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LIVING CONDITIONS OF FEMALE FARMERS IN AUSTRIA

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ABSTRACT

Understanding living conditions is critical to the understanding of female farmers' way of life. They are fundamental to people's lives and vary from person to person. Normatively speaking, a good life cannot be achieved without having good living conditions. The term living conditions, either as a target of different political interests, agendas and priorities or closely related to private spheres of life, refers to the circumstances surrounding an individual's life. This paper: (I) aims to define the multidimensional concept of living conditions, (II) considers the importance of the concept and its application, and (III) offers statistically proven insights into the living conditions of female farmers in Austria. The analysis is based on select data from the female farmers' surveys in Austria. All in all, the results reveal the development from 2006 to 2016. The areas examined (education, financial independence, civic engagement, work-life balance, social network and job satisfaction) show a positive development but there is also room for improvement – especially in the cases of financial inclusion and work life balance. Moreover, there is clearly a need to learn more about the living conditions of female farmers – especially in connection with their lifestyle, and other concepts such as quality of life, social inclusion and standard of living. Finally this paper tries to elaborate on the need for further research and future perspectives.

Keywords: *female farmers, living conditions, Austria.*

INTRODUCTION

In line with the Europe 2020 strategy (European Commission, undated) for smart, sustainable and inclusive growth, the consideration and evaluation of statistics on living conditions has increased (Eurostat, 2014, undated; OECD, 2017; International Forum on Rural Women, undated; O'Neill, 2018; Statistics Austria, 2017; Statistics Sweden, undated). As farming continues to change living conditions in agriculture have been increasingly in the spotlight over the last decades (Källlström, 2002; World Bank Group 2018). This interest in female farmers has largely developed as a critical response to the way in which women's labour had been treated in agricultural research (Friedland, 1991; Haugen, 1990; Whatmore, 1991). It reflects a shift to a more nuanced view of agricultural life. Inherent in this literature is an anticipation of change in favour of female farmers.

The concept of 'living conditions' emerged as an important policy concept in Europe in the 1940s (International Forum on Rural Women, undated). It was a response to the growing social divides that resulted from labour market conditions and the inadequacy of existing social welfare provisions to meet the changing needs of more diverse populations. Living conditions is not, however, just a response to unequal conditions of life. Although many of the papers use living conditions as the starting point for their discussions, they share with us the view that the concept of 'living conditions' has a value on its own as both a process and a goal. Living conditions are about making sure that all humans are able to live a valued, good life. It is, therefore, a normative (value based) concept – a way of raising the bar and understanding how we want to live and how to get there.

An assessment of living conditions can provide a more detailed picture of the progress in economic and social development than a development analysis of traditional economic indicators. Indicators of living conditions offer useful information about important issues, *e.g.* income, education and job opportunities (Eurofound, 2010).

Statistics on female farmers' living conditions currently are few and far between. Basically, this paper broaches the issue of the living conditions of female farmers in Austria. The result of a development analysis is based on the most important indicators recommended by Eurofound (2010), Statistics New Zealand (2009) and Statistics Sweden (undated). With this in mind, this paper firstly frames the concept of living conditions by identifying the main criteria and characteristics. In the second part, there is a description of material and data used. The presentation and evaluation of the findings follow. Finally, we conclude and look at further areas of research.

THEORETICAL ORIENTATION

Throughout history female and male farmers have shaped nature and have maintained food production and other related amenities. Recognizing the importance of difference and diversity has become central to new understandings of identity at both a national and professional level. Female farmers play a key role on the family farm and thus in agriculture (Brandt, 2002; Brasier *et al.*, 2014). The concept of living conditions is crucial in recasting female farmers' lives.

Living conditions form one of the precepts for a conception of a sustainable livelihood as well as the achievement of an adequate quality of life or a good life (c.f. Quendler, 2014). Hereby the term living conditions refers to the fulfilment of (basic) needs in the long-term as well as the achievement of eudaemonistic values – as for example outlined by Maslow's Hierarchy of Needs (Maslow, 1954). Living conditions build on the different material and immaterial needs according to resources and services available within a specific environment. The concept is defined in terms of access to resources in the form of money, possessions, knowledge, mental and physical energy and social relationships – through which individuals can control and consciously direct their living conditions. This is, in part, based on the Swedish approach – c.f. Erikson and Aberg, 1987. (Eurofound,

2010) In terms of Maslow's Hierarchy of Needs this paper presupposes the fulfilment of the first two levels of needs *i.e.* the physiological and safety needs have been attained.

This view of living conditions recognises that the value of a given set of possibilities to use resources and services depends on the context in which they are used. The decision when, how and to what extent to exploit any given set of possibilities depends solely on the individual's attitude, life strategies and choice which however are all, in turn, influenced by a multitude of factors making up the surrounding environment. (DFID, 1999; Eurofound, 2010) Much in the way of a feedback gain in an operational amplifier. The ability of the next generation to enjoy the same possibilities as a result of the living conditions enjoyed and 'decided on' by the current generation forms the critical link to the notion of sustainable livelihood.

There are several barriers that limit the living conditions. For one thing, the notion of protecting present and future generations seems remote in the face of the pressures of contemporary standards of living, financial crises and the limited resources available. On the other hand, the concept is often associated with resource constraints and the maintenance of status quo rather than with opportunities for continued innovation, growth, and prosperity. Living conditions that go beyond the basic minimum are sustainable only if conditions everywhere have regard for long-term sustainable development. Furthermore, living conditions are often misinterpreted as just a goal to which we should collectively aspire as a desirable state. In fact, specific living conditions are not an end state that humans have or can reach; rather, they are a characteristic of a dynamic, evolving process. (*c.f.* Quendler, 2014; Statistics New Zealand, 2009)

MATERIAL AND METHODS

The analysis presented in this paper refers to the Eurofound (2010), Statistics New Zealand (2009) and Statistics Sweden (undated). Literature reviews state that living conditions are a complex and challenging concept that cannot be reduced to only one dimension. The description of the components and indicators used to describe a socially inclusive good life is shown in Table 1. These have been ordered corresponding to their relationship to Maslow's Hierarchy of Needs. While there is no perfect match between the indicators chosen here and Maslow's Hierarchy of Needs the parallels are obvious. Unfortunately there is no data available for female farmers on income and income inequality.

Table 1. Description of the components of living conditions in the sense of a socially inclusive good life.

Components/indicators [Maslow's level]		Description	
Satisfaction with the job female famer [Self-actualisation]		It measures the percentage of respondents that would call themselves "female farmer" or make the same choice of profession again.	Valuing and recognising the profession Promoting satisfaction and happiness in life
Work-life-balance [Self-actualisation]	Time for holiday	The proportion of respondents that have time for holiday and have time also to relax everyday.	Basic needs met with options to meet more Promoting satisfaction and happiness in life
	Time for relaxation		
Education level [Self-actualisation Esteem]		It measures the percentage of respondents successfully completing upper secondary school.	Ensuring opportunities to attain knowledge and skills Developing knowledge and skills to meet economic needs
Financial independence [Esteem]	Off-farm job	It measures the percentage of respondents with an off-farm job.	Ensuring that economic activity meets the needs of individuals and society effectively Ensuring that female farmers have a sense of identity and independent Promoting social participation
	Bank account	The proportion of respondents that have an account at a bank.	
Civic engagement [Love/belonging]		The proportion of respondents that are engaged in voluntary activities.	Promoting civic and political participation Promoting social participation
Social Network [Love/ belonging]	Moral support	It measures the percentage of respondents that are embedded in a social network in case of illness, financial and personal problems.	Basic needs met with options to meet more Strengthening partnership and social participation
	Financial support		
	Illness		

*Source: Eurofound (2010), Maslow (1954), Statistics New Zealand (2009) and Statistics Sweden (undated).

In order to get a picture of female farmers' living conditions select data from two surveys taken on female farmers throughout Austria (Geserick *et al.*, 2008; KeyQUEST Marktforschung, 2017) is analysed. The two surveys in question took place in 2006 and 2016 and deployed different methods. In 2006, the total number of Austrian farms formed the overall population, but in 2016 the number of farms according to Austrian IACS farm data constituted the population for the survey. While in the 2006 survey the return number of questionnaires was 1,127 (on a

target of 1,000) in 2016 the response rate came to 2,200 female farmers due to the online nature of the survey. For the purposes of standardisation the survey populations of the two years were considered using the characteristics: age, location of farm (federal province) and farm size (UAA**)**. The nominal and ordinal data sets were based on answers of either yes or no and scales, for example 'frequency and duration of holidays', satisfaction with the job. For example the best score (very good) is calculated with 1, good with 0.67, less well with 0.33 and the worst one with 0. Thus, criteria such as satisfaction can be expressed by a single measure, the index value. The index can range from 0 to 1. The nearer the calculated value of the indicator is to 1 (or 100%) the more it is fulfilled, *i.e.* the better off in the context of the indicator defined (Bartle, 1964).

RESULTS AND DISCUSSION

Compared to the year 2006 the living conditions for female farmers have improved (Figure 1). The results pertaining to social networking are very positive; most of them have someone in case of problems. However, all other indicators show room for improvement when female farmers aim to improve their living conditions as defined in this paper. It may happen that a female farmer is happy to work on the farm and there is no need, for example, for a holiday or off-farm job. Figure 1 shows the values calculated for each indicator.

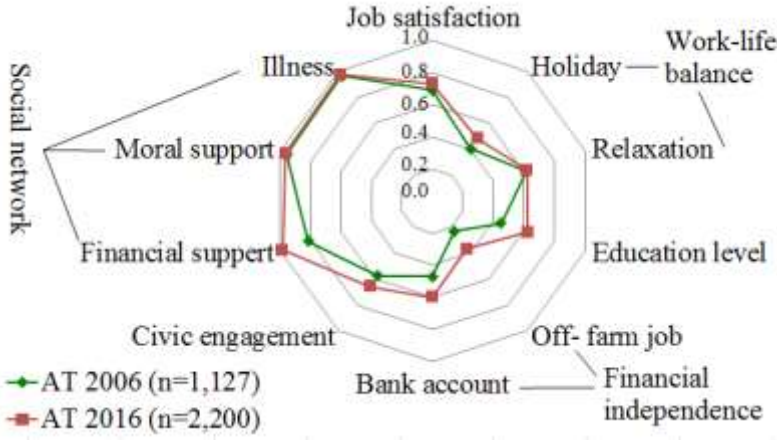

Figure 1. Living conditions of female farmers in Austria, 2006 versus 2016.

*Source: own visualisation according to Geserick, Kapella, Kaindl (2008), KeyQUEST Marktforschung (2017).

Satisfaction with the job female famer

In 2006 79%, in 2016 72%, of the female farmers surveyed identified themselves with the job of female farmer. In each case the reminder are women that identify themselves with the off-farm job. This notwithstanding the answer to the question as to whether the female farmer would take up her job again serves as a general

measure of satisfaction. In 2006, 69% of respondents said they would return to the same farm work, and by 2016 satisfaction steadily rose to 73%. These results show the identification and satisfaction of female farmers with the job of female farmer. (KeyQUEST Marktforschung, 2017)

Work-life-balance

63% of the female farmers have time for rest and relaxation in everyday life – in 2006 it was 62%. If in 2006 the figure was 41%, in 2016 already 71% of female farmers had taken a vacation for at least a week. Of those in 2016, 45% (2006: 41%) of them go on holiday occasionally and only 26% of regularly. Of the 29%, who had never been on holiday, 7% would like to go on holiday. (KeyQUEST Marktforschung, 2017)

Education

The education level of the female farmers surveyed has risen between 2006 and 2016. In 2016, 63% of respondents had completed secondary education whereas in 2006 it was 45% (KeyQUEST Marktforschung, 2017). The number of those respondents who have a Matura (A-level) or have graduated from a university of applied sciences or university has increased. Importantly, post-secondary education or tertiary education reflects the level of human capital development resulting in higher returns to education thereby also contributing to higher rates of economic growth and additional employment opportunities.

Financial independence

The proportion of female farmers who work off-farm has increased by 15 percentage points over the past 10 years. This corresponds to an increase of 41%. In 2016, 37% of female farmers work in their trained profession, usually more than 20 hours or up to 20 hours per week all year round. The main motivation of female farmers for off-farm work is to be financially independent (55%). This is followed by 'use of the skills learned' (53%) and 'additional income' (49%). The motives 'contact with other people' (46%) and the 'change from the farm' (42%) receive less approval. (KeyQUEST Marktforschung, 2017)

Access to a personal account has increased since 2006. In 2006, 47% of respondents had a personal account, compared to 60% of women in 2016 (KeyQUEST Marktforschung, 2017). This and the development of the indicator 'off-farm employment' not only ensure but also strengthen the financial independence of female farmers. This goes hand in hand with the increasing proportion of female farmers who work off-farm.

Civic engagement

Honorary appointments play an important part in the lives of female farmers. In 2016, 66% of the female farmers were voluntarily engaged in at least one organisation (see Figure 1). In 2006, the figure was 58%. In comparison, 24% of all women in Austria undertook voluntary work in 2012 (2006: 23%) (Bundesministerium für Soziales und Konsumentenschutz 2008, Bundesministerium für Arbeit, Soziales und Konsumentenschutz 2013). When

female farmers take on duties in clubs, in communities and in politics, they know that such activities involve additional burdens (but also enrichments) and require time and energy, and the help of the whole family. In order to engage more easily, female farmers gave the following assessments regarding support wanted: 40% would like to have more time. 32% say they expect more support from society. 29% require no support. 14% would like to be more supported by the family (KeyQUEST Marktforschung, 2017).

Social networks

Social networks are important for female farmers in daily life in case of financial, personal and health problems. Female farmers are most likely to report that they have a perceived source of financial support in their social network, with 98% in 2016, compared to 81% in 2006. Evidently, the response behaviour was similar in the case of illness. In 2016, 98% of female farmers have someone who cares about them in the case of illness, compared to 97% in 2006. In the case of moral support, almost equal proportions of respondents in 2016 and 2006 indicated that they have someone when they need someone to talk to. In 2006 96% of female farmers had someone and in 2006 it was 95%. (KeyQUEST Marktforschung, 2017)

CONCLUSION

'Living conditions' is a fundamental concept to understanding female farmers' specificity, conditions of existence, and also the close relation between their behaviour and the structure of opportunities and constraints in which they occur. The results give insight into the development of living conditions connected to female farmers between 2006 and 2016. Overall, the development is a positive one. But it is likewise clear that there is still a need for future action. This further reinforces the need to learn more about the living conditions of female farmers in comparison to male farmers or Austrian women or the population overall. No doubt, gender ideology has played a crucial role in masking the living conditions of female farmers.

In this respect the following points are offered in conclusion: (I) Defining and measuring living conditions: Living conditions remain an elusive concept in this analysis of wellness and welfare research. It is evident that well-being and welfare despite the given policy have the potential to influence living conditions, but far too often living conditions are assumed as a consequence of being successful in the job. The emphases in this paper have been on the living conditions of female farmers. There is a need for action in the case of income and income inequality. This raises several questions: How might we deal with or measure living conditions in order to derive and introduce appropriate measures where necessary? Long term studies are necessary in order to determine the effects on female farmers but also on male farmers or Austria as a whole. In this context the definition of living conditions must be continually revisited and revised in the light of such research but should also include concepts such as lifestyle, quality of life, standard of living, social inclusion, degree of sustainability at different levels, etc. (II) Superstructure and procedures: Policies regarding equity and social support have to be respected.

In order to maximize equal opportunities hierarchical structures must give way to widespread consultation, equal representation, positive and community based action, empowering female farmers to make their own choices and keep control of programmes.

Despite being highly developed in many areas, Austria can also do more in its recognition and support for living conditions. This is not merely a gender equity issue. The Austrian agricultural sector faces challenges from both environmental change and a competitive global market which may be detrimental to the given or further development of living conditions.

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THE TENDENCY CONCERNING THE EVOLUTION OF OILSEED MARKET IN ROMANIA

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ABSTRACT

Agriculture is the main branch of Romania's national economy, an important sector, assuring agro-food product for export, materials for the industry and also, food for population. In the growth a country, an important role plays the international trade because it express the capacity of providing certains services and to produce goods. Romania's agricultural production in 2016 compared to members states of the EU, places Romania, for both cultivated area and output for the sunflower crops, on the first place. Also, rapeseed production has recorded a continuous increasing trend in the analyzed period. The increased price is justified by demand/offer ratio. The main purpose of the paper was to analyze the foreign trade activity of Romania with oilseeds and the trends between 2007-2016. Were used statistical data referring to land surfaces sown with oilseeds, productions, the average yield per hectare, prices, import and export and also, the imports coverage degree by exports.

Keywords: *oilseeds, production, export, import, price.*

INTRODUCTION

Agriculture is an important economic sector assuring food for population, raw materials for processing industry and agro-food products for export. Its contribution to GDP is 5.6%. It registered a continuous development in the last decade, and its future depends on a modern technical endowment, investments, employment of high qualified persons, a corresponding farm structure able to assure a higher productivity, economic efficiency and competitiveness (Popescu, 2015). The Romanian agricultural competitiveness is a debate full topic in the context of the late sectorial reforms during the country accession and convergence to the EU-28 agricultural model (Popescu et al., 2017). The agricultural sector holds a major place in the Romanian economy, with an important contribution to Gross Domestic Product (GDP) creation and also a key role in international trade. The importance of agriculture in Romanian economy results from its share in GDP, labour force and rural community's impact (Ciutacu et al., 2015). The analysis of the foreign trade activity is, like in the case of the other economical branches, of a

major importance for establishing efficiency, identifying trades and the justification of specific decisions in this activity (Anghelache, 1999). The external trade has a determinant role in Romania's trade balance, for both exports and imports, especially due the accentuated dependence for the imported food products. In Romania, the land is cultivated with crops which are competitive on world market: maize, wheat, oil crops and barley. These four categories accounts for almost 80% of arable land and have high competitiveness indices of 7.94, 7.52, 3.51 and, respectively, 9.81. Triticale and tobacco are also competitive on world markets, with Balassa indices of 6.65 and, respectively, 4.8, but their shares in arable land are lower 0.87% and, respectively, 0.01. The same products: maize, wheat, oilseeds and barley account for significant shares in agro-food exports: wheat holds the main share of 19%, followed by oilseeds, with 15%, and maize with 14.3%. This structure of exports contains almost the same agro-food products as other studies report (Gheorghe et al., 2017). Vegetables and fruits are foods of plant origin with an important role in the diet, because of their sensory characteristics and precious nutrients they contain, in the form of carbohydrates, vitamins, organic acids, mineral salts, etc. (Cîrstea et. al, 2013). As (Arghiroiu et. al, 2015) Romania was a net importing country of agricultural products. In 2013 the total trade balance has become a surplus. However, we can say that Romania has become conjectural self-sufficient, because we are surplus to only 5 of the 24 groups of agro-food products. We know a positive balance for cereals, oil seeds and oleaginous fruits, tobacco, live animals, products with raw material nature, and for the remaining agro-food groups we import massive, especially meat, sugars and sugar confectionery, fruits etc. The situation seems to be improving in recent years in terms of the total balance of trade balance, due to the major influence exerted by cereals and oil seeds and oleaginous fruits trade. An important indicator that influences the world, demand and supply is represented by the price over the international market. It has a strong informational consignment, being the basis of economic agent decisions (Angelescu et. al., 2010; Bordean et. al., 2010; Ursu, 2010). Romania's main trading partners in trade with oilseeds are the EU States members, but also we can observe that we import soya beans from Argentina, Brazil and Canada, linseeds from Turkey, India and we export sunflower in South Africa and Pakistan (Armenița Arghiroiu et. al., 2015). Romania is an important pawn over oilseeds market because it produces a significant quantity of sunflower for export. One of the main risk factors in obtaining sunflower crop with stable production is the appearance and evolution of the broomrape. In Romania, more than 60% of the sunflower cultivated area is infested with broomrape. The three more spread broomrape populations in the largest area cultivated with sunflower, are very different regarding the virulence and dissemination of the parasite The race G was definitely found in Tulcea and Constanta counties in Romania and latest surveys showed possible appearance of even more virulent race (Pacureanu et. al., 2009b). As (Pricop et. al., 2011) the race identification must be a continuous process to support farmers, by recommending sunflower hybrids based on the information concerning the parasite spread and virulence throughout the territory.

The identification of the parasite physiological races also supports breeders to develop strategy for improvement programs. An evaluation should be made in this context as follows: when the domestic demand of raw materials for processing increases, exports will be reduced (www.agravista.md, 2013). As (Balasu et al., 2014) the production losses caused by soybean bacterial burning (*Pseudomonas savastanoi* pv. *Glycinea*) are major when seed treatment is ignored and the environmental conditions are favorable for the attack.

MATERIAL AND METHODS

In order to make this research, were used statistical data referring to land surfaces sown with oilseeds, productions, the average yield per hectare, prices, import and export and the import coverage degree by export (an indicator of economic competitiveness) (Anghelache C., 2008).

$$GA = \frac{E}{M} x100$$

GA - represents the imports coverage degree by exports;
E - values of exports;
M - values of imports.

This indicator shows the percentage of the value of imported goods covered by the value of exported goods, showing the surplus, the equilibrated or deficit trade balance.

Multiannual average (A): $A = \frac{X1 + X2 + X3 + ...Xn}{n}$;

Growth rate (%):

$$G(\%) = \frac{Xix100}{A} \quad 100$$

G (%) - Growth rate;
Xi - the main indicator used in the analysis as cultivated area, yield, production, etc.;
A - Multiannual average (A).

These data were given by FOASTAT and the National Institute of Statistic and also obtained from the Ministry of Agriculture and Rural Development.
There were also consulted a series of books, magazines and special studies in order to show as concise as possible the evolution of the oilseeds market.

RESULTS AND DISCUSSION

As an European Union member since 2007 and a NATO member since 2004, Romania is currently one of the most dynamic large markets in Europe and plays a unique and important part in European agriculture.

Table 1. Surface evolution for the main oilseeds in Romania, during 2007-2016 (thousand ha)

Specification	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Average (thousand ha) 2007-2016 growth rate (%)
Sunflower	835.9	813.9	766.1	790.8	995.0	1067.0	1074.6	1001.0	1000.0	1016	936.0
	growth rate (%)										
	-10.7	-13.0	-18.2	-15.51	+6.3	+14.0	+14.8	+6.95	+6.84	+8.6	100
Rapeseed	364.9	365.0	419.9	537.3	392.7	105.3	276.6	406.7	383.0	471.0	366.8
	growth rate (%)										
	-0.5	-0.5	+14.5	+46.49	+7.1	-71.3	-24.6	+10.9	+4.4	+28.4	100
Soybean	133.2	49.9	48.8	63.9	72.1	79.8	67.7	79.9	128.1	127	85.0
	growth rate (%)										
	+56.7	-41.3	-42.6	-24.8	-15.2	-6.1	-20.4	-6.0	+50.7	+49.4	100

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Main oilseed crops cultivated in the EU are rape and turnip rape, sunflower and soya. The production was 31.1 million tonnes in the EU in 2016 which is in line with the 5-year average (– 0.8 % if compared to the 5-year average). In 2016, the rape and turnip rape seeds production was 20 million tonnes and it was the most common oilseed crop in the European Union despite its sharp decline since 2014 (-17.1%). The EU-28 sunflower seed production in 2016 was 8.8 million tons and decreased by -14.8% compared to 2014, followed by increase of 10.7% between 2015 and 2016. In 2016, the EU-28 soya production accounted for 2.5 million tons and it is steady increase since 2012 (Statistical Books, Eurostat, 2017 Edition). In Romania, oilseeds crops register a high weight of the total cultivated area. In Table 1. is presented the evolution of oilseeds surface in Romania, between 2007- 2016. There were analyzed three oilseeds crops, such as: sunflower, rapeseed and soybean. Between 2007-2016, the sunflower cultivated area varied between 766.0-1074.6 thousand ha. The largest surface cultivated with sunflower was of 1074.6 thousand ha, in 2013. During this year, the sunflower cultivated area increased with 14.8% than multiannual average (936.0 thousand ha). Concerning the area cultivated with rapeseed, had an oscillatory evolution, the largest surface was in 2010 with 537.3 thousand ha. In 2012 decreased over the total with 71.3% than multiannual average (366.8 thousand ha). Rapeseed has

become a more and more attractive crop for farmers due to the EU subsidy (Euro 45/ha) provided since 2005 for encouraging bio fuel production (Zahiu et al., 2010). Soybean cultivated area has varied from a period to another but generally it has continuously increased from 48.8 thousand ha in 2009 to 128.1 thousand ha in 2015, with 50.7% than multiannual average (85.0 thousand ha).

Table 2. The evolution of oilseed production (thousand tons) and medium production per hectare (kg/ha) in Romania, between 2007-2016

Specification		2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Average 2007-2016
Sunflower	thousand tons	546.9	1169.7	1098.0	1262.9	1789.3	1398.2	2142.1	2189.3	1758	1954	1530.8
	growth rate (%)	-64.3	-23.6	-28.3	-17.5	+16.9	-8.7	+39.9	+43.0	+14.9	+27.7	100%
	kg/ha	654	1437	1433	1597	1798	1310	1993	2187	1758	1923	1609
	growth rate (%)	-59.4	-10.7	-10.9	-0.7	+11.8	-18.6	+23.9	+35.9	+9.3	+19.5	100%
Rapeseed	thousand tons	361.5	673.0	569.6	943.0	739.0	157.5	666.1	1059.1	959.0	1336	746.4
	growth rate (%)	-51.6	-9.8	-23.7	+26.3	-1.0	-78.9	-10.8	+41.9	+28.5	+79.0	100%
	kg/ha	991	1844	1357	1755	1882	1496	2408	2604	2530	2836	1970.3
	growth rate (%)	-49.7	-6.4	-31.1	-10.9	-4.5	-24.1	+22.2	+32.2	+28.4	+43.9	100%
Soybean	thousand tons	136.1	90.6	84.3	149.9	142.6	104.3	149.9	202.9	262.0	262.0	158.5
	growth rate (%)	-14.1	-42.8	-46.8	-5.4	-10.0	-34.2	-5.4	+28.0	+65.3	+65.3	100%
	kg/ha	1021	1817	1726	2345	1980	1308	2216	2539	2045	2047	1904.4
	growth rate (%)	-46.4	-4.6	-9.4	+23.1	+4.0	-31.3	+16.4	+33.3	+7.4	+7.5	100%

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Sunflower production has increased from 546.9 thousand tons in 2007 to 2189.3 thousand tons in 2014, with 43.0% more than the multiannual average (1530.8 thousand tons). Rapeseed production has recorded a continuous increasing trend in the analyzed period. In comparison with 361.5 thousand tons carried out in 2007, in 2016, Romania achieved 1336 thousand tons, with 79% than the multiannual average (746.4 thousand tons). Rapeseed production started increasing since 2007 at the moment when the European Union decided to expand energetic crops for bio fuel. Therefore, production performance has been determined both by the increased cultivated surface as well as by the increased yield (Table 2). Concerning the soybean production, had an oscillatory evolution during the analyzed period. In comparison with 84.3 thousand tons carried out in 2009, in 2015 and 2016, Romania achieved 262.0 thousand tons, with 65.3% more than the multiannual average (158.5 thousand tons). The medium production of sunflower per hectare varied between 654-2187 kg/ha. In 2014, medium production of sunflower increased over the total with 35.9% in comparison with the multiannual average. Rapeseed medium production varied between 991 and 2836 kg/ha. In 2016 it recorded an increase of 43.9% than in 2007. Soybean medium production varied between 1021 and 2539 kg/ha. The large production was reached in 2014. Table 3. shows the evolution of Romanian oilseeds export, during 2007-2016. The exported quantity of sunflower seeds varied between 382.6 and 1420.1 thousand tons. In terms of value, the year 2013 registered the highest income from sunflower seeds export (550.7 EUR millions). Rapeseed quantitative export varied between 68.2 in 2012 and 1461.9 thousand tons in 2016. The most significant soybean exported quantity was registered in 2016 with 108.9 thousand tons.

Table 3. Evolution of Romania's oilseed exports, during 2007-2016

Specification	2007		2008		2009		2010		2011	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	382.6	105.4	471.3	192.2	564.2	146.1	557.4	214.8	1182.8	508.3
Rapeseed	279.1	77.7	564.0	246.0	782.1	223.7	1052.3	334.0	577.2	273.2
Soybean	22.0	4.7	38.9	13.5	10.4	3.0	36.9	13.2	72.7	28.4

Specification	2012		2013		2014		2015		2016	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	652.4	335.6	1420.1	550.7	1321.9	452.5	1099.3	452.2	1183.7	489.9
Rapeseed	68.2	41.8	471.9	192.5	989.1	338.3	773.4	773.4	1461.9	549.0
Soybean	89.5	41.8	38.8	21.9	40.0	21.4	92.9	40.3	108.9	43.0

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

In Table 4. is presented the evolution of Romania's oilseeds imports, during 2007-2016. The quantitative import of sunflower varied between 66.6 and 1972 thousand tons, and the value oscillated between millions euro 32.5 and 138.4. Rapeseed quantitative import varied between 241.0 and 9.7 thousand tons. The quantitative import of soybean oscillated between 15.6 and 168.3 thousand tons.

Table 4. Evolution of Romania's oilseed imports, during 2007-2016

Specification	2007		2008		2009		2010		2011	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	66.6	32.5	89.5	52.2	141.0	72.9	208.2	109.7	237.3	142.6
Rapeseed	9.7	7.9	76.3	35.8	70.4	28.1	241.0	88.1	70.6	50.2
Soybean	68.5	23.7	94.3	38.0	20.7	7.9	15.6	5.9	34.3	12.9
Specification	2012		2013		2014		2015		2016	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	131.2	108.9	93.3	101.3	118.9	99.2	189.2	127.7	197.2	138.5
Rapeseed	59.4	42.8	28.8	21.3	38.9	29.7	38.3	26.5	28.1	32.2
Soybean	63.3	29.8	117.2	53.7	102.6	41.8	168.3	65.8	122.3	52.5

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

In Table 5., the import coverage degree by export for sunflower, varied from 324.71% in 2007 to 200.46% in 2009, to 543.81% in 2013 and 456.41% in 2014, for rapeseed, except for the year 2012, when trade is highly low compared to the rest of the analyzed period, the coverage is 97.65%, the exports value covering in the year 2014 is 1138.51% out of imports value. For soybean, the imports coverage degree by exports varied from 19.79% in 2007 to 40.8% in 2013, to 220.87% in 2010 and 219.35% in 2011.

Table 5.The imports coverage degree by exports (G_a(%)) for sunflower, rapeseed and soybean, during 2007-2016

Specification	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
	%									
Sunflower	324.71	368.15	200.46	195.77	356.48	308.12	543.81	456.41	354.14	353.8
Rapeseed	983.47	686.93	794.45	379.12	544.32	97.65	903.81	1138.51	2912.4	1703.2
Soybean	19.79	35.55	38.48	220.87	219.35	140.15	40.80	51.11	61.3	82.04

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Table 6. Average purchasing prices for oilseed, during 2007-2015 (RON/kg)

Specification	2007	2008	2009	2010	2011	2012	2013	2014	2015	Average (RON/kg) (%)
Sunflower	0.84	1.12	0.86	1.19	1.58	1.84	1.59	1.26	1.5	1.31
	growth rate (%)									
	-35.9	-14.5	-34.4	-9.2	+20.6	+40.5	+21.4	-3.8	+14.5	100
Rapeseed	0.79	1.20	0.97	1.25	1.62	1.83	1.57	1.34	1.64	1.36
	growth rate (%)									
	-41,9	-11.8	-28.7	-8.1	+19.1	+34.6	+15.5	-1.5	+20.6	100
Soybean	0.78	0.97	0.96	1.23	1.3	1.71	1.83	1.43	1.33	1.29
	growth rate (%)									
	-39.5	-24.8	-25.6	-4.7	+0.8	+32.6	+41.9	+10.9	+3.1	100

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Oilseeds price presented in Table 6. reflected a large variation from a year to another, but mainly a continuous increase starting from 2007. The increased price is justified by demand/offer ratio.

CONCLUSIONS

During 2007-2016, the sunflower cultivated area varied between 766.0-1074.6 thousand ha, with an increased production from 546.9 thousand tons in 2007 to 2189.3 thousand tons in 2014. The exported quantity of sunflower varied between 382.6 and 1420.1 thousand tons, the highest income from sunflower seeds export being reached in 2013 with 550.7 millions euro.

Concerning the rapeseed cultivated area, had an oscillatory evolution, the largest surface was reached in 2010 with 537.3 thousand ha. Rapeseed production has recorded a continuous increasing trend in the analyzed period, compared with 361.5 thousand tons carried out in 2007, Romania achieved in 2016, 1336 thousand tons. Rapeseed quantitative export varied between 68.2 in 2012 and 1461.9 thousand tons in 2016. Rapeseed exports had values situated between 41,8 EUR millions in 2012 and 549,0 in 2016. Soybean cultivated area has varied from a period to another but generally it has continuously increased from 48,8 thousand ha in 2009 to 128,1 thousand ha in 2015. Concerning the soybean production, in 2015 and 2016, Romania achieved 262.0 thousand tons. The most significant soybean exported quantity was registered in 2016 with 108.9 thousand tons.

Oilseeds prices reflected a large variation from one year to another but mainly a continuous increase starting from the year 2007.

The imports coverage degree by exports for sunflower, varied from 324.71% in 2007 to 200.46% in 2009, to 543.81% in 2013 and 4456.41% in 2014, for rapeseed, except for 2012, the trade was low compared to the rest. For soybean, the imports coverage degree by exports varied from 19.79% in 2007 to 40.8% in 2013, as a deficit balance, to 220.87% in 2010 and 219.35% 2011 reaching a surplus.

As a conclusion, in the coming years Romania will continue to become a more and more important oilseeds producer and exporter in the European Community.

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ANTIBACTERIAL ACTIVITY OF DOMESTIC APPLE CIDER VINEGAR

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ABSTRACT

In recent years, interest in examining the chemical composition and pharmacological properties of apple cider vinegar in synergy with the application of natural products in the pharmaceutical, food and cosmetics industry has been growing. The apple vinegar shows a wide range of biological activities (antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, antihypertensive, immune-stimulatory, anticancer) and it has been used in traditional medicine for a long time. It consists of 8 essential amino acids (phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine), organic acids, enzymes, minerals (potassium, calcium, phosphorus, copper, grape and boron) and vitamins (provitamin C, vitamin A, E, B1, B2, B6, vitamin P and provitamin Beta-carotene). The aim of the study was to examine the antibacterial activity of traditionally produced apple cider vinegar and to determine whether it exhibits bactericidal or bacteriostatic activity. The results of the study confirmed the antibacterial activity of apple cider vinegar produced with the traditional method. Its antibacterial activity is in the range of 11.33mm to 14mm.

Key words: *bactericidal, bacteriostatic, antibacterial, therapeutic effects, vinegar*.

INTRODUCTION

Resistance of microorganisms to antimicrobial drugs is globally expanded and its not a new problem. The number of pathogens exhibiting multiple resistance to antimicrobial drugs has been increased. The World Health Organization (WHO, 2017) predicts that antimicrobial resistance could jeopardize the ability to cure many of today's curable diseases. In immunocompromised individuals and risky patients, microbial infections can lead to sepsis, which can lead to systemic inflammation and death. As a result, in recent years there is considerable interest to employ natural antimicrobials. Apple vinegar is one of the natural products for which interest in examining its chemical composition and pharmacological

properties in synergy with the application of natural products in the pharmaceutical, food and cosmetics industry has been growing in recent years.

The use of apple vinegar has been known since ancient times, both in cooking and in traditional medicine. According to some data, the use of vinegar dates back more than 10,000 years (Tan, 2005). Old Romans used apple vinegar as a refreshing beverage while some ancient nations put it into the elixir of youth and health. Hippocrates (400 years BC), the father of modern medicine, used vinegar and a mixture of vinegar and honey for various diseases, including cough and colds, but also as an elixir, a tonic that raises energy. The apple juice was used in wars as a remedy for wound healing (Chan et al., 1993.; Tan, 2005).

In traditional medicine, apple vinegar is used in many ways because it is believed to have an antimicrobial and antiseptic effect and to help fight viruses, eliminates bacteria, fungi and other microorganisms. Likewise, in traditional medicine, apple vinegar is used in chronic fatigue, headache, insomnia, sinus pains, throat irritation, rheumatic pain in joints, extended veins, eczema, burns, excessive weight, etc. (Darzi et al., 2013).

Although the use of apple vinegar for medicinal purposes has an extremely long tradition, the chemical composition and positive influence on human health has been systematically listed and scientifically described in 1958 (Folk Medicine) (Jarvis, 1985). It has been scientifically proven that apple juice maintains vitality and refreshes the body, triggers metabolism and strengthens immunity.

Bacteria and yeasts break down natural apple sugars during fermentation. During the first phase of fermentation, sugar is converted into alcohol, and in the second, further fermentation alcohol is converted into vinegar. The whole process lasts 3-4 weeks, depending on temperature (18-25^{0}C). Vinegar obtained in this way retains all the nutrients that the apple has (pectin, beta-carotene, potassium) with the addition of acids and enzymes produced during fermentation. It is a natural product based on the acidic acid that gives it acid taste and which acts as a natural antiseptic and preservative. This product is rich in minerals needed by our body including magnesium, phosphorus, calcium, chloride, potassium, phosphorus, sodium, trace minerals such as copper, fluoride, as well as vitamin A, B1, B2, B6, C and E, bioflavonoids, and pectins (Del Campi et al., 2012).

Vinegar made from wild or organic apple trees has the greatest beneficial effects. Also, it must be vinegar obtained by cold apple pressing, which is naturally naturalized, with no chemical additives (Del Campi et al., 201).

Apple vinegar is used in food products and is commonly used as a preservative for fruit and vegetable salads, as a supplement to fresh salad, in preparation of mayonnaise, mustard etc. Functional nutritional properties of vinegar have been published in various scientific and professional publications, while health benefits are still being exemined. (Turker, 1963; Tan, 200; Mazza and Murooka, 2009; Ou and Chang, 2009.).

It has been scientifically proven that apple vinegar maintains vitality of the organism, stimulates metabolism, strengthens immunity, cleanses blood from toxic substances and the formation of clots, protects the heart and blood vessels, helps

regulate cholesterol and blood sugar levels. Due to its disinfection and antibacterial activity apple vinegar has a good effect on digestion, stimulating it and protecting it from various disorders, especially infections. It is beneficial for inflammation of the throat, gums, hoarseness, asthma etc. Therefore, apple vinegar has antibacterial, antioxidant, anti-cancer, antifungal, antidiabetic, anti-inflammatory, antihypertensive, immune-stimulatory effects (Nishidai et al., 2000; Ogawa et al., 2000a; Kondo et al., 2001a; Shimoji et al., 2002; Sugiiama et al., 2003a; Nishikawa et al., 2001; Iriti and Faoro 2010; Fern'andez Mar et al., 2012; Ramadan and Al-Ghamdi, 2012).

The man's aspiration to natural substances and the fear of illness caused by resistant bacteria to antimicrobial medication prompted scientists to find alternatives in the field of cattle breeding. The positive effect of apple vinegar has been proven in the prevention and in treatment of animals. It has been observed that apple vinegar has a beneficial effect on the gravidity cows, it also alleviates calving, causes an increase in milkiness, as well as a more favorable chemical and bacteriological composition of milk. The use of apple juice significantly improves the fattening and potency of bulls, reduces acute and chronic mastitis, prevents inflammation of the joints, improper heart muscle work due to thermal stress, increases appetite, reduces mortality, and according to some experiments it is a justified form of antimicrobial substitution.

Microorganisms such as *E. coli, S. aureus, C. albicans* form an integral part of human and animal micro-population. They are normal inhabitants of the skin, digestive and urinary tract as long as they are in balance with the immune and homeostatic system. If balance disorder occurs these microorganism can become pathogens that cause various diseases of the blood, urinary tract, gastroenteritis, endocarditis, soft tissue infections etc. Antimicrobials used in various diseases caused by various bacterial viruses become ineffective, ie bacteria become resistant. Various studies have shown that apple vinegar can be used to inhibit the growth of pathogenic bacterial species (Avci et al., 2007; Pourmouzaffar et al., 2017; Nazioğlu,et al., 2014; Chang and Fang 2007).

The aim of the paper is to examine the antibacterial activity of apple cider vinegar produced in traditional way from wild apples and to examine the type of action on the tested bacterial species.

MATERIAL AND METHODS

Material

The research was done on a sample of local apple vinegar made from wild apples harvested in the forest in the area of Ribnik municipality (Entity of Republic of Srpska, Bosnia and Herzegovina). The process of obtaining vinegar is traditional and takes place in three phases:

1. Washed apples are pressed in order to separate the fruit juice.
2. Squezeed apple juice is filtered and placed in containers covered with gauze for 20 days;

3. After that, the juice is again poured into pots that are covered with gauze for another 20
4. days and after which the juice is squeezed and poured in glass containers.

Test culture

The study of the antibacterial activity of apple vinegar was performed on four clinical isolates (*Enterobacter kobei, Enterobacter cloacae, Staphylococcus aureus* and *Escherichia coli)* from the collection of the laboratory of bacteriology, mycology and parasitology laboratory JU Veterinary Institute of RS "Dr. Vaso Butozan. The cultures were grown in a nutrient broth and incubated 18 h at 37^0C. Petri plates with the appropriate medium (Müeller - Hinton agar)
are seeded with 0.1 mL of a bacterial suspension with a concentration of 10^5cfu / mL.

