposed to pay for a service which does not seem to give the farmers freedom in terms of quantity and timing of water delivery" (Bucknall *et al*., 2001; cited in: Wegerich, 2008, p.46). After that, the items "undesirable experiences of previous activities of water organization ($\overline{X}$= 2.47)" and "no need to establish the WUA due to existence of parallel rural production cooperatives ($\overline{X}$= 2.24)" ranked at the second and third places, respectively. However, these items were not considered as the potentially strong barriers, our respondents explained when farmers are not paid compensation instead of losses they experience because of inefficient irrigation services, they will not trust to the water organization or any entities which would be in charge of water distribution.

From the attitudinal perspective, "farmers' preferences to instant individual advantages instead of future common advantages ($\bar{X}$= 4. 28)" following by the "negative attitude of farmers toward efficacy of local associations ($\bar{X}$= 3.95)" were perceived as the most principal barriers (Table 1). However agricultural extension and education programs could make great changes in rural areas of Iran, the current attitude dominated among rural trace the Rogers' thoughts which marked farmers as those who prefer dependency to governmental authority and instant advantage instead of future advantages.

Lastly, the most significant management barriers which negatively affect farmers' willingness to participate in a water entity were the "inability of farmers to combat with those who illegally extract water ($\overline{X}$= 4. 42)", and "inability of farmers to equal and justice-based allocation of water to different users ($\overline{X}$= 4.38)", respectively (Table 1). These findings are strongly supportive of those stated as the social, attitude and to somewhat cultural barriers.

A precise examination of the findings indicated in Table 1 revealed that management barriers ($\bar{X}$= 4. 07 out of 5) were ranked at the first place, as the most powerful barrier, followed by cultural ($\bar{X}$= 3.79 out of 5), attitude ($\bar{X}$= 3.72 out of 5), social ($\bar{X}$= 3.71 out of 5), financial ($\bar{X}$= 3.19 out of 5) and organizational ($\bar{X}$= 2.45 out of 5) ones, respectively. Although cultural, attitude, and social barriers gained almost a same mean score showing a moderate importance, the financial and organizational barriers revealed a relatively weak mean score in terms of importance.

Table 1. Respondents' view regarding different categories of barriers

	Item	Mean	SD	Rank
Social barriers	lack of motives to stimulate users into participatory system of water management	4.47	0.60	1
	farmers' reluctance to financial participation in protecting of canals and infrastructures	3.92	0.92	2
	illegally water extracting from river and canals	3.85	1	3
	inter-personal conflicts between farmers over water	3.71	0.71	4

	weakness of farmers in operating collective actions	3.61	0.74	5
	conflicts among neighbor rural areas	3.57	0.74	6
	farmers' non-commitment to pay water charge	2.85	1	7
	Mean	**3.71**	**-**	**-**
Financial barriers	farmers' low income	3.66	0.65	1
	no need to establish an entity to distribute water due to enough access to available water	3.28	0.84	2
	insufficient land ownership amount	3.14	1.38	3
	lack of credits and financial facilities	3.04	1.02	4
	diversity of current water resources which obviate need to establish a WUA	2.85	0.91	5
	Mean	**3.19**	**-**	**-**
Cultural barriers	lack of informative opportunities for making farmers aware of the WUAs' benefits	3.90	1.04	1
	low educational level of farmers	3.80	1.16	2
	farmers' ignorance about the function and tasks of WUA	3.76	1.26	3
	lack of access to and contact with information sources	3.71	1.10	4
	Mean	**3.79**	**-**	**-**
Organizational barriers	non-autonomy of WUAs in water management due to government interference in affairs	3.04	1.11	1
	undesirable experiences of previous activities of water organization	2.47	1.07	2
	no need to establish the WUA due to existence of parallel rural production cooperatives	2.24	1.23	3
	opposition of water organization authorities for establishing WUA	2.04	1.20	4
	Mean	**2.45**	**-**	**-**
Attitude barriers	farmers' preferences to instant individual advantages instead of future common advantages	4.28	1	1
	negative attitude of farmers toward efficacy of local associations	3.95	0.92	2
	lack of the proper perception of regional water scarcity among farmers	3.80	0.98	3
	elite's misinterpretations about the status of WUAs	3.61	0.92	4
	farmers' negative attitude toward the function of ex-associations in the region	2.95	1.39	5
	Mean	**3.72**	**-**	**-**
Management barriers	inability of farmers to combat with those who illegally extract water	4.42	1.07	1
	inability of farmers to equal and justice-based allocation of water to different users	4.38	1.07	2
	inability of farmers to protect canals and infrastructures from probable damages	3.90	1.17	3
	the management inability of farmers to administer the established entity	3.57	0.97	4
	Mean	**4.07**	**-**	**-**

To find which categories of barriers interrelated with others, a Pearson Correlation test was run. As Table 2 demonstrated, there were strong positive relationships between management barriers with cultural (r =0.654), attitude (r =0.626) and social barriers (r = 0.903). Expectedly, there were also strong positive correlations between cultural with attitude (r = 0.665) and social barriers (r = 0.787). As descriptive analysis of barriers revealed, there were some interrelationships between these variables; which means that they can affect each other positively.

Table 2. The relationship between different categories of barriers

Variable	X_1	X_2	X_3	X_4	X_5	X_6
Management (X_1)	1					
Cultural (X_2)	0.654**	1				
Attitude (X_3)	0.626**	0.665**	1			
Social (X_4)	0.903**	0.787**	0.625**	1		
Financial (X_5)	0.112	0.038	0.378**	0.063	1	
Organizational (X_6)	-.169	-0.277	-0.144	-0.117	0.071	1

** significant at 0.01 level

CONCLUSIONS

This study revealed that management barriers were perceived as the most significant factors which potentially hamper the establishing of WUAs among farmers. After that, cultural, attitude and social barriers were recognized as the moderate important barriers in compare to financial and organizational barriers which ranked as the relatively weak factors. In detail, lack of motives to stimulate farmers into participatory interventions regarding water management, some inabilities of farmers, for example to combat with those who illegally extract water or to equally water allocation, farmers' willingness toward instant individual advantages, negative farmers' attitude efficacy of local entities, and finally, lack of informative opportunities to aware farmers regarding the WUAs' benefits were recognized as the strongest barriers, respectively. The results also showed that four categories of barriers including management cultural, attitude and social were interrelated and any intervention to change one could affect the others. Hence, the evidence seems to suggest that to initiate involvement of the local people into decentralized systems of water resources management, salient attempts are needed to empower farmers for removing the barriers, mainly management and social.

ACKNOWLEDGEMENT

The authors wish to gratefully thank all the staff working in the irrigation and drainage network of Northeast Ahwaz who kindly agreed to participate in this study.

REFERENCES

Ataei, P., Izadi, N. (2014). Analysis of water user associations' responsibilities and establishing barriers from the users' point of views. Research on Water in Agriculture, Vol. 28(4), pp. 737-748 (in Persian).

Golabi, M., Karami, B., Albaji, M. (2017). Evaluation the performance of irrigation and drainage networks using analytical hierarchy process (AHP) (Case study; Zohreh-Jarahi and Gotvand utilization companies in Khuzestan province). Iran Water Research Journal,Vol 11 (1), pp. 73-65 (in Persian).

Gholamrezaei, S., Mousavi, Z., Rahimiyan, M. (2014). Study of policy and administrative barriers in beneficiary's participation in Keznar Dam Water use cooperative. Journal of Rural Development, Vol. 5(2), pp. 103-116. DOI: 10.22059/jrd.2013.50586

Khanal, P. R. (2003). Participation and governance in Local water Management. Irrigation and Water Engineering Group. University of Wageningen, Netherlands.

Lopez-Gunn, E. (2003). The Role of Collective Action in Water Governance: A Comparative Study of Groundwater User Associations in La Mancha Aquifers in Spain. Water International, Vol. 28(3), pp.367-378, DOI: 10.1080/02508060308691711.

Mustafa, D., Altz-Stamm, A., Mapstone Scott, L. (2016). Water User Associations and the Politics of Water in Jordan. World Development, Vol. 79, pp. 164–176.

Omid, M. H., Akbari, M., Zarafshani, K., Eskandari, Gh. H., Fami, H. Sh. (2012). Factors influencing the success of water user associations in Iran: A case of Moqan. Tajan, and Varamin. Journal of Agricultural Science and Technology, Vol. 14, pp 27-36.

Qiao, G. Zhao, L., Klein, K. (2009). Water user associations in Inner Mongolia: Factors that influence farmers to join. Agricultural Water Management, Vol. 96, pp. 822-830.

Wegerich, K. (2008). Blueprints for water user associations' accountability versus local reality: evidence from South Kazakhstan. Water International, Vol. 33, No. 1, March 2008, 43–54. DOI: 10.1080/02508060801928034.

Original scientific paper
10.7251/AGRENG1802114E
UDC 632.951

INSECTICIDAL AND ANTIFEEDANT ACTIVITY OF THE ETHANOLIC EXTRACTS FROM *ALLIUM ROTUNDUM* L.

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ABSTRACT

The species of the genus *Allium* L., one of the largest genera of higher plants, occupying a significant place in the modern plant world, are of great interest both from the theoretical and from the practical point of view. Plants of *Allium* spp. accumulate a large amount of carbohydrates, phenolic compounds (flavonoids and its glycosides, coumarins, anthocyanins, catechins), amino acids and organosulfur compounds, such as allicin – a precursor for alkaloids and saponins formation. Secondary metabolites of *Allium spp*. have been successfully used during the past few decades in plants protection against pests and pathogens. We have earlier reported about high insecticidal and antifeedant properties of extracts from *A. subhirsutum* L., *A. narcissiflorum* Vill. and *A. ramosum* L. The highest insecticidal properties against imago (20.0%) and larvae (60.0%) of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidaea) were demonstrated by extract from aerial part of *A. subhirsutum.* The purpose of this investigation was to determine the insecticidal, antifeedant and repellent properties of *A. rotundum* L. extracts against various species of insects. As a result, it was found that the ethanolic extracts from the aerial part possessed the moderate level (40.0%) of insecticidal properties against the larvae of the *L. decemlineata* and low – against the imago (6.7-13.3%). Moreover, the extracts of *A. rotundum* showed moderate level of insecticidal, antifeedant and repellent activity against the larvae of lepidopterans (cotton budworm *Helicoverpa armigera* and wax moths *Galleria mellonella*).

