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BIOMASS PRODUCTION OF MORINGA (*Moringa oleifera* L.) AT VARIOUS SOWING DEPTHS IN A COARSE TEXTURED SOIL

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ABSTRACT

Different parts of Moringa (*Moringa oleifera* L.) plant, especially leaves, pods and flowers, are usually processed for human and animal consumption as these parts have been reported to be rich in nutrient elements that are essential for human and animal health. Consequently, a 19-month field experiment was conducted to determine effect of seed sowing depth on biomass production by moringa plant at the Teaching and Research Farm of the University of Ibadan, Nigeria. Moringa seeds were sown at five sowing depths of 1.5, 3.0, 5.0, 7.0 and 9.0 cm. The results showed that sowing depth significantly ($P \leq 0.05$) affected emergence of seeds 14 days after sowing in the following order: 1.5 > 3.0 > 5.0 > 7.0 > 9.0 cm sowing depths. Also, number of branches, height, canopy cover and dry biomass of the resulting seedlings were significantly influenced by seed sowing depths. Number of branches, heights and dry biomass of seedlings from seeds sown at 1.5 and 3.0 cm depths were at par but were significantly superior to those seedlings from seeds sown at 5.0, 7.0 and 9.0 cm depths. However, canopy cover was not in a definite order. Data obtained in this study seemed to indicate that sowing moringa seeds beyond 3.0 cm depth in the field may not be beneficial to moringa plant for organic biomass production.

Keywords: *Moringa, sowing depth, seed emergence, organic biomass.*

INTRODUCTION

Moringa oleifera L. is one of the most widely cultivated tropical tree species of the monogeneric family Moringaceae (Order Brassicales) which consists of 13 species distributed in sub-Himalayan ranges of India, Sri Lanka, Africa, Madagascar and Arabia (Fahey, 2005), Central America and the Caribbean (Foidl et al., 1999). *Moringa oleifera* tree is a fast growing and drought resistant plant with a tuberous taproot. In the wild, moringa plant ranges in height from 5 to 12 m with a straight trunk (10 - 30 cm thick) with corky whitish bark and umbrella shaped canopy (Fuglie, 2005). It is a perennial tree well known for its multi-purpose attributes,

wide adaptability and ease of establishment. Nutritionally, its leaves, pods and flowers which are rich in nutrients, minerals and vitamins are commonly consumed by both humans and animals (Fuglie, 2000). Moringa plant tolerates a wide range of soil type and responds well to mulch, water and fertilizer application (Manh et al., 2005). It thrives in a well-drained sandy or loamy soil with a slightly acid pH of 6.2 to neutral 7.0 (Aregheore, 2005).

Moringa could either be propagated by stem cuttings or seedlings obtained by sowing dry matured seeds (Fuglie, 2000). Seed germination takes up to two weeks. However, sowing depth of Moringa seeds has been reported to affect the emergence of the seeds, and proper anchorage, growth performance, time of flowering, fruiting and harvesting of the resulting plants (Ugese et al., 2010; Karayel et al., 2008; Allah et al., 2012).

Therefore, for optimum crop performance, Moringa seeds must be sown at the appropriate depth (Aikins et al., 2011) where the emerging seedlings could easily access moisture, oxygen and nutrients. The objective of this field study is to determine appropriate seed sowing depth for optimum shoot biomass yield in moringa plant grown on a coarse textured soil.

MATERIALS AND METHODS

The experiment was conducted at the Teaching and Research Farm of the University of Ibadan, (Latitude 07° 27′ N Longitude 03° 53′ E), Nigeria. The experimental field was cleared mechanically by slashing followed by ploughing and harrowing. The size of the main plot (field) was 36 m x 22 m (792 m^2) that was further sub-plotted, each measuring 6m x 6 m (36 m^2), with a space of 1 m in between each subplot. The experiment was completely randomized with three replications for sowing depths of 1.5, 3.0, 5.0, 7.0 and 9.0 cm. Two seeds were sown per hole with the aid of a calibrated stick at a spacing of 1 x 1 m. The soil was minimally tilled to allow accurate seed placement at the depths calibrated on the stick. After two weeks of complete germination, moringa seedlings were thinned down to one stand per plot.

Soil samples were collected at 0 – 30 cm depth prior to clearing of the site, air-dried, sieved through 2-mm and 0.5-mm sieves for physical and chemical properties respectively. Soil texture was determined using Bouyoucous hydrometer method (Gee and Or, 2002) while bulk density was determined by the core method (Grossman and Reinsch, 2002). Saturated hydraulic conductivity was determined using the Constant Head Permeameter as described by Darcy (1984). Soil pH was determined with the pH meter using a soil/water ratio of 1:1. Organic carbon, total N, available P and exchangeable Na, Mg, K and Ca were determined using routine methods (IITA, 1982) while Fe, Mn, Cu and Zn were determined using Atomic Absorption Spectrophotometer. The selected physico-chemical properties of the experimental soil are presented in Table 1. Data on emergence of Moringa seeds and seedling parameters such as number of branches, stem girth, plant height, and canopy cover were measured from one month after sowing (MAS) to nine MAS. Above-ground total biomass was determined at nineteen MAS by cutting the entire

Moringa tree at 1.5 meter above soil level. The cut portion, which was regarded as the dry matter yield, was then chopped into small bits, packed in a sack and weighed to obtain the fresh weight. The dry weight was determined by drying the chopped bits in the oven for 24 hours at 65 ^{0}C.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA), means were tested and separated by Least Significant Difference (LSD) at 5% level of probability (Gomez and Gomez, 1984) using PROC. GLM and CONTRAST procedure.

RESULTS AND DISCUSSION

Sowing depth effect on seed emergence

Seeds sown at 1.5 cm depth showed significantly highest mean percent emergence while lowest seeds percent emergence at 5 DAS was noticed at 7.0 cm and 9.0 cm depths (Figure 1). The reason behind this trend could be attributed to the longer distance that needed to be covered by emerging plumule from 7.0 and 9.0 cm before it can appear at the soil surface (Karayel and Ozmerzi, 2008), implying that the deeper the sowing depth, the higher the number of days required to emerge at the surface of the soil. Percent emergence at 7 DAS was in order of 1.5 cm › 5.0 cm › 7.0 cm › 9.0 cm sowing depths. The same trend was maintained at the 10 DAS. Between 5 and 7 DAS, seeds sown at 1.5, 3.0, 5.0, 7.0 and 9.0 cm depths had total percent emergence of 93, 82, 70, 78, and 59%, respectively. This infers that moringa seeds can be sown in the field at depths not more than 5.0 cm if quick emergence of plants between 5 to 7 days is required. In adverse climate conditions such as drought period, moringa seeds can however be sown up to 9.0 cm depth where moisture level is reportedly higher than what is found in the top soil.

Table 1. Analysis of physico-chemical properties of the soil (0 - 30 cm) at the experimental site prior to sowing

Property	Value
pH (H_2O)	6.3
Total Nitrogen (g kg^{-1})	1.38
Organic Carbon (g kg^{-1})	13.3
Available Phosphorus (mg kg^{-1})	10.03
Exchangeable acidity	0.30
Exchangeable Bases (cmol kg^{-1})	
K	0.16
Ca	4.89
Na	0.61

Micro nutrients (mg kg^{-1})	
Cu	3.86
Zn	1.03
Particle Size Distribution (g kg^{-1})	
Fine sand	110.0
Coarse sand	642.0
Silt	80.0
Clay	168.0
Textural Class	Sandy Loam

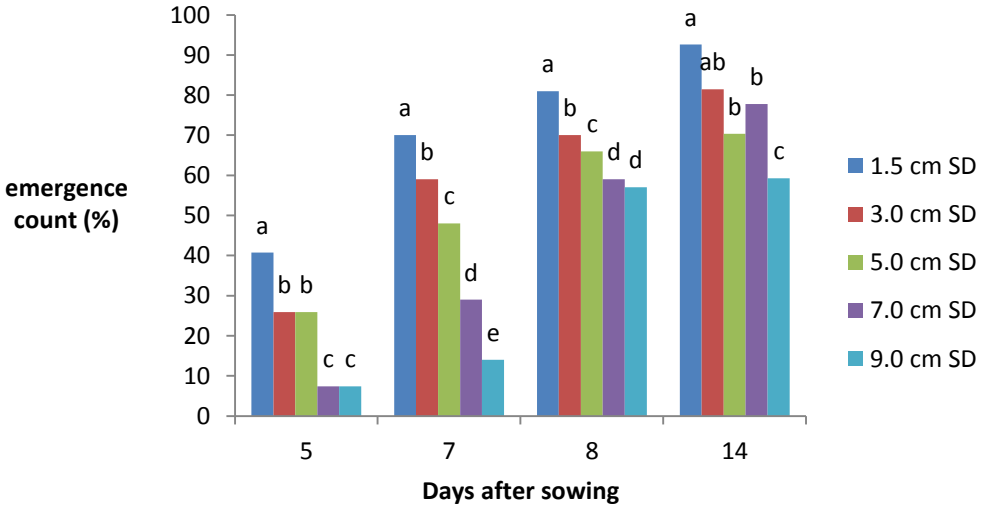

Figure 1. Effect of seed sowing depth on the percent emergence of moringa seeds on the field

Al- Kaisi (2000) reported that deeper sowing depth is preferable in a dry condition to ensure good moisture availability for successful seed germination. At 14 DAS, percent emergence values for seeds sown at 1.5 cm and 3.0 cm depths were significantly higher than seeds sown at other three depths of 5.0, 7.0 and 9.0 cm depths. Liu *et al.* (2007) working in a dry condition discovered that deep sowing might be necessary for crop emergence. This was attributed to the gradual depletion of moisture at the soil surface as the dry season persists. Therefore, sowing depths of 7.0 cm and 9.0 cm might be preferable in dry season while sowing at 3.0 cm is suggested for stand establishment of moringa in wet season.

Effects of sowing depths on plant growth and biomass yield

Sowing depths effect the number of moringa leaves from 2 to 10 WAS (Figure 2). At 2 WAS, seeds sown at 1.5 cm depth showed significant ($p<0.05$) number of moringa leaves produced compared to other sowing depths. Seeds sown at 3.0, 5.0 and 7.0 cm depths were not significantly different ($p<0.05$) for number of leaves produced but significantly different from seeds sown at 9.0 cm depth at 2 WAS. This can directly imply that the deeper the sowing depth, the more the distance needed to be covered by emerging plumule. Therefore, plants that emerge earlier are likely to produce more leaves for photosynthesis. However, sowing depth has no effect on moringa number of leaves from 4 WAS onward.

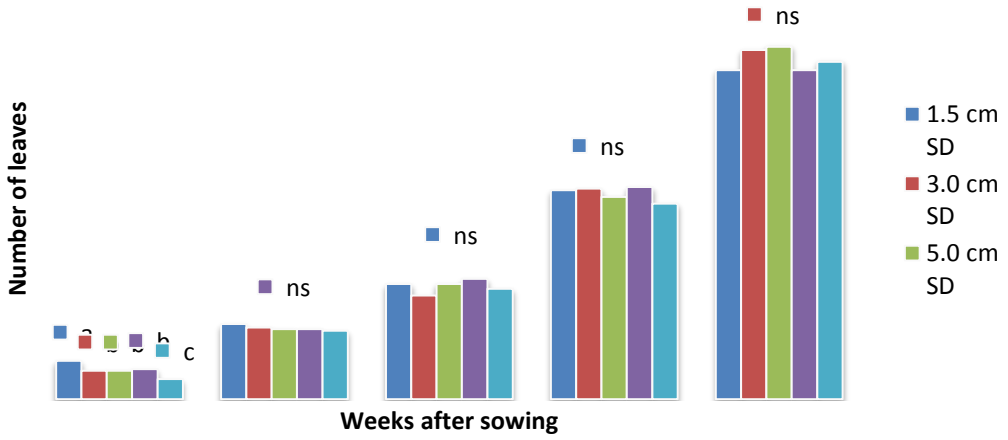

Figure 2. The effect of seed sowing depth on the number of leaves produced by resulting moringa plant

Moringa plant height revealed gradual increase for a period of nine months (Table 2). Between one and two MAS, seeds sown at 1.5 cm and 3.0 cm depth produced taller plants compared to other sowing depths, which implies a faster establishment due to early emergence of moringa seedlings from 1.5 and 3.0 cm sowing depths. Though, there was no significant difference in plant heights among all sowing depths between three and four MAS, seeds sown at 1.5 cm and 3.0 cm depths produced significantly higher plant height than other sowing depths from five to nine MAS.

Table 2. The effect of seed sowing depth on the height (cm) of moringa plant

Seed Sowing depth(cm)	Months after sowing (MAS)								
	1	2	3	4	5	6	7	8	9
1.5	6.4	26.8	64.4	98.2	124.0	150.3	205.6	258.7	303.3
3.0	8.1	29.6	68.4	95.8	120.2	147.2	202.8	258.5	303.6
5.0	7.4	31.1	71.5	90.3	104.2	121.3	160.8	201.5	250.3
7.0	5.1	23.9	57.5	80.2	93.5	113.9	163.1	209.3	247.1
9.0	6.2	27.0	64.3	84.9	103.3	130.0	176.0	220.7	263.5
$LSD_{(0.05)}$	2.9*	5.1*	14.3^{ns}	19.4^{ns}	23.0*	27.6*	29.6*	28.3*	44.4*

*= Significantly different at $p \leq 0.05$; ns = not statistically significant at $p \leq 0.05$.

However, there was no significant difference in the plant heights obtained from seeds sown at 1.5 and 3.0 cm throughout the study period. This indicates that moringa seeds could be sown at 3.0 cm for proper establishment, stability and anchorage. Seedlings obtained from seeds sown at 1.5 cm and 3.0 cm depths attained higher mean stem circumference values than others sowing depths at one MAS (Figure 3), indicating that nutrient concentration decreases with increase in soil depth. Surface soil had higher nutrient concentrations (C, N, K, P and Mg) than subsoil (Oshunsanya, 2014). However, no significant difference in stem circumference of moringa plants was noticed from two to nine MAS.

The moringa plants branched irrespective of the sowing depth for the first five MAS, after which there was significant difference among the sowing depths from six to nine MAS. Emerging plants from seeds sown at 1.5 cm and 7.0 cm depths branched more than plants emerged from 3.0, 5.0, and 9.0 cm at seven MAS. The same trend was maintained throughout the study period (Figure 4).

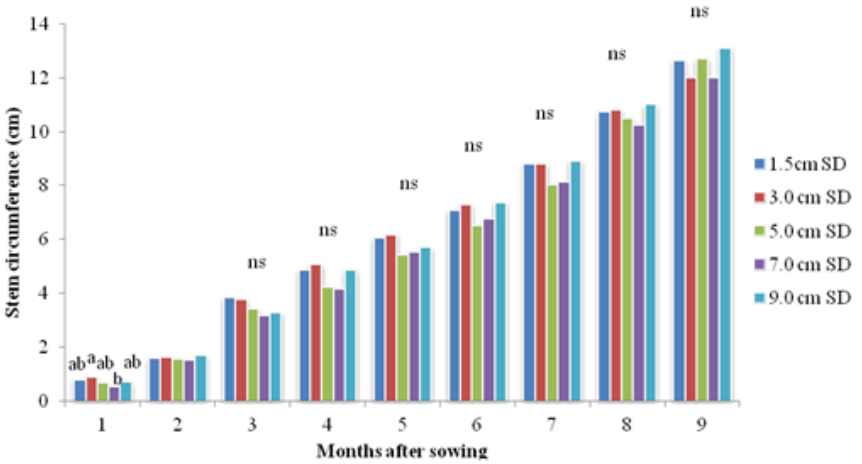

Figure 3. Effect of sowing depth on the stem circumference of Moringa oleifera on the field

*Where: SD = Sowing depth; ns = not significant at $p \leq 0.05$

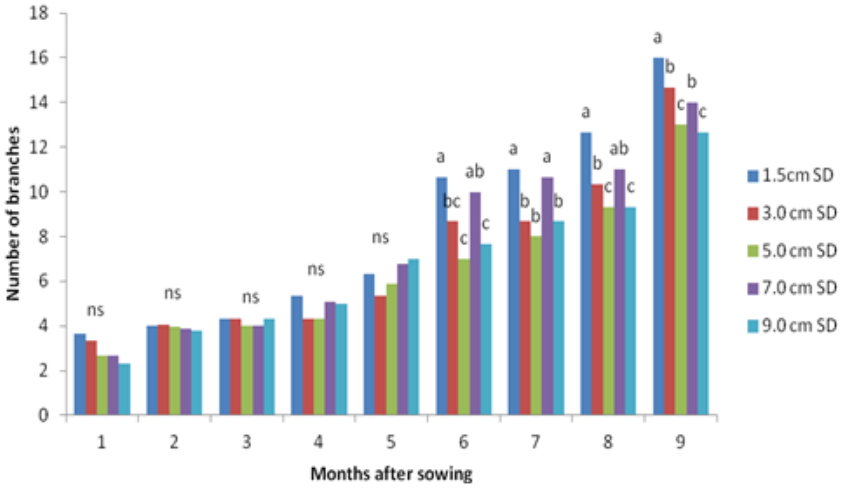

Figure 4. The effect of seed sowing depth on number of branches produced by resulting moringa plant

Moringa plants canopy covers produced from seeds sown at various depths showed significant differences ($p \leq 0.05$). However, canopy covers showed no specific trend with respect to sowing depths (Table 3). It is worthwhile that fresh biomass yield was significantly in the order of 1.5 > 3.0 = 5.0 > 7.0 = 9.0 cm sowing depth (Table 4). This trend is similar to the number of branches and plant height exhibition, reflecting the beneficial effect of early emergence exhibited by seeds sown at 1.5 cm and 3.0 cm depths rather than seeds sown at deeper depths. Early emergence could also bring early leaf and branch formation leading to higher biomass production. High fresh biomass of moringa (leaves and stem) could be used for human and animal nutrition (CTA, 2008).

Table 3. The effect of seed sowing depth on canopy cover (cm) of resulting moringa plant

Seed Sowing depth(cm)	**Months after sowing (MAS)**				
	9	10	11	12	13
1.5	351.8	364.9	390.3	446.8	492.0
3.0	310.0	319.9	342.0	390.0	434.8
5.0	326.2	336.7	386.2	441.6	521.1
7.0	322.0	364.6	391.4	468.4	515.4
9.0	346.7	356.3	380.6	437.5	478.3
$LSD_{(0.05)}$	30.0*	30.0*	31.4*	35.8*	39.6*

*= Significantly different at $p \leq 0.05$; ns = not statistically significant at $p \leq 0.05$.

Table 4. Effect of seed sowing depth on dry biomass yield (t ha^{-1}) of 19-month old moringa plant

Sowing Depth (cm)	**t ha^{-1}**
	Dry biomass yield
1.5	7.1
3.0	6.4
5.0	5.4
7.0	5.1
9.0	4.9
$LSD_{(0.05)}$	0.8*

*= Significantly different at $p \leq 0.05$; ns = not statistically significant at $p \leq 0.05$.

Moringa seeds sown at different depths showed that seeds sown at 1.5, 3.0, 5.0, 7.0 and 9.0 cm produced 7.12, 6.4, 5.41, 5.07 and 4.90 t ha^{-1} dry biomass yields respectively. It is important to note that dry biomass yields from seeds sown at 1.5 and 3.0 cm depth were not significantly ($p \leq 0.05$) different but significantly higher than that of 5.0, 7.0 and 9.0 cm depths. This implies that higher dry biomass content can be obtained from seeds sown between 1.5 and 3.0 cm depths in a coarse textured soil.

CONCLUSION

Sowing depth significantly influenced the field establishment of *Moringa oleifera.* Moringa plant height, number of branches and leaves, fresh and dry biomass yields were highly obtained from seeds sown at 3.0 cm soil depth than 5.0 and 9 .0 cm sowing depths. Therefore, sowing moringa seeds beyond 3.0 cm may not be advisable for quick field establishment and high dry biomass production in a coarse texture soil.

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Original scientific paper
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THE MOST COMMON DISEASES OF ORNAMENTAL PLANTS IN THE ENTITY OF REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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ABSTRACT

During five year period (2011-2015) on territory of Republic of Srpska the continuous monitoring was carried out on different ornamental flowers for the presence of plant diseases. For laboratory analysis following species *Alyssum* sp., *Aster* sp., *Bacopa* sp., *Begonia* sp., *Bellis perennis*, *Calceolaria* sp., *Chrysanthemum* sp., *Cyclamen* sp., *Dahlia* sp., *Dianthus* sp., *Fuchsia* sp., *Gazania* sp., *Gloxinia* sp., *Lobelia* sp., *Myosotis* sp., *Pelargonium* sp., *Petunia* sp., *Petuniaxhybrida*, *Phlox* sp., *Plectranthus* sp., *Portulaca grandiflora*, *Primula* sp., *Ranunculus* sp., *Rusmarinus officinalis*, *Salvia* sp., *Sansevaria* sp., *Tagetes* sp., *Impatiens* sp., *Verbena* sp. and *Viola* sp. were taken. Determination of causal pathogens was carried out on the basis of studying their morphological, pathogenic, biochemical and physiological, serological and molecular characteristics. The most common causal pathogens were *Alternaria* sp., *Botrytis* sp., *Golvonomyces* sp., *Pestalotia* sp., *Phomopsis* sp., *Peronospora* sp., *Phytophtora* sp., *Puccinia* sp. and *Septoria* sp. Also, in much less extent, bacterial and viruses diseases were present. The most common bacterial diseases appear to be genera *Pseudomonas* and *Erwinia*, while the most frequent plant viruses were *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV).

Keywords: *ornamental plants, common diseases, Republic of Srpska*.

INTRODUCTION

In recent years production of ornamental plants in the area of Republic of Srpska (RS) is in a significant increase. However, in this production together with modernization and increasing the production of ornamental plants appeared certain problems. Different types of flowers are often hosts of many plant parasites (fungi, bacteria and viruses), which often lead to their complete extinction, in a stronger occurrence intensity. Therefore an extremely important part of disease control is proper identification of causal pathogen, which was the main goal of this study.

MATERIAL AND METHODS

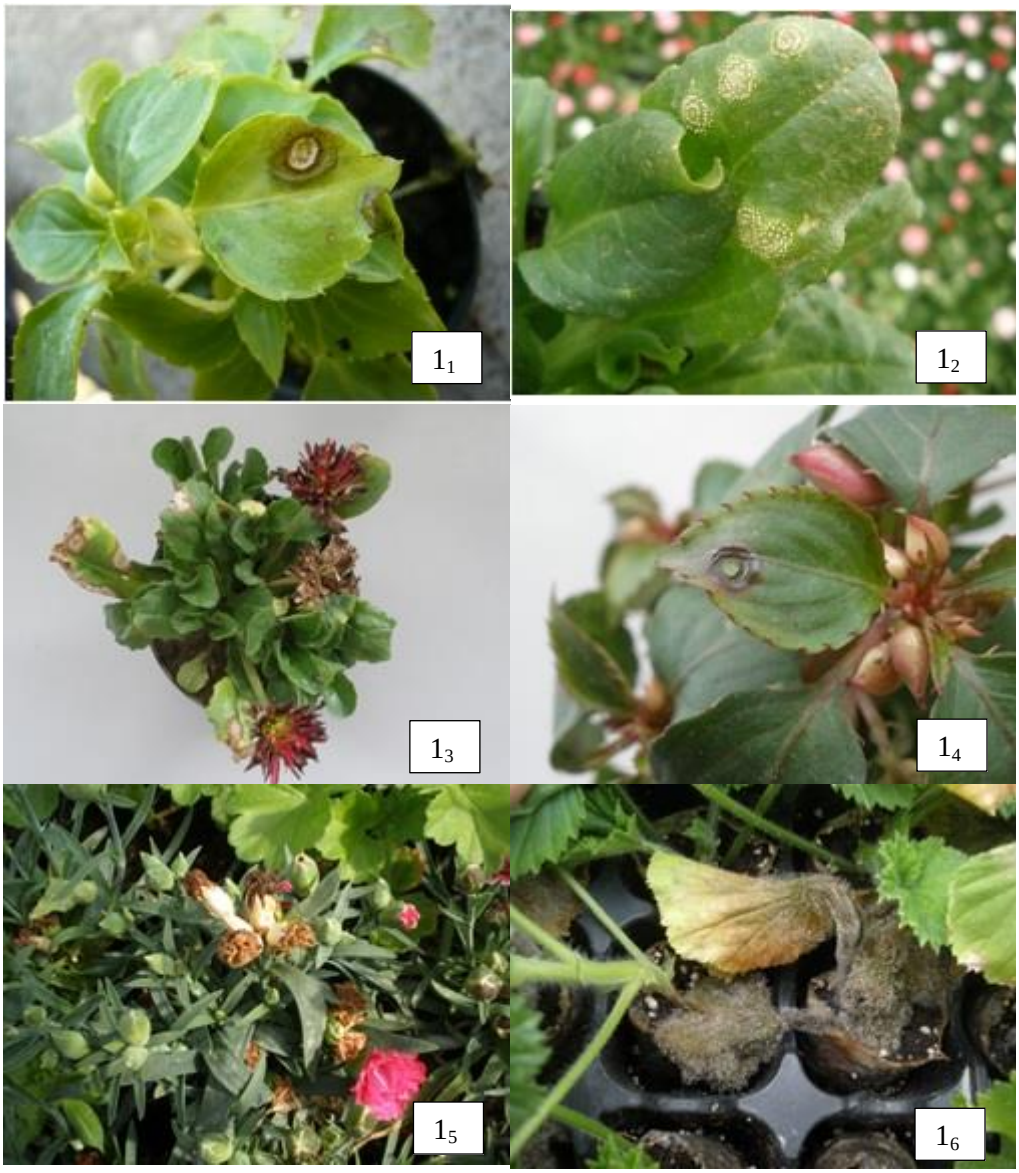
During five year period (2011-2015) on territory of Republic of Srpska the continuous monitoring was carried out on different ornamental flowers for the presence of plant diseases. For laboratory analysis following species *Alyssum* sp., *Aster* sp., *Bacopa* sp., *Begonia* sp., *Bellis perennis*, *Calceolaria* sp., *Chrysanthemum* sp., *Cyclamen* sp., *Dahlia* sp., *Dianthus* sp., *Fuchsia* sp., *Gazania* sp., *Gloxinia* sp., *Lobelia* sp., *Myosotis* sp., *Pelargonium* sp., *Petunia* sp., *Petuniaxhybrida*, *Phlox* sp., *Plectranthus* sp., *Portulaca grandiflora*, *Primula* sp., *Ranunculus* sp., *Rusmarinus officinalis*, *Salvia* sp., *Sansevaria* sp., *Tagetes* sp., *Impatiens* sp., *Verbena* sp. and *Viola* sp. were sampled. Plant material with visible symptoms (photo $1_{1\text{-}16}$) was collected and grouped according suspicion to fungal, bacterial or virus diseases.

A first group of ornamental plants was cut into fragments, 5 mm long, and surface sterilised with 5% sodium hypochloride (ACE) for 30 sec. Then fragments were washed under tap water and placed into disinfected paper to remove water from their surface. After surface disinfection plant fragments (5 per dish) were placed on PDA (Difco, pH 6.5) and incubated at 25°C. Identification of chosen fungal isolates was carried out after 14 to 21 days of incubation based on morphological and cultural characteristics, as well as on pathogenicity test.

Selected tissue of second group of ornamental plants was washed under tap water and placed into disinfected paper to remove water from its surface, and then air dried on room temperature. Small fragments of tissue were cut, surface sterilised with 96% ethanol and then crushed with sterile water. The suspension was placed on mesopeptonagar in Petri dishes (Arsenijević, 1992, 1997). After 48 hours of development in the incubator at a temperature of 26°C, single colonies of developed bacteria were placed on mesopeptonagar. In this way, pure cultures were obtained by a large number of isolates. Vitality of isolated strains was maintained with frequent growing on media at intervals of 20-30 days. For better preservation of culture 24 hours old pure culture were sealed with paraffin oil and kept in a refrigerator at a temperature of 4°C. Chosen bacterial isolates were investigated for pathogenicity in inoculation test and identification was performed based on morphological and biochemical - physiological characteristics.

Third group of symptomatic plants were analysed by serological method, DAS-ELISA test to detect the presence of phytopatogenic viruses using double-antibody sandwich (DAS)-ELISA kit (AG Bioreba, Reinach, Switzerland). The procedure was performed according to the manufacturer's instructions. ELISA (enzyme-linked immunosorbent assay) method is based on the reaction of the antibody–antigen and has been used for the diagnosis of the presence and amounts of specific molecules in the mixed sample. Primary antibody (specific for the protein being tested) was adsorbed onto a solid substrate in the wells of the ELISA plate, after which the known amount of sample was added in the same wells. In the case of the presence of the corresponding virus in the plant material, the entire antigen in the sample binds to the antibody. Another specific enzyme–labelled antibody has been added to the second place in the test protein. The enzyme causes

a colour change in the presence of a substrate reagent, with different intensity depending on the concentration of virus in the test sample. Reading the intensity of wells coloration on ELISA plates was carried out on the MULTISCAN FS instrument (Thermo Scientific, Germany). This instrument registers the transience of the light beam, wave length 405 nm through wells of ELISA plates. The reaction was considered positive if the absorption of light at 405 nm was at least twice as high compared with the absorption of the corresponding control (commercially available negative and positive control).



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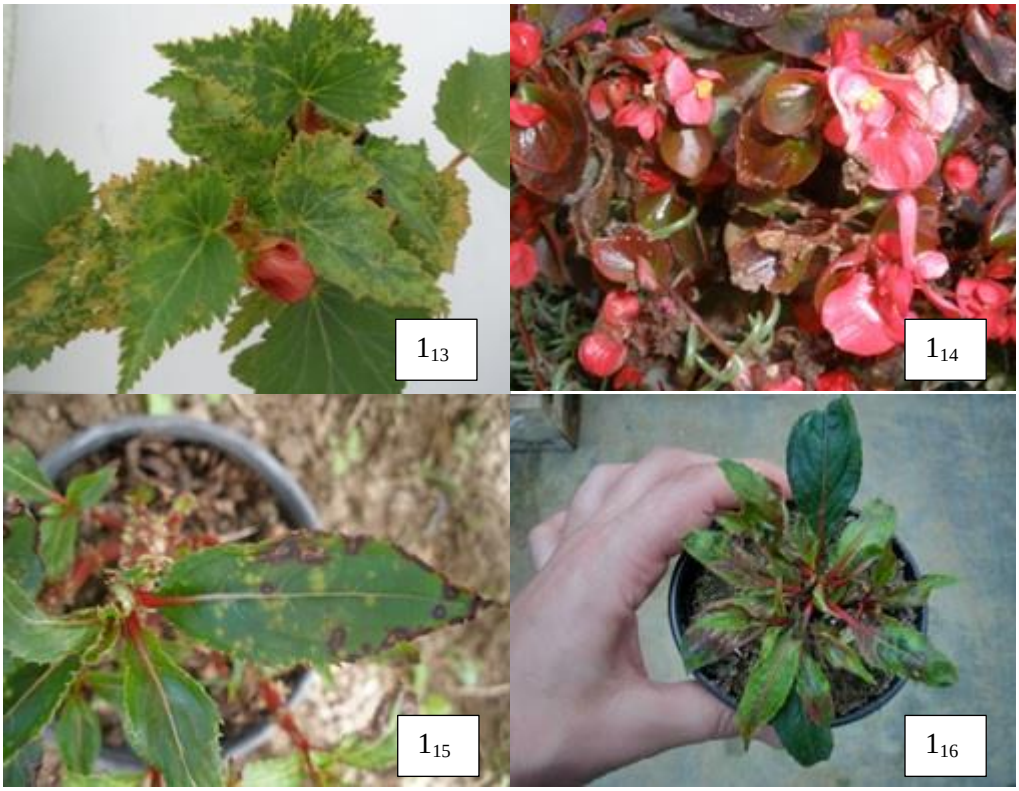


Photo $1_{1\text{-}16}$. Plant material with visible symptoms of different ornamental plants collected and grouped according suspicion to fungal, bacterial or virus diseases.

RESULTS AND DISCUSSION

According to the conducted research during five years period (2011-2015) on territory of Republic of Srpska, studied pathogenic, morphological and biochemical-physiological characteristics, as well as serological and molecular methods of chosen disease simptomatic ornamental plants the most common causes of diseases appear to be phytopatogenic fungi and viruses, while in much less extent bacterial diseases seem to be present in ornamental plants.

Chosen fungal isolates were investigated for pathogenicity in inoculation test on ornamental host plants. Morphological characteristics were studied under the light microscope magnification of 40x, while cultural characteristics of tested isolates, appearance, structure, color, mycelial growth and fructification were also studied. According to the identification of chosen fungal isolates based on morphological and cultural characteristics and pathogenicity in inoculation test, five year monitoring showed that the most common phytopatogenic fungi on ornamental plants were species from the following genera: *Alternaria*, *Botrytis*, *Golvonomyces*, *Pestalotia*, *Phomopsis*, *Peronospora*, *Phytophtora*, *Puccinia* and *Septoria* (photo $2_{1\text{-}8}$).

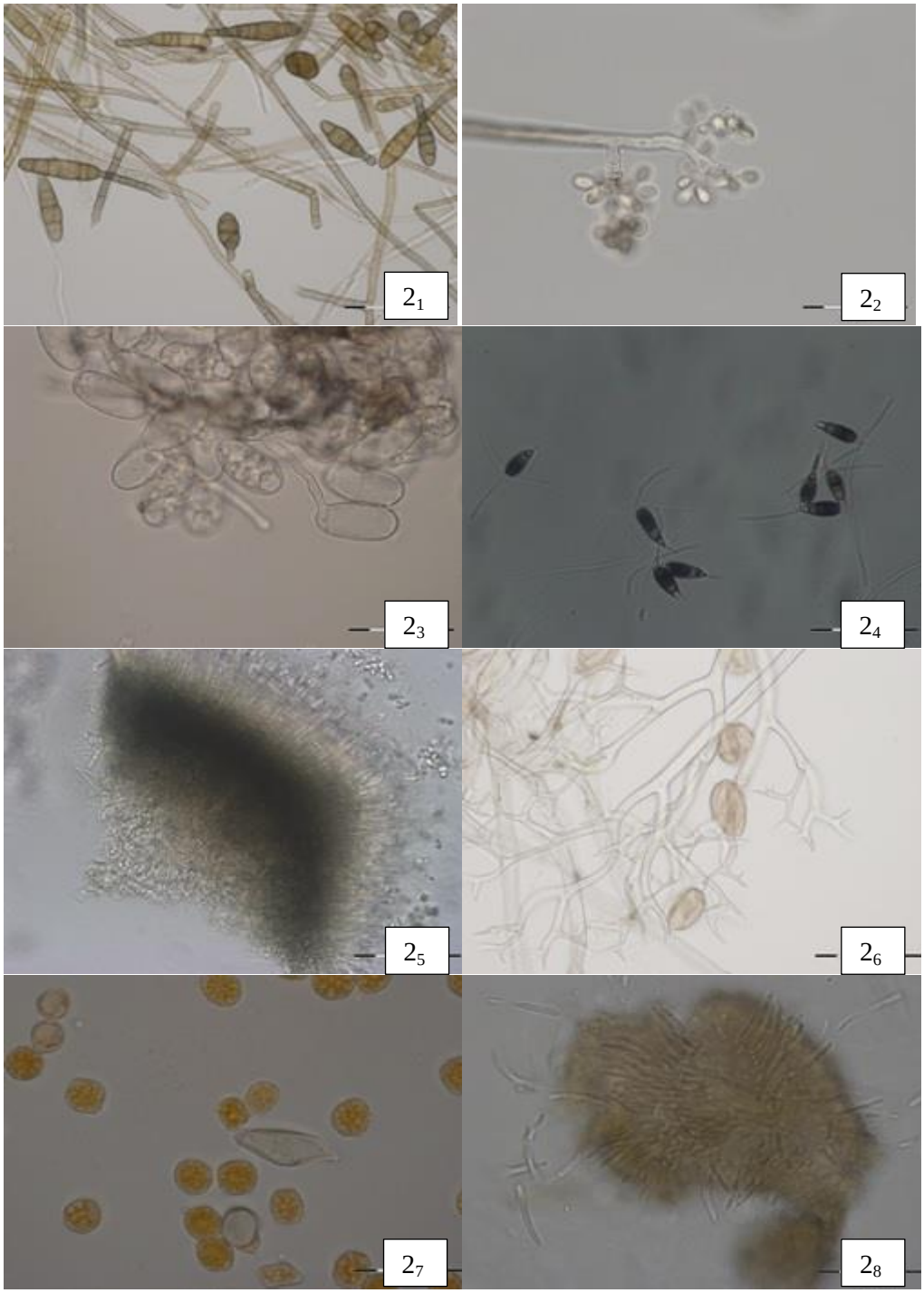
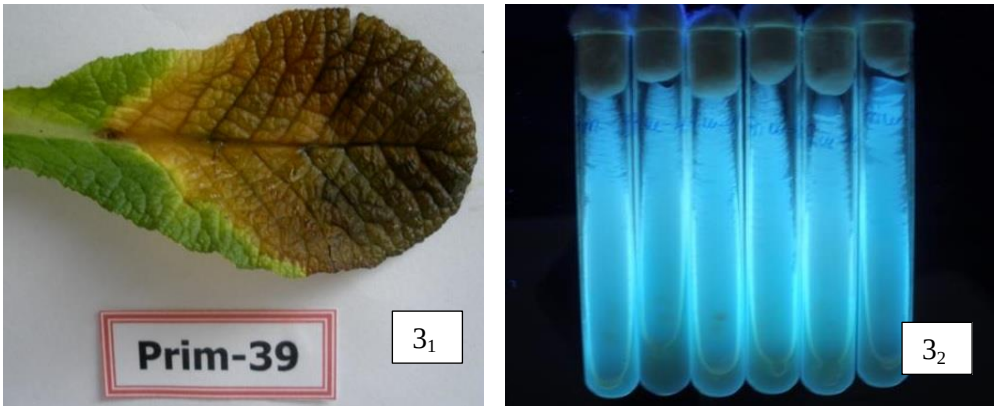


Photo 2_{1-8}. The most common phytopatogenic fungi were species from the following genera: *Alternaria*, *Botrytis*, *Golvonomyces*, *Pestalotia*, *Phomopsis*, *Peronospora*, *Puccinia* and *Septoria*.

Bacterial isolates were investigated for pathogenicity in inoculation test on ornamental host plants. Morphological characteristics of chosen bacterial isolates were studied under light microscope, while Gram staining is confirmed with 3% KOH (Arsenijević *et* Jovanović, 1995). Cultural characteristics, development on mesopepton media and King B (fluorescence), as well as development on Levan and YDC media were studied. Among a biochemical and physiological characteristics creation of catalase, oxidase and arginine-dihydrolase and O/F test glucose metabolism were studied (Fahy and Persley, 1983; Lelliott and Stead, 1987; Arsenijević, 1992, 1997). According to the studied pathogenic, morphological and biochemical-physiological characteristics of chosen bacterial isolates the most common causes of bacterial diseases in ornamental plants appear to be *Pseudomonas* sp. (Photo $3_{1\text{-}2}$) and *Erwinia* sp.



Photo$3_{1\text{-}2}$. Symptoms on the leaves primrose (artificial inoculation) and creation of a fluorescent pigment on King B medium

According to serological test, DAS–ELISA, of third group of symptomatic plants it is confirmed the presence of *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). TSWV was confirmed in genera *Begonia, Impatiens* and *Gloxinia,* while INSV was confirmed only in *Begonia* and *Impatiens* genera.

CONCLUSION

The most important fungi on ornamental plants confirmed during five year monitoring (2011-2015) on territory of Republic of Srpska are species from the following genera: *Alternaria, Botrytis, Golvonomyces, Pestalotia, Phomopsis, Peronospora, Phytophtora, Puccinia* and *Septoria.* Some species of ornamental plants like *Chrysanthemum* sp. and *Primula* sp. were confirmed as hosts of *Erwinia* spp., which on them can cause a mushy, brown, smelly, soft rot or leaf spots. These bacteria can cause symptoms on a wide range of different ornamental plants (Daughtrey *et al*., 1995; Moorman, 2016). Among *Pseudomonas* species according to Trkulja *et al*. (2012), *Pseudomonas marginalis* (Brown) Stevensis reported as causal agent of bacterial spot and soft rot of primrose leaves and flowers. It is

polyphagous bacteria and can cause damp rot on lettuce as well as on a number of other hosts, such as Chinese cabbage (Choi *et* Han, 1989), garlic (Choi *et* Han, 1990a), ginger (Choi *et* Han, 1990b), broccoli (Canaday *et al*., 1991), rice (Cottyn *et al*., 1996) and onions (Kim, 2002). During the five year monitoring (2011-2015) by serological tests confirmed the presence of two viruses - *Tomato spotted wilt virus* and *Impatiens necrotic spot virus* in ornamental plants on territory of Republic of Srpska (Trkulja *et al*., 2013a,b).

Considering research results it is necessary to take control measures for pathogens of ornamental flowers, in order to prevent the spread of the pathogens on numerous other hosts which are grown indoor and outdoor in the Lijevce field, Posavina and Semberia.