Test method

The agar diffusion method was used on a solid sterile nutrient medium (Müeller-Hinton agar-MHA) to test the antibacterial activity of apple cider vinegar on selected bacterial cultures.
Metal cylinders of 9 mm in diameter are placed on the surface of a solid nutrient medium to which a certain pure bacterial culture has previously been sown. Using micropipette 10 μl of apple vinegar was poured into in the cylinders. The antimicrobial drug ceftriaxone was used as a control.
The principle of this method is based on the fact that the antimicrobial agent diffuses in the medium and it radially expands, with its concentration decreasing as distance from the edges of the cylinder increases. If the bacteria is susceptible to the action of the tested antimicrobial agent, it will not grow in the zone of its action. Therefore, after incubation, the zones of absence of growth are observed around the cylinder,so called the inhibition zones. The zones of growth inhibition were measured using a ruler and the sensitivity of the bacterial strain to the tested propolis solution was determined. Petri plates were incubated for 24 hours at a temperature of 37°C
Three repetitions were made for each bacterial culture and the mean value for each bacterial culture was calculated.

Type of activity

Also, the type of action of apple vinegar is determined. To see if vinegar has bactericidal or bacteriostatic activity, a small piece of agar is taken from the inhibition zone and added to the nutrient broth. Incubation was carried out 24h at 37ºC. If, after incubation, there is cloudy broth, it is considered that the vinegar is bacteriostatic, while if, after incubation, the broth remains clear, the effect of vinegar is bactericidal.

RESULTS AND DISCUSSION

The antibacterial properties of domestic apple vinegar were tested on four clinical isolates *E. kobei*, *E. cloacae*, *S. aureus*, *E. coli*, and the results are shown in the graph (Chart 1).

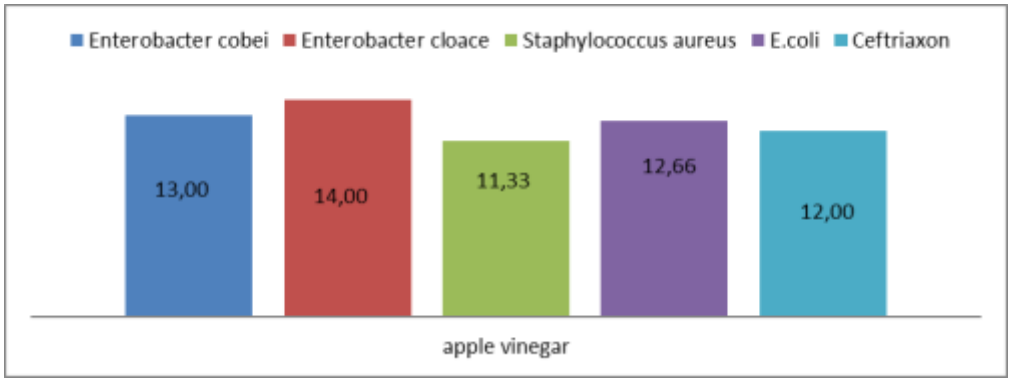

Chart 1. The antibacterial activity of apple vinegar according to the selected bacterial species (inhibition zone in mm)

As it can be seen from the chart, apple vinegar has exhibited strong antibacterial activity against the clinical isolates used in this paper. The antibacterial activity of domestic apple vinegar ranged from 11.33 to 14.00 mm, depending on the bacterial species. Various studies have suggested that apple vinegar can be used to inhibit the growth of pathogenic bacterial species in food products (Sengun and Karapinar, 2004; Wu et al 2000; Rhee et al. 2003; Chang and Fang, 2007) and that acetic acid has high inhibitory effect on Escherichia coli O157: H7. Likewise lactic, malic and citric acid have inhibitory effects on E. coli, Salmonella typhimurium (Yagnik et al., 2018; Entani et al., 1998; Ryu et al., 1999; Chang and Fang, 2007; Sengun and Karapinar, 2004). Apple cider vinegar acts on the bacteria by penetrating the cell wall of the bacteria and destroying their DNA and disabling their reproduction. Apple vinegar also has positive effect in gastrointestinal tract because it reduces the pH which limits the propagation of pathogenic bacteria in already consumed foods. (Yagnik et al., 2018). The inhibitory, or antibacterial effect of apple vinegar varies for each bacterial species, and the results of this work have confirmed that apple vinegar produced in a traditional way has good antibacterial potential and results are consistent with the results of other researchers who have shown that apple vinegar has multiple antibacterial potential with clinical therapeutic implications (Yagnik et al., 2018). To see if vinegar has a bactericidal or bacteriostatic effect, a small piece of agar is taken from the inhibition zone and added to the nutrient broth, and the results are shown in the chart (Chart 2.)

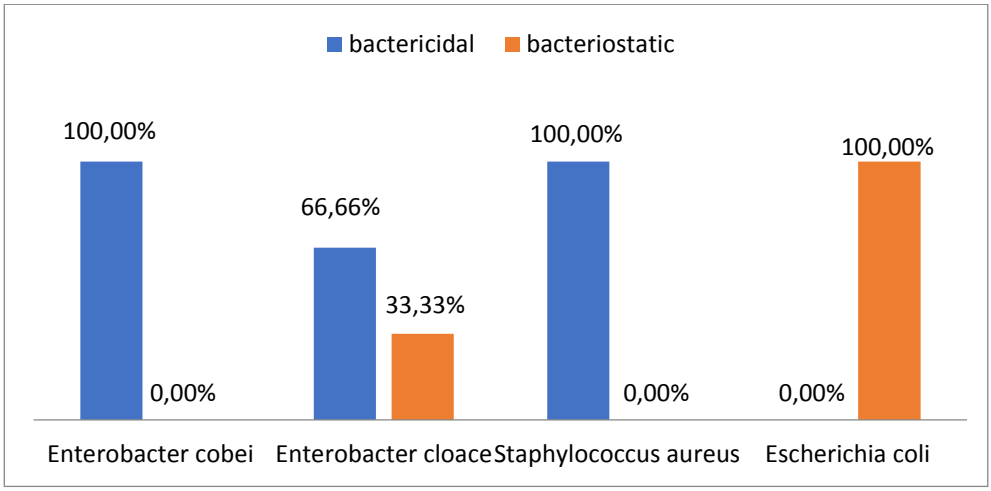

Chart 2. The type of apple vinegar activity

As it can be seen from the chart, apple vinegar displayed 100.00% bactericidal activity against *E. cobei* and *S. aureus*, and 100.00% bacteriostatic activity against *E. coli*. When it comes to *E. cloace*, 66.66% (2 reps), apple vinegar was bactericidal, and 33.33% (one repetition)it was bacteriostatic, which would be somewhat consistent with other published studies (Entani et al., 1998). Whether apple vinegar will have bactericidal or bacteriostatic activity depends to a large extent on the acetic acid concentration in the vinegar, the incubation time and the number of surviving bacteria. The bactericidal activity of apple vinegar increases with temperature. According to the results of some studies, the combination of apple vinegar with sodium chloride using the appropriate temperature proved to be very effective in preventing food poisoning. (Entani et al., 1998).

CONCLUSION

The results of the work confirmed the antibacterial activity of apple vinegar produced in the traditional way. The inhibition zone for Gram-negative bacteria ranged from 12.66 mm to 14.00 mm, while the inhibition zone for Gram-positive bacterial species was in the range of 11.33 mm. The apple vinegar had a bactericidal activity of 100.00% against *E. cobe* and *S. aureas*, and 100.00% bacteriostatic activity against *E. coli*. Due to its positive activities, its impact on improving food taste, improving digestion, and the ability to increase the body's immune response, apple cider vinegar has great potential for use in human and veterinary medicine. This is further contributed by its antimicrobial activity, which is considered a natural and acceptable replacement for antimicrobial drugs.

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PRODUCTIVITY AND COMPETITIVENESS OF RWANDAN AGRICULTURE: A CASE STUDY OF THE MAIZE SECTOR

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ABSTRACT

This study investigated the level of productivity and competitiveness of Rwandan agriculture by focusing on the case study of the maize sector. The data were collected through close monitoring of maize production activities on a sample of 50 producers from five maize producers' cooperatives selected in the districts of Huye, Rusizi, Gasabo, Burera and Bugesera (Rwanda) during two agricultural seasons of 2013/2014 and 2014/2015. They were supplemented by direct observation, interviews with targeted resource persons and secondary data on maize imports and exports retrieved from FAOSTAT website. The analysis was conducted using the comparative analysis, the farm-level economic performance indicators, the Net Export Index (NEI) and the Grubel-Lloyd (GL) measure. The results revealed that the yield was very low compared to theoretical expected yields for about 80% of producers. The analysis showed that the rational use of improved seeds, chemical and organic fertilizers improved the yield. The analysis of the NEI and the GL measures for maize flour and maize grain revealed that Rwanda was a net importer. For these staple foods, the results revealed that if Rwanda managed, through policy and institutional actions, to remove or alleviate the bottlenecks that prevent farmers from producing enough for export, it could have had a competitive advantage on regional markets.

Keywords: *Agriculture, competitiveness, maize sub-sector, productivity, Rwanda.*

INTRODUCTION

Rwanda is a landlocked country with an area of 26,338 km^2 with an estimated average population density of 434 inhabitants per km² in 2015 (NISR, 2016). It is ranked among the most densely populated countries in the world. The poverty and extreme poverty levels are estimated at 39.1 and 16.3% of the population

respectively (NISR, 2015). Agriculture continues to be the leading employer with more than 80% of the economically active population (Musabanganji, 2017) and remains the engine of economic growth in Rwanda. It provides more than 70% of exports in value and accounts for 30% of the Gross Domestic Product (GDP) (NISR, 2017). This economic context, in which agriculture contributes significantly to the country's economic growth, is a peculiarity of many developing economies characterized by a low level of industrialization. For many years, Rwanda has been aware of the role of the agricultural sector in combating poverty and improving the livelihood of its population. Along with the agricultural global and regional commitments, Rwanda developed a policy and strategic framework fostering agricultural and economic development. These include the socio-economic development program known as 'Vision 2020' aiming at transforming Rwanda into a middle-income country by 2020 and the national agricultural policy adopted in 2000 and 2004 respectively. Thereafter, all sectoral programs that have been implemented are a direct consequence of this vision by defining its operationalization frame in various sectors in order to achieve the expected socio-economic development. In this context, besides the growth and agricultural marketing pointed out by Huggins (2013) as key elements of economic expansion, regional trade is also perceived by Rwanda as one of crucial elements enabling agricultural development and boosting its economy. The later allows increasing the share of agricultural production on regional markets and easing goods and services transfer between regional integration agreements partner countries. This has been evidenced by the results that followed its accession to the East African Community (EAC) in July 2007. Since then, the country revised its trade policy and initiated several agriculture related programs and strategies to improve the production capacity, and then guarantee a satisfactory supply, and integrate in both domestic and regional markets for both cash and food crops. Thus, as the ability of the Rwandan primary sector to deal with increasing competition from trade liberalization and its membership to EAC has a significant effect on the macro and micro levels of the national economy, it should be noted that the country needs an agriculture sector which is not only competitive in domestic and regional markets but also able to ensure the food security of the population and generate income for producers. For more than a decade, Rwanda has adopted a series of policies and strategies aimed at economic development and the improvement of the living conditions of its predominantly agricultural population. In agriculture, implementation of these strategies and related programs has been accompanied by a series of advances in the adoption of farming technologies and increasing agricultural production. These initiatives have mainly targeted six priority food crops, namely, the maize, potato, bean, soybean, rice and wheat sectors to make them more productive and competitive. For the maize sector, which is the subject under study, available data reveal that the cultivated area has doubled (about 2.2 times) from 102 000 ha in 2007 to 223,414 ha from 102 000 ha to 223,414 ha in 2011. In season A of 2017, in terms of cultivated area, maize was the fourth largest crop after banana, beans and cassava with 16% which is 23% increase from 2016

while in season B, it was the fifth largest crop with 7.1% corresponding to 27% increase in cultivated area from 2016 (NISR, 2018). The yield has gradually increased from an average of 0.96 tons per hectare in 2006 to 2.35 tons per hectare in 2013 (MINICOM, 2014). However, notwithstanding the increase in cultivated area, yield and produced quantity of maize observed in recent years reflecting the combined effect of the agricultural intensification program and land consolidation policy, the country is continuously depending on imports to meet domestic demand, and maize processing plants always operate below their productive capacity (RDB, 2014). This research aims at examining the level of productivity and competitiveness of this sector in the current production and marketing conditions in order to identify existing constraints and formulate the strategies that can help improve the level of economic performance of maize production and maize marketing system.

MATERIALS AND METHODS

The data used to study the economic performance of maize production come from close monitoring of maize production activities of a sample of 50 farmers selected from five maize producers' cooperatives namely KOAGIMPA, KOTEMIBU, COODAKI, ABAKUNDAKURIMA and INDAKUKI operating respectively in the districts of Huye, Rusizi, Gasabo, Burera and Bugesera during two growing seasons of 2013/2014 and 2014/2015. They were supplemented with direct observation, interviews with targeted resource persons and secondary data on the value of maize (flour and grain) imports and exports retrieved from FAOSTAT website and National Bank of Rwanda (NBR). The analysis was conducted using the yield as the farm-level economic performance indicator. The Net Export Index (NEI) and the Grubel-Lloyd (GL) measure were used to analyse the level of trade performance for maize sector from 2000 to 2017. Note that these indices are calculated for maize grain and flour in trade between Rwanda and trade partner countries of the world. The choice of these two trade indices was based on the fact that they take into account all marketing and transport related costs, and consider simultaneously responses from both demand and supply sides (Frohberg and Hartmann, 1997). Based on Latruffe (2010), these indicators are given by the following equations in the case of a single country and multiple sectors trade analysis:

$NEI_i = \frac{X_i - M_i}{X_i + M_i}$ (Banterle and Carraresi, 2007), where X are exports; M are imports; i denotes the sector or product considered. The NEI values lie between -1 (when a country imports only) and 1 (when a country exports only), with a value of 0 in the case of the equality of imports and exports.

$GL_i = 1 - \frac{|X_i - M_i|}{X_i + M_i}$ (Banterle and Carraresi, 2007), where X are exports; M are imports; i denotes the sector or product considered. GL measure has a range

between 0 and 1, with the value 0 indicating inter-industry trade, while the value 1 indicates an intra-industry trade only.

RESULTS AND DISCUSSIONS

Analysis of maize productivity and inputs use in Rwanda

Studies have shown that the types of seeds and fertilizers used in the required proportions, favorable climatic conditions and the control of harmful organisms combined with the application of improved farming methods play an important role in agricultural productivity (*e.g.* Kpedzroku and Didjeira, 2008, 2011). The results shown in Figure 1 reveal three scenarios: (i) there are maize producers who performed well with a high yield while they have relatively invested less means in the acquisition of inputs, and (ii) other farmers who invested much money in inputs but have registered lower maize yield per hectare (ha). Compared to the theoretical mean yield of 5 tons/ha, the results showed that around 80% of farmers registered lower yield. The reasons behind this are multiple. In Rwanda where land is generally considered as deteriorated, and therefore, lacking enough mineral salts necessary for plant growth, this situation revealed by the study is on one hand grounded in the fact that maize growers would have invested all available resources in the acquisition of a single input such as improved seeds or chemical fertilizers for instance, whereas the use of a single input cannot ensure the best land productivity. On the other hand, the other reason that would be behind such a fact would be the possible failure of producers to respect the dosages prescribed for such or such other input whereas the use of a given input must be done optimally in accordance with prescribed rules and dosages requiring to use only sufficient quantities to meet needed nutrients in a manner that ensures the productivity, the quality and the desired growth rate in maize growing. In this regard, FAO (2005) underlines that an adapted fertilization level is a must to achieve the production level required by the genetic potential of a given species. Hence, the fertilization requires to be properly evaluated so as to achieve economic optimum. In case of over dosage, the productivity reduces due to toxicity effect (FAO, 2005). In addition, the study by Musabanganji *et al.* (2016b) has documented that most of smallholder farmers in cooperatives in Rwanda are experiencing the lack of financial means and technical assistance. Then, it seems evident that maize growers have difficulties acquiring all necessary inputs, and even those who are capable fail to use them in convenient and rational manner due to lack of required knowledge.

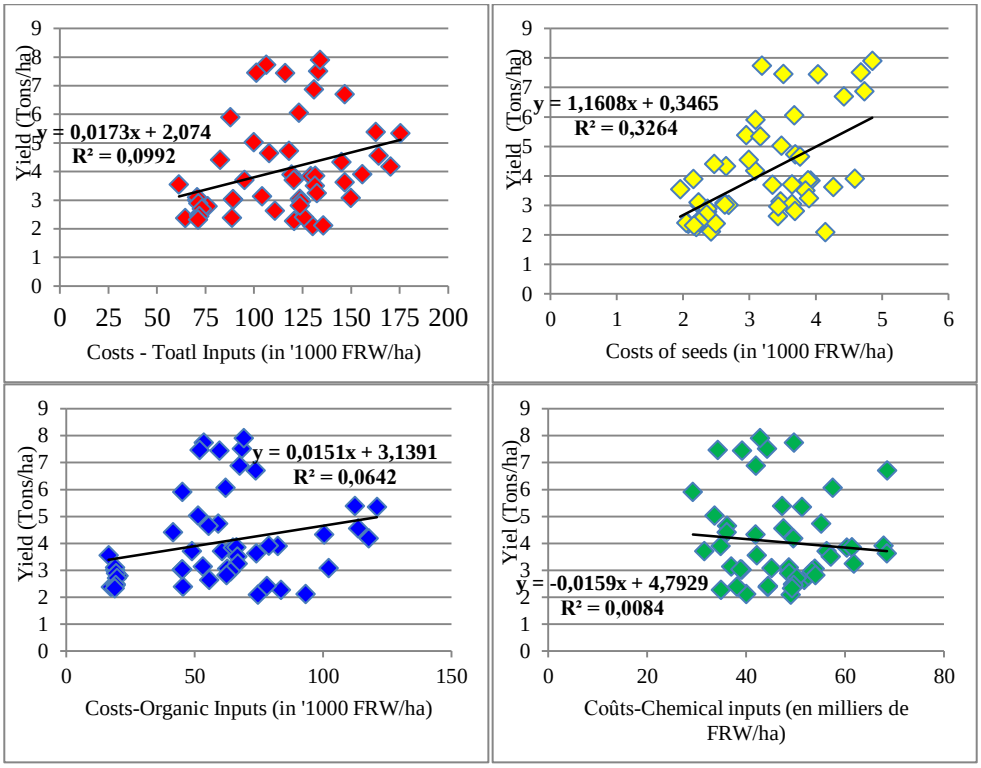

Figure 1: Relationship between cost inputs and the productivity (Tons/ha)
Source: Authors based on 2014 and 2015 research findings

It is worth noting that in addition to 'input' factor proven as playing a crucial role in increasing productivity, there are other factors to which the variation of productivity observed among different operating sub-groups is attributable. They include (i) soil conditions or geographic potential related to maize growing, varying from one region to another ; (ii) the level of adoption of agricultural technologies and innovations varying from one producer to another and from one production zone to another (the varying level of fertilizers utilization for the improvement of soil quality and plant protection products for fighting against parasites and diseases attacking maize in the fields) ; (iii) the variability of various seeds used by the producers ; and (vi) technical support serves received by the producers differing from one zone to another, playing a crucial role in the growing process pattern.

Analysis of Rwandan agricultural trade performance: A case of maize sector

The analysis of agricultural trade performance of a given sector can be carried out by assessing trade indices of competitiveness (Latruffe, 2010). There is a list of neoclassical economics based indicators focusing on trade success and measuring competitiveness with the real exchange rate, comparative advantage indices, and export or import indices (Latruffe, 2010). Comparing with indices based on accounting data, trade indices offer two main advantages: (i) the costs of marketing and transport to and from the port of entry are also taken into account, and (ii) demand and supply responses are considered simultaneously (Frohberg and Hartmann, 1997). The results reported in Table 1 reveal that NEI index is mostly negative for maize grain and maize flour indicating that Rwanda is a net importer of maize grain and maize flour while the GL index is positive and close to zero for the study period, and using a threshold of a GL measure of 0.5 (Banterle and Carraresi, 2007), the results attest that Rwanda is exhibiting a strong inter-industry trade for many years out of 14 considered for the study period. This is explained by the fact that, even though Rwanda has improved its agricultural production following the implementation of various strategic plans for agricultural transformation, it has not yet managed to produce enough for its population hence the increase of imports. In addition, as mentioned by Musabanganji (2017), the rise in imports is also due to the fact that Rwanda has relatively high production costs for many agricultural products in the East African community region. The study results also attest that, in addition to being an importer of maize grain and maize flour, Rwanda is an exporter of some of its agricultural products. Indeed, as USAID/EAT (2013) points out, Rwanda is the main source of agro-food products including corn flour, grain corn, beans, dairy products and livestock formally or informally imported by the eastern region of the Democratic Republic of Congo inhabited by more than 2 million inhabitants (including 1.8 million for Bukavu and Goma) who cannot be fed only by local production. The Akagera region in Tanzania, Burundi and Uganda are also the importing regions of Rwanda's agricultural products and are the main markets for its agricultural production (Musabanganji *et al.*, 2016a). These trade flows result from its integration into the EAC since 2007 which has facilitated access to these markets. Moreover, it should be noted that following its accession to the EAC, Rwanda can develop its export potential, but success will depend more on the increased accompanying measures to develop a dynamic commercial network that can provide specific products in the identified markets. It is worth noting that during the period from 2000 to 2007, data on imports and exports of maize flour are not available.

Table 1.Trade indicators for Rwandan maize flour and maize grain

Year	*Maize grain*		*Maize Flour*	
	NEI	***GL***	***NEI***	***GL***
2000	-1,00	0,00	NA	NA
2001	-1,00	0,00	NA	NA
2002	-1,00	0,00	NA	NA
2003	-0,88	0,12	NA	NA
2004	-1,00	0,00	NA	NA
2005	-1,00	0,00	NA	NA
2006	-0,85	0,15	NA	NA
2007	-0,64	0,36	NA	NA
2008	-0,99	0,01	0,02	0,98
2009	-1,00	0,00	-0,98	0,02
2010	-0,97	0,03	-0,83	0,17
2011	-0,96	0,04	-0,78	0,22
2012	-0,68	0,32	0,4	0,6
2013	-0,65	0,35	0,69	0,31
2014	-0,98	0,02	0,76	0,24
2015	-0,88	0,12	0,42	0,58
2016	-0,87	0,13	0,64	0,36
2017	-0,87	0,13	0,61	0,39

Source: Own calculations based on FAOSTAT and NBR data.

CONCLUSION AND RECOMMENDATIONS

The results of this study show that although farmers sometimes do better in farming, it should be noted that, in the absence of adequate technical assistance, their knowledge regarding inputs use is not certain. However, in addition to putting in place actions to improve the economic conditions of maize producers like easing access to credit, it is also necessary to improve technical assistance for maize producers and increase sensitization sessions on the use of inputs. The monitoring of farmers' activities on regular basis, the training on farming techniques and basics of accounting analysis of agricultural production (for at least those with a primary level or team leaders), the adoption of technological package at the level of each cooperative seem to be necessary measures that would contribute to the increase of productivity at the level of maize producers' cooperatives. The analysis of NEI and GL trade performance indicators for maize flour and maize grain reveals that Rwanda is a net importer of maize flour and maize grain. For these two foodstuffs, it is clear that if Rwanda manages, through policy and institutional actions, to remove or alleviate the bottlenecks ─ among which the financial difficulties and lack of access enough technical knowledge ─ that prevent farmers

from producing enough for export, it can have a competitive advantage in the neighboring countries' markets whose access is facilitated by its accession to the EAC. Low productivity implies low trade performance. Thus, increasing the competitiveness of agriculture throughout modernization of farms, precisely more investment in new technology of maize production can lead to increasing of productivity and improving trade performance. In this regard, it should be noted that non government organizations, institutions of higher education and scientific research and government agencies as the Rwanda Agriculture Board should work in synergy to improve their support to agricultural cooperatives especially in the transfer of knowledge and modern technology through demand-tailored training and demonstration sessions to help increase the agricultural output and that of the maize sector in particular.

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SOIL NITRATE AND ORGANIC CARBON IMPROVEMENT BY USE OF LEGUMINOUS PLANTS AS COVER CROPS IN AN ORGANIC OLIVE ORCHARD

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ABSTRACT

The organic olive orchard represents about 0.5 Mio ha in EU and 36% are located in Spain. This production system implies an economic opportunity for rural areas. The use of cover crops between the rows of olive trees is an agricultural practice that farmers are progressively adopting since they reduce soil erosion while improve soil quality. Due to an usual fertilisation strategy in organic farming is the use of leguminous plants, three legumes (*Vicia sativa*, *Vicia ervilia* and *Vicia villosa*) used as cover crops were compared to study their capacity to protect the soil and improve soil fertility during 4 years. Two soil managements were considered after mowing cover crops: plant residues left on surface or incorporated into the soil. Soil nitrate and coverage were monitored monthly and soil organic carbon (SOC) was analysed at the end of every growing season. Despite not being the best species to protect the soil, the coverage at the end of decomposition period increased by 32% in 4 years in the management without incorporation, providing values over 30% of cover. The soil nitrate recorded in May, when the demand for N by the olive tree is greater, increased in the study period by 70% with the residues left on surface and by 50% when the residues were buried. A carbon sequestration rate of 1.08 Mg C ha^{-1} y^{-1} was reached by *V. villosa* without incorporation. Where residues were incorporated, *V. sativa* obtained the highest carbon fixation with 1.21 Mg C ha^{-1} y^{-1}.

Keywords: *Soil protection, soil nitrogen fixation, carbon sequestration, mulching.*

INTRODUCTION

Olive orchards are currently grown in areas with Mediterranean climate covering approximately 10 Mio ha globally (FAOSTAT, 2016), mainly located in Mediterranean basin. Spain is the greatest producer country and has a surface of 2.65 Mio ha cultivated, 1.6 Mio ha located in the region of Andalusia (South Spain) (MAPAMA, 2017). This crop is not very demanding in term of soil fertility

and depth. Historically, olive orchards have occupied hilly areas with shallow soils (Taguas and Gómez, 2015). These soils are very susceptible to be degraded due to erosion, which is favoured by some inappropriate practices like the intensive tillage (Rodríguez-Entrena *et al*., 2014). Cover crops (CC) in the inter-row of olive trees have proven to be an efficient practice to reduce soil and nutrient losses (Francia *et al*., 2006; Ordóñez-Fernández *et al*., 2007).

Organic farming represents an economic opportunity for rural areas, which are generally unproductive, offering a quality product very demanded in Europe (FAO, 2000). The European organic olive orchards represent about 0.5 Mio ha of which 36% are planted in Spain (EUROSTAT, 2016). Concretely, over 75000 ha are located in Andalusia (MAGRAMA, 2016). This production system is being promoted by political plans, since it produces food of high quality respecting the environment. The use of conventional fertilisers of high solubility is limited in organic production, therefore the nutrition of plant can be a significant problem (Rodrigues *et al*., 2006). A usual fertilisation strategy in organic olive orchards is the use of leguminous plants as green manure. Legumes, through symbiosis with *Rhizobium*, can fix atmospheric N, which is incorporated into the soil improving fertility and microbial activity (Stagnari *et al*., 2017). Thus, the use of leguminous plants could reduce soil and nutrients losses (Sastre *et al*., 2017) while the olive trees are nourished by the decomposition of their residues (Rodrigues *et al*., 2013). Few studies compare between different leguminous species assessing the amount of mineral nitrogen in soil. In addition C sequestration through cover cropping has been studied in this work. The potential of CC to mitigate climatic change has been studied by some authors (Repullo-Ruibérriz de Torres *et al*., 2012) but the most of works are focus on comparing different soil managements.

Three different leguminous plants have been tested and compared to spontaneous vegetation to assess the capacity to protect the soil and improve soil nitrogen and carbon. Furthermore, two soil managements: mowing (M) and mowing plus incorporation (M+I) of residues into the soil, have been studied.

MATERIAL AND METHODS

The experiment was conducted for 4 growing seasons (2012-2013 to 2015-2016) in an organic olive orchard sited in the experimental station 'Alameda del Obispo' belonging to IFAPA (Andalusian Institute for Research and Training in Agriculture) near Guadalquivir River in Córdoba (Spain). The olive trees belong to "Picual" variety, 15 years old, and the plantation pattern is 6×5 m. Three leguminous commonly used as CC in Mediterranean areas, were sown: common vetch (*Vicia sativa* L.), bitter vetch (*Vicia ervilia* L.) and hairy vetch (*Vicia villosa* Roth.). They were studied and compared to vegetation that grew spontaneously in the field, *Medicago polymorfa, Bromus* sp, *Diplotaxis virgata*, *Hordeum leporinum* and *Anagallis arvensis* were identified as the most abundant species. The legumes were seeded at a rate of 200 kg ha^{-1} of cover every year in November to ensure their establishment.

The experimental design was split-split plot with five replicates. Each block included two inter-rows with the aim of comparing to soil management after the mechanical mowing of the CC. In one of the inter-rows plant residues were buried by a discs harrow pass (M+I), in the other inter-row the residues were left on the surface to decompose as mulch (M). The mowing was performed by a hammer cutter at the end of April every year.

The biomass of the CC and their residue after mowing was measured in a metal frame of 0.25 m^2 randomly placed in each subplot, which served to mark out the sampling points for soil samples. After mowing, the monitoring was just carried out in the M area. The soil cover of residues was also measured following the subjective valuation per sector method (Agrela *et al*., 2003), using a frame of 1 m^2, divided into one hundred grids. One permanent point per block and soil management was selected to assess the evolution of coverage.

The soil was sampled in one point per block and soil management at depth of 0-5, 5-10 and 10-20 cm with an Edelman auger every month. The soil samples were taken in the points selected for biomass when it was taken. In addition, core cylinders of known volume were used to measure the bulk density. The soil samples were air-dried and sieved through a 2 mm mesh sieve for their subsequent analysis. Soil nitrate was analysed monthly according to the method described by Griess-Illosvay (Bremner and Keeney, 1965) and soil organic carbon (SOC) was analysed at the end of every growing season by Walkley-Black chromic acid wet oxidation method (Sparks *et al*. 1996).

RESULTS AND DISCUSSION

The evolution of the biomass of CC was monitored every season (Fig. 1). In the developing stage the growth of plants was similar in M and M+I managements. After mowing, the decomposition period only was able to be studied in M system, since the residues were buried in the other management. The decomposition pattern was similar for all leguminous species. However, the spontaneous vegetation had smaller amount of biomass except in the last season. Due to the soil had been managed without weeds before the beginning of the experiment, the seed bank was scarce in this treatment. In the last season, the subplots of spontaneous vegetation obtained a large amount of biomass, which was greater than *V. sativa* and *V. ervilia*. Another leguminous plant, *Medicago polymorfa*, was the most abundant species of the spontaneous flora. It was the treatment with the highest amount of residue at the end of the decomposition period in the last season (Fig. 1). This is a critical point since the risk of erosion increases with the intensive rainfall events in autumn. From an environmental point of view, the selection of a cover crop that maintains the soil protected at that period until the growth of CC in the new season is critical (Rodríguez-Lizana *et al*., 2018). The maintenance of cover is the most effective way to protect the soil against erosion. The table 1 shows the percentage of cover in the M system from mowing to the end of decomposition period.

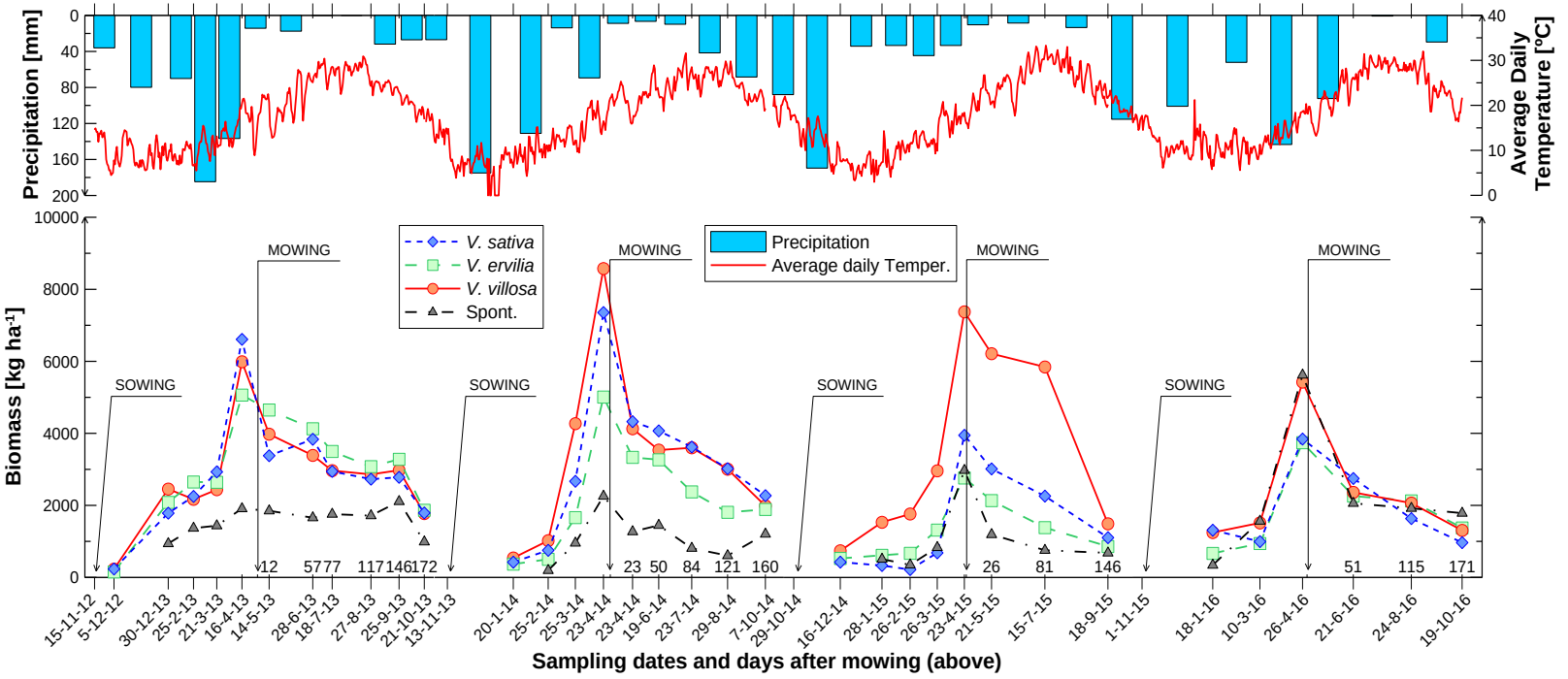

Fig. 1. Evolution of CC biomass and residue after mowing of CC during 4-season study period. The average of both soil managements (M and M+I) is represented before mowing, after mowing, M system is represented. Average daily temperature and precipitation between sampling dates is also shown.

Table 1. Cover of the studied CC after mowing (M management). Different letters indicate significant differences according to LSD test ($P \leq 0.05$). DAM: Days After Mowing.

Season	Date	DAM	*V. sativa*	*V. ervilia*	*V. villosa*	Spont.
1	14/05/2013	12	89.6 a	87.0 a	86.2 a	59.6 b
	28/06/2013	57	86.8 a	83.4 a	82.8 a	51.4 b
	18/07/2013	77	82.2 a	75.6 a	78.8 a	48.2 b
	27/08/2013	117	75.6 a	70.8 a	75.0 a	44.0 b
	25/09/2013	146	49.4 a	46.6 a	47.6 a	36.4 a
	21/10/2013	172	36.8 a	35.2 a	30.4 a	25.0 a
2	23/05/2014	23	91.6 a	88.4 a	92.2 a	38.2 b
	19/06/2014	50	84.8 a	70.6 a	85.2 a	27.4 b
	23/07/2014	84	83.6 a	61.4 bc	73.6 ab	30.8 c
	29/08/2014	121	76.4 a	52.4 b	69.2 ab	25.4 c
	07/10/2014	160	59.4 a	36.0 bc	58.0 ab	28.0 c
3	21/05/2015	26	88.8 ab	85.2 b	98.4 a	57.6 c
	15/07/2015	81	59.4 b	51.2 b	84.4 a	37.2 c
	18/09/2015	146	49.4 a	33.8 b	62.8 a	22.0 b
4	21/06/2016	51	62.4 ab	64.0 ab	69.6 a	48.2 b
	24/08/2016	115	61.8 a	65.0 a	61.4 a	57.4 a
	19/10/2016	171	39.0 b	57.0 a	44.2 b	42.2 b

V. villosa was the species with the highest amount of biomass in the second and third seasons. In the last season, *V. ervilia* was not established properly and this treatment was composed partially by spontaneous vegetation, which had a slower decomposition than legumes due to the low C:N ratio (Quemada, 2004). Other species commonly used as CC such as grasses or crucifers are more suitable for erosion control since they have lower decomposition rate than legumes (Repullo-Ruibérriz de Torres *et al*., 2012). However, the cover at the end of decomposition period provided values over 30%, threshold internationally considered in conservation agriculture (González-Sánchez *et al.*, 2015). In average, all treatments increased the cover at the end of decomposition period in the last season with regard to the first one (Table 1). Legumes are mainly recommended for improving soil fertility either in M or M+I systems. When the residues are buried, the decomposition is faster due to soil moisture and the metabolic activity of soil microorganisms (Gómez-Muñoz *et al.*, 2014). In this experiment, soil nitrate was usually greater in M+I than in M management during the study period. Table 2. indicates the soil nitrate concentration at May of every year, since spring is considered the period when the demand for N by tree is greater.

Table 2. Soil nitrate (kg ha^{-1}) at the several depths studied in the month of May every season.

		Mowing				Mowing + Incorporation			
Season	Depth (cm)	*V. sativa*	*V. ervilia*	*V. villosa*	Spont.	*V. sativa*	*V. ervilia*	*V. villosa*	Spont.
1	0-5	17.67	18.43	27.46	2.90	8.94	8.57	11.64	6.04
	5-10	8.29	7.63	12.15	2.94	7.20	9.47	9.45	7.48
	10-20	5.90	10.16	15.06	3.52	12.55	18.77	13.94	8.74
	0-20	31.87	36.22	54.66	9.36	28.69	36.80	35.03	22.26
2	0-5	21.42	67.32	82.03	11.60	72.07	24.54	114.45	12.99
	5-10	18.37	17.93	66.49	11.04	76.17	25.88	170.01	14.04
	10-20	26.29	29.41	36.23	17.63	122.26	51.77	222.88	22.72
	0-20	66.07	114.67	184.75	40.27	270.50	102.20	507.34	49.75
3	0-5	2.82	3.01	24.48	2.41	1.97	4.54	14.06	2.96
	5-10	2.71	3.42	16.87	2.61	2.45	4.63	15.98	2.71
	10-20	4.35	3.70	22.98	4.31	3.74	6.25	25.17	3.70
	0-20	9.89	10.12	64.33	9.33	8.17	15.42	55.21	9.37
4	0-5	65.20	27.59	92.21	53.95	21.73	19.80	37.06	23.70
	5-10	28.29	13.76	23.06	27.57	16.60	15.48	27.04	9.78
	10-20	39.64	14.55	43.27	24.36	19.31	26.36	33.78	17.17
	0-20	133.13	55.90	158.54	105.87	57.64	61.64	97.88	50.66

In the first season, similar nitrate values were obtained between management systems except for spontaneous vegetation that was higher in M+I. In the second season, the CC had a good development and more biomass was obtained. This led to obtain better increment of soil nitrate, mainly in M+I. *V. villosa* reached over 500 kg nitrate per ha in the first 20 cm in that season. In the third season, lower concentrations were obtained due to a worse growing of CC in the developing stage, although *V. villosa* provided a great amount of soil nitrate in both managements. M was the best management systems in term of soil N in the last season. It was due probably to the precipitation recorded between May, which was scarce in the other seasons. The soil unprotected in M+I facilitated the lixiviation of nitrates in comparison to M system. In all cases *V. villosa* was the species that produced the greatest amount of biomass and provided the highest soil nitrate concentration. Regarding SOC, all seeded CC increased the SOC in the first 20 cm (Table 3). However, the tillage performed at sowing every year produced C emissions, the low biomass reached by spontaneous vegetation in both management, was not enough to improve the SOC in this treatment along the study period. The highest carbon sequestration rate was reached by *V. villosa* without incorporation. Nevertheless, where residues were incorporated, *V. sativa* obtained the highest carbon fixation with 1.21 Mg C ha^{-1} y^{-1}.

Table 3. SOC fixation in 4-season study and annual fixation for that period.

Species	Mowing		Mowing + Incorporation	
	SOC fixation (4 seasons)	Annual SOC fixation	SOC fixation (4 seasons)	Annual SOC fixation
	kg ha^{-1}	*Mg ha^{-1} yr^{-1}*	*kg ha^{-1}*	*Mg ha^{-1} yr^{-1}*
V. sativa	2264.1	0.57	4857.1	1.21
V. ervilia	790.3	0.20	1408.9	0.35
V. villosa	4327.6	1.08	2035.0	0.51
Spont.	-772.0	-0.19	-1937.4	-0.48

CONCLUSIONS

Legumes CC are useful to improve soil fertility through increments in nitrate and SOC. The M+I management system accelerated the supply of soil nitrate by residue, but M soil management maintained the soil covered and protected from erosion for a longer period. The use of CC allows obtaining agronomic and environmental benefits which is an opportunity for organic farming.