Keywords: *Allium rotundum L., extract, insecticidal, antifeedant, repellent.*

INTRODUCTION

The species of the genus *Allium* L. (Alliaceae J. Agardh.), one of the largest genera of higher plants, occupying a significant place in the modern plant world, are of great interest both from the theoretical and from the practical point of view. There are more than 900 species, which naturally grow in the temperate regions of the Northern Hemisphere. Representatives of the genus grow in meadows, steppes, forests (The Plant List, 2018; Block E., 2009). Wild onions are promising for use in

human economic activities; among them there are food, medicinal, honey, ornamental plants. Many of them occupy a limited area.

Plants of the *Allium* genus have long attracted the attention of a large number of researchers due to the high the content of biologically active substances (BAS) which possess a wide spectrum of action. Due to the high content of valuable BAS – steroidal glycosides and unsaturated fatty acids that were found in onions species such as: *Allium nutans* L., *A. narcissiflorum* Wells., *A. giganteum* Rgl., *A. jajlae* Vved., *A. komarovianum* Vved., *A. schoenoprasum* L., *A. schoenoprasum* L., *A. porrum* L., *A. angustifolium* L., *A. ramosum* (*odorum*) L., and because they use as a raw material for the production of valuable medicines, the active substances of which have steroid origin (Shirshova, Volkova, 2005). Many representatives of the Allium genus have demonstrated an insecticidal and antifeedant effect on phytopatogens (Elisovetcaia et al., 2012).

On the territory of Republic of Moldova, there are about 10 species of Allium, including Allium *oleraceum* L. (onion field), *Allium rotundum* L. (Valdstein's onion), *Allium ursinum* L. (onion bear) and some others that are spread wildly in natural ecosystems.

Round-headed leek (or purple-flowered garlic) *Allium rotundum* L. (Amaryllidaceae: Alliaceae) is a perennial herbaceous rhizome-bulbous plant, originally from the steppe and Mediterranean regions of Europe, Anatolia and North Africa. It was distributed in Western Asia, Central and Southern Europe, Central and Southern regions of the European part of Russia, the North Caucasus (Chadaeva, 2016). The species grows in dry sabulous steppes, on coastal sands, rocks, fields and vineyards. In the Republic of Moldova *A. rotundum* is commonly found. It is cultivated as decorative, vegetable, but can inhibit the crops of winter wheat, because its seeds fall easily to the ground. The round garlic has stems of up to 90 cm in height, bearing pink or purple flowers. Its name derives from the large rounded umbels, which are 2-3 cm across. The umbels lack bulbils, but the underground bulb is usually surrounded by purplish-black bulbils with long stalks. In addition to their nutritional effects, the antibacterial, antifungal and antioxidant activities is extensively investigated due to the content of steroidal saponins, sapogenins, steroidal glycosides and others chemicals (Assadpour et al., 2016; Isman, 2006; Maisashvili et al., 2008, 2012; Sobolewska et al., 2016). However, there is no report on pesticidal ability of extracts from this species, growing on the territory of the Republic of Moldova. For this reason, the purpose of our investigation was to evaluate the insecticidal and antifeedant activity of the ethanolic extracts from *Allium rotundum* L.

MATERIALS AND METHODS

Plant material and preparation of ethanolic extract.

Allium rotundum L. was collected from Central zone of the Republic of Moldova. Gathering of phytogenic raw materials has been made according to methods approved in botanical and biochemical researches. Aerial parts of the plant were collected during flowering stage when plants accumulated the highest quantities of

biologically active substances. Phytogenic raw materials were dried off at the temperature of 28-30°C until the hydroscopic moisture concentrations were reached 7-10% according to standard methods. Before extraction the dry materials were crushed using an electric laboratory mill (Type: MRP-1, asynchronous motor). Crushed materials were extracted by maceration using 96% ethanol for 24 hours (shaking on a laboratory horizontal shaker ARMED HY-2B). After that, the extract was separated from the residue by filtration through filter paper. The resulting extract was concentrated under vacuum to a crude solid extract which was then dissolved in 96% ethanol to obtain the alcohol extract containing 20% of solid extract. The concentration of the tested extract of A. rotundum was 2.5%.

Test objects and laboratory testing

Experiments have been made for larvae and imagoes of potato beetle – Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae) a natural population and larvae of lepidopterans – cotton budworm Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) and wax moths Galleria mellonella L. (Lepidoptera: Pyrolidae) laboratory populations.

Contact, intestinal and contact-intestinal effects of extracts have been studied. Each variant consisted of nine replications using 5 insects per replication. Young potato leaves of standard size served as a substrate for feeding L. decemlineata. Artificial diet was used to feed the larvae H. armigera and G. mellonella. In variants with the determination of intestinal and contact-intestinal effects, leaves were treated by immersion into an extract and then were stored in an exhaust box for 1 hour to complete evaporation of solvent. The leaves, treated using 12.0% alcohol solution, were served as the standard. For Lepidoptera, an artificial nutrient medium was mixed with the extract (100 µl of extract per 2 g of medium). As a standard, the mixture of 12.0% water alcohol solution with artificial medium was used. After that, prepared substrates were placed into double (Petri) dishes with insects. During the determination of contact and contact-intestinal effects the tested extract was applied topically on dorsal area of insects. Insecticidal activity was evaluated by counting determined using number of dead insects for three days in comparison with the standard according to accepted equation (Elisovetcaia, 2010, Elisovetcaia et al., 2012). Antifeedant activity of extracts was estimated three days after beginning of each experiment according to the point scale (tab. 1).

Table 1. Scale of antifeedant activity.

Browsing of leaf surface, %	Level of antifeedant activity	Points
0 to 5%	very high	1
6 to 25%	high	2
26 to 50%	moderate	3
51 to 75%	low	4
76 to 100%	very low (zero)	5

The presence or absence of the repellent activity of the extract of *A. rotundum* was established according to standard techniques (Elisovetcaia, 2010) - on the movement of insects in the opposite direction from the artificial nutrient medium mixed with the extract.

Statistical analysis of obtained data has been made according to the one-factor dispersion method (Dospehov, 1979) using Microsoft Excel software.

RESULTS AND DISCUSSION

As a result of laboratory testing, it has been revealed that the extract of *A. rotundum* is the most effective against larvae of potato beetle and lepidopterans. It has been established that the character of effects of this extract on insects, in the main, is intestinal and contact-intestinal. For the imago *L. decemlineata* and larvae *G. mellonella* no contact action of the ethanolic extract *A. rotundum* was found (tab. 2).

Table 2. Insecticidal activity of ethanolic extract from *Allium rotundum* L. against *Leptinotarsa decemlineata* Say, *Helicoverpa armigera* Hbn. and *Galleria mellonella* L.

Tested effects of extracts	Insecticidal activity, %			
	Leptinotarsa decemlineata		*Helicoverpa armigera* Larvae of age II-III	*Galleria mellonella* Larvae of age II-III
	Larvae of age II-III	Imagoes		
Contact	13.3	0	6.7	0
Intestinal	26.7	6.7	40.0	26.7
Contact-intestinal	40.0	13.3	46.7	40.0

It should be noted that observed intestinal insecticidal activity of extract against imago (6.7%) and larvae (26.7%) of *L. decemlineata* and against larvae of *G. mellonella* (26.7%) is also very low and insufficient to reduce the number of these pests to an economically advantageous level. The same effect was obtained in variants with topical treatment or contact action of the ethanolic extract from *A. rotundum* against larvae *L. decemlineata* (13.3%) and *H. armigera* (6.7%) (tab. 2). At the same time, it has been discovered that larvae of potato beetle are more vulnerable to the effects of extracts in comparison with imagoes. The death of larvae *L. decemlineata* in the variant with contact-intestinal treatment reached 40.0%, while the number of imagoes decreased by no more than 13.3%. Thus, it has been established that the ethanolic extract from the aerial part of *A. rotundum* has both intestinal and contact-intestinal action against to both the coleopterans and lepidopterans larvae, mortality of which ranged from 40.0 to 46.6%.

The results of insecticidal activity of *A. rotundum* extract are in good agreement with the data obtained earlier on the predominantly intestinal action of extract from

onion *Allium odorum* L. (Elisovetskaya et al., 2012). It was found that extract from aerial parts of the *A. rotundum* showed the same level of insecticidal activity on the larvae of Colorado potato beetle as extract of *A. narcissiflorum* (46.6%) and was significantly poorer compared to *A. subhirsutum* extract (80.0%) (Elisovetskaya et al., 2012). The higher mortality of larvae under influences of *A. subhirsutum* extract is apparently explained by the fact that the extract has both an intestinal and a contact action in contrast to *A. rotundum* extract which has only intestinal action on the *L. decemlineata*.

It was established that the highest insecticidal properties of the ethanolic extract from the aerial part of *A. rotundum* was demonstrated against larvae *H. armigera*: contact activity consisted 6.7%, intestinal – 40.0% and contact-intestinal – 46.7%.

The antifeedant properties of *A. rotundum* L. ethanolic extracts against various species of insects were on the moderate level and reached 3 point for larvae lepidopterans (*H. armigera* and *G. mellonella*) and imago *L. decemlineata.* The higher level was against larvae *L. decemlineata* and reach 2 point (tab. 3).

Table 3. Antifeedant and repellent activity of ethanolic extract from *Allium rotundum* against *Leptinotarsa decemlineata*, *Helicoverpa armigera*. and *Galleria mellonella.*

Tested effects of extracts	Antifeedant (point) and repellent activity (presence or absence)			
	Leptinotarsa decemlineata		*Helicoverpa armigera* Larvae of age II-III	*Galleria mellonella* Larvae of age II-III
	Larvae of age II-III	Imagoes		
Antifeedant Repellent	2 +	3 –	3 +	3 +

Note: "+"effect presence, "–"effect absence.

It was found that ethanolic extract from *A. rotundum* had a repellent effect on the tested species of coleopterans and lepidopterans insects, except for the imago of the Colorado potato beetle (tab. 3). The insects (larvae) moved from the treated food in the opposite direction, long circling in the Petri dishes, apparently until the action of the volatile components of the onion was reduced, or because of hunger.