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TOWARDS AN INTEGRATED ANALYTICAL FRAMEWORK TO MAP SUSTAINABILITY TRANSITIONS IN FOOD SYSTEMS

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ABSTRACT

Transitions to sustainable food systems are considered necessary to address sustainability challenges in industrial food systems – but also to achieve food and nutrition security especially in countries of the South. To facilitate such transitions, we need a thorough analytical understanding of change processes in food systems. Different transition frameworks have been suggested in the literature, with the Multi-Level Perspective (MLP) on socio-technical transitions being the most prominent. While MLP has proven to be a useful heuristic, earlier studies have identified weak points (e.g. regarding agency, power, landscape factors and institutional innovations) calling for the integration of complementary concepts. This paper proposes a framework for the analysis of sustainability transitions in food systems that integrates elements of the Social Practices Approach, Transition Management, Strategic Niche Management and Innovation Systems. The starting point of the suggested analytical process is to map emerging sustainable food systems along the MLP levels of niche, regime and landscape. To better understand processes of creating and developing initiatives in food systems, our mapping relies on Innovation System approaches (e.g. identifying actors and their networks), Transition Management (e.g. niche stabilization and expansion processes) and Strategic Niche Management (e.g. breakthroughs). As wider transitions require a reconfiguration of relevant regimes, interactions across levels are of particular interest. The Social Practices Approach helps to make niche-regime interactions explicit. Finally, by looking at the impacts and outcomes of change initiatives, we can make statements about the type of transition pathway taken – and whether an initiative has transformative potential or is an incremental adaptation. Further work is needed to refine and test the framework in different contexts.

Keywords: *sustainability transitions, transition framework, multi-level perspective, food systems, agriculture*.

INTRODUCTION

The identification and understanding of trajectories that can navigate societies towards sustainability have become a focus of socio-ecological research (Lachman,

2013). Complexity, ambiguity, interconnectedness and multidimensionality of sustainability problems imply that selective change will not be sufficient - there is a need for transformative systemic change (STRN, 2010). Markard *et al.* (2012) defined sustainability transitions as "*long-term, multi-dimensional and fundamental transformation processes through which established socio-technical systems shift to more sustainable modes of production and consumption"*.

The early research on sustainability transitions tended to focus on energy and mobility systems (Hinrichs, 2014). Accordingly, current transition frameworks are better suited for understanding such systems. Sutherland *et al*. (2014) considered the inherent complexity of food systems as the main factor that complicates the analysis of food systems. Our interest was to identify elements of different conceptual approaches that would help to accommodate the particularities of food systems – other authors have suggested such an integration of perspectives (e.g. Markard & Truffer, 2008; STRN, 2010; Geels, 2011; Geels *et al*., 2015; Spaargaren *et al*., 2016).

Lachman (2013) reviewed the most prominent transition frameworks including the Multi-Level Perspective (MLP), Strategic Niche Management, Transition Management, and Innovation Systems. We therefore reviewed these frameworks, integrated selected elements with the purpose of developing an integrated framework which is better suited to the particularities of food systems.

THE MULTI-LEVEL PERSPECTIVE AS A CONCEPTUAL FOUNDATION

We propose to structure the mapping of transition dynamics in food systems along the levels of the Multi-Level Perspective (Box 1). The MLP has proven to be a helpful construct for making meaning of complex processes – MLP comprises landscape factors, i.e. trends that create opportunities or exert pressures on systems to change. Social behaviour and rules are structured in regimes – these regimes are affected by landscape factors and may be challenged by niches. New practices and ideas emerge from niches – such as more sustainable ways of producing, processing or consuming food. The degree of interaction between the MLP levels determines the impact of niche initiatives towards transformative change.

Box 1. The Multi-Level Perspective (MLP) on socio-technical transitions.
MLP is a widely-used approach to analyse socio-technical transitions. According to the MLP (Rip & Kemp, 1998; Geels, 2002; Smith *et al*., 2005; Geels, 2010; Schot & Geels, 2008; Smith *et al*., 2010; Geels, 2011) transitions come about through interactions within and between three levels: *niches* (micro level; locus of radical innovations); *regimes* (meso level; locus of established practices and associated rules); and *landscape* (macro level; exogenous trends). In MLP, niche-innovations build up internal momentum, changes at the landscape level create destabilising pressure on the regime, and regime destabilisation creates windows of opportunity for radical niche innovations (Markard & Truffer, 2008; Geels, 2011). MLP emphasises that processes at niche, regime and landscape levels should be aligned for a transition to be successful (Geels, 2011). While the MLP has proven to be a useful heuristic to characterize transition processes, it was criticised for the lack of conceptualisation of agency, power and politics; the superficial specification of regimes; its bias towards bottom-up change models; the vague role of landscape

Considering the criticisms of MLP (Box 1) and the particularities of food systems, we further operationalize the analysis of niche, regime and landscape level using elements of Transition Management, Strategic Niche Management, Innovation Systems and Social Practices Approach (Figure 1).

NICHES: CHALLENGING BUSINESS-AS-USUAL

The niche and the activities within it are vital for sustainability transitions (Hinrichs, 2014). When they attempt to bring about change, niches may focus on alternative technologies and practices, new configurations of actor groups, new networks, new beliefs and values, or new policies. Depending on the character of the niche, it may challenge or eventually replace the incumbent regime (Lachman, 2013; Darnhofer, 2015). To usefully characterize niches, we follow the recommendations by Sutherland *et al.* (2014), asking: what exactly is the niche that is analysed? In what way is the novelty it proposes different from the current regime? What regime(s) does it aim to transform?

Transition Management (Rotmans and Loorbach, 2009), and *Strategic Niche Management* (Kemp *et al*., 1998) help to better understand mechanisms and processes of niche creation and development: Has the niche been supported by government policies? How were niche-initiatives organized internally? How did the niche develop rules, adapt in learning processes and stabilized its networks?

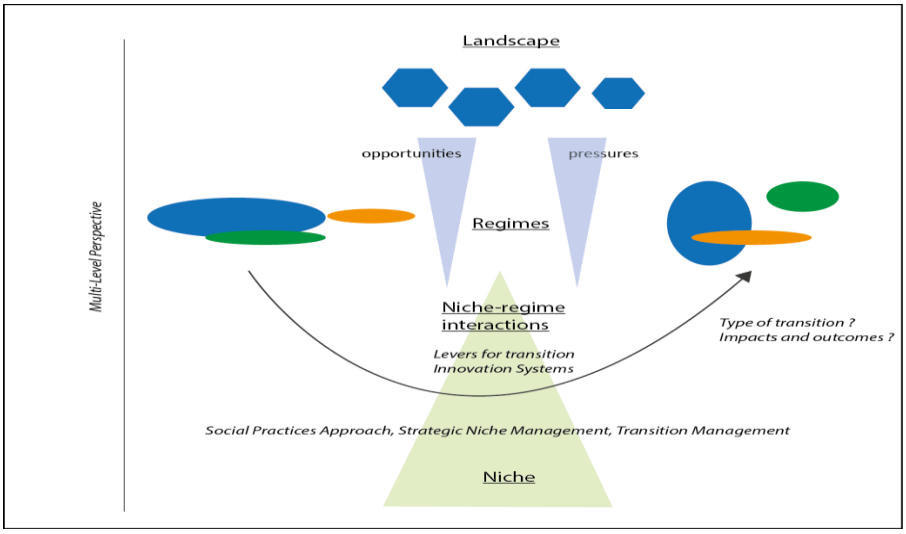

Figure 1. Proposed sustainability transition analytical framework.

Source: Adapted from Geels (2011), Darnhofer (2014), Geels & Schot (2007), Shove & Walker (2010), Hekkert *et al*. (2007), Sutherland *et al*. (2014), Kemp *et al*. (1998).

REGIMES: ESTABLISHED RULES AND INSTITUTIONS

The analysis of the elements of a food system regime will have to include the network of involved actors and social groups; the set of formal and informal rules that guide the activities of actors; and material and technical elements (Geels, 2004). We can distinguish between regulative, normative and cognitive components (Table 1).

Table 1. Types of rules and institutions in socio-technical regimes.

Type	**Examples**
Regulative	Laws, formal rules, incentive structures, sanctions, governance systems, power systems, protocols, standards, procedures
Normative	Norms, values, role expectations, authority systems, codes of conduct
Cognitive	Beliefs, priorities, problem agendas, bodies of knowledge (paradigms), models of reality, jargon/language

*Source: Sterrenberg *et al*. (2013).

Food subsystems (e.g. production, consumption) are shaped by different sub-regimes (e.g. science and education, culture). Related regimes that impact food systems are energy and recreation regimes. The drawing of boundaries between different food subsystems and between various regimes is often difficult and has to be explicitly justified. As for interactions between regimes, Raven and Verbong (2009) proposed to focus the analysis on four types: competition, symbiosis, spill-over and integration.

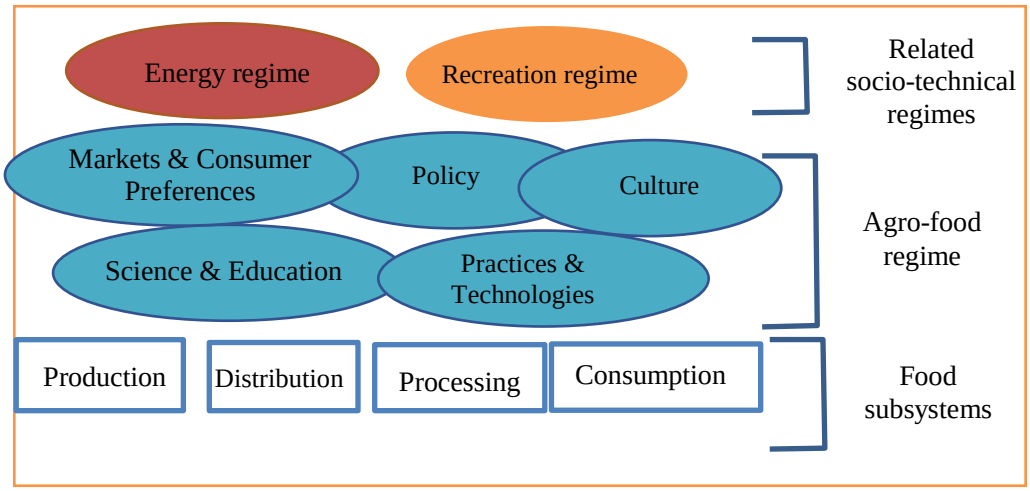

Figure 2. Food sub-systems governed by the agro-food regime and shaped by closely related socio-technical regimes.
*Source: Adapted from Geels (2002), Geels (2011) and Darnhofer (2014).

LANDSCAPE: EXTERNAL TRENDS

The analysis and characterization of the landscape considers external trends, changes and exogenous factors that create pressure or opportunities in food systems (Figure 3). The various trends are interrelated and linked through reinforcing feedback loops.

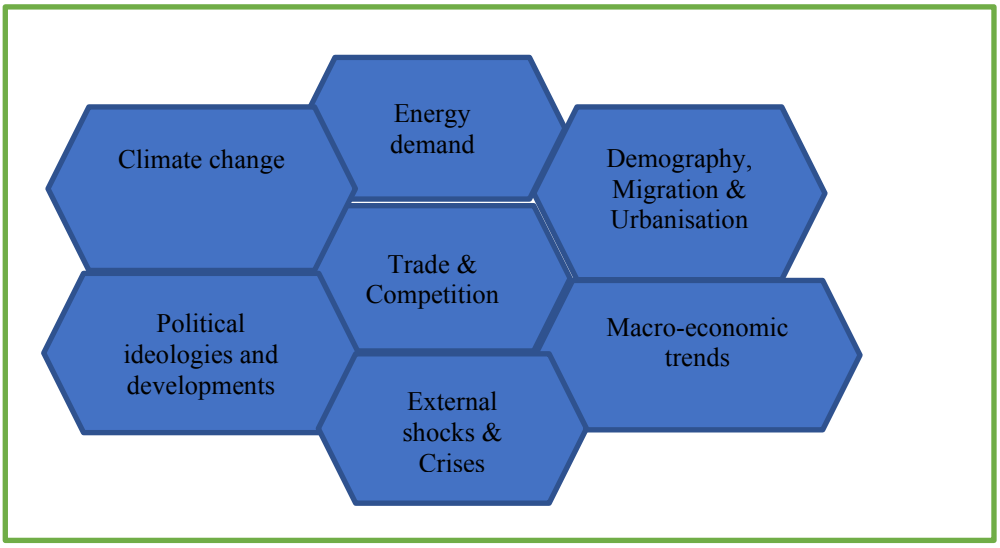

Figure 3. Examples of landscape elements shaping food systems.
*Source: Adapted from Geels (2002), van Driel & Schot (2005), Lachman (2013) and Darnhofer (2014).

IDENTIFYING LEVERS FOR SUSTAINABILITY TRANSITIONS

A deeper transition to sustainability requires a reconfiguration of relevant regimes. To identify potential levers for initiating change, we thus have to look closely at niche-regime interactions and regime-regime interactions. Niche-regime relations do not necessarily have to be opposed – regime structures can overlap or align with niche proposals. A characteristic way how niches interact with regimes is to 'anchor' (cf. Elzen *et al*., 2012) by proposing new rules/institutions, technical systems (technology, infrastructure, processes, practices) or networks (social groups, human actors, organizations). Anchoring can also take the form of legitimization: scientific, policy/politics, legal, practical, civic (Montenegro de Wit & Iles, 2016). Leverage points for transition are thus found at the interfaces where interactions occur.

TYPE OF TRANSITION

Transitions may differ regarding the interactions between niches, regimes and landscapes. To facilitate transformative change, it is important to understand different transition pathways. Geels and Schot (2007) proposed the following categorization, according to timing and nature of multi-level interactions: transformation, technological substitution, reconfiguration, de-alignment and re-alignment. Transition pathways can also start in one pathway but later shift to another. De Haan and Rotmans (2011) identified three basic patterns of regime shifts: re-constellation (top-down constellation change), empowerment (bottom-up constellation change), and adaptation (internally-induced constellation change).

ASSESSING TRANSITION IMPACTS AND OUTCOMES

The analysis of transition impacts requires the definition of a desirable state of a system that we want to achieve – such as food and nutrition security through sustainable food systems. Accordingly, we need to specify sustainable food systems, and assess how transition initiatives increase (or decrease, or have no impact on) different system sustainability dimensions (environmental, human, social, cultural, political, financial, physical). The higher the number of dimensions being driven towards sustainability, the higher is the impact of such an initiative and its contribution to a wider food sustainability transition. Principally, we can assess the outcome of an initiative by comparing the current to the previous state (cf. longitudinal analysis with a historical retrospective) or by comparing it to the conventional food system in the same area (cf. horizontal analysis).

CONCLUSIONS

This paper proposes an integrated analytical framework for understanding transitions in food systems. We rely on the Multi-Level Perspective to structure the analysis and integrate additional frameworks to further specify the analytical categories (niche, regime, landscape, levers for transition, type of transition and

impact). These frameworks include Innovation Systems, Strategic Niche Management, Transition Management and the Social Practices Approach. We believe the integrated framework can help to better understand food system transition dynamics both from vertical – landscape to regime to niche – and horizontal – in-between niches, within regimes – perspectives. Further work is needed to refine and test the framework in different contexts in industrialized food systems and those of the Global South.

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STUDIES ON THE ADAPTATION OF QUINOA (CHENOPODIUM QUINOA WILLD.) TO EASTERN ANATOLIA REGION OF TURKEY

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.), an Andean crop, is native food plant of high nutritional value and its cultivation is increasing rapidly in the World. Background studies should be conducted on the determination of appropriate quinoa varieties for different ecologies for its cultivation to become widespread in a healthy way. This study was conducted in 2015 and 2016 in Erzurum and Igdir non-irrigation conditions in the Eastern Anatolia of Turkey. The locations have different ecological characteristics from each other. The field experiments were conducted with 9 varieties in each location in a randomized complete blocks experimental design with four replications. The grain yield and some related characteristics were examined in the study. The grain yield and related characteristics of quinoa varied significantly depending on varieties and locations in the study. According to the two-year results, quinoa cultivation is a risky in Erzurum which has a high altitude and short vegetation period. The grain yields ranged between 110-599 kg ha^{-1} and was found to be quite low. In the Igdır location, 933-1646 kg ha^{-1} of seed yield was obtained from the varieties. The earliest cultivar is Q-52, it has matured in Erzurum at 102 days, Igdir at 118 days. The latest maturation (138 days) was determined in the Oro de Valle cultivar in Igdir conditions. According to these results, moqu Arrochilla, Q-52, Oro de Valle, French Vanilla and Rainbow cultivars can be recommended for non-irrigated conditions of these locations provided that it is seeded early in the spring. However, Erzurum and similar locations are not suitable for the agriculture of this plant due to short plant growing period.

Key words: *Chenopodium quinoa, adaptation, seed yield, highlands, dry conditions.*

INTRODUCTION

The quinoa (*Chenopodium quinoa* Willd.), which originated from the South America, has become highly popular among health-conscious consumers throughout the world. For this reason, studies have been started on adaptation and breeding in many countries. The importance of quinoa is that the seeds have a high

nutritional value. The average percentage of protein in the seeds varies from 7.5% to 22.1% depending on the variety (Cardozo and Tapia, 1979; Wright *et al.*, 2002). The quinoa seeds, which contain all the amino acids needed by the human body, are in the full protein category (Johnson and Aguilera, 1980). Another important feature of the quinoa is that it is gluten-free. In this regard, it is an important source of food for celiac patients (gluten allergy) to meet protein and carbohydrate needs (Jacobsen, 1993). Its seeds are rich in minerals such as Ca, Mg, K, Fe, Cu and Mn and A, B, C and E vitamins (Repo-Carrasco *et al.*, 2003).

Quinoa seeds with high nutritional value are used in human nutrition in many different forms. Its consumption is increasing day by day, especially in USA and EU countries. For this reason, the importance of quinoa agriculture and production is increasing. More scientific research is needed in different regions in order for quinoa farming to spread healthily. One of the most common failures in quinoa cultivation is the lack of appropriate genotypes. There are thousands varieties or ecotypes of quinoa both cultivated and wild in South America. It is important to test these materials in various ecologies to determine their suitability. Because the yield varies greatly depending on genotypes and locations (Miranda *et al.*, 2012).

There are many records that quinoa is resistant to abiotic stress factors and is grown at high altitudes (Jacobsen, 2003; Bhargava *et al.*, 2007). But, adaptation studies have to be carried out in order to determine appropriate varieties in different ecologies. Eastern Anatolia Region is the highest altitude region of Turkey and the plant growing season is very short. However, there are microclimates in the region that have different ecological characteristics. It is expected that the performances of the different genotypes vary in different microclimates within the region. The main goal of this study is to evaluate the adaptability of some quinoa cultivars in two different locations of Eastern Anatolia Region.

MATERIALS AND METHODS

The study was conducted in non-irrigated experimental areas of Ataturk University Faculty of Agriculture (Erzurum), and Igdir University Faculty of Agriculture (Igdir) in 2015 and 2016. Nine quinoa (*Chenopodium quinoa* Willd.) cultivars obtained from different sources at two different locations were evaluated for seed yield and related characteristics. Field experiments were established on March 29th, 2015 and April 6th, 2016 in Igdir; May 5th, 2015 and May 10th, 2016 in Erzurum. In the each experiment, 9 quinoa cultivars were sown in a randomized complete blocks experimental design with four replications. During the seeding, 2500-3000 g ha^{-1} seeds were spread with 35 cm row spacing by hand at 1.5-2 cm sowing depth on parcels (Tan and Yondem, 2013; Geren *et al.*, 2015). Plot size was 6 rows of 4 m, with an inter-row spacing of 0.35 m. Nitrogen was supplied at sowing (75 kg N ha^{-1}) and again during vegetative growth before flowering (50 kg ha^{-1}). Phosphorus was applied at a dose of 80 kg P_2O_5 ha^{-1} for one time when the seed bed was prepared (Jacobsen *et al.*, 1994; Schulte auf˜m Erley *et al.*, 2005; Yazar *et al.*,2013; Tan and Yondem, 2013; Geren *et al.*, 2015). The weed control in the parcels is

done by mechanical methods like cutting and picking. Harvests by hand in the end of September were done at physiological maturity, which was defined as the date when seeds from the main panicle become resistant when pressed (Bertero *et al.*, 2004). The maturation period, plant height, seed yield and harvest index were determined for the cultivars that came to harvest stage.

The data obtained from the study were subjected to the variance analysis according to the randomized complete blocks experimental design. Differences between the averages were determined with the LSD multiple comparison test. Since the first year data cannot be obtained in the Erzurum location, the results are one-year.

Table 1. Monthly temperature and precipitation during the study months of 2015, 2016 and long year average in Erzurum and Igdir locations.

Months	Temperature (^{0}C)						Precipitation (mm)					
	Erzurum			Iğdır			Erzurum			Iğdır		
	LYA	2015	2016	LYA	2015	2016	LYA	2015	2016	LYA	2015	2016
April	5.5	5.3	7.1	13.3	16.4	14.7	51.4	88.6	39.4	34.5	44.1	20.1
May	10.6	10.6	10.5	17.8	21.3	18.7	70.3	81.5	64.8	47.1	41.5	23.5
June	14.9	17.0	14.8	22.1	28.5	23.0	46.3	28.5	88.6	33.0	27.8	26.9
July	19.3	21.2	19.0	25.9	31.8	26.2	26.0	5.8	17.8	13.8	3	32
August	19.4	21.2	21.0	25.1	30.2	27.5	15.9	38.8	13.6	9.6	14.3	7.8
September	14.5	18.3	12.7	20.0	27.2	20.2	22.7	3.9	17.6	11.1	1.4	19.8
Total/Mean	14.0	15.6	14.2	20.7	25.9	21.7	232.6	247.1	241.8	149.1	129.4	110.1

LYA: Long Year Average

Locations where the studies are conducted have very different ecological characteristics (Table 1 and 2). Erzurum is the province which has the highest altitude in the Eastern Anatolia Region with its location 1860 m above sea level. Winter period is long, cold and snowy, summer months are relatively cool and short. Last frosts of spring may extend to May and first frosts of autumn start in September. Therefore, the plant cultivation season is shorter in Erzurum than in other provinces. Igdir is the province which has the lowest altitude in the region with its 876 m altitude. The plant cultivation season is long in Igdir which has the characteristics of microclimate in the Eastern Anatolia Region. Summer months are hot and dry, evaporation is high. Thus, agricultural soils usually have saline characteristics. While locations are similar in terms of the soil texture class, they are different with regard to electrical conductivity (EC), pH, $CaCO_3$ and available phosphorus and potassium for plants. The soils in Igdir location have the characteristics of being slightly saline, slightly alkaline and mid-calcareous, differently from Erzurum location (Table 2).

Table 2. Some physical and chemical properties of soils in research areas

Soil Properties	Erzurum	Igdir
Texture class	Clay-loamy	Clay-loamy
EC (ms cm^{-1})	0.48	2.00
pH	7.1	7. 9
CaCO3 (%)	2.5	6.5
K (kg K_2O ha^{-1})	1380	3430
P (kg P_2O_5 ha^{-1})	74	80
Organic matter (%)	1.4	1.6

RESULTS AND DISCUSSIONS

Days to harvest of quinoa varied significantly with regard to varieties and locations, and the variety x location interaction was found to be statistically significant (Table 3).

Table 3. Days to harvest and plant height of some quinoa cultivars grown in Erzurum and Igdir non-irrigated conditions.

Cultivars	Days to Harvest (days)			Plant Height (cm)		
	Erzurum	Igdir	Mean	Erzurum	Igdir	Mean
Q-52	102	118	110 F	55.4	90.4	72.9 BC
Rainbow	128	136	132 A	80.6	100.2	90.4 A
Red Head	124	132	128 BC	79.8	100.7	90.3 A
Sandoval Mix	121	133	127 C	73.3	100.5	86.9 A
Cherry Vanilla	125	134	129 ABC	71.5	100.4	86.0 A
French Vanilla	109	137	123 D	64.3	99.4	81.9 AB
Mint Vanilla	129	135	132 A	78.2	94.2	86.2 A
Oro de Valle	122	138	130 AB	62.0	101.0	81.5 AB
Moqu Arrochilla	110	118	114 E	57.3	77.3	67.3 C
Mean	119 B	131 A	125	69.2 B	96.0 A	82.6
Cultivar x Location	*5.3***			*10.1**		

*: 0.05, **: 0.01, Upper case within the same column and row are significantly different at 1%.

The earliest cultivar is Q-52, it has matured in Erzurum at 102 days, Igdir at 118 days. The latest maturation (138 days) was determined in the Oro de Valle cultivar in Igdir conditions. Although the location of the Igdir is warmer than that of Erzurum, because of early seeding, cultivars have been maturing longer. Quinoa is a plant that reaches flowers under short day conditions. For this reason, the plants bloom without completing their growth and development in late sowing. The maturation period was shorter in Erzurum conditions, because the seeding was

made late. Differences in the maturation period due to genotypes and locations are also revealed in other studies (Berterao *et al.,* 2004; Szilagyi and Jornsgard, 2014). The plant height of the quinoa cultivars showed significant differences due to genetic characteristics and different ecological factors (Table 3). Because of the longer development period in the Igdir location, the plants were longer than the Erzurum location. Oro de Valle (101.0 cm) in Igdir and Rainbow (80.6 cm) in Erzurum were found as the tallest cultivars. The differences in the plant heights of cultivars may have resulted from their genetic properties and different reactions to the environment conditions. Similar results have been found in studies conducted in other regions with different ecological characteristics using different genotypes (Pulvento *et al.,* 2010; Bhargava *et al.,* 2007). Seed yields in the study showed significant changes depending on the locations and cultivars (Table 4). Moqu Arrochilla (976.8 kg ha^{-1}) was the most productive cultivar as the average of the locations. Seed yield in the Igdir location (1362.4 kg ha^{-1}) is significantly higher than the Erzurum location (252.3 kg ha^{-1}). The low seed yields in the Erzurum location are quite obvious (110.2-599.0 kg ha^{-1}). Cultivar x location interaction was found to be important in seed yield ($P<0.05$). The highest seed yield was determined in the Moqu Arrochilla cultivar grown in Igdır conditions, followed by Q-52, Oro de Valle, French Vanilla and Rainbow varieties grown in the same location.

Table 4. Seed yield and harvest index of some quinoa cultivars grown in Erzurum and Igdir non-irrigated conditions.

Cultivars	Seed Yield (kg ha^{-1})			Harvest Index (%)		
	Erzurum	Igdir	Mean	Erzurum	Igdir	Mean
Q-52	208.3	1592.5	900.4 ab	6.5	26.7	16.6 AB
Rainbow	211.3	1342.8	777.0 abc	6.9	21.9	14.4 ABC
Red Head	110.2	1230.8	670.5 bc	3.1	20.6	11.8 BC
Sandoval Mix	242.4	1409.9	826.1 ab	5.7	22.1	13.9 ABC
Cherry Vanilla	599.0	1137.3	868.1 ab	13.7	19.9	16.8 AB
French Vanilla	357.1	1399.1	878.2 ab	11.2	24.7	18.0 A
Mint Vanilla	140.0	933.3	536.6 c	4.8	15.6	10.2 C
Oro de Valle	178.8	1486.0	832.4 ab	5.7	23.3	14.5 ABC
Moqu Arrochilla	223.7	1729.8	976.8 a	6.6	30.0	18.3 A
Mean	252.3 B	1362.4 A	807.4	7.1 B	22.8 A	15.0
Cultivar x Location	*409.1**			*6.3**		

* and lower letters: 0.05, ** and upper case: 0.01, Different letters within the same column and row are significantly different at 1%.

The Igdir location has a temperate climate than Erzurum (Table 1). Plants in this area were planted earlier and had a longer development period. Also, the fact that the soil of the region has a slightly saline character may be effective for high yields. Because the quinoa performs better in slightly saline soils (Jacobsen, 2003; Wilson *et al.,* 2002). In Erzurum, seed yields were found to be very low because

the plants could not find a sufficient growth period. Although plants have technically reached harvesting stage, the majority of their panicles have not been seeded. Studies conducted with quinoa varieties in different parts of the world have shown that the seed yield varies between 250-5000 kg ha^{-1}. These results are due to the different performance of the varieties in different ecological conditions (Miranda *et al.,* 2012). The harvest index values of quinoa varieties varied significantly between 10.2-18.3% (Table 4). Moqu Arrochilla, French Vanilla, Cherry Vanilla and Q-52 cultivars have higher harvest index than others. Since the location of Igdir is a more suitable for seed production, harvest index values in this locality are found high. Bhargava *et al.* (2007) and Bertero *et al.* (2004) found significant differences in quinoa harvest index according to varieties and locations.

CONCLUSION

The results of this study revealed that quinoa can be grown in the non-irrigated conditions of Eastern Anatolia, a high altitude region in Turkey. Quinoa has a high yield potential in Iğdır and similar locations which have low altitude and long cultivation season. Moqu Arrochilla, Q-52, Oro de Valle, French Vanilla and Rainbow cultivars can be recommended for non-irrigated conditions of these locations provided that it is seeded early in the spring. However, Erzurum and similar locations are not suitable for the agriculture of this plant due to short plant growing period.

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MICROMORPHOLOGY OF POLLEN GRAINS FROM BISEXUAL AND FUNCTIONAL MALE FLOWERS OF POMEGRANATE

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ABSTRACT

Modern taxonomy uses pollen grain morphology as an important tool due to its specific and diverse features. Pomegranate is an andromonoecious species having two type flowers on the same plant, in other words, hermaphrodite flowers (bisexual) and male flowers (functional male flowers) that develop on the same plant. The length of polar axis (*P*) and the equatorial diameter (*E*) as well as *P/E* ratio of pollen grains, collected from both bisexual and functionally male flowers of the cultivar 'Caner I', were analyzed using scaning electron microscopy (SEM). The length of polar axis (*P*), the equatorial diameter (*E*), and *P/E* ratio was compared between the two grain. The sculpturing pattern of the exine of both type flowers was striate, with more parallel longitudinal ridges Pollen from both bisexual and functional male flower types is similar in size (≈21 μm). In contrast to the divergent pistil development observed between bisexual and functional male pomegranate flowers, no differences in pollen morphology were detected. The pollens had prolate shape (P\E=1.65 vs 1.59) in both types of flowers.

Keywords: *Punica granatum, Pollen Grains, Morphology, SEM.*

INTRODUCTION

Due to the fact that pollen grains have a definite shape, size, color, and structure for each species, genus, and family, research into the morphological characteristics of pollen is of great significance in taxonomy, phylogeny, and paleobotany. Electron microscopy is a very powerful technique for testing the morphological characteristics of pollen in the various species of plants (Evrenosoğlu and Mısırlı, 2009). Sotonyi et al. (2000) stated that dimensions of a pollen grain are to a large extent genetically determined and they can be of great importance for cultivar characterization. Morphological characteristics such as differences in size and surface structure of pollen grain are a useful tool for distinguishing species and cultivars of fruit trees (Moore et al., 1991; Geraci et.al., 2012). Research showed that exine surface, aperturation, shape and size of the grain are all useful features to distinguish rosaceous pollens (Hebda et al., 1988; Mert, 2009; Nagy, 2011). Pollen morphology in apples, peaches, cherries, pears and quince demonstrated species-

specific variation in size and exine sculpturing (Chung et al., 2010; Radovic et al., 2016).

Pomegranate (*Punica granatum* L.) has two types of flowers, a condition referred to as andromonoecy. In other words, hermaphrodite flowers (bisexual) and male flowers (functional male flowers) develop on the same plant. Sometimes, the same pomegranate tree can carry three types of flowers, namely hermaphrodite, male and intermediate forms (Engin et al., 2015). Wetzstein et al. (2011) characterized two types of flowers on the same tree: hermaphroditic bisexual flowers and functionally male flowers. Male flowers are smaller, with a campanulate calyx and a rudimentary ovary. Bisexual flowers (long-styled perfect flowers) are larger, have larger ovaries, and set more fruit than short style types. Information on the morphological characteristics of pollen grain to two types of flowers of pomegranate can be very advantageous to identify cultivars, which is of great benefit for the certification of germplasm and breeding purposes. In addition, the form of pollen grains and protuberances on them play a very important role in the pollination of fruit trees, i.e. pollen transfer (either entomophilous or anemophilous) (Dokic, 1988).

The present work aims to describe the morphology and anatomy of bisexual and functionally male flower types in pomegranate to better understand developmental differences between the flower types. In addition, histological evaluations of pollen grains from bisexual and functional male flowers were conducted using SEM to assess structure and size and shape of the pollen grain.

MATERIAL AND METHOD

The orchard site was located at the Çanakkale Onsekiz Mart University's Horticulture Experimental Farm, 5 m above sea level. 'Caner I' cultivar of pomegranate was selected for the research. Flowers at the same stage (before at the open petal stage) of both functional male and bisexual flowers were collected from 13-year-old trees, planted at distance of 3x5 m and taken to the laboratory. Bisexual and functional male flowers were separated based on the size of the pistil, which in male flowers is expressed as a shortened style. Morphological differences of both bisexual and male flowers were photographed digitally. Their anthers were detached slowly using a forceps and then put on a paper at room temperature of 22°C for about 12-18 hours to waste some of their moisture, split and release pollen. The pollen was further dried up at room temperature for 10-12 hours, then put into brown glass vials. Vials were stored in a refrigerator at +4 °C until examined.

Bisexual and functional male flowers were dissected and prepared using methods described by Engin and Unal (2007) for scanning electron microscopy (SEM). For the SEM study, samples were mounted directly on metallic stubs using double-sided adhesive tape and coated with gold in a sputtering chamber (BAL-TEC SCD 005 Sputter Coater). Observation of the prepared samples was carried out with a scanning electron microscope (SEM) JEOL JSM-7100F (Tokyo, Japan) at 15 kV. Sixty pollen grains from

both bisexual and functional male flowers from the 'Caner I' cultivar were examined for morphological characters; shape, length of the polar axis (*P*), length of the equatorial diameter (*E*) and ratio of polar axis to equatorial axis (*P/E*).

The experiment was carried out in three replicates. In each replicate, ten pollen grains were analyzed. The statistical analysis was performed using MINITAB software (Minitab Inc., ver.16) to determine differences in *P*, *E*, and *P/E* ratio. Significant differences between the mean values were determined using Duncan's multiple range test for significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

Under field conditions, bisexual and functional male flowers ratios in pomegranate can impact crop productivity and yield. Functional male flowers drop and generally fail to set thus, fruits develop exclusively from bisexual flowers. The percentage of flowers that are male in pomegranate can be significant and more than 60% to 70% depending on variety and season (Engin et al., 2015; Mars, 2000).

'Caner I' cultivar of pomegranate characterized two types of flowers on the same tree: hermaphroditic bisexual flowers and functionally male flowers. Male flowers are smaller, with a campanulate calyx and a rudimentary ovary (Fig. 1, left). Bisexual flowers (long-styled perfect flowers) are larger, have a discoid stigma covered with copious exudates, elongated stigmatic papillae, a single elongated style and numerous stamens inserted on the inner walls of the calyx tube (Fig. 1, right).

Based on the analysis conducted by SEM, pollen from both bisexual and functional male flower types is similar. In contrast to the divergent pistil development observed between bisexual and functional male pomegranate flowers, no differences in pollen morphology were detected (Fig. 2). Pollen grains can be characterized as spheriodal with a smooth exine surface. In view of the number, position and type of the apertures, the grains are prolate.

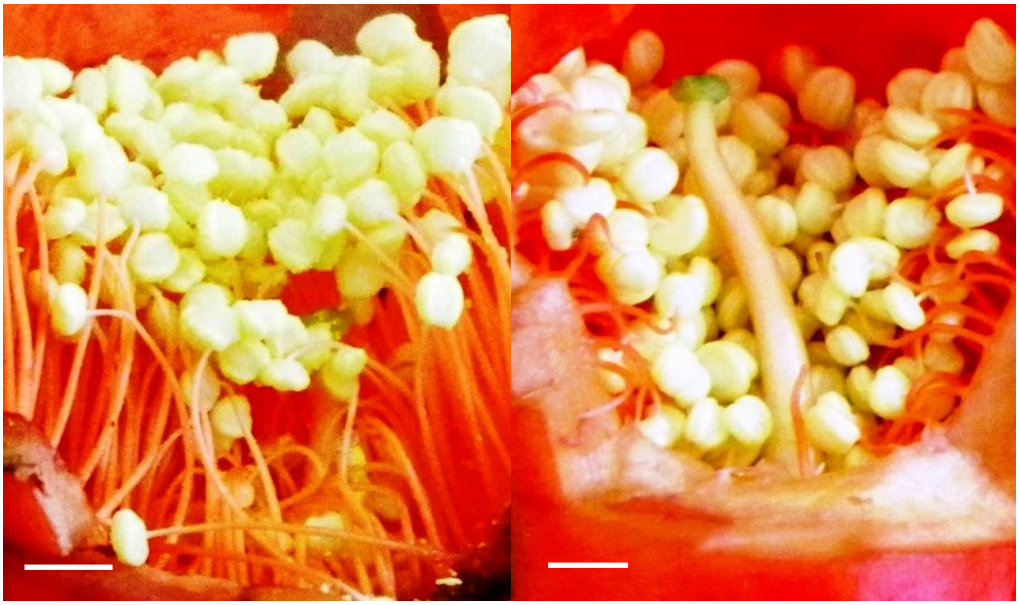

Figure 1. Close-up view of generative organs of pomegranate (*Punica granatum* L.) cultivar 'Caner I' (left: functional male, right=bisexual) Scale bar: 5.0 mm

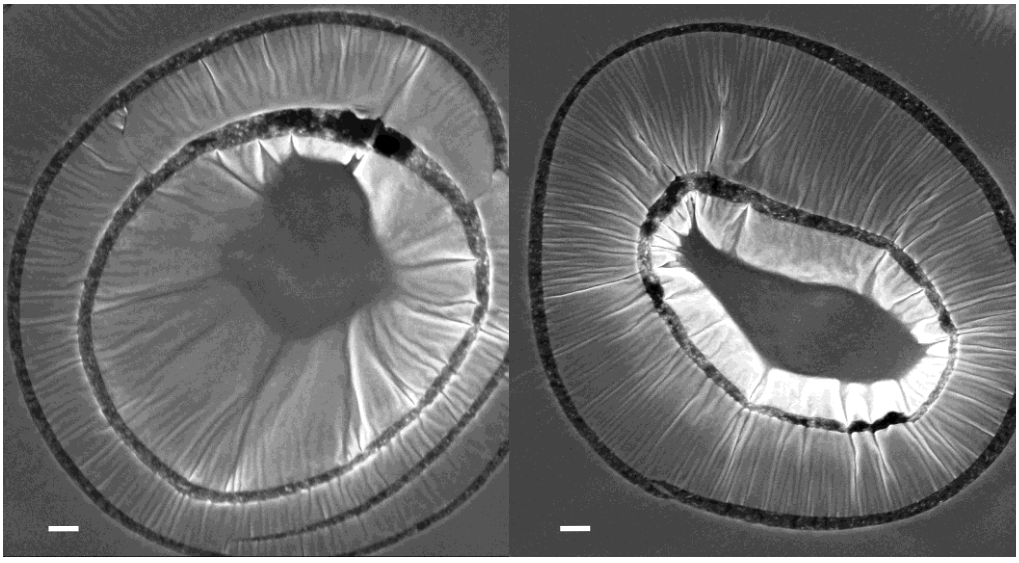

Figure 2. Scanning electron microscopy (SEM) of pollen grain (equatorial view) of pomegranate cultivar 'Caner I' (left: functional male flower and right: bisexual flower). Bars: 1 μm.

Length of the polar axis (*P*), length of the equatorial diameter (*E*) and ratio of polar axis to equatorial diameter (*P/E*) of pollen grains from bisexual and functional male flowers were demonstrated in table 1.

Table 1. Morphological characteristics of pollen grains from functional male and bisexual flowers of pomegranate (*Punica granatum* L.) cultivar 'Caner I' (Mean±SE).

	Polar axis (P) μm		Equatorial axis (E) μm		P/E ratio	shape
	Variation range	Mean value	Variation range	Mean value		
Functional male	27.1-28.2	27.70±1.02 a	15.8-17.2	16.70±1.32 a	1.65±0.07a	prolate
Bisexual	26.6-28.2	27.44±1.08 a	15.4-17.7	17.16 ±1.04 a	1.59±0.09 a	prolate
Pollen (Mean)		27.57		16.93		

*Means within a column (comparing pollens from bisexual versus male flowers) followed by different letters are significantly different at P ≤0.05 using Duncan's multiple range test.

The investigated pollen grains in bisexual and functional male flowers of 'Caner I' cultivar did not differ in their size and shape. Polar length was maximum (28.2 μm) in both sexual morphs and minimum (26.6 μm) in the bisexual flower (Table 1). The width of pollen grains ranged from 15.4 μm to 17.7 μm in both types. The pollen is about the same size (about 21 μm). In relation to the results of Wetzstein et al (2011), pollen size of 'Wonderful' pomegranate cultivar is about 20 μm. This result show that pomegranate pollen is small sized. Shangshang et al. (2015) reported that P/E ratio of 55 indigenous pomegranate cultivars ranged between 1.54 and 2.05. The investigated pollen grain of pomegranate cultivar in bisexual and functional male flowers was classified as prolate, according to the classification of Erdtman (1969). Varasteh and Arzani (2009) characterized the shape of the pollen grains of 14 Iranian pomegranate cultivars as prolate based on *P*/*E* ratio. Evrenosoğlu and Mısırlı (2009) pointed out that in the species of the Mediterranean fruits, length of pollen grains varies from 26.2 to 54.2 μm and width from 15.2 to 29.6 μm. The length of the pollen grain in apple ranged from 40.1 to 43,8 μm and width from 20.9 to 23.2 μm (Currie et al., 1997). In Japanese pear the length was between 43.4 and 45.1 μm and the width between 21.8 and 22.9 μm (Matsuta et al., 1982). Pollen size generally ranges from 15 to 100 μm (Robertson, 2008). Bisexual and functional male flowers of 'Caner I' cultivar had small pollen (15.4-28.2 μm) with grooves on the surface. The sculpturing pattern of the exine is striate, with more parallel longitudinal ridges.