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EFFECT OF NITROGEN ON THE ACCUMULATION AND REUTILIZATION OF DRY MASS IN GRAIN SORGHUM

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ABSTRACT

Accumulation and reutilization of dry mass until anthesis and during a grain filling period of sorghum in response to nitrogen fertilization in rates 0, 60, 120, 180, 240 and 300 kg N.ha^{-1} was studied in a field experiment. Grain sorghum hybrid EC Alize was grown under not- irrigated conditions in the experimental field of Agricultural University of Plovdiv, Bulgaria. The experimental design was a randomized, complete block design with four replications with a size of experimental plots of 20 m^2 after wheat as predecessor. Standard farming practices for the region of Southern Bulgaria were applied. It was established that nitrogen fertilization significantly increased the amount of accumulated dry mass at anthesis and total above ground dry mass at maturity compared to N_0. Not significant effect of higher rates (180, 240 and 300 kg N.ha^{-1}) on the dry mass accumulation of sorghum was found. Average post anthesis net dry mass accumulation was 3291 kg.ha^{-1} and its amount increased in parallel with the nitrogen rate up to N_{180}. The highest dry mass translocation, translocation efficiency, and contribution of pre-anthesis assimilations of the grain was established at nitrogen rate N_{120} with values 2073 kg.ha^{-1}, 25.0 % and 41,8 %, respectively. Growth of sorghum at higher nitrogen rates N_{180}, N_{240}, N_{300} significantly decreased efficiency of dry mass translocation and contribution of pre-anthesis assimilations of the grain. Nitrogen fertilization had very strong negative correlation with dry mass translocation efficiency (-0.860*) and contribution of pre-anthesis assimilations of the grain (-0.863*). Very strong positive correlation (0.988**) was found between dry mass translocation efficiency and contribution of pre-anthesis assimilations of the grain.

Keywords: *Grain sorghum, Nitrogen, Dry mass, Reutilization.*

INTRODUCTION

Yield of cereals crops is mainly determined by the source of assimilations, the irrigation capacity or collimated by both (Borras *et al.*, 2004; Dordas, 2009). The supply of assimilation of the grain may originate from current assimilation and matter assimilated before anthesis and is stored temporarily in the leaves, culms,

chaff, and other vegetative plant parts (Van Sanford and MacKown, 1987). Many factors can affect the source–sink relations during the different growth phases including genotype, temperature, rainfall and fertilization (Miralles and Slafer, 2007; Mohammadi and Amri, 2008).Nitrogen is the main nutrient that affects the assimilation production and distribution and influence directly or indirectly the source–sink relation (Arduini *et al*., 2006; Muchow, 1988). The most active acceptor for assimilations in anthesis and after this phase is grain. Sorghum has better ability to tolerate drought stress compared with other crops and is known as an index for drought resistance of agronomic crops (Beheshti, 1997). Sorghum is mainly grown under non-irrigated fields where stressful conditions during grain filling can limit productivity and increase the dependence of the yield of spare assimilations. In Bulgaria studies were focused on studying wheat (Kostadinova and Panayotova, 2014) and barley (Kostadinova and Ganusheva, 2013; Kostadinova, 2014) and there is not enough information about grain sorghum, especially about the contribution of pre- and post-anthesis assimilation for grain production. A better understanding of the relationship between vegetative and grain reserves in this culture is important for establishing physiological and agrochemical characteristics suitable for adaptation to adverse external effects, mainly related to climate changes such as frequent droughts and other external factors that lead to the modification of grain yield (Borras *et al*., 2004; Beheshti and Behboodi, 2010). The aim of this study was to study the effect of nitrogen fertilization rates on the parameters of dry mass accumulation and reutilization in grain sorghum plants.

MATERIAL AND METHODS

The investigation was carried out on the experimental field of Agricultural University of Plovdiv, Bulgaria in 2017 under non-irrigated conditions. Accumulation, translocation and reutilization of dry mass until anthesis and during a grain filling period of grain sorghum hybrid EC Alize in response to nitrogen fertilization in rates 0, 60, 120, 180, 240 and 300 kg $N.ha^{-1}$ was studied. The experimental design was a randomized, complete block design with four replications with a size of experimental plots of 20 m^2 after wheat as predecessor. Total nitrogen as NH_4NO_3 was applied as pre-sowing fertilization on the background $P_{50}K_{50}$ fertilization as triple superphosphate and potassium chloride, respectively. Standard farming practices for the region of Southern Bulgaria were applied. The soil type of the experimental field is alluvial-meadow *Mollic Fluvisols* (FAO, 2006) with slightly alkaline reaction pH_{H2O}=7.80. The content of available nutrients in the soil before sowing of the sorghum was mineral N - 27.6 mg $Nmin.kg^{-1}$; available phosphorus (Egner-Ream) 158 mg $P_2O_5.kg^{-1}$ and exchangeable potassium 210 mg $K_2O.kg^{-1}$. Meteorological conditions during vegetation period of sorghum were recorded daily in the experimental area and are given in Table 1, together with the long-term average of temperature and precipitations. The values of temperature and precipitations during the vegetation of sorghum characterized hydro-thermal conditions of the period as warm and dry.

Table 1. Hydro-thermal conditions during sorghum vegetation period.

	Temperature (^oC)					Precipitation ($L.m^{-1}$)				
	April	May	June	July	August	April	May	June	July	August
2017	12.7	17.6	23.7	25.1	25.4	26.1	52.7	15.4	29.8	9.2
Long-term norm	12.2	17.2	20.9	23.2	22.7	45	65	63	49	31

At about mid-anthesis and at maturity the above ground dry mass of sorghum plants of each plot was analyzed by representative sampled areas of 1 m^2. Whole plants (leaves + stems + flowered panicles) were analyzed at anthesis. At maturity the plant samples were separated in two components - grain and straw (leaves + stems + chaffs). After cutting and separation at anthesis and maturity the samples were oven-dried at 70 °C for 48 h. Accumulation and reutilization of dry mass within the sorghum plants were studied on the base of parameters referring to dry matter translocation. The parameters were calculated as follows according to different authors for cereals (Cox *et al*., 1985ab; Abeledo *et al*., 2008; Dordas, 2009):

- Pre-anthesis and post-anthesis dry mass accumulation (DM, $kg.ha^{-1}$);
- Dry mass translocation (DMT, $kg.ha^{-1}$) = dry mass at anthesis - dry mass of straw at maturity;
- Dry mass translocation efficiency (DMTE, %) = (dry mass translocation / dry mass at anthesis) × 100
- Contribution of pre-anthesis assimilates to the grain (CAVG, %) = (dry mass translocation / grain yield) × 100 (Papakosta and Gagianas, 1991)
- Post anthesis net dry mass accumulation, or the increased biomass during the period of grain filling, were estimated as the difference between total DM at maturity and the total DM at anthesis (Przulj and Momcilovic, 2001).

An overall analysis of variance (ANOVA) was performed to evaluate the effect of the experimental treatments on the referred variables. In order to establish the difference among the means Duncan's multiple range test at level of significance $p \leq 0.05$ was used. Pearson correlation coefficient was calculated for assessment of the dry mass translocation efficiency and contribution of pre-anthesis assimilates to the grain with some parameters of dry mass reutilization. Analyses were performed with a personal computer using the SPSS™ (SPSS Inc., IL, USA) statistical program.

RESULTS AND DISCUSSION

Nitrogen fertilization in rates N_{60} – N_{300} significantly increased the accumulated dry biomass of sorghum at anthesis compared to the control N_0 (Table 2). The highest amount of dry mass was obtained at N_{300}, which was by 1871 $kg.ha^{-1}$ more than N_0. Growing of hybrid EC Alize at higher levels of nitrogen rates N_{180}, N_{240} and N_{300} did not significantly change dry mass at anthesis, vegetative plant parts (leaves+stems+chaff) and total DM at maturity. The nitrogen rates 60, 120, 180, 240 and 300 kg $N.ha^{-1}$ demonstrated higher grain yield by 7.1, 17.6, 25.8, 19.3 and

17.1 %, in respect to control plants without nitrogen fertilization. Harvest index slightly depended on nitrogen fertilization, but its value proven decreased at N_{300}.

Table 2. Dry mass at anthesis and maturity in dependence of nitrogen fertilization rate.

N rate	DM at anthesis, kg.ha^{-1}	DM grain, kg.ha^{-1}	DM of straw, kg.ha^{-1}	Total DM at maturity, kg.ha^{-1}	HI, %
N_0	7630 d*	4572 d	5826 c	10398 d	44.0 ab
N_{60}	8390 c	4897 c	6407 bc	11304 c	43.4 ab
N_{120}	9000 b	5378 b	6751 b	12129 b	44.4 a
N_{180}	9492 a	5750 a	7419 a	13168 a	43.7 ab
N_{240}	9380 ab	5455 b	7535 a	12989 a	42.0 bc
N_{300}	9501 a	5355 b	7795 a	13150 a	40.7 c
Average	***8899***	***5234***	***6955***	***12190***	***43.0***

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

Table 3. Post anthesis net dry mass accumulation, kg.ha^{-1} and ratio of pre- to post anthesis accumulated dry mass of sorghum in dependence of nitrogen fertilization.

N rate	Post anthesis net dry mass, kg.ha^{-1}	Ratio of pre- to post anthesis accumulated dry mass
N_0	2768 c*	2.76
N_{60}	2914 bc	2.88
N_{120}	3129 b	2.88
N_{180}	3676 a	2.58
N_{240}	3609 a	2.60
N_{300}	3649 a	2.60
Average	***3291***	***2.72***

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

The difference between total dry mass at maturity and the total dry mass at anthesis was positive value in all nitrogen treatments (Table 3). Consequently, the plants increased biomass during the period of grain filling. Average post anthesis net dry mass accumulation was 3291 kg.ha^{-1}. kg.ha^{-1}. It was changed from 2768 kg.ha^{-1} (N_0) to 3676 kg.ha^{-1} (N_{180}). Post anthesis net dry mass accumulation of sorghum increased in parallel with the nitrogen rate up to N_{180}. The results showed that higher than 180 kg N.ha^{-1} rates did not increase the quantity of post anthesis net dry mass accumulation. The ratio of pre- to post anthesis accumulated dry mass was higher than one of all studied nitrogen rates. This indicated that plants accumulated more dry mass in the pre-anthesis period than after anthesis. The ratio of pre- to post anthesis accumulated dry mass varied in a range 2.58 – 2.88. A similar effect of increased biomass during the period of grain filling was found for grain sorghum (Ramazanzadeh *et al*., 2012; Beheshti and Behboodi, 2010) and sunflower (Koutroubas *et al*., 2004).

Table 4. Dry mass translocation, dry mass translocation efficiency and contribution of pre-anthesis assimilations of the grain in dependence of nitrogen fertilization.

N rate	Dry mass translocation, kg.ha^{-1}	Dry mass translocation efficiency, %	Contribution of pre-anthesis assimilates to the grain, %
N_0	1804 c	23.6 ab	39.5 a
N_{60}	1983 b	23.6 ab	40.5 a
N_{120}	2249 a	25.0 a	41.8 a
N_{180}	2073 ab	21.8 b	36.1 b
N_{240}	1845 c	19.7 bc	33.8 bc
N_{300}	1706 d	18.0 c	31.9 c
Average	***1943***	***22.0***	***37.3***

*Values in each column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

The contribution of pre-anthesis assimilations of the grain may be crucial for maintaining grain yield when adverse climatic conditions reduce photosynthesis, water and mineral uptake (Arduini *et al*., 2006). The highest dry mass translocation, translocation efficiency, and contribution of pre-anthesis assimilations of the grain was established at nitrogen rate N_{120} with values 2073 kg.ha^{-1}, 25.0 % and 41,8 %, respectively. Growth of sorghum at higher nitrogen rates N_{180}, N_{240}, N_{300} significantly decreased efficiency of dry mass translocation and contribution of pre-anthesis assimilations of the grain.

Table 5. Correlation of dry mass translocation efficiency and contribution of pre-anthesis assimilations of the grain with parameters of dry mass reutilization

Parameters	Dry mass translocation efficiency, r	Contribution of pre-anthesis assimilates to the grain, r
Nitrogen fertilization	-0.860*	-0.863*
Grain DM	-0.389	-0.461
Straw DM	-0.796	-0.817*
Maturity (Grain+Straw) DM	-0.673	-0.714
Anthesis DM	-0.609	-0.643
Post anthesis net DM accumulation	-0.768*	-0.821*
Dry mass translocation	0.724	0.679
Dry mass translocation efficiency	1	0.988**

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

The correlation analysis was represented in Table 5. It was shown that nitrogen fertilization very strong and negatively correlated with dry mass translocation efficiency (r = -0.860*) and contribution of pre-anthesis assimilations of the grain

(r = -0.863*). Very strong positive relationship (r = 0.988**) was found between dry mass translocation efficiency and contribution of pre-anthesis assimilations of the grain.

CONCLUSIONS

Nitrogen fertilization significantly increased the amount of accumulated dry mass at anthesis and total above ground dry mass at maturity compared to N_0. Not significant effect of higher rates (180, 240 and 300 kg $N.ha^{-1}$) on the dry mass accumulation of sorghum was found. Average post anthesis net dry mass accumulation was 3291 $kg.ha^{-1}$ and its amount increased in parallel with the nitrogen rate up to N_{180}. The highest dry mass translocation, translocation efficiency, and contribution of pre-anthesis assimilations of the grain was established at nitrogen rate N_{120} with values 2073 $kg.ha^{-1}$, 25.0 % and 41,8 %, respectively. Growth of sorghum at higher nitrogen rates N_{180}, N_{240}, N_{300} significantly decreased efficiency of dry mass translocation and contribution of pre-anthesis assimilations of the grain. Nitrogen fertilization very strong negatively correlated with dry mass translocation efficiency (-0.860*) and contribution of pre-anthesis assimilations of the grain (-0.863*). Very strong positive correlation (0.988**) was found between dry mass translocation efficiency and contribution of pre-anthesis assimilations of the grain.

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EFFECT OF DN1 BACTERIAL STRAIN APPLIED BY DIFFERENT METHODS ON SOME MORPHOLOGICAL CHARACTERISTICS OF STRAWBERRY CV. SAN ANDREAS (*Fragaria X ananassa* Duch.)

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ABSTRACT

There have been quite intensive studies on the use of Plant Growth-Promoting Rhizobacteria (PGPR) in agriculture. *Acidovorax facilis* strain DN1 is one of the PGPR commonly used. The effect of DN1 bacterial strain on some morphological characteristics of strawberry cv. San Andreas was investigated. The DN1 bacterial strain was applied via soil, leaf, and soil + leaf, for 3 months (once a month) to strawberry plants. The DN1 spores were prepared with 0.2% boron, 10% corn starch and distilled water. The bacterial solution was applied to plants at the following day with a hand pump (to leaves; 50 cc) and graduated cylinder (250 cc each 5-liter pot). After 3 treatments, plants removed from pots and data collected.
According to the results, DN1 bacterial strain often had a positive effect on the morphological and fruit characteristics. Spraying treatment was the most effective way for the stem and root traits we evaluated (crown diameter: 36.87 mm; stem fresh weight: 63.64 g; leaf number: 38.69; root fresh weight: 34.89 g). In addition, soil + leaf treatment had a positive effect on mean fruit weight (23.57 g) and fruit diameter (27.64 mm). The effect on other properties was also positive, but the root length (26.34 cm) was reduced in leaf treatment compared to the control (29.69 cm). It is expected that the most effective treatment is the combined (leaf + soil) treatment, while the leaf treatment may be the most effective method on soils with boron toxicity.

Keywords: *PGPR, DN1 (Acidovorax facilis) strawberry, and boron (B*)

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is an herbaceous and perennial fruit which has an important place among the berries (Ağaoğlu, 1986). Strawberry is a

species belonging to the genus *Fragaria*, the family *Rosaceae*, and the order *Rosales*. Commercially produced species *Fragaria x ananassa* Duch. has a history of 250 years (Hancock, 1999). Strawberry plants can grow in many parts of the world thanks to short, neutral and long day cultivars (Yılmaz, 2009).

Recently, researchers have been searching for various ways to reduce the use of chemical fertilizers and pesticides in agricultural production. These searches are particularly focused on the use of microorganisms (Adesemoye et al., 2008; Ekici et al., 2015). Plant Growth-Promoting Rhizobacteria (PGPR) can be used as a biocontrol agent and/or biofertilizer to stimulate plant growth. Most of these microorganisms belong to genus *Alcaligenes*, *Azotobacter*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Glick, 1995; Burdman et al., 2000; Romerio, 2000; Somers et al., 2004; Tuzlacı, 2014). The use of PGPRs to replace chemical fertilizers which causes soil and water pollution is increasing year by year (Çakmakçı, 2005; Ahemad and Kibret, 2014; Bashan et al., 2014). PGPRs increase plant growth and productivity by reducing harmful effects of the phytopathological microorganisms or give substances to the plant environment (rhizosphere and phyllosphere) which they produce **(**Altın and Bora, 2005; Saleem et al., 2007; Glick, 2012; Bashan et al., 2014). PGPRs are used as biological fertilizers, phytostimulators, rhizoremidators, phytoremediators and biopesticides (Lucy et al., 2004; Somers et al., 2004; Aontoun et al., 1998; Siddiqui, 2006). It has been reported by many researchers that PGPRs can be treated to plants by root inoculation and leaf spraying (Kokalis-Burelle, 2003; Eşitken et al., 2006; Malusa et al., 2006; Aslantaş et al., 2009; Eşitken et al., 2009; Eşitken et al., 2010; Pırlak and Köse, 2010; Ertürk et al., 2012). In strawberry studies, it has been suggested that bacterial treatments increase seedling number and quality (Aslantaş et al., 2010), fruit yield and quality (Pırlak and Köse, 2009; Ertürk et al., 2012), as well as plant growth (Kokalis-Burelle, 2003; Eşitken et al., 2010).

DN1 is a strain of *Acidovorax facilis* (AY581467) and isolated from bermudagrass roots by Wang and Skipper (2004). In this study, cv. San Andreas plants were treated 3 times (once a month) with DN1 bacterial strains through soil inoculation, spraying, and spraying + soil inoculation. The effects of DN1 bacterial strain on some morphological and fruit characteristics were investigated.

MATERIAL AND METHODS

This study was carried out in the greenhouse of the Department of Horticulture on the Faculty of Agriculture of Selcuk University. The plants were planted with a mixture of peat-perlite (2: 1) in 5-liter pots (February 5, 2015). Bacterial solutions (*Acidovorax facilis* strain DN1) were prepared by adding 1 g DN1 spore, 0.2 g boron, and 10 g of cornstarch with 1-liter of distilled water. After 24 h of incubation at room temperature, the solution was treated 3 times (once a month) with a hand pump (spraying treatment: 50 cc to each replicate) and graduated cylinder (soil inoculation: 250 cc to each plant).

The plants were removed in November, and data for crown diameter (CD), plant height (PH), stem fresh weight (SFW), root fresh weight (RFW), crown number (CN), leaf number (LN), root length (RL), chlorophyll content (CC), mean fruit weight (MFW), fruit length (FL), and fruit diameter (FD) were collected. The data were analyzed with One Way Analysis of Variance (ANOVA) and the Duncan Multiple Comparison Test ($p \leq 0.05$) with the IBM SPSS v.20 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY) statistical software package.

RESULTS AND DISCUSSION

The effect of *Acidovorax facilis* strain DN1 on fruit and morphological characteristics was found statistically significant ($p \leq 0.05$). While the spraying DN1 treatment (36.87 mm) maximizes the crown diameter (CD), soil inoculation (26.92 mm) is the treatment that minimizes plant height (PH) (Table 1). However, spraying + soil inoculation (31.88 mm) reduced the CD compared to the control (33.18 mm). In addition, while spraying + soil inoculation (23.10 cm) has been the most beneficial treatment on PH, the shortest plants were obtained from the control (21.76 cm). On the other hand, the most effective treatment for stem fresh weight (SFW) was spraying (63.64 g), while soil inoculation (61.36 g) was the second. Spraying + soil inoculation (53.85 g) was not as effective on SFW as other DN1 treatments, but it is more effective than control (51.17 g). The most effective treatment on the leaf number (LN) associated with SFW was spraying (38.89), but minimal LN was obtained from the control (26.00). However, unlike SFW, soil inoculation (34.90) was less effective than spraying + soil inoculation (34.61).

Soil inoculation and spraying + soil inoculation treatments may be more ineffective than spraying treatment because of the toxic effect of boron used when preparing the bacterial solution. Bacterial treatments reported having a positive effect on the above-mentioned properties on previous studies (Tahmatsidou et al., 2006; Pırlak et al., 2007; Ertürk et al., 2010; Karlıdağ et al., 2013). In a study conducted by Ekici et al. (2015) on broccoli (*Brassica oleracea L. var. italica*) seedlings, it was reported that bacterial treatments increased PH, CD, and SFW compared to control. In another study, it was reported that bacterial treatments increased the length of shoots and shoot diameters in apple (Pırlak et al., 2007). The use of *Bacillus subtilis* strain FZB24-WG in strawberry increased SFW (Tahmatsidou et al., 2006), and bacterial treatments increased the root diameter of the Hayward kiwifruit seedling cuttings (Ertürk et al., 2010) to a considerable extent. In another study, Karlıdağ et al. (2013) reported that the treatment of bacteria to strawberry plants under salt stress increased the SFW.

Table 1. Effects of *Acidovorax facilis* strain DN1 on the means of crown diameter (CD), plant height (PH), stem fresh weight (SFW), and leaf number.

	Crown Diameter (mm)	Plant Height (cm)	Stem Fresh Weight (g)	Leaf Number
Control	$33.18 \pm 0.37^{b*}$	$21.76 \pm 0.15^{c*}$	$51.17 \pm 0.50^{d*}$	$26.00 \pm 0.25^{d*}$
Soil inoculation	26.92 ± 0.57^{d}	23.10 ± 0.08^{a}	61.36 ± 0.39^{b}	34.90 ± 0.36^{c}
Spraying	36.87 ± 0.34^{a}	22.34 ± 0.29^{b}	63.64 ± 0.26^{a}	38.89 ± 0.34^{a}
Spray. + s. inoc.	31.88 ± 0.52^{c}	22.73 ± 0.28^{ab}	53.85 ± 0.19^{c}	34.61 ± 0.35^{b}
p	0.000	0.000	0.000	0.000

*Significant at $p \leq 0.05$ and the value after ± is the standard deviation.

DN1 bacterial treatments used in this study positively affected crown number (CN), root length (RL), root fresh weight (RFW), and chlorophyll content (CC). The most favorable effect on CN and RL was observed in soil inoculation treatment, while the spraying treatment had the best results in terms of RFW (Table 2). On the other hand spraying + soil, inoculation treatment had the best results on CC (Table 2). In addition, all treatments increased CN, RFW, and CC values compared to the control. However, spraying and spraying + soil inoculation on RL were not as effective as a control (Table 2). This might be due to the stronger effect of mineral and water search of roots than the effect of DN1 treatments. According to previous observations, bacterial treatments have a positive effect on above mentioned traits. In some studies, bacterial treatments increased the CN **(**Aslantaş et al., 2010), RL (Ertürk et al., 2010; Ekici et al., 2015), RFW (Tahmatsidou et al., 2006; Karlıdağ et al., 2013; Ekici et al., 2015), and CC (Karlıdağ et al., 2013; Ekici et al., 2015) compared to control. When considering the effects of bacteria on traits except for RL, it is understood that there are similarities between the previous studies and the present study. On the other hand, the boron used while preparing the solution in spraying + soil inoculation treatment may have been toxic to the strawberry plants. As a result of this, the root length may have been shorter than the control. But, it cannot be clearly understood why the roots obtained from spraying treatment were shorter than the control.

Table 2. Effects of *Acidovorax facilis* strain DN1 on the crown number (CN), root length (RL), root fresh weight (RFW), and chlorophyll content (CC).

	Crown Number	Root Length (cm)	Root Fresh Weight (g)	Chlorophyll Content ($\mu g/cm^{2)}$
Control	$4.85 \pm 0.13^{b*}$	$29.69 \pm 0.62^{a*}$	$27.57 \pm 0.43^{d*}$	$42.67 \pm 0.39^{c*}$
Soil inoculation	5.25 ± 0.05^{a}	29.70 ± 0.34^{a}	31.41 ± 0.32^{b}	45.50 ± 0.41^{b}
Spraying	4.93 ± 0.12^{b}	26.34 ± 0.41^{b}	34.89 ± 0.39^{a}	46.16 ± 0.45^{ab}
Spray. + s. inoc.	4.87 ± 0.12^{b}	26.72 ± 0.27^{b}	29.36 ± 0.27^{c}	46.57 ± 0.13^{a}
p	0.006	0.000	0.000	0.000

* Significant at $p \leq 0.05$ and the value after ± is the standard deviation.

It is understood that the effect of DN1 strain on other traits as well as on the fruit characteristics is positive (Table 3). However, the most effective treatments differ

for mean fruit weight (MFW), fruit length (FL), and fruit diameter (FD). While spraying + soil inoculation was the most effective treatment on MFW (13.57 g) and FD (27.64 mm), the most effective treatment for the FL was spraying (35.53 mm). On the other hand, the lowest values for all three fruit characteristics were obtained from the control (MFW: 11.51 g, FL: 33.98 mm, and FD: 26.05 mm). It can be said that spraying and spraying + soil inoculations were effective treatments on fruit characteristics in general.

Table 3. Effects of *Acidovorax facilis* strain DN1 on mean fruit weight (MFW), fruit length (FL), and fruit diameter (FD).

	Mean Fruit Weight (g)	Fruit Length (mm)	Fruit Diameter (mm)
Control	$11.51 \pm 0.07^{d*}$	$33.98 \pm 0.06^{c*}$	$26.05 \pm 0.09^{c*}$
Soil inoculation	12.29 ± 0.16^{c}	35.19 ± 0.07^{b}	$26.94 \pm 0.,12^{b}$
Spraying	12.48 ± 0.06^{b}	35.53 ± 0.15^{a}	26.82 ± 0.12^{b}
Spray. + s. inoc.	13.57 ± 0.11^{a}	34.99 ± 0.14^{b}	$27,.64 \pm 0.09^{a}$
p	0.000		0.000

*Significant at $p \leq 0.05$ and the value after ± is the standard deviation.

In previous studies, bacterial treatments had positive effects on fruit characteristics. Ipek et al (2014) reported that bacterial treatments have a positive effect on MFW. Some researchers reported that the efficacy is mixed (Tuzlacı, 2014; Ağgün, 2018), while others reported statistically insignificant effects (Tahmatsidou et al., 2006; Eşitken et al., 2010; Pesakoviç et al., 2013). The results obtained from the present study were in agreement with the study conducted by Ipek et al (2014). According to Pesakoviç et al (2013), bacterial treatments (depending on the bacterial species) have different effects on the FL. The effect on FD was insignificant according to the same study. In the present study, the DN1 strain was positively affected both FL and FD as opposed to the study mentioned above.

CONCLUSION

Given the results obtained from this study, the *Acidovorax facilis* strain DN1 positively affected the morphological and fruit characteristics of San Andreas strawberry cultivar. Particularly spraying has been the treatment that increases most of the features. However, the ineffectiveness of soil inoculation and spraying + soil inoculation treatments may be due to boron toxicity. Consequently, because of the possible boron toxicity, an optimum DN1 treatment was not identified. The DN1 strain dose, number of treatments and the amount of boron in the DN1 solution should be determined in future studies.

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MECHANIZED MANAGEMENT OF PRUNING RESIDUES IN SWEET CHESTNUT ORCHARDS

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ABSTRACT

In Italy, *Dryocosmus kuriphilus* is a major insect pest of chestnut orchards causing gall formation and significant yield losses. The use of the parasitoid wasp *Torymus sinensis* is an effective biological control method that requires the appropriate management of the pruning residues, to preserve the woody galls in which *T. sinensis* adults overwinter. An innovative tractor-pulled combined mechanical shredder was tested for treating the residues, once the pruning operations are completed. The machine processes the residues in a single-step, picking-up the prunings from the orchard floor, shredding them to appropriate size, and collecting them into a rear container. The shredded residues may then be concentrated into a few piles for the long-term field storage, until next spring. The aim of the study was to evaluate the machinery's operative performance, the quality of the shredded biomass and the impact on gall integrity. In the field trials, the machinery effectively shredded the residues achieving an average operative capacity of 1.19 $ha \cdot h^{-1}$ and a work capacity of 1.79 $t \cdot h^{-1}$. Moisture content, apparent bulk density, and particle size distribution of the shredded biomass were determined. Over 95% of wood chips were included in the 3.15-45 mm size range, making them suitable for use in residential biomass furnaces. The calorific power of the chestnut residues was similar to that reported for other hardwood species (18-20 $MJ \cdot kg^{-1}$). Also, the mechanical action of the shredder preserved at least two thirds of the winter galls, safeguarding the parasitoid's life cycle.

Keywords: *Gall wasp, Castanea sativa, mechanical harvesting, biomass quality, gall integrity.*

INTRODUCTION

Biomasses are an important energy source, that could be further exploited to reduce fossil fuel dependency in energy production. This energy source can play an important role in increasing the share of renewable energy in the global energy mix. Biomass is a widely available energy resource at a local scale, therefore it would allow a delocalized energy production at limited costs and with simple

power facilities (McKendry, 2002). At present, bioenergy (energy from organic matter), represents the fourth largest energy source (after coal, oil and natural gas) and, globally, bioenergy (including waste) accounted for 14% of the world's energy consumption in 2012 (WBA, 2014). Recent improvements in combustion technology have increased the efficiency of biomass use. These technological upgrades drastically reduce pollutant emissions and increase furnace combustion efficiency to more than 85%. Biomasses for energy use can be obtained from different sources, as the following: purposely grown woody plantations, forest maintenance operations, wood processing waste, and orchard pruning residues (FAO, 1997). However, pruning residues are rarely removed from the orchard for use in energy production. The main obstacles are the persistence of unresolved technical issues associated with the harvesting operations and, more importantly, the lack of a comprehensive information concerning the quantity and quality of the residual biomass obtainable from different orchards (Velázquez-Martí and Fernández-González, 2009). In this context, the recovery of residual biomass from sweet chestnut orchards would represent a significant contribution on the small scale of a local energy sector. In this paper, we present the results of a test conducted in a chestnut orchard in central Italy (Canepina -VT), in which the pruning residues were collected using the tractor-pulled mechanical shredder 'COMBY TR200', manufactured by FACMA L.t.d. (Italy). With a single pass of the machine along the orchard rows, the pruning residues are mechanically picked up from the orchard floor, fed into the chipping unit for shredding into chips, and loaded into the trailed container. The machine can move the collected biomass towards a desired site in the orchard, to concentrate the shredded wood into piles between the trees. The aim of the field trials was to evaluate the operative performance of the combined machine and to assess the quality of the biomass obtained in the process, in particular to determine if it complies with the quality standards established by the current legislation regarding solid biofuels (wood chips). In addition, the specific chestnut orchard is included in a specialized chestnut growing area in Central Italy, that has been infested by the gall wasp *Dryocosmus kuriphilus* Yasumatsu. In this area, the parasitoid *Torymus sinensis* Kamijo has been introduced for the biological control of the pest. The parasitoid feeds on the gall wasp larva developing in the green galls and later remains in the galls for the rest of the year (overwintering in the withered galls), emerging only the following spring. For this reason, the specific biological control protocol establishes that the pruned wood, carrying the galls, must be left in the orchard until the following spring, so that the parasitoid may complete its biological cycle. The management of the pruning residues in the orchards must be conducted accordingly. In our tests the machine was used to collect the pruning residues in the fields, after having completed the pruning operations in autumn. The aim was to evaluate if the mechanical shredding operations would safeguard a sufficient number of galls within the shredded residues. This method was proposed to collect the pruning residues to keep the field clean. The shredded material could be gathered into few piles, distributed in the orchard, to allow the dispersal of *T.*

sinensis from the intact galls, the following spring. This would favour the "biocontrol" action the parasitic wasp on the one hand, while on the other hand it would allow to keep the orchard floor clean for an efficient mechanization of the succeeding cultural operations.

MATERIALS AND METHODS

The filed test was carried out in a 50-year-old chestnut orchard in October. In this orchard, the pruning operations are managed over a three-year interval, with the major pruning work occurring every third winter. The test field had an irregular rectangular shape, extending over 1.26 ha, with irregular plant spacing. In total 67 plants were pruned in the field trial. During the pruning operations, the pruners leave the pruned material spread out, all over the ground. Once pruning is finished, the plant material lying on the ground is gathered into heterogeneous heaps, either placed along the edge of the field or between the trees along the inter-rows (Figure 1a).

Figure 1. Chestnut prunings gathered in piles (a) and clean orchard floor (b).

In general, the residues are arranged in piles to facilitate the other cultural practices in the orchard, especially the harvesting operations in autumn. These piles can remain in the field for some time, but they must be removed before fruit drop to allow mechanical fruit harvesting. Commonly the residues are simply pushed to the edge of the field, using a tractor with a fork. In our tests, a method is proposed to collect the residues mechanically, using a combined single-pass machinery (pick-up, shredder, loader) to collect the residues and then move the shredded material to selected areas in the orchard, while the volume of the piles is greatly reduced by shredding. This method leaves the orchard clean and ready for harvest (Figure 1b).
The equipment used in the field tests was the COMBY TR 200, which is a high capacity combined machine for picking, shredding and loading the residues onto the integrated trailer. The tractor used in the tests was the TRIFRUT 85 HP, a custom made fully hydrostatic-drive tractor (Figure 2a). Both machines are manufactured by FACMA S.R.L. (Italy). The machinery is connected to the tractor via a drawbar, attached to the tractor's rear hook. The main characteristics of the equipment (length, height, width, weight) are indicated in Table 1.

Table 1. Principal characteristics of the COMBY TR 200 shredder used in the field trials.

Parameter		Quantity
Length (mm)	*With drawbar*	4870
Length (mm)	*Without drawbar*	3720
Height (mm)		1800
Width (mm)		2230
Flail hammers (n.)		27
Weight (kg)		2200
Power take-off (Rpm)		540
Total volume of trailed container (m^3)		5

The machine assembly consists of the following 3 sections:

- a front pickup device provided with a horizontal rotor carrying metal tines to lift the prunings from the ground and convey them towards the following shredding rotor;
- a shredding unit having a horizontal rotor with 27 flail hammers, to shred branches up to 90 mm in diameter, and a metal grid placed behind the rotor in the opening into the rear container, to adjust the size of the shredded material;
- a rear container in which the shredded material is loaded, having a gross capacity of 5 m^3, that can be raised for unloading up to 2.5 m, via a pantograph lift (Figure 2b).

Figure 2. Machinery at work in the field (a), trailer unloading a pile (b).

After the mechanical treatment, the shredded biomass was analysed to determine its quality for use as a solid biofuel in energy production. To start, measurements were made on the height, width and length of the piles of shredded material left in the chestnut orchard, to estimate the overall volume of the piles. Then from each pile, 0.1 m^3 samples of the shredded material were weighed using a field digital dynamometer (D = 1 gr), in order to estimate the total amount of biomass obtainable per hectare in the chestnut orchard.

Samples of the biomass were collected for the assessment of moisture content, using the method described by Uni-En 14774-2: 2010. The shredded samples were

weighed and dried in a muffle at 105 ± 2 °C for 24 hours. After removal from the muffle, they were weighed to determine the moisture loss. The apparent bulk density, i.e. the mass per unit of volume expressed in $kg \cdot m^{-3}$, was assessed according to the Uni-En 15103: 2010 standards, collecting the shredded material in a known volume cylinder (0.025 m^3) and then weighing with a field dynamometer. The granulometric distribution of the shredded material was analysed in conformity with UNI-EN 14961: 2010 standards. The dimensional analysis of the shredded material was used to determine the quality of the shredded material for its use as a solid biofuel, according to the technical characteristics required for the use of solid biomass in the furnace. The ash content of the samples was verified in compliance with the UNI-EN 14775: 2010 standard, which applies to all kinds of solid biofuels. The ash content determines the quality of biomass for use in energy conversion. A high ash content reduces the calorific value of the biomass and the combustion performance in the furnace. The calorific value of the pruning residues was measured according to the UNI-EN 14918: 2010 method, to assess the energy value of the biomass. The energy yield of the biomass depends on the calorific value of the material. The calorific value also determines the energy density of the biomass, with obvious implications on the transportation and supply logistics of residues. The assessment of the mechanical action on the integrity of the galls in the pruning residues was determined by taking samples of pruning wood, before and after the mechanical processing and shredding. The percentage of intact galls remaining in the shredded biomass, accumulated in the piles, was calculated by counting the number of intact galls in samples of pruned wood and shredded wood (same weight samples). The operative performance of the machinery in the field work was established according to the recommendations of the Italian Association of Genio Rurale (A.I.G.R.) III^a R.1 (Manfredi, 1971), which is based on the official methodology of the International Commission of the Organization of the Scientifique du Travail en Agriculture (C.I.O.S.T.A.).

RESULTS AND DISCUSSION

The total amount of biomass obtained from the pruning operations conducted in the chestnut orchard used in the field trials was approximately 1.90 t of pruning residues, which corresponds to a unitary yield of 1.51 $t \cdot ha^{-1}$. The bulk density and the moisture content of the shredded biomass are shown in Table 2.

Table 2. Characteristics of the shredded material.

Parameter	Quantity
Total area of the chestnut orchard (ha)	1.26
Bulk density at field moisture content ($kg \cdot m^{-3}$)	185.9
Fresh shredded production (t)	1.90
Fresh shredded production per hectare ($t \cdot ha^{-1}$)	1.51
Moisture content of the shredded residue (%)	52.4
Yield in dry matter ($t \cdot ha^{-1}$)	0.72

Results of the granulometric analysis of the mechanically shredded material showed that more than 70% of the chips are included in particle size classes ranging from 6 to 25 mm. Therefore, the shredded biomass can be considered suitable for use in energy plants, according to the parameters indicated in the UNI-EN 14961-1: 2010 regulation (Febbi *et al*., 2013; Acampora *et al*., 2013). Moreover, more than 95% of wood chips were included in the 3.15-45 mm size range, making them suitable for use in residential biomass furnaces.

The assessment of gall integrity was another important objective of this work. The results of analysis were positive and supported the idea behind the experimental work. Analysis of the samples taken before the shredding operations showed that the galls accounted for about 12% by weight of the entire sample. Later, the analysis of samples of shredded biomass, randomly picked from the different piles, showed that 8% by weight of the material was represented by intact galls. This percentage (8% by weight) corresponded to about two thirds of the galls initially present in the pruned residues (before shredding).

The calorific value of the shredded biomass was measured on the shredded wood and on the galls alone. the calorific value of the biomass to be used for energy production. The calorific values were 18.33 and 18.08 $MJ \cdot kg^{-1}$, for the wood and the galls, respectively. These calorific values are similar to the values relating to other hardwood species, which are normally ranging from 18 to 19 $MJ \cdot kg^{-1}$.

The ash content of the woody portions of the prunings was 6.26% (± 0.07%), while that of the galls was 6.09% (± 0.029%). The value appears to be slightly above the average, but it is quite plausible considering that the wood/bark ratio for chestnut biomass is generally lower than that of other tree species. Also, the wood taken from young twigs and young branches is typically characterized by a higher percentage of ashes than that found in the wood of the trunk and of the larger tree branches. This is also explained by the fact that the wood/bark ratio characterizing thinner diameter shoots is lower than that of the older and thicker shoots.

The work time for the field operations was considered satisfactory, although some unproductive time was recorded, especially in the initial phases of the pile shredding, which required the frequent repositioning of the machine, mainly due to the excessive height of some heaps. The field operations were made easier by the excellent manoeuvrability of the special three-wheeled hydrostatic-drive tractor used in the tests, also for the presence of a wide spacing in the chestnut orchard, so that the driver could reduce the number of turns between the chestnuts.

The operative performance of the machinery is indicated in the different time categories in Table 3. The work capacity achieved by the machinery in the chestnut orchard is similar to the performance achieved by similar machines in the harvesting of orchard prunings and in other mechanized cultural practises (Colorio *et al* 2009; Spinelli *et al* 2012; Acampora *et al* 2013).

Table 3. Work time analysis and machinery operative performance

Parameter	Quantity
Actual working time (%)	60.94
Time to turn (%)	21.52
Discharge time (%)	14.42
Inevitable dead times (%)	3.12
Working time per surface unit ($h \cdot ha^{-1}$)	0.84
Work capacity ($ha \cdot h^{-1}$)	1.19
Productivity ($t \cdot h^{-1}$)	1.79

CONCLUSION

Chestnut orchards can provide significant amounts of good quality wood for energy purposes, on an annual basis. The biomass obtained from pruning is characterized by high values in apparent bulk density, but the ash content is slightly higher than the average values measured for wood biomass, due to the low wood/bark ratio and to the high moisture content.