As a result, it was established that the ethanolic extracts from the aerial part possessed the moderate level of insecticidal (40%), antifeedant (2 point) and repellent properties against the larvae of the *L. decemlineata* and low – against the imago (6.7-13.3%, 3 point). Moreover, the extracts of *A. rotundum* showed moderate level of insecticidal, antifeedant and repellent activity against the larvae of lepidopterans (cotton budworm *H. armigera* and wax moths *G. mellonella*).

Some differences in the degree of insecticidal and antifeedant activity of extracts from *A. rotundum* and other species of onions can be explained by their diverse chemical composition. Thus, aerial parts of the plant *A. rotundum* contains

essential oil, ascorbic acid, saponins (steroidal glycosides), saponegins (taperogen, diosgenin, hekogenin, gytogenin, b-chlorogenin, yukkagenin, agigenin) and others (Maisashvili et al., 2007; Sobolewska et al., 2016). It is known that aboveground parts of *A. subhirsutum* and *A. narcissiflorum* contain steroid glycosides (0.2-0.3%), but *A. narcissiflorum* – saponins, phenols and coumarins as well (Kintea, Degteareva, 1989; Selyutina, 2007). In addition to steroid saponins and glycosides, a plant of *A. odorum* also accumulates 0.1 to 0.3% of alkaloids (Stearn, 1992). It is most likely that the various chemical compounds in extracts explain the different nature of the action of the extracts against *L. decemlineata, H. armigera* and *G. mellonella*.

CONCLUSION

On basis of laboratory testing, it has been proved that ethanolic extract obtained from aerial parts of the plant *A. rotundum* possess insecticidal, antifeedant and repellent properties against imagoes and larvae of Colorado potato beetle (*L. decemlineata*) and larvae of lepidopterans – *Helicoverpa armigera* and *Galleria mellonella*. The character of properties and activity level directly depend on nature of biologically active substances accumulated by plants of the species *Allium* as well depend on the age of phytophagous. Taking into account the obtained data, plant *A. rotundum* can be recommended as biological insecticide for reducing the number of pests.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Chisnicean Lilia, research-coordinator of the Institute of Genetics, Physiology and Plant Protection for helping us in botanical identification of Allium species. The publication was prepared with the active participation of researchers from international network AgroBioNet, as a part of international program "Agricultural biodiversity to improve nutrition, health and quality of life". Author Dina Elisovetcaia are thanking to the International Visegrad Scholarship Fund for financial support by project no. 51700164 given possibilities to realize these researches at the Institute of Biodiversity Conservation and Biosafety and Agrobiotech laboratories, Faculty of Agrobiology and Food Resources, Slovak Agricultural University in Nitra.

REFERENCES

Assadpour S., Nabavi S.M., Nabavi S.F., Dehpour A.A., Ebrahimzadeh M.A. (2016). In vitro antioxidant and antihemolytic effects of the essential oil and methanolic extract of Allium rotundum L. Eu. Rev. Med. Pharmacol. Sci. 20(24): 5210-5215.

Block E. (2009). Garlic and other Alliums: The lore and the science. Royal Society of Chemistry. Cambridge.

Chadaeva V.A. (2016). Strategies for the life of wild species of the genus Allium L. of the Russian part of the Caucasus. Diss ... Doctor of biology. Makhachkala:

FGBUN "Mountain Botanical Garden" of the Dagestan Scientific Center of the Russian Academy of Sciences. [in Russian]

Dospehov B.A. (1979). Methodology of field experience. Kolos. Moscow. [in Russian]

Elisovetcaia D. (2010). Plant extracts as a means of reducing the population density of the Colorado potato beetle (Leptinotarsa decemlineata Say). Thesis PhD, plant protection. Chisinau. [in Russian].

Elisovetskaya D., Nastas T., Cherney E. (2012). Plants of the genus Allium L. as a source of biologically active substances with insecticidal and antifeedant effects. Intern. scientific Symposium „Conservation of plant diversity", ASM Botanical Garden (Institute), Chisinau. Republic of Moldova; „Al. I. Cuza" University, Iasi; „A. Fatu" Botanical Garden, Iasi, Romania. p. 374-380.

Isman M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology. 51: 45-66.

Kintea P.K., Degteareva L.P. (1989). Steroidal glycosides of bulb onion seeds. Structure of zephnozide D. HPS. 1: 139-140 [in Russian].

Maisashvili M.R., Eristavi L.I., Gvazava L.N., Gugunishvili D.M. (2007). Steroidal sapogenins from Allium rotundum. Chem. Nat. Comp. 43(6): 756–757.

Maisashvili M.R., Kuchukhidze D.K., Gvazava L.N., Eristavi L.I. (2008). Steroidal glycosides from Allium rotundum. Chem. Nat. Comp. 44(4): 545–547.

Maisashvili M.R., Kuchukhidze D.K., Kikoladze V.S., Gvazava L.N. (2012). Steroidal glycosides of gitogenin from Allium rotundum. Chem. Nat. Comp. 48(1): 86–90.

Selyutina I. Yu. (2007). Chemical constituents of species of the genus Allium L. (Alliaceae). Phytochemistry. Siberian Botanical Vestnik: electronic journal. 2(2): 79-86 [in Russian].

Sobolewska D., Michalska K., Podolak I., Grabowska K. (2016). Steroidal saponins from the genus Allium. Phytochem. Rev. 15:1-35.

The Plant List. (2018). http://www.theplantlist.org/tpl1.1/search?q=allium

Original scientific paper
10.7251/AGRENG1802121K
UDC 631.4:615.849.2

STRONTIUM CONTENT IN SANDY SOILS IN AGRICULTURE FIELDS (CASE STUDY: MOUNDOU, CHAD)

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ABSTRACT

During evaluation of physical and chemical properties of sandy soils and their fertility in Southern part of Republic of Chad it has been revealed that some soils have very high content of strontium. Its content varies from 10 to 270 mg/kg of soil depending on type of soil, depth of soil layers, clay and organic content. Strontium content negatively correlates with total content of calcium and phosphorus in layers of soil. Low CEC (CEC - Cation-exchange capacity) of soil may be a reason of possible translocation of strontium from higher to lower layers of soils. Strontium content in soils do not relates with level of radioactivity of soil measured. The highest content of strontium has been found in soils developed on some eolian and colluvio-alluvium deposits. Some researchers hypothesize that some endemic and chronic diseases such as Kashin-Beck disease, `Dysostosis enchondralis endemic`, endemic hoiter, osteoarthritis might be caused by high content of strontium in water and plant foods contaminated with it. Absence of consensus on etiological factors of these diseases confirms that it is worth considering necessity of further studies of different affects of high content of strontium in water and foods on human health directly or indirectly through causing misbalance in mineral nutrition.

Keywords: *strontium, sandy soil, Ca/Sr ratio, radioactivity, eolian deposit.*

INTRODUCTION

Analyzing physical, chemical composition and level of fertility of sandy soils on agricultural fields around city of Moundou in Southern part of Republic of Chad it has been found that some soils have extremely high content of strontium. Soils of this area have very specific physical and chemical properties for they are have been formed on eolian and colluvio-alluvium deposits. Along the river Logon in some places may be found hydromorphic clay soils. On sandy soils at higher places farmers usually grow corn, peanut, cassava, and taro. On lower places along the

river they cultivate rice, root vegetables, banana, and green vegetables. Commercial produce of these crops compose main part of people`s diet. Analytical data obtained and known general information on etiological factors which may cause Kashin-Beck disease and other diseases, mostly bone abnormalities, led us to analyzed more deeply strontium status of sandy soils in mentioned area.

Chemical properties of strontium are very similar to those of barium, calcium, as may form the same salts and basis, but being heavier strontium forms less movable hydroxide, what leads to its accumulation in soils and plant and live organism tissue. This fact is supported by high content of this element in all kind sediments. For example, rests of sea acantarium (radiolarium) mainly composed from $SrSO_4$. Sea weeds contain 26-140 mg per 100 g of dry matter, whereas grasses contain around 2-3 mg/100 g d.m. Main forms of strontium salts in sea sediments are carbonate and phosphate. In all geochemical and biochemical processes calcium and strontium accompanies each other. Their ration (Ca/Sr) in soils formed on mother rock or eolian and alluvial sediments unavoidably determines content of strontium in plants. Of cause, it worth mentioning that soils and plants may be polluted by radioactive ^{90}Sr precipitated after nuclear explosions or accidents at nuclear objects. In any case, while evaluating qualities of soil, as an agricultural object, it worth paying attention to total content of strontium and its ratio to calcium. It will be helpful in finding coincidence with of such diseases as Kashin-Beck and other bone abnormalities and finding means of its prevention (Bowen, Dymond, 1955; Петренко, 2008; Вощенко, Смекалов, 2001). Strontium-isotope ratios vary in nature because one of the strontium isotopes (^{87}Sr) is formed by the radioactive decay of the naturally occurring element rubidium (^{87}Rb). The $^{87}Sr/^{86}Sr$ ratios are mainly used as tracers of water–rock interaction (Blum at al., 1994; Negrel et al., 2001). The primary sources of Sr in groundwater are atmospheric input, dissolution of Sr-bearing minerals, and anthopogenic input (Negrel, Petelet-Giraud, 2005). Due to the physico-chemical similarities of caesium (Cs^+) to potassium (K^+) on the one hand and strontium (Sr^{2+}) to calcium (Ca^{2+}) on the other hand, both elements can easily be taken up by plants and thus enter the food chain. This could be detrimental when radionuclides such as ^{137}Cs and ^{90}Sr are involved (Kanter et al., 2010).

MATERIAL AND METHODS

City Moundou (8°34′00″N, 16°05′00″W, fig. 1) is a capital of the Southern province in Chad. All fields around the city are allocated for crop production and pastures. Soils may be considered as very young as they are formed by periodic wind-driven and alluvial deposits.