CONCLUSION

The analysis of morphological characteristics of pollen did not show significant differences among bisexual and functional male pomegranate flowers.

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DIFFERENCE IN CUTICLE COMPONENT AND IMMUNOCOMPETENCE IN NURSE AND FORAGER WORKER HONEYBEE (*APIS MELLIFERA L)*

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ABSTRACT

The aim of this work is to study the difference of physiology between the worker bee nurse and forager (*Apis mellifera intermissa*). The chosen physiological characteristics were the component of the cuticle (protein-chitin content) and the measure of the efficiency of immune system (the total number of haemocytes (THC), the normal haemocytes and the relative mass of fat body). The THC is widely used as an indicator of cellular immunocompetence of insects. The normal haemocytes, also referred to immunocytes, indicate the integrity of cellular immune system. The fat body is an indirect measurement of induced humoral immunocompetence. The THC and the normal haemocytes were determined by the method described by Amdam *et al.,* (2004). For the estimation of the cuticular abdominal protein-chitin content, the method described by Berghiche *et al.,* (2007) was employed. The relative mass of fat body was determined using an ether extraction method according to Doums *et al*., (2002) and Wilson-Rich *et a*l., (2008).

The results show that a considerable percentage of a cuticular protein and a decrease of chitin was observed in nurse compared to forager. The older bees exhibited a strong reduction in the immun parameters.

Keywords: *Apis mellifera intermissa, cuticle component, immunocompetence*.

INTRODUCTION

Honeybee, like insects, are known to possess physiological defenses to combat pathogens. The primary defense include the cuticle as the first line of defense and the effectif immune system resolved into broad categories: cellular and humoral components (Jiravanichpaisal *et al*., 2006). The arthropod cuticle is a remarkable and versatile biological material commonly composed of chitin (polymer of N.

Acetyl glucosamine) and proteins (Cribb *et al.,* 2010). The cellular components of insect immunity, named heamocytes, are able to phagocyte, nodulate and encapsulate (Lavine and Strand, 2002; Jiravanichpaisal *et al*., 2006). The total number of haemocytes (THC) is widely used as an indicator of cellular immunocompetence of insects. The normal haemocytes, also referred to immunocytes, indicate the integrity of cellular immune system (Amdam *et al.,* 2005). The humoral reactions involve induced synthesis of antibacterial proteins (by fat body and haemocytes), coagulation and melanisation (Hoffmann *et al.*, 1996; Lavine and Strand, 2002; Lemaitre and Hoffmann, 2007), which is catalysed by the (propheno-) phenoloxidaese (PO). This PO-mediated melanin synthesis plays a major role in an insect's immune defense and in cuticular sclerotisation and quinone production (Lavine and Strand, 2002). According to Andersen (2010), the quinines react with cuticular proteins stabilizing cuticle structure.

Honey bees, the important social insects, exhibit age division of labor, a consequence of individual bees changing jobs as they grew older. The division of labor and the transition of the nurse bees to perform foraging tasks were suggested to be impacted by changes in brain chemistry, brain structure, endocrine activity and gene expression (Robinson *et al*., 1987, 1989; Huang *et al*., 1994; Huang and Robinson,1996; Lass and Crailsheim, 1996; Ben-Shahar *et al.,* 2000; Schulz and Robinson, 2001; Robinson, 2002; Remolina *et al.,* 2007; Heylen *et al*., 2008; Liu *et al.,* 2011; Greenberg *et al*., 2012). The changes physiological functions have been extensively studied in worker honeybees through the ages. In recents years, significant interests have been shown in studying honeybee humoral, cellular immune response (Bedick *et al*., 2001; Amdam *et al.,* 2004, 2005; Klaudiny *et al.,* 2004; Lourenço *et al.,* 2005; Yang and Cox-Foster, 2005; Evans, 2006; Wilson-Rich *et al.,* 2008; Alaux *et al.,* 2010) and characteristcs of integument (Thompson and Hepburn, 1978; Nemtsev *et al*., 2001; Elias-Neto *et al.,2009,* 2014; Seehuus *et al.* 2013; Kaya *et al.*, 2015). We have a few information about the component of cuticle (Thompson and Hepburn, 1978) and cellular immune component of honeybee age-related division labor (Amdam *et al.,* 2005; Schmid *et al*., 2008). Thompson and Hepburn (1978) used abdominal tergites for determination the chemical and mechanical properties of the pharate adult honeybees cuticle. To our knowledge, the report is the first work, which study the cuticle component using the abdominal integument (tergite and sternite) of two ages (nurse and forager). Seehuus *et al.*, (2013) studied the genes encoding cuticle proteins in nurse and forager. A little work is known about another measure of humoral immune defense, the relative percentage of fat body in the abdomen (Wilson-Rich *et al.,* 2008).

The aim of this study is to investigate the difference of the cuticle component and the immunocompetence between the nurse and forager honeybee (*Apis mellifera intermissa*). The chosen physiological parameters were the cuticular abdominal protein-chitin content, the total number of haemocytes (THC), the normal haemocytes and the relative percentage of fat body.

MATERIAL AND METHODS

The experiments were carried out in an apiary of honeybees derived from *Apis mellifera intermissa* during the summer 2015 in northern Algeria (Isser 36° 43' N., 3 ° 40' E). Bees were determined to be foragers if they returned with pollen loads in their corbicular or had a distended abdomen (signifying nectar or, less likely, water foraging) (Huang and Robinson, 1996). The nurses were collected when they entered into the cells and were nursing the larvae (Liu *et al*., 2011). For the estimation of the cuticular abdominal protein-chitin content, the head and thorax of nurse and forager were removed as well as all body appendages before being dissected. The abdomen was carefully cleaned from all adhering tissues. The abdominal integument were washed for 24 h in ether-chloroform (1:1, v/v) and dried to constant weight at 60 °C. Chitin and total soluble protein were assessed in the cuticle of nurse and foragers according to the method described by Berghiche *et al., (*2007). The total number of haemocytes (THC) was conducted by the method described by Amdam *et al.,* (2004). The cells counts using light microscope were done 5–10 min after filling the neubauer hemocytometer. The haemolymph was drawn with a calibrated microcapillary inserted dorsally into the bee between the second and third abdominal tergite of honeybee worker. Clear and slightly yellow hemolymph was drawn out by capillary action. The normal haemocytes count was determined by the method described by Amdam *et al.,* (2004). The fat body mass was estimated using an ether extraction method according to Doums *et al.,* (2002) and Wilson-Rich *et a*l. (2008). The percentage values are given as dry mass of fat body / dry mass of the abdomen.

Results are expressed as means ± standard deviation (s). The number of honeybee tested per series is given with the results. The significance between the 2 group (nurses and foragers) was estimated using Student's t test at 5% level.

RESULTS AND DISCUSSION

This study examined the abdominal cuticle components (protein-chitin) and an effectif immune parameters of the two ages of worker honeybee, nurse (young bees) which do job in the hive such feeding larva and forager (oldest bees) which perform tasks in a colony forage for nectar and pollen outside the hive. The effective cellular and humoral immune include respectively the measures of the total haemocyte count (THC), the normal haemocytes (immunocytes) and the relative mass of fat body. The results of the component of cuticle, the first line of defense and immunocompetence parameters of nurse and forager *(Apis mellifera intermissa)* are presented in the Fig 1.

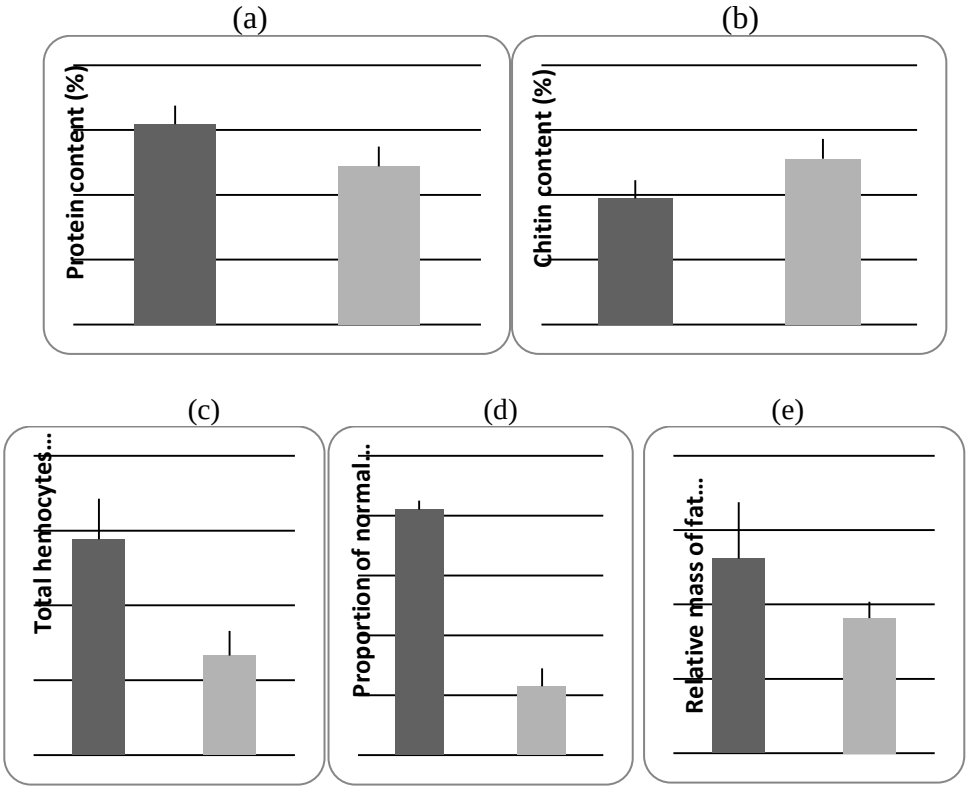

Figure 1. Components of abdominal cuticle and the measures of immunocompetence parameters of nurse and forager bees (*Apis mellifera intermissa*). a and b: Protein-chitin content respectively (n=13), c: Total haemocyte count (n=10), d: Normal haemocytes (n=4), e: Relative mass of fat body (n=20).

The exoskeleton of cuticle of arthropods is an important feature that contributes to their great success in colonizing numerous habitats on earth (Moret and Moreau, 2012). The cuticle is an important assembly of chitin and proteins (Charles, 2010). In the honeybees, ontogenetically, two basic types of exoskeleton can be identified in the honey bees; the flexible and colorless (unpigmented) larval and pupal exoskeletons and the rigid (highly sclerotized) and pigmented adult exoskeleton (Elias-Neto *et al*. 2014). According to Vincent *et al*., (2004**),** the protein is responsible for the stiffness and elasticity of the cuticle. In general, the soft cuticle has a much content of chitin (50% dry weight) than the stiff cuticles (15-30% dry weight). According to Willis (1999), the physical properties of insect cuticle depend on several things, cuticular lipids, the proportion of chitin, the nature and quantity of sclerotizing agents and the sequence of the constituents proteins. Charles (2010) suggested that the physical properties are determined largely by the proteins it contains and vary widely with developmental stages and body regions. The corpses of dry dead bees, gathered after wintering, contained 50-80% protein,

10-20% chitin, 20-30% melanin and 2-3% mineral substances but the dead bees after CO_2 extraction contained the 45-50% protein, 20-22% chitin, 20-22% melanin and 2-3% mineral substances (Nemtsev *et al.*, (2001).

Our results show that the mean protein content in the abdominal cuticle of nurse and forager (*Apis mellifera intermissa)* was 61.83 ± 5.69 % and 48.8 ± 6.1 % respectively (Fig 1a). The chitin level of nurse and forager was respectively 38.91 ± 5.59 % and 51.18± 6.11% (Fig 1b). Thompson and Hepburn (1978) noted that the percentage of abdominal chitin for the honeybee pharat adult was found to be around 19.3 %. However, the chitin content of whole body of honeybee as about 19% (Nemtsev *et al*., 2004). Kaya *et al.*, (2015) found a differences amoung the body parts of honeybee, legs (13.25%), thorax (6.79%), 8.9% for head, 8.61% for abdomen and 7.64% for wings.

Cuticular protein-chitin content from others insects varied with species and their developmental stages. The percentage of protein in the cuticle of the fifth instar nymphs of *Schistocerca gregaria* and the pupal integument of *Tenebrio molitor* is around 70% respectively (Berghiche *et al.*, 2007; Tail *et al.*, 2015). The dry weight chitin contents of the adult *Colorado potato* beetle and larvae were determined as 20% and 7% respectively (Kaya *et al.*, 2014). The chitin content from *Holotrichia parallela* is around 15% (Liu *et al*., 2012). Kaya *et al.*, (2015) found that the level of these component of *Vespa crabro, Vespa orientalis* and *Vespa germanica* were respectively 8.3%, 6.4% and 11.9%.

As is known, physicochemical properties of cuticle are highly affected by extraction method (Thomson *et al.* 2004). The exoskeleton (cuticle) of insects varies widely in shape, biochemical properties and fuctions, which are inherent to the biological species, developmental stage, besides showing, wealth of architectural specialization and nuances in the different body regions (Elias-Neto *et al.*, 2014).

From our results, two main differences were observed. First, we noted that the content of protein was significantly greater in nurse than in forager ($P < 0,0001$). The second, the percentage of chitin content of nurse was significantly lower than in forager ($P < 0,0001$). This is in agreement with previous results showing that nurses have a high investment in cuticular protein, chitinase and chitin metabolic process with 36 expressed genes in comparison to foragers. The older bees also possessed a lowest number of expressed genes in lipid metabolism and a higher number in carbohydrate metabolism (Seehuus *et al.*, 2013). The same authors reported that the difference between foragers and nurses in cuticular component correlate with changes in proteosynthetic activity of fat body. The tissue, a functional homologue of the mammalian liver (Lemaitre and Hoffmann, 2007), is the main site of energy, antimicrobial peptides, protein storage (Lemaitre and Hoffmann, 2007; Roma *et al.*, 2010). It is also one of the most important tissues of maintenance and reproduction process (Roma *et al.*, 2010).

Our results show that nurse bees had greater fat body (52.32 ± 15.13 %) mass than foragers (36.36 ± 4.37 %)(Fig 1e). Our observations are consistent with Wilson-Rich *et al*., (2008)**.** The fat body forager was used as a source of energy involved in stress response, behavior, sensory, learning and memory (Seehuus *et al.,* 2013). It known that the somatic maintenance machinery in forager is a physiologically expensive option cost (Alaux *et al*., 2010; Seehuus *et al.,* 2013). In order to save energy costs, the plausible strategy for the forager, the superorganisme, was also to abandon hemocytic immunity (Seehuus *et al.,* 2013).

As seen in figure 1, the young bees show that the mean of the THC (14 420 ± 2718.2 cells / µl) was significantly higher than in forager (6 660 ± 1644.7 cells / µl) (Fig 1c). The similary observation was found by Amdam *et al.,* (2005); Schmid *et al.,* (2008); Wilson-Rich *et al.,* (2008). As for THC, the nurse possess a higher number of the proportion of immunocytes than forager ($p<0,0001$)(Fig 1d). The similar disruptions of host immune functions have been reported by Amdam *et al.,* 2005; Schmid *et al.,* 2008). Amdam *et al*., (2005) suggested that the juvenile hormone level, which accompanies onset of foraging behavior, induces extensive haemocyte death through nuclear pycnosis. According to Schmid *et al.,* (2008), this loss of immune competence has been regarded advantageous with respect to an already high mortality rate due to foraging and to redistribution of energy costs at the colony level**.** The older worker bees were still significantly less resistant to the three physiological stressors (starvation, thermal and oxidative stress) than the younger bees. Khater *et al.,* (2011) in Abou-Shaara (2014), the forager bees have different n-alkane profiles than the nurse bees with a higher quantity of n-alkane which may help the forager bees to tolerate the ambient conditions. Remolina *et al*., (2007) reported that the forager would be more susceptible to direct attacks from predators and benefit from more protection in the form of stronger cuticle.

CONCLUSIONS

Several remarkable features distinguish nurse, which do job inside the hive from forager, which leave the colony for foraging activity. Pronounced differences between the two ages were found in the abdominal cuticular content of protein-chitin. The forager exhibited a strong reduction of the THC and the number of functional haemocytes. The relative mass of fat body decrease dramatically in older bees compared with the young bees. The differences are due to intrinsic physiological differences, the differential exposure to extrinsic factors such as predation, thermal stress and physical exhaustion. Possessing the strongest cuticle would be beneficial to forager lacking effective escape responses.

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THE IMPORTANCE OF WOODY PLANT INTRODUCTION FOR FOREST TREES IMPROVEMENT

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ABSTRACT

The history of woody plant introduction is closely linked with that of transportation and the European exploration of the planet (16^{th}–19^{th} centuries). Each colonial power established major botanical gardens and experimental stations in various parts of the world. By the 20^{th} century, the purpose of introductions shifted from food plants to timber and other species yielding non-agricultural products. Finally, during the latter part of the 20^{th} century the importance of ornamental species increased dramatically, especially to the more developed and wealthier regions. Over the past two centuries many species have started to spread in their introduced ranges. Until relatively recently the majority of introduced woody species have been highly beneficial, if not essential, to humanity's development, but now ever-increasing numbers of species are becoming detrimental to the maintenance of the earth's biodiversity and to the well-being of human societies. Throughout the 19^{th} and specially in 20^{th} century the large-scale planting of trees for timber production has been one of the main reasons for the introductions of a large number of species, especially conifers e.g. *Pinus, Picea. Pseudotsuga* and Poplars and Willows species. By using methods of mass and individual selection and by establishing of provenances tests, as well as by half and full sib lines of selected exotics test trees, genetical potential productivity and adaptability of introduced species have been tested in numerous experimental plots in areas where introduced have been done. This paper attempts to unravel the relationships between humans and woody plants by looking at the changes in the introduction of species, the way they are perceived by different human groups and the impact these non native species have on forest trees improvement and other human activities.

Key words: *introduction, forest trees, improvement.*

INTRODUCTION

Woody plants have undoubtedly been transported by humans for millennia and were an essential component of early agricultural societies. Over the past 500 year, the geographic barriers that had maintained an almost static distribution of the world's biota for millions of years have been eroded by human activity, and wild species have consequently moved beyond their natural range, (Richardson et al., 2000). During the discovery and conquest of the world by western European powers, fruit trees and ornamental plants were widely dispersed around the globe (Haysom and Murphy, 2003).

Over the past two centuries many species have started to spread in their introduced ranges. Until relatively recently the majority of introduced woody species have been highly beneficial, if not essential, to humanity's development, but now ever-increasing numbers of species are becoming detrimental to the maintenance of the earth's biodiversity and to the well-being of human societies. This paper attempts to unravel the relationships between humans and woody plants by looking at the importance of introduction of woody plant species for different human activities and especially for forest trees improvement (Isajev and Tucovic, 1986). The location of introduction was identified, in full or in part, for 388 of the 458 forestry tree species known to occur outside their native range (85 percent). Figure 1, shows the number of forestry species that were recorded as introduced, intentionally or by accident, into each of seven geographic regions (Europe, Africa, Australasia, North America, South America, Pacific and Asia). Introductions of forestry species were recorded for all regions.

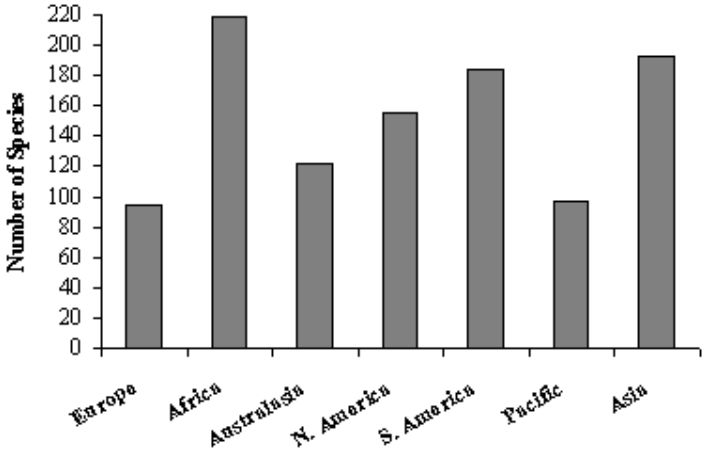

Figure 1. Number of forestry species encountered that having been introduced into each of seven geographic regions, Haysom K.A and Murphy S.T., 2003.

In the majority of cases, there was a lack of information on the mode of introduction (deliberate or accidental) and the country of origin of the introduced material. There were few reports of tree species (from any of the economic use

categories) having been introduced accidentally, and several reports expressed uncertainty concerning the nature of the introduction event.

Historical perspective of woody plant introduction

The history of woody plant introduction is closely linked with that of transportation and the European exploration of the planet (16^{th}–19^{th} centuries), Crosby, 1986. The transport of a species from one biogeographical region to another was carried out with a particular purpose. Once introduced to a new region, many of these species have been spread un-intentionally by humans within the new biotic regions.

Each colonial power established major botanical gardens and experimental stations in various parts of the world, first in the home country and on tropical islands, later in coastal areas and finally in more inland locations. By the 20^{th} century, the purpose of introductions shifted from food plants to timber and other species yielding non-agricultural products. Finally, during the latter part of the 20^{th} century the importance of ornamental species increased dramatically, especially to the more developed and wealthier regions.

Ornamentals have been widely introduced in every part of the world. In the past, botanic gardens and individuals were responsible for these introductions. Botanic gardens in all parts of the world have been responsible for the introduction of a large number of species and in every case species have started to spread into the surrounding vegetation. Erosion control has been a common reason for the introduction of plant species in many parts of the world. Species providing a rapid and thorough cover such as *Lonicera japonica* and *Pueraria lobata* have been favoured but these have become major pests (Williams, 1994). *Elaeagnus angustifolia* was commonly planted as a windbreak and *Lonicera japonica* was planted by game managers for wild deer (Blaisdell, 1967). In countries such as Britain shrub species such as *Symphoricarpos albus,* were introduced to provide ground cover for game birds (Gilbert, 1995).

Overview of domestication and conservation approaches of poplar (*Populus* L.) And willows (*Salix* L.)

The natural range of *Populus* and *Salix* spans impressive ecological amplitude, primarily across the North American, European and Asian land masses – from the subtropics to the boreal forests and arctic tundra, riparian to montane ecosystems and the man-made environment of modern agriculture. As a consequence, poplar and willow geneticists – those responsible for conserving and domesticating germplasm of *Populus* and *Salix* – have an especially broad mandate: to study the genetic diversity of natural populations and be familiar with all the modern tools for genetic improvement in order to serve specific societal needs (Kuzovkina and Quigley, 2005; Kuzovkina *et al*., 2008; Kuzovkina and Volk, 2009, Stanton *et al*., 2010).

Populus domestication has a history of nearly 100 years, beginning with Henry's work-1914, at the Royal Botanic Gardens, Kew, in the UK, and the work of Stout and Schreiner (1933) and Stout *et al*. (1927) at the New York Botanical Garden in

the USA. Other early domestication efforts include those of Wettstein-Westersheim 1933 in Germany, Al'benskii and Delitsina 1934 in Russia, Heimburger 1936 in Canada, and Houtzagers 1952 in the Netherlands. *Salix* domestication traces to the hybridization studies of Heribert-Nilsson in Sweden, Heribert-Nilsson, 1918, along with Nilsson and Hakansson's cytological work in the 1930s, Nilsson, 1931; Hakansson, 1933, 1938. In the UK, H.P. Hutchinson began work in willow conservation and breeding in the 1920s at the Long Ashton Research Station that was continued by K.G. Stott for the following 30 years, Newsholme, 1992; Stott, 1992. Most of this work involves 12 species in the genus *Populus* that are noteworthy for their commercial and ecological values. They are the North American species *P. balsamifera*, *P. deltoides*, *P. trichocarpa* and *P. tremuloides* and the Eurasian species *P. alba*, *P. cathayana*, *P. ciliata*, *P. euphratica*, *P. maximowiczii*, *P. nigra*, *P. simonii* and *P. tremula*, Stanton, J.B. et al 2014.

Within the genus *Salix*, 10 species − *S. caprea*, *S. dasyclados*, *S. eriocephala*, *S. koriyanagi*, *S. miyabeana*, *S. purpurea*, *S. udensis*, *S. schwerinii*, *S. triandra* and *S. viminalis* − are being utilized in developing the world's renewable energy industry, while three others − *S. alba*, *S. babylonica* (synonym *S. matsudana*) and *S. nigra* − are favoured for timber products (Stanton et al., 2014).

Experience with some conifer exotics in Europe

Tree species, particularly conifers, have been introduced from all over the northern hemisphere into Europe, and this is an unsurpassed region in which to obtain information on the behavior of exotics. It is notable that, after more two centuries of experience, the majority of foresters in Western Europe are inclined to be pessimistic regarding exotics, because they have not yet found a completely successful introduced tree, even though certain species showed great initial promise. The native European trees grow slowly, so there has been a search for faster growing species. Eastern white pine (*Pinus strobus* L.) grows more rapidly than the native European conifers with which it has been associated. In northern Germany near Eberswalde, the writer has seen 45-year-old Douglas fir (*Pseudotsuga taxifolia* (Lam.) Br.) of the same size as 100-year-old Scots pine. Near Tharandt, 55-year-old Douglas firs were from 14 to 20 inches (36 to 51 cm.) in diameter at breast height, exactly twice the size of Norway spruce (*Picea abies* Karst.) of the same age mixed with them. In southern Germany, near Munich, 45-year-old planted Douglas fir was the same height as 77-year-old naturally reproduced silver fir (*Abies alba* Mill.).

Eastern white pine (*Pinus strobus* L.)

The first exotic to be extensively planted in continental Europe was eastern white pine, introduced in 1705. At first of great promise and hailed with enthusiasm, it finally encountered an unpredictable pathogenic factor - white pine blister rust caused by the rust fungus, *Cronartium ribicola* Fisch., a migrant from Asia - with the result that the tree was all but abandoned throughout Europe. The wide range of soils on which white pine will grow vigorously in Europe is impressive. In Switzerland also he saw superb mature trees which had developed in mixture with

beech (*Fagus sylvatica* L.), Scots pine and a little Norway spruce. In southern Germany the tree was seen in its fourth generation, the first two generations having been planted and the second two naturally reproduced.

Douglas fir (*Pseudotsuga taxifolia* (Lam.) Br.)

The first seeds were introduced in Europe by David Douglas in 1827 and then planted at Dropmore Park (Buckinghamshire, UK), where there is a tree which is usually considered the oldest Douglas fir of Europe. Initially planted as ornamental, Douglas fir started to be used as a forest species by the end of the nineteenth century. This fir became a major reforestation species in Western Europe after the Second World War, mainly with the support of national or regional forest grants. In Europe, 80 % of the total Douglas fir area is to be found in three countries: France (half of the European area), Germany and United Kingdom. Outside Europe, Douglas fir has also been introduced in several countries of the southern hemisphere (South Africa, South America, New Zealand and Australia). Following World War I, Douglas fir found high favor and was planted extensively, although enthusiasm diminished somewhat in time because of the low quality of wood produced. Planting of the species has been given up in much of southern Germany, while in Switzerland it is being used in mixture only, not with the idea that the mixed stand as such will check the disease, but merely to have other species to take the place of Douglas fir in the stand should the disease prove as catastrophic as is now feared. The first pathogenic factor to threaten the tree was Phomopsis canker caused by a European fungus, *Phomopsis pseudotsugae* Wilson. This turned out to be not as serious as first feared, being largely connected with frost. Next was Rhabdocline needle cast caused by *Rhabdocline pseudotsugae* Sydow - the causal fungus coming from the native home of the tree, but with its virulence apparently increased by the damper European climate during the growing season. Fortunately, only the intermountain and Rocky mountain or blue forms of the tree were attacked, the coast or green form, the really valuable kind for Europe, being unmolested.

Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

In view of the difficulties besetting Douglas fir, it was natural that Sitka spruce (*Picea sitchensis* (Bong.) Carr.) should increase in favor and by 1935 it was thought that this species might supplant Douglas fir for extensive planting. Plantations of the same species which have failed at 20 to 45 years old with no pathogen responsible have been seen in Germany, Switzerland and Great Britain, and it would seem that failure has clearly resulted from an unsatisfactory site. Sitka spruce is exacting in its site requirements, and too often has been planted on unsuitable soil, where the annual precipitation is too low, where temperatures are too severe or where some other adverse factor occurs. Unfortunately, the bad effects of an unsuitable site frequently do not appear until some years after a stand has been established, growth of the earlier years being vigorous. Sitka spruce on the whole is better adapted to Great Britain, where the climate is nearer that of its native habitat, than to most of continental Europe. Even so, care must be exercised in selecting the areas where it is to be established.

Grand fir (*Abies grandis* Lind.)

Grand fir (*Abies grandis* Lind.) is now being most favorably regarded by many foresters in western Europe because of its rapidity of growth and, up to the present, freedom from disease. It is thought that it may ultimately take the place it was first hoped that Douglas fir would occupy, and next Sitka spruce, neither of which is fulfilling their original promise. Since grand fir occurs over a wide geographical range in its native habitat with marked variations in temperature and precipitation, and since consequently there are climatic races within the species, it would be possible to find a race adapted to drier conditions, but such a race would probably grow so slowly that it would have no advantage over native European conifers. The grand fir which the author saw in Europe was all of the fast-growing coastal form. Severe attacks on conifers by (*Armillaria mellea* (Vahl.) Quel.) following drought are characteristic.

Western red cedar (*Thuja plicata* D. Don.)

Western red cedar (*Thuja plicata* D. Don.) is little seen in continental Europe, probably because in Great Britain the tree had so much difficulty in even getting a start that it was quickly discredited. The cedar leaf blight fungus (*Keithia thujina* Durand), which came with the trees from North America, finding the moist climate of Great Britain so much to its liking has reduced the cedar to a slow-growing tree, at least during its younger stages.

Experience with black locust, *Robinia pseudoacacia* L.

The North American black locust has been widely introduced throughout Europe as a source of high quality timber and for erosion control. Some now regard it as a permanent member of the flora, Gams, 1967. The main uses of *R. pseudoacacia* have been somewhat variable in different parts of Europe and have changed over time. In parts of France and Switzerland the young coppice wood was extensively used in vineyards to support the vines, Monnier, 1992, but in recent decades it has been replaced by metal posts and wire, and now the species is hardly used. Although the tree produces a highly durable timber, it is disliked by German foresters because the wrong strain, a shrubby variety, was introduced to that country. In Hungary the tree has remained a key timber and is the main source of honey (Keresztesi, 1977). Even new uses for the groves of this species have recently been found. The Hungarian Formula 1 Grand Prix brings over 100,000 spectators once a year but also attracts hundreds of prostitutes whose trade, over the past few years, has disturbed the tranquillity of local villages. This has now been restored as the local *R. pseudoacacia* groves have been put to new uses, Thorpe, 2000.

Testing and evaulating of introduced exotic species

Differences among species are ordinarily very large as compared with differences among races within a species or as compared with differences among individual trees within a forest. Thus the introduction of exotic tree species has been the single most important aspect of forest tree improvement for some areas. On the

other hand, some regions have such excellent native species or such harsh growing conditions that trees from other lands have proved of little value. There are various intermediate regions in which tree introduction is on of several improvement methods which should be considered.

The procedures and designs used to test exotics are the same as used in the study of individual tree inheritance and racial variation. Exotic testing should be done in two or three stages (Wright, 1993). The first preliminary test should include several scattered plantations on different soil types and with different climates, with few blocks per plantation and small plots. These first tests may well include a few hundred seedlots of several different species or even genera. They may be established over a period of years, with separate plantations for species with different growth rates and growth habits.

The second-stage test should concentrate on those races or species that grew best in the first-stage tests. There should be more replication and perhaps larger plots at each test site, and increased attention can be given to individual tree variation. The second-stage trails may often be considered as semicommercal, designed for the production of wood as well as data. The third-stage trails can consist commercial plantations designed primarily for wood production.

In working with exotic species for the first time, one lacks the background information of site adaptability, pest problems and silviculturals management that is usually available for a native species. This is the reason for suggesting preliminary testing n a variety of site conditions and moderate-scale second-stage testing,

Identification and monitoring of introduced species should be particularly supported in those areas where there is currently little documentation. A number of case studies should be conducted in collaboration with countries that have a high degree of dependence on forestry. Such case studies should cover a range of forestry situations (commercial, developmental and environmental) and include the development and promotion of tools for ecological and economic impact assessments. These could also be incorporated into more general decision support systems that include socio-economic factors as well as biological risk.

CONCLUSIONS

There is a need for further research and monitoring that will provide information on the management processes in planted systems and take account of the scale (i.e. land area) of plantings and of the area occupied by introduced species.

- Introductions should only be considered if clear and well-defined benefits to man or natural communities can be foreseen and demonstrated.
- Introductions should only be considered if no native species are suitable for the purpose for which the introduction is being made.
- Introductions should not be made into pristine natural or semi-natural habitats, reserves of any kind or their buffer zones and, in most cases, oceanic islands.
- The taxonomic identification of the proposed introduction needs to be confirmed.

Only if these first four conditions are met should further assessment be undertaken.

Generally, it may be accepted conclusion, that introduced species can fulfill a gradually increasing role in certain ecological or industrial niches but will probably not replace natives over large areas.

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BIOMONITORING OF ATHMOSPHERE AIR POLLUTION IN THE FOREST ECOSYSTEMS AND ECO-TONE ZONE

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ABSTRACT

Within our research, the air quality of 62 forest ecosystems from Republic of Moldova was assessed, taking into consideration the lichens indicator species specific diversity, abundance and toxitolerance. It was established that the Moldavian forest ecosystems do not contain reserves concerning critical loads for SO_2 pollution, the annual average for the vegetation season for dendrological species being 0,02 mg/m^3 air, and for communities of lichens and cyanobacteria, organisms sensitive to pollution, represented only 0,01 mg/m^3. Lichen indication demonstrated that the current level of pollution is between 0,05 and 0,5 mg/m^3 SO_2 air, thus long-term harmful effects are manifested in all 62 studied forest ecosystems and the ecosystems from the eco-tone zone. We believe that for the Republic of Moldova lichen, indicator species can provide a scale of 6 levels: 5 levels characterized by species with different toxitolerance degree and the last step being an area in which lichens are completely missing, thus the most polluted area. Within the Republic of Moldova territory, there were reported 3 forest ecosystems in which the air is evaluated as clean air, 11- low polluted air, 31- moderate polluted air, 12- polluted air, 3- high polluted air and those with critical polluted air was missing.

Keywords*: licheno-indication, air quality, forest ecosystems, eco-tone zone.*

INTRODUCTION

The application of lichen indication in monitoring the quality of environment is one of the most indicated methods in speciality literature (Nylander,1865; Hawksworth and Rose, 1970; Tracc, 1984, Мэннинг. Федер,1985; Bartok, 1985; Кондратюк, Мартиненко, 2006; and Crisan, 2002)]. According to the synthesis analysis of scientific publications, concerning the Republic of Moldova lichens diversity, conducted by the author, currently, there are about 200 known species of lichens, covered in 12 orders, 35 families and 76 genus, which systematic belonging has been exposed according to *The Ainsworth & Bisby' s Dictionary of the fungi* (Hawksworth et al., 1995)] and nomenclature proposed by [8 (Kondratyuk, Khodosovtsev, Zelenko, 1998)], which constitutes a sufficient biodiversity basis to perform air quality monitoring.

The multitude of air quality assessment scales based on lichens toxitolerance degree form scales from 3 to 12 levels. Typically, those with 10 to 12 levels are applied in England, Pribaltic, Canada (Boreal region), which are areas very rich in lichen flora. For conditions of France, this has already been reduced to 7 levels [(Van Haluwyn et Lerond, 1986)]. We believe that for the Republic of Moldova lichen flora, indicator species can provide a scale of 6 levels: 5 levels characterized by species with different toxitolerance degree and the last step being an area in which lichens are completely missing, thus the most polluted area. The 20 scales analyzed by us were applied in different climatic regions (boreal, temperate, subtropical). Certain species were common in the testing of several authors, but were attributed different degrees of toxitolerance. Thus, our test, comprising gassing, transplantation and research in the field, have enabled us to select 40 bioindication species to be applied in monitoring of air quality in forest ecosystems: 3 species with toxitolerantion degree I, 15 species – II, 16 species – III, 4 species – IV and 2 species – degree V. High frequency of these species within the Republic of Moldova forest ecosystems, ensure and provide for the use of the same species by the European Monitoring Network, as many of these species are common for European space.

MATERIAL AND METHODS

Given the fact that at present there is rich information on the concentration of toxic pollutants in the atmosphere, which cause disturbances in lichens vital activities [1 Atlas, Schofield, 1975; 4 Burton, 1986; 12 Richardson, Nieboer, 1980; 21Tracc, 1977; 14 Блюм,1986; 18 Михайлова, Воробейчик, 1995], some scale authors specify degrees to SO_2 concentrations [6 Hawksworth and Rose, 1970; 9 LeBlanc et Rao,1972; 17 Лийв et al., 1982]. Indicated concentration vary widely from one author to another, perhaps this is due to the fact that some data has been obtained in laboratory conditions whereas other in the field, as well as due to different emissions structure, climate conditions, research methodology etc. Analysis of gradations allows us to see that at most authors the SO_2 concentration <0,05 mg/m^3 air is indicated for zone with clean air and the harmful effects start at 0,1 up to 0,3 mg/m^3 air, some indicating level> 0,3 mg/m^3 as very polluted air, others indicating that fatal for lichens is the concentration SO_2 > 0,5 mg/m^3 air. Thus, based on data published by other authors and based on our testing by gas and transplantation, we suggest Lichens Toxitolerance Scale (LTS) in respect to different concentrations of SO_2 in air (Table 1).

Considering that not any presence of lichens is a factual criterion of indication [15 Викторов, 1962], fact previously exposed for higher plants, in the case of lichen indication, air quality assessment will be true when the thallus of the indicator species comprehends the substrate coverage in a range of over 10% of the total area. This threshold is very important, especially for toxitolerance degree I and II because we can not say that the air is clean, when the investigated sector displays only one sample (or even 2-3) which are indication species which are very sensitive to air pollution, by covering only a very small surface of the substrate and

by having a diminutive feature of development. Thus, given the bioindicators abundance, the following criterion is proposed for application within air quality assessment works (Table 2).

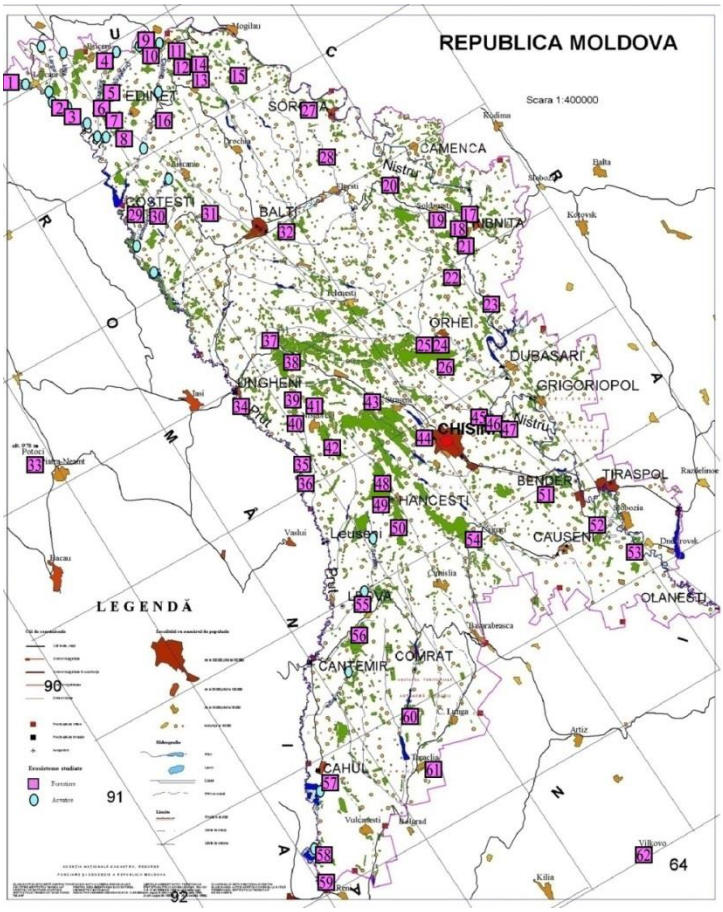
Table 1. Lichens Toxitolerance Scale (LTS) in respect to different SO_2 concentration in the air

Toxitoleranc e	Zone Characteristics	SO_2 concentration in the air, mg/m^3 air	Presence of lichens with different degree of sensibility to pollution
I	Not polluted	< 0,05	Very sensible
II	Low polluted	0,05 – 0,1	Sensible
III	Moderate polluted	0,1 – 0,2	With moderate resistance
IV	Polluted	0,2 – 0,3	With increased resistance
V	High polluted	0,3 – 0,5	With high resistance
VI	Critical polluted	> 0,5	Complete absence of lichens

Table 2. Air Quality Assessment Scales (AQAS) based on the abundance of lichen species with different toxitolerance degree to SO_2

The quality of atmosphere air	SO_2 concentration in the air, mg/m^3	Lichens species abundance with different degree of toxitolerance, % from substrate	Conventional colour
Clean	<0,05	I > 10 or I < 10 and II > 75	Blue
Low polluted	0,05–0,1	I – 0–10 or II – 50–75	Light blue
Moderate polluted	0,1–0,2	II – 10–50 or III > 50	Green
Polluted	0,2–0,3	III – 10–50 or IV > 50	Orange
High polluted	0,3–0,5	IV – 10–50 or V – 1–100	Red
Critical polluted	>0,5	Complete absence of lichens	Brown

As objects for our research served 62 forest ecosystems, in which the sources of pollution and the state of ecosystem components: such as air, water, soil and biota have been assessed. The location scheme of objects includes and spreads over 8 sectors with high environmental risk (large cities, industrial centres), deployed in all five landscape regions of the Republic of Moldova (Figure 1).