The machine tested in this study was suitable for efficiently processing the pruned material by shredding and concentrating the biomass, unloading in selected areas of the orchard. Actual working time (60.94%) and working capacity (1.19 $ha \cdot h^{-1}$) may not appear high, but the data is related to the irregular distribution of plants in the experimental site and to the high amount of biomass to be shredded. The presence of more than 70% wood chips between 6 and 25 mm, makes this shredded material suitable for use as biofuel since it complies with requirements of UNI-EN 14961-1: 2010. For the purpose of the biological control of the gall wasp, the number of intact galls remaining after the mechanical shredding operations was assessed. In our tests, about two-thirds of the initial number of galls present in the pruned material remain intact after shredding. Therefore, the mechanical solution proposed safeguards the presence of *Torymus sinensis* in compliance with the provisions indicated by the Regional Phytosanitary Service of Lazio Region (Italy), that prescribe that the pruning residues must be left in the field and processed only from the end of June, because by that time the adults of *Torymus sinensis* will have already left the galls, to start a new cycle, so that biological control is safeguarded.

Through some modifications to be implemented on the rotor, a higher percentage of intact galls may be obtained. Also we estimate that by decreasing the number of hammers on the rotor, the size of the wood chips could be increased, therefore better preserving the integrity of the galls, still achieving the appropriate dimensional characteristics set by the biofuels standards.

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LYMPHOCYTE TRAFFICKING FOLLOWING ACUTE STRESS AND ALTITUDE HYPOXIA IN LOW AND HIGH HEMATOCRIT SHEEP

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ABSTRACT

The object of the present study was to investigate small and large lymphocytes trafficking in sheep with low and high hematocrit values following shearing, exposure to moderate altitude and transport to low altitude. Twenty out of 101 Ile de France ewes (1-7 years old) were used in the present experiment. All ewes of the flock were artificially inseminated in May 2015 following estrus synchronization. The animals were allocated into two groups following threefold measurements of hematocrit in all ewes as follows: I- low hematocrit group (n=10) and II - high hematocrit group (n=10). The ewes were transported to the Petrohan Pass (1440 m above sea level) in June 2015 immediately after shearing, conducted at the experimental farm of the Institute of Animal Science, Kostinbrod (500 m above sea level). Blood samples were collected before shearing, immediately after shearing, 3 h after shearing, at 14 d following exposure to moderate altitude, immediately after transport to low altitude and following 7d of stay at low altitude. All leukocyte subpopulations were counted microscopically. In the current study we presented the percentage of lymphocytes only, including small and large (reactive) lymphocytes. High and low hematocrit ewes had different percentage of small lymphocytes when exposed to various acute and chronic stress stimuli. There were significant differences in the percentage of large (reactive) lymphocytes between low and high hematocrit ewes following blood collection and immediately after shearing. The observed difference in small lymphocyte dynamics among the groups in response to different stress stimuli was attributed to hematocrit related differences in the time course and magnitude of lymphocyte distribution at early and late phases of stress. The results were interpreted to mean that the differences in lymphocyte trafficking between the two groups of sheep in response to stress were related to possible difference in the share of aerobic and glycolytic pathways for energy supply.

Key words: *small lymphocytes, large lymphocytes, hematocrit, sheep, stress.*

INTRODUCTION

There is now substantial evidence that immune function may be altered after exposure to acute or chronic stress. Phenotyping analysis, based on the size of lymphocytes revealed that both, small and large lymphocytes are present in sheep. The mobilization of leukocytes is selective and primarily affects, among others, effector CD8+ T cells and NK cells (Benschop et al., 1996, Kruger and Mooren, 2007). There is evidence that acute immunologic reactions may be mediated by concomitant sympathoadrenal activation, as evidenced by acute rises in heart rate, blood pressure, and plasma catecholamine concentrations (Bachen et al., 1992; Manuck et al., 1991; Zakowski et al., 1992).

Stress hormones have been identified as the major endocrine mediators in leukocyte distribution during acute or chronic stress (Dhabhar, 2006). The endocrine and autonomous nervous systems regulate immune functions not only directly via hormones and neural innervation, but also indirectly via influences on blood flow, blood pressure, lymph flow (Ottaway and Husband, 1992; Maestroni, 2001) and the supply of substrates like glucose, fatty acids and oxygen (Fox et al., 2005; Straub et al., 2010; Besedovsky and Rey, 2011).

There is evidence that the basal values of hematocrit are closely related to the adrenal response and the metabolic pathway for energy supply (aerobic, anaerobic) (Evans and Whitlock, 1964; Jones et al., 1967; Grace et al., 1992, Mason, 2000; Stark and Schuster, 2012; Shirasawa et al., 2013). In the recent years immunologists have accentuated on the possibility of using bioenergetic profiles of leukocytes, including lymphocytes, with the realization that leukocytes metabolism is closely tied to immunity (Kramer et al., 2014). Recent findings support the concept that circulating leukocytes can serve as early sensors of mitochondrial function under conditions of metabolic stress (Chacko et al., 2014).

The object of the present study was to investigate small and large lymphocytes trafficking in sheep with low and high hematocrit values following shearing, exposure to moderate altitude and transport to low altitude.

MATERIALS AND METHODS

One hundred one Ile de France ewes (1-7 years old) were used in the present experiment. All ewes of the flock were artificially inseminated in May following estrus synchronization. The animals were allocated into two groups following hematocrit measurement in all ewes. Group I comprised individuals with low level of hematocrit (low hematocrit group; n=10) and group II comprised individuals which had high level of hematocrit (high hematocrit group; n=10). Two additional measurement of baseline hematocrit at intervals of 10 days were performed in the sheep of both groups to verify hematocrit values of both groups, since hematocrit is known to be influenced by many factors and fluctuates from day to day. The average age of the ewes in groups I and II was 3.9± 0.795 and 3.1± 0.745 years respectively. The ewes were shorn on June 2nd and were immediately transported from the experimental base of the Institute of Animal Science, Kostinbrod (500 m above sea level) to the Petrohan Pass region (Balkan mountains), located at 1440 m

above sea level. Minimum and maximum temperatures on that day were 13.9 and 25^{o}C for the region of Kostinbrod (low altitude) and 8.2- 13.6^{o}C for the region of Petrohan Pass (high altitude) respectively. The animals remained at high altitude for 4 months where they were on pasture for 10 h during the day. At night they stayed in a barn. The ewes had free access to a NaCl licking stone and water. In addition to pasture, they were offered concentrate once per day. Mean air temperature range in the region of Petrohan pass during the summer months of 2015 was 12 to 20^{o}C. At the end of the grazing season the ewes were transported back to low altitude. At that time the ewes in groups I and II were at 131±6.652 and 140± 4.015 d of gestation respectively as estimated by the day of parturition.

Blood samples were collected before shearing, immediately after shearing, 3 h after shearing, at 14 d following exposure to moderate altitude, immediately after transport to low altitude and following 7d of stay at low altitude.

All samples were taken via jugular venipuncture within 3 min in the morning before feeding in order to minimize handling stress and avoid possible interference caused by cortisol diurnal variation. Differential white blood cell count was performed. All leukocyte subpopulations were counted microscopically in smears made after staining with Giemsa-Romanovsky. In the current study we presented the percentage of lymphocytes only, including small and large (reactive) lymphocytes. The results of one factor analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

RESULTS AND DISCUSSION

Baseline number of small lymphocytes was significantly higher in low hematocrit ewes as compared to that in high hematocrit ewes (Fig.1). In contrast, the number of large lymphocytes was significantly higher in high hematocrit ewes relative to low hematocrit ewes (Fig.2). It is well known that small, mature lymphocytes are the most common lymphocyte in peripheral blood (EclinPath, 2017). Large lymphocytes have been found to be infrequent in healthy animal blood (Cotter, 2015). Consequently, the increased baseline number of large lymphocytes in the high hematocrit ewes could be due to the act of sheep handling and restraint during blood collection. This view is consistent with the reported significant increase in T cell and natural killer cells immediately after the jump in parachutists (Dhabhar, 2006). Similar results have been reported by Rinner et al. (1992) who found that short stressor (1-min handling) caused an increase in mitogen – induced proliferation of T and B cells obtained from peripheral blood. The authors suggested that the modulation of immune cell distribution by acute stress may be an adaptive response designed to enhance immune surveillance and increase the capacity of immune system to respond to challenge in immune compartments. We are prone to believe that the above mentioned increase in blood T and B lymphocytes following short stress involved mainly large (reactive) cells, since the presence of reactive cells is considered to be an immune response in progress to stress (Cotter, 2015). Our view is consistent with the reported change in the percentage of large CD4 and CD8 cells 6 weeks before written examination, one

day before the examination day and 12-14 days after the examination (Halvorsen and Vassend, 1987). Also, this view is supported by the reported significant increase in the diameter of activated CD8-T cells relative to resting cells diameter suggesting that activation was accompanied by an increase in cell volume (Du et al., 2017). Our results suggest that stress, caused by blood sampling, may modulate both small and large (activated) lymphocyte trafficking to their respective compartments. However, low hematocrit ewes, unlike high hematocrit ewes, had relatively low baseline percent of large lymphocytes which could be due to delayed immune response or higher stress threshold in these animals.

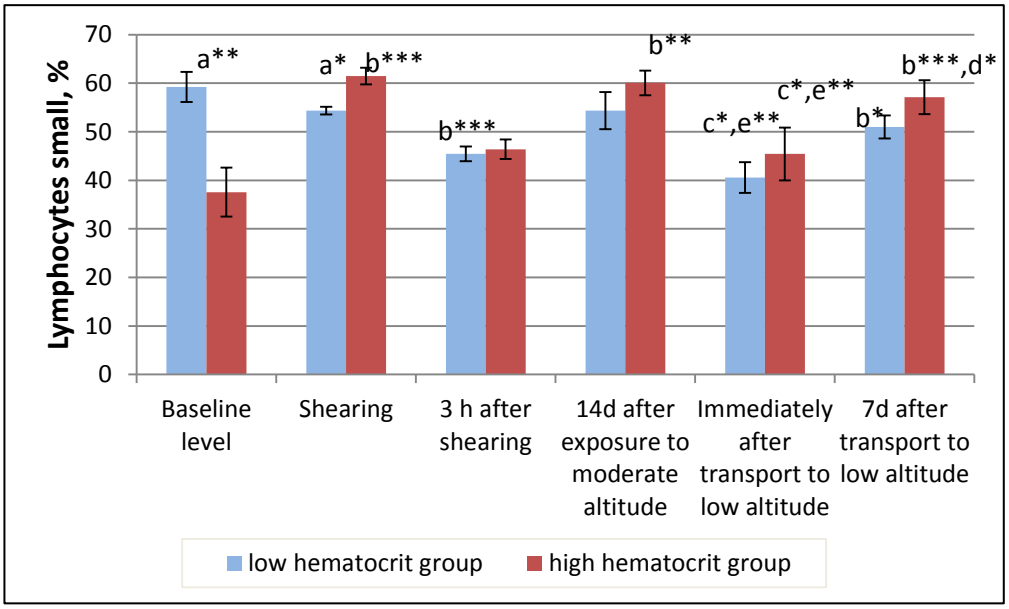

Fig. 1 Lymphocytes (small) in sheep with low and high hematocrit values following shearing, exposure to moderate altitude and transport to low altitude. P*<0.5, ** P<0.01,***P<0.001

a- significantly different among the groups
b- significantly different versus baseline level
c- significantly different versus 14 d after exposure to moderate altitude
d- siginificantly different versus immediately after transport to low altitide
e- significantly different versus shearing

The observed opposite dynamics of large lymphocytes during shearing, which is considered to be an acute stress, confirms once again the established difference in lymphocytes trafficking among the groups, caused by blood sampling. Although less prominent the difference in small lymphocyte percentages between the two groups caused by shearing was still significant. Furthermore, small lymphocyte percentage increased in high hematocrit ewes whereas it was unchanged in low hematocrit ewes as compared to the respective blood collection values. Besides, the percentage of large lymphocytes in low hematocrit ewes in response to shearing

(Fig.2) was significantly lower as compared to that observed in high hematocrit ewes during blood sampling. Shearing stress, unlike the stress caused by blood collection, caused further increase in the percent of small lymphocytes in high hematocrit ewes, while the percentage of small lymphocytes in low hematocrit ewes declined insignificantly (Fig.1). The observed decline in the percentage of large (reactive) lymphocytes in high hematocrit ewes when exposed to shearing stress versus blood sampling stress could be due to faster mobilization and trafficking of lymphocytes and their distribution among different body compartments. If we assume that the effect of shearing stress is more severe compared to the effect of blood collection stress than it is more plausible to accept that the observed decline of large lymphocytes in high hematocrit ewes was due to higher speed in large lymphocyte mobilization to specific target organs which leads to decrease in blood large lymphocyte numbers that occurs later during acute stress (Dhabhar et al., 2012). These results suggest that immune response in low hematocrit ewes was less pronounced than that in high hematocrit ewes which could be due to higher stress threshold in low hematocrit ewes.

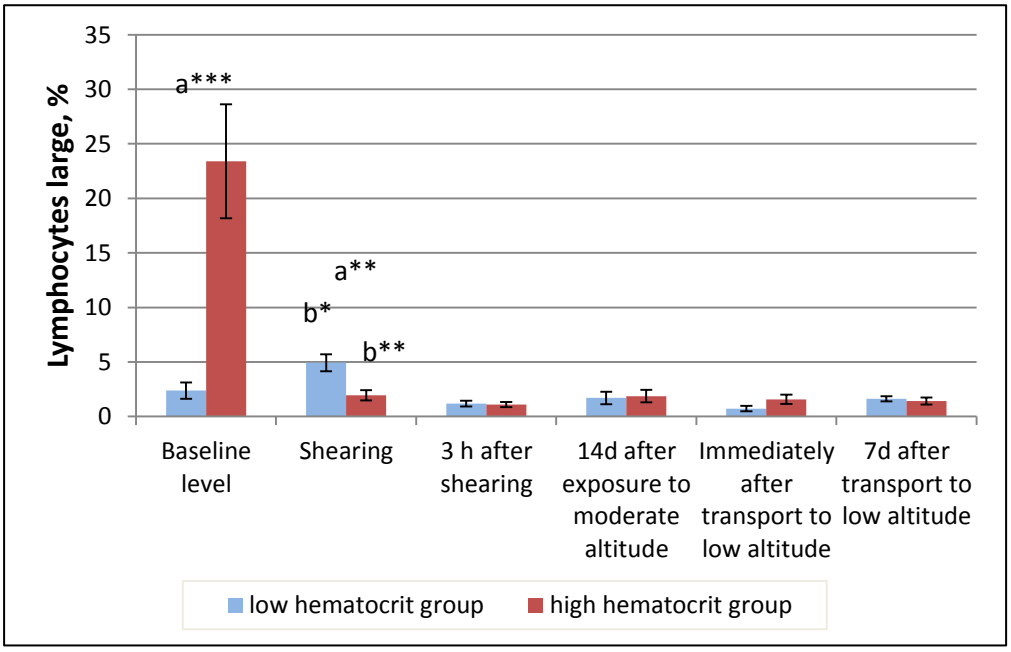

Fig. 2 Lymphocytes (large) in sheep with low and high hematocrit values following shearing, exposure to moderate altitude and transport to low altitude. *P<0.05, **P<0.01, ***P<0.001

a- significantly different among the groups

b- significantly different versus baseline level

Small lymphocyte numbers in both groups were similar at 3 hrs following shearing stress (Fig.1). However the percentage of small lymphocytes in low hematocrit ewes, unlike that in high hematocrit ewes, was significantly lower relative to the respective baseline levels. According to Dhabhar et al. (2012) the kinetics of leukocyte subpopulations in response to stress at early time points would mainly reflect mobilization of cells into the blood from certain compartments (e.g. spleen, bone marrow, lung, lymph nodes), while late time points would mainly reflect trafficking of cells out of the blood to target organs (decreased number). The small lymphocyte percentage at 3 hrs after shearing is consistent with Dhabhar's view. The percentage of large lymphocytes at that time was similar in both groups. It is interesting to note that large lymphocyte percentage in high hematocrit ewes (Fig.2) was significantly lower than the respective baseline value (Fig.2). These results are in agreement with the reported decline in total lymphocyte percentage and lymphocyte subpopulations (helper T cells, cytolytic cells, B cells, natural killer cells) in restraint rats following an initial increase of these lymphocytes (Dhabhar et al, 2012).

Small lymphocyte percentage in high hematocrit ewes, unlike that in low hematocrit ewes was significantly higher relative to the baseline value following 14 d exposure to moderate altitude (Fig.1). We assume that the observed percentage of small lymphocytes at that time displayed lymphocyte trafficking caused by adaptation to altitude. This view is in agreement with the results of our earlier finding showing that cortisol level at 14 d following exposure to moderate altitude is similar to baseline cortisol levels (Moneva et al., 2016). Also, the difference in baseline cortisol levels among the groups persisted following 14 d adaptation to moderate altitude. The principal stress hormones have been found to be the major endocrine mediators of a stress-induced leukocyte distribution (Dhabhar et al., 2012.). Therefore, the established differences in small and large lymphocytes percentage between the groups is in agreement with the difference in cortisol levels among the groups following blood collection and exposure to moderate altitude, found in our earlier study.

The percentage of small lymphocytes in both groups declined significantly immediately after transport to low altitude when compared to lymphocyte percentage at 14 d following exposure to moderate altitude (Fig.1) while the percentage of large lymphocytes remained unchanged (Fig.2). There was no difference between the groups in the percentage of small and large lymphocytes at that time despite the higher cortisol level immediately after transport in low hematocrit ewes as compared to cortisol level at14d following exposure to moderate altitude, found in our earlier study (Moneva et al., 2016). The observed discrepancy between lymphocyte numbers and cortisol levels is consistent with Dhabhar's hypothesis (Dhabhar et al, 2012) that specific combinations of stress hormones (epinephrine, norepinephrine and cortisol) would mediate distinct aspects of stress-induced leukocyte redistribution.

There were no significant differences in the percentage of both small and large lymphocytes among the groups at 7 d following transport from moderate to low

altitude (Fig.1,2). However the percentage of small lymphocytes in low hematocrit ewes declined while it increased in high hematocrit ewes relative to the respective baseline levels (Fig.1). The observed difference in small lymphocyte dynamics among the groups in response to different stress stimuli once again indicate that baseline hematocrit level is most probably related to the time course and magnitude of stress-induced lymphocyte distribution at early and late phases of stress as well as during exposure to acute and chronic stress episodes.

Sheep exposed to confinement and isolation stress were found to change their complex behavioral pattern, cortisol secretion and the numbers of various types of lymphocytes (Degabriele and Fell, 2001). In their animal model the authors found that the severity of the stressor in behavioural (and possibly physiological) terms tended to parallel the ability of CD5 components of the immune system to recover from an adverse reaction to stress.

In our earlier study (Moneva et al., 2016) we suggested that hematocrit level depends on hemoglobin type (hemoglobin's affinity for oxygen) which on its turn is related to the ratio between glycolytic and oxidative muscle fibers. It is well known that oxygen demand exceeds oxygen supply during stress. Also, the fast glycolytic system is a key contributor to the total energy requirements for moderate to high intensity stress (Baker et al., 2010). It is consequently reasonable to expect different energy contribution of the two metabolic pathways in response to stress between high and low hematocrit ewes.

Resting lymphocytes do not have large internal glycogen stores and are highly dependent on the import of extracellular glucose and glycolysis for the production of ATP (Fiorucci et al., 2004). Therefore, the observed difference in lymphocyte trafficking between the two groups of sheep in response to stress could be due to the expected difference in blood glucose levels between low and high hematocrit ewes that is crucial for extracellular glucose uptake by lymphocytes.

CONCLUSIONS

High and low hematocrit ewes had different percentage of small lymphocytes when exposed to various acute and chronic stress stimuli.

There were significant differences in the percentage of large (reactive) lymphocytes between low and high hematocrit ewes following blood collection and immediately after shearing.

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MICROBIOLOGICAL QUALITY, ANTIOXIDATIVE AND ANTIMICROBIAL PROPERTIES OF SLOVENIAN BEE POLLEN

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ABSTRACT

Bee pollen can be considered as perfect food with a great nutritional value, high protein and essential amino acid content, vitamins and minerals. It can be a source of healthy nutrients, but as an animal product also of harmful microbial contaminants. The aim of our study was to determine potential health risks and benefits of Slovenian bee pollen. We determined its i) microbiological burden: aerobic mesophilic microorganisms, yeast, molds, and coliform bacteria in CFU/g; ii) polyphenolic content: the Folin-Ciocalteu method (mgGA/g); iii) antioxidative potential (AOP): DPPH$^{\bullet}$ scavenging assay (EC50 in mgGA/L); and iv) antimicrobial activity (MIC): microdilution method on *Escherichia coli*, *Listeria monocytogenes* and *Campylobacter jejuni*. We analyzed 14 samples of bee pollen gathered from 7 Slovenian geographical regions, from April until May 2017. The microbiological burden was high, with all indicator tests reaching up to 6.78 $\log_{10}$CFU/g of bee pollen, but the number of coliform bacteria in all samples from 2.00 to 4.48 $\log_{10}$CFU/g. The polyphenolic content and AOP of the samples was good, with up to 13.1 mg GA/g and as low as 2.4 mgGA/L (EC50), respectively. Interestingly, antimicrobial activity was not always in correlation with polyphenolic content, but always strongly against *E. coli*, substantial against *C. jejuni*, and negligible against *L. monocytogenes*. Our results show a great health potential of bee pollen for human health, but also the need of bee pollen processing improvement for its standardized quality and safety.

Key words: *Bee pollen, polyphenols, antioxidant, antimicrobial activity, microbiological safety.*

INTRODUCTION

Pollen is a microscopic structure produced in the anthers of plants and typical for every single botanical type. It presents the plants male reproductive organs that are the basis for sexual reproduction in plants. Pollination of plants is carried out by the wind or by insects, where bees play an important role. In the process of pollination when a bee touches the anthers its body becomes covered with pollen

dust. They gather this pollen dust, moisturize it with saliva and nectar, compress it into two pollen baskets on their hind legs, bring it to the beehive and store it in combs for their needs (Almeida Muradian et al., 2005; Bogdanov, 2012; Kieliszek et al., 2018).

Bee pollen can be considered as perfect food with great nutritional value. For human consumption beekeepers collect bee pollen using bee pollen traps placed at the entrance or on the bottom of the bee hive (Lilek et al., 2015). The presence of proteins, essential amino acids, carbohydrates, saturated and unsaturated fatty acids, dietary fibers, vitamins and minerals makes bee pollen very useful in human nutrition - in supplementary and alternative diets with functional and therapeutic properties. It has health promoting effects: anti-inflammatory, antimicrobial, antifungal, antioxidant and antitumor activities (Almeida-Muradian et al., 2005; Campos et al., 2010; Münstedt and Bogdanov, 2009; Bogdanov 2012; Kieliszek et al., 2018), with increasing attention among consumers.

Important functional ingredients of plant food are antioxidants. Bee pollen shows high contents of polyphenolic substances, mainly flavonoids and phenolic acids with potential antioxidant activity and thus preventive in cancerous diseases, cardiovascular diseases, inflammatory processes, neurological disorders and aging (Gómez-Caravaca et al., 2006; Campos et al., 2008; Kieliszek et al., 2018).

To achieve microbiological stability, beekeepers dry bee pollen under controlled conditions. This process will decrease the water activity of bee pollen from 0.7 to 0.3 and thus increase the stability of the product. Chemical, biological and sensory properties of bee pollen are better preserved if bee pollen is consumed fresh. Thus are the high hygienic standards and proper handling of the product in every stage of production crucial for its quality and safety. Opposite activities can cause the product becoming undesirable and harmful for human health (Deveza et al., 2015; Beev et al., 2018; Kieliszek et al., 2018).

In this investigation we examined i) the microbiological quality of bee pollen by determining the total aerobic count, yeast and mold count, and coliform bacteria, ii) the total phenolic content, ii) antioxidative potential (AOP), and iii) antimicrobial activity of bee pollen gathered from 7 different Slovenian statistical (geographical) regions.

MATERIALS AND METHODS

Samples collection: Fourteen samples of bee pollen from Carnolian bees (*A. mellifera carnica*) were collected in beekeeping season of 2017 from seven different geographical regions in period from April until May - from regions Goriška (n=1), Zasavska (n=1), Obalnokraška (n=1), Gorenjska (n=3), South-East (n=2), Pomurska (n=1) and Central (n=5), and refrigerated until analysis. For the microbiological analyses whole bee pollen grains were used, prior to chemical analyses bee pollen pallets were ground.

Microbiological analysis: Bee pollen samples were prepared and diluted in saline solution according to standard procedures (ISO 6887-1:1999). The total aerobic count was enumerated on PC agar (Merck, Germany), coliforms on VRBL agar

(Biolife, Italy), and molds and yeast on DRBC agar (Biolife, Italy). All samples were analyzed in triplicates and results are presented as the $\log_{10}$ number of colony forming units in 1 gram of bee pollen (CFU/g).

Preparation of bee pollen extracts: The pollen extracts were prepared by solid/liquid extraction using 96% ethanol as a solvent (1g/3mL), for 5 h on a shaker in the dark at room temperature with intermediate treatments in the ultrasonic bath. Further the samples were filtered and centrifuged for 10 min at 4000 rpm. At least three extracts for each pollen sample were obtained. The repeatability of extraction of phenolic compounds was within 5%.

Total phenolic content and antioxidative potential determination: The total phenolic compounds were determined according to the Folin-Ciocalteu method (Gutfinger, 1981) according to Terpinc et al. (2012). The content of total phenolic compounds in pollen samples was expressed as mg gallic acid (GA, Sigma Aldrich, Germany) per gram of dry weight of pollen (mg GA/g). All analyses were carried out in at least three repetitions. Determination error was less than 3%. The results for the content of phenolic compounds in pollen samples are given as the average value ± standard deviation.

The AOP of extracts of phenolic compounds from pollen was determined by the method of determining the effectiveness of scavenging radical $DPPH^{\bullet}$ (Brand-Williams et al., 1995) according to Terpinc et al. (2012). The analysis was done in 3 to 5 repetitions. AOP was expressed as the concentration of phenolic compounds in the reaction mixture, which reduced the initial $DPPH^{\bullet}$ content by 50% (EC50). A lower EC50 value means a better AOP.

Bacterial culture preparation: Cultures were grown on Mueller Hinton Agar (MHA, Biolife, Italy), *E. coli* and *L. monocytogenes* for 24 h at 37 °C aerobically, and *C.jejuni* at 42°C microaerobically (5% O_2, 10% CO_2 in N_2). Overnight cultures were prepared in Mueller Hinton broth (MHB, Sigma Aldrich, Germany). For antimicrobial activity testing the optical density at 600 nm (OD_{600}) of the overnight cultures was adjusted to 0.1 in MHB. Culture suspensions were further diluted to the concentration of $5x10^5$ CFU/mL.

Antimicrobial activity of bee pollen extracts: Extract of phenolic compounds (4 mL) was dried prior to antimicrobial testing. The solvent was evaporated in a centrifugal vacuum evaporator HT-4 series II (GeneVac Technologies). Dry residue was dissolved in 0.3 mL of dimethyl sulfoxide (DMSO, Sigma, Germany) and further in MHB. The MIC was determined for *E. coli* and *L.monocytogenes* according to Klančnik et al. (2009) and for *C. jejuni* according to Kovač et al. (2015). MIC is presented as mg of dried bee pollen extract per mL of solution (mg/mL).

Statistical analysis: The statistical analysis was carried out in the SPSS software version 21 (IBM Corp., Armonk, NY, USA). The correlation analysis was determined using the Pearson correlation coefficient. The statistical significance of the phenolic content, radical scavenging and antimicrobial action was determined using one-way ANOVA.

RESULTS AND DISCUSSION

Microbiological quality of bee pollen

Bee pollen is an animal product, gathered by bees and therefore exposed to conditions that enable a high microbial contamination. Although the composition of bee pollen is defined by the origin and mixture of plants pollinated by bees and can thus be very variable, this was not reflected in the microbiological variability of samples. The total aerobic count and the number of coliform bacteria were similar in all samples. In general, the microbiological load of samples from different geographical regions (Fig. 1) was similar. Only the Obalnokraška region stands out with the lowest average number of molds (<1 $\log_{10}$CFU/g) and the highest average number of yeast (7.59 $\log_{10}$CFU/g).

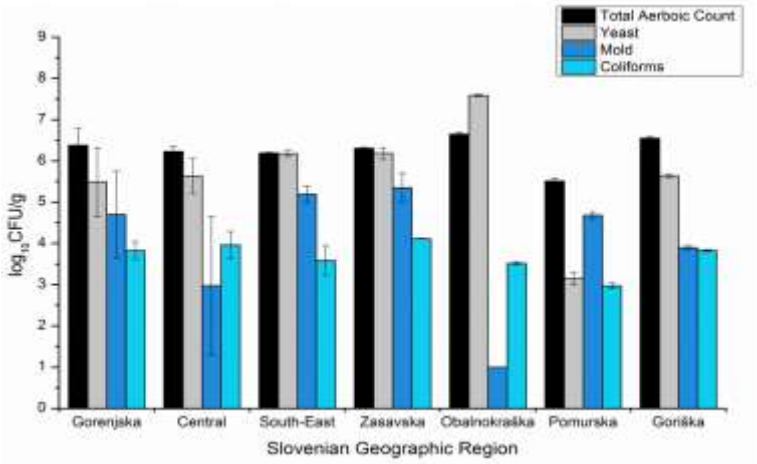

Figure 1. Total aerobic count, yeasts, molds, and coliforms in samples of fresh Slovenian bee pollen from 7 geographical regions, presented as the average $\log_{10}$CFU/mL ± standard deviation of all samples from one region. Samples with a value of <1 $\log_{10}$CFU/g are presented as 1 $\log_{10}$CFU/g in the graph without standard deviations.

The high microbial contamination of the fresh bee pollen samples in this study is similar to the samples observed by Mauriello et al. (2017) and Beev et al. (2018). Beev et al. (2018) reported water activity of fresh bee pollen of 0.7 and more, which enables the proliferation of yeast and molds. Bee pollen can thus be a subject of spoilage by these microorganisms or a vector for pathogenic fungi. Although this and lower water activity would not enable most pathogenic bacteria to proliferate, it can be a vector for the transfer of pathogenic bacteria (Sancho-Madriz, 2003; Beuchat et al., 2013). Coliform bacteria are usually considered an indication of fecal contamination and unsanitary conditions in food production, but it is also a diverse group of microorganisms that can be found in the natural environment (Martin et al., 2016). In a fresh food product such as bee pollen the presence of coliforms should be interpreted carefully as it cannot be considered as proof of either the presence of fecal pathogens or unsanitary conditions.

Phenolic content and antioxidative potential of bee pollen extracts

Results show that among samples there are notable differences ($p<0.05$) in the content of phenolic compounds (Table 2, Fig. S1). The content of phenolic compounds in investigated bee pollen ranged from 6.5 to 13.1 mg GA/g (Fig. S1). The highest phenolic content was observed in samples C2 (Central region 2) and P (Pomurska region), which were significantly higher compared to other samples ($p<0.05$). The lowest phenolic content was observed in the sample C5 (Central region 5). No correlation was found between the phenolic content and the location of bee pollen collection, although more sampling is needed for the confirmation of this observation. The content of total phenolic compounds in the investigated pollen of Slovenian origin coincides with the literature values for pollen originating from Brazil as reported by Carpes et al., (2007) of 7 mg GA/g in extract obtained by 90% ethanol. Our results are also in accordance with the values for the Portuguese bee pollen with total phenolic content ranging from 13 to 20 mg GA/g as reported by Feas et al. (2012) and with results of Morais et al. (2011) who obtained values from 10 to 17 mg GA/g. On the other side Pascoal et al. (2014) who prepared their extracts with methanol determined somewhat higher total phenolic content in bee pollen from Spain ranging from 18 to 29 mg GA/g. The differences can be explained by different solvents used for phenolic compounds extraction from bee pollen.

Table 2. Average, median, minimum and maximum content of total phenolic compounds expressed as mg gallic acid per gram of dry matter of pollen (mg GA/g) and antioxidative potential expressed as the concentration of phenolic compounds in the reaction mixture which reduced the initial $DPPH^{\bullet}$ content by 50% (EC50) for 14 samples.

	Content of total phenolic compounds (mg GA/g)	EC50 (mg GA/L)
Average	9.9	10.7
Median	9.8	10.6
Min	6.5	2.4
Max	13.1	22
No. of samples	14	14

The obtained results show that all investigated bee pollen extracts expressed the $DPPH^{\bullet}$ radical scavenging activity. However, the EC50 values considerably deviate between 14 analyzed samples (Fig. S2). The Pomurska region sample (P) expressed the highest AOP (EC50 = 2.4 mg GA/L; $p<0.05$) while the AOP of the Zasavska region sample was the poorest (EC50 = 22 mg GA/L) and differed significantly from all other samples ($p<0.05$). Campos et al. (2003) in their investigation determined appreciably lower AOP with EC50 ranging from 40 to 330 mg/L. The values for AOP of phenolic compounds in the investigated pollen samples of Slovenian origin are in accordance with the values of AOP, which were

determined in our previous research for phenolic compounds from propolis (Mavri et al., 2012), oil seeds (Terpinc et al., 2012), rosemary (Klančnik et al., 2009) and the EC50 value for the synthetic antioxidant BHT.

Antimicrobial activity of bee pollen extracts

The antimicrobial activity of bee pollen extracts was tested on two gram negative bacteria, *E. coli* and *C. jejuni*, and one gram positive bacterium, *L. monocytogenes*. The extracts showed no antimicrobial activity against *L. monocytogenes*. The antimicrobial activity of most samples (Table S2) was substantial against *E. coli* and *C. jejuni*. Samples G1 (Gorenjska region 1), C2 (Central region 2), P (Pomurska region), and GO (Goriška region) showed a better antimicrobial activity against *E. coli*, compared to other samples ($p<0.01$). Samples P and C2 also showed the highest antimicrobial activity against *C. jejuni* compared to other samples ($p<0.01$). A significantly higher antimicrobial activity ($p<0.01$) against *C. jejuni* was observed also in samples C1 (Central region 1) and SE1 (South-East region 1).

Table 3. Average, median, minimum and maximum antimicrobial activity of bee pollen extracts presented as the MIC in mg of dry extract/ mL, tested on *E. coli, C. jejuni* and *L. monocytogenes* from 14 samples.

	Minimal inhibitory concentration (MIC) in mg/mL		
	E. coli	*C. jejuni*	*L. monocytogenes*
Average	2.68	9.93	>6.25
Median	3.13	12.50	-
Min	1.56	0.78	-
Max	3.13	12.50	-
No. of samples	14	14	14

The antimicrobial activity of the bee pollen extracts against *E. coli* and *C. jejuni* showed a significant correlation to the total phenolic content of the extracts. When the total phenolic content was higher, the MIC was lower for *E. coli* ($r= - 0.634$, $p=0.015$) and *C. jejuni* ($r= - 0.537$, $p=0.048$). Extracts from bee products such as honey, propolis and bee pollen with high polyphenolic content have shown good antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and other organisms (Grange and Davey, 1990; Estevinho et al., 2008; Mavri et al., 2012). The topic of antimicrobial activity of bee pollen is not widely researched, and the information available reports antimicrobial activity with the agar diffusion method rather than the microdilution method, making the comparison of results a challenge. Fatrcová-Šramková et al., (2016) and Morais et al. (2011) reported an antimicrobial activity against *E. coli* comparable to ours, but a better activity against Gram positive bacteria than Gram negative, although our results show the opposite.

SUPPLEMENTARY INFORMATION

Table S1. Microbiological contamination of fresh Slovenian bee pollen from 7 statistical regions presented with the $\log_{10}$CFU/g average ± standard deviation of total aerobic count, yeast, mold, and coliforms.

Sample designation	Statistical region	Average total count in $\log_{10}$CFU/g ± standard deviation			
		Total Aerobic Count	Yeast	Mold	Coliform bacteria
G1	Gorenjska	6.65 ± 0.03	5.98 ± 0.02	5.50 ± 0.20	3.52 ± 0.12
G2	Gorenjska	6.70 ± 0.00	4.30 ± 0.10	3.22 ± 0.22	3.95 ± 0.01
G3	Gorenjska	5.80 ± 0.02	6.18 ± 0.10	5.39 ± 0.39	4.01 ± 0.01
C1	Central	6.03 ± 0.03	6.17 ± 0.06	5.15 ± 0.15	3.63 ± 0.05
C2	Central	6.33 ± 0.02	5.46 ± 0.02	3.80 ± 0.20	4.07 ± 0.05
C3	Central	6.12 ± 0.48	4.92 ± 0.02	3.94 ± 0.10	4.52 ± 0.02
C4	Central	6.28 ± 0.02	5.84 ± 0.06	<1.00 ± - *	3.62 ± 0.15
C5	Central	6.38 ± 0.01	5.77 ± 0.07	<1.00 ± - *	3.97 ± 0.01
SE1	South-East	6.21 ± 0.06	6.10 ± 0.02	5.39 ± 0.09	3.95 ± 0.09
SE2	South-East	6.18 ± 0.02	6.26 ± 0.02	5.00 ± 0.30	3.23 ± 0.03
Z	Zasavska	6.31 ± 0.03	6.18 ± 0.14	5.35 ± 0.35	4.11 ± 0.01
OK	Obalnokraška	6.65 ± 0.06	7.59 ± 0.02	<1.00 ± - *	3.52 ± 0.04
P	Pomurska	5.51 ± 0.01	3.15 ± 0.15	4.68 ± 0.09	2.97 ± 0.07
GO	Goriška	6.55 ± 0.04	5.64 ± 0.04	3.90 ± 0.05	3.83 ± 0.02
-	All	6.26 ± 0.32	5.68 ± 1.00	3.88 ± 1.65	3.78 ± 0.38

*No standard deviation is presented as all tested samples had less than 1 $\log_{10}$CFU/g.

Table S2. Antimicrobial activity of bee pollen extracts presented as mg of dry extract/ mL, tested on *E. coli, C. jejuni* and *L. monocytogenes* of 14 samples from 7 geographical regions.

Sample designation	Minimal inhibitory concentration (MIC) in mg/mL		
	E. coli	*C. jejuni*	*L. monocytogenes*
G1	1.56	12.50	>6.25
G2	3.13	12.50	>6.25
G3	3.13	12.50	>6.25
C1	3.13	6.25	>6.25
C2	1.56	0.78	>6.25
C3	3.13	12.50	>6.25
C4	3.13	12.50	>6.25
C5	3.13	12.50	>6.25

SE1	3.13	6.25	>6.25
SE2	3.13	12.50	>6.25
Z	3.13	12.50	>6.25
OK	3.13	12.50	>6.25
P	1.56	0.78	>6.25
GO	1.56	12.50	>6.25

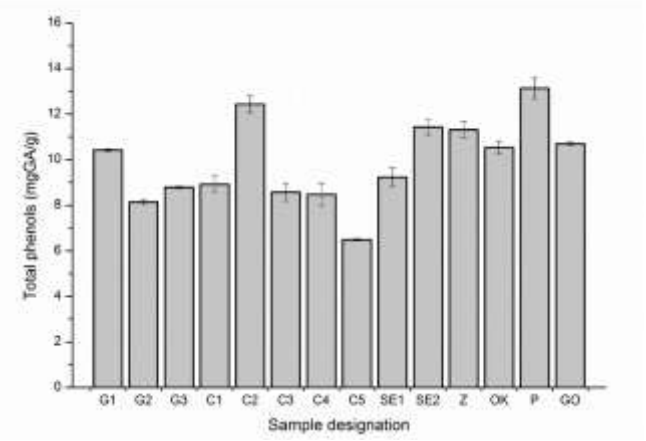

Figure S1. The content of total phenolic compounds in Slovenian bee pollen expressed as mg gallic acid per gram of dry matter of pollen (mg GA/g).

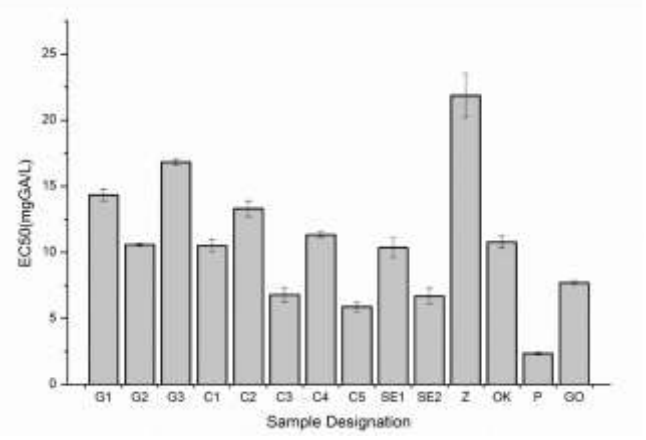

Figure S2. The antioxidative potential of Slovenian bee pollen expressed as the concentration of phenolic compounds in the reaction mixture which reduced the initial DPPH$^{\bullet}$ content by 50% (EC50).

CONCLUSION

The high microbiological burden of fresh bee pollen raises concerns about the suitability of bee pollen as a fresh product. It highlights the need for improvement

of bee pollen production and demands, alongside with a good manufacturing practice, additional steps to decrease the contamination. Despite the challenges in bee pollen production it has great potential as a nutritional supplement with an anti-oxidative effect and a high polyphenolic content that translates into good antimicrobial effect against enteric pathogenic bacteria, although this effect may vary between samples collected from different regions. These bioactivities together with a nutritional benefit make bee pollen a valuable product.

