ISSN: 2490-3442

UNIVERSITY OF EAST SARAJEVO
FACULTY OF AGRICULTURE

Agrofor

# AGROFOR International Journal

AGROFOR International Journal, Vol. 6, Issue No. 3, 2021

agrofor.ues.rs.ba

# AGROFOR International Journal

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



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

**CONTENT**

**Original Scientific paper**
10.7251/AGRENG2103005E
UDC 631.147(64)

# TRANSITION TO ORGANIC AGRICULTURE IN MOROCCO

Hamza EL GHMARI[1], Rachid HARBOUZE[2], Hamid EL BILALI[1]*

[1]International Centre for Advanced Mediterranean Agronomic Studies – Mediterranean Agronomic Institute of Bari (CIHEAM-Bari), Valenzano (Bari), Italy
[2]Hassan II Institute of Agronomy and Veterinary Sciences, Rabat, Morocco
*Corresponding author: elbilali@iamb.it

## ABSTRACT

Agriculture is a vital sector in Morocco; it contributes 13% to the gross domestic product and employs around 30% of the total workforce and 70% in the rural areas. This puts agriculture in a sensitive spot where it affects the livelihoods of a great proportion of the population. Moroccan agricultural development programs (viz. Green Morocco Plan 2008-2019 and Green Generation 2020-2030) aim to enhance the livelihoods of farmers by focusing on the valorisation of small farms and rural areas through the development of protected geographic indications (PGI), cooperatives, and organic farming. This review paper describes the dynamics and development process of the organic agriculture niche in Morocco through the lens of the Multi-Level Perspective (MLP) on socio-technical transitions. The MLP is a widely used framework that bases its analysis on transitions being the result of the interaction of niches, socio-technical regimes, and socio-technical landscape. Niches represent novelties, regimes are the incumbent and dominant systems, and the landscape includes the external factors that affect both regimes and niches and shape their development. Results show that although the organic niche is well established (11,000 ha of organic land area in 2019), it is still developing at a slower rate than expected. While organic farming does solve many sustainability challenges that Moroccan agriculture faces, it still lacks the infrastructure and human capital to succeed as a niche. All in all, organic farming is still in the first transition stages and can follow a multitude of pathways before becoming relevant in the current agri-food system.

**Keywords:** *sustainability transitions, Multi-Level Perspective, agri-food system, organic farming, Morocco.*

## INTRODUCTION

During the first two decades of the millennium, the Moroccan economy has experienced a remarkable growth. This economic prosperity had been translated to a demographic development, which in turn increased the pressure on Morocco's natural resources (Benmansour *et al.*, 2013). In particular, agriculture knew a tremendous evolution through the modernisation of practices and the intensification of production in selected areas where farm area and resources were

more abundant, while other areas remained traditionally managed with little inputs (Errahj, 2017). This situation has created a two-level agricultural system in Morocco where small farmers produce to survive and big firms invest in intensive production systems to compete. This has made dealing with sustainability challenges difficult, as the governmental programs have to consider two different production systems at once (Haut Comissariat au Plan, 2007).

Currently, Morocco is facing the outcome of years of unsustainable use of resources as well as climate change's negative effects on the environment such as irregular rainfall, droughts, degradation of soil, desertification and pollution (Salhi *et al.*, 2020). The complexity of such challenges does not allow solving them using incremental solutions that address one aspect at a time. Instead, they require radical solutions in the form of sustainability transitions, which change the entire system into a more sustainable one (Sustainability transitions research network, 2017). The transition towards organic agriculture is an example of such transitions that aims to enhance the environmental and socio-economic performances of the Moroccan agri-food system. In this review paper, we will describe the different components at play in this transition (i.e. niche, regime and landscape) as well as propose a pathway that the transition is likely to follow.

## MATERIAL AND METHODS

Sustainability transitions are a wide research field where multiple frameworks are used. In our case, we will study the ongoing transition in the Moroccan agri-food system through the lens of the Multi-Level Perspective (MLP) framework. In this context, we will consider the Moroccan agri-food sector as a socio-technical system consisting of actors, institutions alongside their underlying regulations and norms, and material artefacts and knowledge. These elements' interaction is what provides specific services for society, in this case, the production of food and fibre (Markard *et al.*, 2012). A sustainability transition occurs when a socio-technical system undergoes deep multi-dimensional change involving multiple actors and spanning for long periods. In order to study such a complicated topic, we need the help of socio-technical approaches to analyse this process (Loorbach *et al.*, 2017)

The MLP is one of the major frameworks used to study transitions through the combination of ideas from evolutionary economics, sociology of innovation and institutional theory (Sustainability transitions research network, 2017). This framework suggests that transitions are the result of the interaction of niches, regimes, and landscapes and the way they happen is through the pressuring factors of the landscape that present opportunities for niches to break through and change the socio-technical regimes (Markard *et al.*, 2012). Niches are considered as the protected space in which revolutionary ideas emerge, nurtured by pioneers and leading entrepreneurs who experiment with new configurations. These revolutionary innovations can spread widely and change the current regime but this requires the regime to be under pressure emanating from landscape developments. The MLP is particularly interested in the systemic dimensions of transitions that

are reflected by the different degrees of structuration of each analytical level (Sustainability transitions research network, 2017).

Additionally, scholars have identified certain types of transition pathways depending on the niche-regime-landscape interaction (Markard et al., 2012). Geels and Schot (2007) described one of the major typologies suggesting that the *nature of interaction* between niche and landscape developments can be a reinforcing one or a disruptive one, and that the niche can be in various stage of development depending on the *timing of the transition*. Transition pathways can be differentiated using a combination of different levels of these criteria. This typology describes four major pathways that are the *substitution*, *transformation*, *reconfiguration*, and *de-alignment and re-alignment* pathways as well as two additional pathways for the case of a stable regime (*reproduction process*) and that of a combination of pathways (*sequence of transition pathways*).

In the present paper, we will be gathering secondary data from literature and analysing it through the MLP framework in order to describe the type and features related to the pathway of transition to organic agriculture in Morocco and to define the environment components, internal and external factors, that affect the organic transition and understanding the relations between them. Secondary data was collected through multiple queries on the databases of Clarivate Analytics-Web of Science from the 10$^{th}$ to the 20$^{th}$ of July 2021. Additional grey literature, such as reports and brochures, was collected through Google research engine. The following section describes the findings in relation to each level of the agri-food sociotechnical system as well as the suggested pathway for the Moroccan agriculture transition into organic agriculture.

## RESULTS AND DISCUSSION

The state of the Moroccan agri-food sector is profoundly marked by the grand challenges that affect agriculture. As a North African country, Morocco deals with major environmental setbacks regarding water and soil resources. These challenges are the natural result of the country's climate and geography, but they are additionally emphasised by the economic growth of the country leading to more urbanization while simultaneously having limited funds and frameworks for resource management. The coupled effect of bio-climatic conditions and intensive agricultural practices puts around 15 million hectares of land under threat of degradation due to lower fertility rates as well as reducing water reservoirs' capacity through siltation mechanisms (Benmansour *et al.*, 2013). Additionally, Morocco is considered to be one of the most sensitive countries towards climate change due to rain-fed agriculture representing more than 90% of the production (El Youssfi *et al.*, 2020). Some of the direct effects of climate change in Morocco are the shift in rainfall patterns that causes yield losses, and the recurring droughts that affect social stability and accentuate the existing inequality between farmers (Schilling *et al.*, 2012). El Assaoui *et al.* (2021) states that rainfall has seen a decrease of 3 to 30 % during the 1960-2000 period, and that the forecasts indicate a decrease of 10 to 30% in cumulative annual rainfall by 2100.

While we showed that climate change threatens Morocco's agriculture, it is important to signal that Moroccan policies and agricultural practices also contribute to the degradation of environmental resources; in fact, environmental degradation costed around 3.5 % of the gross domestic product (32.5 billion MAD) in 2014 (Belghazi and Sarraf, 2017). Over the last decades, Morocco's economic growth led to a significant increase in greenhouse gas emissions, 21% of which are linked to agriculture (Belghazi and Sarraf, 2017). Moroccan agriculture started becoming more intensive since the middle of the 20$^{th}$ century with the adoption of nitrogen fertilisation, new irrigation systems, and the introduction of mechanisation (Schilling *et al.*, 2012). Pesticide use has also been proven to cause biodiversity loss and health issues especially with the inadequate use by farmers (Farahy *et al.*, 2021).

Another pressuring factor for the agri-food system is the Moroccan diet, which relies on cereals to provide 60% of food energy supplies with an average consumption of 200 kg/year/capita of cereals (Saidi and Diouri, 2017). Cereal production being highly dependent on rainfall makes it difficult to achieve self-sufficiency; therefore, Morocco has to import up to 40% of its national needs of cereals (El Youssfi *et al.*, 2020). The dependence on the external market goes beyond imports and includes exports as well. Moroccan policies encouraged more crop production via the Green Morocco Plan (2008-2018) and did succeed in increasing the yields of many crops. The downside being the low efforts put into commercialisation resulting in difficulties in exporting excess production (El Youssfi *et al.*, 2020). Thus, Morocco is depending on the international market to import crucial staple food and to export high added value crops such as tomatoes and citrus. Unfortunately, this exchange is unfavourable for Morocco as fruits and vegetables require more water to grow than cereals, thus increasing Morocco's sensitivity to water shortage even further (Saidi and Diouri, 2017).

In the midst of this situation, Moroccan policies shifted towards more sustainable alternatives that preserve non-renewable resources by adopting newer systems of irrigation, encouraging ecologically friendly cropping methods such as conservation agriculture and organic farming, and valorising agricultural products through labels and production standards (Errahj, 2017). Indeed, it is difficult to solve the aforementioned challenges through incremental change because of their complexity and the way they are deeply rooted in the current agricultural regime. Thus, it is better to opt for a deeper and more radical transition that changes the current configuration into a more sustainable one through a proper sustainability transition, in our case, the transition to organic agriculture.

Organic agriculture can be defined as "a production system that sustains the health of soils, ecosystems, and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation, and science to benefit the shared environment and promote fair relationships and good quality of life for all involved" (IFOAM, 2005). Organic farming has proven to be able to mitigate the negative effects of climate change and to conserve soil and water resources by

maintaining a high level of organic matter in the soil (Pimental *et al.*, 2005). Additionally, organic farming practices such as crop rotations and cover crops limit soil erosion (Pimental *et al.*, 2005), and help reducing greenhouse gas emissions as they boost the soil carbon sequestration process (Azadi *et al.*, 2011). Organic farming systems can help build flexible food systems as they encourage diverse cropping systems in a single farm (Azadi *et al.*, 2011). In the Moroccan context, organic farming has the potential to provide a better living for farmers while answering the environmental challenges in a sustainable way.

Organic farming was introduced for the first time in Morocco in 1986 through private operators growing olives and citrus in Marrakech and Benslimane regions respectively. However, the organic sector will not truly become relevant until 2011 when the first program promoting organic agriculture was signed between the government and the Moroccan association of organic production sector (AMABIO). The objectives of the program were to expand the organic cultivated land to reach 40,000 ha and increase the organic production to 400,000 T, to create the equivalent of 35,000 job opportunities, increase the national consumption of organic products, and put in place a legislation for organic agriculture within 9 years. The total investment foreseen for these actions was 1,121 billion MAD, of which 286 million MAD were covered by the state while the rest was covered by the inter-professional federation of the organic sector (FIMABIO) (Ministère de l'agriculture de la pêche maritime du développement rural et des eaux et forêts, 2020a).

Recent surveys in 2017/18 revealed that Morocco has an area of 9,500 ha of organic certified cultivated farmland, 980 ha of farmland in transition, and 273,000 ha of wild collection areas, and a total organic production of 104,600 T in 2018 (Ministère de l'agriculture de la pêche maritime du développement rural et des eaux et forêts, 2020a). The surveys clearly show that the organic niche in Morocco is not progressing as planned by the 2011 program. Some of the reasons behind this slow progress are the absence of adequate infrastructure dedicated to the handling and commercialization of products on the internal market, the unavailability of qualified technicians in the organic sector, and the shortage in subsidies of production, export, processing (Ministère de l'agriculture de la pêche maritime du développement rural et des eaux et forêts, 2020b).

In order to define which pathway the organic niche is more likely to follow, we need to figure out the nature of the niche-regime and regime-landscape interaction as well as find the timing or stage at which the niche is currently accordingly with the typology of Geels and Schot (2007).