Silvosteppe zone:

A. Plateaus and silvosteppe plateau region includes*: I) 8 ecosystems located within the influence area of Lipcani and Edinet towns within a radius of 80 km, with forests near the settlements: 1-Criva, 2-Pererata, 3-Teţcani, 4-Trebisăuţi, 5-Trinca, 6-Fetesti, 7-La Castel, 8-Zăbriceni; II) 8 ecosystems located within the influence area of Edinet and Mogilau town, within a radius of 100 km, with forests near the settlements: 9-Clocusna, 10-Ocnita-Hădărăuţi, 11-Lipnic, 12-Cernoleuca, 13-Donduşeni, 14-Climauti, 15-Călărăşăuca-Mosana, 16- Chertroşica Noua; III) 10 ecosystem within the influence area of Ribnita and Rezina towns, within a radius of 90 km with forests near the settlements: 17-Ciorna, 18-Popauti, 19-Şoldăneşti, 20-Cuhurestii de Sus, 21-Saharna, 22-Pohribeni, 23-Lopatna, 24-Orhei, 25-Seliste, 26-Ivancea.*

Figure 1. Map representation of investigated areas

B. Plateaus and plains with grassland regions from Balti steppe: *IV) 6 ecosystems located within the influence area of Balti and Floresti towns, within a radius of 70 km with forests near the settlements: 27-Rublenita, 28-Rădulenii Vechi, 29-Stanca Mare, 30-Hâjdieni, 31-Iabloana, 32-Mândreştii Noi.*

C. Plateau region with Codri forest: *V) 10 ecosystems in the influence area of Ungheni and Iasi towns, within a radius of about 120 km, with forests near the settlements: 33-Potoci (Romania),34-Valea Mare, 35-Neamteni,*

36-Cotul Morii, 37- Corneşti, 38-Bahmut, 39- Bălăneşti, 40-Seliste Leu, 41-Cobac, 42-Bujor, VI) 8 ecosystems located within the influence area of Chisinau and Hincesti towns, within a radius of 60 km with forests near the settlements: 43-Capriana, 44-Durlesti, 45-Tohatin, 46-Budeşti, 47-Cimişeni, 48-Logăneşti, 49-Sarata-Mereşeni, 50- Sărata Galbenă.

Steppe zone:

D. Steppe region of the lower Nistru flood plain: *VII) 4 ecosystems under the influence area of Bender, Tiraspol and Dnestrovsc towns, within a radius of about 40 km, with the forests around the settlements: Hârbovăţul Nou-Balmaz, 52–Copanca, 53–Cioburciu-Răscăieţi, 54–Cărbun.*

E. Fragmented plains region from Bugeac steppe*: VIII) 8 ecosystems located under the influence of the Cahul and Comrat towns, within a radius of about 170 km, with forests near the settlements: 55–Sărata Nouă, 56–Codrii Tigheci, 57–Crihana Veche, 58–Văleni, 59–Giurgiuleşti, 60–Congaz, 61–Taraclia, 62–Vilcovo (Ukraina).*

RESULTS AND DISCUSSION

The assessment of air quality in 62 forest ecosystems throughout the Republic of Moldova was carried out taking into account the specific diversity, abundance and indicator species toxitolerance, applying the Lichens Toxitolerance Scale (LTS) and Air Quality Assessment Scale (AQAS) developed by us [3Begu, 2008].

Within the Republic of Moldova territory, there were reported 3 forest ecosystems in which the air is evaluated as clean air, 11- low polluted air, 31- moderate polluted air, 12- polluted air, 3- high polluted air and those with critical polluted air was missing. The quality of air in ecosystems evaluated as clean (SO_2 <0,05 mg/m^3 air) is confirmed by the presence of species sensitive to pollution, with coverage of the substrate over 10% (i.e. *Usnea hirta* – at Ocniţa–Hădărăuţi, *Peltygera canina* – at Bahmut et *Ramalina fraxinea* – at Selişte Leu). Ecosystems with low polluted air (SO_2 = 0,05 to 0,1 mg/m^3 air) are located primarily in the north (6 – Trebisăuţi, Feteşti, La Castel, Zăbriceni, Lipnic, Donduşeni) and in the centre part of the country – Codri region (3 – Bujor, Cimişeni, Logăneşti), and 2 respectively located in the middle course of the Nistru River (Lopatna) and Prut River (Cotul Morii). Ecosystems with moderate polluted air (SO_2 = 0,1 to 0,2 mg/m^3 air) are the largest in number (31) and have a wide distribution in northern, central and southern part of the country, often being subject to impact from local sources (i.e. – Hâjdieni, Criva, Orhei, Selişte, Durleşti, Budeşti, Văleni, Giurgiuleşti etc.) or cross-border sources of pollution, particularly via acid precipitation (i.e.– Bălăneşti, Cobac). Others probably are subject to common effects, because they are slightly away from the sources of pollution (i.e.– Teţcani, Clocuşna, Rubleniţa, Stânca Mare, Sărata Galbenă, Sărata Mereşeni, Cărbuna etc.). The share of ecosystems with polluted air (SO_2 = 0,2 to 0,3 mg/m^3 air) is 12, these primarily dominating the surrounding areas of pollution outbreaks, such as Bălţi, Rezina–Râbniţa, Chişinău, Tiraspol, Cuciurgan (i.e.– Trinca, Chetroşica Nouă, Mândreştii Noi, Păpăuţi,

Şoldăneşti, Tohatin, Hârbovăţ, Cioburciu etc). High polluted air (SO_2 = 0,3 to 0,5 mg/m^3 air) is evaluated for 3 ecosystems – Criva, Saharna, Copanca which have a dislocation in the immediate vicinity of the pollution sources and are located on the path of dominating winds, which move the emitted pollutants. Ecosystems with critical polluted air (SO_2> 0,5 mg/m^3 air) were not recorded.

Certainly, the air quality in the investigated ecosystems, may be influenced and be subject to a range of factors, via direct dependence effects, emission of certain pollutants, but also by synergistic effects of these. Important are orographic parameters (altitude, exposition), climatic (precipitation, wind), and the effects of air pollution – type and quantity of emitted pollutants, the location/distance of the ecosystem in respect to source of pollution, frequency and emission cycles and the effects of transboundary pollution. Classically, ecosystem with low polluted air are located at altitudes above 200 m whereas those with polluted air – less than 200 m. Nevertheless, there are some exceptions and these are subject to the ecosystem location in respect to the source of pollution and the direction of prevailing winds (i.e. Trinca, Călărăşeuca, Chetroşica Nouă, Mândreştii Noi, Saharna – located at altitudes above 200 m, but rather polluted). The majority of forest ecosystems in the Republic of Moldova are of hilly type (200-600 m), rarely plains (0-200 m). More pronounced are the effects of pollution on lowland ecosystems surrounding localities Valea Mare, Nemţeni, Crihana Veche, and for Criva, Hâjdieni, Orhei, Tohatin, Copanca, Hârbovăţ, Cioburciu, in which the decisive role has been played by the distance of the ecosystem from the sources of pollution (distance from the source and the direction of the prevailing winds).

Thus, the highest SO_2 emissions from local sources in 2005 were typical for the areas located in the southeast part of the Republic of Moldova, pollution outbreaks Tighina–Tiraspol–Cuciurgan, over passing Chisinau about 20 times, 100 times Bălţi and over 700 times Cahul. This outbreak has led to the pollution of the country southeast ecosystems, mainly due to the northwest winds rose direction towards southeast, fact conformed as well by us via bioindication. SO_2 emissions from Soroca – 154 t/year and Balti – 85 t/year were determinative in the degradation of Hâjdieni ecosystem, whereas geological explorations in Criva and Trinca, the later being affected as well by unauthorized burning of tire for lime production, placed these ecosystems in the category of ecosystems with high polluted air. High emissions of SO_2 are characteristic to Hincesti town (332 t/year), which have left its mark on the state of atmospheric air in surrounding ecosystems – Sărata Galbenă and Sărata Mereşeni. Chisinau emissions have obviously contributed to the pollution of Balmaz–Hârbovăţ ecosystem, towards southeast and Tohatin–Budeşti towards east. To a large extend the effects of pollution from the outbreak Rezina–Râbniţa was expressed only in the immediate vicinity on Saharna, Ciorna, Păpăuţi and this is due to wind rose northwest towards southeast, thus did not affect Pohrebeni and Lopatna ecosystems. Possible adverse effects on vegetation from the outbreak Cuciurgan were more pronounced for Copanca and slightly less for Cioburciu–Răscăieţi, as well due to rose wind northwest towards southeast.

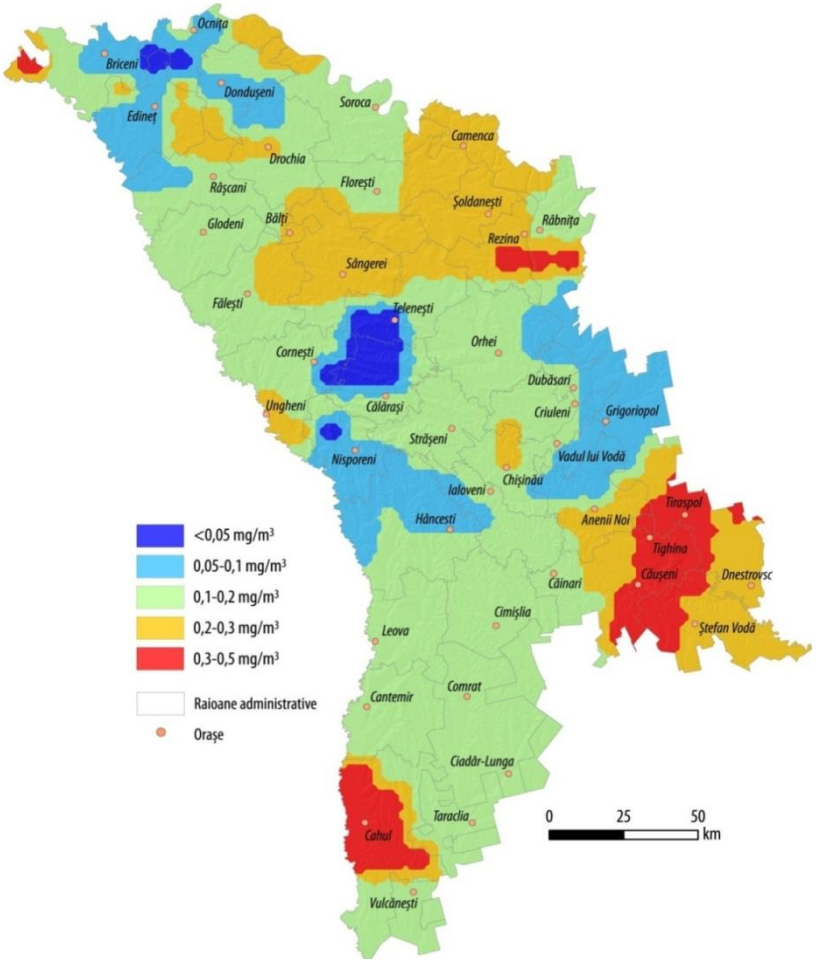


Figure 2. Spatial distribution of air pollution with SO2, based on lichen indication

The south part of the country is affected by transboundary pollution (Galati town, Romania), especially Crihana Veche and Giurgiulesti and Valeni. . Effects of pollution from sources located in Iasi town, inseparable from those originating from Ungheni, strengthen the pollution in ecosystems located in eco-tone zone – Valea Mare, Nemţeni, to a slighter extend Bălăneşti, Cobac, however not crossing Bahmut and Cornești situated in the northwest part from the outbreak and protected by the landscape dominated with high altitude. According to EMEP Report 1/2003 [11 Rewiew and Revision, 2003] developed by the Norwegian Meteorological Institute, transboundary pollution remains an issue for many European countries, including Moldova, which is situated in the annual deposition of SO_2 700-1000 mg/m^2. As well, in the last five years a trend appears in relation to increased

atmosphere emissions due to increasing economic potential of south east European countries. The lichen indication in connection to the 62 investigated ecosystems, and the EMEP 50x50 km grid, reveals that the real environmental situation is camouflaged, probably because the effects of pollution are more pronounced up to about 25-30 km from the source of pollution. In the case of EMEP network, the ecological situation is levelled throughout the 50x50 km grid which does not correspond to reality.

The foundation of a sustainable environmental balance in the functioning of forest ecosystems in the Republic of Moldova, serve the critical levels established by the Geneva Convention (1979) for SO_2, NOx and NH_3. Thus, forest ecosystems from the Republic of Moldova do not provide reserves concerning the critical tasks of SO_2 pollution, as dendrospecies annual average or growing season, being 0,02 mg/m^3 air, and for lichens communities and cyanobacteria – organisms sensitive to pollution, representing only 0,01 mg/m^3. Lichen indication have demonstrated that the current level of pollution is between 0,05 and 0,5 mg/m^3 SO_2 air, thus long-term harmful effects are manifested in all the 62 investigated forest ecosystems. Broadly speaking, we can say that there are only 3 ecosystems, assessed by lichen indication, with clean air (SO_2 <0,05 mg/m^3air) and which have a good structural stability and functionality (Ocniţa-Hădărăuţi, Bahmut and Selişte-Leu). Thus, increasing industrial activities require us to maintain control and monitor this index to identify ways to mitigate the impact on forest, agricultural and urban ecosystems deployed in the area of eco-tone.

CONCLUSIONS

Based on the performed research there were established the premises of ecobioindication in the Republic of Moldova, expressed by the presence of 40 species of lichens, which form certain associations sensitive to air pollution with SO_2 and accumulate in their body concentrations of heavy metals.

The results obtained via passive and active biological monitoring allowed us to argue on the theoretical possibility and effectiveness of ecobioindication application in monitoring of air quality within forest, agricultural and urban ecosystems deployed in the area of eco-tone.

Parmelia sulcata species, common in forest ecosystems, has proved to be most responsive to air chemical pollutants, particularly with SO_2, registering obvious morphological and biochemical changes (colour change, degradation of thalus and photosynthetic pigments).

Recommendations

Widespread, high frequency and dominance of *Parmelia sulcata* species prevalent in forest ecosystems, as well as the possibility of being easy transplanted into urban, agricultural and industrialized ecosystems, allows its use as a standard in geographical mapping of air pollution with SO_2 and heavy metals, a requirement introduced by European Committee for Standardization Programme, Measurement and Testing.

In order to assess the environmental state of forest, agricultural and urban ecosystems, ecobioindication method is recommended – efficient and easily realizable method by applying the Air Quality Assessment Scale (AQAS), which has been developed taking into consideration the specific diversity of lichens, the indicator species toxitolerance degree and substrate coverage.

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IN VITRO SCREENING OF INSECTICIDAL EFFECT OF PLANT AQUEOUS EXTRACTS ON THE COWPEA APHID *APHIS CRACCIVORA*

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ABSTRACT

The research of new control methods that have low negative effects represent an alternative aiming to limit disadvantages of chemical control of pests. This study aimed to test the effect of 11 aqueous plant extracts belonging to four species (*Santolina africana, Juniperus thurifera, Artemisia herba-alba* and *Pituranthos scoparius*), on the larvae of the aphid *Aphis craccivora* installed on the leaves of *Robinia pseudoacacia* under laboratory conditions. The insecticidal effect of aqueous extracts on the mortality of aphids was evaluated by the introduction of acacia leaflet into an extract and infestation of them by 10 larvae of the aphid. Later, a counting of dead larvae after 3, 6, 12 and 24 h was performed. In addition, we investigated the effect of the aqueous extracts on the orientation of the aphids by putting the latter in a position of choice between six leaflets, each treated with a different extract. In addition, a phytochemical screening was carried out to detect the presence of saponins and polyphenols in the extracts studied. Through our study, the extract obtained from *S. africana* at a concentration of 15% was the most effective with a mortality rate higher than 80% after 24 h of artificial infestation. As for the effect of the four extracts on the orientation of the cowpea aphid, they did not attract the larvae massively. Regarding phytochemical screening, differences in the chemical composition of the analyzed extracts were observed. These differences may explain in part the variation of aphicidal effects of tested plant extracts.

Keywords: *Santolina africana, orientation, mortality rate, polyphenols, saponins.*

INTRODUCTION

Chemical control is the most common way to limit aphid infestations. In addition to its high cost, this method presents a potential danger to the environment (Bhatia *et al*., 2011). Some pesticides pollute surface and groundwater (Horrigan *et al*., 2002). On the other hand, Sullivan (2008) mentioned that non-selective pesticides

can destroy auxiliaries. In addition, massive use of insecticides resulted in the development of resistant strains of aphids (Harmel *et al*., 2008) and other pest species (Brévault *et al*., 2003). As a result, scientists have begun to look for other ways to control these insects (Jordan, 2013). Plants represent a vast storehouse of potentially useful natural products, and indeed, many laboratories worldwide have screened thousands of species of higher plants not only in search of pharmaceuticals, but also for pest control products (Isman, 1997).

Aphis craccivora (Koch) colonizes the young shoots of many plants, in particular those of Leguminosae (Blackman and Eastop, 2006). Attacks on shrubs and trees are rarely of economic importance, except on *Robinia pseudoacacia* (L.), where outbreaks can sometimes affect its ornamental value (Fraval *et al*., 1997). Furthermore, this aphid is involved in the transmission of about 30 persistent and semi-persistent phytoviruses (Blackman and Eastop, 2007).

The aim of this work is to study the effect of some aqueous extracts of plants on the larvae of the cowpea aphid (*Aphis craccivora*) found on acacia locust (*Robinia pseudoacacia*) under laboratory conditions.

MATERIALS AND METHODS

Aqueous extraction and preparation of treatments

The aerial part (particularly the leaves) of four plant species (*Santolina africana*, *Artemisia herba-alba*, *Juniperus thurifera* female and *Pituranthos scoparius*) were dried in the open air and then freed from dust. Then, they are finely ground using an electric grinder. With regard to extraction, two methods have been adopted: maceration and infusion.

In our case, a quantity of 10 g of powder from the aerial part of each of the 4 plants was diluted in 100 mL of cold distilled water. Each of the four mixtures obtained was agitated for a few minutes and then left for 24 hours. Each mixture was then filtered and diluted with distilled water to obtain two concentrations (5 and 15%).

As for the thuriferous juniper, an infusion extract was made. In our case, an amount of 10 g of *J. thurifera* powder was diluted in 100 mL of hot distilled water. The mixture obtained was agitated for a few minutes and then left for 2 hours. It was filtered and diluted with distilled water to obtain two concentrations (5 and 15%).

Collection of aphids

The animal material consisted of larvae of the aphid *Aphis craccivora* taken from a tree of *Robinia pseudoacacia* in the Batna region (eastern Algeria).

Evaluation of the insecticidal effect of aqueous extracts on the mortality of aphids

The various bioassays were carried out under laboratory conditions. The uninfested leaves of *R. pseudoacacia* and larvae (L3 and L4) of *A. craccivora* located in the same tree were used. 36 Petri dishes were prepared. Each one contains 2 leaflets treated with false acacia. A total of 12 treatments were tested with 3 repetitions for each: distilled water (control), 8 solutions obtained by maceration, 2 solutions

obtained by infusion and treatment by mixing the 4 solutions 5% obtained by maceration. Each leaflet is inserted into the container containing the corresponding treatment in such a way that the foliage is well imbibed. 10 larvae / box were placed at a rate of 5 individuals / leaflet. Dead larvae were counted 3, 6, 12 and 24h after the artificial infestation.

Evaluation of the repellent effect of aqueous extracts on the orientation of aphids

Three repetitions were prepared. Each repetition consisted of a plate and six leaflets arranged at its periphery. One leaflet was submerged in distilled water, another in the extract 5% of *S. africana*, another in the extract 5% of *A. herba-alba*, another in the extract 5% of *J. thurifera*, another in the extract 5% of *P. scoparius*, and the last one in a mixture of the four extracts 5%. At the center of each plate 20 larvae of *A. craccivora* were introduced.

Larvae installed on each treated leaflet were counted 12 and 24 h after deposition of the aphids.

Phytochemical screening

For the characterization of compounds belonging to the group of polyphenols, the reaction with ferric chloride was used. A drop of alcoholic solution of ferric chloride ($FeCl_3$) at 2% was added to 2 mL of each solution. The appearance of a blackish or greenish coloration more or less dark indicates the presence of polyphenolic derivatives (Soro *et al*., 2009).

To search the saponosides, we poured 10 mL of the aqueous extract into a test tube. The tube was agitated for 15 seconds, then allowed to stand for 15 min. A persistent foam superior than 1 cm, indicates the presence of saponins (N'guessan *et al*., 2009).

Statistical analysis

In order to compare the aphid mortality averages of each treatment and the orientation of the aphids to the treated leaves, ANOVA one way was used. When there is a significant difference, a Student-Newman-Keuls test is used to show the homogeneous groups. These analyzes were performed using SPSS software for Windows 10.0.5 (SPSS, Inc.)

RESULTS AND DISCUSSION

The ANOVA analysis revealed a highly significant difference between treatments during the 4 inspections (Table 1). The extract of *J. thurifera* 15% obtained by maceration was the most effective during the counts of 3, 6 and 12 h, with a mortality rate of 50%. While the *S. africana* 15% extract recorded the highest mortality rate after 24 h. The concentration 15% gave generally the best results for the 4 plants.

Table 1. Comparaison of mortality rates of aphids on treated leaflets.

Extract	After 3h	After 6 h	After 12 h	After 24 h
Distilled water	3,33 a	6.66 a	10 a	30 ab
Mixture	6.66 ab	6.66 a	20 ab	20 a
Artemisia 5 %	10 ab	10 a	13.33 ab	33.33 ab
Artemisia 15 %	16.66 ab	20 ab	20 ab	46.66 b
Pituranthos 5 %	13.33 ab	23.33 b	30 ab	60 bc
Pituranthos 15 %	33.33 b	33.33 b	43.33 ab	53.33 b
Juniperus 5 %	46.66 c	46.66 bc	46.66 b	60 bc
Juniperus 15 %	50 c	50 c	50 b	60 bc
Juniperus 5 % infusion	36.66 bc	46.66 bc	50 b	56.66 bc
Juniperus 15 % infusion	23.33 b	23.33 b	26.66 ab	50 b
Santolina 5 %	23.33 b	30 b	46.66 b	76.66 c
Santolina 15 %	33.33 b	43.33 bc	50 b	83.33 c
Signification	0.000	0.000	0.001	0.000

* Values indicated with different letters are significantly different at P<0,05

In general, the aqueous extract of *S. africana* and *J. thurifera* showed a remarkable insecticidal effect on aphids *A. craccivora*. On their part, Attia *et al*. (2011 and 2012) revealed that *S. africana* oils can provide valuable acaricidal activity. These oils cause high mortality of *Tetranychus urticae*. In addition, *J. Phoenicea* essential oils showed a mortality of *Myzus persicae* aphids that exceeded 50 % (Hakimi *et al*., 2015).

As for the aqueous extracts of *A. herba-alba*, they gave less insecticidal effects. Similarly, Nia *et al*. (2015) found that the aqueous extract of desert wormwood did not show a significant insecticidal effect against *Myzus persicae* compared to ethanolic and etheric extracts. Furthermore, Chermenskaya *et al*. (2010) mentioned that extracts from many species belonging to the genera Artemisia (not including *A. herba-alba*) were effective against aphids.

Concerning the mixing of the extracts, it was noticed that it is less effective in comparison with the effect of the extracts of the plants alone. Also, a study conducted by Ali *et al*. (2015) found that some plant mixtures tested had lower mortality rates of the aphid *Diuraphis noxia* compared to these plants alone.

Evaluation of the effect of aqueous extracts on the orientation of aphids

Statistical analysis of the number of aphids installed on each treated leaflet showed that there was a significant difference during the 2 observations ($P < 0.05$). The mixture of the 4 plants studied was the most attractive followed by the control. While other plant extracts did not attract any individual (Table 2). This can be explained by the absence of attractants to the aphids in the composition of these extracts.

Table 2. Mean percentage of aphids installed on each treatment.

	After 12 h	After 24 h
Distilled water	8,35 b	13,35 b
Mixture	20 a	21,65 a
Artemisia herba-alba	0 b	0 c
Pituranthos scoparius	0 b	0 c
Juniperus thurifera	0 b	0 c
Santolina africana	0 b	0 c

* Values indicated with different letters are significantly different at P<0,05

Phytochemical screening

Phytochemical screening revealed the presence of saponosides in desert wormwood only (Table 3). Likewise, Nia *et al*. (2015) reported the presence of saponins besides the terpenoids in the aqueous extract of *A. herba-alba*. Saponins can be useful as natural aphicides and deterrents. Furthermore, the insect midgut epithelium is suggested to be a primary target of saponin activity (De Geyter *et al*., 2012).

Table 3. Phytochemical screening of the aqueous extracts

Extract	Saponins	Polyphenols
Artemisia herba-alba	+	+
Pituranthos scoparius	-	-
Juniperus thurifera	-	+
Santolina africana	-	+

On the other hand, polyphenols were present in the three analysed extracts, and they were absent only in *P. scoparius* (Table 3). Allelochemics which affect the behavior or population biology of insects can be extremely important factors in host plant resistance (Berlinger, 2008). Phenols are the principal class of secondary metabolites (Lattanzio *et al.*, 2006). It is likely that the quantity of some molecules belonging to these chemical families have the greatest effect on aphids, rather than the total amounts of polyphenols and saponosides. According to Dreyer and Jones (1981), most flavonoids, which represent a group of phenols, are strong deterrents.

CONCLUSION

In this study, the insecticidal and repellent effect of plant extracts of four plant species on the aphid *Aphis craccivora* was tested.

In general, the extract obtained from *Santolina africana* at a concentration of 15% was the most effective with a mortality rate higher than 80% after 24 h. As for the effect of these extracts on the orientation of the cowpea aphid, these extracts alone did not induce larval attraction.

As regards phytochemical screening, differences in the chemical composition (saponosides and polyphenols) were observed between the analyzed extracts, which may explain differences in the efficacy of each extract.

These preliminary results pave the way for further research to confirm the results obtained under field conditions on one side; and to know by what mechanism the plant extracts influence the biology and ecology of pests on the other side.

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NEEDS OF TEA GROWERS FOR PARTICIPATING IN TEA PRODUCTION INSURANCE: A CASE STUDY IN PHU THO PROVINCE, VIETNAM

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ABSTRACT

The research has evaluated the situation of tea production of tea growers in Phu Tho Province, Vietnam. Average tea plantation area among the largest group of households is 0.61 ha. In production, the types of risks that tea growers encounter include: unfavorable weather (33.4%), diseases (13.2%), insects and worms (2.3%), capital (0.3%) and price which is the most major risk (50.8%). The survey of 1,000 tea growers identified that 46.7% of the households are in need to participate in tea production insurance. The average willingness to pay was estimated to be 2,407.07 (thousand VND/ha/year).If the agricultural program is successfully implemented in the province, it is estimated to have a total agricultural insurance fund of 34-35 billion VND/year. The survey results show that gender, education level, tea growing area and location are factors that affect the level of willingness to pay for agricultural insurance for tea trees. There are four solutions which are proposed to enhance the participation of tea growers in agricultural insurance for tea: (1) Development of an insurance product for the production of fresh tea leaves for easier access from farmers; (2) Active communication on agricultural insurance policy to local authorities, farmers' union, agricultural expansion stations and especially the farmers; (3) Close collaboration between local authorities and enterprises in the review on current mechanisms and policies to timely propose adjustments and supplements to meet the needs of farmers on agricultural insurance; (4) Strengthening coordination between the State, insurance companies, credit and financial institutions and farmers.

Keywords: *agricultural insurance, tea growing households, tea production.*

INTRODUCTION

Phu Tho is a midland province in the north of Vietnam which suitable terrain and climate for tea trees. Recognizing this strength, since 2000, the People's Committee of Phu Tho Province has put tea tree into the key agricultural programs of the

province. Therefore, Phu Tho tea trees play an increasingly important role in the development of local agriculture.

Currently, tea is the main export items of Phu Tho province. In 2010, the province exported tea with worth of $7.3 million; in 2011, the amount was increased to over $13 million (Khuong, 2012). The tea sector has a significant contribution to job creation and income generation for people in many parts of the province. Except for households with small-scale and new production, most tea growing households have a stable income. With the purchase of VND 3,000-4,000/kg of fresh bud, households with a growing area of 1,000 to 5,000m2 can have tens of millions dongs of income; many households have become rich thanks to the tea tree (Hoan, 2011). Besides, the price of tea in recent years has been relatively stable, so the life of the tea growers has been firmly guaranteed. However, tea production in Vietnam in general and in Phu Tho province in particular in recent years have been influenced by many factors such as natural disasters, disease outbreaks and market prices which have negative impacts on the productivity, output and income. Therefore, farmers need a cure from agricultural insurance to limit the damage.In Vietnam, the pilot agricultural insurance under Decision No. 315/QD-TTg dated 01/3/2011 by the Prime Minister was implemented. As of 30/04/2013, the pilot of agricultural insurance was been implemented throughout the country with the participation of 234,235 households and the contract insurance value for crops, livestock, fisheries of VND 5,437 billion; total original premium revenue reached over VND 303 billion (Dinh, 2014). The types of agricultural insurance are mainly for rice, cattle, pigs, poultry and seafood. Insurance for tea trees has not been reported in any locality in the country. Besides its new formation and development, agricultural insurance is facing difficulties and obstacles in its pilot implementation: (i) Agricultural insurance is a new insurance type with complexity; there has not been much experience in its implementation; (ii) wide range of insured products and large insured geographical coverage make it difficult for the control, risk limit, monitoring processes for procedures, production standards, farming, aquaculture; assessment, identification of diseases, epidemics and loss; (iii) natural disasters and epidemics sometimes cause huge financial losses exceeding the financial capacity of the insurance business; (iv) direction and communication in some localities are still confusing; implementation has been carried out in some localities but the number of contracts is still limited; (v) a number of farmers, agricultural organizations have participated in agricultural insurance as an exploratory activity (Son, 2009). Due to the important role of tea tree in economic development of rural households in Phu Tho province and the contribution of the agricultural insurance in minimizing damage to agricultural production, research: *"Needs of tea growers for participating in tea production insurance: A case study in PhuTho province, Vietnam"* is urgently needed to help tea farmers reduce the damage caused by risks in the production process.

MATERIAL AND METHODS

In Phu Tho province in Vietnam, Thanh Son, Yen Lap, Doan Hung and Thanh Ba are the four districts having largest area of tea and most developed tea production. Therefore, between 2014-2015 the research selected 1,000 households in the 4 districts to conduct survey on their needs for agricultural insurance participation through prepared questionnaires. The questionaires consists of 40 questions focusing on households' characteristics and their tea production activities. In particular, in order to collect information about the needs for agricultural insurance participation of tea growers, the research used Contingent Valuation method (CVM) which allows the survey on needs and requirement assessment and analysis of the basic factors affecting needs and requirement of tea growers for agricultural insurance (Dung, 2011). The basis of this randomized evaluation method is to study the willingness to pay (WTP) of customers for the change of the insurance policy. In addition, the research also conducted in-depth interviews of 50 managers at provincial, district and commune level in the research area; 3 – 5 persons for each level were interviewed.

After having been aggregated and analyzed using descriptive statistical and comparative statistical methods, the data is used in the regression model to provide more clearly factors affecting the level of tea growers' willingness to pay for agricultural insurance. The model is described as follows:

$$\mathbf{WTP = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_{12} X_{12} + u}$$

Dependent variable WTP is willingness to pay of each tea grower for agricultural insurance. Independent variable X represents the factors affecting the willingness to pay of tea growers including factors from within the tea growers as age, gender, education level, economic conditions, area, form of ownership, productivity of tea; and external factors such as level of risk encountered in the production of tea and tea growing localities.

RESULTS AND DISCUSSION

General situation of tea production of the surveyed households

Tea tree is a perennial plant; in production, tea tree needs a certain amount of care each year. Investment volume for tea tree (capital, labor, machinery for production) also depends on economic conditions, production scale of each household.nIn terms of investment, the capital investment in tea growing of households having less than 0.5 ha of area is the lowest with VND 12.83 million. Capital investment for tea growing of households is raised from two main sources of family budget or borrowings (usually from a relative or credit institutions in the locality). In terms of labor, tea growing can be considered as the main occupation of many households, the income of many families have to depend entirely on the tea gardens. Therefore, labors in tea growing are mainly family members. In addition to family labor, many households also hire extra workers during harvest, spraying, tea cutting time. In terms of risks encountered in the process of growing tea, it can be said that agricultural production is heavily dependent on natural conditions and weather, thus being very risky. For the tea tree, the harvest time is short (about 45

days/harvest) and there are several harvest during the year (starting from March to October, November), so the risks faced are greater with more serious damage. In recent years, the tea growers often face the risk of natural disasters, pests which cause decreased productivity and quality of tea leaves. In addition, the risks of input market price and capital also affect the productivity of tea. Among the five risks listed above, the risk of market price (including input prices and output prices) was considered as greatest impact by 50.8% of the households, followed by the risk of weather which was identified by 33.4% of the households. Risks related to pests, diseases were believed to reduce productivity and output by 15.5% of the households.

Needs for agricultural insurance for tea trees of surveyed households

The need for participation and the willingness to pay

Survey data shows that 467 households, accounting for 46.7% of the surveyed households, are willing to participate in agricultural insurance. 533 households, accounting for 53.3%, do not have the needs for participating in agricultural insurance for tea trees with the most common reasons that they have no idea agricultural insurance for tea trees (100%) and that their small tea plantation area is not worth buying agricultural insurance (26.34%).

The findings on the willingness to pay to participate in agricultural insurance of the surveyed households are shown in Table 2. The aggregated data from CVM method show that 138 out of 467 households agree to participate at VND 1,350,000/ha/year, accounting for 29.55%. With participation fee of VND 2,025,000/ha/year and VND 2,700,000/ha/year, there are 101 and 97 households agreeing to buy, respectively. With higher fee of VND 3,375,000/ha/year, there are 86 households agreeing to participate (in total of 467 participating households), accounting for 18.42%. With highest participation fee of tea agricultural insurance of VND 4,025,000/ha/year, there are only 45 households participate. From here, we determine the average willingness to pay (average WTP) of the surveyed households according to the weighted average method: average WTP is 2,407.07 (thousand VND/ha/year).

Table 2. The level of willingness to pay to participate in tea agricultural insurance

WTP (1,000 VND/ha/year)	Demand for insurance with productivity index	
	Number of participants	Percentage (%)
1,350	138	29.55
2,025	101	21.63
2,700	97	20.77
3375	86	18.42
4025	45	9.64
Total	467	100.00

*Source: Authors' own calculations from survey data in 2015

The demand curve in Figure 1 is in line with the law of supply and demand as other commodity markets. Demand on the willingness to pay also has tendency that the higher compensation rate of the agricultural insurance is, the less people accept. However, the chart shows that when the purchase prices of agricultural insurance is higher; there is not much change in the number of participants.

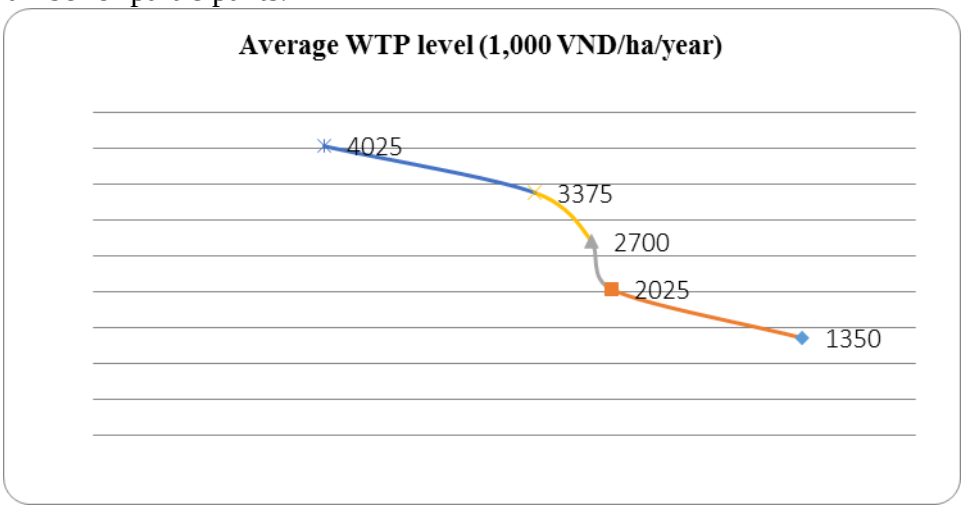

Figure 1. The level of average WTP for participation in agricultural insurance for tea trees

*Source: Authors' own calculations from survey data in 2015.

The lowest and highest levels given by the people were VND 1,350 thousand/ha/crop and VND 4,025 thousand/ha/year, respectively. The reason for the large difference between these two values is that CVM is a random survey methodology; the level of the individual WTP is completely dependent on the awareness, knowledge and subjective opinion of the respondents on the agricultural insurance for tea tree. The high level was given by those with better knowledge and income while the lowest was given mainly by low-income people.

On this basis, the research can estimate the total insurance funds collected annually if agricultural insurance is successfully implemented in Phu Tho province: Total insurance fund is estimated to reach 34 to 35 (billion VND/year) based on the total productive tea growing area of 14,483.8 hectares in the province.

Needs for insurance evaluating agency

Through the data Table 3, we can see that the majority of households expect that when risk occurs it is necessary to have a combination of agricultural experts, local authorities and insurance agency to evaluate the loss of tea production. According to the households, it would be more accurate and more transparent in evaluation to do so and their rights and interests are better protected.

Table 3. Needs for co-agencies for loss evaluation

Agency	No. of household	Percentage (%)
Commune authority	33	7.07
Agricultural experts	5	1.07
Both	429	91.86
Total	467	100.00

*Source: Authors' own calculations from survey data in 2015

When being interviewed, there were 91.86%, equivalent to 429 households out of a total of 467 households wishing to participate in tea production agricultural insurance, said that it was required for the participation of commune people's committee, district department of Agriculture and Rural Development or farmers' union in loss evaluation together with insurance agencies. There were 33 households, equivalent to 7.07%, said that only commune authority and the households were enough to evaluate loss when risk occurred.

Needs for insurance payment forms

Upon occurrence of risk, the interviewed households have different needs on how to compensate, some wanted to be compensated in cash, some wanted household supplies such as fertilizers, pesticides and other wanted to be paid back in tea seedlings in the case of death tea trees. Needs for compensation payment mechanisms are shown in Table 4.

Table 4**.** Needs for compensation forms of surveyed households

Forms of payment	Number (households)	Percentage (%)
Payment through the bank (card, account)	13	2.78
Payment in cash (direct payments)	378	80.94
Payment in fertilizer (direct payment)	49	10.49
Other forms (pesticides, seedlings ...)	27	5.78
Total	467	100.00

*Source: Authors' own calculations from survey data in 2015

Data collected from the survey shows that the majority of producers wish to be compensated directly in cash (80.94%). When being asked about the reasons, these households said that they needed to receive compensation in cash to pay for the loans as investment in tea production or cover other expenses of their families. Generally, upon receipt of cash compensation, producers can use them in a flexible manner for many different purposes.

There was 10.49% equivalent to 49 out of 467 households agreed to participate in agricultural insurance with compensation in fertilizers. And only 5.78% of the households wanted to be compensated in other forms, such as pesticides, seedlings ... These are households wishing to receive compensation for investment to overcome the loss caused by risk. If the loss was caused by the risk of pests and diseases, they would like to get pesticide; if tea trees die, they want to get seedlings and fertilizer as they believe that the supplies (seedlings, fertilizers, pesticides) they

receive are evaluated by agricultural experts, therefore they would be better than those purchased by them.

Needs for the State's support for agricultural insurance payment

Table 5. Needs on the level of Government's support for agricultural insurance payment

Target group	Respondents (%)	Government regulations (%)
1. Poor households	100	100
2. Nearly poor households	93.33	90
3. Not poor and nearly poor households	67.87	60
4. Agricultural organizations	36.67	20

*Source: Authors' own calculations from survey data in 2015

Currently, the Government has policies supporting farmers to participate in agricultural insurance. The Government supports 100% for poor households, 90% for nearly poor households, 60% for other households and 20% for agricultural organizations. The interviewees desired support levels as follows:

According to the survey on the time to pay agricultural insurance fee, the majority of households said that it should be paid on a quarterly basis (47.32% of the total households agreeing to participate in agricultural insurance for tea tree). This group believed that due to the change in the weather and diseases over time, the risks encountered in tea production is different for each season. Therefore, they want to participate mainly quarterly. Amongthe467 households, 134 households (representing 28.69%) wish to participate in agricultural insurance for tea trees yearly which would be more reasonable. Yearly agricultural insurance payment would save time for purchasing and avoid cumbersome, complicated procedures. Payment could be done once a year. Besides the above opinions, there were 112 remaining households desiring to pay agricultural insurance fee monthly.