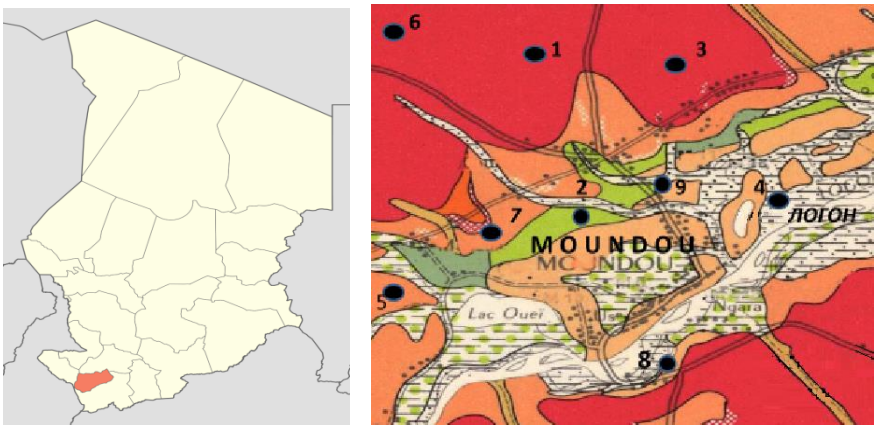

Fig. 1. Location of city Moundau and pedons

Locations of pedons excavated (fig. 1., table 1) were purposely chosen to study influence of height above sea level, hydrology, notable difference in soil profiles, mode of soil use (arable, pasture, crops cultivated).

Table 1. Location and principal properties of soils studded in province Moundou (Chad)

Pedon	Coordinates	High above sea level *, m	Total content in 30 cm layer, mg/kg		Ca/Sr ratio		Level of diation, *μSv/h**
			Sr	Ca	mass	atomic	
1	2	3	4	5	6	7	8
1	8º 37’ 26.94’’N 15º59’33.28’W	4748	15	469	31	69	20
2	8º35’57.96’’N 16º03’33.34’W	400	235	827	4	7,7	16
3	8º35’22.52’’N 16º06’20.28’W	389	16	714	45	99	15
4	8º37’49.24’’N 16º05’51.60’W	411	14	851	61	125	9
5	8º33’58.79’’N 16º00’18.10’W	412	11	422	38	82	18
6	8º39’52.64’’N 16º01’38.36’W	481	8	500	63	139	14
7	8º34’30.40’’N 16º00’38.06’W	409	12	347	29	62	13
8	8º32’59.59’’N 16º05’49.36’W	396	9	381	42	95	13
9	8º36’14.68’’N 16º04’46.58’W	3961	273	2310	9	19	8

*Level of natural radiation = 9-10 *μSv/h*

NB: Level of water above sea level in the river = 380 m.

Soils at different locations are represented by pedons excavated at different fields around the city Moundou. Some fields along the river Logon experience periodic but prolong flooding (pedons 2, 8 and 9). All pedons were excavated up to 100-120 cm depth. As there no distinguished genetic soil horizons soil sample were taken from regular layers 0-30, 30-50 and 50-100 cm. All agrochemical properties such as pH of salt solution, CEC, content of total and exchangeable phosphate, calcium, magnesium, potassium for each soil sample were determined by appropriate techniques (Nagornyy, 2013). Total content of P, Ca. Sr, Fe, Mn, Mg, K was determined using by X-ray spectrometer `Spectroscan Max G`. According to Atlas Cartographique (2003) these soils pertain to sandy ferritique and ferrallitic groups (pedons: 1, 3, 4, 5, 6, 7). Upper layers of sandy ferrallitic soils have light brown or gray-brown color. Soils with some hydromorphic features (pedons: 2, 8, 9) have dark gray color. All soils have sandy granulometric composition. Clay content in soils is in the range 2-3%, cation exchange capacity varies from 1 to 2 meq./100g. Soil acidity measured in KCL extraction was in the range 3.9 – 5.,2. Content of exchangeable aluminum was very low (0.2 – 0.5 meq./100 g). Organic matter content in soils on higher places was in the range 0.6 -0.8%. In the soils with hydromorphic features OM (OM - soil organic matter) content was in the range 1.1 – 1.3%.

RESULTS AND DISCUSSION

The main goal pursued in our research was to evaluate physical, chemical, hydrological, and agronomical qualities of soils used for crop production in the province Moundou in Chad. The Data obtained revealed very wide variation in content of strontium in different soils of the province (table 2). Nowadays it is recognized that high accumulation of strontium in human body may cause Kashin-Beck disease osteoarthritis and different metabolic disorders (Вощенко, Смекалов, 2001; Ильин, 2000). Russian researchers have accumulated big volume of information on nature of strontium content in soils and biological tissues, its mobility in different conditions, and its influence on health of people. They classified soils on basis of strontium content and established level of ratio Ca/Sr in soils which may be dangerous for human being, and find the way for soil remediation. More over on basis of this data a special State regulation has been adopted (Петренко, 2008). Having this in mind we trying to evaluate data obtained on strontium content in sandy soils of the province Moundou in Chad.

Main part of soils in the province may belong to three main groups, Arenosols, Ferrasols, Flivisols (Atlas…, 2003). Eolian nature of soil formation at the area and very high content of sand allow us to expect high risk of mobility of strontium in soil profiles and high content of strontium in ground water. As other researchers found these parameters directly depend on granulometric composition of soil, OM content, soil acidity, calcium and phosphate content (Khaleghpanah et al., 2010; Shalex et al., 2013). Total strontium content and other properties of soils influence uptake of strontium by plants what may be determined by value of transfer factor (TF).

Table 2. Level of content of OM, clay, sand and selected elements in soils at different locations and depth of soil pedons

Pedon	Layer, *cm*	OM,%	Sand,%	Clay,%	Silt,%	pH		Total content, mg/kg		
						2O	KCl	P	Ca	Sr
1	0-30	0.79	98.67	0.8	0.6	5.3	4.8	0.075	469	75
	30-50	1.18	96.6	1.6	1.8	5.3	5.0	0.067	664	105
	>100	0.99	95.8	3.2	1.0	4.8	4.8	0.063	796	125
2	0-30	1.13	943.0	2.6	3.4	4.8	4.5	0.086	827	1175
	30-50	0.78	97.2	1.4	1.4	4.8	4.1	0.095	510	1170
	>100	0.45	96.6	1.6	1.8	4.6	4.2	0.068	880	1035
3	0-30	1.43	99.2	0.4	0.4	5.3	5.2	0.066	714	80
	30-50	0.53	98.6	0.6	0.8	5.0	5.4	0.078	246	65
	>100	0.59	96.2	1.6	2.2	4.8	4.1	0.074	389	105
4	0-30	0.84	97.6	1.0	1.4	5.2	4.9	0.109	851	70
	30-50	0.64	97.6	0.8	1.6	4.9	4.5	0.104	536	105
	>100	0.96	99.2	0.6	0.2	4.8	4.0	0.081	263	130
5	0-30	0.58	98.6	0.4	1.0	5.1	4.6	0.096	422	55
	30-50	0.71	98.2	0.8	1.0	4.6	4.0	0.080	274	85
	>100	0.51	99.2	0.6	0.2	4.3	3.9	0.083	137	130
6	0-30	0.65	99.6	0.2	0.2	5.4	4.9	0.064	499	40
	30-50	0.51	99.0	0.2	0.8	5.3	4.7	0.080	370	55
	>100	0.72	98.2	0.4	0.6	4.8	4.2	0.080	290	75
7	0-30	0.85	97.8	0.8	1.4	5.3	4.5	0.087	347	60
	30-50	0.45	97.8	1.2	1.0	5.1	4.2	0.076	312	75
	>100	0.26	97.2	2.2	0.6	4.7	3.9	0.044	279	145
8	0-30	1.04	99.4	0.4	0.2	5.6	5.0	0.084	381	45
	30-50	0.45	98.3	0.6	1.2	5.1	4.3	0.072	187	50
	>100	0.52	98.0	1.2	0.8	4.8	4.0	0.068	470	95
9	0-30	1.54	95.0	2.2	2.8	5.5	4.8	0.136	2310	1365
	30-50	0.84	98.0	1.2	0.8	5.3	4.3	0.075	1322	1260
	>100	0.51	94.3	2.7	3.1	5.0	4.1	0.101	1249	1185

Data presented in the Table 3 shows that the range of strontium content in soils varies very much: from very low (8-12 mg/kg) to extremely high (235-273 mg/kg). Low content of native strontium prevail in profile layers of most soils studded. Let it be noted that in some soils lower layers have higher content of Strontium, what may be explained by (a) different content of strontium in wind-brought material in previous times, and (b) by lixiviation of strontium together with silt into lower layers of soil. But as it is suggested by soil scientists (Andersen, 1973; Khaleghpanah et al., 2010) abundance of strontium are to be compared to that of its homologous element which is calcium. Wide variation in total content of Strontium and Calcium has been found in parent rocks and minerals (Kate et al, 2011, Twining et al., 2003). Whereas content of extractable form of these elements in

soils is less variable due to use of the same salt solution for extraction of exchangeable forms of both elements. It has been found (Khaleghpanah et al., 2010), that mobility of strontium highly depends on mass or atomic ratio Ca/Sr (see table 3). Value of these ratios matters for assessment of the strontium status of the soils, and this value have been used in Russia for classifying soils and drinkable water. For example, water which contains 7 mg and more of strontium is per one liter is not to portable and not to be used in kitchen. Soils which have wide ratio Ca/Sr (more than 100) are not to be used for production of food crops (Петренко, 2008). The reason is much recognized: such levels of strontium in water and wide ratio Ca/Sr may cause Kashin-Beck disease, osteoarthritis, `strontium-caused rachitis`, other physiological abnormalities (Вощенко, Смекалов, 2001). It is accepted that these diseases are a consequence of misbalance between Ca and Strontium in water and food diet, what causes displacement of Ca by Sr (Худяев, 2008).