As demonstrated above, the Moroccan organic niche is lagging behind relative to the goals set by the program for various reasons. Despite the niche having the support of the government since 2011, it is still in its early stages, as the national organic law, developed in 2013, would not come into force until late 2018. Additionally, the government recently revoked the recognition from the inter-professional federation of the organic sector (FIMABIO) in December 2020 (Agrimaroc, 2021) in a clear indication that the sector is still struggling to operate

in an optimal way. Thus we can describe the niche as underdeveloped. Based on the prior descriptions, the Moroccan agri-food regime faces multiple pressures emanating from the landscape. Environmental challenges are constantly threatening the present and future productivity, and by consequence, they stir social unrest especially considering the proportion of the population relying on agriculture for their income. It is important to note that the pressure presented by these factors is not focused on a singular moment in time, but rather it is a "disruptive" pressure. In other words, the landscape poses little pressure in the short term but it is continuously present and building up to become more imposing in the long term, as resources are depleted, climate change is accentuated, and social inequalities grow wider (Geels and Schot, 2007). Concerning the niche-regime relation, it is clear that the current agri-food regime encourages organic farming through contract programs and adopts it as a possible alternative towards a more sustainable production system. This is also true if we consider that the current regime presents a "low use of pesticides in multiple crops as well as cheap and abundant labour and a high integration between crop production and livestock which satisfies the farmer's need for manure" (Alaoui, 2009). Therefore, we can conclude that the niche is synergised with the regime. Therefore, the possible pathways with regards to the timing and nature taken by the transition in question can be either the *transformation pathway*, the *reconfiguration pathway*, or *a sequence of transition pathways*.

The *transformation pathway* (Figure 1) is especially interesting for our context as it occurs when "Moderate landscape pressure occurs early in disruptive landscape change. Niche-innovations cannot take advantage […] because they are not sufficiently developed" (Geels and Schot, 2007). The initially moderate pressure can be difficult to perceive from within the regime, which is why it is important to have outsiders to draw attention to these pressures. In our context, worldwide experts are the actors drawing the Moroccan regime's attention to environmental pressures. Additionally, outsiders can demonstrate promising alternatives and inspire the regime actors to adopt them, which is precisely the case for the organic niche as an external innovation that the Moroccan regime is starting to adopt. The *transformation pathway* achieves transition through cumulative reorientations by the niche actors who adjust themselves and pressure the regime to change its rules. Through this process, the actors of the regime might remain the same but the social network they belong to might change.

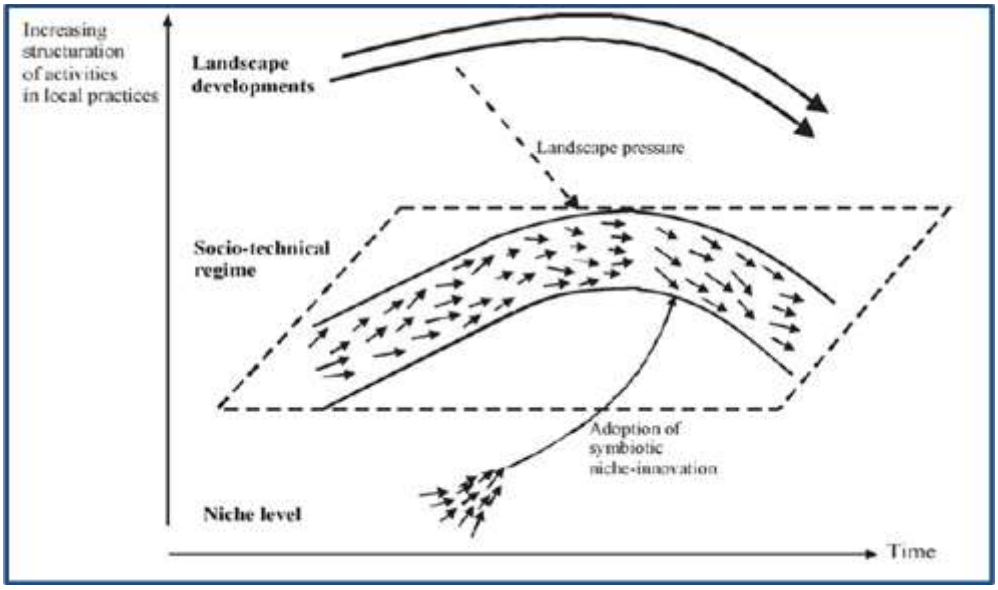


Figure 1. Transformation pathway.
Source: Geels and Schot (2007).

The *reconfiguration pathway* (Figure 2) is a similar case of *transformation pathway* where the niche innovations are symbiotic with the regime and are easily adopted. The difference between the two is that while the *transformation pathway* does not necessarily cause deep changes in the regime's architecture, the *reconfiguration pathway* causes deep changes when the adopted innovations keep causing further changes requiring further adjustments, to a point where other niche innovations are introduced to the regime as the regime actors explore new possibilities. This is especially true in our case since we are dealing with the agri-food system which is known for having multiple interlaying subsystems where newer innovations are likely to add up after a major regime change. In this type of pathway, the transition is the result of multiple niche innovations that breakthrough around the initial innovation that sparked the change.

A *sequence of transition pathways* applies in the event where the landscape pressure begins by being moderately disruptive but later becomes significantly pronounced, as in climate change posing environmental pressure that causes civil unrest or a deep economic setback. Regime actors start perceiving moderate change at first and the system begins the transition under a transformation pathway, later on as the pressure increases, more changes are encouraged and the transition becomes a reconfiguration. Finally, when the pressure becomes too high, the regime "collapses" and many niche innovations fill the "vacuum" until eventually one of them resurges as the new regime, in what we call a de-alignment re-alignment pathway. Alternatively, if the pressures happen at a timing where a niche-innovation is well developed, a substitution occurs where the innovation replaces the old regime.

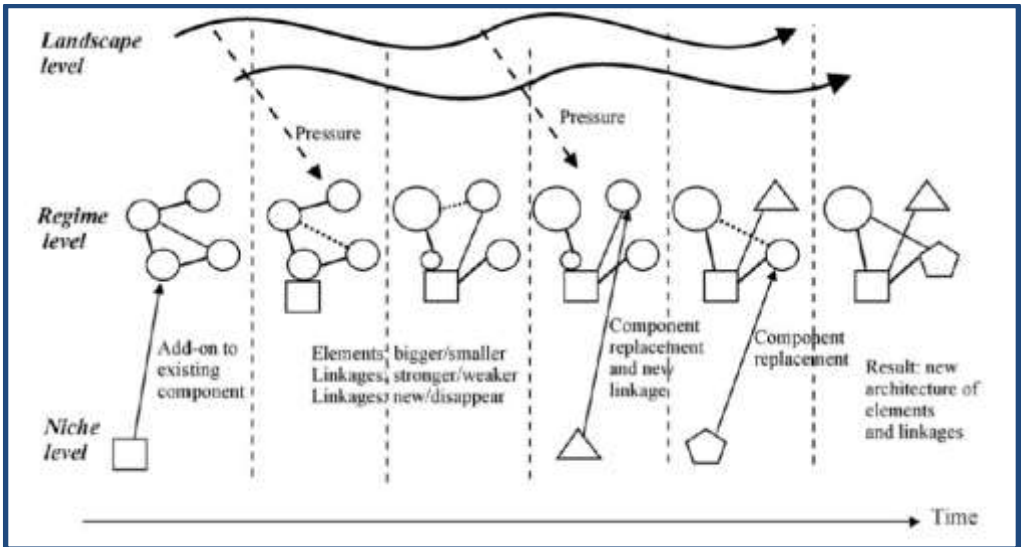


Figure 2. Reconfiguration pathway.
Source: Geels and Schot (2007).

## CONCLUSIONS

The current state of the Moroccan agri-food system has proven to be lacking in sustainability be it environmental, economic, or social sustainability. As it stands now, Morocco's agriculture is depleting non-renewable resources, such as soil and water, and causing social unrest because of its dependence on a fluctuating climate without being able to reach self-sufficiency. This situation calls for a meaningful sustainability transition guided by efficient government programs and carried out by the actors of the current regime. As we have shown in this paper, organic farming can be a reliable alternative to alleviate the effects of intensive farming and enhance the performance of the agri-food system sustainability-wise. The organic niche in Morocco has a great potential to achieve this since it benefits from governmental support alongside favourable climatic conditions and a growing interest in agro-ecology. However, the organic niche is still struggling to take-off for a number of reasons mostly related to governance and awareness of the value of organic farming. Throughout this paper, we have shown how the organic niche benefits quite efficiently from the pressures that the landscape is putting on the Moroccan agri-food regime, and we suggested pathways that this transition might take based on the relationships between the different socio-technical levels and the state of the niche itself.

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**Original Scientific paper**
10.7251/AGRENG2103015N
UDC 338.5:631.1/.7(497.11)

# TENDENCIES AND PREDICTION OF PRICES OF INDUSTRIAL CROPS IN SERBIA

Nebojša NOVKOVIĆ[1]*, Beba MUTAVDŽIĆ[1], Ljiljana DRINIĆ[2], Snežana JOVANOVIĆ[3], Dragana TEKIĆ[1]

[1]Faculty of Agriculture, University of Novi Sad, Serbia
[2]Faculty of Agriculture, University of Banja Luka, Bosnia and Herzegovina
[3]Institute of Maize, Zemun Polje, Serbia
*Corresponding author: nesann@polj.uns.ac.rs

## ABSTRACT

The research in this paper deals with the prices of the most important industrial crops in Serbia – soybean, sunflower, sugarbeet, rapeseed and tobacco. The main aim of the research was to perform aquantitative analysisto determine the trends in the prices and to predict the trends for the following period. The analysis was based on the average annual prices for the period 2005-2019. The quantitative analysis was performed by using methods of descriptive statistics and the average annual rate of change to discover the trendsfor the analyzed period and to predict the trends for the following five years (2020-2024). The average annual price of soybean was 311.86 EUR/t. The price ranged between 189 and 527 EUR/t. The annual change rate of soybean price in Serbia in the analyzed period was 2.76%. The average annual price of sunflower was 264.78 EUR/t. The price ranged between 163 and 455 EUR/t. The annual change rate of sunflower price in Serbia was 2.13%. The average annual price of sugarbeet was 31.60 EUR/t. The price ranged between 24.36 and 40.31 EUR/t. The annual change rate of sugar beet price was 1.85 %. The average annual price of rapeseed was 299.58 EUR/t. The price ranged between 145 and 447 EUR/t. The annual change rate was 5.84%. The average annual price of tobacco was 1,749 EUR/t. The price ranged between 1,068 and 2,159 EUR/t. The annual change rate of tobacco price in Serbia was 3.90%.The predictions show that the expected prices for the industrial crops in 2024 in Serbia will be as follows: soybean 337, sunflower 266, sugar beet 35.33, rapeseed 425 and tobacco 2,208 EUR/t.

**Keywords:** *industrial crops, soybean, sunflower, sugarbeet, rapeseed, tobacco, prices prediction, Serbia.*

## INTRODUCTION

Industrial crops include a group of crops whose fruits, leaves or other plant parts are used as raw materials in the processing and textile industries. By processing of these plants we obtain important food products: sugar, edible oil as well as

numerous by-products used in livestock nutrition includingsugarbeet pulp and meal.

Industrial crops are grouped according to botanical classification, or the products obtained from these plants:

- Oil crops–crops used for the production of edible oils obtained from fruits or seeds (e.g. sunflower, rapeseed, soybean, castor oil plant, flaxseed).
- Fiber crops – fiber is obtained from the stem, fruit or leaves (e.g. cotton, flax, hemp, jute, manila).
- Crops for production of starch, sugar and alcohol –swollen underground organs are used for obtaining sugar, starch and alcohol (e.g. sugarbeet, sugarcane, potatoes).
- Aromatic and spice plants.
- Medicinal plants – plant species in which the leaf, flower, fruit, root or stem contains a substance used for therapeutic purposes or cosmetic products (e.g. chamomile, sage, lavender).
- Other industrial crops– tobacco and hops.

Production of industrial crops depends on the agro-ecological conditions required for their cultivation. The Republic of Serbia has favorable natural conditions for the cultivation of industrial crops. These crops are grown on an area of 400 thousand hectares, which accounts for around 8% of the arable land in the Republic of Serbia. The most important crops produced in the country include sugar beet, sunflower and soybean.

The research in this paper is an analysis of the tendencies and prediction of the prices of the most important industrial crops in Serbia: soybean, sunflower, sugar beet, rapeseed and tobacco.

The aim of the paper is to use time series analysis of the prices of the industrial crops from the past period as a basis for predicting the absolute and relative prices of these industrial crops, and forecasting economic (market) conditions for the production of these crops.

There are numerous examples of applying quantitative methods in analyzing, modeling, forecasting and planning of economic characteristics of agricultural products and inputs in agriculture (Mutavdžić et al. 2007; 2010; 2017; Novković et al. 2006; 2020; Novković, Mutavdžić, Ivanišević et al. 2016; Mihajlović et al. 2018).

## MATERIAL AND METHODS

The research methods applied in this paper were selected based on the described subject and aim of the research. The statistical methods included descriptive statistics and time series analysis.