Analysis of factors affecting the willingness to pay for agricultural insurance

Table 6. Estimated results of factors affecting the level of WTP

Description	Coefficient	Value	Level of significance	P-value
Coefficient of freedom	β_0	-510.06	ns	0.767
Age	β_1	13.92	ns	0.385
Sex	β_2	15.43	**	0.023
Academic level	β_3	12.27	*	0.073
Economic conditions 1	β_4	652.29	**	0.041

Economic conditions 2	β_5	230.05	***	0.003
Tea growing area	β_6	1182.24	***	0,001
Ownership	β_7	480.27	ns	0.906
Location 1	β_8	40.53	*	0.068
Location 2	β_9	20.49	*	0.080
Location 3	β_{10}	-12.60	**	0.037
Tea productivity	β_{11}	127.57	NS	0.109
Risk level	β_{12}	73.99	NS	0.205
$R^{2:}$ 0.535 F: 6.39; Sig F: 5,55E-6				

**Source: Authors' own calculations from survey data in 2015 and model running*
**** Confidence 99%; ** Confidence 95%; * Confidence 90%; Ns No statistical significance*

The analysis on the status of the willingness to pay for agricultural insurance for tea trees of tea growers showed that the households would like to pay different amounts depending on many factors such as level of education of the household head, household head sex, economic conditions, tea growing area or risks encountered in the production process. In order to quantify these effects, the research used linear regression models as is described in the research methods.

The results of the regression model describe factors that influence, in a statistically meaningful way, the willingness to pay for agricultural insurance for tea trees include: household head sex, education level, economics conditions, tea growing area and location. The levels of influence are as follows:

In terms of gender: the average willingness to pay to join the of agricultural insurance market for tea tree of men is VND 15,430 higher than that of women, which is explained that household head men are often more decisive in spending and have better awareness on production risk prevention.

In terms of education: education levels are included in the model as a continuous variable. The model shows that with every each year increase, the willingness to pay of tea growers tend to be VND 12,000 higher. In fact, people with high education levels are often aware of the production risks better than those with lower education levels.

In terms of economic conditions: economic conditions of the tea growers are recognized on 3 types of households: rich, medium and poor households; in which the poor are considered subject for comparison. Results of the regression model analysis indicates that those with better economic conditions are likely to pay higher agricultural insurance, around VND 652,000/ha/year higher than the poor households; also following this trend, the medium households are willing to pay VND 230,000 VND/ha/year higher compared to poor households. It can be said that the income of the family decides the ability to pay agricultural insurance fee of tea growers; those with better economic conditions have needs for agricultural insurance and will provide a higher willingness to pay.

In terms of growing area: In our research, the households' tea growing area is divided into 3 groups: small (031ha); average (0.74ha) and large (1.63ha). The

regression model results show that when the tea growing area of the households increases by 1 ha, the level of willingness to pay for agricultural insurance in tea production will grow by VND 1,182,24 thousand/ha/year.

In terms of growing locations: as mentioned above, the research selected four districts with large area of tea growing in Phu Tho province which are Doan Hung, Thanh Ba, Thanh Son and Yen Lap. Yen Lap is mountainous and the most remote district, so it was chosen for comparison. It can be seen that the willingness to pay of tea growers in the various districts also differ considerably; tea growers in Doan Hung district pay the highest rates, VND 40,000/ha higher than those in Yen Lap district and people in Thanh Son pay the least, VND 12,600 dong/ha less than those in Yen Lap.

CONCLUSION

Through studying and direct interviewing 1,000 tea growing households, the research identifies that there are 46.7% of the households are in need to participate and 53.3% of the households have no wish to join the agricultural insurance market. The level of the average willingness to pay is estimated to be 2,407.07 (thousand VND/ha/year). If agricultural insurance is successfully implemented in the province, it is estimated that the total agricultural insurance fund would be VND 34-35 billion/year.

Besides, the research has synthesized a number of people's recommendations for the agricultural insurance market participation: (i) 91.86% of the participating households requires agricultural experts and local authorities to be present at the time of loss evaluation; (ii) about 70% of the households want to buy agricultural insurance and receive compensation for loss at the office of commune authorities; (iii) the majority of the participating households (80.94%) want to receive compensation in cash for loss; (iv) tea growers expect the Government to support up to 93% of insurance fee for poor households.

The research has also analyzed a number of factors affecting the needs for agricultural insurance participation of tea growers in Phu Tho province. The factors being analyzed are gender, education level, tea farming area, economic conditions of tea growers and geographical factors. The survey shows that the average willingness to pay to join the market of agricultural insurance for tea of men is higher than that of women; the willingness to pay of tea growers with high educational levels tend to rise; the willingness to pay depends on the area of tea growing of households, households with greater area have higher level of willingness to pay; the willingness to pay for participating in agricultural insurance for tea trees of tea growers in Doan Hung district is the highest.

To attract tea growers to participate in agricultural insurance for fresh tea leaves production:

(1) It is a need to develop an insurance product for the production of fresh tea leaves which is suitable and accessible for farmers, thus creating basis for the confidence of producers in the existing products. (2) Actively disseminating general agricultural insurance policy to local authorities, farmers' union,

agricultural extension stations and especially farmers. (3)Agricultural insurance policy must be specific and in accordance with the production conditions, habits and mentalities of tea growers. (4) Opening training courses for farmers; compensate expenses for enterprises to implement agricultural insurance; supporting tea growers to participate in agricultural insurance. (5) Functional agencies need to cooperate closely with local authorities, businesses to regularly review the mechanisms and policies, , to attract the participation of households on the basis of ensuring the harmony among the interests of people, insurance agencies and the government. (6) Strengthening the coordination between the Government, insurance, reinsurance agencies, credit and financial institutions and the people.

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IMPACT OF FOLIAR FERTILIZATION ON PRODUCTIVITY OF NEW INTRODUCED CULTIVARS OF PADDY RICE

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ABSTRACT

The interest to the introduced rice cultivars (*Oryza sativa* L.) is explained by the search for more suitable varieties with good adaptability and high yield to be grown under the agro-climatic conditions in Bulgaria. The new foliar treatment products on the market - Folur, Amalgerol and Lithovit, have also provoked our interest. The survey was carried out in 2013 - 2015, applying the two-factor field experiment following the split plot method. Two Turkish - Osmanchik 97 and Gala, along with four Italian cultivars – Lince, Cameo, Puma and Brio were set as factor one. The foliar product Folur (2 l/da), Amalgerol (1.2 l/da) and Lithovit (0.300 kg/da) were set as factor two. The following factors were studied: phenological development of the cultivars, productive tillering capacity and yield. It has been specified that Osmanchik 97 and Gala had a shorter vegetative period. Lince stood out against the 6 tested cultivars by highest tillering results – 4.70 number of tillers/plant and was sharply outlined from the standard – Osmanchik 97, Puma and Brio. The tested products Folur and Lithovit had proven their effect on the formation of more productive tillering capacity – by 7.7% and 13.1% respectively. The highest yield was observed with Cameo (954.5 kg/da) and Brio (949.1 kg/da), which surpassed the Osmanchik 97 (control) by 8.6% and 8% respectively. The Amalgerol treatment proved to have the strongest positive impact on productivity and increased the average yield of the tested cultivars by 11.3%.

Keywords: *Rice (Oryza sativa L.), cultivars, foliar treatment products, development and productivity.*

INTRODUCTION

As a result of various objective (climate, limited suitable soils) and subjective reasons, (constant reorganizations, reconstructions, change of property, etc.), temporary reductions and subsequent recoveries of cultivated areas have been observed during the last two decades in Bulgaria. After the analysis of harvested areas, average yield and total production in this country, it has become clear that despite all fluctuations, harvested areas and average yields are steadily growing. By 2015, harvested areas amounted to 124 000 da and production for the same period increased almost 2.9 times – from 20 000 to 67 000 tons. Average yields were

comparatively steady - from 448.0 kg/da (2005) to 545.4 kg/da (2015), depending mainly on the biological characteristics of the cultivars and agro-meteorological conditions throughout the whole year. The immediate task for ensuring competitive power of Bulgarian rice production on the European market is yield increase and high quality production (Angelova, 1999). Additional options for raising productivity via foliar treatment products (Bari *et al.*, 2009; Dong *et al*, 2012) are being explored. The objective of the present study is to make a comparative characteristics of perspective Turkish and Italian rice cultivars, regarding phenological development, tillering capacity and productivity in the agro conditions of South Bulgaria, as well as the influence of Folur, Amalgerol and Lithovit foliar products on them.

MATERIAL AND METHODS

In the period 2013-2015 a two-factor field experiment using the split plot method was carried out in the town of Saedinenie, Plovdiv region, Bulgaria. The basic studied factor (big plots) in the experiment was the genotype. Six introduced cultivars were tested – two of Turkish origin (Osmanchik 97 and Gala) and four of Italian origin (Cameo, Lince, Puma and Brio), whereas Osmanchik 87 was used as the control. The tested foliar products (Folur, Amalgerol and Lithovit) were applied on the small plots. A non-treated control for each cultivar served for comparison. The experiment was set after rice as a fore-crop in four repetitions, with reported size of the harvested plot of 14.85 m^2. The statistical procession of the test data was made through SPSS V.9.0 for Microsoft Windows. Variation proofs were indicated at significance level P 5% (Fowler *at al.*, 1992). The experiment was carried out on alluvial-meadow soil with humus content below 2.5%, well-stored with phosphorus and potassium and insufficient total nitrogen. The reported pH defined the soil as acidic (pH 5.81 to 5.18). The meteorological conditions for the vegetation year 2013 were favourable for rice growing (Figs.1 and 2). The high temperatures which followed after sowing, preconditioned fast germination and intensive plant growth in terms of height. The average temperatures from May till October were by +0.2°C to +3.8°C higher, compared with those in the long-term period. The latter contributed to: growth and development acceleration, formation of maximum productivity and shortening the vegetation period. The agro-conditions in 2014 were completely different from those in 2013. The average daily temperature results for the first two months of the vegetation period (May – June) showed, that at this stage rice was in less favourable conditions than the previous year. In May temperatures were considerably lower (by 2.7°C), compared with the long-term period which had a negative effect on the duration of the germinating period and initial development. In the process of tillering and reaching full maturity, the registered temperatures were comparatively close to those in the long-term period. On the other hand, compared to 2013, they were lower by 0.5°C to 1.2°C. The average monthly rainfall for the whole vegetation period in 2014 was dramatically heavier compared to the long-term period and 2013 (Fig.2). The registered heavy rains proved to be unfavourable for rice development as they changed the regulated

water regime and microclimate in the rice enclosure. The amount of rainfall was lower only in August and the registered temperatures reached 35.6°C, which did not facilitate normal rice flowering.

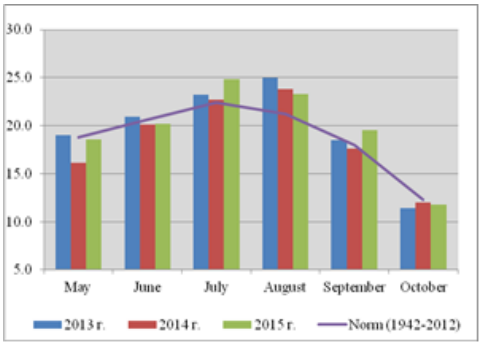

Figure 1. Average monthly temperatures (°C) for the research period, 2013-2015

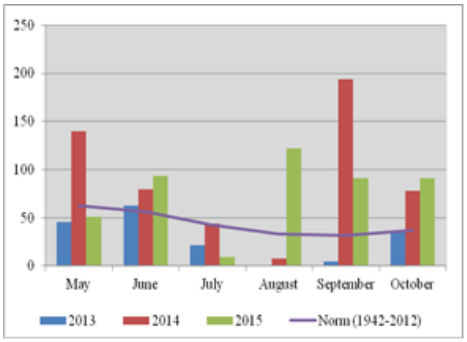

Figure 2. Total sum of rainfall (mm/m^2) for the for the research period, 2013-2015

RESULTS AND DISCUSSION

Phenological development. It is extremely important to realize and observe the biological characteristics of the crop and coordinate them with the environmental growing conditions towards achieving maximum manifestation of biological crop potential. Some factors, such as temperature, are limited under the agro-conditions in Bulgaria. The rice plant development strongly depends on the sowing date and environmental factors (Moldenhauer *et al*., 2003). Each cultivar, according to its origin, is significantly different in terms of time required for passing through the separate phenological phases and reaching fruiting, thus performing its full potential capacity.

Table 1 indicates the length of the interphase periods in days. The presented data show no difference among the cultivars from emergence until tillering. This period lasted for the longest (39 days) in 2013, followed by 2015 (32 days) and 2014 (24 days).

Depending on the agro-meteorological conditions, between 20 (average for all cultivars) (2013) and 35 (2014) days were needed from tillering until the panicle initiation. This stage lasted for the longest with the Italian cultivars (from 25 to 40 days for the different years), while the Turkish cultivars required from 9 (Osmanchik 97, 2013) to 33 days (Gala, 2014).

The average stage duration since panicle initiation till heading continued 20 - 25 days (average for all cultivars) for the three years of the study. For the time of the experiment, the interphase proceeded longer with the Turkish cultivars (21-32 days) compared with the Italian (18-25 days). The aforementioned stage is of great importance to plants, as this is the time for panicle formation and differentiation. It is considered to be the most critical stage for rice. Extreme temperatures at this

stage, both high and low, combined with other factors, may lead to a number of disturbances in plant development (Moldenhauer *et al.*, 2003).

Table 1. Duration of inter-phase periods of the tested rice cultivars, 2013-2015

Year	Rice cultivar	Inter-phase period (duration in days) of rice								
		S-E	E-TL	TL-T	T-PI	PI-H	H-MR	MR-DR	DR-M	VP
2013	Osmanchik 97	12	16	23	9	32	4	21	10	115
	Gala	12	16	23	14	31	7	14	12	118
	Linche	12	16	23	25	20	7	23	12	126
	Kameo	12	16	23	25	21	6	23	8	122
	Puma	12	16	23	25	18	9	23	8	122
	Brio	12	16	23	25	21	6	23	12	126
2014	Osmanchik 97	20	8	16	28	24	8	21	12	117
	Gala	20	8	16	33	21	8	23	13	122
	Linche	20	8	16	40	17	8	26	10	124
	Kameo	20	8	16	37	21	9	27	10	128
	Puma	20	8	16	37	19	9	26	10	125
	Brio	20	8	16	37	20	9	26	10	125
2015	Osmanchik 97	12	12	20	15	31	7	18	18	121
	Gala	12	12	20	20	29	8	17	17	123
	Linche	12	12	20	28	22	10	20	18	130
	Kameo	12	12	20	31	20	10	20	19	132
	Puma	12	12	20	26	24	9	21	15	127
	Brio	12	12	20	26	25	8	21	18	130

Legend: Sowing (S), Emergence (E), Third leaf (TL),Tillering (T), Panicle Initiation (PI), Heading (H), Milky Ripening (MR), Dough Ripening (DR), Maturity (M), Vegetation period (VP)

The duration of the vegetation period is a complex function, combining the interaction between genotype and growing conditions. It was hard to make the connection between deadline for sowing and deadline for the reported full maturity in the course of the experiment. Vegetation could have ended up as early as the second half of September (from 16.09. till 25.09 in 2015) under the created sowing conditions (on 04.05.2015), but it could have also continued until 05-08.10.2014, if sowing had taken place 9 days later. Sowing was done the latest in 2013 (16.05), nevertheless, at sufficiently high temperature sum, the seed entered full maturity until 01.10.

After tracing the growing processes of the variants treated with Folur, Amalgerol and Lithovit, it became clear that there was no difference in their phenological development compared with the non-treated control. The duration of each stage can rather be defined by the growing conditions. There is no connection between deadline for sowing, germination and duration of the subsequent inter-phase periods. According to these results and the classification used by Arraudeau *et al.* (1988), all the tested cultivars come under the category of cultivars with average vegetation duration of 120 – 140 days.

Table 2. Impact of main factors (variance analysis) on productive tillering capacity of rice

Tillering capacity (TC: number per plant), and Duncan's test (DT)											
	TC	DT	%		TC	DT	%		TC	DT	%
Year effect				Cultivar effect				Foliar treatment effect			
				Os. 97	4.13	a	100.0				
2013	4.41	a	100.0	Gala	4.44	ab	107.5	Contr.	4.13	b	100.0
2014	4.97	b	112.7	Linche	4.70	a	113.8	Folur	4.45	a	107.7
2015	3.63	c	82.3	Kameo	4.34	ab	105.1	Amal.	4.37	ab	105.8
				Puma	4.27	b	103.4	Litovit	4.67	a	113.1
				Brio	4.14	b	100.2				

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test

Productive tillering capacity. Productive tillering capacity has always corresponded more directly with yield. In the conditions of strongly reduced number of plants in the paddy field for objective reasons (low field germination, stress), the number of the productive tillers is an incredibly significant component of yield. Table 2 represents the summarized results of the three-year period, as well as the statistically reported influence of the respective year. The data analysis shows proved differences throughout the years, regarding the studied indicator. Best tillering capacity of plants was observed in 2014, followed by 2013 and 2015.
Among all tested cultivars, Lince proved itself to have formed the highest number of tillers during the three years of the experiment. The other cultivars also outmatched the control – Osmanchik 07. Puma and Brio only kept closer values to the control.After reporting the effect of the foliar treatment products, it was found out that all of them improved productive tillering capacity by 5.8% to 13.1%, whereas Folur and especially Lithovit were statistically outlined. *Yield (paddy rice).* The agro-meteorological conditions in 2013, 2014 and 2015 were highly specific and created prerequisites for obtaining proven different yields. The tested cultivars most successfully realized their potential in 2013, reaching average values for harvested paddy rice of 1112.6 kg/da (Table 3). The climatic situation throughout the same year was extremely favourable for rice growing (Figs.1 and 2), emphasizing the effect of high yield values. The years 2014 and 2015 witnessed proven lower yields as a result of cooler weather and heavier rainfall, reaching values of 819 kg/da and 750 kg/da respectively. These values were lower by 27% in 2014 and by 33% in 2015, compared with the favourable 2013.

Table 3. Impact of main factors (variance analysis) on paddy rice yield

Grain yield (kg/da) of paddy rice and Duncan's test (DT)											
	kg/da	DT	%		kg/da	DT	%		kg/da	DT	%
Year effect				Cultivar effect				Foliar treatment effect			
				Os. 97	879.2	a	100				
2013	1112.6	a	100	Gala	878.9	a	99.9	Contr.	846.0	b	100.0
2014	819.0	b	73.6	Linche	862.1	a	98.1	Folur	887.0	ab	101.3
2015	750.0	c	67.4	Kameo	954.5	a	108.6	Amal.	942.0	a	111.3
				Puma	839.5	a	95.5	Litovit	898.9	ab	102.5
				Brio	949.1	a	108.0				

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test.

The data analysis for the independent effect of the cultivar on productivity throughout the three consecutive years showed that highest seed yielding was performed by Cameo and Brio – average yield of 954.5 kg/da and 949.1 kg/da which was by 8.6% and 8.0% respectively higher than the control – Osmanchik 97. The cultivars performing lower yield than the control (by 2% to 5%) were Lince – 862.10 kg/da and Puma – 839.5 kg/da, while considering the huge differences throughout the years, the complex assessment of the factors showed that all tested cultivars came within the same range as the control.

Averagely for the period, the tested foliar treatment products had comparatively stronger effect on yield. A statistically proven effect on the variants treated with Amalgerol was definitely observed throughout the whole 3-year period. Increase of paddy rice yield was noted by 7.5% on the average. Bearing in mind that 2014 and 2015 were not very favourable years, the tested products proved to be most suitable for paddy rice crop under the conditions of changeable agro-meteorological parameters. On the average, the variants treated with Folur and Lithovit for the whole duration of the experiment, also outstanded the control, although by lower percent.

CONCLUSION

The cultivars of Turkish origin Osmanchik 97 and Gala developed faster in the agro conditions of Saedinenie compared with the tested Italian cultivars – Lince, Cameo, Puma and Brio. The duration of the vegetation period for the Turkish cultivars was 115-123 days, (making them more adaptive to the Bulgarian agro-conditions), while for the Italian cultivars it was from 122 to 130 days.

The complex conditions throughout the years had proved their effect on tillering productive capacity. Out of the six tested cultivars in the process of the research period, Lince had highest tillering capacity – 4.70 number of tillers/plant and was proved to stand out against the control – Osmanchik 97, Puma and Brio. The tested foliar treatment products Folur and Lithovit had a proven effect on the formation of higher number of productive tillers – by 8% and 13% respectively.

The most favourable conditions for the realization of rice productive potential were in 2013. The average harvested yields of paddy rice for all tested cultivars were 1112.6 kg/da, standing higher than 2014 - by 26.4% and higher than 2015 - by

32.6%. The cultivars with highest mean values of yields for the period were Cameo (954.5 kg/da) and Brio (949.1 kg/da), which surpassed the control Osmanchik 97 by 8.6% and 8.0% respectively.

The most positive effect on productivity was reported after treating with Amalgerol, which increased tested cultivar yield by 11.3% on the average. The average effect of Lithovit and Folur for the period was insignificant – 2.5 and 1.3% respectively.

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SIMULTANEOUS SELECTION OF MOST STABLE AND HIGH YIELDING GENOTYPES IN BREEDING PROGRAMS BY NONPARAMETRIC METHODS

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ABSTRACT

Explaining genotype by environment (GE) interaction is important in breeding programs because environmental effects are very often greater than genotypic effects in multi-environment trials. Statistical methods that select for high yield and stability have been proposed, but have not been compared for their usefulness especially for nonparametric methods. We compared fourteen nonparametric methods used for analyzing GE interaction at a set of experimental lentil data (11 genotypes at 20 environments). Nonparametric methods consist of six Huehn's statistics (S1, S2, S3, S4, S5 and S6), four Thennarasu's statistics (NP1, NP2, NP3 and NP4), tow Sabaghnia's statistics (NS1 and NS2), Kang's RS and nonparametric method of Fox et al. (1990). Considering mean yield versus nonparametric stability values via their plotting in a plot, indicated four different sections as A, B, C and D. The genotype fall in the section D were the most favorable genotypes due to high mean yield as well as high stability performance. Plot of the most nonparametric methods showed that genotypes G1 (1.21 t ha^{-1}), G2 (1.34 t ha^{-1}) and G5 (1.38 t ha^{-1}) were the most favorable genotypes and so these genotypes considered both yield and stability simultaneously. Although, most of the nonparametric methods have static (biological) concept of stability and measure the real concept of stability but plotting them versus mean yield and selecting the genotypes of section D, could identify relatively the high mean yield genotypes as the most stable ones.

Keywords: *GE interaction, static stability, plotting, mean yield.*

INTRODUCTION

Multi-environment trials (MET) are important in plant breeding for investigating yield stability of genotypes across environments. There are nonparametric statistics versus the parametric methods as an alternative strategy which are unaffected by data distribution and they are based on ranks and a special genotype is considered stable if its ranking is constant across test environments. Several nonparametric

stability statistics have been developed to explain the GE interaction in multi-environment yield trials (Huehn, 1979; Thennarasu, 1995; Sabaghnia, 2015). The nonparametric strategy is based on ranks of genotypes across test environments and provides an alternative to the parametric strategies. These methods separate genotypes based on their similarity of response to a range of test environments and has some advantages over the parametric strategies such as: reduction of the bias caused by outliers, no need for assumptions about the data distribution, easy to use, and not influencing by additions or deletions of few genotypes or environments (Huehn, 1996), Furthermore, for many applications such as selection, the rank order of genotypes is the most essential information. The good ability of the nonparametric methods for detecting the most stable genotypes as well as the GE interaction investigation have been reported in different crops such as lentil (Sabaghnia et al. 2006), chickpea (Ebadi-segherloo et al. 2008) and durum wheat (Sabaghnia et al. 2012b). The objective of this investigation was an estimation of yield stability performance of genotypes in environments via two new nonparametric stability statistics and their comparison with the existent methods.

MATERIALS AD METHODS

The lentil multi-environmental trials dataset of Sabaghnia et al. (2006) was used in this research and its two-way layout of yield performance for 11 autumn lentil cultivars at 20 different environments. Several nonparametric stability statistics were computed for each of lentil genotypes. The six statistics were based on yield ranks of genotypes in each environment and calculated as (Huehn, 1979). Kang's (1988) rank-sum (RS) was another nonparametric statistics where and computed via mean yield and stability variance. Nonparametric stability indices as Top, Mid and Low were calculated as Fox et al. (1990) using stratified ranking of the genotypes at each environment separately and the number of environment at which the genotype occurred in the top, middle, and lower third of the ranks was computed. Four nonparametric statistics of Thennarasu (1995) were based on the corrected ranks. The two nonparametric stability statistics as $NS_i^{(1)}$ and $NS_i^{(2)}$ which are proposed by Sabaghnia (2015) computed as:

$$NS_i^{(1)} = (Q_3 - Q_1) / M_{di}$$

$$NS_i^{(2)} = (D_9 - D_1) / M_{di}$$

where, $Q_3 - Q_1$ was the inter-quartile range which is a nonparametric index of statistical dispersion. M_{di} is the median of the genotypes' ranks in the test environments, $D_9 - D_1$ was the inter-decile range which is another nonparametric index of statistical dispersion of the values in a set of data. For simultaneous selection of most favorable genotypes (stable and high yielding), the calculated values of each of above mentioned nonparametric methods were plotted against mean yield performance separately. Each generated plot can be divided into four distinct sections as section A: low stability and low mean yield; section B: low stability and high mean yield; section C: high stability and low mean yield and section D: high stability and high mean yield. Therefore, the genotypes which were

in the section D could be regarded as most favorable genotypes (stable and high yielding). All of mentioned stability statistics were computed via Microsoft EXCEL program and plots were generated via STATISTICA version 10.0.

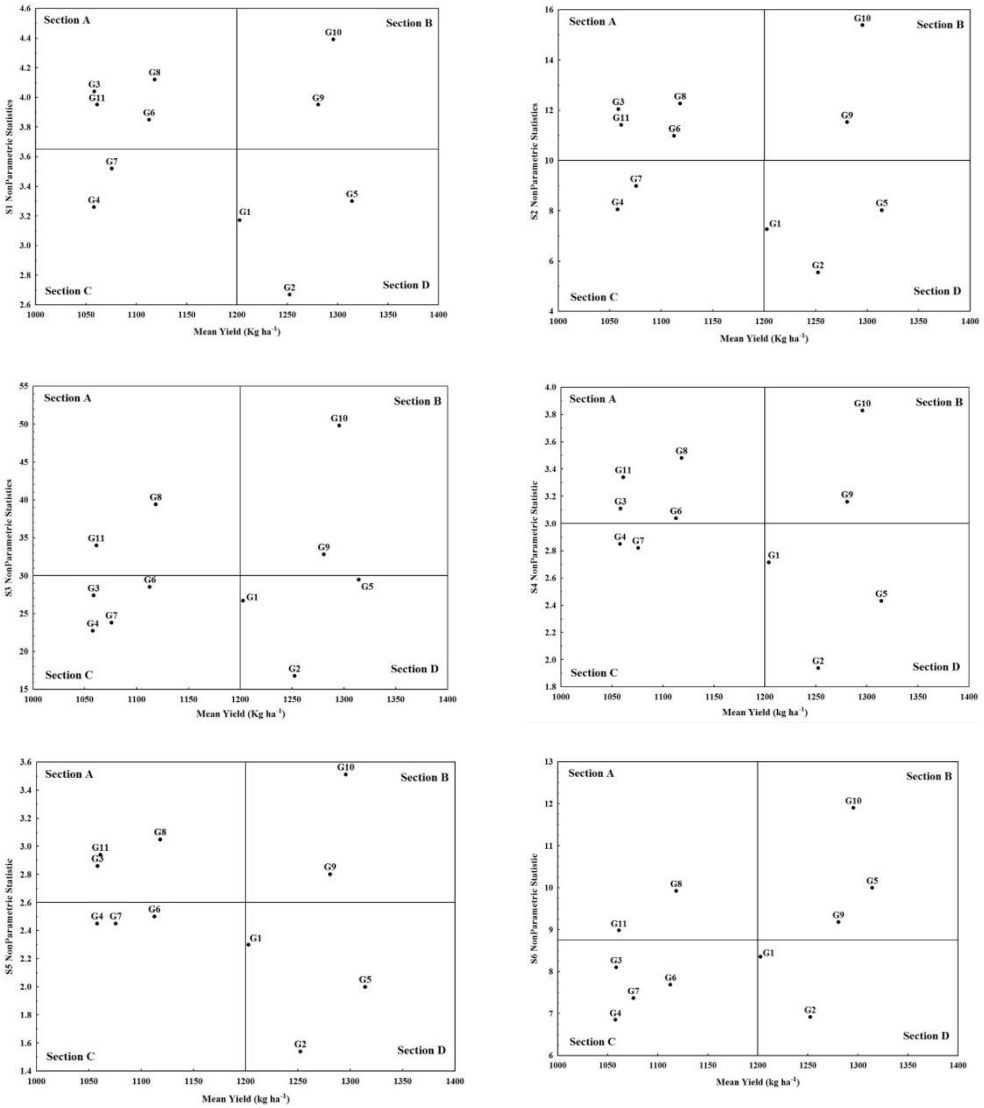

Figure 1. Plot of S1, S2, S3, S4, S5 and S6 stability statistic versus mean yield using yield data from 11 lentil genotypes grown in 20 environments.

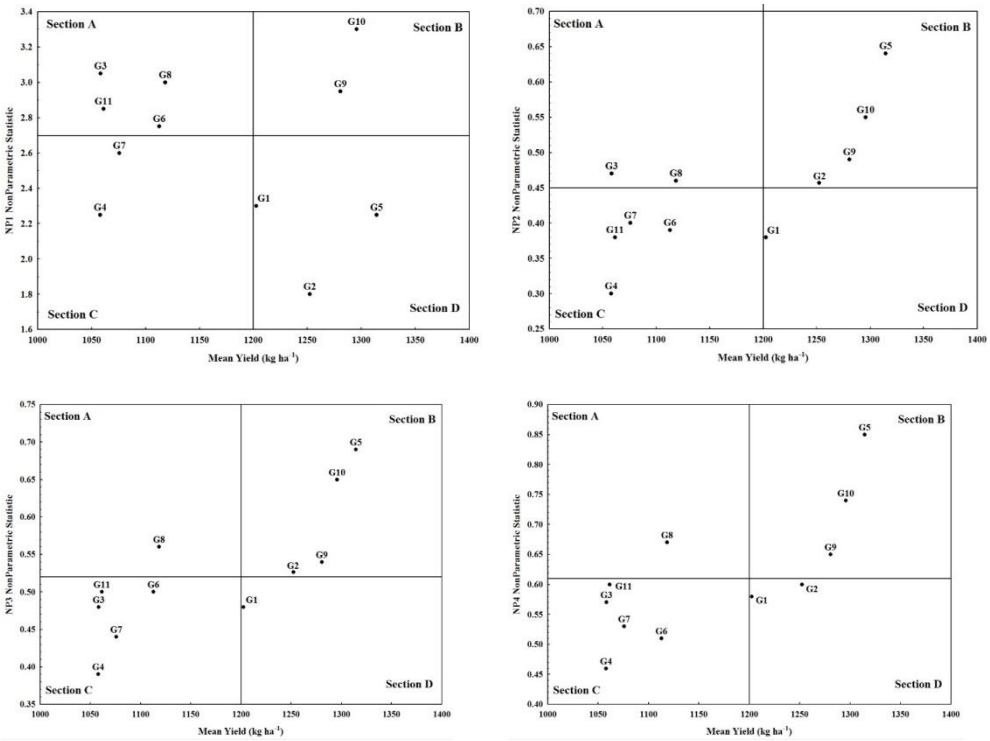

Figure 2. Plot of NP1, NP2, NP3 and NP3 stability statistic versus mean yield using yield data from 11 lentil genotypes grown in 20 environments.

RESULTS AND DISCUSSION

According to Fig. 1, genotypes G1, G2 and G5 were in the section D of related plots to five nonparametric statistics of Huehn (1979) as S1, S2, S3, S4 and S5. In the sixth statistics of Huehn (1979) as S6, only genotypes G1 and G2 fall into section D and could be regarded as the most favorable genotypes. Based on Fig. 2, genotypes G1, G2 and G5 were in the section D of NP1 while only genotype G1 was in the section D of NP2 and NP3. Also, genotypes G1 and G2 were in the section D of NP4. Plots of NS1 and NS2 (Sabaghnia, 2015) revealed that genotypes G1 in NS1 and genotypes G1, G2 and G5 in NS2 were the most favorable genotypes due to high mean yield and high stability performance (Fig. 3). Kang's (1988) rank-sum (RS) indicated that genotypes G1, G2 and G5 were in the section D while based on three nonparametric stability indices of Fox et al. (1990), genotypes G1, G2, G5 and G9 were in the section D (Fig. 3).Crop breeders have used the yield stability to characterize a genotype which acts a constant yield, ignoring of environmental changes, and so they have explored for genotypes with a less variation for yield performance over different environmental conditions. This idea of yield stability is similar to the homeostasis in quantitative genetics and is regarded as a biological or static concept of stability (Becker, 1981), which a genotype having a constant performance in all environments does not necessarily respond to changes of conditions. The above type of yield stability, thus, is not

suitable to most farmers, who would prefer an agronomic or dynamic concept of stability (Becker and Leon, 1988). For each test environment, the performance of a stable genotype based on static concept corresponds to the estimated level but in the dynamic stability, it is not required that the genotypic response to environmental changes should be equal for all studied genotypes.

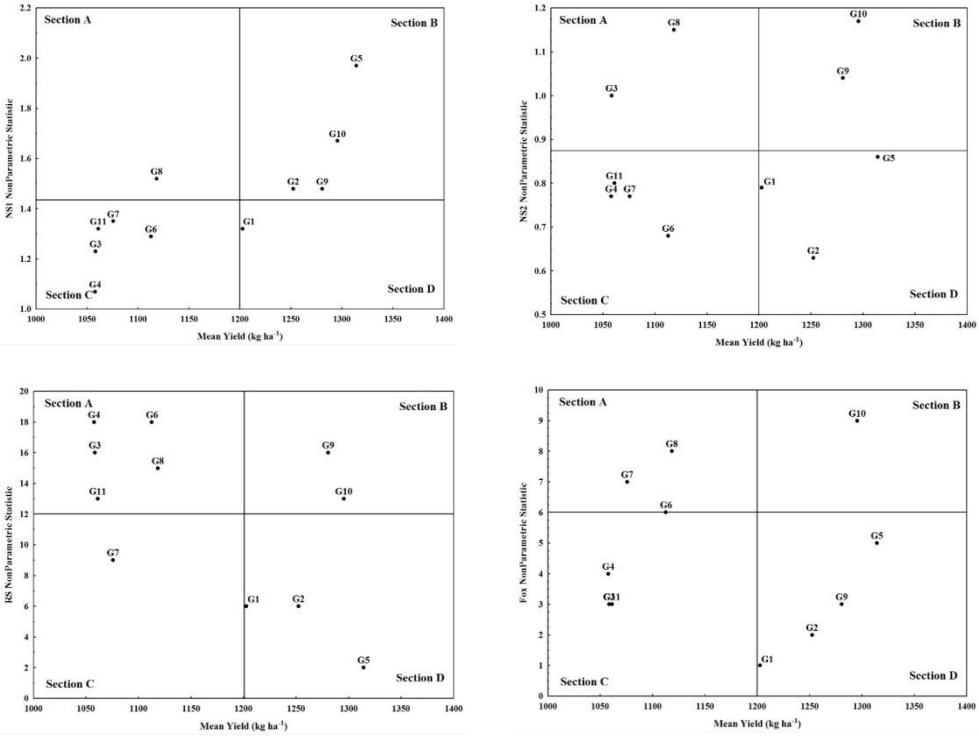

Figure 3. Plot of NS1, NS2, RS and Fox stability statistic versus mean yield using yield data from 11 lentil genotypes grown in 20 environments.

Flores et al. (1998) pointed out that the nonparametric statistics of S1 and S2 are associated with the static or biological concept of stability. Sabaghnia et al. (2006) noted that the nonparametric measures of stability, S1, S2, S3 and S6 are similar in concept to genotypes by environment interaction measures as they define stability in the sense of homeostasis. Sabaghnia et al. (2012) pointed out that the nonparametric statistics of NP1, NP2, NP3 and NP4 and are associated with the static or biological concept of stability. Sabaghnia et al. (2015) noted that the nonparametric measures of stability, S1, S2, S3, S4, S5 and S6 are similar in concept to yield stability measures as they define stability in the sense of static concept. Thus, the nonparametric statistics corresponding to the biological concept of stability. The fact that stability is of economic importance for a genotype was recognized as the variance across environments and such stability parameters follow a static concept meaning that a stable genotype is defined as one having an

unchanged performance regardless of any variation in the environmental conditions.

It is desirable that target traits such as yield performance should be maintained through all environments but the yield performance of a genotype usually reacts like other quantitative characters to favorable or unfavorable environmental conditions thus, varies in its performance. It is unrealistic to expect the same level of performance in all environments and yield performance thus follow an agronomic or dynamic concept of stability, meaning that the performance may change from environment to environment, but in a predictable way. A genotype is therefore regarded to be economically stable if its contribution to the genotype by environment variance is low. Although, a wide range of stability parameters resulting from univariate, parametric and nonparametric, and multivariate methods have been described and advantages and disadvantages as well as the relationships between them have been reviewed by several authors (Lin et al. 1986; Flores et al. 1998; Sabaghnia et al. 2006).

Though several statistical strategies for yield stability analysis have been proposed, they each reflect different aspects of stability nature and maybe no single method can adequately explain genotype performance across test environments but we found that using plot of a stability statistic versus mean yield performance can detect the most favorable genotype (high mean yield and high stability) even this stability statistic had a static concept. This a simple method for selecting the best genotypes via traditional methods like S1 or environmental variance. The nonparametric stability statistics seem to be useful alternatives to parametric methods (Huehn, 1990), because they do not supply information about genotype adaptability and for several reasons, the use of nonparametric stability statistics is preferred. For making practical recommendations, it is necessary to study the effectiveness of the other statistics (univariate, parametric and multivariate method) and compare their powers for detection of the most favorable genotypes.

CONCLUSION

The NS1 and NS2 nonparametric statistics which used are similar to the nature and concept of environmental coefficient of variation (Francis and Kannenberg, 1978), because they use the central tendency of ranks is the median and its related measures of dispersion are inter-quartile or inter-decile range. It seems that there are good poetical in the introduced strategy in distinction of favorable genotypes in plant breeding programs because it provides some flexibility in the hands of plant breeders for simultaneous selection for yield and stability.

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COMPARISON OF WATERMARK SOIL MOISTURE CONTENT WITH SELYANINOV HYDROTHERMAL COEFFICIENT

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ABSTRACT

Agricultural producers to determine irrigation scheduling practices for crop's water requirement better when the soil water content of their fields is known. Selyaninov Hydrothermal Coefficient (*HTC)* coefficient is used for identifying droughts during the active vegetation period, based on the water balance equation. For farmers to make measurements of soil moisture is simply with humidity sensors, for example *Watermark* type. Soil humidity values established using *Watermark* type humidity sensors, value interpretations are based on manufacture indications, however they have not been adapted to Lithuanian conditions. Soil moisture was measured with *Watermark* soil moisture sensors placed at 20 and 50 cm depths. After analysing the values taken throughout the whole period and summarizing the results it has been identified that plant growth condition period evaluation according to *HTC* and factual soil humidity reserves (*W*) differs by 20%. *HTC* meaning dependencies during vegetation period using *Watermark* measured humidity, strong or averagely strong interrelation is determinate, in most cases – statistically significant. When evaluating soil humidity reserves based on soil texture, it is recommended to keep the critical *Watermark* level in light texture soil (sands) at 80 cbar, and in all other types of soil - at 150 cbar. The results clearly indicate that soil composition could be factors limiting the success of identifying droughts in agriculture carried by *Watermark* type humidity sensors.