Table 3. The Pearson`s correlation matrix among measured levels of OM, clay and selected elements

	OM,%	Sand,%	Clay, %	Silt,%	pH		Total content, mg/kg		
					H2O	KCl	P	Ca	Sr
OM,%	1								
Sand,%	0,2397	1							
Clay,%	0.1511	0.3455	1						
Silt,%	0.2065	0.5019	0.6737	1					
pH-H2O	0.0600	-0.0384	-0.0878	0.0469	1				
pH-KCl	0.5074	0.0118	-0.1815	-0.0668	0.0104	1			
P	0.3669	0.0516	0.0434	0.4845	0.0654	0.1132	1		
Ca	0.5484	0.0975	0.5260	0.5851	-0.1074	0.1914	0.5556	1	
Sr	0.2512	0.3501	0.55558	0.6445	-0.1143	-0.1904	0. 4085	0.7481	1

Data presented in Table 1 show that at some places should be of big concern as cultivation of food crops may bring problems with health of people living there. Higher content of strontium in low layers of some soils may be explained at least by two obvious factors. Firstly, it may be caused by downward movement of strontium in sandy soils during rainy season [Twinnong Netta salex]. Sandy soils with low content of clay and silt and, as consequence, with low CEC are not able to hold basic elements in upper layers of soil. Wind-translocation of weathered material from Northern part of the country (Tibetsy area) is the second factor. That area is rich in strontium-containing material originated from ocean deposits of Pleistocene period. Lowest layers of soil may be formed from send deposits brought by the North-West wind decades before, whereas other layers has been formed later by sand and dust delivered by wind from other directions.

Strontium translocation along the soil profile and sorption of this element by soil depend on prevailing chemical composition of soil (salt: sulphate, carbonate, chloride, and phosphate; OM, clay and silt content). All these suggest possible measures of soil remediation. Such measures may include enrichment of soil with in organic material (Andersen, 1973; Bowen, Dymond 1955; Khaleghpanah et al., 2010), use of phosphate fertilizers (Kate at al., 2011; Twining et al., 2003). This measures may reduce transfer factor of Strontium from 0.2-0.3 to as low as 0.01-0.008 (Худяев, 2008).

CONCLUSIONS

Grate majority of soil of Moundou province in Chad pertain to group of light sandy Arenosols, Ferralsols, Fluvisols of low fertility. Some soils have comparatively high content of strontium and calcium due to their formation from wind-brought materials originated from ocean deposits of Pleistocene period. Such soils have bigger Ca/Sr ratio what may cause higher transfer of strontium from soil to plant produce. Future many-side and versatile research is needed to establish correlation between Strontium content in soils, rate of transfer of this element to plant produce, and frequency and severity of diseases thought to be caused by high accumulation of strontium in human body.

REFERENCES

Andersen J.A. (1973). Plant Accumulation of Radioactive Strontium with Special Reference to the Strontium-Calcium Relationship as Influenced by Nitrogen. Danish Atomic Energy Commission of Research Establishment Report No. 278.

Atlas Cartographique (2003). Schema Directeur de l`eau et de l`assainissement du Tchad, 2003-2020. P. 90.

Blum J.D., Erel Y., Brown K. (1994). $^{87}Sr/^{86}Sr$ ratios of Sierra Nevada stream waters: implications for relative mineral weathering rates. Geochimica et Cosmochimica Acta, 58, p. 5019-5025.

Bowen H.J.M., Dymond J.A. (1955). Strontium and Barium in Plants and Soils. Proceedings of the Royal Society. Biological Sciences. DOI: 10.1098/rspb.

Kanter U., Hauser A., Michalke B., Dräxl S., Schäffner A.R. (2010). Caesium and strontium accumulation in shoots of *Arabidopsis thaliana*: genetic and physiological aspects, Journal of Experimental Botany, Volume 61, Issue 14, 1 September 2010, p. 3995–4009, https://doi.org/10.1093/jxb/erq213

Kate B., Grimes V., Niven L., Steele T.E. (2011). Strontium isotope evidence for migration in late Pleistocene Rangifer: Implications for Neanderthal hunting strategies at the Middle Palaeolithic site of Jonzac, France. In Journal of Human Evolution. No. 61, p. 176-185.

Khaleghpanah N., Roozitalab M.H., Majdabadi A., Mirkhani R. (2010). The adsorption of Strontium on soils developed in arid region as influenced by clay content and soluble cations. 2010 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1 – 6 August 2010, Brisbane, Australia.

Nagornyy V.D. 2013. Soil and Plant Analysis, Moscow, RUDN, p. 140.

Negrel Ph., Casanova J., Aranyossy J.F. (2001). Strontium isotope systematics used to decipher the origin of groundwaters sampled from granitoids: the Vienne case (France) Chemical Geology, 177, p. 287-308.

Negrel, P., Petelet-Giraud, E. (2005). Strontium isotopes as tracers of groundwater-induced floods: the Somme case study (France). Journal of Hydrology, *305*(1-4), p.99-119.

Shalex N., Lazar B., Halicz L. (2013). Strontium isotop fractionation in soils and pedogenic processes. In Procedia `Earth and Planetary Science`, 7, p 790-793.

Twining J., Shotton P., Tagami K., Payne T., Itakura T., Russell R., Wilde K., McOrict G., Wong H. (2006).Transfer of radioactive caesium, strontium and zinc from soil to sorghum and mung beans under field conditions in tropical Northern Australia. Classification of soil systems on the basis of transfer factors of radionuclides from soil to reference plants. In Proceedings of a final research coordination meeting organized by the oint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and held in Chania, Crete, 22–26 September 2003, p. 21-39.

Вощенко А.В., Смекалов В.П. 2001. Уровская (Кашина-Бека) болезнь. Малая энциклопедия Забайкалья: Здравоохранение и медицина. Наука, 630 p. *(in Russian)*

Ильин В.Б. (2000). Оценка существующих экологических нормативов содержания тяжёлых металлов в почве. Агрохимия. 9, p. 74-79. *(in Russian)*

Петренко Д.В. (2008). Влияние производства фосфорных удобрений на содержание стронция в ландшафтах. Дисс. Канд. биол наук, 158 p. *(in Russian)*

Худяев С.А. (2008). Стронций в компонентах ландшафтов юга Обь-Иртышского междуречья: Дис. канд. биол. Наук. Новосибрск, 130 p. *(in Russian)*

Original scientific paper
10.7251/AGRENG1802129S
UDC 512.04:556.18(55)

A BLUEPRINT FOR ELEMENTARY REPRESENTATIVE WATERSHED SPECIFICATION

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ABSTRACT

Provision of ecosystem services is necessary for our quality of life. In this vein, it is critical to develop a baseline to monitor the ecosystem behavior using monitoring, mapping, and modeling indicators of landscape condition. The representative watersheds (RWs) can therefore be considered as such decision blueprint to implement the best management practices to conserve and consequently improve ecosystem services. There are few researches for the selection of RWs. However, the comprehensive and of course applicable methodologies still are lacked particularly in developing countries where concentration and proper directing of conservation measures are further needed owing to investments constraints. Therefore, in the present study, an intensive watershed selection process was undertaken to identify RW across the Gharesoo-Gorgan River Basin (ca. 12987 km^2), Golestan Province, Iran. This study aimed to adopt the Laize's approach in Gharesoo-Gorgan Watershed for identifying the representative sub-watershed. Towards this, four GIS-based layers of elevation, slope, rainfall erosivity and land use were selected for sub-watersheds characterization. The representative watershed index (RWI) was then calculated and mapped using MATLAB 2016 and ArcGIS 10.3 softwares, respectively. The RWI was calculated pixel wise for all nine individual sub-watersheds and the entire watershed as reference area with the help of matrix combinations of four study layers. Ultimately, RWIs obtained from 21.3 to 62.6 with mean of 44.94±14.49 and coefficient of variation of 32.25% were used for sub-watershed prioritization. Accordingly, the sub-watershed 8 in north east with RWI of 56.8 was proposed as the final RW for the whole Gharesoo-Gorgan Watershed. The results of the study is helpful to be used by authorities for launching monitoring systems in the RW to collect behavioral indicators leading to designation of reasonable eco-environmental restoration strategies.

Keywords: *Environment protection, geographic information system, integrated watershed management, watershed prioritization.*

INTRODUCTION

Nowadays, the world is facing set of ecosystems problems caused by unsustainable development, demand increasing, limited resources and raising pressures on ecosystems. The effect of these problems in connection with adverse impacts of natural disasters and climate change undermine the natural resources and ecosystem services (Shotadze and Barnovi, 2011; Webb, 2012; Debnath, 2016). Globally, the focus of managerial strategies has shifted from high resources usage in terms of unsustainable development to understanding and quantifying the ecosystems benefits in order to reach sustainable management goals (Pickard *et al*., 2015; Campbell, 2016; Raum, 2018). In respect to achieve these goals, developing a decision blueprint to identify the representative watersheds (RWs) as a benchmarking and monitoring baseline to implement the best management practices (BMPs) is very valuable to conserve and consequently improve ecosystem services (Shotadze and Barnovi, 2011, Montenegro *et al*., 2014; Jackson *et al.*, 2016). RW is introduced as a typical and instrumented watershed, as well as candidate of the general situation having a general stability in all factors of the reference areas aiming at monitoring natural changes (AGU, 1965; Toebes and Ouryvaev, 1970; Arbor, 2010; Hillman and Rothwell, 2016). There are few researches in regards to developing approaches for representative watershed selection. Whist, more comprehensive and applicable methodologies are needed worldwide particularly in developing countries where concentration and proper directing of conservation measures are further required owing to investments constraints. Towards this, the present study was therefore formulated to characterize the representative watershed index (RWI) based on four common and important criteria of elevation, slope, rainfall erosivity and land use for the Gharesoo-Gorgan Watershed, Iran.

MATERIAL AND METHODS

The study was carried out for the Gharesoo-Gorgan Watershed, Golestan Province, in north-east of Iran with nine sub-watersheds. The Gharesoo-Gorgan Watershed is located in eastern part of Alborz range (55° 00′ to 56° 29′ E longitude and 36° 36′ to 37° 47′ N latitude) with some 12987 km^2 in area. The maximum and minimum temperatures are +49 and –28 °C, respectively. Moving from the north toward the south of study watershed, the temperature decreased. The average monthly humidity varies between 47 to 89 %. The rainfall in mountainous parts of the study area is about 574.8 mm (Bordbar *et al*., 2018). Figure 1 illustrates general location and distribution of sub-watersheds, climatologic and hydrometric stations of the Gharesoo-Gorgan Watershed.