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AGROBIOLOGICAL FEATURES OF MUSTARD (*Brassica juncea* L) IN UKRAINE UNDER CURRENT CLIMATE CHANGE CONDITIONS

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ABSTRACT

The market for oilseeds in Ukraine is a large segment of the general market for agricultural products. Mustard (*Brassica juncea*L) is an oilseed crop that can restore the optimal ratio of crops in crop rotation and not reduce the rates of economic activity. Ukraine is among the top ten world leaders in its cultivation. The warming trends observed over the last 30 years in the world and in Ukraine, allow growing of mustard throughout the country. Consequently, it became necessary to develop varietal technologies for growing mustard for specific soil and climatic conditions. The objective of this research was to develop cultivation technology for *Brassica juncea*L in the conditions of the north-eastern forest-steppe of Ukraine for the first time. We studied the influence of weather conditions on the peculiarities of growth, development, formation of biomass, photosynthetic activity, productivity indices, yield, seed quality, oil production, depending on the variety, sowing time, seeding rates and fertilizer for growing bluish mustard. According to research results, for the formation of 1.71-1.91 t/ha of bluish mustard seeds in the conditions of the north-eastern forest-steppe of Ukraine on typical black soil, the cultivation technology should include: use of seeds of modern high-yielding adapted varieties; start sowing at soil temperature of 4-5 ^{o}C at a depth of 10 cm; the rate of fertilizer $N_{30}P_{30}K_{30}$ (during pre-sowing cultivation) and seeding rates of the varieties Prima - 1.5 million pieces/ha, and Retro -2.0 million pieces/ha.

Keywords: *bluish mustard, sowing date, fertilizer rates, seeding rates, yield.*

INTRODUCTION

Brown mustard (*Brassica juncea L.*) – trowse mustard or Indian mustard – is grown extensively for oil in India, Pakistan, China, Canada and in other countries (Masierowska, 2002; Melnik et al., 2015).

The market for oilseeds in Ukraine is a large segment of the general market for agricultural products. Mustard is an oilseed crop that can restore the optimal ratio

of crops in crop rotation and not reduce the rates of economic activity. Ukraine is among the top ten world leaders in its cultivation. The warming trends observed over the last 30 years in the world and in Ukraine, allow growing of mustard throughout the country. Consequently, it became necessary to develop varietal technologies for growing mustard for specific soil and climatic conditions (http://www.ukrstat.gov.ua/). Mustard is the third important oilseed crop in the world after soybean (Glycine max) and palm (Elaeis guineensis Jacq.) oil. Mustard is a multi-vector industrial crop due to its diverse use. It is very important as an oilseed crop; quality of the oil produced from its seeds is not inferior to the sunflower oil. Mustard oil is widely used for food, as well as in many industries - canning, baking, confectionery, margarine, soap and pharmaceutical. In addition to oil, the seeds of bluish mustard contain essential oil, which is used in cosmetics and perfumes. Bluish Mustard is used to produce mustard powder necessary for toast mustard in food industry and mustard plasters in medicine (Shekhawat et al., 2012; Melnik et al., 2015; Zhuikov O.G., 2014). The objective of this research was to develop cultivation technology for *Brassica juncea*L in the conditions of the north-eastern forest-steppe of Ukraine for the first time.

MATERIALS AND METHODS

Studied the influence of weather conditions on the peculiarities of growth, development, formation of biomass, photosynthetic activity, productivity indices, yield, seed quality, oil production, depending on the variety, sowing time, seeding rates and fertilizer for growing bluish mustard.

The experimental part of the work was conducted at the training and practical center of Sumy National Agrarian University (Ukraine) for three years (2015, 2016 and 2017). It is situated at 50 52.742N Latitude, 34 46.159E Longitude at an altitude of 137.7 m above mean sea level in the north-eastern forest-steppe of Ukraine**.**Experiments were laid on black soil, characteristic for coarse-medium loam. Two sorts were sown at plant densities 1,5 million plants/ha with 10 rows in each plot and 15 cm between rows.

To determine the dynamics of linear growth, the pre-marked plants were studied. The area of the leaf surface was determined by the method of "carving", and the net productivity of photosynthesis and photosynthetic potential were determined according to the methods of A. O. Nychiporovich. The content of chlorophyll in the leaves was determined by means of preparing the solution in an alcohol extract with the further determination by a spectrophotometer ULAB 102 (Grytsaenko, 2008). Harvesting of the crop was carried out plot by plot by means of the Massey Ferguson 307 direct combining, when the color of the main stem and the pods was yellow, and the leaves fell off, simultaneous weighing the seeds according to the options of the experiment and sampling to determine the moisture and purity. Harvesting was brought up to 100% of purity and 10% of moisture content of the seeds.

Hydrothermal coefficient (HTC) (G. T. Selyaninov) were determined by the formula: $HTC=\sum \frac{K}{\sum T \times 10}$, where ∑K is the amount of precipitation, mm, for a period with an average daily air temperature above 10 ° C; ∑T is the sum of the temperatures, ° C, for the period with the average daily air temperature above 10 ° C.

The oil content of the seeds was determined using the device Spinlock Magnetic Resonance Solutions. Data were subjected to ANOVA at 5% level of probability with the statistical software STATISTICA (version 8).

RESULTS AND DISCUSSIONS

The success of its cultivation mustardis largely determined by the changing environmental conditions, that is, weather and climate. Observations by the metrological network of Ukraine testify to the fact that regional climate change, especially temperature rise, has already affected a number of meteorological characteristics. The average annual air temperature has increased, the terms of formation and duration of snow cover have changed, the heat supply of the growing season gradually increased, and the number and intensity of adverse meteorological phenomena (drought, heavy rain, etc.) has increased (Melnik et al., 2015). For example, in the Forest-steppe of Ukraine we considered the main meteorological parameters for the period from 2000 to 2017. Thus, according to the results of the analysis of meteorological conditions for 18 years, it has been established that for the period of the growing (farming) season (April-August), there was an average of 281.9 mm of rainfall, with fluctuations from 171.4 to 461.7 mm. In this region, the average temperature during this period amounted to 2710.8 ° C with fluctuations ranging from 2311.0 °C to 3090.7 °C. Increase in the heat supply of the growing season was noted at 285.8 ° C and decrease in the amount of precipitation by 12.1 mm. Based on HTC which for the past 18 years has decreased from 1.21 to 1.05, it was established that the conditions of this region correspond to the conditions of the Steppe zone of Ukraine. Analysis of weather conditions during the investigated years found that for the hydrothermal coefficient (HTC) in 2015, there was a severe drought in April (HTC = 0.08), weak drought in July and August (HTC = 0.76 and 0.77), excessive moisture in May (HTC = 2.5), and sufficient (adequate) moisture in June (HTC = 1.24). The vegetation period in 2016 was characterized by a sufficient level of humidification in April and June (HTC = 1.45 and 1.02). In July, there was a weak drought (HTC = 0.86), and in May and August, excessive moisture were observed (HTC = 3.04 and 1.87). It should be noted that in 2017, the dry conditions were different for most months, particularly in April and August which observed a very severe drought (HTC = 0.06 and 0.21), while strong and average drought respectively were observed in May and June (HTC = 0.43 and 0.57). Only July was sufficiently humid (HTC = 1.19). According to the Ukrainian State Register for Plant Varieties (SRPV), in 2017, the agricultural producer presently have 10 varieties of bluish mustard.

An important segment of the market in Ukraine is occupied by high-performance adapted hybrids of domestic breeding: the Instituteof Oil seeds of NAANU, Instituteof arable farming of NAANU, Precarpa thian State Agricultural Experimental Station of NAANU and a number of other institutions. According to results of research conducted in 2015-2017 at the Department of Crop Productionin Sumy National Agrarian University in Ukraine, a regional technology for bluish mustard cultivation, which involves the selection of adapted hybrids and optimization elements was developed. Having analyzed the grey mustard varieties in terms of yield formation and their suitability for the cultivation in the Northeastern Forest-steppe of Ukraine, we concluded that the period of vegetation of the grey mustard varieties varies from 88 to 95 days. Demeter, Prima and Chornyava varieties had the longest vegetation period. We recorded the shortest period of vegetation for Rosava variety. The average area of the leaf surface at the flowering stage was 42.8 thousand m^2/ha, determined by the method of carving. The varieties of Prima, Mriya, Retro and Demetra had the above average value. An indicator close to the average value was characteristic for Felitsia variety. Rosava, Roksolana and Chornyava had the smallest leaf surface area.The content of chlorophyll "a" and "b" among the studied varieties, determined on the ULAB 102 spectrophotometer, varied from 0.97 to 1.14 in mg/g of the fresh weight.

The varieties of Prima (1.91 t/ha), Retro (1.83 t/ha) and Demetra (1.81 t/ha) formed significantly higher seed yield capacity. Average yield was recorded in the varieties of Mriya (1.69 t/ha), Felitsia (1.58 t/ha) and Roksolana (1.33 t/ha). The yield of the varieties of Rosava (1.25 t/ha) and Chornyava (1.07 t/ha) was significantly lower.

With the help of the infrared analyzer Spinlock Magnetic Resonance Solutions., we determined the maximum content of oil in the Prima variety seed - 40.1%. Retro and Felitsia varieties (38.9%) showed the above average value. The varieties of Mriya (37.3%) and Rosava (31.0%) had significantly lower values.The average oil yield over the years of research was 0.60 t / ha (Fig. 1).

In order to study the nature of the influence of morphological parameters on productivity, a regression analysis of the main indicators was carried out. A number of authors argue that there is a strong connection between the length of the pod and the number of seeds in it (Zhyikov, 2014). However, the results of studies by other scientists indicate that the length of the pods is not a direct element of the structure in the seed productivity (Vyshnivsky, 2011). The results we have received confirm this very trend. Thus, the calculated correlation coefficient is not significant ($r = 0.05$), and the regression line is in a horizontal position.

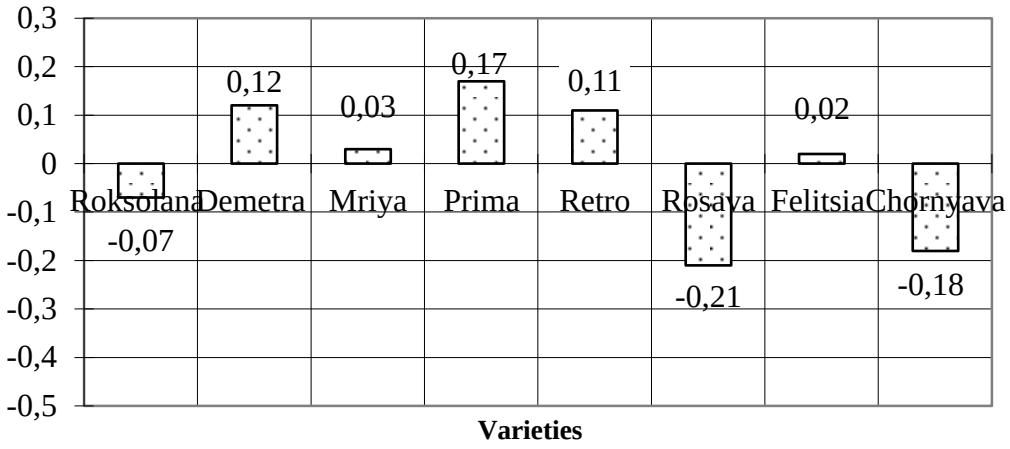

Fig. 1. Deviation from the average (0.6 t/ha) for the oil production of the bluish mustard varieties, depending on the variety, t/ha (average over 2015-2017)

The timing of sowing determine the level of moisture and nutrients availability for the plants. A properly established term will enable the formation of highly productive plants of the spring mustard (Kurmi 2002; Singh 2002). The change in climate conditions in Ukraine over the past decades has had an impact on the soil maturity and allowed sowing all the crops as well as mustard at earlier dates (Melnik et al., 2015; Zhykov, 2014). Sowing of the grey mustard varieties of Prima and Retro in the first sowing term (the soil temperature was 4-5 °C) contributed to the maximum period of vegetation 92 and 90 days respectively, the second (the soil temperature was 6-7 °C) and the third (the soil temperature was 8-9 °C) terms the vegetation period was reduced by 3 and 6 days respectively on the average. The first term of sowing ensured the formation of the leaf surface area in the flowering phase of Prima and Retro varieties at the level of 44.2-45.6 thousand m^2/ha. The second term sowing reduced the figure by 8% and for the third term by 15% compared with the first sowing term. The highest yields were typical for Prima variety - 1.86 t/ha in the first sowing term. During the second sowing term, there was a slight decline in the yields up to 1.75 t/ha. The third sowing term significantly reduced the yields to 1.53 t/ha. Retro variety recorded a similar tendency to decrease yields for the late sowing. The first term is 1.76 t/ha, the second - 1.67 t/ha, and the third - 1.46 t/ha. Late sowing also caused a decrease in the content of oil by 0.3% and 0.4% respectively (Fig. 2). According to the results of a dispersion analysis, a rather significant impact of the factor "conditions of the year" (55.0%) was established. In addition, the factor "sowing times" (36.6%) had a rather significant impact on the yields of mustard. The influence of the factor "variety" was 3.9%, the factor "others" - 4.3%, "factor interaction" only - 0.1%. Consequently, weather conditions (moisture reserves, temperature mode, etc.) make adjustments in determining the optimal timing of sowing and all together affect the mustard yields.

We would like to note the influence of the studied norms of mineral fertilizers on the duration of the varieties' vegetation period. Introduction of mineral fertilizers contributed to an increase in the duration of the vegetation period: prima variety had $N_{30}P_{30}K_{30}$- 5 days longer, and $N_{60}P_{60}K_{60}$- 7 days longer, Retro variety - 6 days longer compared with the control.

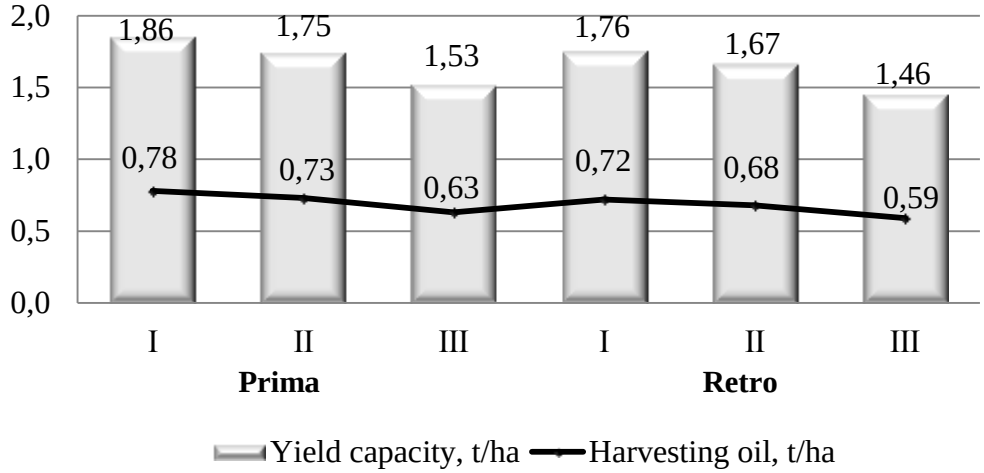

Fig. 2. Yield capacity and harvesting of the bluish mustard oil depending on the variety and timing of sowing, t/ha (average over 2015-2017)

Improvement of the mineral nutrition level in the studied varieties increased the leaf surface area. The maximum value of the leaf surface area was fixed in the flowering phase in the variant with the norm of fertilizers $N_{60}P_{60}K_{60}$ - 47.9-51.2 thousand m^2/ha, which exceeded the indicator in the control variant of 14.7-16.0 thousand m^2/ha, on the variant with the norm of fertilizers $N_{30}P_{30}K_{30}$ the leaf surface area was 44.2-45.6 thousand m^2/ha, which exceeded the control variant by 10.4-11.0 thousand m^2/ha. The analysis of experimental data of P. S. Vyshnivsky and others as to the influence of mineral fertilizers on the formation of the yield capacity of mustard under the conditions of the Northern Forest-steppe of Ukraine showed that the application of nitrogen fertilizers on the background of phosphoric-potassium (P45K45) contributed to the increase in yield capacity of the mustard seed of the variety from 0.08 to 0, 73 t / ha, with the indicators at the control of 0.90 t/ha. The efficiency of nitrogen fertilizers was 0.11-0.65 t / ha. The maximum yield capacity rate of 1.78 t / ha provided the maximum dose of mineral fertilizers $N_{60}P_{60}K_{60}$, where the gain to control was 0.88 t/ha (Vyshnivsky, 2011).
We observed a significant increase in the yields of Prima variety when applying $N_{30}P_{30}K_{30}$ on 1.89 t/ha, which is 0.47 t/ha more than the control variant. The maximum yields were obtained on the variant with the norm of fertilizers $N_{60}P_{60}K_{60}$ - 2.03 t/ha, which is 0.61 t/ha more than the control variant. Retro variety also showed a significant increase in the yields by 0.41 and 0.53 t/ha in the variants

with fertilizer rates of $N_{30}P_{30}K_{30}$ and $N_{60}P_{60}K_{60}$ compared with the control variant. We would like to note that in both varieties, the difference in the yields between fertilizer standards is negligible. The highest content of oil in Prima variety was on the control variant (40.4%). Application of fertilizers in the rate of $N_{30}P_{30}K_{30}$ reduced the content of oil in the seeds by 0.3% and fertilizers in the rate of $N_{60}P_{60}K_{60}$ caused a decrease in the content of oils by 0.7%. In Retro variety, we also recorded the highest oil content in the control variant, which reached 39.7%. Fertilizing in the rate of $N_{30}P_{30}K_{30}$ and $N_{60}P_{60}K_{60}$ reduced the oil content by 0.5% and 0.6% compared to the control. At the same time, proceeding from higher levels of the yields, application of fertilizers contributed to an increase in oil production: $N_{30}P_{30}K_{30}$ by 0.15-0.19 t/ha, $N_{60}P_{60}K_{60}$ by 0.20-0.24 t/ha compared to the control.

One of the important components of cultivation technology is the determination of optimal sowing standards, as to acquire high yields it is necessary to ensure a rational amount of crops and productive branches per area unit.

We determined that a longer period of vegetation was at the sowing rate of 1.5 million pp./ha - 90-92 days, further increase in the sowing rate contributed to a decrease in the period of vegetation in the studied varieties by 3-7 days on an average. The leaf surface area of Prima variety in terms of seed sowing rate of 1.5 million pcs/ha was at the level of 44.5 thousand m^2/ha, which is 9.2 and 17.0 thousand m^2/ha less than the sowing variant of 2.0 and 2.5 million pieces/ha. Retro variety had the leaf surface area of 40.5 thousand m^2/ha in the variant with the sowing rate of 1.5 million pieces ha. Increasing the sowing rate up to 2.0 and 2.5 million pcs/ha increased this figure to 46.7 thousand m^2 ha and 54.8 thousand m^2/ha respectively. The net productivity of photosynthesis calculated by A. A. Nychyporovych in the interphase period of budding - flowering in the variant with the sowing rate of 1.5 million pcs./ha was 4.34 g/m^2/day an increase of the sowing rate up to 2.0 and 2.5 million pcs/ha contributed to the reduction of the net productivity to 3.58 and 2.60 g/m^2/day, respectively. Retro variety had a similar tendency.

An optimum sowing rate for Prima variety was found to be 1.5 million pcs/ha, which ensured the highest yield of 1.89 t/ha. By increasing the seed rate to 2.0 and 2.5 million pounds per hectare, yields dropped significantly to 1.81 and 1.75 t/ha respectively. Retro variety's yield was 1.71 t/ha with the sowing rate of 1.5 million pounds per hectare. A maximum yield was obtained at an optimum sowing rate of 2.0 million pounds/ha - 1.77 t/ha, an increase in the sowing rate up to 2.5 million pounds per hectare significantly reduced the yields to 1.69 t/ha (Fig. 3).

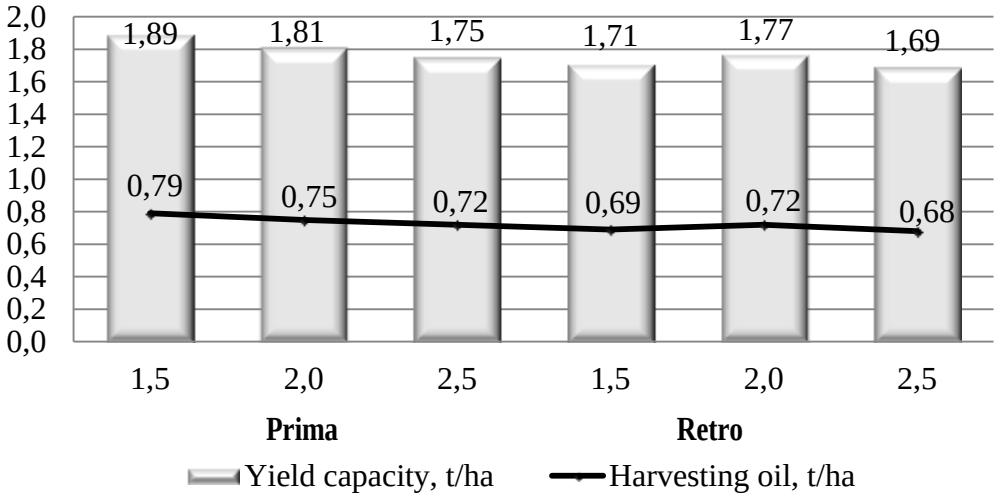

Fig. 3. Yield capacity and harvesting of the bluish mustard oil depending on the variety and seeding rates, t/ha (average for 2015-2017)

Analysis of the indices of economic and energy efficiency of Prima variety production, depending on the studied elements of cultivation technology, showed that the highest indicators of the level of profitability of seed production are 101-120%, the coefficient of energy efficiency of the production - 3.17-4.82.We recorded the maximum values in the first sowing term in a non-fertilized variant with the sowing rate of 1.5 million pcs./ha. Retro variety's maximum profitability indicators were within the range of 89-112%, the energy efficiency ratios - within the range of 3.07-4.69. They formed the maximum indices in the first sowing term in a non-fertilized variant with a sowing rate of 2.0 million pcs./ha.

CONCLUSION

According to research results, for the formation of 1.71-1.91 t/ha of bluish mustard seeds in the conditions of the north-eastern forest-steppe of Ukraine on typical black soil, the cultivation technology should include: use of seeds of modern high-yielding adapted varieties; start sowing at soil temperature of 4-5 ^{o}C at a depth of 10 cm; the rate of fertilizer $N_{30}P_{30}K_{30}$ (during pre-sowing cultivation) and seeding rates of the varieties Prima - 1.5 million pieces/ha, and Retro -2.0 million pieces/ha.

According to the results of the analysis, trends in the meteorological parameters caused the expansion of the range of area under bluish mustard cultivation in Ukraine, which contributed to the increase in total national production of oilseeds. Having all the opportunities (natural, climatic, logistical and human), Ukraine will increase its presence in the world market of oilseeds.

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GENOMIC AND PEDIGREE-BASED INBREEDING IN SLOVAK SPOTTED CATTLE

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ABSTRACT

The objective of this study was to evaluate the level of inbreeding in population of Slovak Spotted cattle and to compare its genomic and pedigree-based estimates. The genomic data have been obtained from in total of 37 AI sires and 50 sire dams genotyped by using Illumina BovineSNP50v2 BeadChip and ICBF International Dairy and Beef v3, respectively. The genealogical information have been obtained from the database of Breeding Services of the Slovak Republic, s. e. The pedigree file consisted of 109,686 individuals (105,229 dams and 4,457 sires), while the reference population included only living animals, AI sires (129) and dams (36,949). The genomic inbreeding (F_{ROH}) was computed as the length of the genome present in runs of homozygosity (ROH) divided by the total length of the autosomal genome covered by SNPs on the chip and the pedigree-based inbreeding (F_{PED}) was calculated based on assumption that inbreeding of an individual reflects the probability that both alleles in one locus are derived from the same ancestor or are identical by descent. The ROH segments greater than 4 Mb ($F_{ROH > 4Mb}$) covered in average 2.09 % of the genome, while the ROH segments greater than 16 Mb ($F_{ROH > 16Mb}$) achieved 0.43 % which indicated in analysed population recent inbreeding. Similarly, the increase of inbreeding across generation signalized the average ΔF_{PED} computed from pedigree information (0.094%). However, the pedigree-based and genomic estimates of inbreeding differ from each other (in average $F_{ROH>4}$=0.02; F_{PED}=0.004). In recent generation, the obtained values of F_{ROH} indicated considerably higher degree of inbreeding.

Keywords: *Cattle, Genotyping data, Inbreeding, Pedigree analysis, Runs of Homozygosity.*

INTRODUCTION

The Slovakia has close to a century of tradition in breeding dual-purpose cattle breeds, namely the Slovak spotted and Slovak Pinzgau cattle. The beginnings of a production type transformation go back to 1972 when cross breeding (mainly combined crossing) with foreign breeds (Lowland Black-spotted and later on also

Holstein) has begun, with the purpose of acquiring a pronounced milk-type cattle population but retaining however a certain hereditary ratio (about 30 %) of Slovak spotted cattle in order to maintain its typically good meat utility value in the target population (Biros, 1997). Currently, the breeding of Slovak Spotted cattle continues in the dual-purpose direction focusing on high milk and beef production. Regular fertility, longevity, good adaptation potential and an ability to consume large amounts of bulk feed in connection with a high utility level are required from the Slovak Spotted cattle mainly with respect to the efficiency and economics on the farms. In 2017, totally 28,747 purebred cows have been registered in Slovak Spotted cattle herd book (ZCHSSD, 2018).

Generally, in livestock the inbreeding coefficient is recognized as one of the most important parameter reflecting the level of genetic diversity, relatedness of animals as well as individual fitness of in a population. The inbreeding coefficient of an individual can be described as the probability that two alleles at a locus in that individual are identical by descent and equals the coancestry between its parents (Howard *et al*., 2015). Thus, the inbreeding coefficient can be considered as a key parameter mainly with respect to understanding of the amount of matings between related animals in a population (Gomez-Raya *et al*., 2015). It has been shown that the high level of inbreeding leads to a reduction in fitness and overall productivity at the phenotypic level as well as to a higher risk of homozygosity for deleterious/lethal alleles at the genotypic level (Curik *et al*., 2014; Howard *et al*., 2015; Forutan *et al*., 2018). Therefore, the periodic control of inbreeding in livestock populations is crucial for the preservation of animal genetic resources mainly in case of small populations and from a wider perspective for the population management and development of mating plans (Gomez-Raya *et al*., 2015). Traditionally, inbreeding coefficient has been estimated based on pedigree-based relationships (Meuwissen and Luo, 1992). Pedigree-based inbreeding is based on Mendelian sampling probabilities, so that the inbreeding coefficients of full-sibs are always identical. Using pedigree information for calculating the level of inbreeding usually underestimates the true inbreeding coefficient, mainly due to incomplete pedigree information, especially for distant generations (Forutan *et al*., 2018). This can be avoided for example by the use of genome-wide data that allow to estimate the level of inbreeding derived from the relative amounts of autozygosity due to recent as well as remote ancestors (Ferenčaković *et al*., 2013a). The best concept to quantify the level of genomic inbreeding based on true or realized autozygosity was suggested by McQuillan *et al*. (2008). The genomic inbreeding coefficient (F_{ROH}) is defined as an individual autozygosity reflecting the proportion of the autosomal genome, in which autozygosity is derived from the assumption that very long stretches of homozygosity (ROH) can only result from inbreeding (Curik *et al*., 2017).

The objective of this study was an analysis of the trend of inbreeding in Slovak Spotted cattle by using pedigree-based (F_{PED}) and genomic (F_{ROH}) estimates with respect to compare both approaches and to obtain more realistic view on situation in current population.

MATERIAL AND METHODS

The pedigree database of Slovak Spotted cattle included in total of 109,686 individuals (105,229 dams and 4,457 sires). The reference population (RP) covered only living animals (129 AI sires and 36,949 dams) that were included in performance testing in 2013. The genealogical information were obtained in cooperation with the Breeding Services of the Slovak Republic, s. e. The genotyping database consisted of totally 87 animals (37 AI sires and 50 sire dams) that were genotyped in commercial lab by using two platforms, Illumina BovineSNP50v2 BeadChip (AI sires) and ICBF International Dairy and Beef v3 (sire dams). Animals for genotyping were selected based on the previous pedigree analyses that were performed to select only most representative animals in terms of the Slovak Spotted population gene pool. The quality of genealogical information expressed as the pedigree completeness was evaluated based on the equivalent complete generations of ancestors and pedigree completeness index described by MacCluer *et al*. (1983) using Endog v4.8 software (Gutiérrez and Goyache, 2005). The quality of genotyping data was tested by using PLINK 1.9 (Chang *et al*., 2015). Because of the two different genotyping platforms used for animals' genotyping, the consensus map had to be firstly constructed. The consensus map file included overall 40,033 markers. Subsequent quality control of genotyping data were performed to remove all of SNPs with unknown chromosomal position or localized on unmapped genomic regions based on the bovine genome assembly Btau 5.0.1 and markers located on sex chromosomes. In the subsequent SNP pruning only samples with lower than 10 % of missing genotypes, autosomal SNPs with call rate higher than 90 % and minor allele frequency higher than 1 % that adhered to mendelian inheritance patterns were retained.

The level of pedigree-based inbreeding (F_{PED}) was characterized by using two measures: increase in inbreeding (ΔF_{PED}) and individual increase in inbreeding (ΔF_{PEDi}). The increase in inbreeding was calculated using Endog v4.8 (Gutiérrez and Goyache, 2005) according to Gutiérrez *et al*. (2009) as follows:

$$\Delta F_{PED} = \frac{(F_t - F_{t-1})}{(1 - F_{t-1})},$$

where F_t and F_{t-1} are the average inbreeding at the i^{th} generation and $1 - F_{n-1}$ is increase of inbreeding in last generation. The individual increase in inbreeding was computed as:

$$\Delta F_{PEDi} = 1 - \sqrt[t-1]{1 - F_i},$$

where F_i is the individual coefficient of inbreeding and t is the equivalent complete generations (Maignel *et al*., 1996).

The genomic inbreeding (F_{ROH}) was calculated for each individual as the length of the genome present in runs of homozygosity (ROH) divided by specified length of the autosomal genome covered by all SNPs that retained in the database after quality control of genotyping data (2,496,829 kb):

$$F_{ROH} = \frac{\sum k\ lenght(ROH_k)}{L},$$

where *k* is the number of ROH identified for each individual in kilobases and *L* is the total length of the genome covered by SNPs in chip (McQuillan *et al*., 2008). The genome-wide distribution of ROH segments were scanned using PLINK 1.9 (Chang *et al*., 2015). The ROH were determined based on following criteria: the minimum number of SNPs included in the ROH segments was fixed to 15; the minimum length of ROH was set to 1 Mb; minimum density of one SNPs on every 100 kb; maximum gap between consecutive SNPs of 1 Mb; one heterozygous call was allowed only for length >16 Mb. In addition, one missing call was allowed for length >4 Mb, 2 for >8 Mb and 4 for >16 Mb. Ferenčaković *et al*. (2013b) showed that the 50K panel is not enough sensitive for the precise determination of segments that are 1 to 4 Mb long, so that only for three ROH length categories were taken into account (ROH > 4 Mb, ROH > 8 Mb and ROH >16 Mb) to differentiate between ancient and recent inbreeding.

RESULTS AND DISCUSSION

As expected, the higher level of pedigree completeness across first five generations was found in reference population (81.37 %) than in pedigree file (44.94 %). The maximum number of generation detected was 12. Even if the dams showed generally lower level of pedigree completeness, taking into account only first 5 generations in both groups more than 80 % of the ancestors were known. In agreement with previous studies the completeness of pedigrees had decreasing tendency with increasing number of known generations (Bernardes *et al*., 2016; Sarmiento *et al*., 2016; Utrera *et al*., 2018).

The average value of pedigree-based inbreeding was in range from 0.14 % (pedigree file) to 0.76 % (group of sires). In group of sires the highest individual increase in inbreeding between generations was found (0.19 %), while the average F_{PED} was almost the same regardless of gender (0.25 %) (Figure 1A). Because of this we can expect in the next generations the increase of inbreeding level with the same intensity in both dams and sires groups. The obtained proportion of inbred animals in reference population of Slovak Spotted cattle (42.77 %) is comparable with Irish Simmental population (< 50 %), but the average F_{PED} in group of inbred animals (0.85 %) was much lower than in Irish Simmental breed (F_{PEDi} = 2.21 %) (McParland *et al*., 2007). Similarly, the proportion of inbred animals found in Slovak Spotted cattle is about half lower than in Slovak Holstein cattle (83 %; Pavlík *et al*., 2012). In reference population, the higher proportion of inbred animals showed the group of sires (Figure 1B) that confirmed study of Pavlík *et al*. (2012) as well. Pavlík *et al*. (2012) reported for Holstein cattle significantly lower proportion of inbred animals in group of dams (82.97 %) compared to sires (98.82 %). This results are logical and clearly reflect the utilization of reduced number of sire lines in breeding practices.

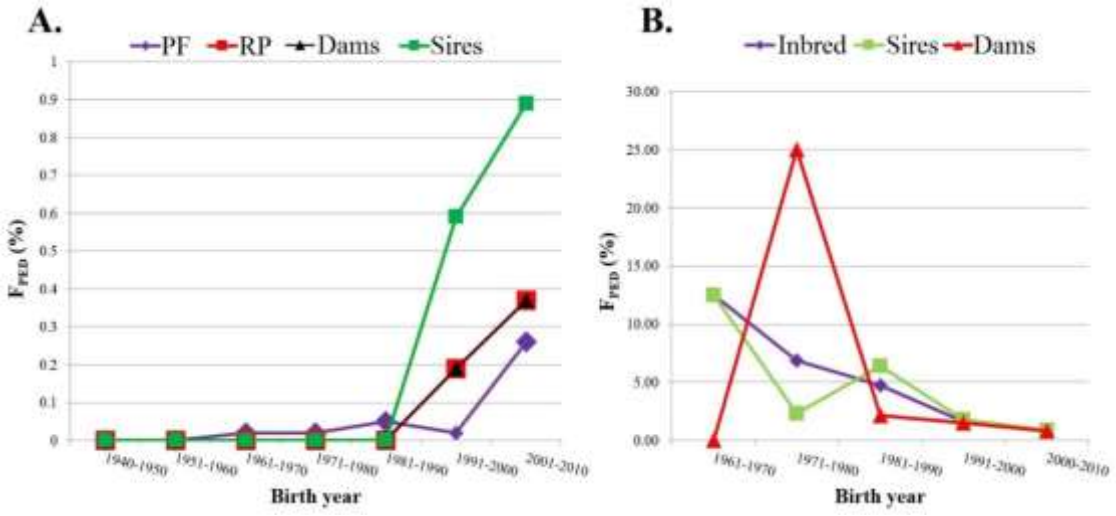

Figure 1. Trend of pedigree-based inbreeding by birth year in Slovak Spotted cattle (A – whole population and B – inbred animals; PF – pedigree file, RP – reference population).

It has been shown that inbreeding coefficients derived from the distribution of ROHs with different length in the genome reflect differently remote common ancestors from the past generations. Various studies reported that the $F_{ROH>4Mb}$ is related to the proportion of autozygosity originating from chromosomal segments present in ancestors that were born 12-13 generations ago. The inbreeding coefficient derived from the distribution of ROH segments greater than 8 Mb ($F_{ROH>8Mb}$) is related to the proportion of autozygosity originating from ancestors that were born 6-7 generations ago and $F_{ROH16>Mb}$ presents in ancestors that were born 3-6 generations ago (Howrigan *et al*., 2011; Ferenčaković *et al*. 2013a; Curik *et al*., 2014). In Slovak Spotted cattle, the ROH segments greater than 4 Mb cover in average 2.09 % of the genome, while the ROH>16 Mb achieved 0.43 %. This signalized that around 0.5 % of its genome is affected by mating of relatives in recent population. Figure 2 shows boxplot distribution of F_{ROH} by each length category.

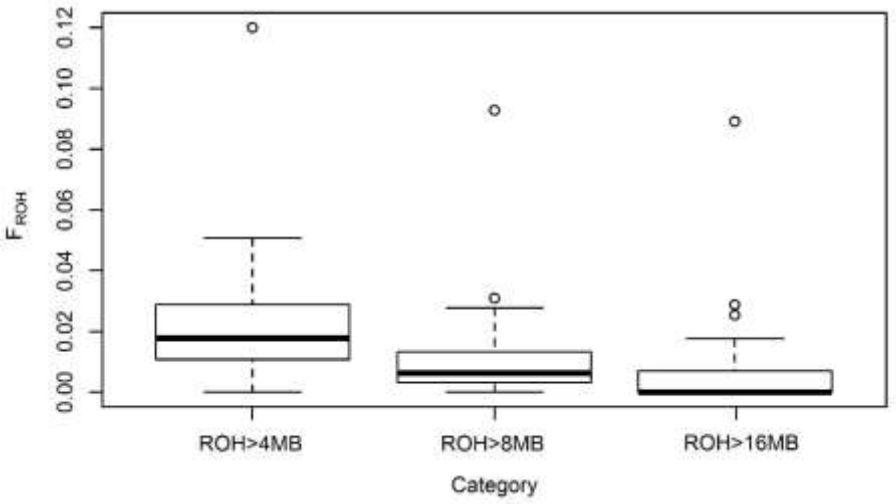

Figure 2. Boxplot distribution of genomic inbreeding derived from different ROH length categories.

As you can see on the figure 2 as well as in table 2 the value of $F_{ROH>4MB}$ indicated considerably higher degree of genomic inbreeding compared to the pedigree-based estimate regardless of gender (F_{ROH} = 2 % vs. F_{PED} = 0.36 %). This also points out that the level of true inbreeding in current population of Slovak Spotted cattle can be significantly underestimated. As previous studies have shown an increase in inbreeding over 1 % can lead to a reduction in fitness as well as overall performance at the animal's phenotypic level. For example, Bjelland *et al*. (2013) reported for daily cattle significant association between increase of 1 % of F_{ROH} and decrease of milk yield, some liner-type traits, increase in days open and maternal calving difficulty. Similarly, Pryce *et al*. (2014) and Ferenčaković *et al.* (2017) revealed inbreeding depression for milk yield and reproduction performance with stronger unfavourable effects for F_{ROH} related to closer ancestors (longer ROH segments).

Table 1. Genomic inbreeding by F_{ROH} category in group of sires and dams

Group	Category	Mean ± SD	Range	Lower 95% CI	Upper 95% CI
Sires	$F_{ROH>4Mb}$	0.020 ± 0.011	0.004 - 0.051	0.016	0.024
	$F_{ROH>8Mb}$	0.007 ± 0.007	0.000 - 0.023	0.005	0.010
	$F_{ROH>16Mb}$	0.002 ± 0.005	0.000 - 0.018	0.001	0.004
Dams	$F_{ROH>4Mb}$	0.022 ± 0.018	0.000 - 0.120	0.016	0.027
	$F_{ROH>8Mb}$	0.011 ± 0.015	0.000 - 0.093	0.007	0.015
	$F_{ROH>16Mb}$	0.006 ± 0.014	0.000 - 0.089	0.002	0.010

SD – standard deviation, CI – confidence interval

CONCLUSION

Both pedigree-based and genomic estimates of inbreeding coefficient per generations indicated risk of increase of inbreeding in current population of Slovak Spotted cattle. The obtained level of inbreeding didn't show significant differences between sires and dams. Thus, in next generations we can expect the increase of inbreeding with the same intensity in both dams and sires group. However, the obtained level of recent inbreeding depended on the applied approach. The comparison between F_{PED} and F_{ROH} values clearly points out that the level of true inbreeding in current population is considerably underestimated most likely due to the incomplete pedigrees in distant generation. The F_{ROH} value around 2 % represents a risk for population especially in view of its production and reproduction performance. Therefore, it would be desirable to take into account not only pedigree data but also genomic information mainly in management of Slovak Spotted population nucleus.

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IMPACT OF AGRICULTURE ON WATER POLLUTION

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ABSTRACT

The impact of agriculture on surface and groundwater is determined as negative. On the other hand, agriculture is negatively influenced by wastewater and polluted groundwater. The aim of the paper is to identify, analyze and assess the impacts of agriculture on water pollution and agriculture as a pollutant. The object of the survey is agriculture, and the subject of research is 1) the impact of water pollution on the agrarian sector and 2) the effects of agrarian activities on water pollution. The methodological framework of the paper includes: 1) literature review of impact of the water resources on agriculture and agriculture as a contributor to the water pollution; 2) analysis of impact of agriculture on water pollution based on statistical information and own survey; 3) conclusions and recommendations for mitigation of water pollution. The used method in the paper is survey method among agrarian, environmental and agroecological experts.