Keywords*: agriculture droughts, humidity, soil moisture, Lithuania.*

INTRODUCTION

Drought or water deficit stress is the major environmental factor that negatively impacts agricultural yield throughout the world (Selote, Khana-Chopra, 2004). Droughts and their consequences cause substantial damages and losses to the sectors of agriculture, energy, nature, they have significant social and other impacts (Wilhite et al., 2000). Climate change affects water availability not only by changing regional precipitation levels and temporal variability, but also by affecting water flows and soil moisture dynamics (Holsten et al., 2009). An efficient irrigation system should meet crop demands for water. A limited water

supply may result in reductions in yield, while excess irrigation is a waste of resources. To investigate water availability throughout the growing season, on-the-go sensing technologies (field elevation and apparent electrical conductivity) were used to analyse the spatial variability of soil relevant to its water-holding capacity (Pan et al., 2013). Soil moisture sensing network is used to monitor the moisture contained in soil and help irrigation decisions in drip irrigation systems. Evapotranspiration is directly linked to soil moisture from one part and to crop yield from the other part (Eagleson, 1994). Irrigation scheduling is crucial to effectively manage water resources and optimize profitability of an irrigated operation. Tools that can be customized to a field's characteristics can greatly facilitate irrigation scheduling decisions (Aguilar et al., 2015). Drought indices have been developed by several generations of researchers during the XX century in the domains of meteorology, hydrology, agricultural research and application, remote sensing, and water resources management. More than 80 drought indices have been easily identified, and probably the total number of drought indices is close to double (Niemeyer 2008). The wireless sensors networks are actually used in the agriculture field to monitor the climate, the crops, the control of the crop inputs and the irrigation supply. A good control of these parameters would make it possible to the farmers to carry out proactive actions in order to better improve the crop yield (Diaz et al. 2011, Garcia-Sanchez et al. 2011, Lopez et al. 2009, Wark et al. 2007). The use of soil moisture sensors, like the granular matrix and those based on the time and frequency domain reXectometry, has increased in the last decades since advances in electronics and computers applied to agriculture enable better soil water monitoring (Hilhorst and Dirksen 1994; Girona et al. 2002). Errors in soil moisture measurements (or other methods of soil moisture determination) require that irrigation commences at a higher threshold of the measured soil moisture content, so that with a given statistical probability (coincidence level), the true value of soil moisture content is not lower than the threshold. A `true value' of soil moisture content needs to be defends. Here, the total amount of water that is in the soil volume within reach of the roots of one plant, divided by that volume, is intended. Scheduling irrigation by soil moisture sensors should not be done without carefully considering the measurement uncertainty (Schmitz, Sourell, 2000). Rapid progress is being made in transmitting sensor data, obtained from different depths down the soil profile across irrigated areas, to a PC that processes the data and on this basis automatically commands irrigation equipment to deliver amounts of water, according to need, across the field (Greenwood et al., 2009). *Watermark* sensors allow for multiplexed, automated, in situ measurements for determining changes in soil water content and the onset of wetting fronts when such occur abruptly in the field. The system described here proved reliable, effective, and cost efficient, exhibiting only minor problems (Light, 1990). The *Watermark* sensor has proven to work quite well in both the clay and sandy soils, however because it does not have an indicator light on the sensor control next to the controller, it took most of the summer to tweak it to operate at acceptable levels (Mecham, 2006). McCann et al. (1992) noted that three to six *Watermark* granular matrix sensors

placed at a given location should yield matric potential within 10% of the actual value with a 90% confidence interval. The dynamic response of the sensors can vary with changing soil moisture. Agricultural producers to determine irrigation scheduling practices for crop‘s water requirement better when the soil water content of their fields is known. G.T. Selyaninov suggested using the hydrothermal index for agrometeorological problems. This index has been widely used in practice till now. It has many different modifications for specific territories. The index considers the water budget input and moisture evaporation from the surface of a territory under study. It is easily calculated. However, it characterizes only wetness, without considering stored soil moisture (Utkuzova et al., 2015). The Selyaninov hydrothermal coefficient (HTC) is used as the primary climate variable, which includes not only precipitation, but also temperature during the vegetative period (Melkonyan, Asadoorian, 2013). In Lithuania HTC is used to identify the droughts. This index also was used in all former USSR territory in previous decades by forestry, agricultural and hydrology specialists for long time (Meshcherskaya and Blazhevich, 1996). *HTC* is used Lithuania for identifying droughts during the active vegetation period, based on the water balance equation. For farmers to make measurements of soil moisture is simply with humidity sensors, for example *Watermark* type. Soil humidity values established using *Watermark* type humidity sensors, value interpretations are based on manufacture indications, however they have not been adapted to Lithuanian conditions. The main goal of this study is to evaluate the *HTC* links with soil humidity measurements carried by *Watermark* type humidity sensors and the possibility of applying it for identifying droughts in agriculture.

MATERIAL AND METHODS

The experimental plots are located across the Lithuania (Figure 1).

Figure 1. Objects area location

Meteorological conditions have been explored in hydro-meteorological stations of the Lithuania Hydro meteorological Institute located in the catchment surroundings. Sampling was done each day May to September during 2013. The object basic characteristics are given in Table 1.

Table 1. Characteristics of objects

Object No.	Soil	Precipitation during the year (mm)	Thermal conditions of summer (ΣT>10°C)	Wilting moisture, %
1.	Dusty, heavy loam	500 - 600	2100 - 2200	9,1-10,2
2.	Averagely heavy gravel loam	500 - 600	2100 - 2200	5,6-6,9
3.	Heavy loam	500 - 700	2000 - 2200	9,1-10,2
4.	Averagely heavy clay	650 - 750	2200 - 2300	9,5-10,2
5.	Sand	700 - 800	2000 - 2200	4,4-6,4
6.	Alluvial clay	700 - 900	1900 - 2000	7,9-9,1
7.	Averagely coarse sand	600 - 700	2100 - 2300	6,4-6,6

Soil humidity measurements have been carried out using *Watermark* (*W, cbar*) humidity sensors. Soil moisture was measured with *Watermark* 200 SS soil moisture sensors (USA) placed at 20 (*W*20) and 50 (*W*50) cm depths.

The Watermark soil moisture block is sold as a qualitative indicator of soil moisture for applications such as irrigation scheduling. It consists of two concentric electrodes embedded in a porous matrix containing a soluble salt (CaSO4), so that the water in the porous matrix is always gypsumsaturated. Lead wires are connected to the electrodes so that the electrical resistance of the porous medium can be measured. The device is encased in a synthetic membrane supported by PVC plastic. This presumably confers a life expectancy longer than that of gypsum blocks, which dissolve over time (Egbert, 1992).

Complex indicators give more comprehensive characteristics. In order to get a more complete analysis of thermal and rainfall conditions, the relative (non-dimensional) indicator known as G. T. Selyaninov's hydrothermal coefficient (HTC) (Gathara at al., 2006):

$$HTC = R / 0.1 \Sigma T,$$

where R is the total precipitation for the period having an average air temperature of greater than 10° C;

ΣT is the sum of average daily air temperatures for the same period, which is divided by 10, giving a figure that characterizes evaporation quite well.

HTC meaning scale is divided into 3 groups which are most relevant to drought, optimal and rainy period soil according to recommended interpretations by *Watermark*. Aiming at identifying interlinked connections the *HTC* meanings have been compared to *Watermark* type sensor results. *HTC* and *Watermark* meaning comparison is presented in Table 2. Correlative analysis is used for statistical evaluation of the study data (5% significance level).

Table 2. *HTC* and soil humidity (based on *Watermark* sensors) value interpretation

Group	*HTC*	*Watermark*
Wet	>1,5 (Wet)	<11 cb (Wet)
	1,0 - 1,5 (Sufficiently humid)	11 – 29 cb (Humid/ averagely wet)
Optimal	0,8 - 1,0 (Insufficiently humid)	30 – 60 cb (Optimal)
Dry	0,6 - 0,7 (Arid)	60 – 100 cb (Arid)
	0,0 - 0,5 (Dry)	100 – 200 cb (Dry)

Differences were considered significant if $P< 0.05$. Correlation analysis (r) was used to determine the relationship between the humidity sensors *Watermark* in 20 cm or 50 cm depth and *HTC*.

RESULTS AND DISCUSSION

When analysing the *HTC* meaning dependency during vegetation period using humidity measurements *W, cbar* quantity at 20cm depth taken using *Watermark*, the average strength interrelationship has been achieved No. 2 ($r = 0.54$), No. 3 ($r = 0.51$), No. 6 ($r = 0.46$) at 20 cm depth, and the average strength interrelationship has been achieved No. 1 ($r = 0.61$), No. 4 ($r = 0.63$), No. 5 ($r = 0.74$), No. 6 ($r = 0.74$), No. 7 ($r = 0.55$), a strong relationship is present at No.3 ($r = 0.78$). The standard variance in all stations contains more than 20% of the arithmetical average, so, the spread of the results is very wide, but the results are statistically important at objects No. 2, No. 3 and No. 6 at 20 cm depth and at all objects - at 50 cm depth (Table 3).

After analyzing the values taken throughout the whole period and summarizing the results it has been identified that plant growth condition period evaluation according to *HTC* and factual soil humidity reserves (*W*) differs by 20% (Fig. 2). McCann et al. (1992) noted that the dynamic response of *Watermark* granular matrix sensors was good during typical soil water drying cycles after complete rewetting, but was poor during rapid drying or partial rewetting of the soil. By Thompson et al. (2006), the general performance of the Watermark sensor under the given environmental and soil water conditions. The best performance of the Watermark sensor was obtained under conditions of moderate evaporative demand in moist soil.

Table 3. The results of regression analysis between *HTC* and *Watermark*

Object No.	Method	Stdev	Average	c_v	*r*		*P*	
1.	W (20)	29.18	76.14	0.38				
	HTC	0.34	0.95	0.36	-0.10		0.26	
	W (50)	13.89	107.94	0.13		-0.61		0.00
2.	W (20)	51.36	56.21	0.91			0.00	
	HTC	0.33	1.32	0.25	-0.54	-0.41		0.00
	W (50)	33.89	107.77	0.31				
3.	W (20)	58.90	93.33	0.63				
	HTC	0.40	1.20	0.33	-0.51	-0.78	0.00	0.00
	W (50)	68.10	86.19	0.79				
4.	W (20)	62.49	126.18	0.50				
	HTC	0.53	1.66	0.32	-0.07	-0.63	0.43	0.00
	W (50)	40.69	167.57	0.24				
5.	W (20)	66.11	117.50	0.56				
	HTC	0.49	1.25	0.39	-0.10	-0.74	0.26	0.00
	W (50)	25.33	69.82	0.36				
6.	W (20)	57.39	49.18	1.17				
	HTC	0.81	1.45	0.56	-0.46	-0.52	0.00	0.00
	W (50)	24.99	15.28	1.64				
7.	W (20)	32.52	38.85	0.84				
	HTC	0.27	1.29	0.21	-0.16	-0.55	0.08	0.00
	W (50)	16.14	48.12	0.34				

The Watermark may be used in soil water monitoring systems, under drier conditions than the ones measured by the tensiometer, contributing to a better understanding of the different hydrological and erosional processes acting on the hillslopes (Bertolino, 2002). Drought occurs when moisture around the roots is so drought but depending on the severity and time of reduced that a plant is not able to absorb enough water, occurrence of the drought, yield reduction could reach or in other words with transpiration of water absorption 80% (Tarighaleslami et al., 2012).

Using an experiment it has been identified that dependently on soil, wilting humidity is reachable when humidity reserved in soil range from 4.4 to 10.2 % based on weight of dry soil, which according to *Watermark* device's calibration curve meets 80 to 160 *cbar* or higher value (Table 4).

After implementing the correlation, taking into consideration the values presented above it becomes evident that plant vegetation period humidity evaluation according to *HTC* and soil humidity values (*Watermark*), the values indicating the beginning of drought are matching in 50 cm depth (Fig. 2).

Table 4. Interpretation for moisture meters estimates of beginning the drought

Object No.	Moisture reserves in the soil from the dry weight of the soil, %	*W, cbar*
1.	4.4- 6.4	≥80
2.	9.1- 10.2	≥100
3.	5.6- 6.9	≥ 120
4.	7.9- 9.1	≥ 100
5.	9.5- 10.2	≥ 160
6.	6.4- 6.6	≥80
7.	9.1-10.2	≥80

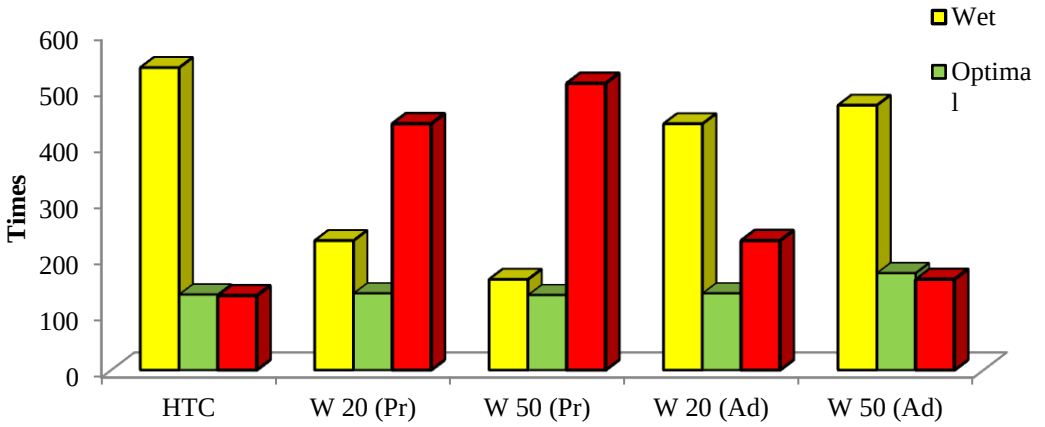

Figure 2. Matching of *Watermark* and *HTC* after a correction is made based on granulomeres texture (*Pr-producer recommendation, Ad- adjusted by soil)*

These trigger points cannot easily be related to different soils, different sensors, and other sources of information such as extension fact sheets and research publications, however, because the actual water content measurements may not be correct (Leib et al., 2003). The Watermark sensors utilized a local soil water retention relationship in order to convert soil water potential into volumetric water content. The results suggests that irrigators can still use uncalibrated sensors to improve their watering schedules by setting irrigation trigger points that may relate only to specific sensor in a specific soil.

CONCLUSIONS

When researching *HTC* meaning dependencies during vegetation period using *Watermark* measured humidity, strong or averagely strong interrelation is determinate, in most cases – statistically significant: statistically important at objects No. 2, No. 3 and No. 6 at 20 cm depth and at all objects - at 50 cm depth. Evaluating soil humidity reserves based on soil texture, and it is recommended to keep the critical *Watermark* level in light texture soil (sands) at 80 cbar, and in all

other types of soil - at 150 cbar. Plant growth condition period evaluation according to *HTC* and factual soil humidity reserves (*W*) differs by 20%. Correlative analysis of the study data (5% significance level) shows that differences were considered significant. The results clearly indicate that soil composition could be factors limiting the success of identifying droughts in agriculture carried by *Watermark* type humidity sensors.

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QUALITY AND TECHNOLOGY OF INDIGENOUS TRADITIONAL ''BIENO'' CHEESE IN THE REGION OF MARIOVO, MACEDONIA

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ABSTRACT

"Bieno'' cheese is an indigenous dairy product in Macedonia with autochthonous traditional production technology which dates back from the Ottoman Empire. The research includes and presents the results of the chemical composition and safety of raw milk used for traditional production of ''Bieno'' cheese, technology and physico-chemical and microbiological quality of ''Bieno'' cheese. The quality of the milk samples was determined within the permissible maximum according to data legislation. It is necessary to respect the hygienic-sanitary norms related to cultivation, preservation and care of the milking herds, and with the right technology of milking. After 45 days of ripening the researchers recorded the average results for the following parameters of the ''Bieno'' cheese: moisture (38.63%), dry matter (61.37%), milk fat (26.89%), milk fat in dry matter (43.83%), proteins (26.53%), ash (9.25%), salt (5.21%) and the average of yield (9.36%). The research specified the microbiological quality of "bieno" cheese after 45 days of ripening in accordance with the special requirements of food safety regarding the microbiological criteria. Nowadays, there is a growing interest of consumers for cheeses produced with traditional technologies, usually based on handmade production, characterized by piquant, unique and specific aromas, atypical for industrial cheeses. The technology, physico-chemical and microbiological quality could be used in the protection of the origin and geographical labelling based on its unique technology. The data obtained serve as the basis for creating standardized production procedures, leading to the uniform quality of these products.

Key words: *Traditional production, raw milk, chemical composition, cheese, microbiological quality.*

INTRODUCTION

Bieno cheese is a typical indigenous dairy product in Macedonia from the central area of Mariovo region whose production dates back from the Ottoman Empire. Bieno cheese is manufactured in industrial conditions, but in some places it is still homemade, formerly produced from sheep milk, but today mostly from cow milk.(Talevski, 2012)

In rural and environmentally unpolluted regions, such as Mariovo, the production of traditional cheese is preserved today. The region of Mariovo is situated in southern part of Macedonia, close to the border with Greece. It is surrounded by high mountains and represents a specific natural area. The region has a diverse flora, due to the specific climate, as well as geologic, geomorphologic and pedologic features (Matevski and Kostadinovski, 1998).

The future of small farmers is to preserve the traditional technologies, production of authentic and traditional products, protection and marketing of domestic and foreign markets. Given its solid consistency and extremely salty taste it is often consumed by the poor and therefore called 'poor cheese'.

Beaten cheese has yellowish color, hard consistence, pleasant aroma and particularly salty taste (5-10%). Diff erent size halls with random arrangement are visible on the cross-section.(Levkov and Kakurinov, 2011)From this perspective, the study finds a particular practical application and economic viability in the new conditions, plans and policies for the development of agriculture and rural development of certain regions in the country. The aim of this study was to present the technology of “bieno cheese” production, and also its physical, chemical and microbiological characteristics.

MATERIALS AND METHODS

Analysis and sampling

This paper presents the results of the milk quality, technology and physico-chemical quality of Bieno cheese. Sampling of milk and cheese, as well as production technology, were done in the Mariovo region, Macedonia.

The technology of Bieno cheese includes the following steps:

Raw milk

↓

Squeezing through cloth lurch

↓

Heating to 35-36^{0}C and coagulation with chymosin CHY-MAX (2080 imcv/g)

↓

Processing of curd and heating of curd (by adding boiling water t = 70-75^0 C)

↓

Kneading and collecting of curd in ball

↓

Self pressing with hanging in the cloth 16-18 h

↓

Ripening at 26-28^0 C, 5 days

↓

Cutting strips (4-5cm)

↓

Dry salting (1-2 days)

↓

Salting in brine (20-22%)

↓

Ripening and storage (45 days) 16-18^0 C

Physico-chemical analysis

Milk samples were analysed for the content of dry matter, fat, protein, lactose and solids non fat by infrared analyser LactoScope. Active acidity was measured with pH meter (Testo 206) and titratable acidity according to the Soxhlet Henkel method (Carić et al., 2000). Total count of somatic cell was analysed with a Somascope, and total count of bacteria with Bactoscan.

For the whey there were conducted the following analysis: Active acidity of the milk with a digital pH meter (testo 206) **;**Titrable acidity (^{0}SH) - by the method of Soxhlet Henkel (Carić et al., 2000)**;**Chemical analisys of whey (milk fat, protein, lactose and dry matter) with an infrared analyzer LactoScope.

The cheese composition was analysed by standard methods: dry matter (AOAC,1995), fat Soxhlet Henkel method (Carić et al., 2000), protein (AOAC, 1995), salt and ash (Inihov method, 1971). The contents of moisture and fat in the dry matter (FDM) were calculated (Codex, 1978), and also the yield of ‘’bieno’’ cheese (Balthadzieva,1993).

Microbiological analysis

The cheese was subject to the following microbiological analysis:ISO 6579, Microbiology of food and feed-Horizontal method for the enumeration of *Salmonella spp* (ISO6579: 2002 / Cor: 2004); ISO 16649, Microbiology of food and feed-Horizontal method for the enumeration of β - glucuronidase - positive *Escherichia colli* ISO 16649 1, 1: Material of counting colonies of 44 0C using membranes and 5 bromo-4-chloro- 3-indolyl β - D- glucoronide (ISO 21528-1: 2001); ISO 21528-2, Microbiology of food and animal feed - Horizontal method for the detection and enumeration of *Enterobacteriaceae*, Part 2: Method of counting colonies (ISO 21528 1: 2004); ISO 11290-1, Microbiology of food and animal feed - Horizontal method for the enumeration of *Listeria monocitogenes*, Part 1: Detection method (ISO 11290-1: 1996/ Amd.1: 2004); ISO 6888-1, Microbiology of food and feed-Horizontal method for the detection of *Staphylococcal koagulasa* positive (*Staphyloccocus aureus* and other species), Part 1: Technique using Baird-Parker agar medium (ISO 6888-1: Amd 1: 2003)

RESULTS AND DISCUSSION

The quality parameters of raw milk used for the production of ''bieno'' cheese are shown in Table 1.

Table 1. Physico-chemical parameters and hygienic quality of milk for ''bieno'' cheese

	Raw cow milk				
Parameters %	$\bar{x}$	**Min**	**Max**	**Sd**	**Cv**
Milk fat	**3,50±0.12**	**3,29**	**3,67**	**0,15**	**4,25**
Proteins	**3,11±0.04**	**3,02**	**3,18**	**0,06**	**1,96**
Lactose	**4,28±0.06**	**4,20**	**4,41**	**0,08**	**1,93**
Dry matter	**12,29±0.16**	**106**	**12,.50**	**0,19**	**1,57**
pH	**6,69±0.08**	**660**	**6,81**	**0,09**	**1,41**
^{o}SH	**6,32±0.10**	**614**	**6,51**	**0,14**	**2,24**
*TCSC/ml	**343600**	**276000**	**368000**	**38474,67**	**11,20**
*TCB/ml	**342000**	**326000**	**363000**	**15313,39**	**4,48**

*TCSC/ml (Total count of somatic cells)
*TCB/ml (Total count of bacteria)

The milk used for the production of indigenous hard cheese has good chemical composition, hygiene is right with allowable number of somatic cells and the total number of bacteria in accordance with the special requirements for raw milk. Our results regarding the chemical composition of cow's milk were in accordance with the results from other authors (Srbinovska,2007; Mateva et al., 2008; Talevski,2012) . In the indigenous technology of production of bieno cheese, must pay particular attention to all the factors that affect the microbiological quality of the milk and the hygienic correctness, namely: hygienic milking, milk cans, cooling devices (tankers) were the milk is held at a temperature of 5°C, transport and storage. It is necessary to respect the hygienic-sanitary norms related to cultivation, preservation and care of the milking herds, and with the right technology of milking, (Dozet and Macej, 2006; Samaržija et al., 2003).

The total number of bacteria in the milk used for the manufacture of "bieno" cheese varies in the range of 2.7 x 10^6 to 1,0 x 10^8 / ml and 2.0 to 3.5 x 10^7 / ml by the author (Kakurinov, 2002), (Levkov and Kakurinov, 2007) and that depends on milking hygiene, storage and transportation of milk. Whey is a product obtained in the manufacture of cheese. It is more or less clear, yellowish liquid with a distinctive sweet flavor.

The chemical composition and properties of whey depend primarily on the quality of milk and the technological process of producing cheese. The results of chemical composition and acidity of the whey can be seen in Table 2. Whey is an important by product in the manufacture of ''bieno'' cheese which can be used to obtain fresh albumin cheese.

Table 2. Chemical composition of whey

	Whey				
Parameters %	$\bar{x}$	**Min**	**max**	**Sd**	**Cv**
Milk fat	0,19±0,03	0,15	0,23	0,04	18,86
Proteins	1,04±0,04	0,98	1,14	0,06	5,88
Lactose	4,46±0,06	4,38	4,57	0,08	1,75
Dry matter	6,42±0,05	6,33	6,51	0,07	1,06
pH	6,49±0,06	6,40	6,58	0,07	1,13
^{o}SH	4,89±0,32	4,60	5,34	0,37	7,59

From the table 2 can be seen that the content of milk fat (0,19±0,03) in whey likely due to the higher heat treatment. In whey, it exceeds about 50% of the dry matter of the milk. The largest% of the dry matter in the whey is lactose, followed by proteins and minerals, and finally, the fat percentage is the lowest.

Protein is an important component in the chemical composition of whey and average mean values ranging from 1,04±0,04%. Most % of the dry matter constitutes lactose whey, followed by protein and minerals and finally, with the lowest number was fat. The active acidity of the whey has an average mean (6,49±0,06) and the titrable acidity has an average mean (4,89±0,32). Similar results are found in whey obtained in the production of cheese and soft white cheese in research of ,(Mateva et al., 2008, Sulejmani et al.,2014)].

From Table 3, the Bieno cheese contains 38,63±0,32 of moisture, 61,37±0.32 of dry matter, 26,89±0,26 fat, 43,83±0,62 fat in dry matter, proteins 26,53±0.93, ash 9,25±0,06 and salt 5,21±0,05 after 45 days of ripening. Those results coincide with results of scientific papers whose topic was hard cheese and that show that moisture content ranges from 38 to 40%, fat 21 - 26% and fat in dry matter 40 - 43%(Kapac-Parkačeva,1974; Kamber and Uelik,2007).

Yield is a complex variable because it depends upon a number of factors, the major effect on the quality of milk, the protein content and milk fat,(Guinee, 2004) . The greater degree of the distribution of the components of milk into cheese, primarily protein and milk fat affect the majority of the cheese dressing percentage. This statement can be seen in our results obtained in our research and average cheese yield of bieno cheese was (9.36 %).

Table 3. Chemical composition of bieno cheese after 45 days of ripening

	“Bieno” cheese				
Parameters %	$\bar{x}$	**Min**	**Max**	**Sd**	**Cv**
Moisture	38,63±0,32	38,29	39,40	0,46	1,18
Dry matter	61,37±0.32	60,60	61,71	0,46	0,74
Milk fat	26,89±0,26	26,43	27,50	0,39	1,46
Fat in dry matter	43,83±0,62	42,84	45,38	0,94	2,14
Proteins	26,53±0.93	25,26	28,33	1,23	4,63
Ash	9,25±0,06	9,14	9,33	0,07	0,80
Salt	5,21±0,05	5,08	5,28	0,08	1,46

The research specified the microbiological quality of "Bieno" cheese after 45 days of ripening in accordance with the special requirements of food safety regarding the microbiological criteria.

Table 4. Microbiological analisys of ''bieno'' cheese after 45 days of rippening

	Entereobacteriaceae cfu/ml	***E. coli*** cfu/ml	***Coagulase positive staphylococcus*** cfu/ml	***Listeria monocytogenes*** cfu/ml	***Salmonella* spp.** cfu/ml
I	680	430	92	/	/
II	2000	1200	102	/	/
III	20	10	124	/	/
IV	1600	720	36	/	/
V	1150	300	180	/	/

From the results of Table 4 can be concluded that it was not determined the presence of Listeria monocytogenes and Salmonella spp. The presence of Enterobacteriaceae set possibly due to the production of bieno cheese from raw milk. For cheeses manufactured from raw milk, according to the Regulation on microbiological quality, allowed the presence of coagulase positive staphylococcus.

In our study, these values are much lower in all variants in all iterations of the experiment; it can be connected with the fact that at the time of mating the cheese dough temperature of 70-75°C comes to the destruction of coagulase positive staphylococci as confirmed in tests of (Lima et al., 2008)and (Gomez-Lucia et al., 1990). E. coli whose limits are allowed (10 to 1200 cfu / ml) was determined in all samples in limits given refer to each tested unit - sample. According to Kakurinov (2002), following the dynamics of coliform bacteria during the whole technological process for hard cheese, 45th day of ripening in brine, found the presence of coliform bacteria in height 10^4 -10^6/ml. In tests of (Levkov and Kakurinov, 2007), 45th day of ripening of hard cheese in brine, the number of coliforms ranged from $1{,}0\times10^6$ to $2{,}8\times10^6$/ ml. The presence of Enterococci under [14] in their ripening, due to their tolerance to temperature of 10 to 45°C, pH value of 4.0 to 9.0 as a result of tolerance to certain concentrations of salt (6.5%).

CONCLUSION

Bieno cheese from the region Mariovo was indigenous product with specific and recognizable properties. The chemical composition and properties of whey depend primarily on the quality of milk and the technological process of producing cheese. Whey is an important by product in the manufacture of ''bieno'' cheese which can be used to obtain fresh albumin cheese.

"Bieno" cheese has a good chemical composition: moisture (38.63 %), dry matter was (61.37 %), milk fat (26.89 %), the content of fat in dry matter (43.83 %), protein (26.53 %), ash (9.25 %), salt (5.21 %). According to dry matter content

cheese belongs with hard cheeses, and according to their storing belongs to the sour brined cheese. Average yield of "Bieno" cheese was (9.36 %).

"Bieno" cheese is hard, low fat cheese with spongy appearance, which matures in brine, with a great diversity in production and non-standard quality. The data obtained may serve as the basis for creating standardized production procedures, leading to the uniform quality of these products. Therefore, traditional dairy should not be seen as a return to the past, but as an effort to preserve the indigenous technology, to gain their organized form, the ethnographic richness of a given region so distinctive, a time stamp to the development of a nation.

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REGRESSION ANALYSIS OF ROTATIONAL INTENSITY, CROP DIVERSITY INDEX, LAND UTILIZATION INDEX AND YIELD EFFICIENCY IN BIO-INTENSIVE AND CONVENTIONAL FARMING SYSTEMS IN NEPAL

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ABSTRACT

This study was conducted in Udayapur district, Nepal. A questionnaire-based survey with 100 household heads along with field visit and personal interaction with the concerned farmers were conducted to gather required information. Data were analyzed to compute rotational intensity (RI), crop diversity index (CDI), land utilization index (LUI), and yield efficiency (YE). Regression analyses were done to reveal relationships among these traits. Bio-intensive farming system (BIFS) farmers were found to practice scientific crop rotation substantially more than conventional farming system (CFS) farmers both in rice-based and maize-based cropping systems. In this study, higher RI, CDI, LUI and YE were found in sustainable bio-intensive farming system (BIFS) as compared to conventional farming system (CFS). The study has revealed strong positive relationship of RI with CDI and LUI in BIFS, CFS and in general. Cropping system both in BIFS and CFS with scientific crop rotation that ensures higher CDI and LUI is recommended for increasing yield efficiency. Direct positive relationship among RI, CDI and LUI has been revealed by this study as a rule; and has suggested to be used in validating yield efficiency of optional farming system as compared to the mainstream conventional farming system.

Key words: r*otational intensity, crop diversity index, land utilization index, yield efficiency, bio-intensive farming system.*

INTRODUCTION

Proponents of High Chemical Inputs Agriculture System (HCIAS) generally argue that developing countries should opt for an agro-industrial model that relies on standardized technologies and ever-increasing use of the chemical fertilizers and pesticides to provide additional food supplies for growing population. In contrast, a growing number of farmers, agro-ecologists, I/NGOs and analysts propose that instead of the capital and petro-chemical input-intensive as well as environment-degrading approach, developing countries should favour an agro-ecological and socio-economic model (Altieri, Rosset, & Thrupp, 1998; Rajbhandari, 2000). Many

of the techniques that comprise the bio-intensive method were present in the agriculture of the ancient Chinese, Greeks, Mayans, and of the Early Modern period in Europe (early 90s). Bio-intensive farming is a system that emphasizes biodiversity conservation, recycling of nutrients, synergy among crops, animals, soils, and other biological components, and regeneration and conservation of resources. In other words, the concept and approaches of BIF system is based on holistic system of sustainable management of natural resources in a given agro-ecosystem with specific cultural and knowledge base (Rajbhandari & Gautam, 1998). The bio-intensive method was further developed by Ecology Action (2001) into a sustainable 8-step food-raising method known as "GROW BIOINTENSIVE".

The principles of BIF system include scientific crop rotation; mixed farming systems; optimization of organic recycling; participatory and sustainable management of natural resources including biodiversity; participatory research and extension; and attainment of high degree of self-reliance of farm households against external techno-economic shocks (Rajbhandari, 2000). The bio-intensive approach is initially more labor-intensive than conventional approaches, and therefore best suites to small scale family centered food production in urban or rural settings. The bio-intensive farming system is a biologically intensive mixed farming system, which relies on intensive engagement of farmers, and organic recycling optimization through intensive scientific crop rotations. It relies on appropriate spatial management of field crops, vegetable crops, fruits and fodder trees as well as livestock and poultry for rational and ecologically non-destructive utilization of lands. Furthermore, it increases the soil fertility, revitalizes the degraded soil, decreases environmental pollution and prevents health hazards to humans and livestock as well as reduces further degradation of the environment, which otherwise might lead to desertification of the land. It is, therefore, not only eco-friendly but also friendly to human and animal health (Rajbhandari, 2010 b).

The technique behind adopting bio-intensive farming is that cropping systems and techniques specially tailored to the needs of specific agro-ecosystems are based on local inputs and techniques with each combination fitting to particular ecological resources by combining different components of the farming system (plants, animal, soil, water, climate and people) in order to optimize the synergistic interaction among the components (Rajbhandari, 2010 b). In this approach, performance criteria include not only increased production but also properties of sustainable food security, biological stability, resource conservation and equity (UNDP, 1995). However, there is not a single and simple method of validating efficiency of the alternate farming system like BIFS as compared to the mainstream conventional (petro-chemical based) farming system.

This study was conducted to estimate and analyze the relationships among rotational intensity, land utilization index, crop diversity index and yield efficiency, and find out the means of validating efficiency of bio-intensive farming as compared to the mainstream conventional farming system.

MATERIALS AND METHODS

Bio-intensive farming system initiative has been implemented in Udayapur, Nepal for the last 15 years; and therefore this district was selected as the study site. This study was conducted covering four Village Development Committee (area) s i.e. Rauta, Triveni, Hadiya and Jogidaha and one Triyuga municipality. Sample households were taken based on the farmer's engagement in bio-intensive farming system (BIFS) and conventional farming system (CFS). The total sample size was 100 households, i.e. 50 households from each system (10 households per VDC/municipality). Purposive random sampling technique was used to select the required number of households from both systems.

The study was based on the primary data collected from the household heads or senior members with the help of semi-structured questionnaire and published secondary information. No obstacles were faced while conducting the survey. The local farmers were quite supportive. The questionnaire was developed to gather relevant information required to meet the specific objectives. The questionnaire was first pre-tested with randomly selected ten farmers from the same communities for its accuracy and clarity. Some of these farmers were also included in the final round of interview. The questionnaire was finalized by incorporating farmers' suggestions. The collected data were grouped, coded and entered into the computer for processing. Computer software MS excel was used to analyze the data. Mean value, standard deviation (SD) and R^2 were obtained to interpret the results. Similarly information was also used to compute Rotational Intensity (RI), Land Utilization Index (LUI), Crop Diversity Index (CDI), and Yield Efficiency (YE). These were computed using the formulae given below. The number of crops grown in 5-year rotation, and respective areas and crop yields were taken into consideration. Regression plains were drawn and R^2 determined to estimate relationship between various parameters.

$$RI = \frac{\text{No. of crops grown in a rotation}}{\text{Duration of rotation}} \times 100$$

$$LUI = \sum_{i=1}^{n} ai\ di\ /A \times 365$$

Where, a_i=Area occupied by i^{th} crop

$_di$ =days occupied by i^{th} crop

A=Total cultivated area available for 365 days (1 year)

n=Total number of crops gown per year

$$CDI = 1 - \sum x^2 / (\sum x)^2$$

Where, x=Area under the individual crop

$$YE = Y_a / Y_l \times 100$$

Where, Ya= Yield per unit area of the farm

Y_l=Yield per unit area of locality

RESULTS AND DISCUSSION

Scientific crop rotation

Scientific crop rotation is one of the important agro-techniques employed in order to reduce the pest incidence in the field as well as sustain crop yield. It was found that 68 percent of the BIFS adopting households followed the scientific crop rotation but in the case of CFS adopting households the case was just opposite. Sixty four percent of the households employing CFS had not followed scientific crop rotation (Figure 1). Those who did not followed the crop rotation usually used to grow the same vegetable (in the rice-based and maize-based cropping systems) in the same season, which actually provided ground for pest incidence.

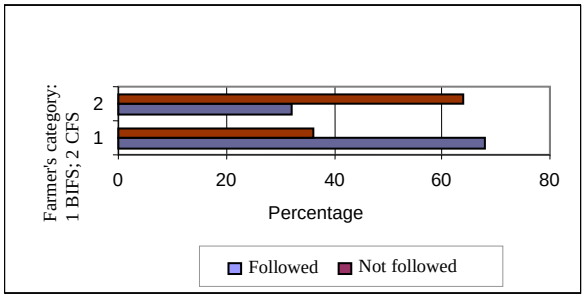

Figure 1: Practice of scientific crop rotation by the respondents (%)

Rotational intensity (RI), CDI, LUI and YE

Computed mean values and standard deviations of rotational intensity (RI), crop diversity index (CDI), land utilization index (LUI) and yield efficiency (YE) in bio-intensive farming system and conventional farming system are presented in Table 1.

Table 1. Mean values and SD of RI, CDI, LUI and YE in BIFS and CFS

Farming systems		RI, %	CDI	LUI	YE, %
BIFS	Mean	440	0.70	0.91	123.00
	SD	54.77	0.054	0.035	12.29
CFS	Mean	260	0.44	0.65	84.00
	SD	54.77	0.051	0.055	11.00

It is evident from the table 1 that RI was only 260 percent in CFS, where farmers used to grow only 2-3 crops in the annual pattern, while in case of BIFS it was 440 percent (increase by 180%). The BIFS adopting farmers used to grow 4 or more crops in the annual cropping pattern. Owing to higher RI the BIFS had higher CDI (0.70) than in CFS (0.44). Likewise, the BIFS had higher LUI (0.91) and YE (123%) as compared to the CFS (LUI = 0.65, YE = 84%). Rajbhandari (2010 a) and Duwal (2008) have shown that CDI and LUI have direct positive relationship with yield efficiency.

BIFS farmers had higher return due to the higher RI, CDI and LUI. This clearly showed the supremacy of bio-intensive farming system based on sustainable agro-ecological approach over petro-chemical based conventional farming system.

Regression analysis of RI, CDI and LUI

Regression plains computed among various pairs of quantitative traits have revealed strong positive relationship of RI with CDI and LUI and that of CDI with LUI in general (in both systems together) and in both systems- BIFS and CFS- separately (Fig. 2 to 10). The computed value of R^2 in all pairs of traits in both systems combined (general) and separately was statistically significant (P= 0.900).

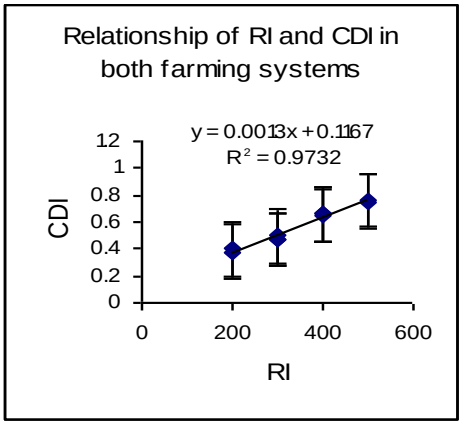

Figure 2: Relationship between RI & CDI in both farming system combined

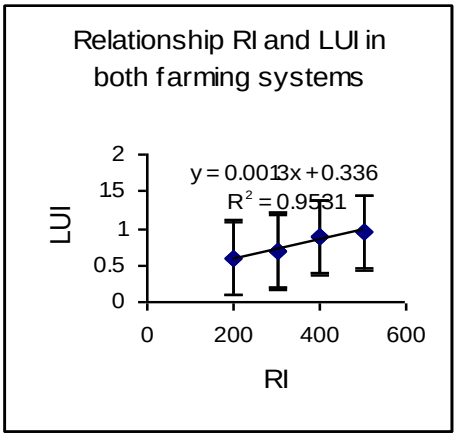

Figure 3: Relationship between RI & LUI both farming system combined

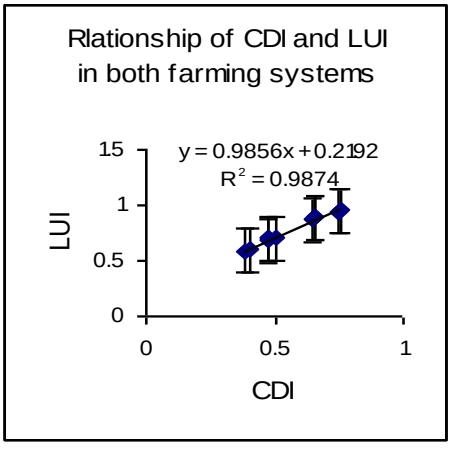

Figure 4. Relationship between LUI & CDI in both farming systems combined

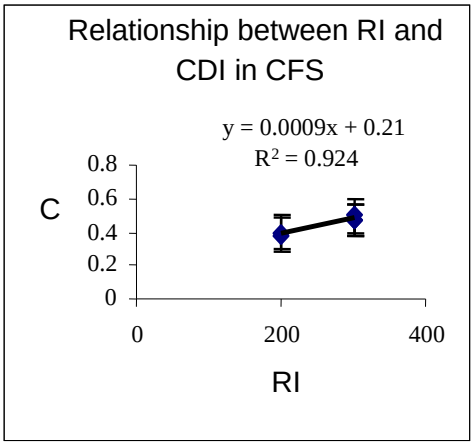

Figure 5**.** Relationship between RI and CDI in CFS in both farming systems combined

Similar trends in the relationship among CDI, LUI and YE were reported by Rajbhandari (2010a). Positive relationship between cropping intensity and CDI was reported by Shahidullah *et al* (2006). The findings of this study were at par to those described by Rajbhandari (2010 a), Duwal (2008) and Shahidullah et al (2006).

Regression analysis has also revealed strong negative relationship of crop rotation and LUI with severity (incidence) of pest damage as reported by Duwal (2008) and Rajbhandari (2010 a). Obviously, in the CFS where RI and LUI are lower, the pest damage to yield is substantial. This is an important reason that yield has been declining in the mono-cropping-based farms, where the use of only chemical fertilizers along with chemical pesticides are continued without following scientific crop rotation. The farmers adopting BIFS had reported lesser incidence of crop damage by pests, and they have higher cropping intensity, CDI and YE (Duwal, 2008).