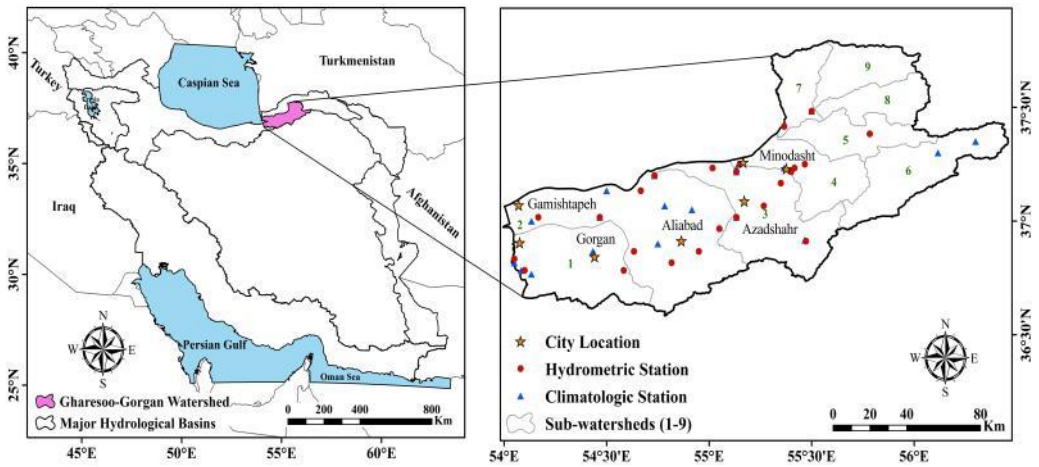

Fig 1. Distribution of sub-watersheds, climatologic and hydrometric stations of the Gharesoo-Gorgan Watershed, Iran

The paper aims to introduce an initiative and practical approach in field of RW determination stemmed on Laize's approach (Laize, 2004). To conduct the present research, firstly, the basic maps of elevation, slope and land use were prepared from previous researches conducted as part of the National Mega Project on the Integrated Watershed Management (Khaledi Darvishan *et al*., 2017). In addition, the rainfall erosivity (t m ha^{-1} cm h^{-1}) as the rainfall potential to generate soil erosion was also provided from Sadeghi and Hazbavi (2015), Zabihi *et al*. (2016) and Sadeghi *et al.* (2017). Secondly, by using Geographical Information System (GIS) software (Version 10.3), the raster map of the mentioned datasets were integrated in order to characterize the relationship between spatial datasets within each individual sub-watershed and reference area (i.e., the entire Gharesoo-Gorgan Watershed). Thirdly, the raster datasets were prepared at the same scales and pixel sizes (30×30 m) and a quadri-partite dimensioned desired category. Fourthly, numbers of combined pixels in different classes of layers were calculated. Fifthly, appropriate matrices were developed between the reference and each sub-watershed on a cell-by-cell basis in order to calculate the RWI of each sub-watershed as described in Eq. (1). To achieve the RWI as seen in Eq. (1), the absolute value of differences (D) of normalized pixels between compound values of reference (j) and sub-watersheds (i) were calculated as explained in Eq. (2). To this end, the normalized value of pixels of the matrices was obtained by dividing every combined class to total pixels of every layer, as well. The RWIs were then calculated for all individual sub-watersheds ranging from zero to 100 and prioritized accordingly.

$$RWI = (1 - 0.5 \times D) \times 100 \quad (1)$$

$$D = \sum_{i,j}\left(\left|V_{i,j}(sub\_watershed) - V_{i,j}(reference)\right|\right) \quad (2)$$

RESULTS AND DISCUSSION

The representative watershed (RW) was identified based on matrices developed for four datasets of elevation, slope, rainfall erosivity and land use for all nine sub-watersheds of the Gharesoo-Gorgan Watershed as summarized in Table 1.

Table 1. Representative Watershed Index (RWI) in quadri-partite dimensional matrix combinations of the study determinant variables for the Gharesoo-Gorgan Watershed, Iran

Sub-watershed No.	RWI (Out of 100)	Area (km^2)
1	36.0	1780.9
2	54.6	3490.2
3	62.6	2060.2
4	42.2	1103.8
5	39.4	879.8
6	22.1	1482.4
7	21.3	658.5
8	56.8	714.0
9	38.4	816.8

For the present study, a threshold of acceptance of 70^{th} percentile of RWIs was also considered to examine any other potential candidates in other different viewpoints. Ultimately, RWIs obtained from 21.3 to 66.2 with mean 44.94±14.49 and coefficient of variation of 32.25% were used for sub-watersheds prioritization. Spatial distribution of the RWIs in the Gharesoo-Gorgan Watershed has been depicted in Figure 2. The higher RWI shows the better status of the sub-watershed for representing the whole watershed. Nonetheless, the RWIs with more than 70^{th} percentile of 46.6 were considered as the basis for final selection of practical RWs in real condition.

According to Figure 2, sub-watersheds 2, 3, and 8 with respective RWI of 54.6, 62.6 and 56.8 stand at top priority of RW candidates in the Gharesoo-Gorgan Watershed. For determination of superior RW, additional criteria viz. hydrological independency, availability of hydrometric and meteorological stations and ultimately the general location of the candidate sub-watersheds were also considered in the selection process. Accordingly, the sub-watershed eight in north-east with RWI of 56.8 was proposed as the final RW for the whole Gharesoo-Gorgan Watershed. In this regard, the result showed watershed size influenced the RWI; because larger watersheds were more likely to encompass the reference area than smaller ones. This finding proved that choosing available datasets and their pixel size were very important in performance of model and reducing of processing time. Allocation further budget in field of instrumentation and monitoring of the proposed superior RW is strongly recommended for the better evaluation of the watershed behaviour to different driving forces. It is expected that the selected RW

could be considered as a baseline for unmonitored watersheds as issues of monitoring deficiency as noted by Laize (2004) and Hannaford *et al*. (2013). Evaluating various alternative criteria like effect of upstream and downstream watersheds against a set of environmental, socio-economic and governance is also suggested for future researches on developing more comprehensive RW determination approaches.

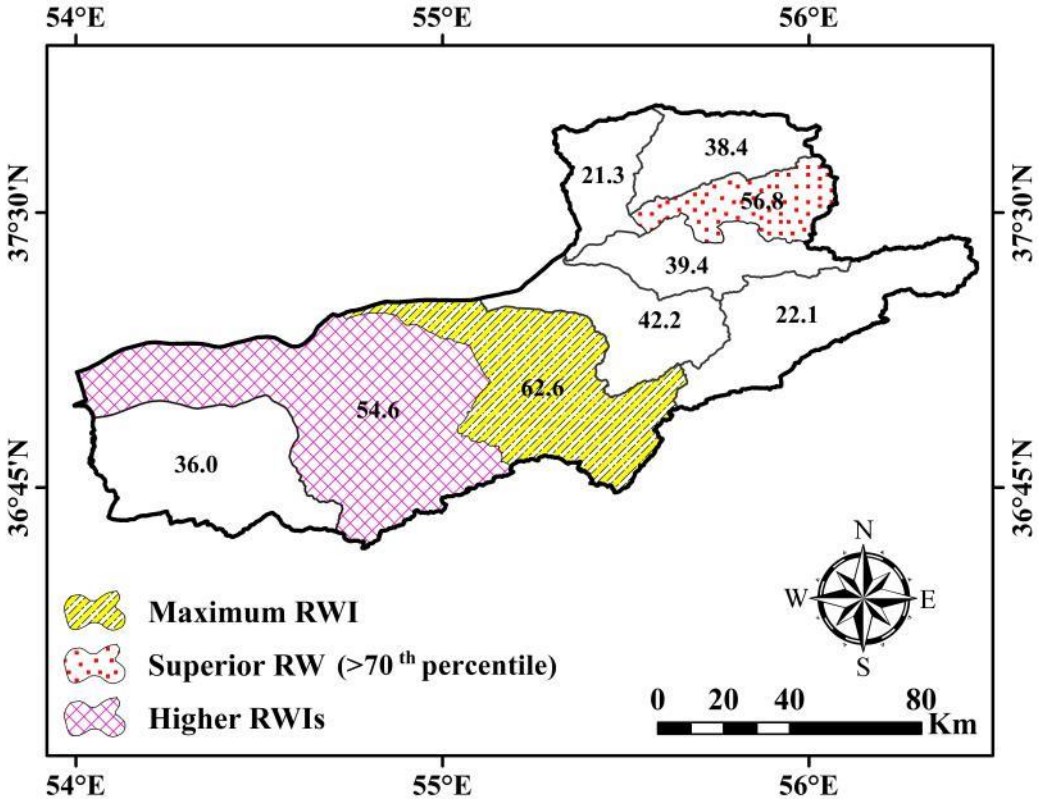

Fig 2. Spatial distribution of the representative watershed index (RWIs) in the Gharesoo-Gorgan Watershed, Iran

CONCLUSION

To get a global picture of identifying the RWs in an area, developing comprehensive method and applying proper and exact variables are necessary to make local issues meaningful for decision makers. The RWI allows watersheds to be ranked according to their level of representativeness and their influence on regionalisation procedures. When RWI scores are combined with indices relating to other characteristics they constitute a powerful decision support mechanism to make proper decision. In this study, the RWI scores were successfully calculated based on overlaying the multi-dimensional matrices namely elevation, slope, rainfall erosivity and land use for the Gharesoo-Gorgan Watershed in Iran. It is accordingly recommended that the watershed management authority at regional and national scales and even the running projects like the National Mega Project on

the Integrated Watershed Management would focus on the behaviour of the selected sub-watershed as a representative area for the Gharesoo-Gorgan Watershed to monitor and assess the effects of natural and anthropogenic driving forces on the outcome of the watershed.

ACKNOWLEDGEMENTS

The present research was collaboratively supported by Tarbiat Modares University and the National Mega Project on the Integrated Watershed Management in Iran whose valuable facilitation and financial supports are greatly appreciated. The authors also wish to express their gratitude to the corresponding authorities for their cooperation in field surveying and providing valuable data. Many thanks to Dr. A. Sadoddin and Dr. A. Khaledi Darvishan and Mrs S. Babaei for their assistances in project planning, providing necessary data and analyses.