Keywords: *agriculture, water pollution, recommendations.*

INTRODUCTION

The impact of agriculture on surface and groundwater is determined as negative. Multiple farming activities as livestock breeding, pesticide use, fertilization and unsustainable land use lead to water pollution. Agricultural practices also damage aquatic ecosystems and river beds. The agricultural sector is responsible for water pollution due to plant and livestock activities. According some authors (Parris, 2011; Wiebe and Gollehon, 2007) pollutants can be classified as nitrate, phosphorus, pesticide, soil sediment, salt, and pathogen pollution. Ivanov (2015) considers agriculture has a negative impact on water resources through pressure on: 1) water quality - the use of fertilizers and pesticides, wastewater, 2) the water quantity through change and destruction of natural riverbeds, 3) water habitats – negative impact because of intensive agriculture, soil erosion and others. According UNEP (2016) agriculture pollutes water resources as a result of the use of agrochemicals, organic substances, saltwater drainage, and pollution threatens aquatic ecosystems and human health. The same opinion is shared by a working group set up by the Global food security programme (2015), considering that agriculture impacts on water quality by the release in water bodies of nutrients and

other chemicals, as well as changes of the physical habitat of rivers. According OECD (2012) water pollutants from agriculture are related to nutrients and pesticides, soil sediments, and other contaminants. The water pollution leads to negative impact on aquatic ecosystems, commercial freshwater and marine fisheries. The pollution also leads to decrease of social benefits of water resources as swimming and waterscapes and has negative impact on human health. Petkova (2012) considers that the main polluters of water from agricultural activities are: organic and mineral fertilizers, soil erosion, and wastewater from livestock farms. The author summarizes the reasons for water pollution with nitrates from agricultural sources such as: receiving of higher yields from cultivated crops, lack of knowledge of soil nitrogen storage, uneven soil fertilization, and improper storage of fertilizers. The other reasons for the negative impact of agriculture on water resources are connected with the population growth. Mateo-Sagasta et al. (2017) consider that water quality is negatively influenced by the intensification of production due to the increasing demand for food. Negative influence stems from cropping and livestock systems and aquaculture. The authors reveal that agriculture has a negative impact on water as a result of the population growth and changes in dietary patterns. The use of polluted water has also negative impact on agriculture because it affects the agricultural production. Sarathamani et al. (2014) make connection between wastewater and water springs. Polluted water springs have negative impact on agriculture and health of the farmers due to contact with the contaminated water. Ongley (1996) also considers that agriculture is a victim of water pollution, because polluted surface and ground water influenced negatively on crops and lead to different disease to consumers and farm workers. Wiebe and Gollehon (2007) consider that water pollution leads to additional costs. The presence of sediment, nutrients, pesticides, salts, and pathogens create costs for users of water resources. The authors stress on the fact that is very complicated to calculate the arised losses by poor water quality. The issue is due to the lack of physical monitoring and the difficulties in assessing the economic costs and benefits of environmental goods and services. An OECD study indicates that most of the costs due to the quality of water resources are related to removing pollutants from water resources, negative impact on ecosystems, fishing, etc. (Parris, 2011). The aim of the paper is to identify, analyze and assess the impact of agriculture on water pollution and agriculture as a pollutant. The conclusions could be used for policy making support and introduction of practices and mechanisms in agriculture that will contribute to the improvement of the environment and in particular the quality of the water resources.

MATERIAL AND METHODS

The methodological framework of the paper includes: 1) literature review of impact of the water resources on agriculture and agriculture as a contributor to the water pollution; 2) analysis of impact of agriculture on water pollution based on statistical information and own survey; 3) conclusions and recommendations for mitigation of water pollution. The object of the survey is agriculture, and the

subject of research is 1) the impact of water pollution on the agrarian sector, and 2) the effects of agrarian activities on water pollution. The analysis is based on national statistical data and own survey. The used method in the paper is survey method among agrarian, environmental and agroecological experts. The sample includes 24 expert interviews with closed and open questions. The survey was conducted in 2018. The respondents evaluate the impact of the agricultural sectors on water pollution and agriculture as a victim of water pollution. The main agricultural practices related to plant and livestock breeding are evaluated to find out their negative impact on water resources. Respondents shared a view about voluntary and restricting measures related to reducing the negative impact of agricultural activities on water.

RESULTS AND DISCUSSION

Statistical data present the increase of waste water from agriculture in Bulgaria for the period 2007-2016 (Table 1). Wastewater increased over 5 times. A large part of the wastewater from agriculture (from 93% to 99%) is discharged into water bodies. Insignificant part of total wastewater (from 2% to 18 %) is discharged of wastewater treatment plant. This reveals the importance of the problem and the need of solutions to reduce the amount of agricultural wastewater discharged into the water bodies without treatment. Table 2 presents a ranking of agricultural sector as a pollutant and affected by water pollution. Respondents consider that polluted water has a high impact on plant sectors (over 60% of experts). The relevantly high impact is supported by a different number of respondents for the plant sectors, their share ranges from 54% to 75%. The most affected sectors by water pollution are vegetables and perennials, and vines, followed by cereals, oil crops, industrial cultures, medicinal and aromatic crops. The highest pollutants according to respondents are grain sector and vegetables (54% and 50% support this statement). This could be explained by intensive agricultural practices and mechanization in grain sector and the high level of pesticide usage in vegetable sector. The experts consider that the low level of influence have the sectors - medicinal and aromatic crops (37.5%), and perennials and vines (25%).

Table 1. Generation and discharge of wastewater from agriculture in Bulgaria, mill. m^3/year

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Wastewater from agriculture, of which:	7.02	9.65	18.39	16.94	16.75	24.59	18.24	23.38	36.62	39.75
Total wastewater discharged into water bodies	6.52	9.18	18.14	16.66	16.31	24.29	18.05	22.00	36.42	39.40
Wastewater discharged without treatment	5.36	8.12	17.49	15.94	15.60	23.50	17.40	21.31	33.44	38.60
Discharged in wastewater treatment plant	1.16	1.06	0.66	0.73	0.72	0.79	0.65	0.69	2.98	0.81

*Source: NSI (2016). Generation and discharge of wastewater - total for the country, by Statistical Region and River Basin District. Statistical data.

Table 2. Sector ranking - Pollutants and affected by pollution - Crops

Sector	Rank - Influenced by water pollution	Rank - Influence on water pollution
Grain production	2	1
Oil crops	3	4
Perennials and vines	1	6
Industrial cultures	3	3
Medical and aromatic crops	3	5
Vegetables	1	2

*Source: Authors' elaboration based on the survey results.

Table 3 presents the ranking of stockbreeding subsectors of agriculture as pollutant and affected by pollution. Experts consider that beekeeping, rabbit breeding, horse breeding are the subsectors that have lowest negative impact on water resources, respectively 87%, 45%, 50 % consider they don't affect on water pollution.

Table 3. Sector ranking - Pollutants and affected by pollution - Livestock breeding

Sector	Rank - Influenced by water pollution	Rank - Influence on water pollution
Cattle-breeding and buffalo-breeding	3	3
Sheep and goat breeding	3	4
Pig breeding	4	1
Poultry rising	4	2
Beekeeping	1	7
Rabbit breeding	2	6
Horse breeding	4	5

*Source: Authors' elaboration based on the survey results.

Sectors as cattle-breeding and buffalo-breeding, pig breeding and poultry rising are considered by the experts with high negative impact on water (respectively 50%, 58%, 54%). Most respondents are on the opinion that stockbreeding sector is highly influenced by water pollution. The evaluations varied from 50% for pig and horse breeding and poultry rising up to 63 % for beekeeping.

Figure 1 presents the consideration of the experts of possible sources of water pollutions from agricultural activities. Wastewater from livestock breeding (75%), plant protection and pest control preparations and non-compliance with the Nitrate Directive (75%) and bad agricultural practices (71%) are pointed as practices with very high influence on water pollution. A third of the respondents (33%) considers that application of intensive agriculture to obtain higher yields of cultivated crops is a source with high influence. With the lowest impact on the water pollution is pointed soil erosion.

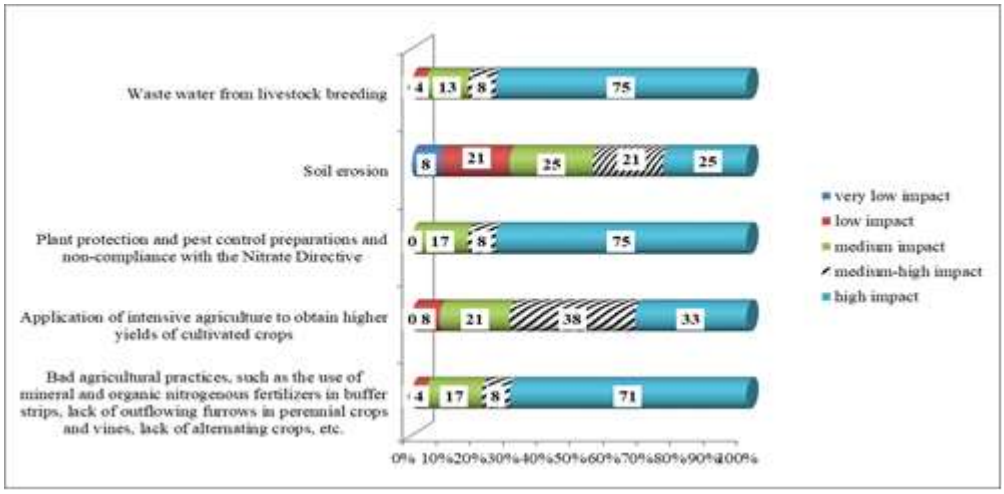

Figure 1. Practices related to water pollution connected with agricultural activities.
*Source: Author's elaboration based on the survey results.

The experts had to evaluate the restrictive mechanisms which could be undertaken to reduce water pollution by agricultural activities. Most of the respondents consider that ecotax for non-compliance with the Nitrate directive and good agricultural practices, implementation of preparation ecotax, will contribute as mechanisms connected with reducing water pollution of agricultural activities (Figure 2).

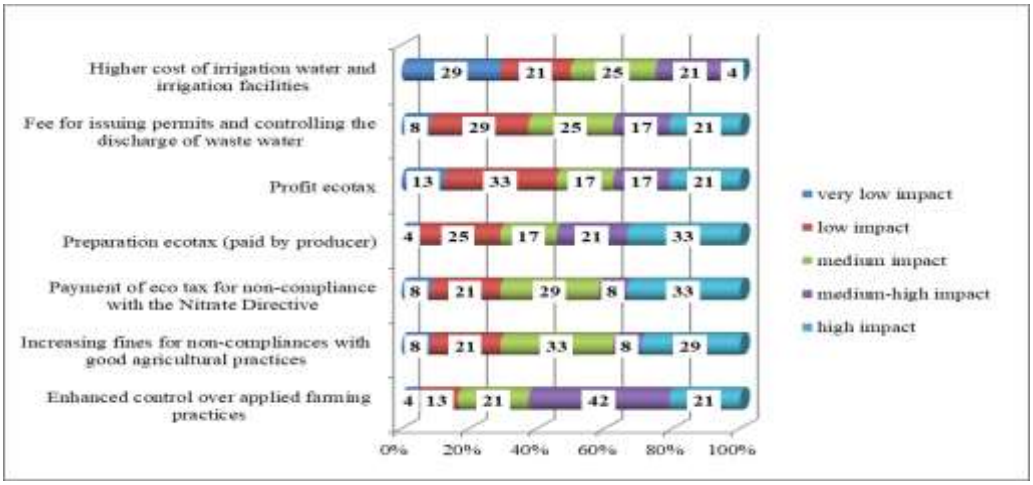

Figure 2. Restrictive mechanisms in agriculture related to reducing the impact of agricultural activities on water pollution
*Source: Authors' elaboration based on the survey results.

Fee for issuing permits and controlling the discharge of wastewater splits the experts into two groups - 37% consider this mechanism would have low impact on water pollution and 35% are on the opinion that the impact will be high. According respondents' view, higher cost of irrigation water and irrigation facilities will have low impact on water resources and will not be helpful for reducing levels of pollution.

The respondents had to evaluate also the voluntary mechanisms for reducing water pollution, by implementing voluntary measurements. Investments in irrigation facilities is pointed as the highest possibility for reducing water pollution, followed by policies which motivate the application of ecological practices. The most of the respondents consider that different type of voluntary mechanisms in agriculture will have a positive influence on water pollution (Figure 3).

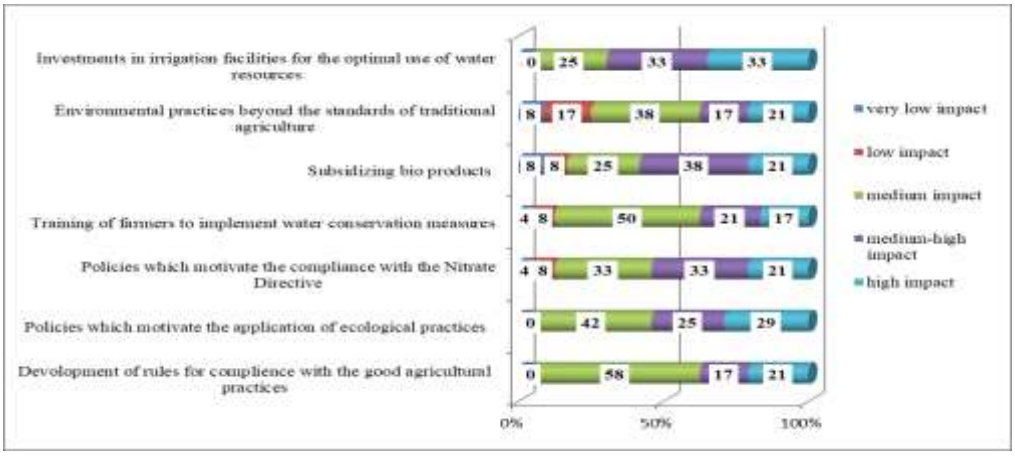

Figure 3. Voluntary mechanisms in agriculture related to reducing the impact of agricultural activities on water pollution.

*Source: Authors' elaboration based on the survey results.

The evaluation of mechanisms related to reducing the impact of polluted water on agriculture shows that construction of treatment facilities to prevent the entry of contaminated water is the measure with highest importance (58%), followed by informing farmers about contaminated water bodies (38%) (Figure 4). All mechanisms related to reducing the impact of polluted water on agriculture are with significant importance.

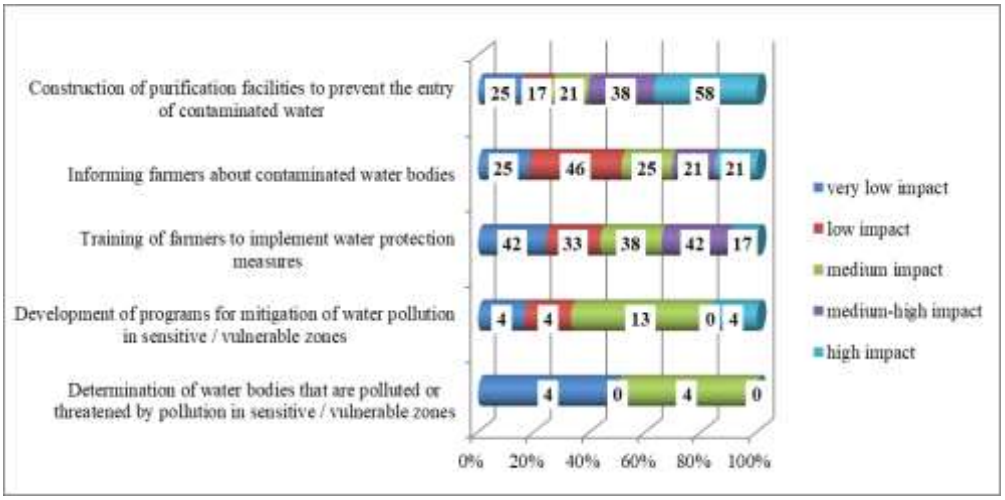

Figure 4. Mechanisms related to reducing the impact of polluted water on agriculture

*Source: Authors' elaboration based on the survey results.

CONCLUSIONS

In order to reduce water pollution, is necessary to develop policies encouraging farmers to apply good agricultural practices such as creating and maintaining buffer strips, creating run-off holding furrows in perennial crops and vines and planting crop strips as well as animal waste management. Technologies and innovation are a driving force and an opportunity to reduce water pollution and negative impact on agriculture. Farmers' attitude for applying more environmental friendly approach and implementation of the principles of sustainable development in their agricultural practices will reduce the negative impact of polluted water on agriculture and also the impact of the agrarian sector on water resources. In this connection, is necessary to stimulate projects in the water sector and for their successful realization to implement eco-innovative approaches and practices in the management of water projects. Reducing the negative impact of agriculture on water resources could also be achieved by increasing farmers' awareness and creating a culture of thinking about the environment and people. The impact of agriculture on water resources and the impact of water on agriculture will be positively influenced by the participation of farmers in training to implement good agricultural practices. This requires participation in specific trainings and awareness programs. They could be related both to mandatory practices as compliance with the national standards for good agricultural practices, Nitrate Directive requirements and to the voluntary mechanisms as erosion reduction activities, integrated pest management, irrigation water management, etc.

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EFFECTS OF CONTROLLED DRAINAGE ON SOIL WATER REGIME AND QUALITY IN LITHUANIA

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ABSTRACT

Lithuania remains one of the most extensively drained of the Baltic and Nordic countries. The overall drained area (ditches plus tile drains) totalled 87% of the agricultural land area. Many nutrients from soil are leached through drainage resulting in polluting streams (drain flow receivers) water. Drain flow is treated as a major determinant of water quality. Therefore, the reduction of nutrients entering the drains is very important. Controlled drainage conception, when the outflow height is increased at the mouth, helps reduce drainage runoff and partially purify water. The aim of the research was to establish controlled drainage influence on the soil moisture regime, nitrogen and phosphorus leaching. Investigations were carried out in sandy loam and loam soils in the Middle Lithuanian Lowland. Based on studies, several tendencies were observed: when drainage outflow began, the amount of soil moisture in subsoil (50-80 cm layer of the soil) of controlled drainage plot was higher than in the conventional drainage plot, and higher moisture supplies stayed for a longer period of time. Controlled drainage had no direct impact on phosphorus and nitrogen concentrations but they were influenced by the leaching quantities of plant usable nutrients. The reason that in many cases lower nitrate nitrogen (54% of all measurements) and phosphorus concentrations (77% of all measurements) were found in the conventional system rather than in the controlled drainage might be connected to the fact that the latter area contained predominantly lighter textured soils (sandy loam) making it easier to wash away the nutrients unused by plant.

Keywords: *drainage water, nitrogen, phosphorus, sandy loam, soil moisture.*

INTRODUCTION

The territory of Lithuania is defined as a zone of excess moisture, because precipitation amount is about 60% higher than the evapotranspiration. During some periods, the soil is too moist for growing agricultural crops. Drainage allowed to regulate rainfall effects on plant growth. A perennial drainage runoff study shows that in former years there was no drainage outflow in the winter or very rarely formed. Nowadays drainage often is functioning almost throughout the winter. An

increase in drainage outflow during cold periods, when the soil is not covered with plants, it can makes easier to leaching nutrients. The concentrations of nitrate nitrogen in drainage water can vary from 3 to 20 mg/l and total phosphorus levels vary from 0.1 to 0.15 mg/l (Povilaitis *et al.*, 2015). Traditional drainage systems work one-sidedly, permanently removing moisture from the soil. In order to reduce this impact on drained areas, groundwater level should be regulated, thus reducing the leakage volume and speeding up chemical compounds transformation into the gaseous form, it should also be implemented during the growing season by adapting it for irrigation. The aim of the research was to establish controlled drainage influence on the soil moisture regime, nitrogen and phosphorus leaching in sandy loam and loam soils in the Middle Lithuanian Lowland.

MATERIAL AND METHODS

The study area was carried out in Lipliūnai village (55°19'N; 23°50'E), Kėdainiai district, in the catchment of Graisupis stream (area 16.6 km^2) which is situated in the Middle Lithuanian Lowland. The area (A=10.3 ha) was drained in 1960, tiles installed at a depth of 0.90-1.10 m with drain spacing of 20-24 m. The drain area reconstruction was carried out in 1999. Having installed a drainage manhole at the junction of two separate systems – 4.9 ha free conventional drainage (CD) and 5.4 ha controlled drainage (CWD) – were arranged. The groundwater table to rise to a maximum of 68 cm above the tiles. The area impacted by groundwater table management covered about 52% of the CWD treatment plot (Fig. 1) (Ramoška and Morkūnas, 2006; Ramoska *et al*., 2011). The research of drainage water regulation renewed in June 2014 is a continuation of study conducted during 2000-2007. The soil is non-acid Endocalcari-Endohypogleyic Cambisol (*CMg-n-w-can*) (IUSS…, 2015). In conventional drainage area, in 0-30 cm layer the soil was sandy loam and loamy sand, in 31-70 cm layer – sandy loam, in the controlled drainage area in the upper layer – loamy sand, and in subsoil – sandy loam and loamy sand.

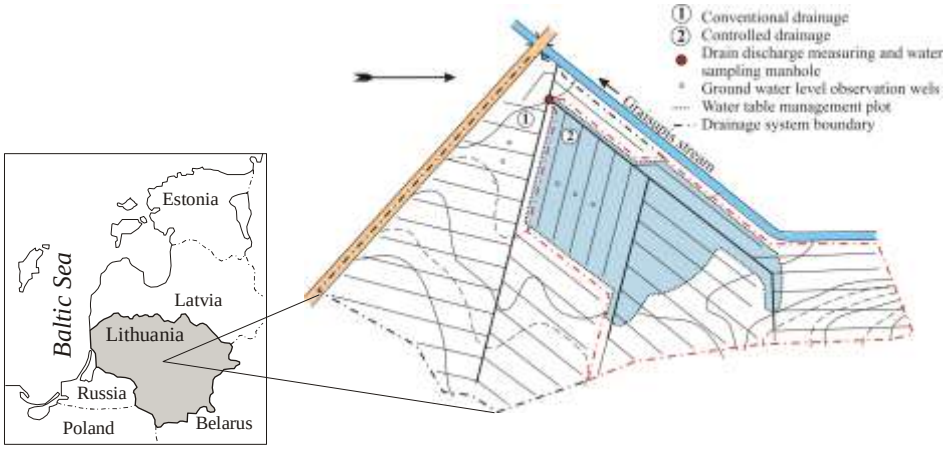

Fig. 1. Scheme of study area

Drainage water quality (NO_3-N (nitrate nitrogen) and TP (total phosphorus)) were determined by spectrometric method according to Lithuanian Standards (LAND 58:2003; LAND 65:2005). The water samples analysis was done at Chemical Analysis Laboratory of the Institute of Water Resource Engineering, Faculty of Water and Land Management of Aleksandras Stulginskis University.

The samples of soil moisture were taken at every 10 cm to 80 cm depth. Soil moisture was determined in all samples by drying at 105^{o}C and reweighing to constant weight. Meteorological condition (precipitation and average air temperature) was described using the data of the nearest Dotnuva Meteorological Station (Kėdainiai district), which is 8 km away from the study area. The mean annual precipitation is 566 mm (using 1981-2010 data) and the annual mean air temperature is 7.0 ˚C. Student's test was used to determine the reliability of differences between the studied data (Čekanavičius and Murauskas, 2001).

RESULTS AND DISCUSSION

The annual precipitation was 560 mm for 2014, 485 mm for 2015 and 721 mm for 2016, thus was approximate to the mean annual precipitation, 14% below the mean annual precipitation, and 27% above the mean annual precipitation, respectively.

During the research period the highest monthly precipitation variations from the perennial average was observed in February 2016 (262% perennial average), in January 2015 (191%), in August 2015 (9%), in October 2015 (14%), in February 2015 (15%), and in September 2016 (19%) (Fig. 2).

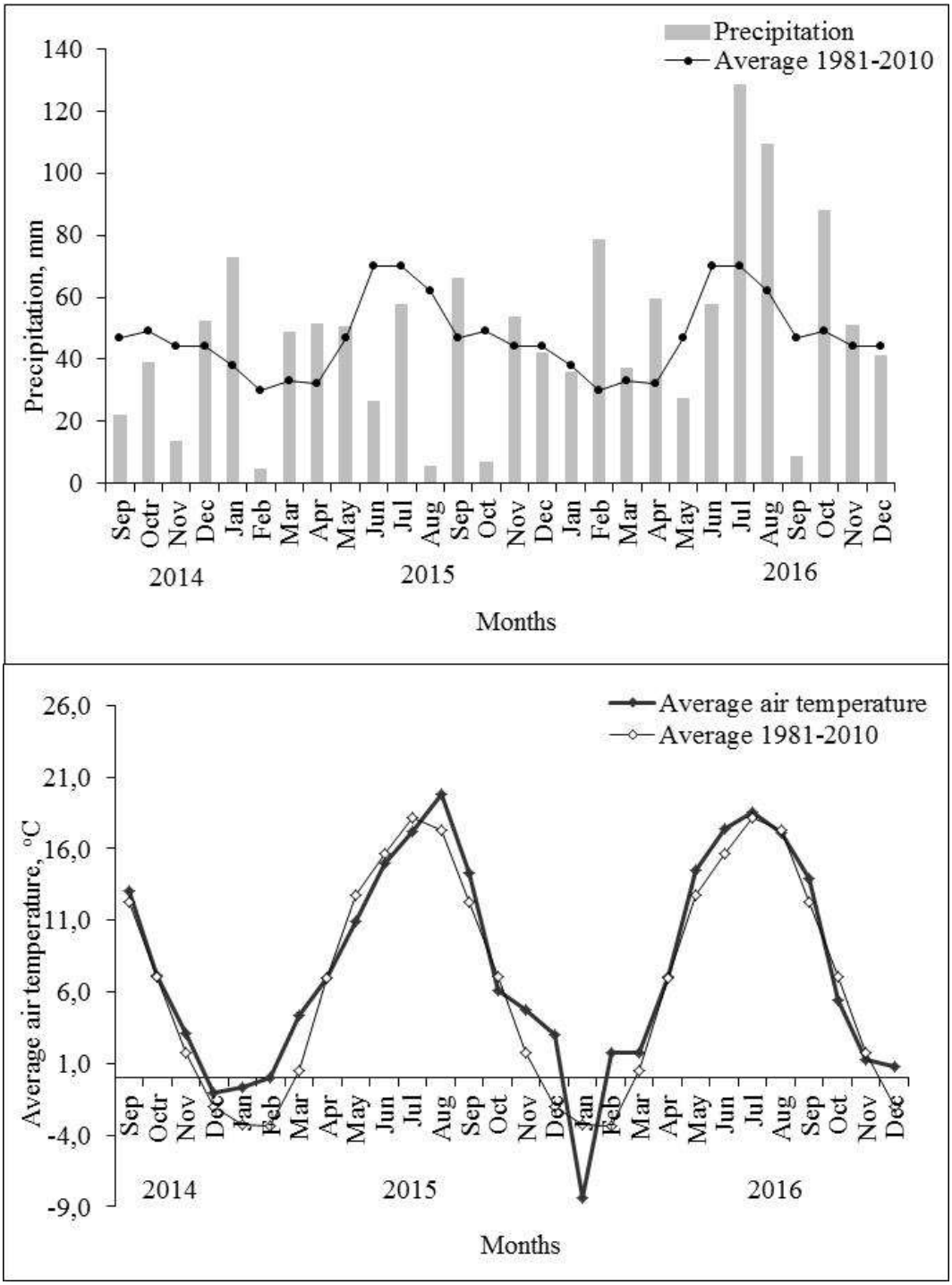

Fig. 2. Monthly precipitation (mm) and monthly air temperature (˚C)

Average annual air temperature 14% (2014), 22% (2015), and 9% (2016) was higher than the perennial average. Average monthly air temperature was 5 ^{o}C higher than the perennial average in December 2015 and February 2016, 3.9 ^{o}C higher in March 2015, 3.3 ^{o}C higher in February 2016, but 5 ^{o}C lower in January 2016.

During the study period, the average soil moisture of 0-80 cm layer was higher in conventional drainage area (12.2-25.6%) than in controlled drainage area (10.4-25.0%), but this difference was insignificant (Fig. 3). The moisture of the soil depended on meteorological conditions and soil texture. Studies (Morkūnas and Ramoška, 2001) shown, that the affluent drainage has impact on soil moisture. It is estimated linear correlation between soil moisture and groundwater table above the collector. In most cases this correlation (correlation coefficient r = 0.41-0.77) varied from weak to strong every 10 cm in 20-70 cm soil layer

In the study area, the moisture in 50-80 cm layer (55 of all measurements) was greater in controlled drainage area. In this drainage area, the moisture supplies obtained in subsoil for a longer period compared to conventional one. Ramoška (2001) states that the controlling of drainage outflow has increased the soil moisture supply in the subsoil and it did not has a greater impact on their changes in the upper soil layers.

In both study plots the highest variation of moisture was in 0-30 cm layer (in the conventional drainage plot standard deviation (SD) was 5.3-6.0, in the controlled drainage plot – 5.2-5.8). Black (1973) maintains that the moisture is more rapidly changing in the upper soil layer compared to the deeper layers. In sandy soil fluctuation of moisture at the top layer depended on meteorological conditions, at the lower layer equalled to the highest capillary moisture (Ivanauskienė, 1976).

The nitrate nitrogen concentration (54% of all measurements) and the total phosphorus concentration (77% of all measurements) were greater in the controlled drainage water (Fig. 4). However, significant differences (Student's test t_{act} = 2.28, p = 0.027) only in TP concentration between the studied drainage systems were confirmed. The TP concentration had the largest difference between the two systems in August 2014. In this month, the precipitation was 111 mm (66% higher than the CN), therefore more soil particles could enter in the water level control device.In the CWD system area prevailing lighter textured soils (sandy loam) which the nutrients can be easier leaching. This could be reason why in many cases lower NO_3-N and TP concentrations were found in the CD system. The literature (Wesström and Messing, 2007; Ramoska et al., 2011; Povilaitis et al. 2018) states that the controlled drainage reduced nutrients load while reducing drainage outflow. Controlled drainage outflow was 21-24% lower than the normally functioning drainage.

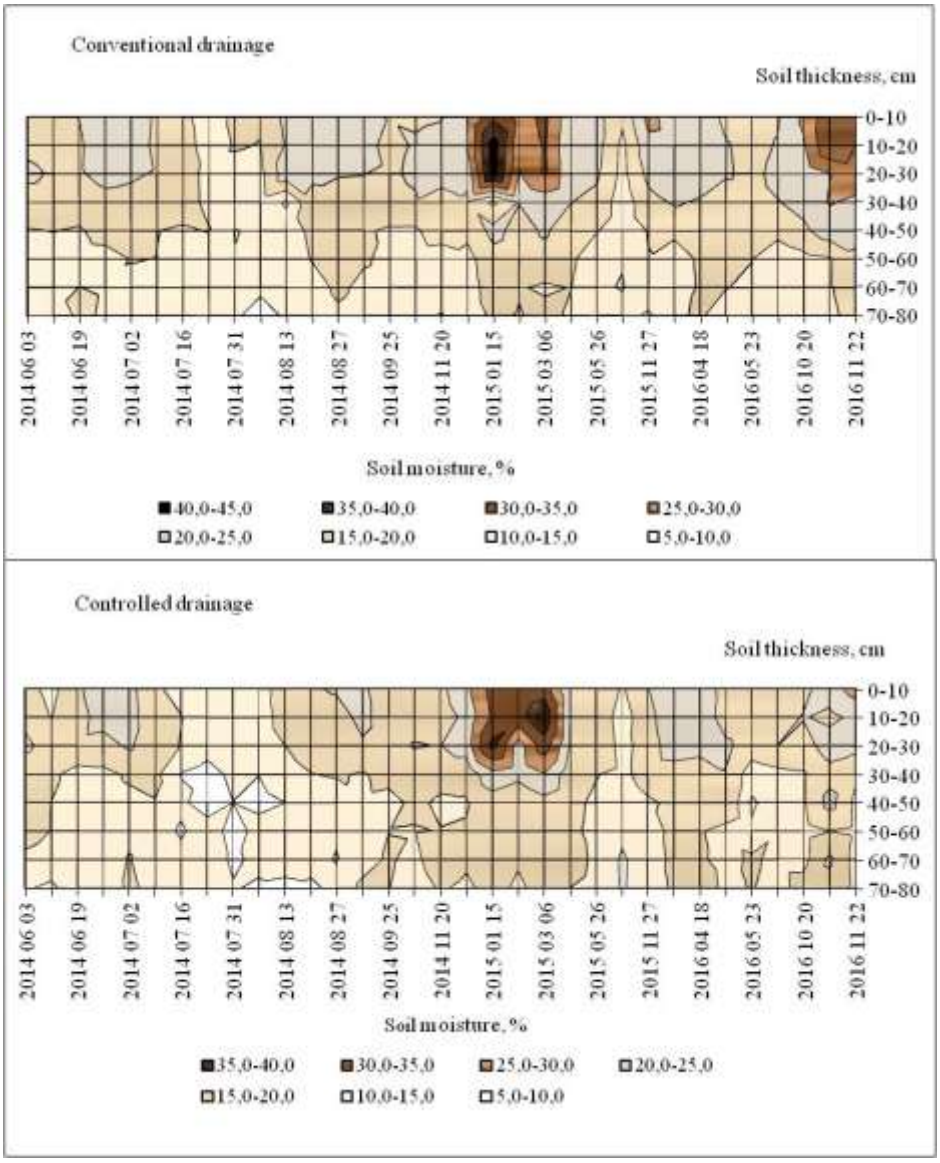

Fig. 3. Dynamics of soil moisture in the conventional drainage and controlled drainage area

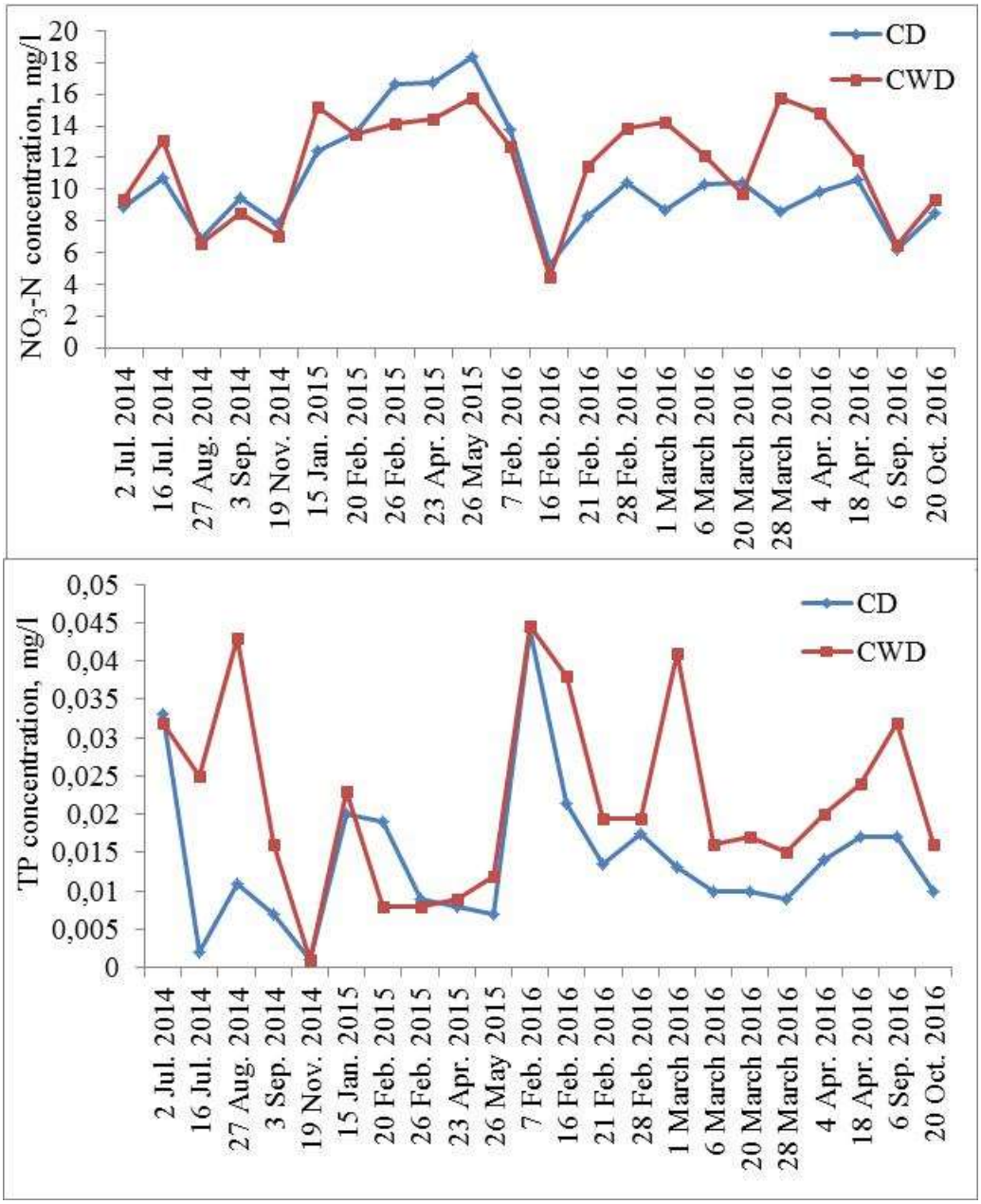

Fig. 4. Nitrate nitrogen (NO_3-N) and total phosphorus (TP) concentration in conventional (CD) and controlled drainage (CWD) water

In both study area, the NO_3-N concentrations was lower in period when average air temperature was about 10°C and precipitation amount was close to the CN (Table 1).

Table 1. Precipitation, average air temperature, NO_3-N and TP concentrations in drainage water estimated in seasons

		2014 Jun-Dec	2015 Jan-May	2016 Jan-May	2016 Sep - Oct
Precipitation	mm	372	228	238	97
	% CN	96	127	132	101
Average air temperature	°C	10.6	4.3	3.3	9.7
	% CN	106	158	122	100
	Conventional drainage				
NO_3- N, mg/l	AVG	8.7	15.4	10.5	7.3
	SD	2.6	2.4	3.5	1.6
TP, mg/l	AVG	0.028	0.013	0.019	0.014
	SD	0.06	0.006	0.011	0.005
	Controlled drainage				
NO_3- N	AVG	9.6	14.8	13.4	7.9
	SD	4.8	0.9	5.2	2.03
TP	AVG	0.03	0.013	0.026	0.024
	SD	0.02	0.006	0.011	0.011

CN – climate normal; AVG – average; SD –standard deviation.

The efficiency of pollutant retention depended on the climatic conditions. In dry years, the difference between controlled and conventional drainage was higher and it was lower in moderate years (Ramoska et al., 2011). Woli *et al.* (2010) reported that soil denitrification was rarely observed during the early period of spring, because of the minimal activities of denitrifying bacteria owing to the cold temperature. Denitrification rate may reflect the amount of nitrate in the water.

CONCLUSIONS

Investigations were carried out in sandy loam and loam soils in the Middle Lithuanian Lowland showed several tendencies: higher moisture supplies stayed for a longer period in the deeper layers of controlled drainage area; controlled drainage had no direct impact on nitrate nitrogen and total phosphorus concentrations.

When the drainage mouth was affluent (retained leakage), at the time chemical compounds with the runoff did not drain into the water receiver, and this had a positive impact on the environment.

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STUDY OF THE COMPRESSION BEHAVIOR OF SUNFLOWER SEEDS USING THE FINITE ELEMENT METHOD

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ABSTRACT

It is known that the phenomena that occur during compression of sunflower seeds are very complex. Comprehension of these phenomena is important for increasing the performance of the equipment in the vegetable oil industry, both for the cracking of shells and for the grinding of kernels. Also for the pressing of oilseed materials it is helpful to understand the compression behavior of sunflower seeds. The major objective of this work is to find an easy way of highlighting how the stresses and deformations propagate in sunflower seeds kernels and shells during the compression process, with the aim of optimizing the energy consumption required for the mechanical processing. Therefore, now days there is and we can use the finite element method. This method is the most advanced engineering tool for computing numerical and mathematical modeling of complex phenomena involving the propagation of stress and strain fields in continuous media. In this paper a two-dimensional FEM model for analyzing sunflower seeds subjected at compression by axial and lateral directions is presented. For experimental validation of FEM model we made uniaxial compression tests on sunflower seeds, using a Hounsfield/Tinius Olsen unit for mechanical tests, H1KS model. The models used in this work highlight that the orientation of the seeds is very important. There are situations when it is desirable that the stresses to be higher (at shelling, grinding, pressing, etc.) or situations when it is desirable that the stresses to be smaller (at transport, storage, etc.).

Keywords: *Finite Element Method, Sunflower seeds, Experimental validation, Oil industry.*

INTRODUCTION

Modeling of stresses and strains propagation of sunflower seeds in the compression process is very important because it allows estimating the energy consumption required to their decortication and pressing for oil extraction. To develop the

mathematical models of seeds behavior at compression it is necessary to know the physical and mechanical characteristics for kernel and the shell, respectively Young's modulus and Poisson's ratio. Figure 1 presents the morphological structure of the one of the most known type of oilseed, i.e. the sunflower seed, composed of the outer shell (seed vessel or pericarp), a thin peel (seed coat or endocarp) and the kernel. In recent years, experimental studies and research were conducted to determine the physical-mechanical properties of seeds. Thus, in their works, Khodabakhshian et al. (2010) and Khodabakhshian (2012), have deducted the mean comparison of elastic modulus of sunflower seed and kernel considering the influence of variety, moisture content and loading rate. It was found that the elastic modulus of sunflower seeds and their kernels decreased with increasing moisture content from 3% to 14% dry basis, and also with increasing the loading rate from 2 to 10 mm/min, regardless seed varieties and size categories. Average Poisson's ratio values of seeds from 0.28 to 0.35, 0.31 to 0.38, 0.33 to 0.42, were obtained for moisture levels ranging from 3% to 14%, respectively. Poisson's ratio increased from 0.281 to 0.357, 0.314 to 0.387 and 0.346 to 0.42 at loading rates of 2 to 10 mm/min. The Poisson's ratio and elastic modulus of sunflower seed and its kernel also exhibit a positive correlation with the size for all studied varieties, moisture content and loading rates.