The absolute prices and the price parities of the most important industrial crops in Serbia were analyzed by means of descriptive statistics for the period 2005–2019. The analysisincluded the basic statistical indicators: average value, extreme value

(minimum and maximum), coefficient of variation and average annual rate of change (r).

*Prediction of the prices for theindustrial crops was carried out using of the*average annual rate of change (r):

$$r = (G - 1)$$

$$G = \left( \frac{Y_n}{Y_1} \right)^{\frac{1}{n-1}}$$

where:

r = the average annual rate of change

G = the average annual index of change

$Y_1$ = the absolute value of the first member of the time series

$Y_n$ = the value of the last number of the time series

n = the length of the series (the number of years).

The average annual prices of the industrial crops in the analysis were converted into euro per ton to enable comparison with foreign countries and to reduce the factor of domestic inflation. Conversion of the prices into euro was carried out according to the average annual exchange rate of euro based on the data of the National Bank of Serbia.

Based on the established parity change rates (r), the prediction was made for the period 2020–2024.

The data used in the analysis refer to the prices in Serbia. The price series in this paper are either taken from or formed on the basis of statistical publications of the Institution of Statistics of the Republic of Serbia.

## RESULTS AND DISCUSSION

### *Descriptive analysis of the prices of industrial crops in Serbia*

Sunflower is the most common industrial crop in Serbia. On average, it is grown on about 210 thousand hectares, with an annual production of around 616 thousand tons. In the analyzed period (2005-2019), the average annual price of sunflower was 264.58 EUR/t. The price ranged from the minimum price of 163.17 EUR/t in 2009, to the maximum price of 455.33 EUR/t in 2012. The coefficient of variation was relatively high – the highest of all analyzed industrial crops, amounting to 28.65%. The price of sunflower shows a trend of a slight increase at an average annual rate of 2.13%.

When observing the relative price movement, i.e. the parity between different industrial crops, the price of sunflower (as currently the most common industrial crop in Serbia) was taken as the standard.

Soybean is the second most common industrial crop in Serbia. It is grown on an average of 170 thousand hectares with an annual production of 598 thousand tons. The average annual price of soybeanwas 311.86 EUR/t, which is by 47.08 EUR/t,

or 17.78% more than the average price of sunflower. The annual price of soybean varied in the interval from 189.28 EUR/t in 2006 to 527.02 EUR/t in 2012. Soybean also had a high coefficient of variation of 25.27%. The price of soybean showed a trend of increase at an average rate of 2.76% per year.

The average relative price of soybean, i.e. soybean/sunflower price parity, was 1.21. This means that one ton of soybean was worth as much as 1.21 tons of sunflower. The price parity varied in the interval from 0.79 in 2010 to 1.76 in 2013. The coefficient of variation was the lowest in relation to all other observed price parities, amounting to 21.62%. The soybean/sunflower price parity had a positive trend in favor of soybean, but it was very low amounting to only 0.61% per year.Therefore, the price parity between these two oil crops in Serbia is considered to be stable.

Rapeseed is the third most important oilseed crop in Serbia. It is much less common compared to the previous two oil crops (produced on 24 thousand hectares, with an annual production of 69 thousand tons). The average annual price of rapeseed in the analyzed period was 299.56 EUR/t. That is around 12 EUR/t, or 4% lower than the price of soybean, or 35 EUR/t or 13% higher than the price of sunflower. The price of rapeseed varied from the minimum price of 144.60 EUR/t in 2005, to the maximum price of 446.93 EUR/t in 2012. The coefficient of variation of the rapeseed price was slightly lower than for the price of sunflower, amounting to 27.15%. The price of rapeseed showed a trend of increase at a relatively high annual rate of 5.84%.

The average price parity of rapeseed in relation to sunflower was 1.16. The most unfavorable parity was recorded in 2007, when a ton of rapeseed was worth 0.68 tons of sunflower, while the most favorable parity was in 2013, when one ton of rapeseed was worth as much as 1.7 tons of sunflower. The coefficient of variation for rapeseed/sunflower price parity in the observed period was 24.19%. The price parity shows a trend of increase in favor of rapeseed at an average annual rate of 3.66%.

Sugar beet is the third most cultivated industrial crop (after sunflower and soybeans) in terms of the harvested area in the Republic of Serbia, with an average harvested area of 46,000 hectares per year. The average price of sugar beet was 31.60 EUR/t. It ranged from 24.36 EUR/t in 2010 to 40.31 EUR/t in 2013. Sugar beet had the most stable prices of all analyzed industrial crops. The coefficient of variation was 15.86%. The price of sugar beet also recorded the lowest growth rate in the analyzed period compared to other industrial crops, amounting to only 1.85% per year.

Sugar beet/sunflower price parity averaged 0.13. This means that a ton of sugar beet was worth as much as 130 kg of sunflower. The parity varied from 0.07 in 2010 to 0.18 in 2013. The coefficient of variation was 25.07%. Sugar beet is the only crop showing a negative trend of the price parity in relation to sunflower. The rate of decline was very low, amounting to 0.53% per year,indicating that the relative price position of these two crops is stable, and that it will remain so in the future.

Tobacco is grown averagelyon less than 6,000 hectares in Serbia. The annual production is around 8 thousand tons. The average annual price of tobacco in the period from 2005-2019 in Serbia amounted to 1,749 EUR/t. The price varied in the analyzed interval from 1,068 EUR/t in 2005 to 2,159 EUR/t in 2013. As was the case with sugar beet, the price of tobacco also had a low coefficient of variation, which amounted to 16.07%. In the analyzed period, the price of tobacco showeda trend of increase at an average annual rate of 3.90%.

The average price parity of tobacco/sunflower was 7.01. It ranged from 4.15 in 2012 to 11.31 in 2009. This parity had the highest coefficient of variation of 28.48%. It showed atrend of increase in favor of tobacco, at an average annual rate of 1.73%.

***Prediction of the prices for industrial crops in Serbia***

Prediction of the prices and the price parities for the industrial crops in Serbia for the period 2020-2024 was conducted by applying the average rate of change calculated for the analyzed period 2005-2019. The rate of change (for the prices or price parities) was applied to the values obtained for 2019,in this way projecting the values for 2020. The values for the remaining prediction period were calculated in the same way (using the same rate of change).

Table 1 shows the projected values of the prices for certain industrial crops in Serbia for the following period.

Table 1. Predicted prices of industrial crops in Serbia (EUR/t) for the period 2020-2024

| *Crop* | *Year* | | | | |
|---|---|---|---|---|---|
| | *2020* | *2021* | *2022* | *2023* | *2024* |
| *Sunflower* | *244* | *249* | *255* | *260* | *266* |
| *Soybean* | *302* | *311* | *319* | *328* | *337* |
| *Rapeseed* | *339* | *359* | *380* | *402* | *425* |
| *Sugar beet* | *32.84* | *33.44* | *34.06* | *34.69* | *35.33* |
| *Tobacco* | *1,895* | *1,969* | *2,046* | *2,126* | *2,208* |

The projected price of sunflower in 2024 will reach a value that is practically at the same level as the annual average from the previous period, while it is by 27 EUR/t or 11.3% higher than the price in 2019.

In 2024, the predicted price of soybean will be by 25 EUR/t or 8% higher than the average price in the analyzed period. The projected price in 2024 will be by43 EUR/t or 14.6% higher than in 2019. The difference between the prices of soybean and sunflower in 2024 will increase from 47 EUR/t, which was the average difference, to 71 EUR/t. This amount is higher even than in 2019, when the difference was 55 EUR/t. The projected average price for 2024 for the third observed industrial crop (rapeseed) will be by125 EUR/t or even 41.7% higher than the average price in the analyzed period. The projected price will be by 105 EUR/t or 32.8% higher compared to 2019.

In the last year of the prediction period, sugar beet will reach a price that is by 3.73 EUR/t or 11.8% higher than the average price in the analyzed period. Compared to 2019, the projected price of sugar beet in 2024 will be higher by 3.09 EUR/t or 9.6%.

The price of tobacco in 2024 will be higher than the average price in the analyzed period by 729 EUR/t, or 26.2%. Compared to the last year of the analyzed period, the price of tobacco will increase by 384 EUR/t or 21.1%.

Predictionsof the price paritiesfor the analyzed industrial crops in relation to the price of sunflower are shown in Table 2.

Table 2. Predictions of price paritiesfor industrial cropsin relation to the price of sunflower in Serbiafor the period 2020-2024

| *Crop* | *Year* | | | | |
|---|---|---|---|---|---|
| | *2020* | *2021* | *2022* | *2023* | *2024* |
| *Soybean* | *1.2375* | *1.2451* | *1.2565* | *1.2603* | *1.2680* |
| *Rapeseed* | *1.3890* | *1.4399* | *1.4897* | *1.5442* | *1.6007* |
| *Sugar beet* | *0.1293* | *0.1283* | *0.1279* | *0.1273* | *0.1266* |
| *Tobacco* | *7.7620* | *7.8963* | *8.0329* | *8.1719* | *8.3132* |

The projected soybean/sunflower price parity in 2024 will be 4.8% higher than the average parity in the analyzed period, and by 3.1% higher than in 2019.

The projected price parity for rapeseed/sunflower in the last year of the prediction period will be by as much as 38% higher than the average parity, and by 19.5% higher compared to 2019.

Sugar beet is the only analyzed industrial cropwhose price parity will slightly decline in relation to the price of sunflower. The projected sugar beet/sunflower price parity in 2024 will be lower by 2.6% compared to the average parity (the same as compared to the last year of the analyzed period – 2019).

In the last year of the prediction period (2024), the average price parity of tobacco/sunflower will be by 18.6% higher than the average parityin the analyzed period, and by 9% higher than the parity in 2019.

## CONCLUSION

Based on the scientific research conducted in this paper, it can be concluded that the prices of all analyzed industrial crops showed a tendency of increase in the period 2005-2019. The fastest growth was recorded for rapeseed (5.84% per year), while the lowest growth was determined for sugar beet (1.85%).

The most stable prices are determined for sugar beet and tobacco (coefficients of variation were 15.9% and 16%, respectively), while the prices of sunflower were the most unstable (coefficient of variation was 28.7%). Among the oilseed crops, soybean had the highest price (312 EUR/t), followed by rapeseed (300 EUR/t), and sunflower (265 EUR/t). However, the predictions show that this order will change. In the future period (2020-2024), the highest price will be achieved by rapeseed (425 EUR/t), followed by soybeans (337 EUR/t) and sunflower (226 EUR/t).

The price parities are very stable for sugar beet and soybean, while rapeseed and tobacco show trends of a significant increase in the price paritiesin relation to sunflower, and thus improvement of their relative price (market) position.

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**Original Scientific paper**
10.7251/AGRENG2103022G
UDC 582.929.4:621.38

# INFLUENCE OF BLUE AND RED LEDs ON DEVELOPMENT AND NUTRITIVE VALUE OF *PERILLA FRUTESCENS* (L.) CULTIVATED IN CLIMATE CHAMBERS

Nikolina GRABOVAC, Michael H. BÖHME*

Humboldt-Universität zu Berlin, Faculty of Life Sciences, Dept. Horticultural Plant Systems, Lentzeallee 75, D-14195 Berlin, Germany
*Corresponding Author: michael.boehme@hu-berlin.de

## ABSTRACT

In this study influence of supplemental blue and red LED lighting on growth, nutrient solution uptake and concentration of secondary metabolites was investigated. *Perilla* plants were cultivated in climate chambers, where main light was provided by fluorescent tubes with PPFD between 123-177 $\mu molm^{-2}s^{-1}$. Additionally, blue and red LEDs were used, providing 11 $\mu molm^{-2}s^{-1}$ or 12 $\mu molm^{-2}s^{-1}$, respectively. Temperature was set on 24/19°C (day/night), relative air humidity on 64/56% (day/night), and day length was 16 hours. Results showed that small portion of supplemental blue light (~9%) increased fresh mass (FM), dry mass (DM), and nutrient solution uptake (NSU) up to 50.33%, 10.85% or 31.80%, respectively. Supplemental red light (~6%) increased nutrient solution uptake up to 23.56%, while fresh and dry mass were higher than control, but without statistical significance. Regarding nutritive value of *Perilla*, supplemental blue light significantly increased carotenoid concentration (Car) (+13.37%), but polyphenols (PP), anthocyanins (Anth) and flavonoid (Fl) concentrations did not differ from the control. In treatment with supplemental red light, only flavonoid concentration was significantly increased (+14.34%). Use of supplemental blue or red LEDs in closed systems with controlled conditions increase or tends to increase plants fresh mass, dry mass, nutrient solution uptake rate, as well as concentration of some secondary metabolites.