REFERENCES

AGU (American Geophysical Union) (1965). Inventory of representative and experimental watershed studies conducted in the United States. Symposium on Representative and Experimental Watershed Studies Conducted in the United States, Under the Auspices of the Section of Hydrology, American Geophysical Union, Budapest, Hungary, Washington, D.C, 156 p.

Arbor A. (2010). Great lakes watershed ecological sustainability strategy (GLWESS): Watershed characterization and selection of candidate pilot watersheds. Great Lakes Protection Fund and the GLWESS Project Advisory Panel (PAP) in collaboration with the Nature Conservancy, August 17, Michigan, 52 p.

Bordbar M., Neshat A., Javadi S. (2018). Vulnerability assessment of Gharesou-Gorganroud coastal aquifer using GALDIT and SINTACS and optimization by SPSA and GIS. Ecohydrology, 5(2), 699-711. (In Persian)

Campbell I.C. (2016). Integrated management of large rivers and their watersheds. Ecohydrology & Hydrobiology, 16(4), 203-214.

Debnath R. (2016). A review of the sustainability of recent watershed management programmes in Bangladesh. Lakes & Reservoirs: Research & Management, 21(2), 152-161.

Hannaford J., Holmes M.G.R., Laize C.L.R., Marsh T.J., Young A.R. (2013). Evaluating hydrometric networks for prediction in ungauged watersheds: a new methodology and its application to England and Wales. Hydrology Research, 44(3), 401-418.

Hillman G. Rothwell R. (2016) Spring Creek representative and experimental watershed project. The Forestry Chronicle, 92(1), 43-46.

Jackson F.L., Malcolm I.A., Hannah D.M. (2016). A novel approach for designing large-scale river temperature monitoring networks. Hydrology Research, 47(3), 569-590.

Khaledi Darvishan A., Adhami M., Katebikord A., Gholami L., Feizi V., Ghasempouri S.M., Rastgar A., Mohammadamini H., Gholamalifard M.,

Ownagh M. (2017). Assessment of the environmental diversity of Iran. A Project as part of Mega Project on the Integrated Watershed Management Project in Iran, Gorgan University of Agricultural Sciences and Natural Resources, 476 p.

Laize C.L.R. (2004). Integration of spatial datasets to support the review of hydrometric networks and identification of representative catchments. Journal of Hydrology and Earth System Sciences, 8(6), 1103–1117.

Laize C.L.R., Marsh T.J. (2006). The use of spatial information to improve hydrometric network design and evaluation. IAHS publication, 308, 56 p.

Montenegro S.M., da Silva B.B., Antonino A.C., Lima J.R., de Souza E.S., de Oliveira L.M., de Moura A.E., Souza R.M.S. (2014). Hydrological studies in experimental and representative watersheds in Pernambuco State, Brazil. Proceedings of the International Association of Hydrological Sciences, 364, 422-428.

Pickard B.R., Daniel J., Mehaffey M., Jackson L.E., Neale A. (2015). EnviroAtlas: A new geospatial tool to foster ecosystem services science and resource management. Ecosystem Services, 14, 45-55.

Raum S. (2018). Reasons for Adoption and Advocacy of the Ecosystem Services Concept in UK Forestry. Ecological Economics, 143, 47-54.

Sadeghi S.H.R., Hazbavi, Z. (2015). Trend analysis of the rainfall erosivity index at different time scales in Iran. Natural Hazards, 77, 383-404.

Sadeghi S.H.R., Zabihi M., Vafakhah M., Hazbavi Z. (2017). Spatiotemporal mapping of rainfall erosivity index for different return periods in Iran. Natural Hazards, 87(1), 35-56.

Shotadze M., Barnovi E. (2011). Selection of pilot watersheds/areas. technical report of integrated natural resources management in watersheds (INRMW) of Georgia Program, Global Water of Sustainability Program, USAID from the American People, 79 p.

Toebes C., Ouryvaev V. (1970). Representative and experimental watersheds. An International Guide for Research and Practice, An International Guide for Research and Practice United Nations Educational, Scientific and Cultural Organization, UNESCO, 348 p.

Webb A.A. (2012). Payments for watershed services and the role of experimental catchment studies. Revisiting Experimental Catchment Studies in Forest Hydrology, 353, 207-216.

Zabihi M., Sadeghi S.H.R., Vafakhah M. (2016). Spatial analysis of rainfall erosivity index patterns at different time scales in Iran. Journal of Watershed Engineering and Management, 7(4), 442-457. (In Persian)

Original scientific paper
10.7251/AGRENG1802136G
UDC 621.375.826:636.2.053

USE OF LOW-INTENSITY LASER RADIATION IN REHABILITATION OF HYPOTROPHIC CALVES

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ABSTRACT

The effect of low-intensity laser radiation on the biochemical, immune and clinical status of hypotrophic calves was studied. The studies were carried out in 2 groups (test and control ones) of 12 animals with symmetric moderate hypotrophy. The calves in the test group underwent low-intensity laser irradiation of blood within the red spectral range (wave length of 630 nm). The animals of the control group (intact) were not treated. Clinical observations of the calves were carried out during 2 months, taking into account the incidence, duration and severity of the course of the disease, as well as the calves' weight gain. Blood sampling for biochemical and immunological studies was performed on the 1st and the 21st days of the calves' life. On the first day the biochemical status and natural resistance in the animals of the both groups did not differ. On the 21st day, the calves of the test group had higher levels of glucose, pyruvic acid, vitamin E, Blood Serum Complement Activity (SCA), Blood Serum Lysozyme Activity (SLA), Leucocyte Phagocytic Activity (LPA), Phagocytic Number (PN), Phagocytic Intensivity (PI), and lower levels of lactic acid, middle molecules and ectoglobular hemoglobin in comparison with the control group. The treatment of the animals with low-intensity laser radiation positively affected their clinical state and productivity. The calves in the test group had mild forms of gastrointestinal diseases with a shorter course duration, and the increase in body weight was significantly higher compared with the control group. The positive effects of low-intensity laser radiation on biochemical status, natural resistance and clinical condition of hypotrophic calves were established, which allows us to recommend it for their rehabilitation.

Key words: *calves, antenatal hypotrophy, low-intensity laser radiation, biochemical status of blood, natural resistance.*

INTRODUCTION

In specialized milk production farms of the Russian Federation antenatal pathology (congenital liver disease, antenatal hypoxia, hypotrophy) is registered in 9-21% of the newborn calves. Antenatal hypotrophy occurs when the fetus has insufficient supplies of oxygen, nutrients, energy and biologically active substances. In order to maintain the vital activity of the fetus, necessary substances are redistributed to the

benefit ofits vital organs. As a result, the development of the musculoskeletal system, the respiratory system and the gastrointestinal tract (and, in severe cases, the liver and the vascular system) is suppressed [Shakhov, 2013].

The perinatal development disorder of calves adversely affects the formation of the immune status and is manifested by immunodeficiency, which is accompanied by the instability of the natural resistance indicators dynamics and their adaptive immunity, which leads to the infection of animals with various pathogens and the occurrence of gastrointestinal and other diseases. The aggravation of hypotrophy in newborn calves results from the decrease in the adaptive capacity of their body, the accumulation of intermediate and final metabolites, the membrane structures disorder, the endogenous intoxication and metabolic acidosis. The subsequent growth and development disorders of such animals and their high disease rate call for rehabilitation activities [Alyokhin, 2013]. To activate the protective mechanisms of the body, various means and methods are used that are divided into three groups based on their origin: biological, originating from cells and tissues of living organisms (animals, humans, microbes, plants), chemical (natural and synthetic) and physical (radiation energy, ultrasound, magnetic field and others). In this aspect, non-medicamentous immunocorrection should be considered. It can be used to treat severe diseases, drug therapy resistance or side effects. Non-pharmacological methods are environmentally safe, since no foreign potentially dangerous chemical substances are introduced into the animal's body. Non-medicamentous immunomodulation includes low-intensity laser radiation [Kataranov, 2005, Golubtsov, 2009].

Research objective was to study the effect of low-intensity laser radiation on the biochemical status and natural resistance of hypotrophic calves and the possibility of using it for their rehabilitation.

MATERIALS AND METHODS

The research was carried out at Agrofirma "Grachevskoe" farm in the Usman district of the Lipetsk region on calves obtained from the red-and-white «Voronezhky» cows. Animals selected for the experiment had the syndrome of the symmetric moderate hypotrophy: low body weight (27,3 ± 0,3 kg), hypothermia tendency (body temperature after 12 hours after birth 38,4 ± 0,05°C), moderate tachycardia (144,0 ± 3,75), tachypnoe (39,5 ± 0,50), inhibition of physiological reflexes, etc.

The animals were divided into two groups by the analog method (n=12). The calves of the control group were intact. The animals of the test group were exposed to the low-intensity laser radiation (LILR). For the procedure, "Matrix" laser therapeutic apparatus with a KO4 radiator (wavelength of 630 nm) attached was used. The calves had their hair cut in the jugular vein and were contact-scanned for 5 minutes with a slight soft tissue compression in the morning; the treatment course had 10 sessions with a 48 hours interval. The radiation power in the first session was 2,5 mW. During the next six sessions, it was increased by 2,5 mW each time, and then kept at the level of 15 mW. When choosing the mode and dose of

radiation, general recommendations on the use of physiotherapeutic methods were followed, as well as the method for evaluating the effect of low-intensity laser radiation of blood on the animal body [Golubtsov, 2014]. Clinical observations of the calves were carried out for 2 months, taking into account the incidence, duration and severity of the course of the disease, and the calves' weight gain. Blood sampling for biochemical and immunological studies was performed on the 1st and the 21st days of the calves' life. To determine the etiology of gastrointestinal diseases, feces were studied for bacteriological culture.

Conventional methods were applied to study the blood morphological composition, the neutrophils absorption activity, the lysozyme, complementary and bactericidal activity of the blood, the vitamin E concentration, glucose, pyruvic and lactic acid, the middle molecules content (MMC), ectoglobular hemoglobin (EGH), the erythrocyte membrane modification coefficient (EMM), bacteriological studies of feces. Statistica v6.1 applications were used for statistical processing of the obtained data, and the Student's t-test – for reliability assessment.