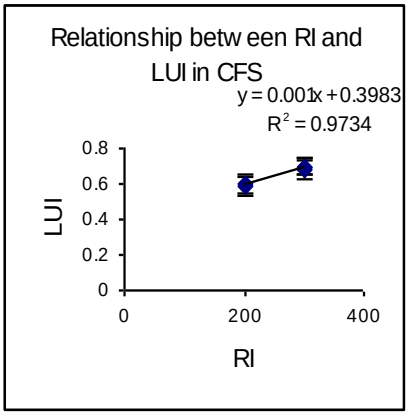

Figure 6. Relationship between RI & LUI in CFS

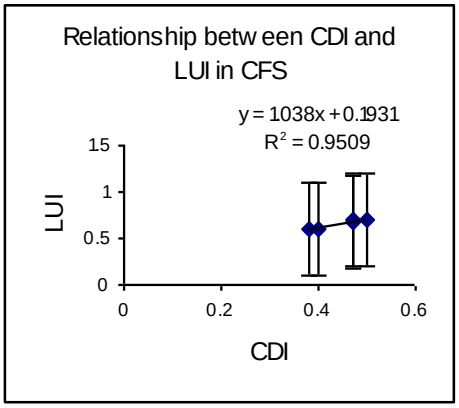

Figure 7. Relationship between CDI and LUI in CFS

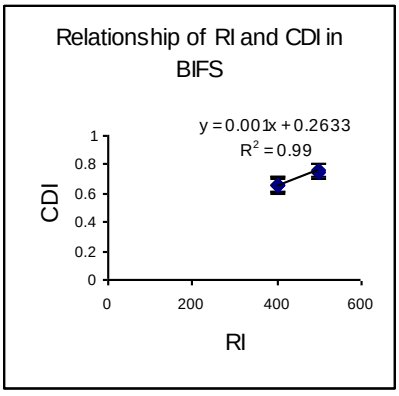

Figure 8**.** Relationship between RI & CDI in BIFS

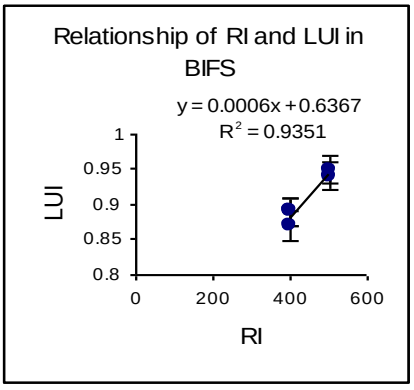

Figure 9. Relationship between RI and LUI in BIFS

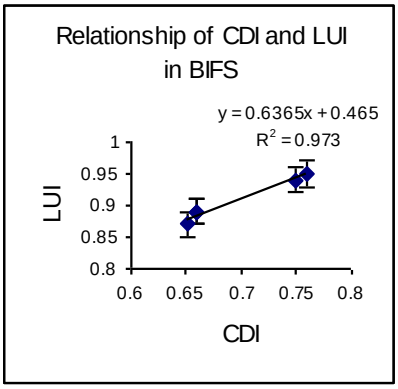

Figure 10. Relationship between CDI and LUI in BIFS

The RI, CDI and LUI had direct positive relationship in general (Figure 11) as well as in both farming systems separately (Figure 12 & 13). Thus direct positive relationship among RI, CDI and LUI has been revealed by this study as a rule that might be used in validating yield efficiency of optional agricultural system, e.g. BIFS as compared to the mainstream conventional agricultural system.

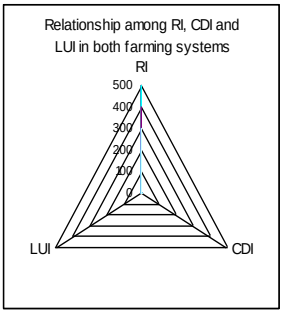

Figure 11. Relationships among RI, CDI & LUI in general (both farming systems combined)

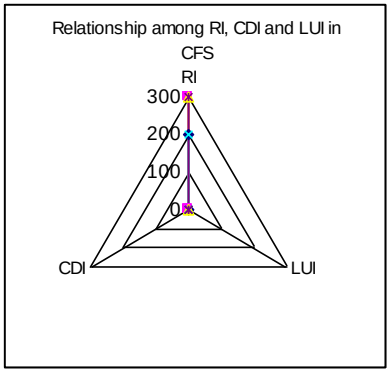

Figure 12. Relationship among RI, CDI & LUI in CFS

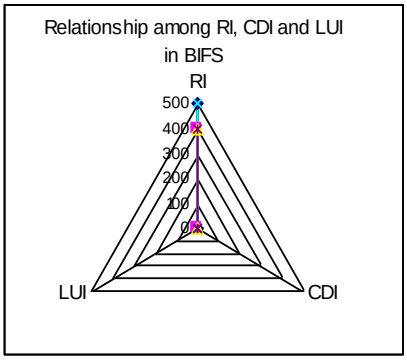

Figure 13. Relationship among RI, CDI & LUI in BIFS

CONCLUSION

It is obvious from the findings that scientific crop rotation increases crop diversity and land utilization, and consequently the total crop yield in a given locality both under bio-intensive and conventional farming systems. Bio-intensive farming may be one of the best options to govern this relationship positively in favour of ecology and environment protection as well as food production and human health.

Direct positive relationship among RI, CDI and LUI has been revealed by this study as a rule that might be used in validating yield efficiency of optional agricultural system as compared to the mainstream conventional agricultural system.

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ASSESSMENT OF EXTENSION AGENTS' KNOWLEDGE AND SKILLS REGARDING PEST MANAGEMENT IN KHYBER PAKHTUNKHWA PROVINCE -PAKISTAN

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ABSTRACT

The research study was initiated in Khyber Pakhtunkhwa province of Pakistan to investigate about the extension agents' knowledge and skills regarding pest management. Key objectives of the study were to know the present levels of their potentials and to judge their interested level of skills and potentials in pest management capabilities needed by Agriculture Extension Officers (AEOs) for their effective job performance. Respondents were asked to report the self perceived level of expertise possessed by them and the level required for smooth performance regarding pest management measures in the field to sustain agriculture. The present and interested levels of potentials were measured both on Likert scale ranging from 1 to 5 such as "very low" as "1" and "very high" as "5". There were significant differences observed in the agricultural officer's potential for sustaining agriculture regarding age, professional qualification and job experience. Age, professional qualification and job experience have significant association on technical efficiency regarding protection of plants from pests for sustainable agriculture. Moreover, required level in said capability was higher in identification of major field crops' diseases as well as the guidance of farmers for herbicides use against weeds. Trainings of AEOs were required in identification of diseases and insect pests of minor crops along with their causes. Hence it is recommended that AEOs may be trained and well prepared in the mentioned parameters of pest management measures to face challenges in the field of agriculture and can face farming community effectively to raise their living standards by sustaining agriculture for their coming generation.

Key Words: *assessment, extension agents, knowledge and skills and pest management.*

INTRODUCTION

Since old times, agriculture has been considered as the most momentous and capable economic activity in Indo-Pak Subcontinent. Agriculture is the substantial segment and dominant impulsive power for increase, progress and also the elementary cause of livelihood for the population of Pakistan (Ali *et al*., 2013).

Agriculture had been vital to fiscal expansion and progress in Pakistan. Agriculture is the most important fragment of the economy of Pakistan and has a share of 19.8% to Gross Domestic Product (GDP) along with provision of employment to 42.3%. Agriculture has an imperative part in achieving foodstuff sanctuary, increasing economic growth, plummeting paucity, and the development of cottage industries (GOP, 2016).

Now a days farming in Pakistan is confronting numerous issues that are directly connected with sustainable improvement in agriculture. The most indispensible among these are the expanded utilization of manures, weedicides and bug sprays and so forth to give a support to profitability of agriculture. The extreme utilization of these inputs has a decaying impact on the dirt and epitomizes a danger to the surrounding. The major challenge to the development of agriculture is to retain sustainable increase in production and also to avoid the degradation of the natural resources as well. The sustainable farming improvement needs practices and technological advances that are scientifically correct, monetarily reasonable, ecologically non-debasing and socially adequate for accomplishing nourishment security and enhanced personal satisfaction for present and future eras. Sustainable development is a continuous process that includes involving utilization of the natural resources base and orientation of technological and institutional changes in such a manner to ensure the attainment and continued satisfaction of human needs for present and future generations (GOP, 2016).

Agricultural Extension is a system of diffusing latest farming methods and ideas to farming community for bettering their conventional farming practices (Abbas *et al*., 2008). The extension institutions consider at utmost that the farmers have to improve their cropping system and their lands and also to motivate them for the judicial use of latest agricultural equipments and to adopt the latest technologies according to their climatic conditions (Safdar, 2005). Farmers have must be made conscious of the latest and updated farming practices prior to their utilization. The vital practical objective of successful extension worker is to teach the farmers how they could improve their land, setup a cropping plan and stimulate the farmers to develop latest farming tools based on recent research (Bajwa, 2004).

Agricultural yield may be rapidly improved by the use of suitable appliance of latest techniques in farming (Farooq *et al*., 2007). For this reason it is indispensible that the farmers have to be made aware of the logical information and enhanced practices and also the techniques regarding farming. This objective is achieved by the government through the dissemination of modern techniques in agriculture with the help of agricultural extension services (Ziaullah, 2005; Ali *et al*., 2008). AEOs' duty is to probe the problems of the farmers and then to forward their solutions to the farming community again. The Agricultural Extension Officer provides information to the farming community regarding various cropping practices, diseases and pest management with application of chemicals along with crops harvesting and threshing techniques as well as the marketing of the produce. Therefore, in this context, agricultural extension can be defined as the valuable mean and organization which enables famers to assist themselves. Farmers have

the opportunities to a wide range of educational programmes to provide and facilitate themselves for bettering farming system, growing techniques, improve yield and ultimately upgrade their livelihood (Safdar, 2005).

Humans and their environment is highly affected by the indiscriminate use of chemical pesticide resultantly crop reduction in crop production. Plant protectionist has developed another concept of Integrated Pest Management (IPM) for sustainable agriculture by developing pest disease resistant indigenous crop varieties. Pest damage can also be compensated by increasing the seed rate, sowing time adjustment to avoid pest damage with no cost and no harm to environment. To be sure, farmers have an unavoidable requirement for different sorts of information to carry out their agricultural activities in a viable way. The information regarding enhanced agro-innovations generated by agricultural researchers and scientists must be diffused in ways that are perfect with the necessities of ranchers and as a result brings satisfaction among the end user due to that information. This space in desired yields and genuine yields is credited to farmers' lack of knowledge about the use of modern and up dated research agricultural methods including pest management measures. The research study was initiated in one among four agriculturally rich provinces with the objective to examine existing and required levels of technical competency in pest management skills of AEOs. This study will help policy makers to devise policies to make agricultural extension service more effective and efficient which will further help to increase agriculture productivity without harming to human and environment and to help in poverty alleviation.

MATERIALS AND METHODS

The present research was carried in the province of Khyber Pakhtunkhwa from all the Agricultural Officers (n=111) who constituted the sample of the study. Primary data were obtained by carefully prepared questionnaire which was pre tested on 20 extension workers including deputy directors and directors that have served the post of AEOs and also on senior field assistant having more than one decade of experience in the field of agriculture extension before actual data collection so that irrelevant questions can be discarded. The research was supported by secondary data got through published and unpublished sources. Well-structured questionnaire was designed with the help of experts in the field of agriculture extension that consists of groups of questions regarding professional competencies (program planning, extension teaching methods, use of audio visual aids, participatory extension methodology, computer skills, supervision and administration and public relations etc.) and of the technical competencies like agronomic practices, pest management and protection measures, horticultural crops, farm mechanization, soil sciences and biotechnology. In addition to the above competencies, questions related to demographic characteristics and satisfaction regarding different facilities of AEOs were included in questionnaires that was based on the official job description of their duties. Questionnaires were sent by hard mail to AEOs and at district level their meetings were called by requesting the Director General of Agriculture Extension (Khyber Pakhtunkhwa) and explained to them any

ambiguity in the research instrument by clarifying their questions. Preferred potentials of AEOs identified were supported as per their job description of them. Following Ali (1991), Randavary and Vaughn (1991), Najingo *et al*. (1991) and Easter (1985) by using Likert scale to capture their potentials intensity. Available and interested potentials were scaled from one to five where one representing very low and five represented very high. Levels were: very low, low, average, high and very high. Potentials relating to pest management skills were assessed. Data were analyzed by SPSS and Microsoft Excel (version 2000). To find out relationship between demographic characteristics and the potentials of Agriculture Extension Officers in pest management skills were divided into different groups. In the present study, scaling index value lies between 0 and 1. The assigned values of scaling for possessed and required or wishing competencies ranged from 0.00 to 1.00. The scaled were assigned values and classified into five categories as expressed by (Qadeer, 1993) by stating very low was 0 - 0.20, 00.21 – 00.40 (low), 00.41 – 00.60 (medium), 00.61 – 00.80 (good) and 00.81 – 1.00 (very good). Descriptive statistics and mean ranks were calculated whereas to find the relation among various studied variables Pearson's correlation coefficient was calculated. The author faced no noticeable problems during the course of this research study.

RESULTS AND DISCUSSION

On-hand and Interested Level of Pest Management Skills:

The Agricultural Extension Officers rated the potentials they possessed in pest management and interested levels of these potentials to perform their job in pest management skills for agriculture sustainability. Their self perceived responses are depicted in Table 1. AEOs rated all fourteen potentials in pest management by wishing and showing high interested (means scores = 4.42–4.62) to perform their jobs effectively (Table 1). Among fourteen potentials, the top three were the job performance for Agriculture Officers as perceived by themselves were 1) identification of disease of major field crops (mean = 4.62, SD = 0.647), 2) farmers guidance in herbicides use for weeds (mean = 4.61, SD = 0.677) and 3) identification of insects/pests of major field crops (mean = 4.60, SD = 0.666). The potentials, which received very low rank on interested scale, included 1) advise the modes of loss of insects of minor field crops (mean = 4.42, SD = 0.695), 2) identifications of weeds of minor field crops (mean = 4.50, SD = 0.673) and 3) identification of disease of minor field crops (mean = 4.51, SD = 0.659).

Table 1. Mean SD and rank of Interested and Available potentials in Pest Management

S.No.	Potentials	Interested Levels			Available Levels		
	The talent to:	Mean	SD	Rank	Mean	SD	Rank
1	Identify disease of major field crops	4.62	0.647	1	3.59	0.948	8
2	Guide farmers for herbicides use against weeds	4.61	0.677	2	3.75	0.967	3
3	Identify the insect/pest of major field crops	4.60	0.666	3	3.64	0.989	5.5
4	Advise the modes of loss of insects of major field crops	4.57	0.683	4	3.61	0.992	7
5	Describe the control measures of insect/pest	4.56	0.710	5	3.70	1.005	4
6	Identify the weeds of major field crops	4.56	0.697	6	3.80	0.932	2
7	Describe the control measures of major field crops	4.55	0.723	7	3.64	0.989	5.5
8	Describe the control measures of minor field crops	4.55	0.629	8	3.57	0.959	10
9	Discuss the causes of disease of minor field crops	4.54	0.658	9	3.49	0.971	11.5
10	Identify the insect/pests of minor field crops	4.54	0.658	10	3.49	0.980	11.5
11	Discuss the causes of disease of major field crops	4.53	0.711	11	3.58	0.949	9
12	Identify disease of minor field crops	4.51	0.659	12	3.38	0.915	14
13	Identify the weeds of minor field crops	4.50	0.673	13	3.83	0.893	1
14	Advise the modes of loss of insect of minor field crops	4.42	0.695	14	3.39	0.926	13
		4.55			**3.60**		

* Source: Author s' elaboration based on the questionnaire survey results.

The self-perceived responses of Agriculture Extension Officers in the potentials they posses presently having mean score of 3.38 to 3.83 on a five-point scale (Likert Scale). The potentials, which were available in Agriculture Extension Officers at maximum were, 1) Identify the weeds of minor field crops (mean = 3.83, SD = 0.893), 2) Identify the weeds of major field crops (mean = 3.80, SD = 0.932) and 3) Guide farmers for herbicides use against weeds (mean 3.75, SD = 0.967). The three potentials, which received lowest mean rating on the scale, were; 1) Identify disease of minor field crops Identify disease of minor field crops (mean = 3.38, SD = 0.915), 2) Advise the modes of loss of insect of minor field crops (mean = 3.39, SD = 0.926) and 3) Discuss the causes of disease of minor field crops and Identify the insect/pests of minor field crops (mean = 3.49, SDs = 0.971 and 0.980). On the whole average of the means of the interested level was 4.55 as against 3.60 in available level (possessed level). The disparity values showing gap on the basis of differences of interested levels of AEOs to perform their pest management skills and the available levels of potentials were calculated. These differences were considered as training needs in the identified potentials. The data regarding these features are available in Table 2..

Table 2. Self-perceived desired level and present level by AEOs with training need and its rank regarding Pest Management

S.No.	Potential	Interested Level	Available Level	Training Need	
	The talent to:	Mean	Mean	Training = IL-AL	Rank
1	Identify disease of minor field crops	4.514	3.38	1.1351	1
2	Identify the insect/pests of minor field crops	4.541	3.49	1.0541	2
3	Discuss causes of disease of minor field crops	4.541	3.50	1.0450	3
4	Identify disease of major field crops	4.622	3.59	1.0360	4
5	Advise modes of loss of insect of minor crops	4.423	3.523	1.0360	5
6	Describe the control measures of minor crops	4.550	3.57	0.9820	6
7	Identify the weeds of minor field crops	4.505	3.52	0.9820	7
8	Discuss the causes of disease of major crops	4.532	3.58	0.9550	8
9	Identify the insect/pest of major field crops	4.595	3.64	0.9550	9
10	Advise modes of loss of insects of major crops	4.568	3.61	0.9550	10
11	Describe the control measures of major crops	4.550	3.64	0.9099	11
12	Guide farmers about use of herbicides for weed	4.613	4.91	0.8649	12
13	Describe the control measures of insect/pest	4.559	3.70	0.8559	13
14	Identify the weeds of major field crops	4.559	3.80	0.7568	14
		4.548	3.674	0.966	

Source: Author s' elaboration based on the questionnaire survey results.

The disparity values showing gap on the basis of differences of interested levels of AEOs to perform their pest management job efficiently and effectively as well as the available levels of potentials were calculated. These disparity values or gaps were considered as training needs in the identified mentioned potentials (Table 2). Among fourteen training needs of Agriculture Extension Officers, the most important top three were 1) identification of disease of minor field crops (Difference = 1.1351), 2) identification of insect/pests of minor field crops (Difference = 1.0541) and 3) Disease causes of minor field crops (Difference = 1.0450). The AEOs training requirements with lowest levels of interest included 1) identification of weeds of major field crops (Difference = 0.7568), 2) describe the control measures of insect/pests (Diff. = 0.8559) and 3) guidance of farmers in herbicides for weeds control (Difference = 0.8649). The disparity and gap based on the mean perception of AEOs for all potentials ranging from the very low level (0.7568) to top level value (1.1351). Agriculture is an industry under open sky and thus faces many environmental vagaries. Thus the plants are always prone to many disease, insects/pest and weeds. Therefore, proper identification of plant pathogens, insects and weeds are required to manage. The only option is the prevention which is possible if the Agriculture Extension Officers are fully aware from the latest information and trends in the pest management skills.

Judgment between possessed and attractive self perceived aptitudes in Pest Management:

Judgment between available and attractive self perceived aptitudes in Pest Management for Agricultural Sustainability assessed by them is presented in a web-radar diagram Fig.1 based on their mean values which is presented in Table 1. The change agents have to be skilled in every fragment of farming that can help the farmers to boost up their income. According to Khan *et al.* (2006) yield of the crops are reduced due to the continuous struggle of the weed with the principle crop for light, space, nutrient and most importantly water. By the utilization of IPM techniques these weeds can be evoked, so there is a need to educate the farmers regarding IPM techniques through competent AEOs in the field of pest management skills. Ahmad (1992) in his study regarding assessment of the competencies of extension agents reported that lack of training opportunities for the extension agents negatively affect their working efficiency.

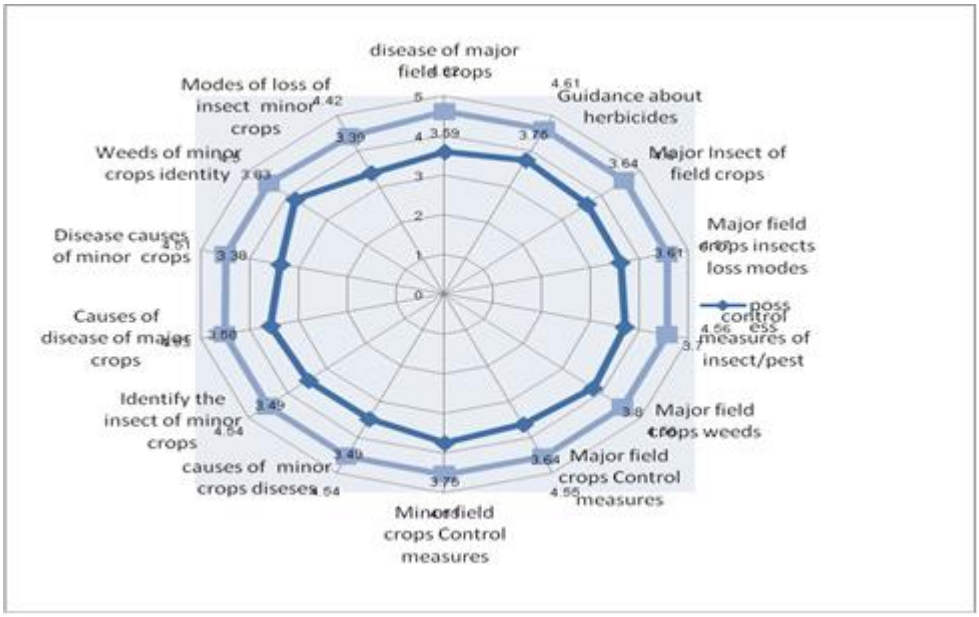

Figure 1. Judgment between available and required self perceived aptitudes in Pest Management

Issues coupled and associated with Pest Management skills for sustainable agriculture: Pest management capability is promoted significantly by job experience in the field of agriculture extension along with age and highly significant with higher professional and academic qualification of the Agricultural Extension Officers. Demography such as Agricultural extension Officers' specialty during student life as major in agriculture education, and domicile have negative relationship with pest management skills along with urban/rural background and personal experience in farming. (Table 3).

Table 3. Issues coupled and associated with Pest Management Ability

Issues (Independent)	Correlation Co- efficient Value	Significant level
Maturity level of AEOs	0.207*	0.030
Job knowledge	0.195*	0.041
Certified Qualifications	0.387**	0.000
Institution specialty during student life	-0.067	0.488
Background of Family	0.110	0.252
Rural/ Urban Living	-0.182	0.056
Length of Experience in Farming	-0.173	0.069
Availed opportunities of Training Program	-0.379**	0.000

*Source: Author s' elaboration based on the questionnaire survey results.

(We have recorded the response of respondents who have not attended the regular training with "1" and those who attended with "0". Therefore, correlation becomes negative.)

Age, job experience, professional qualification and availing the opportunities of training programs are considerably contributing to enhance the pest management competencies of AEOs rather than other demographic factors who are not significantly contributing to their competency. This shows that young AEOs of enthusiastic attitude in agricultural field having greater pest management capacity that increase with experience in the field with the passage of time. More exposure in the university education up to PhD level contributing a lot by reviewing more literature for their dissertation and formal sitting with university professors also give more exposure to him in the said competency. According to Darkenwald and Merriam (1982) statement the importance of staff progress to excite intelligence, to increase understanding, to keep eyes on new progress and development to increase organizational effectiveness could be availed through trainings**.** Department of Agricultural Extension facilitate farmers how to progress their productivity, profits and how to be cooperative with one another. Accordingly these programs are changing of laying down from scientific methods to spotlighting more on capacity building of the farmersfor community development in order to identify and take assistance from the current situation, together practical as well as financial. In order to play such a diversified role, the change agents are required to be trained in areas further than scientific farming to make ability in farmer's motivation (World Bank, 2008). Lodhi (2003) reported the lack of training facilities for the change agents of Punjab and also affirmed that department of extension is not up to mark and extension workers are not considered knowledgeable for their job performance.

CONCLUSIONS

• The study indicates that there are significant gaps between the level possessed and the level required in the potentials of pest management for smooth performance of their responsibilities.
• The professional qualification, job experience and professional trainings during service have improved the capabilities of AEOs.
• Demographic characteristics of AEOs like age and length of experience in job have significant effect on their competency in pest management.
• The Agricultural Extension Officers lack the mandatory information about pest management and mostly rely on outdated extension technologies.
• Most of the AEOs does not possess the expected technical competency level which seems supportive in raising the production of farmers.

RECOMMENDATIONS

Pre-service training for newly recruited AEOs must be arranged for efficient outcome of AEOs especially in pest management skills.
In-service trainings on regular basis ought to be provided to make them acquainted with updated technologies in field of agriculture with special emphasis on pest management skills.
Rural women can play their best role especially in storage of grains in their homes, so must be taken into consideration to train them best in pest management skills to save the stored grains at homes. Therefore, it is also recommended that rural women must be trained and to include this component in agriculture extension department.
It is also suggested that there is strong need to revamp training programmes as well as refresher courses for Agriculture Extension Officers in order to make them efficient, effective and competitive to meet the emerging challenges in crop protection measures and pest management skills as the problem with the insects/pests and diseases are changing with changes in the climates and their importance cannot be ignored in crop production.

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DETERMINATION OF *PINUS SYLVESTRIS* L. TRUNK DIAMETER BASED ON STUMP DIAMETER IN DIFFERENT BOREAL CONIFEROUS FOREST CONDITIONS OF PERMSKII KRAI (RUSSIA)

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ABSTRACT

Sometimes there are situations when it is necessary to determine the size of cut logs in the logging area in its absence. Mostly such situations arise in the detection of illegal logging. They can also occur in the case legal harvesting, when the tenant of a forest plot does not receive the scheduled volumes of timber for which he paid the money. In this case, the diameters of the felled trees are determined by the tree stumps. Recalculation of diameters is carried out on special tables developed in the early 20th century. Studies conducted in Krasnoyarsk, Khabarovsk, Bryansk (Russia), Gomel (Belarus), showed the need to refine the data of scaling tables for local conditions. Large discrepancies between actual and tabular trees appear with the increasing diameter of the tree. Studies of the relation of the formation of the butt log of pine (*Pinus sylvestris* L.) in the Perm region was carried out in 2015-2016. Forest plots were selected in different forest types of the middle taiga (Nirobskii forestry) and southern taiga (Perm urban forestry). Measurement of trees was carried out in pure pine stands at the age of 75-130 years. The stand density of the plantings was of 0.6-0.8. In each forest type the replication of studies was threefold. Studies have shown that trunks of pines formed a fuller bole in the Perm region, than the established scaling tables. For trees 40cm in diameter, recalculation leads to underestimation of the pine tree trunk diameter by 1-2 diameter class. That is understating the actual volume of felled tree by (16-20 %). There were no significant differences in the formation of the pine bole between the forest zones or by the corresponding types of forest. Also, there were no significant differences between forest types, which allows using a single conversion scale for the taiga part of the Perm Territory. Due to the fact that the relative completeness of the comparative stands were close, the influence of the distance between the trees on the development of the butt of pine trunks was not detected.

Key words: *illegal logging, diameter of pine stump, forest type, middle taiga, southern taiga, boreal coniferous forest.*

INTRODUCTION

Forests are about 45 % of the territory of Russian Federation. The logging and the wood processing are traditional field of activities in our country. The large forest territory and the lowering of forest control after late forestry reforms are causes of the illegal logging incident increase. The wood volume of illegal logging is 10-35 % of the harvesting volume in Russia. In some regions the illegal logging or the dubious wood origin are 50 % (Golovan 2015). According to unpublication date of Ministry of native recourses and forestry of Permskii krai the illegal logging is 8295 incidents from 2008 to 2015. The volume of this wood is 463000 $м^3$ and the loss is more 3 billions rubles. The clearing of crime of illegal logging is increase. If culprits of illegal logging were revealed 39 % of incidents in 2008, that this index was 75 % in 2015. But imperfect proof base lengthen court investigations. The investigation of illegal logging has four stages. The first is establishment of the placement and the measure of the logging plot. At this stage an expert uses geodetic tools. The second is the wood volume measurement by the stumps. An expert measures stump diameter and determines wood volume by specific tables (Vice 2011.). The third is the clarification of the logging time. Used dendrochronogical method enables to determine year of the logging. Also scientists look for another methods of the time identification. The fourth is the tool and mechanism recognizing by leave traces. The information about these researches are absent in publications. The object of research is a correlation between pine (*Pinus sylvestris* L.) trunk and butt diameters for the successive determination of wood volume. Used standard tables are not correct for different regions of Russia(Golovan 2015, Klennikova 2012 Vice 2011.). The big difference are displaying for mature and overmature forest.

MATERIAL AND METHODS

Researches were fulfilled in 2015-2016 in pine stands of Permskii krai (subzones middle and southern taiga) in Russian Federation. Trunk and butt diameters of pines were measured on plots with different forest type. They are lichen pine forest, cowberry pine forest, green-moss pine forest, sorrel pine forest, bilberry pine forest and polytric pine forest. The age of pine forests are about 90-130 years. Diameters were measured at heights 10, 20 and 130 cm above soil surface (Kishenkov 2009). The length to near-by trees was measured for the study of their influence. The stand density of the plantings was of 0.6-0.8. Researches pine-trees had diameters 20, 40 and 60 cm at height 1.3 m.

RESULTS AND DISCUSSION

Facts of table 1 show a small difference between forest types in taiga subzone and between subzones. But pines growing in optimal soil conditions have a less pronounced butts then pines growing in dry and wet soils. Pines growing in Middle taiga have a less pronounced butts then pines growing in similar conditions of Southern taiga. These inconspicuous differences enable to use one computational table for diameter trunk determination. Any way the real correlation between pine

trunk diameter and stump diameter is big then facts of standard table. The larger the pine trunk, the tighter the difference! Other authors write about this problem too (Kishenkov 2009).

Facts of table 2 show the divergence of damage account if we use different tables for pine diameter trunk determination. The expert measures same stumps and computes their diameters to trunk diameters using standard table. He gets low diameter class then it is real. The industrial wood yield declines. The cost of the felled tree declines about 33 %. The account of a cost of felled pine tree was done according to the rates of the charge for unit of wood and coefficients for a rates of the charge used in Russia [5, 6].

Table 1. Correlation $D_{1,3}/D_{0,2}$ for pines growing in different forest type of southern and middle taiga

Forest type	Diameters of pine trunk ($D_{1.3}$)		
	20 cm	40 cm	60 cm
Southern taiga			
Сосняк брусничный (Сбр) cowberry pine forest	0.847±0.010	0.868±0.012	0.928±0.008
Сосняк зеленомошный (Сзм) green-moss pine forest	0.882±0.006	0.939±0.009	0.956±0.004
Сосняк кисличный (Ск) sorrel pine forest	0.903±0.007	0.916±0.006	0.954±0.007
Сосняк черничный (Сч) bilberry pine forest	0.852±0.011	0.877±0.007	0.924±0.006
С долгомошный (Сдм) polytric pine forest	0.875±0.017	0.898±0.007	0.936±0.004
Middle taiga			
Сосняк лишайниковый(Слш) lichen pine forest	0.890±0.010	0.870±0.010	0.920±0.010
Сосняк брусничный(Сбр) cowberry pine forest	0.850±0.004	0.900±0,004	0.950±0.004
Сосняк черничный(Сч) bilberry pine forest	0.850±0.010	0.890±0.010	0.940±0.010
Сосняк долгомошный(Сдм) polytric pine forest	0.860±0.010	0.890±0.010	These trees are absent
Standard table for European part of Russia	**0.800**	**0.820**	**0.830**

Table 2. Difference of damage accounted by standard and new tables

Index	Own value			Standard table		
	Small stump	Middle stump	Large stump	Small stump	Middle stump	Large stump
Diameter of real stump ($D_{0.2}$), cm	24	44	63	24	44	63
Diameter of trunk after computation ($D_{1.3}$), cm	20	40	60	19,1	35,9	51,9
Diameter class, cm	20	40	60	20	36	52
Trunk volume, m^3	0.249	1.221	2.876	0.249	0.969	2.137
Volume of large timber, m^3	0	0.899	2.447	0	0.582	1.825
Volume of middle timber, m^3	0.154	0.158	0.032	0.154	0.248	0.038
Volume of small wood, m^3	0.057	0.020	0	0.057	0.022	0
Volume of fireplace wood, m^3	0.005	0.012	0.063	0.005	0.010	0.034
Cost of one felled pine tree for 2017 (27/06/2017), euro	0.43	3.39	8.22	0.43	2.55	6.16

The declining of wood volume is the problem of state forestry. The first the damage of the illegal logging is low then it is real. The second the wood volume examined by the expert on logging plot is lower then it is describe in the mensurational description for that plot. These situations took place when the leaseholder felled the bought forest, account the timber volume and sow its discrepancy to bought wood volume. According to expert facts the state forestry put up the overstated wood volume to the leaseholder.

CONCLUSIONS

The determining of wood volume by stumps needs the differentiated approach in each region of Russia.

There were no significant differences in the formation of the pine bole between the forest zones or by the corresponding types of forest in Perm region. Also, there were no significant differences between forest types, which allows using a single conversion scale for the taiga part of the Perm Territory.

The mistakes of trunk diameter determine is cause of the reducing of actual wood volume (at 1.15-1.26 once). The damage of illegal logging is reduced at 33 %.

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O koeffitsientakh k stavkam platy za edinitsu ob''ema lesnykh resursov i stavkam platy za edinitsu ploshchadi lesnogo uchastka, nakhodyashchegosya v federal'noi sobstvennosti (Postanovlenie Pravitel'stva RF ot 17 sentyabrya 2014 №947). About coefficients for a rates of the charge for unit of forest source volume and the rates of the charge for the unit of a forest plot square is in federal property. Decree of the Government of the Russian Federation No 947 of 17 September 2014

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EFFECTS OF TILLAGE SYSTEMS AND FERTILIZATION LEVEL ON THE WEEDINESS OF WINTER WHEAT

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ABSTRACT

This paper deals with results of the effects of three tillage systems and different nitrogen fertilizer level on floristic composition of weed community in winter wheat in investigated period on the chernozem-luvic soil type. The trial was carried out on the estate experimental field of the Faculty of Agriculture - Zemun "Radmilovac" near Belgrade in Serbia. Tillage systems and fertilization with nitrogen fertilizers have a big influence on weed control and floristic composition, number of species and individuals and biomass of weeds in winter wheat. The weed community in winter wheat crops was composed of 14 weed species in both year of investigation, respectively, with dominancy of terophytes. The obtained results showed that the dominant weed species were *Avena fatua* L., *Papaver rhoeas* L., *Sinapis arvensis* L. and *Stellaria media* (L.) Vill. from annuels and. *Convolvulus arvensis* L. from perennials prevailed in the weed community in winter wheat crops.Conventional tillage system with nitrogen level in top dressing 120 kg ha^{-1} had better effect in weed control than both of both investigated conservation tillage systems (MTS and NTS) had. However, mulch tillage has not lagged behind the conventional tillage increased total weed number of individuals annual and perennial species especially fresh biomass. No-tillage system makes weed control difficult.

Keywords: *tillage systems, nitrogen, weed community, winter wheat.*

INTRODUCTION

Conventional small grain seeding equipment has been designed to operate most efficiently in a tilled, firm, residue-free seedbed. Unfortunately, the tillage operations required to prepare the soil for conventional seeding are expensive, leave land susceptible to erosion and enhance seedbed moisture loss through evaporation. Recent farming technology has provided various combinations of

reduced tillage systems capable of cutting costs, conserving moisture and reducing erosion. Tillage systems are generally categorized in conventional tillage using a mouldboard plough, conservation tillage using chisel plow, disk plow, harrow disk or cultivators, and no-till systems using direct drilling in untilled soil. There is increasing worldwide interest in soil conservation systems due to their economic and environmental benefits. Economic benefits of no-till systems may arise from lower drought susceptibility due to higher plant-available soil water content, resulting in more stable yields and savings of labor and fuel. Ecological benefits include an increase of soil organic carbon, biotic activity, soil porosity, agro-ecological diversity, less soil erosion and lower carbon emissions (due to less fuel consumption) (Derpsch et al. 2010). However, because these systems retain large amounts of residue on the soil surface, they require different seeding equipment. The transition from conventional farming systems that use large quantities of mineral fertilizers and pesticides to sustainable systems like organic production for example leads through "low-input" technology (Barberi et al., 1997; Koocheki et al., 2009; Kovačević et al., 2010a; Dolijanovic et al., 2014). The floristic composition and structure of the weed community in winter wheat crops depends to a large extent on the way cultivation is done, where the tillage system and fertilization have a frequent influence. Such systems can only be achieved by a more flexible cultural practices in harmony with agroecological conditions and soil types in a particular region. Adaptation, means first of all adaptation to natural and also economic conditions. Reduction of production costs is one strategy for increasing farm profitability (Leibman et al., 2001; Kovacevic et al., 2010b).

The aim of the present study was to assess the influence of three soil tillage systems, and two level of nitrogen dressing on weed infestans in winter wheat under Pannonian climate conditions on a chernozem luvic soil type in central part of Serbia.

MATERIAL AND METHODS

Field experiments were conducted during 2012/13 and 2013/14 winter wheat growing seasons to examine the effect of three tillage systems and two level of nitrogen in top dressing (60 and 120 kg ha $^{-1}$) on weed floristic composition in winter wheat. The experiment was carried out at the Radmilovac experimental farm of the Faculty of Agriculture University of Belgrade, near Belgrade, R. Serbia (44°45'N, 20°35'E; 152 m a.s.l.).The field is located east of Belgrade, Serbia.

The following tillage systems (A) were included:

Conventional tillage system - (CTS) - moldboard ploughing to the depth of 25 cm + presowing preparation by disking and harrowing;

Mulch tillage (MTS)-chisel ploughing to the depth of 25 cm + presowing preparation by disking and harrowing and

No-tillage system - (NTS). The whole amounts of chopped residues where lefted on the ground after harvesting of maize on the MTS and NTS plots. Since more than 30 percent of land surface were covered with organic residues those tillage systems could be marked as conservation tillage practice.

The main plot treatment was split into two subplot treatments (B): control-without N fertiliser (N_0) and two level of nitrogen in top dressing (N1-60 kg ha^{-1} and N_2 - 120 kg ha^{-1} nitrogen). The nitrogen fertilizer (Urea, 46% N) was applied. Plots for each tillage system were arranged in a split plot design. Plots were divided into subplots and were subjected to tillage treatments with four replications.

The experiment was conducted in a 4-year rotation (maize-winter wheat-spring barley+red clover-red clover) where red clover acted as nitrogen source.

Wheat was sown in mid-October (between 12^{th} and 24^{th}) with 600 germinable seeds/m^2. Six Serbian winter wheat cultivar Pobeda were used. Weed control was generally performed with 2, 4 D herbicides (Monosan 1, 5 l ha^{-1}) in mid-April on all plots. Combine harvest was performed in July (between 5^{th} and 10^{th}).

Weediness in winter wheat was evaluated in both investigated years 2013 and 2014 at the end of second decade of May in cvs Pobeda one month from the application. The evaluation, which consisted of weed counting, was using the weight–counting method on the quadrats (l m^2 area) in each replication. At the end of second decade of May a destructive sample was taken in the quadrants and the weeds were identified, grouped into perennials and annual and counted. These groups were counted separately. Weeds were identified to species level and counted were taken and fresh biomass weights were recorded. After drying in the air, the dry biomass of weeds weights were recorded.

The data for number of weeds species, individuals and weed biomass in investigated tillage systems and top dressing treatments, as well as their interactions, were subjected to an analysis of variance test using the statistical software (STATISTICA 5.0 for Windows), while the least significant difference (LSD) test was used for individual comparison of differences between means.

Meteorological data (temperatures and precipitation) in Belgrade during the period of investigation

Figure 1 presents weather conditions in the experimental field during studies on weed infestation in winter wheat. Both investigation years were favourable for weed winter wheat. On the territory of Serbia, 2013, with a mean air temperature of 11.6ºC, it is the seventh hottest year in the period from 1951. According to the distribution of percentile, the amount of precipitation in 2013 was in the category of normal average for this continental climate. The dominant climatic feature of 2014 in the area of Belgrade is the phenomenon of extremely high precipitation. It was the worst year since 1888, and at the same time the second hottest (after 2000) the hottest year since systematic meteorological measurements. This meteorological conditions were with a more favorable for the growth and development of weeds in winter wheat are performed. The second year were extremely wet in a long period (from mid May to August) and affected to dominant weeds on the observed area.

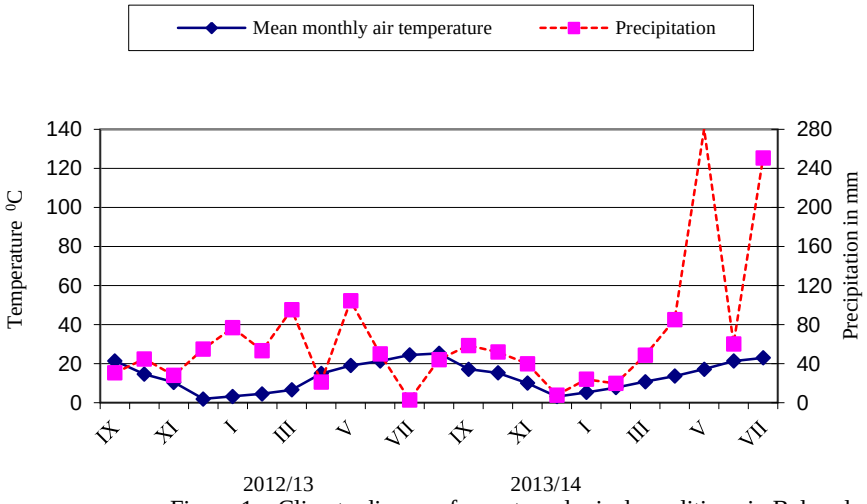

Figure 1. Climate diagram for meteorological conditions in Belgrade according Walter

RESULTS AND DISCUSSION

Tables 1 and 2 present effects of different tillage systems and top dressing on weed weediness in the winter wheat crop. The differences between the two years of investigation were, mainly a result of different weather conditions, especially precipitation sums, and distribution during the growing season of winter wheat. According to data presented in Tables 1 and 2, the weed community of winter wheat was composed of 14 weed species in both year of investigation. *Avena fatua* L., *Sinapis arvensis* L. and *Stellaria media* (L.) Vill. prevailed among annual weed species, while *Convolvulus arvensis* L. were dominant perennial weed species in first investigated year. In the second investigated year hot and wet conditions in second part of May were favourable, especially for *Papaver rhoeas* L., *Avena fatua* L., and *Stellaria media* (L.) Vill. from annuals and *Convolvulus arvensis* L.