**Keywords**: *Perilla, blue and red LEDs, climate chambers, growth and nutritive value.*

## INTRODUCTION

*Perilla frutescens* (L.) Britt. (Lamiaceae), known as Beefsteak plant, Shiso in Japan, Tía tô in Vietnam, is an Asian herbaceous plant native to mountainous areas from India to China, but mainly cultivated and consumed in Korea, Japan, Thailand and Vietnam. *Perilla* is an annual plant adopted to warm, humid climates and grows well on semi-shade or sun. Except for culinary use, its fresh leaves and seeds are well-known for treatments of various diseases like tumor, heart diseases,

diabetes, anxiety, depression, infections and intestinal disorders. The health promoting effects of *Perilla* have been attributed to its high content of secondary metabolites such as polyphenols, flavonoids and anthocyanins (Grbic et al., 2016a). Sato et al. (2002) found a total phenolic content of 727 mg/ 100 g FW. Depending on the cultivar, between 700 and 1200 mg polyphenol content/100 g FW were detected by Müller-Waldeck et al., 2010. Hong and Kim (2010), detected total flavonoid content in *Perilla* of 7.23 mg/ g DW.

Secondary metabolite synthesis can be significantly affected by light intensity and temperature, causing morphological and physiological changes in plants (Hwang et al., 2014). Some observations were already made in *Perilla* plants regarding influence of light on ingredient contents. Depending on light conditions, Park et al. (2013) found an anthocyanin content of 100-400 mg/100 g FW. Nishimura et. al (2009) investigated influence of different light conditions on growth and content of secondary metabolites of red-leafed *Perilla* cultivated in climate chamber. According to their study, dry weight and growth were positively affected by red-enriched light treatments (red alone, blue and red, green and red), while anthocyanin content was 1.3-1.7 times higher in red light treatment than in any other treatments. Importance of light spectra on plant development and concentration of secondary metabolites was also shown in the study of Grbic et al. (2016a), where *Perilla* plants were grown under greenhouse light conditions manipulated by means of colored plastic films. Light intensity can also affect *Perilla* leaf color, which vary from green to purple.

As the blue and red spectral ranges are supposed to be primary energy sources for photosynthesis and mainly absorbed by important photoreceptors as phytochromes, cryptochrome and phototropin, aim of this study was to investigate influence of these specific light spectra on plant growth, nutrient solution uptake rate, and content of secondary metabolites of *Perilla frutescens* cultivated under controlled growing conditions.

## MATERIAL AND METHODS

Plant material and cultivation

In both experiments *Perilla frutescens* var. *crispa* (Thunb.) H. Deane was investigated. Bronze-leafed "H&V" *Perilla* seed from Vietnam was used. The main leaf colour is green, later partly turning bronze or purple.

Seeds were sown into plug trays and after the young plants had 4-6 fully developed leaves, they were transplanted into 18 Mitscherlich pots (Bergmann, 1958) filled with a substrate mixture (6 l). Continuous supply with nutrient solution was ensured by textile wicks, placed in the substrate of these pots so that its lower end was immersed in the pot saucer with nutrient solution, while upper end was in the plants root zone. In each pot four plants were cultivated.

Then all plants were acclimatized for one week under artificial light provided by fluorescent tubes. After acclimatisation pots were placed in two climate chambers. One was proposed as control (CC2), where plants grew under fluorescent tubes; in another one (CC1) growth conditions were the same as in CC2, but beside

fluorescent tubes as a main light source, four LED bars (length 120 cm) were horizontally placed 20 cm bellow fluorescent tubes in order to influence the light spectrum. There were 16 plants per treatment. Experiment with supplemental blue LEDs took four and experiment with red LEDs took three weeks.

Growth conditions

Climate parameters in chambers were constant and controlled by computer. Temperature was set to 24/19°C (day/night), relative air humidity to 64/56% (day/night), and day length was 16 hours. As main light source in climate chambers 14 fluorescent tubes F58W/827 (Sylvania, Luxline Plus, Germany) were used, placed 1 m above the bottom of the chamber. Light emitting diodes used in climate chamber experiments were monochromatic blue (443 nm) and red light (629 nm), providing 11 $\mu molm^{-2} s^{-1}$ or 12 $\mu molm^{-2} s^{-1}$, respectively. Wavelengths were measured with the Avantes Ava Spec NIR 256 spectrometer.

Photosynthetically active radiation (PPFD) was measured with the micro quantum/temperature sensor 2060-M of PAM-2000 portable chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) at height of 40 cm from the chambers bottom. Average PPFD values during experiments are given in Table 1. As it is shown, there are some differences in value between CC2 and CC1 during one experiment, what was result of unequal aging of fluorescent tubes.

Table 1. Average PPFD in $\mu mol\ m^{-2}\ s^{-1}$ measured in climate chamber CC1 (Control) where plants grew under fluorescent tubes (FT), and in CC2 where plants grew under fluorescent tubes (FT) and supplemental blue (first Experiment) and red (second Experiment) LEDs

| Climate chambers | First Experiment | Second Experiment |
|---|---|---|
| CC1 (FT-Control) | 123.29 | 177.33 |
| CC2 (FT+supplemental LEDs) | 125.62 | 197.31 |

Growing place, media and nutrients

Experiment was carried out from April to July 2013 in climate chambers of the Humboldt University of Berlin, Research station Berlin-Dahlem.

Growing substrate used in the experiments was consisting of white peat (65%), black peat (20%) and perlite (0.2-6 mm) (15%), produced by the company Gramoflor GmbH & Co. KG (Vechta, Germany).

The basic composition of nutrient solution used during experiment was: N (110 ppm), P (50 ppm), K (225 ppm), Ca (120 ppm), Mg (80 ppm), $HCO_3$ (90 ppm), $SO_4$ (60 ppm) and microelements, of which Fe (10 ppm). The EC value equalled 1.5 mS $cm^{-1}$ and pH value 5.8, whereas pH value was adjusted with 85% $H_3PO_4$. The HYDROFER computer programme compatible with an Excel programme (Böhme, 1993) was used to calculate the required amounts of fertilizers, salts and acids.

Plant growth determination

Plant height was measured once a week, beginning after acclimatization and from then on in seven-day-intervals till harvest.

Fresh and dry mass were determined at the end of experiment. All leaves were removed from stems and weighed without petioles, which was figured as fresh mass (FM). After that these leaves were dried at 60°C to constant weight and figured as dry mass (DM) in %.

Nutrient solution uptake rate

Immediately after potting, two litres of nutrient solution were poured in the Mitscherlich pot saucers. From then on, every second day the nutrient solution left in saucer was recorded, poured away and new two litres were added. At the end of experiment all differences recorded between nutrient uptake and solution left in saucers were summed and figured as nutrient solution uptake rate (NSU).

Determination of secondary metabolites

Secondary metabolites were determined once a week. For the analyses, first fully developed leaves from the top of the plants were taken and whole leaf area without main leaf vain was used. Photosynthetically active pigment carotenoid was determined using spectrophotometer (Unicam UV/Vis Spectrometer UV2) according to Lichtenthaler and Buschmann (2001).

Total polyphenols were determined spectrophotometrically with Folin-Ciocalteu reagent as described previously (Zheng and Wang, 2001). After the extraction with 80% methanol and 5% HCl, 100 µl of each sample was filled into test cuvettes. 1 ml of Folin-Ciocalteu`s reagent was added and after five minutes 1 ml $Na_2CO_3$. The absorbance was measured after one hour at 760 nm. For the calibration gallic acid has been used. Contents of anthocyanins were measured using pH-differential method as described by Wrolstad et al. (2005) and expressed as shisonin equivalent calculated using the formula according to Meng et al. (2006). Extraction was as same as for phenols. 2x250 µl of each sample was filled into test cuvettes and mixed with potassium chloride (pH 1.0) or sodium acetate (pH 4.5), respectively. Both mixtures were measured at 520 and 720 nm. Total flavonoids were also determined spectrophotometrically according to method of Bahorun et al. (2004) using quercetin as a standard solution. After extraction with 80% methanol, 500 µl of each sample was filled into test cuvettes. 2 ml of 2% aluminium chloride was added and after 10 minutes, absorbance was measured at 367 nm.

Data evaluation

All data were evaluated with statistical software SAS. Mean values and standard deviations were calculated and analysed using ANOVA (Tukey test, significance level $P \leq 0.05$). Before analysis of variance, data were tested for normality with Shapiro-Wilk test.

## RESULTS AND DISCUSSION

In climate chambers influence of blue and red LED light could not be investigated at same time, hence both blue and red light treatment cold only be compared with respective control.

Plants cultivated under additional blue and red LED lighting were significantly lower than control (Tables 2 and 3). FM and DM were significantly higher in FT+B treatment, while FT+R treatment did not differ from the control (Tables 2 and 3). Positive influence of additional blue light on FM of *Perilla* cultivated in greenhouse was already reported by Grbic et al. (2016b). Noguchi and Amaki (2016) showed similar effects of blue light on growth and FM of Mexican mint (Lamiaceae). In their study elongation of main and lateral shoots was reduced, but FM was increased under blue light. Positive effect of supplemental blue light might be explained by its enhancing impact on amount of guard cells and stomata opening (Frecilla et al., 2000; Kang et al, 2009). Therefore, a higher $CO_2$- uptake can be achieved. In combination with more photosynthetic usefully radiation, higher biomass accumulation might be a result.

Results from the Tables 2 and 3 show also some tendencies regarding light intensity and cultivation period - smaller light intensity and longer cultivation period could increase plant height, FM, DM, and NSU.

Table 2. Plant height, fresh and dry mass, and nutrient solution uptake rate (NSU) of the *Perilla* plants after four weeks cultivation in climate chambers under fluorescent tubes (FT) and additional blue LED lighting (FT+B). Control plants were grown under PPFD of 123.29 µmol $m^{-2}$ $s^{-1}$ and plants with additional blue LEDs under PPFD of 125.62 µmol $m^{-2}$ $s^{-1}$. Values in brackets relate to respective control (100%).

| Treatment | Plant height (cm) | Fresh mass (g/plant) | Dry mass (g/plant) | NSU (l/plant) |
|---|---|---|---|---|
| Control (FT) | 51.53±1.43 a | 24.74±1.14 b | 3.91±0.25 b | 3.71±0.12 b |
| FT+B | 40.28±1.72 b (-21.82%) | 37.19±1.29 a (+50.33%) | 4.34±0.21 a (+10.85%) | 4.90±0.03 a (+31.80%) |

Different letters indicate significant difference among treatments (Tukey-test, $P \leq 0.05$, n=16)

Nutrient solution uptake rate was significantly affected by both additional lighting (Tables 2 and 3). Blue light increased it up to 31.80%, red light up to 23.56%. Sams et al. (2016) investigated impacts of light quality on nutrient uptake. In their study, additional blue and red light increased nutrient uptake in marigold plants grown in greenhouse. Higher nutrient uptake can be explained by higher needs due to the enhanced biomass formation, especially for plants cultivated under blue light. Hwang et al. (2014) investigated influence of different light intensities and cultivation periods on Perilla plants and found out that increased light intensity or longer cultivation period under increased light intensity decrease plant height.

Table 3. Plant height, fresh and dry mass, and nutrient solution uptake rate of the *Perilla* plants after three weeks cultivation in climate chambers under fluorescent tubes (FT) and additional red LED lighting (FT+R). Control plants were grown under PPFD of 177.33 μmol $m^{-2}$ $s^{-1}$ and plants with additional red LEDs under PPFD of 197.31 μmol $m^{-2}$ $s^{-1}$. Values in brackets relate to respective control (100%).

| Treatment | Plant height (cm) | Fresh mass (g/plant) | Dry mass (g/plant) | NSU (l/plant) |
|---|---|---|---|---|
| Control (FT) | 27.61±0.94 a | 15.80±1.81 | 2.32±0.26 | 1.76±0.17 b |
| FT+R | 23.00±1.12 b (-16.72%) | 18.08±2.52 (+14.46%) | 2.86±0.39 (+23.57%) | 2.18±0.23 a (+23.56) |

Different letters indicate significant difference among treatments (Tukey-test, P≤0.05, n=16)

Additional blue light showed positive influence on carotenoid concentration, but FT+R did not differ from the control (Tables 4 and 5). Sams et al. (2016) showed that even low PPFD of supplemental blue light may increase total carotenoid concentration. Key enzyme of carotenoid biosynthesis pathway is phytoene synthase. Previous studies demonstrated an enhancing effect of blue light on activity of phytoene synthase (Spurgeon, 1979; Li, 2008).