RESULTS AND DISCUSSION

The clinical status of the hypotrophic calves in the control and test groups in the first days of life was almost identical. They independently rose after 2,6±0,18 and 2,8±0,12 hours, the manifestation of the sucking reflex was recorded after 2,0±0,27 and 2,3±0,37 hours, the body temperature was at 38,9±0,09 and 38,8±0,14°C, the pulse was 120,0±2,0 and 120,7±2,0 / min, the respiratory rate was 38,0±2,0 and 36,8±0,5 per minute, and the meconium was released after 9±1,5 and 10,0±1,5 hours, respectively.

The blood chemistry values in the calves of the both groups in the first day of life did not differ (Table 1).

Table 1. The blood chemistry values in hypotrophic calves on the first day of life

Value	Unit of measurement	Control group	Test group
Glucose	M/L	6,99±0,30	7,01±0,43
Pyruvate	mM/L	180,0±20,8	178,0±19,0
Lactate	M/L	3,3±0,17	3,4±0,21
EGH, g/L	g/L	1,62±0,04	1,68±0,02
Vitamine E	μM/L	4,97±0,69	4,36±0,63
MMC_{237nm}	cond. unit	0,61±0,011	0,76±0,005
$MMC_{254\ nm}$	cond. unit	0,36±0,001	0,36±0,002
EMM		1,60±0,02	1,62±0,01

Note: *p≤0,05; **p≤0,01; ***p≤0,001; numerator – control group, denominator – test group.

On the day 21st the glucose content in the animals of the test group, compared with the control group, was 25,0% higher, the pyruvic acid content - 30,2 higher, and the lactic acid content - 9,9% lower. The glycolysis activation with the

dominance of the aerobic pathway is confirmed by the optimal lactate/pyruvate ratio (12,3±3,33). In the intact calves, it was 30,5% higher (17,7±0,53), indicating a higher activity of anaerobic glycolysis (Table 2).

Table 2. The blood chemistry values in hypotrophic calves on the twenty-first day of life

Value	Unit of measurement	Control group	Test group
Glucose	M/L	4,36±0,27	5,45±0,43*
Pyruvate	mM/L	97,0±4,98	126,3±8,13**
Lactate	M/L	1,72±0,06	1,55±0,04*
EGH, g/L	g/L	1,04±0,01	0,35±0,02***
Vitamine E	μM / L	6,43±1,08	9,20±0,62*
MMC_{237nm}	cond. unit	1,17±0,024	0,50±0,013***
MMC_{254nm}	cond. unit	0,34±0,004	0,21±0,021*
EMM		1,54±0,03	1,32±0,02***

Note: *p≤0,05; **p≤0,01; ***p≤0,001; numerator–control group, denominator–test group.

In the pathogenesis of the autointoxication, an important role belongs to the defect of the membrane structures. The indirect reflection of the membrane structures state are the values of ectoglobular hemoglobin (EGH), the erythrocyte membranes modification coefficient (EMM), as well as the content of vitamin E and middle molecules content (MMC).

The level of the ectoglobular hemoglobin in the hypotrophic calves was 2,7-2,8 times higher than the values typical of healthy normothrophic calves (0,60 g/l) on the first day of life. On the day21st, the intact hypotrophic calves showed a decrease in the destructive phenomena in the erythrocyte membranes, as indicated by a 36,0% decrease in EGH, while it did not exceed the norm (0,40 g/l) in the animals of the test group, due to the restoration of the membrane structure.

Of the calves with mild and moderate congenital hypotrophy, a distinctive imbalance of the autonomic nervous system with the sympathetic division dominance is typical, which is confirmed by a higher level of adrenoreceptor activity on the surface of erythrocyte membranes. Our studies showed that the erythrocyte membranes modification coefficient under the action of epinephrine in the experimental calves at the diurnal age was 1,6 (1,1-1,4). The EMM in the animals of the control group decreased with the increase in age, but remained higher (1,54) than in healthy calves (1,4). The low-intensity laser radiation of calves' blood promoted the normalization of themembrane-receptor complexes of erythrocytes, and as a result the EMM decreased to the physiological values (1,32).

The low-intensity laser radiation decreased the number of the middle molecules by 38,2% (λ=254 nm) and 57,3% (λ=237 nm) in the animals, which indicates the decrease in the level of both metabolic and resorptive endogenous intoxication. At the same time, the resorptive auto-toxication progressed in the intact calves with age (1,17 cond. units).

One of the indicators reflecting the state of the blood antioxidant system is the concentration of vitamin E. Its content in the calves of the control group increased by 29,4%, and in the animals of the test group - by 2,1 times on the day 21st. The higher level of vitamin E in the animals treated with the low-intensity laser radiation lends an indirect evidence of the decrease in the intensity of the processes of lipid peroxidation and the attenuation of the destructive processes in their body membrane structures.

Studying of the natural resistance in the calves of the both groups on the first day revealed no significant differencesin values (Table 3).

Table 3. The natural resistance values in hypotrophic calves on the first day

Value	Unit of measurement	Control group	Test group
SBA	%	77,3±2,19	74,2±2,79
SCA	% hem.	15,4±0,30	16,1±0,51
SLA	mg / ml	1,6±0,05	1,8±0,11
LPA	%	78,6±3,81	76,0±4,34
PN		6,20±0,75	6,46±0,77
PI		7,8±0,64	7,37±0,65

Note: *p≤0,05; **p≤0,01; ***p≤0,001; numerator – control group, denominator – test group.

Most of the natural resistance values in the animals of the control group decreased in comparison with the background data on the day 21st : SBA by 5,5%, SCA by 8,9, LPA by 3,4, PN and PI by 6,9 and 1,8% while SLA increased by 31,3%. The calves under the influence of the radiation had a more significant increase in SLA (2 times), as well as LPA by 3,4%, PN and PI by 22,3% and 23,5%, respectively, and a less prominent decrease in SBA (by 1,4%) and SCA (2,3%). Comparing the natural resistance values in the intact animals and the test group calves within the indicated period, it should be noted that the latter had 2,1 times higher SCA, 1,7 times higher SLA, 4,2% increase in LPA, 36,9% and 18,8% increase in PN and PI respectively, which indicates a stimulating effect of the radiation on thenonspecific protection (Table 4).

Table 4. The natural resistance values in hypotrophic calves on the twenty- first day

Value	Unit of measurement	Control group	Test group
SBA	%	71,8±2,89	72,8±1,74
SCA	% hem	6,5±0,60	13,8±0,62***
SLA	mg / ml	2,1±0,46	3,6±0,13**
LPA	%	75,2±1,78	79,4±1,04*
PN		5,77±0,67	7,90±1,02*
PI		7,66±0,42	9,1±0,57*

Note: *p≤0,05; **p≤0,01; ***p≤0,001; numerator–control group, denominator–test group.

The clinical studies in the calves of the both groups recorded gastrointestinal pathology during the colostral period, but the severity and the duration of the

disease progression varied. In all the animals of the control group during the colostral period, the disease progressed in a severe form for 4-6 days, with the first signs registered on the second day in 50% of the cases. From the feces of the diseased calves, the enteropathogenic E. coli of the serovariants 02; 04; 026; 0103; 0138; 0141 were excreted. The relapse of the gastrointestinal diseases was registered on the day 9th of life; the duration of the pathology was an average of 6 days. From the feces of the sick animals, E. coli of the serovariants: 02; 026; 033; 0103; 0138; 0115 and the coronavirus gene were excreted. The mean daily weight gain during 2 months was 340,7 ± 20,0 g. Gastrointestinal diseases were also recorded in all the animals of the test group, but they proceeded in a mild form for 3 days. Diarrheal syndrome was recorded in 50% of the animals from the second day of life, with an average duration of 2 days. The diarrheal syndrome manifested itself on the days 4th-6th of life with an average duration of 3 days in 50% of the animals. From the feces of the sick animals, enteric-pathogenic E. coli serovariants 02; 04; 026; 0103; 0138; 0141 were excreted. The mean daily weight gain during 2 months was 550,5 ± 24,0 g.

CONCLUSION

Low-intensity laser radiation of blood in hypotrophic calves increases the natural nonspecific resistance, the stability of the membranes of the body cellular elements, it stimulates synthetic and metabolic processes, reduces autointoxication, which allows us to recommend it for rehabilitation activities.

REFERENCES

Alekhin, Yu. N. Perinatal Pathology in Cattle and Pharmacological Aspects of its Prevention and Treatment/ Yu. N. Alekhin: Extended abstract of Veterinary Science Dr. Dissertation. - Voronezh: GNU VNIVIPFIT, 2013. - 48 p.

Kataranov, A. A. Clinical and Immunological Characteristics of Newborn Calves and Non-drug Methods for Correcting their Immunodeficiency/ A. A. Kataranov: dis. ... Cand. Vet. Sciences: 16.00.07.- Saratov, 2005. - 131 p.

Golubtsov, A.V. Crystallization Evaluation of Changes in the Conformational Properties of Blood Serum under the Influence of Low-Intensity Laser Radiation / A.V. Golubtsov // Pressing Issues of Veterinary Biology. - 2013. - №2 (18). - P. 3-7.

Golubtsov, A.V. Application of Low-Intensity Laser Radiation to Increase the Natural Resistance of Hypotrophic Calves / A.V. Golubtsov, V.V. Vasilisin // Vestnik of the Voronezh State Agrarian University. - 2010. - №2 (25). - P. 51-59.

Shakhov, A.G. et al. A Methodical Guide for the Diagnosis and Prevention of Abnormalities of Antenatal and Intranatal Origin in Calves. / A.G. Shakhov et al. - Voronezh: Istoki, 2013. - 92 p.

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- INTRODUCTION

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used, etc.

- RESULTS and **DISCUSSION**

Results and Discussion should be combined into a single section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

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

- ACKNOWLEDGMENTS

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

- REFERENCES (LITERATURE)

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. Please see "INSTRUCTIONS FOR AUTHORS" / Bibliographic style on the web page of the Journal for more details: http://agrofor.ues.rs.ba/page.php?id=8

Short communication should include the following sections: Title, Abstract, Key words, Main text, Acknowledgments, References, Tables and Figures with captions.

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