Table 1. The effects of tillage systems on floristic composition weed sinuzia in winter wheat (2013)

No.	Weed species	(A) Tillage systems								
		CTS			MTS			NTS		
		(B) N level in dressing (N_0- control, without N; N_1 60 kg ha $^{-1}$; N_2 120 kg ha $^{-1}$)								
		N_0	N_1	N_2	N_0	N_1	N_2	N_0	N_1	N_2
1.	*Avena fatua* L.	15	7	10	11	6	10	4	-	-
2.	*Bilderdykia convolvulus* (L.) Dum.	-	-	-	1	2	-	1	-	-
3.	*Capsella bursa pasteris* L. Med.	-	-	-	-	-	-	-	-	3
4.	*Convolvulus arvensis* L.	-	1	-	2	5	-	3	2	-
5.	*Consolida regalis* Gray	-	-	-	1	2	1	-	2	1
6.	*Daucus carota* L.	-	-	-	-	-	-	-	1	-
7.	*Matricaria chamomilla* L.	-	-	-	-	2	-	-	-	-
8.	*Papaver rhoeas* L.	-	-	-	-	-	-	1	-	2
9.	*Polygonum aviculare* L.	-	-	-	2	1	-	1	-	1
10.	*Sinapis arvensis* L.	6	7			2	5	2		1
11.	*Stellaria media* (L.) Vill.	2	2	3	2			3	5	3
12.	*Sonchus oleraceus* L.	-	-	-	-	1	-	5	5	5
13.	*Stenactis annua* (L.) Ness.	-	1	-	-	1	2	5	1	2
14.	*Veronica persica* Poir.	1	1	-	1	1	2	-	1	-
Total number of weed species		4	6	2	7	10	5	9	7	8
Total number of individuals m^2		24	19	13	20	23	20	25	17	18
No. of individuals annual weeds		24	18	13	18	18	20	22	14	18
No. of individuals perennial weeds		-	1	-	2	5	-	3	3	-
Fresh Biomass g m $^{-2}$		72.2	129.8	61.2	140.4	47.4	31.6	149.0	53.2	80.1
Air-dry Biomass g m $^{-2}$		27.4	51.3	41.0	43.3	24.4	12.1	34.1	16.6	22.9

Table 2. The effects of tillage systems on floristic composition weed sinuzia in winter wheat (2014)

No.	Weed species	(A) Tillage systems								
		CTS			MTS			NTS		
		(B) N level in dressing (N_0 - control, without N; N_1 60 kg ha $^{-1}$; N_2 120 kg ha $^{-1}$)								
		N_0	N_1	N_2	N_0	N_1	N_2	N_0	N_1	N_2
1.	*Avena fatua* L.	1	4	2	-	-	-	4	6	7
2.	*Ambrosia artemisiifolia* L.	-	-	1	-	-	-	-	-	-
3.	*Bilderdykia convolvulus* (L.) Dum.	4	-	1	-	2	1	-	-	1
4.	*Chenopodium album* L.	-	-	-	-	-	-	-	1	-
5.	*Convolvulus arvensis* L.	1	-	1	-	1	-	7	6	2

6.	*Consolida regalis* Gray	-	-	-	-	-	-	4	2	2
7.	*Daucus carota* L.	-	1	-	-	-	-	-	-	-
8.	*Papaver rhoeas* L.	11	8	4	7	3	8	13	10	10
9.	*Polygonum aviculare* L.	-	-	1	-	-	-	-	-	-
10.	*Sinapis arvensis* L.	1	2	1	2	1	3	1	1	5
11.	*Stellaria media* (L.) Vill.	4	-	3	4	1	4	1	3	-
12.	*Sonchus oleraceus* L.	-	1	-	-	-	-	-	1	-
13.	*Stenactis annua* (L.) Ness.	-	-	-	3	1	-	-	-	-
14.	*Veronica persica* Poir.	1	1	-	-	1	1	-	-	2
Total number of weed species		7	6	8	4	7	5	6	8	7
Total number of individuals m^2		23	17	14	16	10	17	30	30	29
No. of individuals annual weeds		22	17	13	16	9	17	23	24	27
No. of individuals perennial weeds		1	-	1	-	1	-	7	6	2
Fresh Biomass g m^{-2}		31.2	67.0	70.1	52.5	119.0	146.0	157.2	86.2	162.8
Air-dry Biomass g m^{-2}		16.5	18.3	12.5	17.3	57.8	23,7	34.3	20.4	44.4

Agricultural practices change the population and composition of weeds and the soil seed bank in agro-ecosystems; although most weed management systems do not consider the impact on weed population dynamics (Davis et al., 2004). If we compared tillage systems annuel weeds dominated in total weed population accounted for 89,29% in 2013 and 91,52% of the total weed population in second year of investigation, respectively.

This could be associated with a lower tillage level applied for seed-bed preparation in these cropping systems. Greater prevalence of perennial weeds in minimum tillage has been reported in the literature (Dawit and David, 1997; Kovacevic et a., 2010b). (Barberi et al. (1998) reported that frequent seed-bed preparation and high level of tillage practices decreased perennial weeds.

The highest number of weed plants and weed plants per species, with a significant number of individuals annual and individuals perennial weed plants, was registered in no-tillage system in both years of investigation (Table 1, 2 and 3).

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Table 3. Statistical significance for investigated parameters – LSD 0.05 and 0.01 values

	2013			2014		
	A	B	AB	A	B	AB
Total number of weed species						
LSD 0.05	0.982	0.982	1.701	2.174	2.174	3.765
0.01	1.445	1.445	2.504	3.197	3.197	5.542
Total number of individuals m^2						
LSD 0.05	0.899	0.899	1.541	1.134	1.134	1.964
0.01	1.309	1.309	2.218	1.669	1.669	2.891
No. of annual weeds						
LSD 0.05	0.956	0.956	1.657	0.982	0.982	1.701
0.01	1.408	1.408	2.439	1.445	1.445	2.504
No. of perennial weeds						
LSD 0.05	0.471	0.471	0.817	0.444	0.444	0.770
0.01	0.694	0.694	1.203	0.654	0.654	1.134
Fresh Biomass g m^{-2}						
LSD 0.05	4.479	4.479	7.758	25.713	25.713	44.537
0.01	6.504	6.504	11.266	37.364	37.364	64.717
Air-dry Biomass g m^{-2}						
LSD 0.05	32.184	32.184	55.744	19.374	19.374	33.557
0.01	46.766	46.766	81.002	28.153	28.153	48.762

The increased number of weed plants per species per area units means enhanced competition for principal factors of the growth and development, hence the crop density is lower, and without the optimum density, there are no optimum yields.
The differences in weed fresh biomass among NT compared with CT and MT were statistically significant. In both years of investigation, the lowest values of weed fresh biomass per area unit were obtained in conventional tillage systems and top dressing with 120 kg ha^{-1}. Our study show that the greatest number of weed plants per species was detected in two conservation systems (MT and NT). Not only was a great number weed species, total weed individuals, detected in all investigated tillage systems especially in second year of investigated period, but also the significantly greatest fresh and air-dry weed biomass was recorded in control variant and dressing with 60 kg ha^{-1} nitrogen level conservation tillage systems in first investigated year MTS 282.3 g m^{-2} and NTS 406.2 g m^{-2} in second year of investigated period).

CONCLUSION

According to studies on the effect of three different tillage systems and top dressing with different level of nitrogen on the weed community in winter wheat conducted on leached chernozem in the experimental agricultural farm of Radmilovac (R. Serbia) during the two-year period, the following can be concluded:

The weed community in winter wheat crops was composed of 14 weed species in both year of investigation, respectively, with dominancy of terophytes.
The annual species *Avena fatua* L., *Papaver rhoeas* L., *Sinapis arvensis* L. and *Stellaria media* (L.) Vill. and. *Convolvulus arvensis* L. from perennials prevailed in the weed community in winter wheat crops.The conventional tillage soil treatment showed greater efficiency in suppression (number of species, individuals and weed fresh and air-dry masses) than two conservation systems (MT and NT).The conventional tillage system showed on chernozem luvic soil type all the advantages compared to both conservation tillage systems (MT and NT). No tillage system makes weed control difficult. However, this results shows that there are interesting interactions between soil tillage systems and top dressing. The statistically lowest values of the number of weed individuals and fresh biomass were recorded in conventional tillage systems with 120 kg ha^{-1} nitrogen in top dressing.

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EFFECTS OF DIFFERENT TYPES OF CYTOPLASM ON THE KERNEL ROW NUMBER OF MAIZE INBRED LINES

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ABSTRACT

The aim of the present study was to determine effects of both, different types of cytoplasm (*cms*-C, *cms*-S and fertile) and environmental factors on the kernel row number of 12 maize inbreds lines. The trial with inbred lines was set up in two locations (Zemun Polje-Selection field and Zemun Polje-Školsko dobro) in 2013 and 2014. Moreover, the three-replicate trials were set up according to the randomised complete block design within each type of cytoplasm. Each plot within the replicate consisted of four rows. Fertile versions of inbred lines were sown in two border rows and they were pollinators for their sterile counterparts. Statistic-biometric data processing was based on mean values per replicate and included the analysis of variance. According to this analysis, significant differences in the kernel row number were established among inbred lines in dependence on the type of cytoplasm, year and the location. The average kernel row number ranged from 10.3 (L_9) to 15.8 (L_5 and L_7). The variation of the kernel row number, related to the source of cytoplasm, was very significant. Differences ($Lsd_{0.01}$) in the kernel row number were not determined in inbred lines L_5, L_8, L_{10} and L_{12} in regard to the type of cytoplasm: *cms*-C, *cms*-S and fertile. The average kernel row number significantly ($P \leq 1\%$) varied in regard to the year of investigation. A higher average value (13.75) was established in 2014 than in 2013 (13.31). The kernel row number per year very significantly varied ($Lsd_{0.01}$) in all inbreds, but the differences were not significant in the inbreds L_2, L_3, L_8, L_9 and L_{12}. Gained results point out to effects of different types of cytoplasm on the kernel row number.

Key words*: cytoplasmic male sterility, inbred lines, kernel row number.*

INTRODUCTION

According to the distribution and the scope of production, maize is, next to wheat, the most essential field crop in our agricultural production. The development of new hybrids of high genetic potential is one of the most important factors of the

maize production increase. Many traits of maize plant and ear that are interesting and important for selection are quantitative in nature, meaning that they are determined by a great number of genes whose effects are modified by environmental conditions. In studying these traits it is essential to establish their genetic background and modes of their inheritance in order to successfully apply breeding methods. Due to its morphology, maize is a plant very suitable for the production of large quantities of hybrid seeds, because hybridisation is relatively easily achieved with sowing parental components in alternate rows and tasselling of male inflorescence on female plants immediately after their emergence. It such a way, only pollen of a male (not detasselled) parent is distributed in the field, hence the seed produced on female (detasselled) plants are hybrid seed. In order to utilise seed superiority within the aforementioned scope, it is necessary to accomplish the full hybridisation between parental components. Failure to achieve the complete hybridisation, the female component, which, as a rule has low yield, occurs in produced seeds and reduces the total yield per area unit, and, consequently results in incomplete utilisation of heterosis. The complete hybridisation may be achieved if detasselling in rows with female plants is done in due time (before pollen release). This operation needs engagement of a large number of workers in a relatively short period of time (10-30 days). Besides manpower, it is necessary to provide control and super control of the quality of performed work, which means engagement of a many qualified workers. Since the beginning of the hybrid maize seed production, mechanical topping of tassels has been imposing as the simplest solution to the problem of detasseling. Experiments with mechanical topping have been performed by numerous researches (Dungan and Wudworth, 1939; Bogeson, 1943; Kiesslbach, 1945; Bauman, 1959; Hunter et al., 1973 and many others), while results of their studies were summarised by Huey (1971) and Trifunović (1975). Huey (1971) has stated that mechanical toppers were not usable under poor weather conditions, and that they did not solve the problem of detasseling on tillers and underdeveloped plants, while leaf loss could not be reduced below 2-3 leaves even with the most attentive operation. The possibility of efficient detasseling problem solving in the hybrid maize seed production has occurred when cytoplasmic male sterility in maize was discovered. The use of male sterile versions of the female component thoroughly eliminates the need to perform detasseling, reduces the number of workers necessary for control, effectively improves production quality and significantly reduces costs and accompanying risks, and ultimately, in this way, the seed production becomes very attractive for producers. The first description of male sterility was provided by Rhoades (1931). Further researches showed that cytoplasmic factors were responsible for sterility. Cytoplasmic male sterile plants of the female component do not consume nutrients and energy to form and shed pollen, but to form the grain. Fertile pollen is a great recipient of mineral nitrogen, much more than any other part of the plant. It was estimated that sterile plants may save approximately 10-30 kg nitrogen ha^{-1}, which is, instead of being used to form pollen, directed into female reproductive organs, thus resulting in the grain yield increase.

Kaeser et al. (2003) consider cytoplasmic male sterility (*cms*) a trait interesting for the maize seed industry, because it leads to lower costs of the hybrid seed production by eliminating of the labour-intensive mechanical emasculation of parental lines. In recent years, many hybrid seeds based on male sterile inbred lines have been produced with major *cms* types, *cms*-C and *cms*-S (*cms*-T is susceptible to maize leaf pathogens). The main goal in the commercial maize production is the highest possible grain yield, along with other favourable agronomic traits. Increasingly strong competition in the market of maize seed requires studies on the effect of the type of cytoplasm and its interaction with a genotype on yield and some morphological traits for the purpose of the production.

MATERIAL AND METHODS

Material and methods in performing field trials

The total of 12 maize inbred lines were used to study the effect of the type of cytoplasm on the kernel row number. Inbred lines were classified into the following three groups: 1) inbreds with the *cms*-C type cytoplasm, 2) inbreds with the *cms*-s type cytoplasm and 3) inbreds with fertile cytoplasm. Seeds of these materials (inbred lines) for this study were produced in the technical isolation, so called manual pollination. Sowing of all trials was always performed on the optimum dates (the third decade of April) with the application of common cropping practices. Two comparative trials were set up under dry-farming conditions in two locations (Zemun Polje - Selection field and Zemun Polje - Školsko dobro) in 2013 and 2014. The tree-replication trials were set up according to the randomised block design. Each plot within the replication consisted of four rows. Fertile versions of inbred lines were sown in two border rows and these inbreds had a role of a pollinator for their sterile counterparts. Each row consisted of 12 hills with 4 seeds each. The within-row hill distance amounted to 40 cm, while the inter-row distance was 70 cm. The elementary plot size was 5.6m². Thinning to two plants per hill was done at the 5-leaf stage. In order to avoid the effect of border plants, only plants from 10 inner hills were used in the analysis of agronomic traits. The 12 observed inbred lines encompass the majority of maize germplasm that is used in the seed production of the Maize Research Institute. The comparison of their possible late break of stability in two observed *cms* types can point out to a more suitable *cms* type for the seed production under conditions of our country.A total number of both, lodged and broken plants, was great in all replications immediately prior to harvest. Broken plants were all those plants that were broken below the upper-ear bearing node, while lodged plants were those in which the angle between the stalk and the ground was less than 45º. Harvest was done at full maturity. Yields of fresh ears for each inbred per replication and each elementary plot were measured at harvest. An average sample of 20 ears was separately measured with the technical balance in the laboratory. After shelling of the average sample, cobs were weighed, while the moisture metre was used to establish grain moisture percentage for each replication.

Methods of experimental data processing

Statistical-biometrical data processing is based on means per replication. Differences among analysed maize inbred lines with various sources of cytoplasm (C, S and fertile), in two locations and during two years as well as their interactions were determined by the analysis of variance for the factorial trial set up according to the randomised block design, as wall as by the LSD test at the probability levels of 5% and 1% (Hadživuković 1991). In order to draw objective conclusions on effects of observed factors on tested traits of maze inbred lines and the possibility of applying parametric tests (ANOVA and LSD-test), homogeneity of variance was tested.

RESULTS AND DISCUSSION

The grain yield is an important and complex trait consisting of a greater number of components of quantitative nature with polygenic genetic base. The kernel row number is one of the yield components. It is a quantitative trait that varies under effects of genetic factors, environmental factors, to a lower extent, and their interaction. This trait is very important for maize grain yield. The average kernel row number of inbred lines ranged from 10.3 (L_9) to 15.8 (L_5 and L_7) (Table 1). In dependence on the type of cytoplasm, the highest average kernel row number (13.6) was established in inbreds with *cms*-S cytoplasm, while this number was somewhat lower in inbreds with fertile (13.5) and *cms*-C cytoplasm (13.4). Variation of average values of the kernel row number of all inbred lines was very significant ($P \leq 1\%$) in both years of investigations. The average kernel row number (13.31) was very significantly lower in 2013 than in 2014 (13.75). Furthermore, a very significantly greater kernel row number was obtained in the first location, Zemun Polje-Selection field (13.55) than in the second location, Zemun Polje-Školsko dobro (13.52), (Table 1). If obtained values are compared with ones achieved by Todorović (1996), it can be concluded that the gained values for the kernel row number were approximately equal ranging from 12.91 to 15.73. The kernel row number does not depend only on genetic background of the trait, but also on many environmental factors and cropping practices (sowing density, soil type, presence of diseases and pest, meteorological conditions...).According to two-year studies carried out by Todorović (1995), the average values for the kernel row number were greater in observed hybrids than in observed inbreds, ranging from 11.84 to 17.88 vs. 13.26 to 19.12.Average values of the kernel row number were significantly higher in hybrid combinations than in parental inbred lines in the study performed by Grčić (2016), which points out that heterosis for this trait was very pronounced.According to everything stated it can be concluded that the kernel row number is a very important trait that affects yield and varies under genetic factors, environmental factors, to a smaller extent, and under effects of their interaction.

Table 1. Average values for the kernel row number over inbred lines, years, type of cytoplasm and locations

Year (Y)	Location (L)	Cytoplasm (C)	Inbred lines (I) 1	2	3	4	5	6	7	8	9	10	11	12	LSD test 5%	1%
Y_1	L_1	C_1	11.6	12.1	14.6	14.6	14.2	13.8	15.0	12.7	10.5	11.8	11.3	11.4	1.284	1.745
		C_2	12.8	11.5	15.8	15.2	16.9	16.0	16.0	15.3	10.6	12.9	12.1	9.9		
		C_3	13.1	12.0	15.6	15.0	16.9	17.1	16.4	17.3	10.6	9.2	11.4	11.3		
	L_2	C_1	11.8	12.5	13.3	13.6	16.3	14.5	15.3	16.3	10.3	12.0	12.1	11.1		
		C_2	12.3	11.9	13.4	13.6	16.6	14.7	15.5	15.3	10.4	12.9	10.5	9.9		
		C_3	11.6	12.3	13.5	13.7	15.8	14.2	15.1	17.2	10.5	10.5	12.7	1.8		
Y_2	L_1	C_1	14.1	11.7	14.5	15.5	14.9	15.6	16.3	14.7	10.0	13.7	12.8	11.7		
		C_2	13.8	11.6	13.8	15.3	17.1	15.6	16.8	15.9	10.0	13.4	12.5	11.0		
		C_3	13.7	11.4	14.0	15.4	14.0	14.3	16.1	13.9	10.0	12.2	12.7	11.0		
	L_2	C_1	13.3	11.9	14.1	15.6	15.3	17.5	15.5	15.7	10.0	13.4	12.9	11.6		
		C_2	13.7	10.9	14.0	14.6	16.4	15.7	16.1	15.7	10.0	14.2	12.7	11.2		
		C_3	13.4	11.6	15.1	15.1	15.0	17.5	16.2	15.8	10.5	12.2	13.2	11.7		
Average for inbreds			12.9	11.8	14.3	14.8	15.8	15.5	15.8	15.5	10.3	12.4	12.2	11.0	0.35	0.46
Average for cytoplasm			C_1	13.4			C_2	13.6			C_3	13.5				

				F test
Average for years	Y_1	13.31	Y_2	13.75**	** P≤1%
Average for locations	L_1	13.55	L_2	13.52	

C_1 -cms-C cytoplasm
C_2 -cms-S cytoplasm
C_3 -fertile (N) cytoplasm
* ≤ 0.05
**≤ 0.01

CONCLUSION

Based on two-year studies of maize inbred lines with different types of cytoplasm the following conclusions may be drawn:

An inbred line, type of cytoplasm, year and the location significantly affect the kernel row number.

The highest kernel row number (15.8) was recorded in the inbred lines L_5 and L_7.

The lowest kernel row number (10.3) was recorded in the inbred line L_9.

The highest (13.6), i.e. lowest (13.4) average kernel row number was detected in inbreds with *cms*-S cytoplasm, i.e. C type cytoplasm, respectively.

The average kernel row number was significantly lower in the first year (13.31) than in the second year (13.75).

The average kernel row number was higher in the first location (13.55), than in the second location (13.52).

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AUTOMATED MAPPING WITH LiDAR AND SPECTRAL CHARACTERIZATION IN MEDITERRANEAN FOREST AGROECOSYSTEMS

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ABSTRACT

Mapping with LiDAR data is not a standardized practice, though LiDAR databases are increasing in all countries in Europe. We develop and test a simple method for automated land-cover mapping. The study area was a farm located at a natural park of southern Spain. It comprises 502 ha covered by Mediterranean forest agroecosystems, like dehesa (a very open woodland of scattered evergreen trees used by grazing animals), woodland and scrubland, and transitions among them, composing a heterogeneous landscape. This heterogeneity is caused by variations in holm and cork oak tree density and a sclerophyllous shrub cover, i.e., 3D structure of woody vegetation. Using aerial photographs digitization, Landsat image classification, and image segmentation of tree crowns, land-cover maps were generated. Besides, other maps were produced from LiDAR-derived canopy cover and height of tree vegetation and shrub stratum. These 3D variables allowed to a wall-to-wall characterization of woody vegetation land-cover classes in the study area, that was completed with a NDVI assessment. The results show that automated mapping with LiDAR is reliable and accurate enough in comparison with other mapping techniques. It outperforms them because its higher spatial resolution, and can be combined with other remote sensing methods to provides an improved understanding of forest landscapes.

Keywords: *canopy, Dehesa, forest structure, GIS, LiDAR, vegetation structure.*

INTRODUCTION

Remote sensing and Gegographic Information Systems (GIS) are used for land-cover mapping, characterization and monitoring land-cover changes at a local, regional and global scale (Rogan & Chen 2004; Giri 2012). Point cloud data from active sensors like LiDAR provide a 3-dimensional (3D) information of features of land-cover classes and, specifically, 3D structure of woody vegetation (e.g. Parent et al. 2015). Land-cover mapping at high or moderate spatial resolution is a

challenge in complex Mediterranean landscapes with mosaic vegetation and high spatial variability of tree and shrub cover. In addition, recent studies combine LIDAR with satellite images, demonstrating its capacity to characterize the Mediterranean vegetation (Maselli et al. 2017; Gouveia et al. 2017). Land-cover mapping with low-density LiDAR point clouds in Mediterranean agroecosystems is not tested yet.

The goal of this research is to develop and to test an automated simple method for land-cover mapping from airborne LiDAR data that can be applied to a heterogeneous landscape. The specific objectives are: (1) to identify and to map the woody vegetation land-cover classes with LiDAR data in a relatively large area, and (2) to characterize the woody vegetation of land-cover classes and their internal spatial structure from 3D variables extracted from LiDAR and multi-spectral satellite data.

MATERIALS AND METHODS

The study area is the farm Zahurdillas, which is part of the Sierra de Hornachuelos Natural Park located in Sierra Morena mountain range (Southern Spain). It comprises 502 ha covered by different Mediterranean forest agroecosystems. Dehesa is the Spanish name of a common type of vegetation similar to savanna. It is a very open woodland of scattered evergreen trees –mainly holm and cork oak– where grasses, tree and shrub biomass, and acorns are used by livestock, sheep and pigs. Besides the dehesa, other woody vegetation were present, such as woodland and scrubland, as well as transitions between them, composing a heterogeneous land-cover landscape. The study area has been divided into a grid of 30x30 m cells. The coordinate system used for the study area was ETRS89 and UTM projection, zone 30.

Two conventional and two more modern mapping methods have been used. High-resolution aerial images (50 cm pixel size) of June 2014, provided by the National Geographic Institute of Spain (IGN), have been used for an accurate photointerpretation and manual digitization This method allowed the identification of six land-cover classes (digitized map). The criteria for the delimitation of these classes with woody vegetation were the tree density, tree cover, and shrub abundance. Landsat 8 satellite images (30 m pixel size and 7 spectral bands) of July 2016, downloaded from the Earth Explorer website (USGSG 2017) were used to generate a map of land-cover classes by the supervised classification method. These images were also used to generate a NDVI (Normalized Difference Vegetation Index) map, which is related to chlorophyll concentration and vigor of vegetation (Glenn et al. 2008).

Segmentation of tree crowns from aerial images was performed using a multiresolution algorithm, and the nearest neighbor algorithm for classification (Aldrich 1997), both implemented on the eCognition software (Trimble 2017). The multiresolution segmentation algorithm is a bottom up segmentation which departs from one pixel and merges neighbor pixels according to a heterogeneity and size parameters (Hamilton et al. 2007). A relatively simple segmentation was carried

out. Compactness and shape parameters were set at default values and scale parameter was defined at 50. The three bands of the image were used as input data for segmentation, assigning the green band a double weight compared to the red and blue bands. Classification was performed using two main classes (vegetation and non-vegetation) to identify objects that were likely to represent tree crowns. Several objects were manually selected for each class to be used as training data for classification. Tree crown area within each cell estimated by segmentation was used to calculate the relative total tree canopy cover ($RTCC_S$) and delimitate land-cover classes.

Airborne LiDAR data was acquired from the IGN, between December 2014 and January 2015. The density of the point cloud was 0.5 points/m^2. Data was reclassified automatically. A Digital Terrain Model (MDT, 1x1 m), based on LiDAR points classified as soil was obtained using the IDW method (interpolation by weighted inverse distance; Watson et al. 1985) to calculate the normalized height of the LiDAR point cloud. In each cell, four LiDAR-derived attributes were estimated: tree canopy height (TCH) defined by the 85^{th} percentile of height, establishing a threshold of 3 m (Gopalakrishnan et al. 2015), after calibration with an empirical model; shrub canopy height (SCH): 95th percentile height of shrub stratum (points below 3 m); tree canopy cover index (TCCI), and shrub canopy cover index (SCCI). These last two indexes have been estimated as the number of high vegetation points and the number of vegetation points below 3 m, respectively, expressing their units in points/ha.

ArcGIS 10.4 software has been used for the spatial data analysis. An additional tree crown segmentation has been performed by the aggregation of LiDAR points of high vegetation in polygons with a smoothed geometry using GIS tools. Relative tree canopy cover derived from this segmentation ($RTCC_L$) was used to evaluate the TCCI significance. From the combination of the TCCI and SCCI maps using GIS tools, an automated map of woody vegetation land-cover classes was produced. The characterization of each vegetation class has been done by limiting the descriptive statistical analysis to those cells which were completely within each vegetation class, avoiding the other cells which contained different vegetation classes. Coefficient of variation (CV) was used as a measure of total spatial variability of a variable within a land-cover class. The automated map was compared to the digitized map, without assuming its inherent quality. For those cells that belonged to two or more land-cover classes derived from the manual digitization, the class which occupied a larger area within the cell was assigned. The agreement between automated and digitized (reference) maps was evaluated using the confusion matrix method and Kappa index, which is a measure of agreement for multinomial data commonly used for thematic mapping accuracy assessment (Rossiter 2014). We have analysed both global accuracy and land-cover class accuracy. R software was used for these data analyses.

RESULTS AND DISCUSSION

Maps derived from digitization and image analysis

Figure 1a-c shows three maps. The first map represents a conventional map based on digitization of aerial photography (Figure 1a). From this map, four main land-cover classes with woody vegetation and different tree density and shrub cover were identified and mapped: Woodland (W); Woodland/Scrubland Complex (W/SC); Dehesa with a sparse shrub cover (D-ss), and Dehesa with a dense shrub cover (D-ds). The relative area of these land-cover classes in the farm was: 42% (W), 4% (W/SC), 48% (D-ss) and 5% (D-ds). Other minor land-cover classes were also mapped but they do not include woody vegetation. The second map was derived from supervised classification of Landsat images (Figure 1b). This map only discriminated approximately between D-ss (48% of total area) and the rest (W, W/SC and D-ds; 52%). The product of segmentation of tree crowns from aerial photography (Figure 1c) produced similar land-cover units: D-ss (56%, 10%<$RTCC_s$<70%); W, W/SC and D-ds (43%; woody canopy cover >70%); besides, Grassland unit (1%) was identified ($RTCC_s$ <10%). Though $RTCC_s$ was estimated and used to delimitate land-cover classes, there was an overestimation of this variable, due to the limitations derived from the segmentation, but also mapping the D-ss and the rest (W, W/SC and D-ds) was done approximately.

Tree canopy cover and height

LiDAR-derived TCCI was useful to discriminate vegetation classes (Figure 1d). There was a strong nonlinear relationship between TCCI and a LiDAR estimation of tree canopy area, $RTCC_L$ (N=4,216; R^2_{aj}=0.86; p<0.001). TCCI was above 2,000 in most parts of W and W/SC, and below 2,000 in most parts of D-ss and D-ds. LiDAR allowed to tree canopy height mapping (Figures 1h and 2a). TCH was 6-8 m in 92% of W area, but was more concentrated, 7-8 m, in W/SC, where represented 73% of area. Average TCH was 7.1 m in W and 7.3 m in W/SC. TCH was lower in dehesa: 6-7 m in most area of dehesa (76% in D-ss and 67% in D-ds). Average TCH was 6.7 in D-ss and 6.5 m in D-ds. The CV in TCH in the four woody vegetation classes was 6-8%.

Spatial internal structure of land-cover classes

Figure 2b-c shows the spatial variability within each woody vegetation land-cover class considering two different LiDAR-derived attributes, SCCI and SCH. Shrub canopy cover was clearly mapped (Figure 1e). W and D-ss showed strong skewness of SCCI values (Figure 2c). The distribution of SCCI was right-tailed in both vegetation classes, which indicates a spatial uniformity with predominance of low and very low shrub cover classes in 75-80% of the total area, being average shrub cover slightly less in D-ss than in W. Oppositely, W/SC and D-ds showed a more symmetric distribution of SCCI values (Figure 2c). SCCI was medium in about a third of total area of both woody vegetation classes, and almost 40% of total area showed higher SCCI in D-ds. Shrub cover map clearly showed spatial variability and differences among woody vegetation classes (Figure 2e). Their

internal variability could be partly the effect of a misidentification of training objects within the vegetation classes (Martin et al. 2001). For example, some isolated areas with SCCI over 400 in northern and southern parts of W area should have been classified as W/SC. W and D-ss showed a left-tailed skewness of SCH values (Figure 2b) with a relatively large variation range in canopy height classes in most part of the total area within these vegetation classes. SCH was between 1.5 and 2.5 m, but SCH in 35% of the total area was below 1.5 m in D-ss, where variability in SCH was greater than in W. Conversely, W/SC and D-ds showed a more concentrated distribution of SCH values (Figure 2b). SCH was between 1.5 and 2.5 m in 99% of total area of these woody vegetation classes, though higher SCH classes were more relevant in D-ds.

Spectral characterization of land-cover classes

NDVI variability was clearly related with the relevance of leaf biomass in woody vegetation (Figure 1g). The higher the TCH, the greater the values of NDVI ($N=4,245$; $R^2_{aj}=0.28$; $p<0.001$). Landsat images dates from June, meaning that during that month, almost all the natural grass vegetation in southern Spain is dry, losing all its chlorophyll (Figure 1c). Average NDVI in the small grassland patches in the farm was the lowest, 0.20. Average NDVI in both types of dehesa were 0.28 and 0.29, reflecting probably both, a large area of dry grass stratum and a low density of tree and shrub leaf biomass, being its overall spatial variability relatively low (CV<10%). NDVI values were below 0.3 in most of the area of dehesa (81% of D-ss and 67% of D-ds); this last class showed NDVI between 0.3 and 0.4 in 34% of total area, probably due to the contribution of shrub leaf biomass. Average NDVI in W and W/SC was 20-25% higher than in dehesa classes, which could be attributed to a greater density of leaf biomass of trees and trees with shrubs, respectively. NDVI range of 0.3-0.4 was observed in 98% of total area of W/SC, while the same range was limited to 75% of W area, which interestingly showed 10% of area in the top values recorded in the farm, range 0.4-0.5. The overall internal variability of W and W/SC classes was also low, with CV of 12% and 6%, respectively. Spectral discrimination through NDVI was useful but not enough to differentiate between dehesa classes nor between W and W/SC. NDVI allows to differentiate among ecosystems (Pettorelli et al. 2005), but not among woodlands with similar species composition as occur in the study area.

Automated map generation and accuracy

Combining two LiDAR-derived indexes related to tree cover (TCCI) and shrub cover (SCCI), allowed to generate and automated land-cover map of woody vegetation (Figure 1f). This map represented a similar image of the land-cover classes area and distribution identified by digitization. In fact, its accuracy can be considered higher than other maps because it was based in high spatial resolution measures of 3D the vegetation structure. The comparison of digitized and automated maps of land-cover classes revealed a reasonable accuracy of the automated mapping method based just in LiDAR point-clouds.

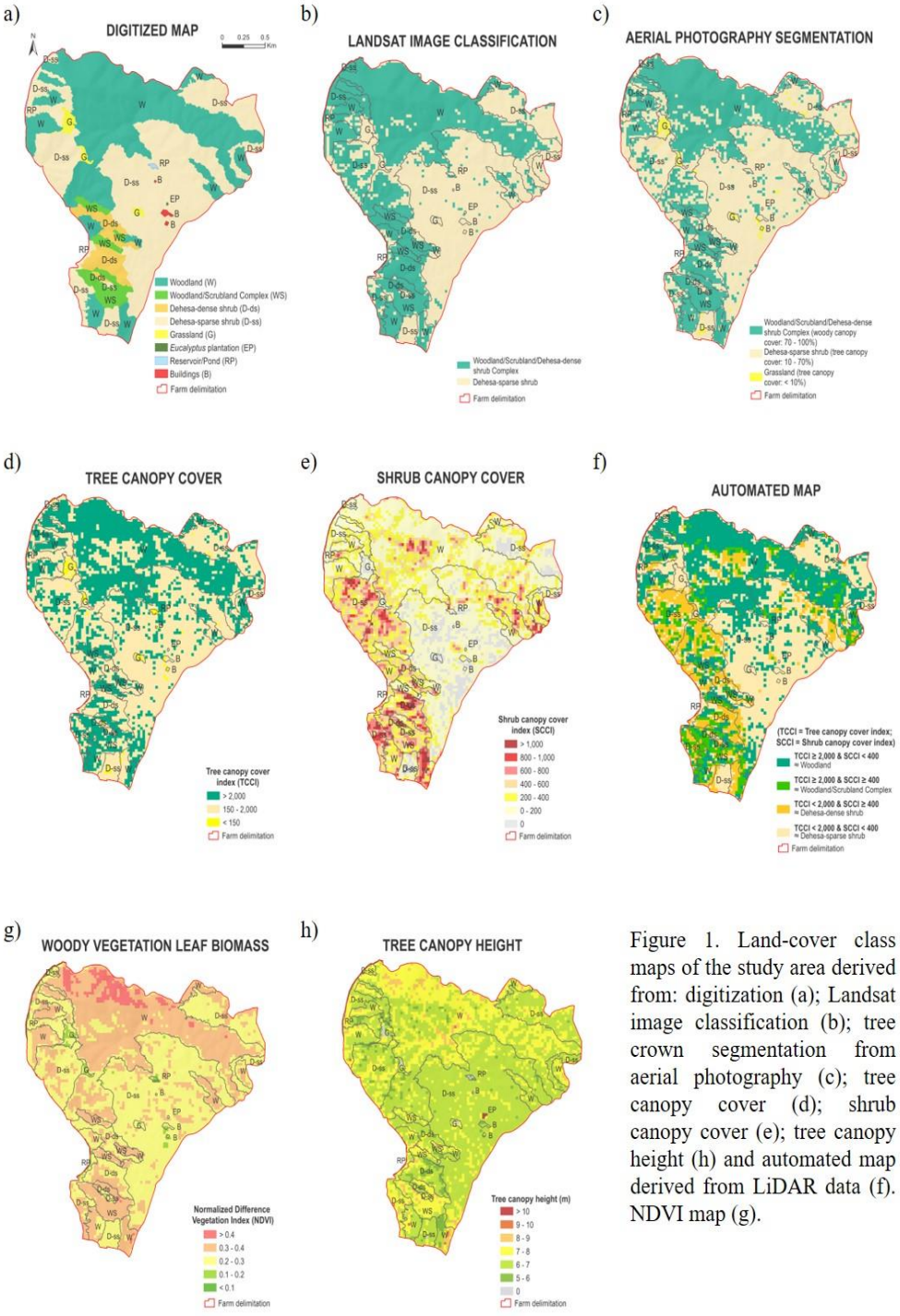

Figure 1. Land-cover class maps of the study area derived from: digitization (a); Landsat image classification (b); tree crown segmentation from aerial photography (c); tree canopy cover (d); shrub canopy cover (e); tree canopy height (h) and automated map derived from LiDAR data (f). NDVI map (g).

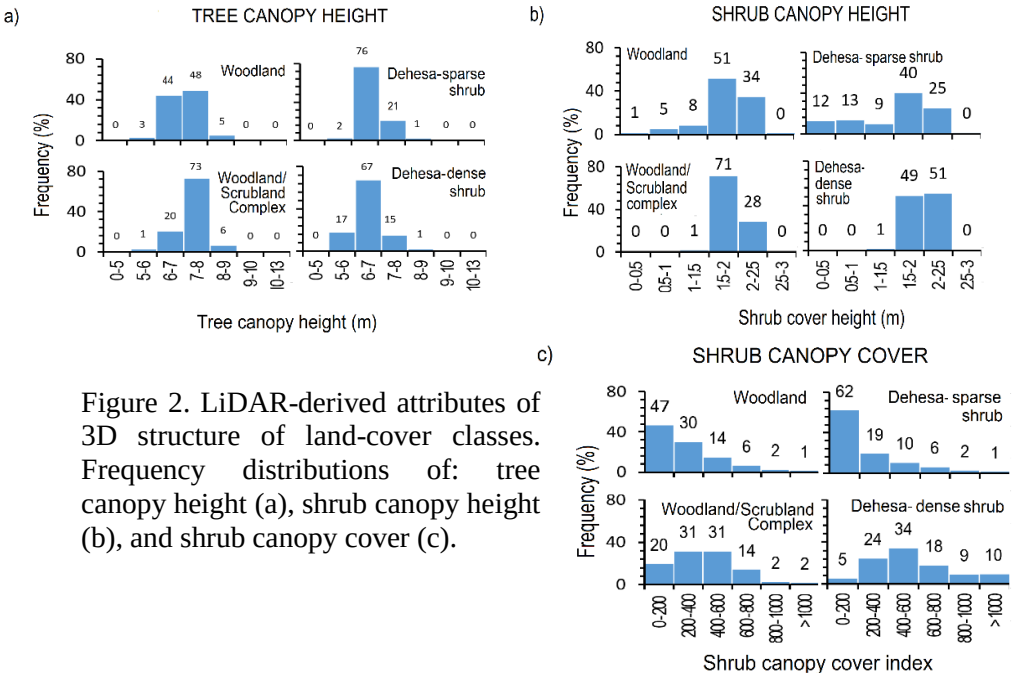

Figure 2. LiDAR-derived attributes of 3D structure of land-cover classes. Frequency distributions of: tree canopy height (a), shrub canopy height (b), and shrub canopy cover (c).

First, the confusion matrix derived had a Kappa index of 0.297. Although it was not very high, it was a statistical evidence of a general agreement of automated and digitized classification of land-cover units. Accuracy in automated identification of W and D-ss was high (Kappa values of 0.415 and 0.412, respectively). These results are relevant because the 65% of the W area and the 71% of the D-ss area were correctly identified. These vegetation classes represented the 90% of total woody vegetation in the study area. The automated identification of the other two classes, W/SC and D-ds, was less accurate. Nevertheless, these apparent incorrect classifications were probably related to the identification errors, caused by the manual digitization of both land-cover classes, that included a mixture of tree and shrub strata, not easily perceived by the human eye. The accuracy of the automated map was the effect of the quality of 3D data and the standardized method applied, with quantitative thresholds. The automated map can be considered better than the real reference digitized map. Besides, the high spatial resolution of the automated map suggested that its quality outperforms the digitized map. These results are consistent with previous applications of LiDAR data for automated land cover mapping in non-Mediterranean areas (e.g. Parent 2015).

CONCLUSIONS

Preliminary results indicate that airborne LiDAR data allows to automate the production of land-cover maps, and to characterize their 3D vegetation structure in relatively large areas of complex Mediterranean vegetation with a high spatial heterogeneity.

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- ACKNOWLEDGMENTS

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

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