Table 4. Concentration of carotenoids (Car), polyphenols (PP), anthocyanins (Anth), and flavonoids (Fl) in fresh *Perilla* leaves after four weeks cultivation in climate chambers under fluorescent tubes (FT) and additional blue LED lighting (FT+B). Control plants were grown under PPFD of 123.29 μmol $m^{-2}$ $s^{-1}$ and plants with additional blue LEDs under PPFD of 125.31 μmol $m^{-2}$ $s^{-1}$.

| Treatment | Car (mg/100 g FM) | PP (mg GAE/100 g FM) | Anth (mg SHE/100 g FM) | Fl (mg QE/100 g FM) |
|---|---|---|---|---|
| Control | 8.78±0.40 b | 958.75±70.70 | 286.51±46.68 | 1124.12±168.11 |
| FT+B | 9.95±0.62 a (+13.37%) | 864.03±84.05 (-9.88%) | 326.97±97.02 (+14.12%) | 1057.55±27.81 (-5.92%) |

Different letters indicate significant difference among treatments (Tukey-test, P≤0.05, n=4)

Concentrations of polyphenols and anthocyanins were not influenced by additional blue and red light (Tables 4 and 5), but their absolute concentrations still correspond to those found by Müller-Waldeck et al. (2010) and Park et al. (2013).

Table 5. Concentration of carotenoids (Car), polyphenols (PP), anthocyanins (Anth), and flavonoids (Fl) in fresh *Perilla* leaves after three weeks cultivation in climate chambers under fluorescent tubes (FT) and red LED lighting (FT+R). Control plants were grown under PPFD of 177.33 $\mu molm^{-2}$ $s^{-1}$ and plants with additional red LEDs under PPFD of 197.85 $\mu molm^{-2}$ $s^{-1}$.

| Treatment | Car (mg/100 g FM) | PP (mg GAE/100 g FM) | Anth (mg SHE/100 g FM) | Fl (mg QE/100 g FM) |
|---|---|---|---|---|
| Control | 9.72±0.95 | 1027.17±182.15 | 439.38±126.61 | 902.29±47.42 b |
| FT+R | 10.01±0.35 (+2.88%) | 988.06±175.04 (-3.80%) | 435.62±121.73 (-0.86%) | 1031.65±34.21 a (+14.34%) |

Different letters indicate significant difference among treatments (Tukey-test, $P \leq 0.05$, n=4)

These concentrations were also higher than concentrations obtained in greenhouse conditions investigated by Grbic et al. (2016b). It can be assumed that controlled environmental condition lead to higher concentration of polyphenols and anthocyanins. But like anthocyanin content is also affected by temperature (Azuma et al., 2012), all growing conditions must be considered. Polyphenols and especially anthocyanins are part of a protection system to avoid oxidative damages. Supplemental light intensity provided by LEDs was moderate. Therefore, higher contents of antioxidants seem not to be necessary.

Regarding flavonoid concentration, it was significantly higher in FT+R treatment, FT+B did not show significant difference (Tables 4 and 5).

## CONCLUSION

Even though additional blue light increased total light intensity only about ~9%, FM, DM and NSU were increased up to 50.33%, 10.85% or 31.80%, respectively. These experiments showed that blue light was more suitable for obtaining more fresh and dry mass than red light. Small portion of blue light was also enough to increase carotenoid concentration, while additional red light (~6%) influenced flavonoid concentration in a positive manner. Therefore, low-energy treatments can be used to increase yields without negative impact on nutritional quality.

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**Original Scientific paper**
10.7251/AGRENG2103031G
UDC 633.1:631.8

# ENERGY EFFICIENCY OF THE MINERAL FERTILIZER APPLICATION IN CEREAL PRODUCTION

Marija GAVRILOVIĆ[1*], Aleksandra DIMITRIJEVIĆ[2], Zoran MILEUSNIĆ[2], Rajko MIODRAGOVIĆ[2], Milan UGRINOVIĆ[1], Radiša ĐORĐEVIĆ[1], Dejan CVIKIĆ[1]

[1]Institute for Vegetable Crops, Karađorđeva 71, SmederevskaPalanka, Serbia
[2]University of Belgrade - Faculty of Agriculture, Nemanjina 6, Belgrade, Serbia
[*]Corresponding author: mgavrilovic@institut-palanka.rs

## ABSTRACT

Cereal grains have represented the principal component of the human diet for thousands of years. Modern cereal production cannot be imagined without the use of mineral fertilizers, particularly in terms of better utilization of biological potential of the yield of growing plants. The aim of this study was to evaluate the energetic efficiency of wheat and barley production, with special reference to the share of the use of mineral fertilizers in total energy consumption. Input data and yield of wheat and barley fields were collected in the experimental trials in Serbia. Results showed that total energy inputs of wheat and barley fields were 22178.04 and15921.16 $MJ \cdot ha^{-1}$, respectively. Total energy outputs for wheat and barley fields were 80037.83 and 104496.08 $MJ \cdot ha^{-1}$, respectively. The results obtained indicate that mineral fertilizers claim a share of the total energy consumption in cereal production ranging from 49.19% in barley to 52.01% in wheat. Specific energy input, energy output–input ratio (energy use efficiency), energy productivity and net energy gain were 5.13 $MJ \cdot kg^{-1}$, 3.61,0.19 $kg \cdot MJ^{-1}$ and57859.79$MJ \cdot ha^{-1}$in wheat system and 2.75 $MJ \cdot kg^{-1}$, 6.56, 0.36 $kg \cdot MJ^{-1}$ and 88574.92 $MJ \cdot ha^{-1}$ in barley system, respectively. According to the results, it seems that barley production is more efficient from different aspects of energy consumption compared to wheat in the studied region. In general, production in barley fields was more sustainable than wheat production because, in view of ecological indices such as amount of energy use and renewable energy consumption, it was more environment-friendly production.

**Keywords:** *Cereal production, mineral fertilizers, energy consumption, energy efficiency.*

## INTRODUCTION

Cereal grains have represented the principal component of the human diet for thousands of years. Their processing comprises an important part of the food production chain, but it is a complex procedure (Zahid *et al*., 2010; Sahabi*et al*.,

2012). Efficient use of energy helps to achieve increased production and productivity and contributes to the economy, profitability and competitiveness of agricultural sustainability of rural communities. Modern agricultural production of cereals cannot be imagined without the use of fertilizers, particularly in terms of better utilization of biological potential of the yield of growing plants (Sãulescu*et al*., 2005; Zengin *et al*., 2009; Dawson and Hilton, 2011; Klikocka *et al*., 2019). Otherwise, the yields would be significantly reduced, regardless of the application of all other agricultural practices such as tillage and crop protection. In current agricultural practice, nutritive value of fertilizers was evaluated on the basis of their impact on crop yield increase and the possibility of improving yield quality (Ryan, 2008). However, with the advancement of all sectors, including agriculture, more and more analyses are dedicated to the energy consumption in the production and application of fertilizers. Also, attention is given exceptionally and other processes in dealing with fertilizers such as transport, storage and handling of it and that also affects on the final energy balance (Ziaei, 2015). Share of agriculture in total energy consumption in some countries is very high, up to 5%. From these values of total energy consumption, the value which is built in fertilizers goes up to 50%. This is one of the key reasons why fertilization needs additional attention and rationalization, with the aim of not only economically effective, but also environmentally friendly production (Ozkan *et al*., 2004; Alluvione *et al*., 2011; Dimitrijević *et al*., 2020).

The aim of this study was to estimate the energy input and output in wheat and barley production and to analyse the distribution of different energy input utilized in the production.With these data it was possible to evaluate the influence of the energy input through the fertilizer on overall production energy efficiency.

## MATERIALS AND METHODS

Energy consumption is defined through the energy input in wheat and barley production from the moment of soil tillage and preparation for sowing until wheat and barley grain leave the field (Roberts, 2008). This means that energy inputs for storage and post-harvest processes are not included in the energy balance calculation. The data were collected during the three-year field trials (2013/15) on the estate of PKB Corporation (Agricultural Corporation Belgrade) "7 July" Farm in Jakovo (Belgrade region, 44º43′06.42″ N; 20º15′37.68″ E, Serbia). It is situated on the left bank of the Sava river into a narrow band. Of the soil types are represented Humic Gleysols and aluvijum Dystric Fluvisols soil. Under the arable land belonging to the farm is 4011 ha that is used for crop production and animal husbandry. As for the seeding structure, the estate is oriented to wheat (535 ha), maize (1472 ha), maize hybrids (109 ha), silage maize (374 ha), barley (200 ha), soybean (553 ha), sunflower hybrids (10 ha), alfalfa (290 ha), sugar beet (400 ha) and meadows (68 ha). In this particular case presented energy investments and the yields of individual plots sown with wheat and barley, three seasons in a row.

The method used for energy efficiency analysis (Ortiz–Cañavate and Hernanz, 1999; Abrishambaf *et al*., 2019;Ghorbany *et al*., 2011) is based on the definition of directand indirect energy inputs, calculation of the energy output for given plant production. Based on these data in wheat and barley production specific energy input, energy output–input ratio, energy productivity and net energy gain were estimated as follows:

EI = EIP/Y,
ER = EOP/EIP,
EP = Y/EIP,
NEG = EOP - EIP

where: EI is the specific energy input ($MJ \cdot kg^{-1}$), EIP is the energy input in the production ($MJ \cdot ha^{-1}$), Y is yield ($kg \cdot ha^{-1}$), EOP is the energy output of the production ($MJ \cdot ha^{-1}$), EP is energy productivity ($kg \cdot MJ^{-1}$) and NEG is net energy gain ($MJ \cdot ha^{-1}$).

The energy inputs were calculated by multiplying the material input by the referent energy equivalent (Ozkan *et al*., 2004). The quantities of the material input were obtained directly from the farm managers. The input energy indices in agriculture are divided into 4 group of energy: direct energy, indirect energy, renewable energy and non-renewable energy (Wang, 2009).

The direct energy requirements are needed for land preparation, cultivation, irrigation, harvesting, post-harvest processing, food production, storage and the transport of agricultural inputs and outputs. Indirect energy needs are in the form of sequestered energy in fertilizers, herbicides, fungicides and insecticides (FAO, 2000). So energy indices divided into following groups (Mobtaker *et al*., 2010; Abdi *et al*., 2012): direct energy - human power, diesel fuel, water and electricity; indirect energy - chemicals, fertilizers, seeds and machinery; renewable energy - human power, seeds, manure fertilizers and irrigation waterand; non-renewable energy - diesel fuel, electricity, chemical fertilizers, herbicides, insecticides, fungicides and machinery.

The energy outputs are determined based on the yield of cultivated plants upon completion of the production cycle and the corresponding energy equivalents.

## RESULTS AND DISCUSSION

Energy inputs and energy outputs in wheat production are shown in Table 1. The values represent the average values of data collected within the three-year period. As it can be seen, average energy consumption was 22178.04$MJ \cdot ha^{-1}$.

Table 1. Energy consumption in wheat production

| Energy | Quantity per unit area (ha) | Total energy equivalent ($MJunit^{-1}$) | Percentage of total energy input (%) |
|---|---|---|---|
| *Input* | | | |
| Human labor (h) | 7.25 | 15.70 | 0.07 |
| Tractor (h) | 7.25 | 664.01 | 2.76 |
| Combine (h) | 0.48 | 42.36 | 0.19 |
| Transport (h) | 1.70 | 50.66 | 0.23 |
| Other Machinery (h) | 6.08 | 381.42 | 1.56 |
| Diesel fuel (l) | 87.38 | 4176.61 | 21.07 |
| Nitrogen (kg) | 174.59 | 11599.54 | 49.23 |
| Phosphate ($P_2O_5$) (kg) | 38.10 | 474.01 | 2.78 |
| Insecticides (kg or l) | 0.19 | 18.89 | 0.10 |
| Fungicides (kg or l) | 0.66 | 142.56 | 0.65 |
| Herbicides (kg or l) | 0.64 | 153.11 | 0.78 |
| Water for irrigation ($m^3$) | 0.07 | 0.07 | 0.01 |
| Seeds (kg) | 303.34 | 4459.10 | 20.57 |
| Total energy input (MJ) | | 22178.04 | 100.00 |
| *Outputs* | | | |
| Seed yield (kg) | 4321.97 | 63532.91 | 79.82 |
| Straw yield (kg) | 1320.39 | 16504.92 | 20.18 |
| Total energy output (MJ) | | 80037.83 | 100.00 |
| Energy use efficiency | | 3.61 | |

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

Based on the data given in Table 1 we see that the largest amount of energy was consumed over fertilizers.The percentage was 52.01%. The chemical composition of a plot was such that nitrogen was most needed. The fuel energy ranked second with a share of 21.07%. Seeding material within energy input had its share of 20.57% in total energy consumption. Water displayed is the water consumed trough the plant protection and therefore percentage is small. There is no irrigation. Based on the energy analysis we have the following results:

$$EI= 5.13\ MJ\cdot kg^{-1};\ ER= 3.61;\ EP= 0.19\ kg\cdot MJ^{-1};\ NEG = 57859.79 MJ\cdot ha^{-1}$$

Energy inputs and energy outputs in barley production are shown in Table 2. The values represent the average values of data collected within the three-year period. As it can be seen, average energy consumption was 15921.16 $MJ\cdot ha^{-1}$.