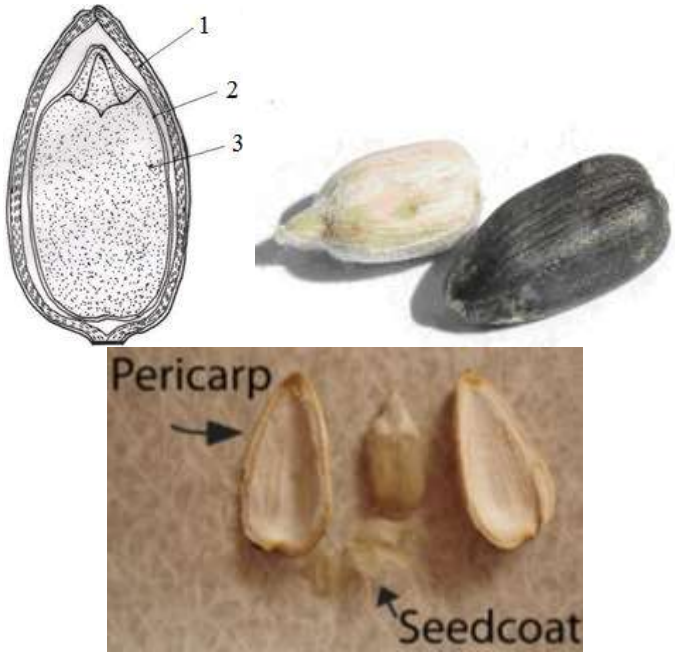


Fig. 1. Morphological structure of the sunflower seed
(1-pericarp; 2-endocarp; 3-kernel)

Gupta and Das (2000) studied the energy consumption for achieving the compression stress of sunflower seeds oriented vertically and horizontally. They concluded that the seeds loaded in a vertically absorbed more energy prior to breakage than those loaded in horizontally. Kernels loaded in vertically required less energy to breakage than those loaded in horizontally.

In their research, Jafari et al., (2010) present interesting experimental results, showing that the average compressive force required to cause seed breakage was 43.36 N for the vertical and 27.37 N for horizontal orientations of loading. Both the deformation and the energy absorbed at the breakage point of the seeds increased with increasing moisture content, regardless the orientation of loadings.

The major objective of this work is to discover and highlight the propagation of stresses and deformations in the kernel and shell of sunflower seeds during compression, aiming to optimize the energy consumption required for seed processing. The most advanced engineering tool for predicting the propagation of stresses and deformations is the Finite Element Method (FEM), widely used for computing numerical and mathematical modeling of complex phenomena involving the propagation of stress and deformation in continuous media.

MATERIAL AND METHODS

The mechanical properties of oilseeds can be determined using the uniaxial compression test. After their proper processing, the load-strain curves obtained by uniaxial compression test can provide important information regarding seed hardness, crushing strength, apparent modulus of elasticity, energy consumption for crushing, force and deformation at various compression moments etc. (ASAE Standards, 2000).

Table 1. presents the measured and calculated physical properties of the sunflower seeds used in this study.

Table 1. Physical properties of sunflower seeds

Dimensions, (mm)			Weight, m (g)	Volume, V (mm^3)	Average diameter, d_m (mm)	Coefficient of sphericity, ψ	Weight of 1000 seeds (g)
l	b	c					
10	6	3	0.06	100	6	0.55	58

Figure 2 shows the KS H1 Hounsfield/Tinius Olsen mechanical testing machine and the two orientations of seeds during compression tests. The characteristic force-displacement curves were obtained from these tests and the values of strain, force, energy consumption and slope to the breakage point of the shell were read at different moments during compression (Fig. 3).

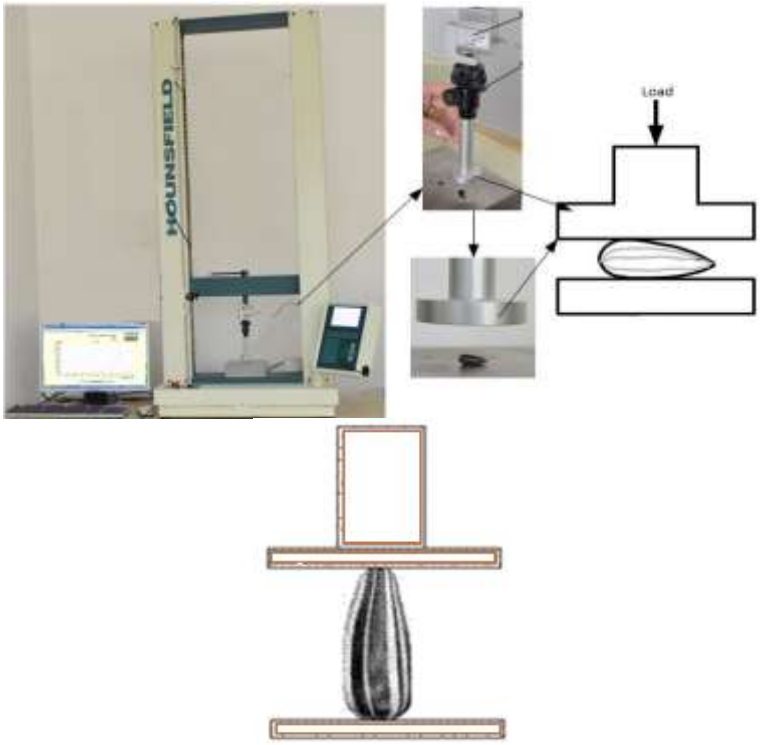


Fig. 2**.** The Hounsfield/Tinius Olsen mechanical testing machine, H1 KS model

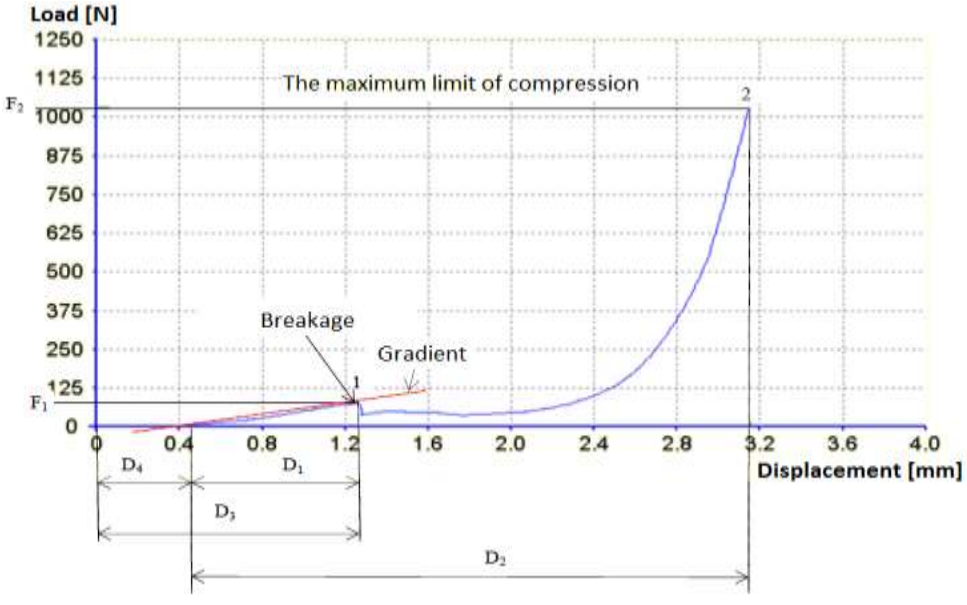


Fig. 3. Force-strain curve and its characteristic points

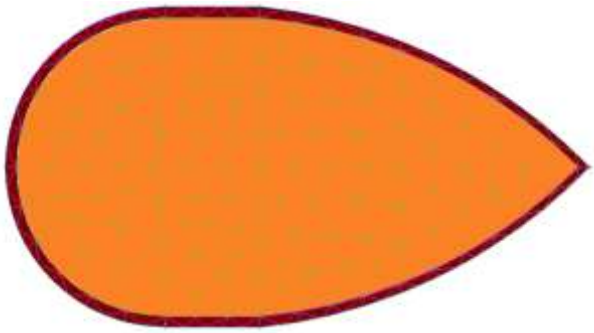


Fig. 4. Meshed model of sunflower seed

Seeds were placed and stabilized on the fixed plate of the machine and the speed of the movable plate was set at 1 mm/min. Force and strain values are given and saved by the software (Qmat) of the Hounsfield/Tinius Olse equipment. In point "1" on the force-strain curve (Fig. 3) occured the shell breakage and a sudden drop of the force can be observed. Beyond that point, the kernel further was subjected to compression, until reaching the maximum force of about 1030 N (in point "2") for 1 kN cell force. The results are used for the meshed model, which is required in the FEM modeling of the propagation of stresses and deformations in sunflower seeds. For numerical solving it was used the QuickField Finite Elements Analysis System, Release 6.0 software. Meshed model of the sunflower seed is shown in Figure 4. Young's modulus for shell had a value of 123 MPa, respectively 107 MPa for the kernel (Khodabakhshian, R., 2012). Poisson's ratio was 0.36 for the shell and 0.3 for the kernel (Khodabakhshian, R., 2012).

RESULTS AND DISCUSSION

Using the QuickField Finite Elements Analysis System, Release 6.0 software, were obtained the distributions of von Mises equivalent stresses and total displacements, for the analysed field of the complex model consisting of kernel and shell. The results obtained when placing the seed horizontally and vertically are presented in Figures 5, 6, and 7.

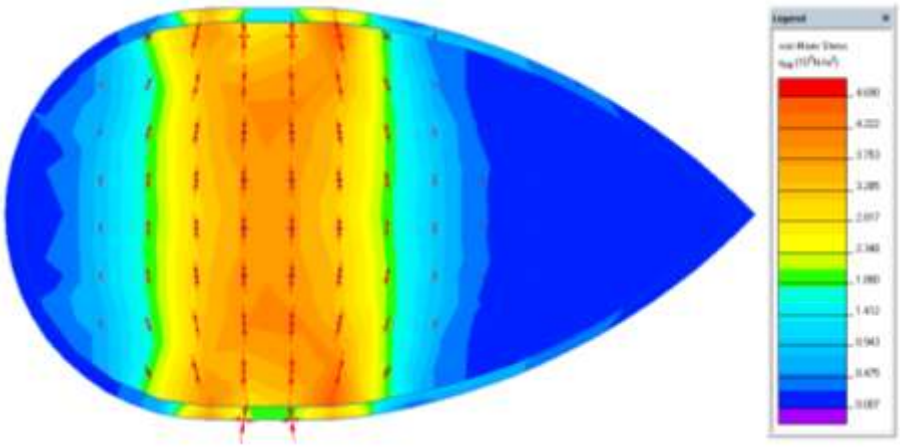


Fig. 5. Distribution of equivalent stresses in the sunflower seed oriented horizontally

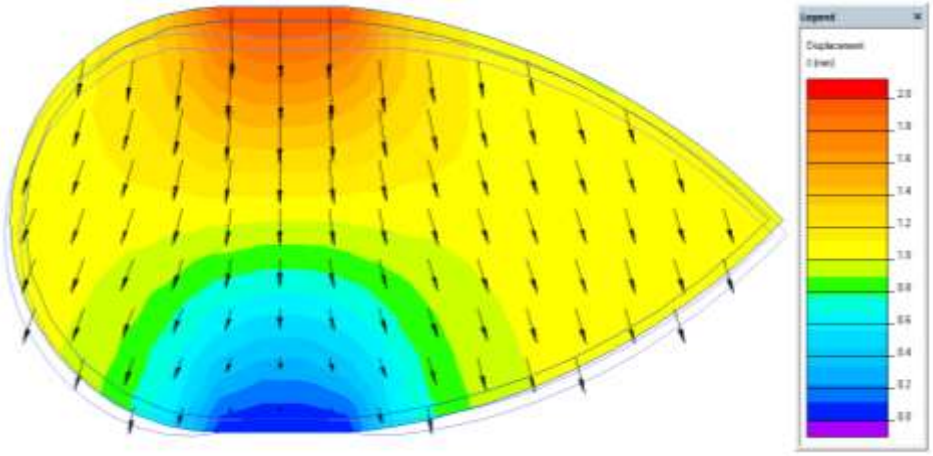
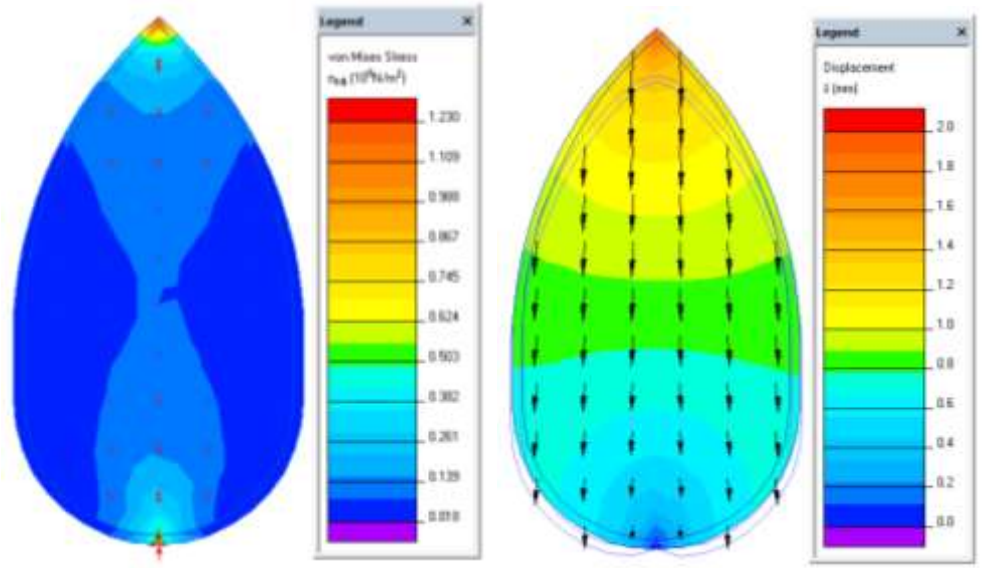


Fig. 6. Distribution of equivalent displacement in the sunflower seed oriented horizontally

In Figure 5. it can be noticed that the highest equivalent stress are found in the contact area between seeds and the plates of the testing machine. There, the maximum values are about 47 MPa. In the vertical-central area, the stress has values ranging between 20 MPa and 45 MPa. In this area, due to these stresses, are met the grinding conditions of seeds kernel. The surface of this area should be as high as possible. The stress in the shells is quite low (18-30 MPa).

Figure 6 shows the distribution of total displacements and how the seeds deform during loading. It can be observed that the highest displacements are found in the contact area between the mobile plane of the testing apparatus and they are oriented in vertical direction.

From Figure 7.. it can be seen that the highest equivalent stresses are found in the area near seed's top (100-123 MPa) which will thus favour seed breaking. It can also be observed that the highest displacements are oriented in vertical direction.



a) *b)*

Fig. 7. Distribution of equivalent stresses (a) and displacements (b) when the sunflower seed is oriented vertically

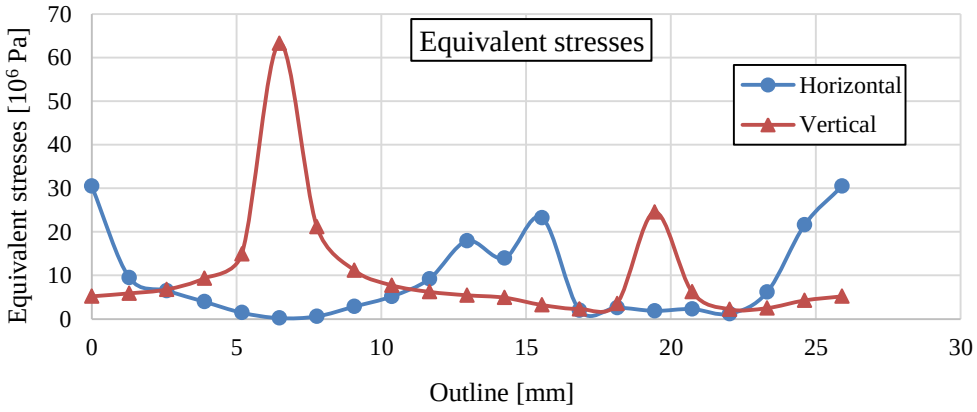


Fig. 8. Comparative analysis of equivalent stresses on the sunflower seed outline for both orientations

Figures 8 and 9 show the comparative analysis of equivalent stresses and total displacements on the sunflower seed outline for horizontal and vertical orientations. It is obvious that the stresses are more evenly distributed if the seed is placed horizontally. For vertical orientations, the highest equivalent stresses are concentrated on the top, respectively on the basis of the seed.

From Figure 8. it can be noticed that if the seed is placed horizontally, the distribution of stress is more uniform on its entire outline, favouring seeds transport and storage situations, but also a more uniform grinding of seed's kernel. Vertical orientation favours an easier breakage of seed shell (which is harder to achieve in practice).

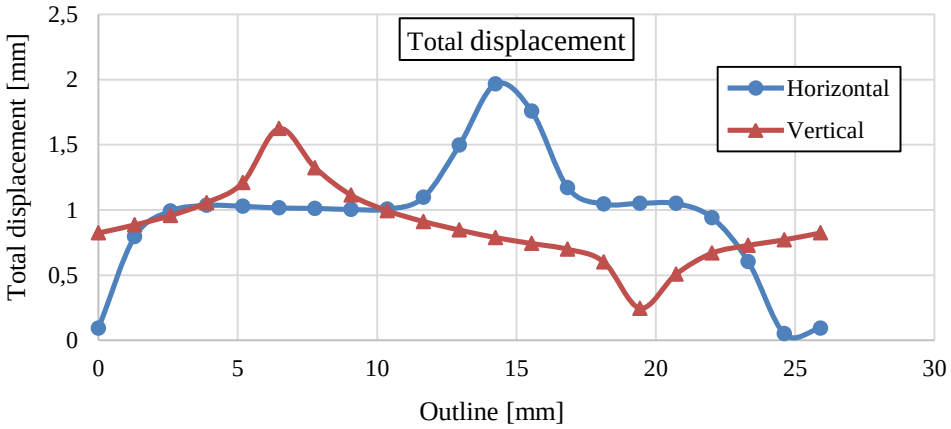


Fig. 9. Comparative analysis of total displacements on the sunflower seed outline for both orientations

CONCLUSIONS

The Finite Element Method is the most advanced engineering tool for computing numerical and mathematical modeling of complex phenomena involving the propagation of stress and displacement (strain) fields in continuous media. Thus, FEM can be an important tool for the modeling of stresses and strains propagation in the kernel and shell of sunflower seeds. The models used in this work highlight that the orientation of the seeds is very important. In some situations, it is desirable that the stresses are higher (at shelling, grinding, pressing, etc.) but there are also situations when the stresses should be smaller (during transport, storage, etc.). FEM models developed in this work facilitate the study of all situations to which the seeds are subjected in the technological processes.

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NEW ROLE OF AGRICULTURAL EXTENSION AND ADVISORY SERVICES BASED ON CURRENT FINDINGS AND FURTHER COLLABORATION FOR IMPROVED NUTRITION

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ABSTRACT

Nowadays, there are a number of projects tackling on challenges around which this study is based. For instance, the ultimate goal of the current project SKIN is to create a permanent stakeholders' association on short food supply chain (SFSC) that works on the joint economic growth of the agricultural sector through the exchange of local food practices and through coaching sessions stimulating innovation. It creates a European network of best practices in SFSC that addresses the fragmentation of knowledge in the agricultural sector and supports bottom-up innovation initiatives. No doubts, boosting innovation through that project in local areas will lead to economic growth in the regions. But, for its sustainable development, it is crucial to create the agricultural extension and advisory services (AEAS), particularly in EU countries and also to modify their role using bottom-up approaches. Despite the fact that the role of AEAS in the EU countries is transforming in the last few years, from a technology transfer paradigm to a demand-driven model, there are still two challenges that should be tackled in the global agenda: 1) facilitate linking of local agricultural sector and nutrition; 2) build a sustainable network of advisors in the EU for improving knowledge flows in national and regional agricultural knowledge and innovation systems (AKIS). According also to the findings of the SKIN project and to our recently developed concept (FENIX), the launch of new initiatives will enable AEAS by gathering of a large amount of information and knowledge from local areas and population, helping all types of stakeholders to improve health, environmental, and economic sectors in targeted regions.

Keywords: *agricultural sector, innovation, extension, food security, personalized nutrition, rural advisory services.*

INTRODUCTION

A look at the current global health and nutrition situation suggests agriculture can make more crucial and invaluable contribution to health and nutrition. Indeed,

leveraging agriculture for health and nutrition has the potential to speed progress toward meeting all of the Millennium Development Goals (Fan *et al*., 2012).

Agricultural development is the only tool to end extreme poverty, boost shared prosperity and feed a projected 9.7 billion people by 2050. The leading cause of death worldwide is associated with poor nutrition. Approximately three billion people are either not eating enough or eating the wrong types of food, resulting in illnesses and health crises. A 2017 report found that 2.1 billion people were overweight and obese, and 62% of them originated from / were living in developing countries (FAO, 2017).

According to the World Health Organization (WHO, 2015) chronic diseases are the second leading cause of death in the world, now especially increased in developing countries (around 80%) and first of all it is various cardiovascular disorders (17 million deaths in 2002), followed by cancer (7 million deaths), chronic lung diseases (4 million), and diabetes mellitus (almost 1 million).

The global prevalence of leading chronic diseases is projected to increase substantially over the next two decades. For example, the number of individuals with diabetes is estimated to rise from 171 million (2.8% of the world's population) in 2000 to 366 million (6.5%) in 2030, 298 million of whom will live in developing countries (Wild *et al.*, 2004). A related problem is the rising number of people who are overweight or obese. One of the leading risk factors for chronic diseases is inappropriate nutrition.

The extent and rapidity of the rise of diet-related chronic diseases led WHO to call for action in its Global Strategy on Diet, Physical Activity and Health (WHO, 2004). The Strategy recognizes agriculture as a key distal determinant, stating that *"National food and agricultural policies should be consistent with the protection and promotion of public health. Governments should be encouraged to examine food and agricultural policies for potential health effects on the food supply. Agricultural policy and production often have a great effect on national diets. Governments can influence agricultural production through many policy measures. As an emphasis on health increases and consumption patterns change, Member States need to take healthy nutrition into account in their agricultural policies"* (WHO, 2004). A growing number of governments, donor agencies, and development organizations are committed to supporting nutrition-sensitive agriculture (NSA) to achieve their development goals.

Demand for empirical evidence of "what works" for nutrition through agriculture has arguably never been higher. In the past few years, there has been a proliferation of interest in how to leverage agriculture to maximize its impacts on nutrition (Webb and Kennedy, 2014). The belief that *"agriculture contributes not just to food production, but also to human nutrition and health"* (Global food policy report, 2011) is widely held, and it underpins ongoing efforts globally to *"make agricultural policies and programs nutrition-sensitive"* (Bill & Melinda Gates Foundation, 2012).

While consensus exists on pathways through which agriculture may influence nutrition-related outcomes, empirical evidence on agriculture's contribution to

nutrition and how it can be enhanced is still weak (Ruel *et al.*, 2018). Ruel and Alderman (2013) identified six pathways through which agricultural interventions can impact nutrition: (1) food access from own-production; (2) income from the sale of commodities produced; (3) food prices from changes in supply and demand; (4) women's social status and empowerment through increased access to and control over resources; (5) women's time through participation in agriculture, which can be either positive or negative for their own nutrition and that of their children; and (6) women's health and nutrition through engagement in agriculture, which also can have either positive or negative impacts, depending on exposure to toxic agents and the balance between energy intake and expenditure.

The question is: who will be the link between governmental, private, public, scientific, producers' sectors and the society as consumers? It should be the player, who came from the local environment with the passion and ability to play a key role in improving the future.

Agricultural extension and advisory services (AEAS) play an important role in agricultural development and can contribute to improving the welfare of farmers and other people living in rural areas. *"Rural advisory services, also called extension, are all the different activities that provide the information and services needed and demanded by farmers and other actors in rural settings to assist them in developing their own technical, organisational, and management skills and practices so as to improve their livelihoods and well-being."* (Christoplos, 2010). "Agricultural extension" describes the services that provide rural people with access to knowledge and information they need to increase productivity and sustainability of their production systems and improve their quality of life and livelihoods. It includes, but is not limited to, the transfer of knowledge generated by agricultural research. It has helped countries move towards meeting food needs, conserving natural resources and developing human and social capital (NRI, 2011). Nowadays, the role of AEAS remains important and potential, but still has a weak influence on the local population. Also, its role should expand from being a transfer of knowledge that links with nutrition to a real link between above-mentioned sectors, a mentor in the innovation.

The argument for it is showed up in the Global Forum for Rural Advisory Services as extension services enable farmers to take up innovations, improve production, and protect the environment. The extension shows positive effects on knowledge, adoption, and productivity. With studies showing very high (13–500%) rates of return to the extension, it is a cost-effective way to improve farmer productivity and income (GFRAS, 2012). AEAS imply more than just the transfer of technologies. In a broader sense, AEAS means the transfer of know-how and information, which will eventually enable the client/farmer to make his/her autonomous decision to change or modify the production and/or adopt innovations. The know-how in the meaning of not only technologies or marketing, but also innovative tools for maintaining/improving health conditions for preventing diseases.

The importance of agricultural extension and advisory services in relation to the fight against food insecurity and poverty in line with the aspirations of the Agenda 2030 for Sustainable Development cannot be over-emphasized. The same we can see in the politics of Horizon 2020 that provided over € 4 bn for agriculture and food research. Despite all efforts of the projects development and implementation, there are still two challenges: absence of uniting project that can tackle named challenges and the lack of consideration the local projects as the key players in the developing regions. As Fanzo (2015) stated - one of the major groups of AEAS providers are agricultural/rural development projects. The formal linkages between the three sectors of AEAS providers – public, private, projects – are emerging and still at an infant stage, but developing.

However, there is a lack of coordination, harmonization and quality assurance (standards). Therefore, there is a need for coordination and guidance to improve efficiency in service delivery. This will avoid duplication of efforts and most importantly cope with the new and dynamic demands of modern agriculture.

This paper aims to present innovative approach for creating new role of AEAS which is based on current findings of the ongoing project SKIN and further collaboration for improved nutrition through expertise described in our recent concept (FENIX). Building a sustainable network of new advisors will avoid duplication of efforts (all sectors remain fragmented) and most importantly cope with the new and dynamic demands of modern agriculture with the linkage with personalised nutrition.

MATERIALS AND METHODS

The paper is based on a mixed methodology, which includes: 1) the systematic literature review covering the following databases: PubMed, Google Scholar, and IBSS for the period from 2012 to 2018; 2) the interviews with smallholder local farmers and traders in Ukraine (Transcarpathian region) and the Slovak Republic conducted during 2017-2018.

Data were collected by structured questionnaires from the 75 stakeholders in the following regions: Transcarpathian (Ukraine), Kosice, Malacky, Littoral, Zilina, Presov, Banska Bystrica, Trencin, Bratislava, Nitra, Trnava regions in the Slovak Republic. Findings of the ongoing project SKIN by an extended review of secondary data are also summarised here.

As background for the FENIX concept, the data from clinical trials, mathematical modelling, and IT-based approaches had been used.

RESULTS AND DISCUSSION

To ensure sustainable agriculture as a basis for solving global problems, promoting strong economies (with the provision of jobs, budget revenues, and the reduction of migration flows), generating innovative ideas for development (e.g. circular bioeconomy), in line with environmental safety, enable improving and further maintaining human health, the extended role of the existing advice service is necessary. The adviser should be the player, who came from the targeted region. It

has the following reasons: common language, being present (physically) in the region for not only giving consultation, but also being a mentor during the whole process of improving knowledge, business, etc. The time of chaotic presentation is gone. So, the new advisor should be trained by the other experienced advisors, and he/she has to come to the region and transfer/support farmers to increase the impact of face-to-face interaction.

New advisors should be as coordinators that will not only control the process of bottom-up innovation, but also be mentors within building linkages between AEAS providers (*figure 1*).

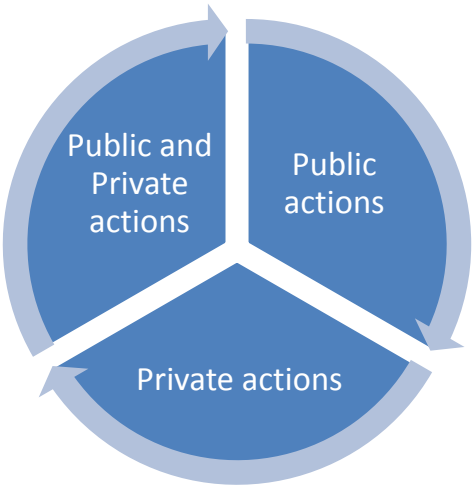

Figure 1. Linkages between AEAS providers

The advisor faces difficult tasks, that need to be solved due to his/her renewed role:

• The public sector is not ready to share the "goods" of the country and allow common collaboration between projects activities and private sectors.

• The project sector, as donors have a weak influence on the private and public sector in the meaning of achieving a common goal. Lack of trust causes separate work and cannot lead to continuous progress and development of results of the projects.

• The private sector tries to monopolize its own business in one way, without consideration of possible growth within a collaboration between above-mentioned sectors.

Nevertheless, all begin in the individual level – changes and adapting of the know-how causing a high level of insecurity at an individual and institutional level. This leads to the paradox situation: institutions (private/public sector), that have resources for implementing innovation don't want to do this (reasons: lack of trust, weak financial assessment); farmers, even they really want to adopt innovation – don't have knowledge and resources for it. With these challenges only advisors, as mentors and mediators can tackle providing knowledge to farmers, building a

trustful relationship, which will cause to accept innovation and promote economic growth.

Based on this study we have found that there are a lot of open questions on how to improve AEAS within new knowledge, what is the precise working approach that will link nutrition and agriculture particularly, and how to tackle the challenges that faced society of 21st century generally. Key opportunities for integration efforts in order to renew the role of AEAS are engaging communities, creating a demand for nutrition, and the use of innovative communications. But how and what knowledge should it be?

To answer the first part of the question, we propose to use the findings of the above-mentioned project SKIN. It is an ambitious initiative of 20 partners in 14 countries in the area of Short Food Supply Chains (SFSCs). It intends to systematise and bring knowledge to practitioners, promote collaboration within demand-driven innovation logic and provide inputs to policymaking through links to The European Innovation Partnership on Agricultural Productivity & Sustainability (EIP-AGRI). SKIN will build and animate a community of about 500 stakeholders, with the strategic objective of setting up, at the conclusion of the project, a European association permanently working for the improvement of SFSCs efficiency and for the benefit of stakeholders and growth in the sector. The community will be built and animated around the identification of good practices in short supply chains across Europe. SKIN puts significant efforts in dissemination, to reach as many stakeholders as possible, and exploitation, to plan post projects developments in the form of a permanent association that would give continuity to the activities launched with the project (community expansion, circulation of good practices, promotion of research-based innovation and linkages with the EIP and policy-making instances). It's an absolutely direct answer to the wide range of the questions that remain open in the reviewed literature regarding how to engage the community and how to promote demand-driven innovation in agriculture and food production. SKIN will identify a vast population of 10,000 stakeholders in the sector, who will be informed, stimulated and targeted as potential new members of the Community.

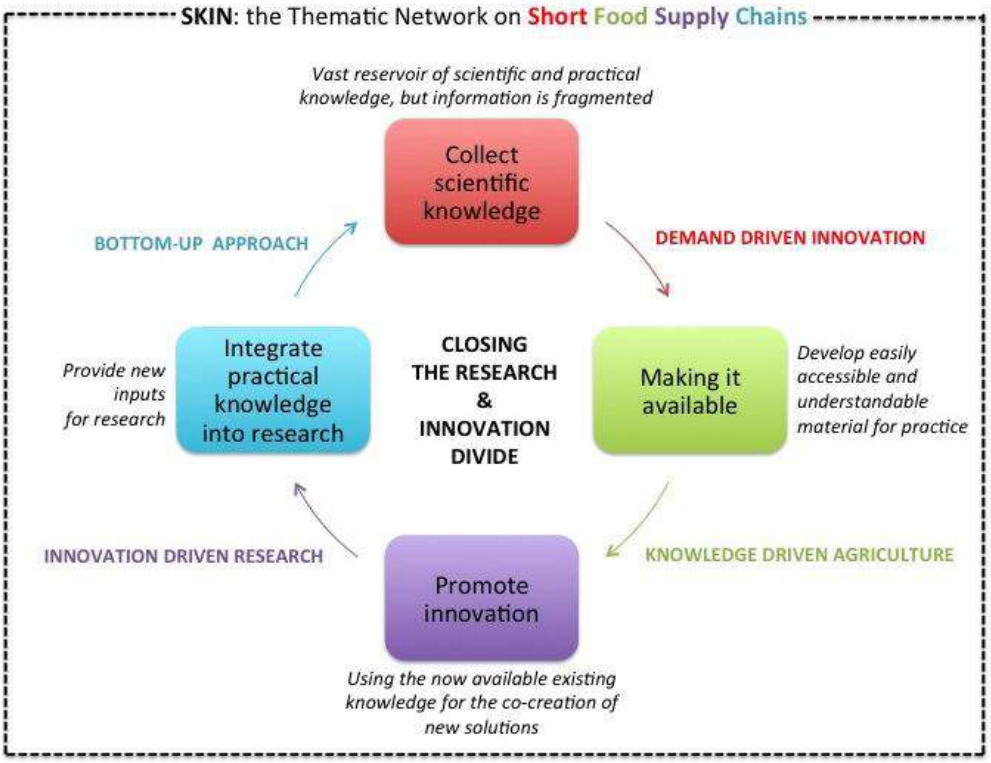

Figure 2: The SKIN Approach to Knowledge Exchange

Short food supply chains have economic, social and cultural benefits for farmers, consumers and rural areas in general. This sector increases the income of farmers and the consumption of fresh and relatively unprocessed food, brings consumers and farmers closer, engages public institutions in its promotion, helps to strengthen rural-urban linkages (particularly in the case of peri-urban agriculture) and contributes to sustainable development. The sector is growing across Europe to meet rising consumer demand. Thanks to the personal interview with the farmers, we can ensure engaging in further collaboration. We precisely know what are the problems that they face and we can ensure the demand in the innovation. Through the coaching session, the innovative projects calls will be developed to improve the situation. But from this point, two challenges remain: who will support the projects implementation and their continuation and will they be linked to the nutrition approach? The renewed AEAS can play the role of mentors in those projects. Nevertheless, they need to get knowledge about working and adapting to challenges of 21st century approach regarding personalised nutrition. It's also answering the above-mentioned challenges regarding presented diet for prevention of diseases and maintaining health conditions. At that point we propose the expertise of the innovative idea described in FENIX, that was mentioned above. This idea was developed by core partners, who are all innovative SMEs in their

particular expertise. FENIX proposes to exploit an easy-to-use coherent tool to make recommendations for personalized nutrition (PN) requirements that meet the precise needs of EU citizens. The beta version of it is already developed by Ediens LLC team. The tool proposed by Ediens is based on measurements and an innovative bioinformatics approach for interpretation of individual microbiome data with other relevant and crucial factors (evidence-based and correlated biomarkers, calculating age, gender and indicating personal health status, personal nutritional requirements, food composition data, lifestyle, cultural preferences, environment conditions) and also considers the available source and analytical characteristics of ethnic foods and innovative food processing approach of further individualisation proposed for local farmers and food producers. Ediens proposes to calculate this PN with consideration of the patented algorithm (correlated microbiome with biochemical / blood parameters – immune indices relevant to detection of inflammation biomarkers for early detected changes / shift between health / diseases condition / balance, to be really able to consider and to calculate all the other personal internal biological characteristic (genes, phenotypes, microbiome). Proposed IT tool / Algorithm / approach will take into account other crucial internal determinants – mental health, physical activity, stress, behaviour, food perception, culture habits, religion and food perception restriction, nutrition intuitive preferences and also all the sets of external factors: social (professional activity, social status) and economic factors (leaving allowance, budget). This will proceed via mathematical modelling PNA based on limited trial studies for adjusting in accordance to all data received.

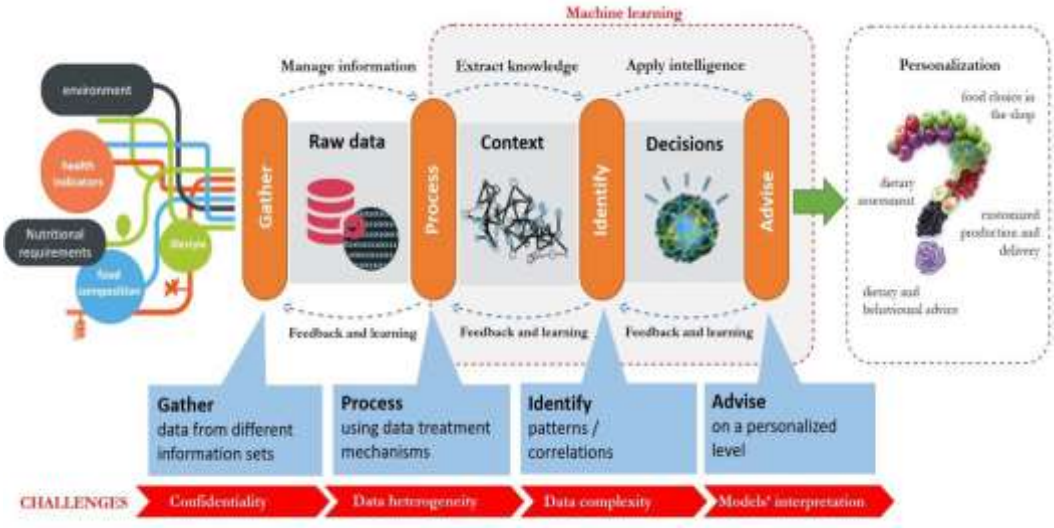

Figure 3: FENIX vision and approach. On behalf of core partners

CONCLUSION

We need to consider the already done research, projects activity to further improving agriculture system. It will avoid duplication efforts and can gather all relevant stakeholders to achieve common, ultimate goal – to build sustainable health, productive, welfare society in a friendly environment. For that reason, we should use a bottom-up and demand-driven approach that will ensure trust, build community relationship, boost needed innovation, create new job places, decrease migration, to provide health conditions to the society, save the environment. In that case, we see that the new advisors can be a driver for those changes. The good practices collected by SKIN farmers, familiar for the local community, can help in it. The information on innovative personalised nutrition approach provided by Ediens and described in FENIX will ensure that local food can be a functional food for maintaining microbiome status particularly, and healthy condition in general.

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DIRECT ORGANOGENESIS OF *STEVIA REBAUDIANA IN VITRO* USING NODAL EXPLANTS

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ABSTRACT

Stevia rebaudiana Bertoni is a medicinal herb belonging to the family of Asteraceae. It is a natural sweetener plant, which is estimated to be 300 times sweeter than cane sugar. In this study, reliable protocol was developed for direct organogenesis of *S. rebaudiana* using *in vitro* derived nodal explants. Seeds were collected from mother plants and they were surface sterilized. To optimize the surface sterilization procedure, dark color (fertile seeds) seeds were surface sterilized using different concentrations and in different exposure time of carbendazim and sodium hypochlorite (Clorox). Out of different combinations 0.2% carbendazim for 5 minutes, 10% sodium hypochlorite for 10 minutes and 70% ethanol each followed by two successive washings in sterile distilled water was found to be the best for surface sterilization. Two sets of seeds (fresh, stored) were cultured on MS basal medium supplemented with different concentrations of GA3 for seed germination. According to the results seed viability was lost with time and it affected seed germination. Seed germination was not affected by GA3, but seedling height was affected by it. Seeds germinated on MS medium supplemented with 3.0 mg/L GA3 showed the highest seedling height after 10 days. MS basal medium supplemented with different concentrations of BAP and Kin were tested for shoot bud and multiple shoot induction. Out of different media Ms basal medium supplemented with 2.0 mg/L BAP was found to be the best medium for shoot bud and multiple shoot induction within 60 days.