Table 2. Energy consumption in barley production

| Energy | Quantity per unit area (ha) | Total energy equivalent (MJunit$^{-1}$) | Percentage of total energy input (%) |
|---|---|---|---|
| *Input* | | | |
| Human labor (h) | 6.66 | 13.06 | 0.08 |
| Tractor (h) | 5.91 | 541.84 | 3.38 |
| Combine (h) | 0.85 | 74.49 | 0.46 |
| Transport (h) | 3.03 | 90.20 | 0.56 |
| Other Machinery (h) | 3.76 | 235.75 | 1.47 |
| Diesel fuel (l) | 82.40 | 3938.88 | 26.23 |
| Nitrogen (kg) | 108.75 | 7225.57 | 45.00 |
| Phosphate ($P_2O_5$) (kg) | 36.66 | 456.01 | 2.80 |
| Potassium ($K_2O$) | 19.56 | 218.13 | 1.39 |
| Insecticides (kg or l) | 0.22 | 22.60 | 0.14 |
| Fungicides (kg or l) | 0.64 | 137.52 | 0.84 |
| Herbicides (kg or l) | 0.62 | 148.02 | 0.96 |
| Water (m³) | 0.18 | 0.18 | 0.01 |
| Seeds (kg) | 191.74 | 2818.91 | 16.68 |
| Total energy input (MJ) | | 15921.16 | 100.00 |
| *Outputs* | | | |
| Seed yield (kg) | 5781.04 | 84981.29 | 86.37 |
| Straw yield (kg) | 1561.18 | 19514.79 | 13.63 |
| Total energy output (MJ) | | 104496.08 | 100.00 |
| Energy use efficiency | | 6.56 | |

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

According to the results presented in Table 2, the share of fertilizers in the energy balance is lower than in wheat production. Percentage was 49.1%. The fuel energy ranked second with a share of 26.23%. Seeding material within energy input had its share of 16.68% in total energy consumption. Water displayed is the water consumed trough the plant protection and therefore percentage is small. There is no irrigation. The analysis of energy consumption gave the following results:

$$EI= 2.75\ MJ\cdot kg^{-1};\ ER= 6.56;\ EP= 0.36\ kg\cdot MJ^{-1};\ NEG = 88574.92\ MJ\cdot ha^{-1}$$

The amount of energy efficiency of barley fields was obtained higher than wheat fields respectively 6.56 and 3.61. This means that per each unit of energy consumption in the fields of wheat and barley, 6.56 and 3.61 yield units is, respectively, achieved. The amount of energy productivity for two different crops has been reported as 0.19 for wheat and 0.36 for barley. Energy productivity is an almost better parameter in comparison to energy efficiency to compare two different crops on the two different plots from the point of the production of a plant. Because difference in energy efficiency can be due to difference in energy

input and yield, it will make it a bit difficult to judge. But energy productivity index calculates the ratio of production yield per kg into consumer energy and better shows the difference between the two crops from same plant family. The amount of specific energy and net energy in wheat production obtained was5.13 $MJ \cdot kg^{-1}$and 57859.79$MJ \cdot ha^{-1}$, respectively; in barley 2.75 $MJ \cdot kg^{-1}$and 88574.92 $MJ \cdot ha^{-1}$, respectively.

## CONCLUSIONS

Task of agricultural production is not just to produce the food. Above all, it must be a cost-effective, profitable and must meet certain environmental standards. It is necessary to constantly increase soil fertility, particularly through application of fertilizers and then watering, as well trough the creation of new varieties and hybrids of plants. We should take into account not only energy efficiency of agricultural production, but also environmental protection.

On the basis of the above research results, it can be concluded that the reduction of use of mineral fertilizers effects on the yield decline. In this case, barley production is more efficient from different aspects of energy consumption compared to wheat in the studied region. In general, production in barley fields was more sustainable than wheat production because, in view of ecological indices such as amount of energy use and renewable energy consumption, it was more environment-friendly production. The fact is that without the use of fertilizers we have not sustainable production. Of course, this practice should not be abolished, but it should be adjusted to the optimal value of application. Losses are not extremely high, production will be energy cost-effective and environmental impact will be significant in a positive way. Energy should be transfered to investments at irrigation, better machinery management technique, integrating the legume crops into the rotation and some other good agricultural practices. All of this may be the options to increase the energy use efficiency of cereal production and way to reduce the environmental pollution.

## ACKNOWLEDGEMENT

The research work was fulfilled and given paper was prepared with the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia, Agreement of the realization and financing of scientific research work of scientific research organizations in 2021. No. 451-03-9/2021-14/200216.

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**Review paper**
10.7251/AGRENG2103038E
UDC 636

# PASTORALISM AND SUSTAINABLE DEVELOPMENT IN THE MEDITERRANEAN REGION

Hamid EL BILALI*, Francesco BOTTALICO, Giovanni OTTOMANO PALMISANO, Gianluigi CARDONE, Antonia ACQUAFREDDA, Roberto CAPONE

International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Valenzano (Bari), Italy
*Corresponding author: elbilali@iamb.it

## ABSTRACT

Pastoralism has a long tradition in the Mediterranean region. However, there is little evidence about its contribution to sustainable development in the region. Therefore, this review analyses the state of research on the multifaceted relations between pastoralism and sustainable development in the Mediterranean with a particular reference to the Millennium Development Goals (MDGs) and the Sustainable Development Goals (SDGs). It draws upon a systematic review of all documents indexed in the Web of Science by June 2021. The analysis of the scholarly literature suggests that (i) there is a divide with most studies performed in Northern Mediterranean countries; (ii) no article has investigated the contribution of pastoralism to the implementation of the sustainable development agendas (MDGs and SDGs) in the Mediterranean; and (iii) most of the selected articles deal with environmental sustainability (cf. biodiversity, land use, land degradation, deforestation) while social, cultural and economic aspects are generally overlooked. The ongoing processes of intensification, specialisation and modernisation of pastoral systems do not only jeopardise the provision of various ecosystem services, but also put at risk the preservation and sustainability of traditional pastoral systems. Such modernisation also leads to the erosion of pastoral culture and the abandonment of some traditional systems such as sylvo-pastoralism and mobile pastoralism. Sustainable development of pastoralism in the Mediterranean implies improving the livelihoods and living conditions of pastoralists while preserving their unique cultural heritage and social capital and ensuring the continued provision of ecosystem systems. Such development pathway should be guided by and aligned with the SDGs.

**Keywords:** *agro-pastoralism, biodiversity, Millennium Development Goals, pasture, Sustainable Development Goals.*

## INTRODUCTION

The Mediterranean Strategy for Sustainable Development clearly shows that the development of the Mediterranean region cannot be sustainable unless the fundamental common goods are protected and regenerated (UNEP/MAP, 2005, 2016). The Mediterranean region is facing unprecedented and interdependent environmental, economic and social challenges that affect food security, health, nutrition and sustainability, and thus the livelihoods of all Mediterranean people (Lacirignola *et al.*, 2014; CIHEAM and FAO, 2015; Dernini and Capone, 2021). At the crossroads of three continents (viz. Africa, Asia and Europe), the Mediterranean is undergoing rapid and drastic changes and is expected to be among the regions most affected by climate change, with an acceleration of land degradation and desertification (MedECC, 2019). Furthermore, significant discrepancies in socio-economic development among countries, together with regional conflicts, raise more challenges for the sustainable future of the Mediterranean. Despite progress made over the last decades, Mediterranean countries face several challenges in their implementation of the 2030 Agenda for Sustainable Development (El Bilali et al., 2020; Riccaboni et al., 2020). The 2020 SDG Dashboards for the Mediterranean region (Riccaboni *et al.*, 2020) show that the Mediterranean has a general score of 73.5 (meaning that SDG targets are achieved by 73.5%); the SDG index shows better performance in Europe West than in Europe East, North Africa and Middle East.

Livestock production and pastoralism play a central role in the social, economic and environmental sustainability and results essential in the implementation of the 2030 Agenda and the Sustainable Development Goals (SDGs) (FAO, 2018a, 2018b; Serra Prieto *et al.*, 2019). Indeed, pastoralism and livestock production have strong linkages with, inter alia, SDG 1 (No poverty), SDG 2 (Zero hunger), SDG 6 (Clean water and sanitation), SDG 13 (Climate action) and SDG 15 (Life on land) (FAO, 2018a). While pastoralism is as old as the Mediterranean civilisation, the opinions about its role diverge among actors and stakeholders (El Bilali *et al.*, 2020). These diverging opinions and worldviews might be due to the absence of clear scientific evidence about the contribution of pastoralism to sustainable development in the region. Therefore, this review analyses the state of research on the multifaceted relations between pastoralism and sustainable development in the Mediterranean with a particular reference to the global development agendas viz. Millennium Development Goals (MDGs) and SDGs.

## METHODS

The article draws upon a systematic review of all documents indexed in the Web of Science (WoS). The review covers all 21 Northern, Southern and Eastern Mediterranean countries considered in the Mediterranean Strategy for Sustainable Development (UNEP/MAP, 2005, 2016). A search was performed in June 2021 using the search query: *(pastoralism OR pastoralist OR pastoral OR grazing*

*OR rangeland OR pasture) AND ("sustainable development" OR "sustainable rural development" OR "development goal") AND (Mediterranean OR "North* Africa" OR "West* Asia" OR "Balkan" OR Albania OR Algeria OR Bosnia OR Croatia OR Egypt OR France OR Greece OR Italy OR Israel OR Jordan OR Lebanon OR Libya OR Macedonia OR Malta OR Montenegro OR Morocco OR Palestine OR Portugal OR Serbia OR Slovenia OR Spain OR Syria OR Tunisia OR Turkey).* The search yielded 48 documents. Three inclusion criteria were considered: geographical coverage (viz. dealing with at least one Mediterranean country), thematic focus (viz. both pastoralism and sustainable development) and document type (viz. research articles, book chapters and conference papers; reviews were excluded). Following the analysis of titles, abstracts and full-texts, 22 documents were excluded (Table 1).

Table 1. Systematic review: Article selection process.

| Selection steps | Number of documents | Number of excluded documents and exclusion reasons |
|---|---|---|
| Search on WoS | 48 | -- |
| Screening of titles | 48 | 1 document excluded because it deals with Canada |
| Screening of abstracts | 47 | 20 documents excluded because they do not deal with the Mediterranean (2 documents), pastoralism (15) and/or sustainable development (3) |
| Scrutiny of full-texts | 27 | 1 review excluded |
| Inclusion in the systematic review | 26 | -- |

Consequently, 26 documents were selected and included in the systematic review (Table 2); the selected documents include 24 journal articles and 2 conference papers.

Table 2. List of the selected documents.

| Year | Number | References |
|---|---|---|
| 2021* | 1 | Venturi et al. (2021) |
| 2020 | 1 | Noll et al. (2020) |
| 2019 | 3 | Alary et al. (2019); Correia and Chaves (2019); Faccioni et al. (2019) |
| 2018 | 2 | Berriet-Solliec et al. (2018); Fetzel et al. (2018) |
| 2017 | 4 | Chalazas et al. (2017); Genovese et al. (2017); Salvati et al. (2017); Statuto and Picuno (2017) |
| 2016 | 4 | Bagella et al. (2016); Jouven et al. (2016); Ocak (2016); Ocak et al. (2016) |
| 2015 | 1 | Gibon et al. (2015) |
| 2013 | 1 | Pantera et al. (2013) |
| 2011 | 2 | Bauer and Bergmeier (2011); Cohen et al. (2011) |
| 2010 | 1 | Barhoumi (2010) |

| Year | Number | References |
|---|---|---|
| 2009 | 2 | Beranger (2009); Le Roux and Bouazid (2009) |
| 2003 | 1 | Marin-Yaseli and Martinez (2003) |
| 2001 | 1 | Plieninger and Wilbrand (2001) |
| 1999 | 1 | De Miguel (1999) |
| 1991 | 1 | Bencherifa and Johnson (1991) |

*As of June 2021.

## RESULTS AND DISCUSSION

The analysis of the geography of the research on the nexus between pastoralism and sustainable development in the Mediterranean region suggests that there is a research divide with more research carried out in the Northern Mediterranean countries (NMCs) with respect to Southern and Eastern Mediterranean ones (Table 3). In fact, the highest number of the selected studies was performed in Greece, France, Italy and Spain. This might imply a higher interest in pastoralism among the research communities in NMCs or, simply and merely, that research systems are more performing and have better funding in these countries, which allows them to get quality results and publish them in journals indexed on WoS (which are often paying journals). Moreover, there is no single article that addresses the relationship between pastoralism and sustainable development in the whole Mediterranean region but there are some multi-country studies that deal with both shores of the Mediterranean. For instance, Alary et al. (2019) analyse the dynamics of agro-pastoral systems in Egypt, France and Morocco. Further articles have a more global perspective and provide comparisons between Mediterranean countries and those from other world regions (Ocak *et al.*, 2016; Venturi *et al.*, 2021). For example, Venturi et al. (2021) investigate the multifunctional role of the features that characterize cultural landscapes of traditional agro-silvo-pastoral systems in the Mediterranean and Latin America viz. the *sabana de morro* in Dolores (El Salvador) and pastures with carob trees in Ragusa (Sicily, Italy).