Keywords: *Stevia rebaudiana, surface sterilization, seed germination, shoot induction, direct organogenesis.*

INTRODUCTION

Stevia rebaudiana is a herbaceous plant belongs to Family Asteraceae. It's also known as sweet leaf herb or honey plant due to its sweetness. It's about 300 times sweeter than cane sugar. The chemical compounds that produce its sweetness are steviol glycosides and there are seven major sweetener compounds and among them Rebaudioside A and Stevioside are the most important two (Singh *et al*., 2017). They are non caloric sweeteners and can be used as an alternative to sugar

and synthetic sweetening agents. Therefore this could be considered as the best alternative natural sweetener for diabetic patients. In addition to its non caloric sweetening property it has many therapeutic values such as anticancer, antimicrobial and anti-inflammatory activity. Therefore Stevia is used as food supplement and sweetener in countries like USA, Japan, Brazil and China (Yadav *et al*., 2011). The demand for sugar in Sri Lanka is likely to go up in coming years. Therefore this will elegantly meet the requirement of sugar in Sri Lanka including demand in pharmaceuticals and soft drink industries.In natural conditions, percentage seed germination is poor and unsuccessful due to small endosperm and infertile seeds. There are reports about propagation of Stevia through stem cuttings, but direct planting of them in field has limitations due to poor rooting (Yadav *et al.,* 2011). Plant regeneration from *in vitro* culture can be achieved by either organogenesis or embryogenesis. Supplement of different plant growth regulators enhances and accelerates the production of *in vitro* plants with good agronomical traits and steviosides content in leaves (Singh *et al*., 2017).

MATERIALS AND METHODS

Mother plants were collected and were maintained in a shade house.

MS medium supplemented with 30.0 g/L sucrose and 8.0 g/L agar was used as the basal medium. The pH of the all media was adjusted to 5.8 ± 0.5. Temperature of the culture room was maintained at 25 ± 1 C° and PAR (Photosynthetically Active Radiation) was provided for 18 hours per day. There were at least 20 replicates in each treatment and growth regulators free MS medium was used as the control. Completely Randomized Design (CRD) was used in all experiments.

Optimizing surface sterilization protocol for seeds

Seeds were collected from mother stock maintained in department shade house and were initially washed with few drops of liquid detergent for 5 minutes followed by running tap water for 30 minutes. After that they were treated with a fungicide (Carbendazim) and Clorox. Each step was followed by two successive washings with sterile distilled water. Then seeds were treated with 70% ethanol for 30 seconds followed by washing with sterile distilled water twice. Finally surface sterilized seeds were cultured on growth regulators free MS medium. To determine the suitable surface sterilization method, effect of 2.0% carbendazim with two different exposure time and Clorox at two different concentrations with different exposure time were tested. Contamination percentage, survival percentage and germination percentage were recorded and best method was used for further experiments.

In vitro seed germination

Seeds were collected from mother plants and two sets of seeds (fresh and ten days stored) were used for the experiment. They were surface sterilized and cultured on MS medium supplemented with different concentrations of (1.00 mg/L- 3.00 mg/L) GA3. Germination percentage and mean shoot length after 10 days were recorded.

Multiple shoot induction *in vitro*

Nodal segments were used as the explants source and taken from two weeks old *in vitro* germinated seedlings. Seedlings were carefully taken out from the culture bottles and approximately 1.0 cm length nodal segments were prepared. They were cultured on MS medium supplemented with different concentrations of (0.5 mg/L - 2.5 mg/L) BAP and (0.5 mg/l-2.5 mg/L) Kin. Number of shoots per explants and mean shoot length after 30 days were recorded.

RESULTS AND DISCUSSION

Optimizing surface sterilization protocol for seeds

After seven days of incubation, seeds in growth regulator free MS medium, it was observed that 0.2% carbendazim with 5 minutes exposure time and 10% Clorox with 10 minutes exposure time (T3) was the most suitable surface sterilization protocol based on the visual observations of the contamination, germination and survival percentage of seeds. It showed low percentage contamination (16.0%) together with highest percentage germination of seeds (61.9%). Though percentage contamination of the seeds treated with 0.2% carbendazim for 10 minutes and 15% Clorox for 10 minutes was zero, none of the seeds were germinated. It was observed that when the Clorox concentration and exposure time increase, percentage contamination decreases, but showed adverse effects on seed viability (Table 1). In addition, treatment T4, T5 and T6 also showed considerably higher germination, which is higher than 45.0% compared to the other treatments.

Table 1: Percentage contamination, percentage survival and percentage germination of seeds with different treatments used for surface sterilization

Treatment code	Conc. Of Clorox (v/v)	Exposure time of Clorox	Exposure time of 0.2% carbendazim (min)	% Contamination	% Survival	% Germination
T1	10 %	5	5	76.0	24.0	16.67
T2	10 %	5	10	68.0	32.0	12.50
T3	**10 %**	**10**	**5**	**16.0**	**84.0**	**61.90**
T4	10 %	10	10	36.0	64.0	50.00
T5	15 %	5	5	28.0	72.0	55.56
T6	15 %	5	10	32.0	68.0	47.05
T7	15 %	10	5	4.0	96.0	0
T8	15 %	10	10	0	100.0	0

Contamination is one of the major problems in plant tissue culture. Therefore determination of suitable sterilization method is necessary for successful establishment of protocol. Urbi and Zainuddin (2015) reported that, effect of Clorox in disinfecting of Stevia is time and concentration dependent and results of the present study confirmed the same fact.

In vitro seed germination

After 10 days of incubation, it was observed that fresh seeds shown higher percentage germination in all media than 10 day stored seeds (Table 2). Seedling height was increased with GA_3 in both types of seeds. Therefore the results indicate that seed storage affect their viability and GA_3 only affect in seedling height. The highest mean seedling height after 10 days was observed in seeds cultured on MS medium supplemented with 3.0 mg/L GA_3 (T4) in both types of seeds (4.38 cm for fresh seeds, 4.10 cm for 10 days stored seeds). Therefore it was the best medium for *in vitro* seed germination (Figure 1).

Figure 1*: In vitro* germinated seedlings on MS medium supplemented with 3.0 mg/L GA_3

Gunasena and Senarath, (2017) reported that the height of the *in vitro* germinated *Punica granatum* seedlings exponentially increase with the increase in GA_3 concentration in MS medium. Present study also showed an increase in seedling height with increase of GA_3 concentration.

Table 2. Percentage seed germination and mean seedling height of the seedlings after 10 days in the presence of GA_3

Treatment code	Seed type	GA_3 (mg/L)	% Germination ± SD	Mean height after 10 days (cm) ± SD
T1	Fresh seeds	0.0	52.0 ± 0.51	1.49 ± 0.01
T2	Fresh seeds	1.0	56.0 ± 0.50	3.41 ± 0.14
T3	Fresh seeds	2.0	64.0 ± 0.49	3.94 ±0.09
T4	**Fresh seeds**	**3.0**	**72.0 ± 0.46**	**4.38 ± 0.14**
T5	10 days stored seeds	0.0	20.0 ± 0.41	1.38 ± 0.08
T6	10 days stored seeds	1.0	20.0 ± 0.41	3.76 ± 0.09
T7	10 days stored seeds	2.0	20.0 ± 0.41	3.90 ± 0.1
T8	10 days stored seeds	3.0	24.0 ± 0.44	4.10 ± 0.17

Multiple shoot induction *in vitro*

After 4 weeks of incubation, all the nodal segments cultured on MS medium supplemented with BAP showed higher number of shoots per explants and higher mean shoot length than nodal segments cultured on MS medium supplemented with Kin (Table 3). According to the results, number of shoots per explants and mean shoot length increase with BAP concentration up to 2.0 mg/L. Results indicated that shoot induction was also affected by Kin, but not vary with its concentration. Therefore the best medium for multiple shoot induction was MS medium supplemented with 2.0 mg/L BAP (T4) and there were 8.20 shoots per explants and mean shoot length was 3.94 cm (Figure 2).

Figure 2: Multiple shoots obtained from nodal segments cultured on MS medium supplemented with 2.0 mg/L BAP

The presence of cytokinins in the medium was essential to induce bud break and shoot proliferation from nodal explants. Out of the two cytokinins used, BAP was found to be more effective than KIN for shoot bud development from nodal explants (Thiyagarajan and Venkatachalam., 2012). Debnath (2008) reported that the maximum shoot proliferation was in 2 mg/L BAP and it confirms the observations in the present study as well.

Table 3. Number of shoots per explants, mean shoot length after 30 days in media with different concentrations of BAP and Kin

Treatment code	BAP (mg/L)	Kin (mg/L)	Number of shoots per explants ± SD	Mean shoot length after 30 days (cm) ± SD
T1	0.5	-	5.78 ± 0.09	2.53 ± 0.12
T2	1.0	-	6.05 ± 0.13	2.67 ± 0.13
T3	1.5	-	6.91 ± 0.12	3.38 ± 0.13
T4	**2.0**	**-**	**8.20 ± 0.12**	**3.94 ± 0.14**
T5	2.5		5.19 ± 0.14	2.54 ± 0.13
T6	-	0.5	4.81 ± 0.12	2.43 ± 0.13
T7	-	1.0	4.52 ± 0.13	2.37 ± 0.12
T8	-	1.5	4.32 ± 0.10	2.28 ± 0.12
T9	-	2.0	4.36 ± 0.14	1.97 ± 0.09
T10	-	2.0	4.25 ± 0.18	1.57 ± 0.11

CONCLUSION

The best surface sterilization protocol for *S. rebaudiana* seeds is 0.2% carbendazim with 5 minutes exposure time and 10% Clorox with 10 minutes exposure time. Fresh seeds have higher seed germination percentage than 10 days stored seeds and it indicates seed storage affect their viability. Tissue culture practices revealed that GA_3 only affect in seedling height and highest mean seedling height can be obtain *in vitro* using MS medium supplemented with 3.0 mg/L GA3. Best medium to obtain highest number of shoots per explant and highest mean shoot length is MS medium supplemented with 2.0 mg/L BAP.

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ASSESSING GREENHOUSE GAS EXCHANGE OF AGRICULTURAL CROPS BY FLUX MEASUREMENTS IN THRACE PART OF TURKEY

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ABSTRACT

Agriculture plays an important role in the global greenhouse gas (GHG) budget and its cycle. CO_2 is one of the most important greenhouse gases, and plants release CO_2 into the atmosphere by respiration and sink it by photosynthesis from the atmosphere. In addition, soil has an essential role in this exchange. Unfortunately, studies on the measurement of greenhouse gases above agricultural crops in internationally accepted methods are not sufficient, especially in developing countries. Thus, it is a clear need to determine carbon exchange of agricultural crops and activities (sink and emission) by taking into consideration of the specific conditions such as climate, crop variety, soil etc. Eddy Covariance (EC) is one of the widely used micrometeorological methods in the world for flux measurement studies. Developments in measurement and analysis by instruments have allowed this method to be applied more by researchers for the studies on GHG exchange. In this research, carbon exchanges (sink and emission) of watermelon grown in Atatürk Soil, Water and Agricultural Meteorology Research Institute located in the Thrace part of Turkey, was measured using the Eddy Covariance method. Finally, estimated gas exchange above crops will be presented.

Keywords: *Carbon, greenhouse gas, micrometeorology, agricultural meteorology, flux.*

INTRODUCTION

Greenhouse gas (GHG) exchange between earth and the atmosphere becomes important with relation to industrial revolution because GHG cause global warming and climate change. Deforestation, forest fire, drought, land use changes and usage of fossil fuels for different purposes result increases in GHG concentration in the

atmosphere. In the terrestrial ecosystem, agriculture has one of the key components of global GHG's budget by means of capturing CO_2 from the atmosphere. Therefore, agricultural and forest areas have critical roles by sinking of carbon for the national global GHG's budget. CO_2 from the atmosphere is captured by photosynthesis, emitted by respiration and stored by sink (Net ecosystem exchange) within the plant organs. There are however few studies on this topic for the agricultural crops when compared to the studies on forests. In most of the developing countries; such as Turkey, carbon budget of agriculture is calculated according to IPCC values. For this reason, representativeness of the calculated carbon budget should be assessed by comparison with measured data and actual emission and sink coefficients.

The purpose of this study is to assess the results of the experimental GHG flux studies, were done in the Thrace part of Turkey, on CO_2 and H_2O above crop (watermelon) using eddy covariance (EC) flux approach. The measurements were carried out from 2012 to 2013. Experimental studies were conducted in the selected fields (KRK) of Atatürk Soil Water and Agricultural Meteorology Research Directorate in the Kırklareli city, Turkey.

MATERIAL AND METHODS

The flux studies had been carried out between 14 May and 9 October 2012 in watermelon planted field in KRK (41.73 ° K, 27.21 ° D) which is shown in Figure 1. The experiment area is about 2 ha. An EC measurement system (3D sonic anemometer, open type gas analyzer) and an agricultural meteorology research station were established in the study area. Figure 2 shows EC and agricultural meteorology measurement stations established in KRK. At the EC station, the 3D components of wind speed by 3D sonic anemometer and the CO_2 gas concentration by open path infrared gas analyzer, were measured. In the agricultural meteorological station, the global solar radiation, the net radiation and photosynthetic active radiation were measured at 2 m, wind speeds at 0.5, 1, 2, 5 and 10 m and wind direction at 2 m, soil temperatures at 2, 5, 10 and 20 cm.

Figure 1. Watermelon planted area in KRK.

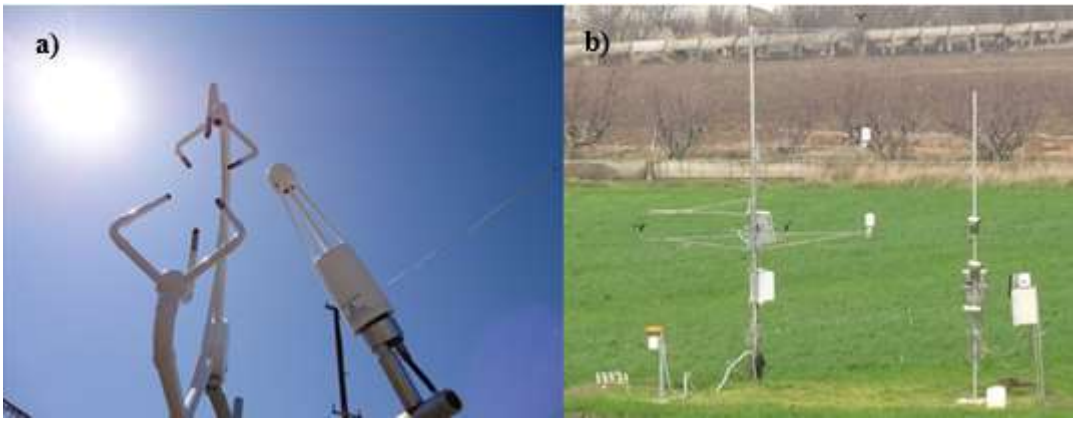


Figure 2. a) EC b) Agricultural meteorological station (Şaylan et al. 2012)

Thanks to the advancement in technology and the development of measuring devices, 3D wind speed and gas flows have been measured in very short time intervals (Burba, 2013). The EC method, in which the fluxes of the interested gas (CO_2, CH_4, H_2O etc.) related to the covariance between the concentration and vertical wind speed in the eddies, has been popular since the 90's. Nowadays, EC is the most widely used method in flux studies.

Equation 1 shows how the CO_2 flow is calculated according to the EC method:

$$F_c = \rho \overline{w'c'} \quad (1)$$

where F_c is CO_2 flux (μmol m^{-2} s^{-1}), w' is deviation of average wind speed (m s^{-1}), c' is deviation of average CO2 concentration, and $\overline{w'c'}$ is covariance of the deviations (Foken, 2008).

The main output of the EC method is Net Ecosystem Exchange (NEE). Negative values of NEE refers the net carbon accumulation of the crop. The NEE obtained as a result of the measurements and calculations is fragmented into the Ecosystem Respiration (R_{eco}) values representing the amount of carbon that the plants give to the atmosphere as a result of respiration, and Gross Primary Production (GPP) that shows total carbon production of plants. The relation of these three variables is given in Equation 2:

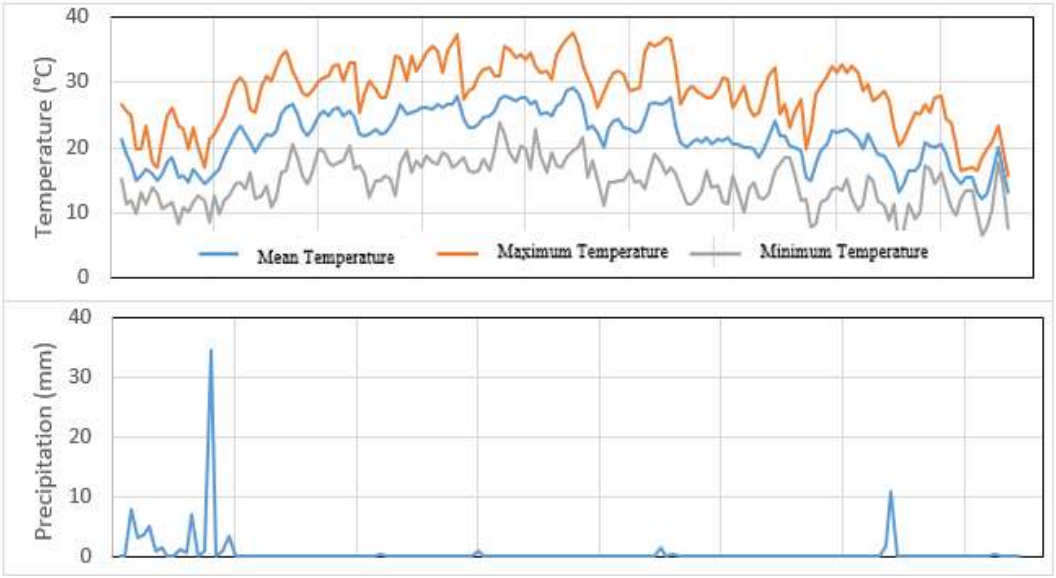
$$R_{eco} - \mathrm{NEE} = \mathrm{GPP} \quad (2)$$

The direct and high resolution of flux measurement capability of EC method has made this method the most widely used in flux studies despite the cost of installation and difficulties in data analysis.

RESULTS AND DISCUSSION

The maximum, minimum and average temperatures, precipitation, relative humidity (RH), wind speed, global and net radiation averages obtained from measurements made at the meteorological station are shown in Figure 3. In this

period including watermelon growing, the daily mean air temperature is 22.7 °C, the average maximum air temperature is 37.6 °C measured on August 8, 2012 and the minimum air temperature is 8.28 °C measured on May 24, 2012. The air temperature generally remained below about 2 °C over the 50-year average temperature recorded by Turkish State Meteorological Service during the development period (Aslan, 2014). The first month of the growing period was quite rainy. A total of 88.2 mm of precipitation during the whole period as 71.4 mm of it fell between 14 May 2012 and 2 June 2012. From the planting of the crop until July 31, 2012 which was the first harvest date, 18 rainy days past and 73 mm of precipitation were observed (precipitation over 0.1 mm was considered). The May which is a rainy month in the City of Kırklareli has caused the relative humidity to be generally high during this month. The daily average relative humidity during the growing period was 61.41%. The maximum and minimum relative humidity values were measured on May 29, 2012, with 96.3%, and on August 28, 2012, with 33.62%. Global and net radiation showed similar changes in the same period. Due to the rainy weather and cloudiness during May, very low values were observed for both variables. Global and net radiation increased during June and began to decline from July. During this period, the lowest daily average global and net radiation values were measured on May 29, 2012 as 71.09 Wm^{-2}, 4.61 W m^{-2}, respectively. The maximum global radiation and net radiation values were measured on June 18, 2012 with 373.2 W m^{-2} and June 17, 2012 with 176.7 W m^{-2}, respectively. The average global radiation and net radiation values were determined as 274.11 W m^{-2}, 126.5 W m^{-2}, respectively. Daily average, maximum and minimum wind speeds were determined as 1.74, 7.36 and 0.46 m s^{-1} respectively while the average wind speed was 1.74 ms^{-1}.



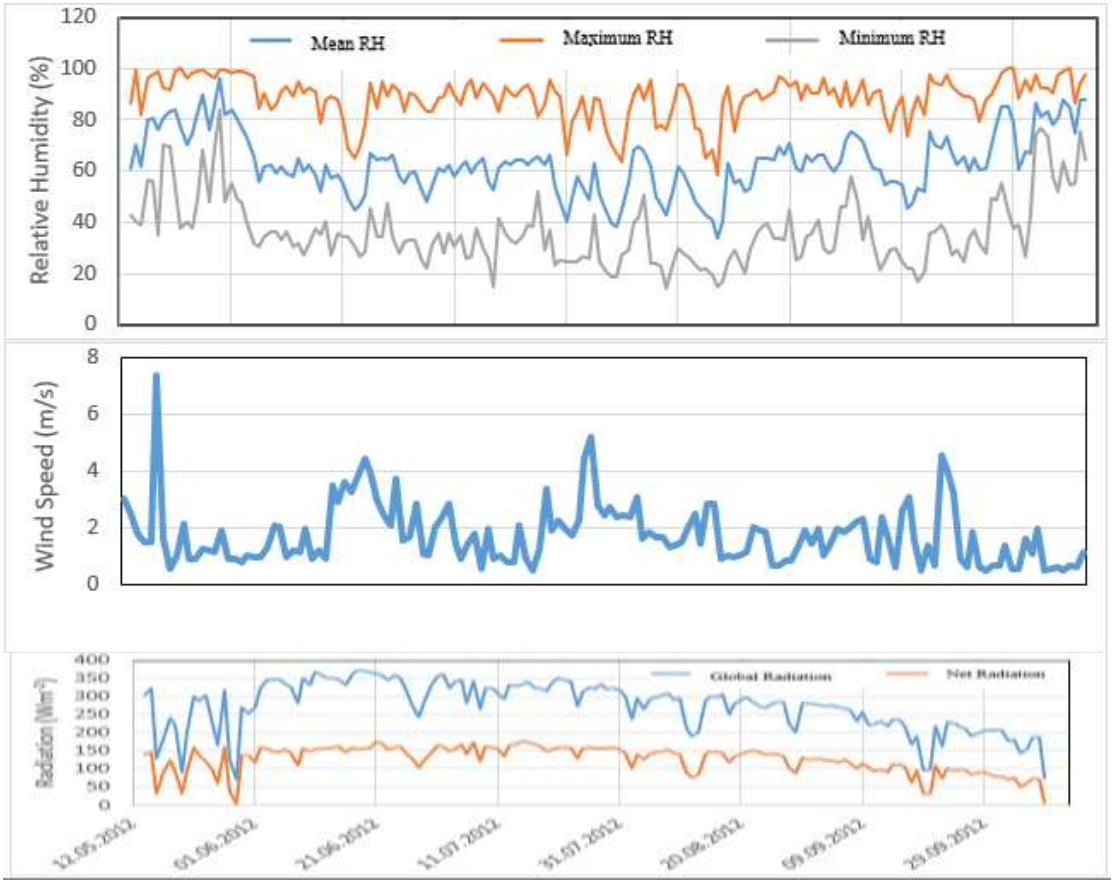


Figure 3. Variation of meteorological parameters during growing period

The variation of the daily values of NEE, GPP and R_{eco} during the growing period is shown in Figure 4. As a result of the measurements and analyzes, cumulated GPP was found as 1160.2 g C m^{-2} (4246.3 g CO_2 m^{-2}), while R_{eco} was 846.35 g C m^{-2} (3097.6 g CO_2 m^{-2}) and NEE was -299.03 g C m^{-2} (-1094.45 g CO_2 m^{-2}). The negative value of NEE shows that the crops act as sink in the carbon budget. The daily mean GPP, NEE and R_{eco} values were 8.99, -2.31 and 6.56 g C m^{-2}, respectively. Daily maximum and minimum GPP were determined as 17.64 and 1.27 g C m^{-2} respectively during the growing period of watermelon. The maximum daily amount of carbon released by watermelon respiration was 10.08 g C m^{-2}, whereas the minimum was found as 2.91 g C m^{-2}. The maximum amount of carbon storage from the atmosphere was measured as -9.09 g C m^{-2}. The daily average emission value of watermelon was determined as 6.56 g C m^{-2} (24 g CO_2 m^{-2}) and the sink value was 2.31 g C m^{-2} (8.45 g CO_2 m^{-2})

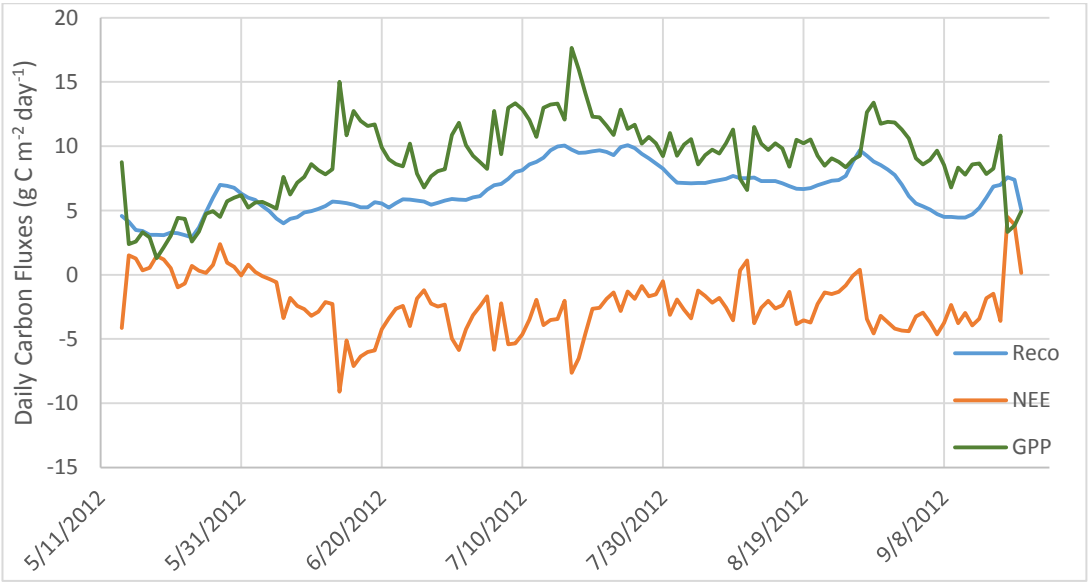


Figure 3. Variation of NEE, GPP and R_{eco} during growing period

CONCLUSIONS

The measurement and analysis results cannot be compared with any study, because this study is the first one in the world including watermelon crop. Coefficients obtained from the developed countries are used in the assessment of the carbon budget of the countries. With the spread of similar studies, it will be possible to determine the national carbon budget by determining the coefficients representing the climate and ecosystem conditions of individual countries.

The carbon fluxes of the crop is under the influence of many meteorological variables. In order to represent the fluxes accurately, it is important to examine the linear or nonlinear relationships between NEE, GPP and R_{eco} and also meteorological variables. The mathematical models using the obtained relations and values by this kind of studies may be able to determine carbon fluxes correctly. However, it is necessary to measure carbon exchange over crops using micrometeorological methods for having actual data, although it is costly and inconvenient.

ACKNOWLEDGMENT

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BOTTLENECK ANALYSIS OF ANATOLIAN BLACK CATTLE (*BOS TAURUS)* USING MICROSATELLITE MARKERS

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ABSTRACT

The present study was conducted in order to reveal the genetic diversity and bottleneck in Anatolian Black Cattle (*Bos Taurus*). Animal material of the study consisted of 75 cattle raised in International Center for Livestock Research and Training. The bottleneck in the cattle breed studied was checked with 10 microsatellites markers, amplified in a multiplex polymerase chain reaction (PCR) were used according to recommendation of FAO (2011). A total of 116 alleles was observed from microsatellites studied. Overall value belongs to average number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), the polymorphic information content (PIC), average heterozygosity ($\hat{H}$), and $F_{IS,}$ known as the inbreeding coefficient, were 11.60, 5.35, 0.80, 0.78, 0.80 and 0.012, respectively. All microsatellite markers except INRA23 and ETH3 deviated from Hardy Weinberg equilibrium (HWE). Bottleneck was analyzed with Bottleneck software according to three different mutation models including the infinite allele model (IAM), two-phase mutation model (TPM) and stepwise mutation model (SMM). It can be said that there is not any ultimate risk in terms of bottleneck considering L–shaped curve showing normal distribution obtained from the analysis.

Keywords: *Bottleneck, microsatellite, Anatolian Black Cattle.*

INTRODUCTION

Anatolian Black Cattle originates from *Bos Taurus brachiceros* and it is the most prominent breed in terms of number and spread. Middle Anatolia is its natural habitat and it is bred almost every place of this region (Alpan, 1993; FAO, 2018). It has a relatively small body and hairs are dark black. Skin is generally thick and stiff. Both males and females have horns and they are slim, crescent shaped. This breed is very resistant to harsh conditions, diseases, pests and parasites. It has an excellent digestion system and can live and reproduce in forests or mountain terrains without any support. Anatolian Black Cattle has a relatively high

adaptation capability to harsh conditions. It is usually hard to manage mainly due to its aggressive nature and tough body. It is bred for milk, meat and body power.
After several genetic characterization studies, breeds with high allelic diversity and heterozygosity haven been accepted as genetic repository for genetic diversity. It has been emphasized that particularly breeds close to domestication regions have to be conserved (Giovambattista et al., 2001; Egito et al., 2007; Sharma et al., 2008; Medugorac et al., 2009; Özşensoy et al., 2010). Extinction of Anatolian domestic animal breed is critical due to close residing to first domestication center (Bruford and Towsend, 2004). It has been reported that Anatolian Black Cattle is under the risk of extinction (Ertuğrul et al., 2000). This breed is being conserved *in situ in vivo* in the International Center for Livestok Research and Training under a conservation program named "Conservation of Local Animal Genetic Resources" initiated by Turkish Ministry of Food, Agriculture and Livestock in 2004.
Microsatellites are used extensively in many species (Ağaoğlu and Ertuğrul, 2011) because they are codominant (Ramamoorthi et al., 2009), specific to loci (Condit and Hubbell, 1991), abundant and evenly distributed along genome (Iamartino et al., 2005; Ramamoorthi et al., 2009), highly mutated (Toro et al., 2009), informative (Ramamoorthi et al., 2009) and PCR based. Microsatellites is one of most preferred markers for genetic studies due to compatibility to PCR (Weber and May 1989; Liu, 1998). Heterozygosity and allele frequency distribution can be used to determine bottleneck effects in populations. Bottleneck effects cause genetic variation losses, fixation of unfavorable alleles and inbreeding depression (Hedrick, 2005; Luikart and Cornuet, 1998).
Bottleneck analysis was conducted first time using microsatellites for the Anatolian Black Cattle herd being held in the International Center for Livestok Research and Training under the conservation program.

MATERIAL AND METHODS

The study was carried out on 75 Anatolian Black Cattle breed is raised in International Center for Livestock Research and Training Institute genetic conservation flock. Blood samples were collected from *vena jugularis* into containing K3EDTA tubes and stored at -20°C until DNA extraction. Genomic DNA was extracted from 200 μL whole blood using QIAamp 96 DNA QIAcube HT Kit (Qiagen, Hilden, Germany) by the standard protocol of QIACube HT extraction robot. Amount and quality of DNA were checked using Titertek® micro amount spectrometer. In the present study, a panel of ten microsatellite markers, recommended by FAO (2011), was used to reveal intra-breed genetic diversity and bottleneck test. Microsatellite loci were amplified by a commercial multiplex kit, "StockMarks® Bovine Genotyping (Thermo Fischer Scientific) in a programmable thermocycler (ABI 9700). Fluorophore labelled amplicons were loaded to ABI Prism 3130 capiller electrophoresis device for fragment analysis with ROX® Gene Scan 500 internal size standard. Results were evaluated using GeneScan® software.

Statistical Analysis

The numbers of alleles (Na), effective alleles (Ne), observed heterozygosity (Ho), expected (He) heterozygosity, mean heterozygosity ($\hat{H}$) and Hardy-Weinberg equilibrium were calculated using the GenAlEx genetic analysis program (Peakall and Smouse, 2006; Peakall and Smouse, 2012). Inbreeding coefficient (FIS) values were obtained using POPGENE statistical software (Yeh et al., 1997) The Cervus 3.0.3 (Marshall et al., 1998; Kalinowski et al., 2007) program was used to calculate polymorphic information content (PIC) and null allele frequency (F(Null)).

Bottleneck events were tested with Sign, Standardized differences and Wilcoxon sign–rank tests under the different mutation models such as Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Model of Mutation (TPM) model in Bottleneck software version 1.2.02 (1 000 simulation) (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998; Piry et al., 1999).

RESULTS AND DISCUSSION

A total 116 alleles were observed from 10 microsatellites used in this study. Computed genetic diversity statistics was given in Table 1.

Table 1. Genetic polymorphism statistics across 10 microsatellite loci

Locus	N	Na	Ne	Ho	He	PIC	$\hat{H}$	F_{IS}*	HWE	F(Null)
BM2113	75	14	8.44	0.80	0.88	0.87	0.88	0.099	***	0.0474
TGLA53	75	11	4.09	0.75	0.76	0.72	0.76	0.019	***	0.0117
ETH10	75	10	4.32	0.69	0.77	0.73	0.77	0.104	*	0.0524
SPS115	74	14	4.56	0.81	0.78	0.76	0.78	-0.032	***	-0.016
TGLA126	75	11	4.15	0.75	0.76	0.73	0.76	0.023	***	0.011
TGLA122	75	17	5.84	0.84	0.83	0.81	0.83	-0.007	**	-0.008
INRA23	74	10	6.52	0.88	0.85	0.83	0.85	-0.031	ns	-0.0196
ETH3	75	10	4.74	0.88	0.79	0.76	0.79	-0.109	ns	-0.0619
ETH225	75	9	3.98	0.75	0.75	0.71	0.75	0.009	***	-0.0082
BM1824	75	10	6.90	0.83	0.86	0.84	0.86	0.040	**	0.0179
Overall		**11.60**	**5.35**	**0.80**	**0.80**	**0.78**	**0.80**	**0.012**		

Na: Number of alleles, **Ne**: Effective number of alleles, **PIC**: Polymorphic information content, **Ho**: Observed heterozygosity, **He**: Expected heterozygosity, **$\hat{H}$**: average heterozygosity, **HWE:** Significance level of Hardy-Weinberg Equilibrium, **F(Null):** Null allele frequency, *****: P<0.05, ******: P<0.01, *******: P<0.001, ns: non-significant

The highest number of alleles and effective number of alleles were obtained from TGLA122 (17) and BM2113 (8.44), respectively. It was seen that the microsatellites used in the present study were highly informative for defining the genetic diversity in the population studied, given that PIC values varied between 0.71 and 0.87. Overall mean of observed heterozygosity value was equal the expected heterozygosity value. According to the diversity parameters we obtained,

high genetic variation was detected in Anatolian Black cattle breed. These parameters were higher than some of cattle breeds raised in different location (Özkan et al., 2009; Mateus et al., 2004; Karthickeyan et al., 2008). This situation may be accepted as evidence of the accumulation of high genetic diversity in domestication centres. The average of F_{IS} value, also known as inbreeding coefficient and described as Wright' F statistics, was 0.012. All microsatellite loci except INRA23, and ETH3 deviated from the Hardy-Weinberg equilibrium ($P<0.05$). Null alleles that is defined as a non-amplifiable allele due to mutations in the PCR binding site, causing only a single allele to peek like a homozygote, thus causing erroneous reading. It has been reported by Dakin and Avise (2004) that the null allele frequency value should be below 20% in order for molecular genetic studies to be performed without errors. When the null allele frequencies obtained are examined, it is seen that the null allele frequency values of 10 microsatellites to be studied are below 0.20. Taking this value into consideration, it has been demonstrated that working locus can be used safely in genetic diversity and bottleneck study in the population studied. It is necessary to understand the processes that cause decreasing genetic diversity such as genetic bottleneck, genetic drift and inbreeding especially in small populations. For this reason, genetic bottleneck analysis was performed to investigate whether there was a bottleneck in Native Black cattle population conserved as a genetic resource. Since the mutation pattern of evolution and microsatellites are not clearly known, the data set obtained was tested with 3 different mutation models, Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two-Phase Model of Mutation (TPM) model reported by Cornuet and Luikart, 1996; Luikart and Cornuet, 1998 and Piry et al., 1999. Sign, Standardized differences and Wilcoxon sign rank tests were used to predict excess of heterozygosity (Table 2).

Table 2. Test for null hypothesis under three microsatellite evolution models for bottleneck analysis

Models		**Sign Test**	**Standardized**	**Wilcoxon rank test** (one tail for H excess)
IAM (The infinite allele model)	**Hee**	6.03	**T2=**2.147 **P=**0.0159	0.00098
	Hed	1		
	He	9		
	P	0.04798		
TPM (Two-phase mutation model)	**Hee**	5.99	**T2=**-0.453 **P=**0.32537	0.61523
	Hed	5		
	He	5		
	P	0.36838		
SMM (The stepwise mutation model)	**Hee**	5.82	**T2=**-5.442 **P=**0.00000	0.99658
	Hed	8		
	He	2		
	P	0.01662		

Hee: Expected number of loci with heterozygosity excess, **Hed:** heterozygosity deficiency, **He:** heterozygosity excess

It was reported that two-phase mutation model (TPM) is the best alternative for bottleneck analysis with microsatellites comparing to other models namely infinite allele model (IAM) and stepwise mutation model (SMM) due to inconsistent results and also reported that TPM was the most useful model with regard to revealing heterozygosity (Piry et al., 1999; Di Rienzo et al., 1994; Luikart et al., 1998). On the other hand, Wilcoxon test with high statistical power was reported to be useful even for bottleneck analysis with limited loci (<20) with high reliability (Piry et al., 1999). Considering the both TPM and Wilcoxon test results it can be said that there is no bottleneck for Anatolian Black Cattle population.
The population studied was found to be bottlenecked by the Wilcoxon test according to the infinite allele model (IAM). But it should not be forgotten that the most suitable model for microsatellites in the Wilcoxon test is the TPM model.
Mode-shift graph was drawn by means of allele frequency classes obtained from the study with ten microsatellites to reveal potential bottlenecks (Figure 1).

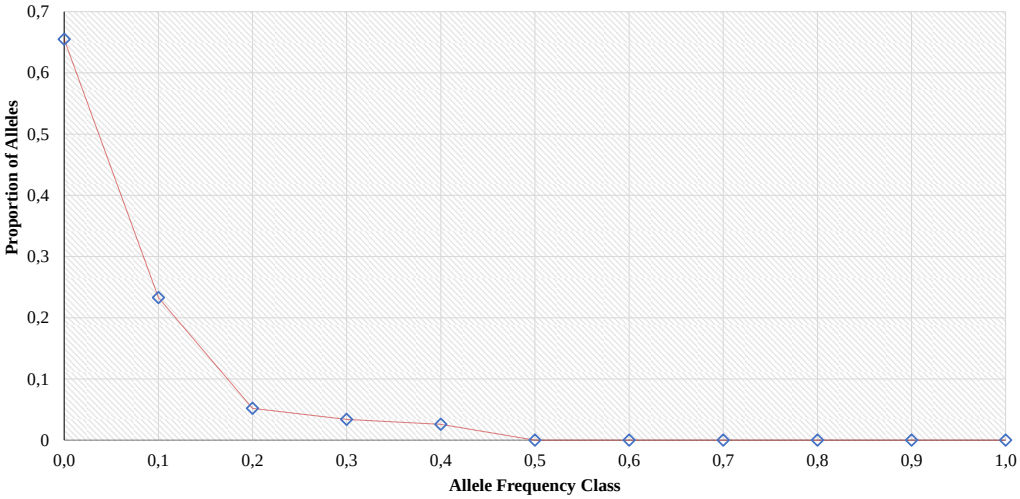

Figure 1. Mode-shift graph for bottleneck in the Anatolian Black cattle breed

As it can be deduced from the figure, L-shaped graph is consistent with the normal frequency distribution intervals. This L-shaped distribution indicates that there isn't any significant genetic bottleneck for the population in question lately (last 40-80 generations).
For Anatolian Black Cattle there are few studies conducted using microsatellites regarding genetic bottlenecks. In an earlier bottleneck study by Özşensoy and Kurar (2014) it was shown that Turkish native cattle breeds' allele frequencies represents L-shaped distribution and Anatolian Black Cattle was not in a potential risk of extinction recently.

Kramarenko et al. reported in 2018 Ukranian Red Steepe (RS) cattle had no bottleneck in near past. In another similar study by TPM assumption it was shown Kherigarh cattle hadn't have bottleneck either (Pandey et al. 2006). Ganapathi et al in 2012 reported Indian cattle breeds had no bottleneck supporting Pandey's results after a study with IAM, SMM and TPM assumptions and furthermore, IAM and TPM asssumptions revealed genetic richness in 25 loci.
In contrary of abovementioned studies, in 2004, Sazaaki et al reported bottleneck in two sublines of Japanese Black Cattle.

CONCLUSION

Consequently, the present study results indicated that despite dramatic decline in Anatolian Black cattle population, genetic diversity was significantly high in the gene pool. Our findings revealed that the microsatellite markers used in this study that can be successfully used in genetic diversity and bottleneck studies for this breed. On the other hand, obtained results will help to interpret the genetic structure of indigenous Anatolian Black cattle and will be of benefit to the efforts for conservation of this breed. The strong inference that the Anatolian Black cattle has not undergone major bottlenecks is also important for cattle breeders and other conservation programs. However, due to decreasing population numbers, conservation programs for these breeds are still necessary.

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Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used, etc.

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The International System of Units (SI) should be used.

- CONCLUSIONS

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

- ACKNOWLEDGMENTS

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

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