Table 3. Geography of research on pastoralism and sustainable development.

| Country or region | Number of documents | References |
|---|---|---|
| Greece | 5 | Bauer and Bergmeier (2011); Chalazas et al. (2017); Fetzel et al. (2018); Noll et al. (2020); Pantera et al. (2013) |
| France | 4 | Beranger (2009); Berriet-Solliec et al. (2018); Gibon et al. (2015); Jouven et al. (2016) |
| Italy | 4 | Bagella et al. (2016); Faccioni et al. (2019); Genovese et al. (2017); Salvati et al. (2017) |
| Spain | 4 | Cohen et al. (2011); De Miguel (1999); Marin-Yaseli and Martinez (2003); Plieninger and Wilbrand (2001) |
| Algeria | 1 | Le Roux and Bouazid (2009) |
| Morocco | 1 | Bencherifa and Johnson (1991) |
| Portugal | 1 | Correia and Chaves (2019) |
| Tunisia | 1 | Barhoumi (2010) |

| Country or region | Number of documents | References |
|---|---|---|
| Turkey | 1 | Ocak (2016) |
| Mediterranean * | 1 | Alary et al. (2019)[1] |
| Northern Mediterranean ** | 1 | Statuto and Picuno (2017)[2] |
| Global *** | 2 | Ocak et al. (2016)[3]; Venturi et al. (2021)[4] |

*This category includes documents dealing with at least one southern/eastern Mediterranean country and one northern Mediterranean country; ** This category includes documents dealing with at least two northern Mediterranean countries; *** This category includes documents dealing with at least another country outside the Mediterranean region.
[1] Egypt, France and Morocco; [2] Italy and Montenegro; [3] Romania and Turkey; [4] El Salvador and Italy.

Most of the selected documents deal with cattle, goats and sheep but a few address other animal species such as horses (Jouven *et al.*, 2016). No article analyses the potential contribution of pastoralism to the implementation of the sustainable development agendas (viz. MDGs and SDGs) in the Mediterranean. Furthermore, studies on pastoralism in the Mediterranean region generally focus on environmental aspects while economic and socio-cultural aspects are generally overlooked.

As for environmental sustainability, most of the selected articles focus on land use and land cover changes (Plieninger and Wilbrand, 2001; Cohen *et al.*, 2011; Salvati *et al.*, 2017) as well as the effects of pastoralism on biodiversity, land and climate. Salvati et al. (2017) found that intensive grazing is one of the main drivers of land-cover changes in Tolfa-Cerite district (Northern Latium, central Italy) and a key cause of deforestation as it determined the forest conversion to pastures and shrublands. Meanwhile, Pantera et al. (2013) argue that valonia oak silvopastoral systems in Greece are being at risk of extinction because of deforestation, illegal logging, overgrazing and forest fires. However, Cohen et al. (2011) found that forest area more than doubled at the expense of non-forest habitats such as pastures and cultivated lands during the second half of the 20$^{th}$ century in the Catalan pre-Pyrenees (Spain).

Both overgrazing and abandonment of grazing can lead to environmental degradation (Noll *et al.*, 2020). Faccioni et al. (2019) show that the processes of intensification of dairy production and abandonment of summer pastures, unfold simultaneously in Alpine agroecosystems in north-eastern Italy. Referring to the Greek island of Samothrace, Noll et al. (2020) and Fetzel et al. (2018) argue that local ecosystems have been severely degraded because of decades of overgrazing by sheep and goats, as the feed demand of small ruminants covered by grazing exceeded available grazing resources. Bauer and Bergmeier (2011) argue that intensive grazing and overgrazing affected the plant communities of mountain woodlands in the western Crete (Greece) and advocate a set of exclosures in

different areas and elevations to allow their regeneration. Also, Plieninger and Wilbrand (2001) posit that livestock production intensification and traditional grazing practices abandonment threaten biodiversity within the *dehesas* agro-silvo-pastoral systems in Cuatro Lugares (Spain).

It seems that the impacts of pastoralism on ecosystems in general and biodiversity in particular depend on the pastoral system. In fact, while many scholars highlight the role of pastoralism in biodiversity loss, others point out that pastures are rich in biodiversity (De Miguel, 1999; Bagella *et al.*, 2016; Venturi *et al.*, 2021). For instance, Venturi et al. (2021) point out that traditional silvo-pastoral systems in El Salvador and Sicily (Italy) help "*creating important microhabitat for many animal and vegetal species and a network of ecological corridors*". Focusing on Central Anatolia (Turkey), Ocak (2016) and Ocak et al. (2016) suggest that by practicing agro-ecological principles over millennia, transhumant pastoralists have helped to shape a complex mosaic of habitats, reduce erosion, improve soil quality and deter the likelihood of forest fires. Also, De Miguel (1999) argues that traditional agro-sylvo-pastoral systems in Spain (*caserios* and *dehesas*) are important in the maintenance of biological diversity as a result of the continued optimization of natural resources management. Furthermore, pastoralism is important for the preservation of High Nature Value (HNV) farmland (Genovese *et al.*, 2017; Noll *et al.*, 2020). Correia and Chaves (2019) suggest that adaptive land management (e.g. rotational grazing system) can contribute to solving some environmental concerns related to livestock production and can regenerate and provide greater resilience to pastoral ecosystems.

Pastoral, agro-pastoral and silvo-pastoral systems provide different ecosystem services (Gibon *et al.*, 2015; Ocak *et al.*, 2016). According to Ocak et al. (2016), ecosystem services provided by Turkish transhumant livestock systems include biodiversity preservation as well as carbon sequestration and $CO_2$ emissions reduction to mitigate climate change. Likewise, Gibon et al. (2015) argue that extensive livestock systems have allowed the preservation of numerous ecosystem services in the Pyrenees National Park (France) and point out that "*The most common strategy seeks the long-term preservation of the different types of services (production, regulation, and cultural) provided by semi-natural ecosystems, from the field level to the landscape level*" (p. 305). The valuation of ecosystem services (Faccioni *et al.*, 2019) is at the interface between environmental sustainability and economic sustainability. In this respect, Faccioni et al. (2019) argue that local stakeholders in north-eastern Italy value positively the services provided by the mountain dairy livestock systems (especially regulation services cf. water quality) and the total economic value of ecosystem services clearly exceeded current expenditure on agro-environmental programmes. However, Chalazas et al. (2017) found that nutrient mitigation capacity is exceeded in a grazed area draining to the Gulf of Kalloni (Lesvos, Greece) with a compensation cost of millions dollars over a 20-year period.

Social and, especially, cultural issues are rarely addressed in studies on pastoralism in the Mediterranean. In this context, Venturi et al. (2021) argue that "*Despite*

*many researches on traditional agro-silvo-pastoral systems tend to focus only on land uses and land use changes, is also important to analyse the different features that characterize cultural landscapes, as well as to produce detailed spatial maps, in order to preserve and valorise these systems as a whole*". Indeed, it is important to recognise and valorise the multifunctional role of traditional pastoral systems such as *sabana de morro* (El Salvador) and Sicilian pastures with carob trees (Italy) (Venturi *et al.*, 2021).

The literature also underlines the paramount importance of local and traditional knowledge in the preservation of pastoralism and its transmission from a generation to another (Ocak, 2016). Bencherifa and Johnson (1991) warn that the cultural determinants behind indigenous resource-use practices (e.g. terracing, mobile pastoralism) do no longer exist in the Moroccan Middle Atlas mountains and natural resources are endangered by the new agro-pastoral patterns. Evidence from the area of Sefiane in Algeria (Le Roux and Bouazid, 2009) shows that the awareness of the community about the impact of desertification and land degradation led them to have a strong desire to be assisted in the development of environmental education initiatives. Such initiatives can combine the existing local, traditional knowledge with scientific knowledge to achieve a better understanding of the complexity of pastoral socio-ecological systems and suggest ways to ensure the sustainable development of pastoral communities.

Migration from pastoral areas can determine labour shortage. For instance, Fetzel et al. (2018) argue that large-scale migration from the Greek island of Samothraki to Germany in the 1950s and 60s caused a lack of labour power, which, in turn, hinders the capacity of local livestock breeders to apply adequate management practices to halt or reverse land degradation. This clearly shows the strong linkages between social dynamics and environmental degradation, especially in remote and isolated territories such as islands.

Studies also highlight trade-offs between the sustainability dimensions. For instance, Alary et al. (2019) suggest that there is an antagonism between social vulnerability and ecological efficiency as "*Crop and livestock integration reduced the risk of biodiversity loss and low environmental efficiency observed in specialized systems, but mixed systems were more socially vulnerable*" (p. 40). Likewise, Berriet-Solliec et al. (2018) identify and analyse trade-offs between short-term agricultural economic strategies of some livestock farmers and long-term environmental and social benefits produced by agro-pastoral systems in the Cevennes National Park (France). Beranger (2009) point to the opposing requirements of productivity and the respect of nature and its diversity, and argue that "*The correct utilization of the permanent pastures and the acknowledgement of the multi-functionality of grasslands are part of the sustainable development and of the protection of the environment*" (p. 465).

Some articles analysed the relationships, antagonistic or synergistic, between tourism and pastoralism in Mediterranean countries (Marin-Yaseli and Martinez, 2003; Genovese *et al.*, 2017; Statuto and Picuno, 2017). Marin-Yaseli and Martinez (2003) argue that the current model of tourism development represents

serious problems in terms of sustainable development as it had negative impacts on extensive livestock farming in Upper Esera (Spanish Central Pyrenees). In fact, tourism development determined a drop in livestock farming (decrease in livestock population and farms). This is due to the competition of tourism for labour and fertile land. The abandonment of pastures, in turn, led to a decrease in landscape diversity and an increase in fire hazard and soil erosion. However, using the example of the Lanzo Valleys, an alpine mountain region in north-western Italy, Genovese et al. (2017) suggest that pasture-based livestock farming systems (PLSFS) and tourism can coexist and put that a system of collaboration between firms and institutions may represent a strong network, able to foster sustainable development for the territory so that "*environment and cultural heritage may be preserved, as well as the economic perspective of farms reinforced, while the PLSFS could become more attractive for the tourism phenomenon*". Similarly, Statuto and Picuno (2017) suggest that the valorisation of vernacular farm buildings in areas of mountain pasture for summer cattle grazing can favour the sustainable development of rural tourism in mountain areas of southern Italy and Montenegro.

The literature also points out the importance of synergies in the sustainability of pastoralism in the Mediterranean area. For instance, Ocak (2016) suggests that there is "*a clear link between social and ecological resilience emphasizing that sustainable development relies on the interconnectedness between biological and cultural diversity*" (p. 439) in the case of mobile pastoralism in Central Anatolia (Turkey). Further elaborating on the necessary synergies in the Catalan pre-Pyrenees (Spain), Cohen et al. (2011) put that "*The sustainable development of this territory should make the objectives of conservation, biodiversity and landscape protection and the preservation of their Mediterranean features compatible, and support agricultural activities that will contribute to this biological diversity and cultural identity*" (p. 79).

Mediterranean livestock farming and pastoral systems have evolved to adapt to pressures such as climate change, demographic growth and urbanization as well as greater competition for land and water resources. In their analysis of the pathways for integrated crop-livestock systems in southern (Egypt, Morocco) and northern (France) Mediterranean countries, Alary et al. (2019) identified two main trends: a centrifugal trend of specialization and a centripetal trend of diversification. Both pathways are affected by the gradient of socio-ecological contexts and the availability of natural resources. The specialization trend is towards cash crops or dairy herds in favourable areas and pastoral systems for meat production in harsher environments. Meanwhile, the diversification trend is based on mixed crop-livestock systems in irrigated areas and agro-pastoral livestock-crop systems in intermediate rain-fed areas.

Some papers highlight the importance of policies in determining not only the environmental sustainability of pastoralism but also the socio-economic sustainability of the livelihoods of pastoral communities (Alary *et al.*, 2019; Noll *et al.*, 2020). In this regard, Noll et al. (2020) put that "*The regional implementation*

*of CAP (Common Agricultural Policy) continues to support excessively high animal numbers, while farmers are highly dependent on subsidies and find themselves in an economic deadlock*" in the island of Samothrace (Greece). Indeed, in the European Union (EU), subsidies play a paramount role in livestock farmers' income but they also determine their financial dependency which can increase their economic vulnerability. Alary et al. (2019) call for dedicated rural development policies that favour the diversification as a lever for sustainable development, take advantage of spatial mobility abilities of livestock farmers, promote collective actions, develop higher value added product chains, while halting or reversing land fragmentation and degradation.

Sustainable development also implies the adoption of inclusive governance arrangements and participatory processes that ensure the active involvement and participation of pastoral communities in the development of their territories. However, experience from north-western Tunisia (Barhoumi, 2010) shows that the leading role played by the public administration in designing and implementing development projects and the population's expectations are among the main difficulties faced when applying participatory approaches.

## CONCLUSIONS

This paper explores how the scholarly literature indexed in WoS addresses the relationships between pastoralism and sustainable development in the Mediterranean. The analysis of the scholarly literature suggests that there is a divide with most studies performed in Northern Mediterranean countries (e.g. Greece, France, Italy and Spain). Furthermore, no article analysed the contribution of pastoralism to the implementation of the sustainable development agendas (viz. MDGs and SDGs) in the region. The analysis also shows that most of the selected articles deal with environmental sustainability (cf. biodiversity, land use, land degradation, deforestation) while social, cultural and economic aspects are generally overlooked. As for environmental sustainability, most of the selected documents focus on land use and land cover changes as well as the effects of pastoralism on biodiversity, land and climate.

Pastoralism is important for sustainable development not only from an environmental viewpoint, as a provider of different ecosystem services, but also from economic and socio-cultural standpoints. Pastoralism contributes to local economies and is central in the livelihoods of many communities in remote, mountainous Mediterranean territories. Pastoralism is also an important asset for developing different income-generating activities. Indeed, pastoralism-related culture, traditions and landscape are important in many rural tourism destinations. Pastoralism in the Mediterranean has been undergoing a deep transformation with social, economic, and ecological changes thus shaping the development of rural territories, especially remote and mountainous ones. The ongoing processes of intensification, specialisation and modernisation of pastoral systems (cf. agro-pastoral, sylvo-pastoral and agro-sylvo-pastoral systems) not only jeopardise the provision of various ecosystem services but also put at risk the

preservation and sustainability of traditional pastoral systems. In fact, these processes lead to an increase in stocking rates which exacerbates the pressure on the natural resources and determines overgrazing and/or grazing abandonment with an increase in reliance on purchased feed as well as disturbance of natural landscapes (cf. extension of shrubland). Such modernisation also leads to pastoral culture erosion and the abandonment of traditional systems such as sylvo-pastoralism and mobile pastoralism (cf. transhumance).

The sustainable development of pastoralism in the Mediterranean region implies halting and reversing biodiversity loss and land degradation, and mitigating climate change while ensuring the livelihoods and preserving the cultural identity and social fabric of pastoral communities. This implies improving the livelihoods and living conditions of pastoralists while preserving their unique cultural heritage and social capital and ensuring the continued provision of various ecosystem systems. Such a development pathway should be guided by and aligned with the principles and philosophy of the SDGs. In this context, more, Mediterranean-wide studies are necessary to better understand and operationalise the relationships between the development of sustainable pastoralism and the achievement of the SGDs in the Mediterranean.

The main limitation of the present research was the choice of the search database. The use of WoS means that the present article considers only quality scholarly literature published in journals with impact factor (cf. Science Citation Index Expanded, Social Sciences Citation Index) or that are under evaluation to get impact factor (cf. Emerging Sources Citation Index) as well as the proceedings of some conferences (cf. Conference Proceedings Citation Index – Science) and some books. This implies that articles published in journals, books and conference proceedings that are not indexed in WoS were not considered. Similarly, the grey literature (e.g. reports) was not considered in this paper.

## ACKNOWLEDGEMENT

This work is financed by the Italian Ministry of Education, Universities and Research (MIUR) in the framework of PACTORES (Pastoral ACTORs, Ecosystem services and Society as key elements of agro-pastoral systems in the Mediterranean) project (http://www.pactores.eu) within ERANET-MED program (project code: ERANETMED2-72-303).

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**Original Scientific paper**
10.7251/AGRENG2103051P
UDC 637.433

# QUALITY OF TABLE EGGS OF WHITE AND BROWN SHELL

Lidija PERIĆ*, Mirjana ĐUKIĆ STOJČIĆ, Sava SPIRIDONOVIĆ

University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Novi Sad, Serbia
Corresponding author: lidija.peric@stocarstvo.edu.rs

## ABSTRACT

Since the white shell eggs are present in the domestic market, it is necessary to examine their quality in order for consumers to be adequately informed, especially because they have certain prejudices against white shell eggs. The purpose of this study was to examine the effects of genotype (brown or white layers) on the table eggs quality after storage of 28 days. Total of 60 eggs from two strains of laying hens (Hisex brown and Hisex white) were sampled and the first set of 15 eggs were examined first day after laying. The second set of 15 eggs per strain was stored in a refrigerator (4°C) and examined after storage period of 28 days. The following egg quality parameters were evaluated: egg weight, shell breaking force, albumen height, Haugh units (HU) and yolk color. The results showed no significant differences in egg quality parameters between white and brown shell eggs neither in fresh eggs nor after storage. However, in both strains the storage significantly affected the albumen height (6.34 vs. 5.46 mm in brown eggs; 6.74 vs. 5.64 mm in white eggs) and HU (76.87 vs.70.40 in brown eggs; 79.11 vs. 71.44 in white eggs). pH values of albumen were not significantly affected by storage (9.14 vs.9.35 in brown eggs; 9.37 vs. 9.42 in white eggs). The results suggest that the albumen height and the HU significantly decreased during storage in both white and brown shell eggs.

**Key words:** *Brown eggs, White eggs, Storage, Quality.*

## INTRODUCTION

Egg quality is very important issue for consumers because eggs are one of the most valuable foods available to humans and they are less expensive than other equivalent animal protein sources (Windhorst, 2006). Eggs are rich in proteins and minerals, containing many essential amino acids with important biological values (Abeyrathne *et al*., 2013) and consumers are very interested in their freshness, quality, and chemical composition. Besides that, shell quality of eggs is of major importance to the egg industry worldwide.

The internal quality of eggs depends on several factors such as strain of hens (Silversides and Scott, 2001; Samli *et al.*, 2005), nutrition (Franchini *et al*., 2002*)*, hen age (Silversides and Scott, 2001; Đukić Stojčić *et al*., 2017), and storage

duration (Roberts, 2004; Jin *et al*., 2011*)*. Storage is one of the most important factors that affect egg quality, especially the albumen quality (Samli et al., 2005). After a longer storage in the refrigerator, table eggs are losing their quality, and this process depends on the duration of the storage period (Jin *et al.*, 2011; Đukić Stojčić and Perić, 2018), temperature of storing (Akter *et al.*, 2014) the age of laying hens (Perić *et al.*, 2018) and genetic factors (Silversides and Scott, 2001; Hanusova *et al.*, 2015)

One of the very important issues for the consumers is the color of the shell. In general, the fact is that eggshell colour has always received more attention from the consumer than it deserves (Hanusova *et al.*, 2015). The fact is that eggshell color does not give an indication of the egg quality. White eggs are produced commercially by lines derived from the White Leghorn breed, whereas brown eggs are produced by hens derived from several dual-purpose breeds (Barred Plymouth Rock, Rhode Island Red, New Hampshire, and others). However, in some parts of the world brown eggs have been perceived by the consumer to be more natural or healthy than white eggs (Scott and Silversides, 2000). In Serbia, as well as in the surrounding countries, brown shell eggs are predominantly represented on the market. Considering that white shell eggs are also appearing more and more on the market, it is necessary to examine their quality for consumers to be adequately informed, especially because they have certain prejudices against white shell eggs.

The purpose of this study was to examine the effects of genotype (brown or white strain layers) on the table eggs quality after storage of 28 days.

## MATERIAL AND METHODS

Total of 60 eggs from two strains of laying hens (Hisex brown and Hisex white) were sampled on the commercial farm. Both strains were of the same age (47 weeks) and the laying hens were fed according to the nutrient requirements suggested in the Hisex white and Hisex brown Management Guide.

The first set of 15 eggs were examined first day after laying. The second set of 15 eggs per strain was stored in a refrigerator (4°C) and examined after storage period of 28 days. The egg quality analysis was performed at the Laboratory for Poultry Meat and Egg Quality, the Department of Animal Science, the Faculty of Agriculture in Novi Sad. The following egg quality parameters were evaluated: egg weight, shell breaking force, albumen height, Haugh units (HU), yolk color, pH of yolk and albumen. The egg weight was measured using a precision scale (0.01 g). Shell breaking force was determined by Egg Force Reader (Orka Food Technology Ltd, Israel). Yolk color was determined using the Roche yolk color fan. Albumen height was measured with a tripod micrometer. Based on egg mass (M) and albumen height (H), Haugh units were calculated according to formula HU=100log (H+7.57-1,7M0.37).

Statistical analyses were done in program STATISTICA (TIBCO, v. 14) using factorial ANOVA. When the effect of the main factor was significant, the means were separated using Duncan's test. Probabilities of less than 0.05 were considered significant for all analyses.

## RESULTS AND DISCUSSION

Effects of the strain of hens and storage on the external egg quality are shown in the table 1.

Table 1. Effects of strain and storage on external egg quality traits

| Strain | Storage time, day | Egg weight, g | Egg weight loss, % | Shell breaking force, (kg) |
|---|---|---|---|---|
| Hisex white | 0 | 65.14 | - | 4.85 |
| | 28 | 63.58 | 2.39 | 4.96 |
| | SEM | 0.530 | 0.231 | 0.290 |
| Hisex brown | 0 | 65.37 | - | 4.91 |
| | 28 | 63.64 | 2.46 | 4.93 |
| | SEM | 0.478 | 0.232 | 0.256 |
| Source of variation | | | | |
| Strain | p value | 0.255 | 0.666 | 0.927 |
| Storage | | 0.135 | <0.01 | 0.758 |
| Interaction | | 0.160 | 0.666 | 0.989 |

SEM – standard error of means

Regarding the egg weight, the results of this research showed no differences between white and brown eggs although other authors reported significant effect of the strain on the egg size. Silversides and Scott (2001) found that eggs from ISA Brown were larger than eggs from ISA-White. Larger egg size in brown hens compared to white hens was also reported by Vits *et al.* (2005) and Joubrane *et al.* (2019). These authors assumed that genotype has a direct influence on egg weight and other egg characteristics.

Significant effect of storage on egg weight loss was found in both strains. However, there was no significant difference between weight loss during storage between white and brown shelled eggs. The effect of storage on egg weight loss was also confirmed by Akter *et al.* (2014), Đukić Stojčić *et al.*, (2017) and Perić *et al.* (2018). The main factors which affect egg weight loss are time of storage and temperature of storage (Akter *et al.*, 2014). Weight loss during storage occurs due to loss of solvents (water and other gaseous products) from the egg content through the shell by evaporation so with increase length of storage, egg weight loss increase (Hasan and Okur, 2009). Shell strength was under no influence neither of strain nor the storage which is in line with the results reported by Jones and Musgrove (2005). Joubrane *et al.* (2019) found no significant differences in shell quality between brown and white shell eggs. Contrary to our results, Đukić Stojčić *et al.* (2017) found that shell strength significantly decreased during storage.

Table 2. Effects of strain and storage on internal egg quality traits

| Strain | Storage time, day | Albumen height, (mm) | Haugh units, (HU) | Yolk color | pH of yolk | pH of albumen |
|---|---|---|---|---|---|---|
| Hisex white | 0 | $6.74^{A}$ | $79.11^{A}$ | 11.73 | $6.83^{B}$ | 9.37 |
| | 28 | $5.64^{B}$ | $71.44^{B}$ | 12.21 | $7.14^{A}$ | 9.42 |
| | SEM | 0.181 | 1.388 | 0.255 | 0.095 | 0.02 |
| Hisex brown | 0 | $6.34^{A}$ | $76.87^{A}$ | 12.00 | $6.73^{B}$ | 9.14 |
| | 28 | $5.46^{B}$ | $69.41^{B}$ | 12.53 | $6.99^{AB}$ | 9.35 |
| | SEM | 0.186 | 1.594 | 0.229 | 0.068 | 0.085 |
| Source of variation | | | | | | |
| Strain | p value | 0.208 | 0.256 | 0.396 | 0.175 | 0.141 |
| Storage | | <0.01 | <0.01 | 0.144 | <0.01 | 0.409 |
| Interaction | | 0.633 | 0.958 | 0.939 | 0.773 | 0.217 |

$^{A-B}$ Different letters indicate significant differences between the means in each column ($P < 0.01$)
SEM – standard error of means

Albumen quality is a standard measure of egg quality that is most often measured as the height of the inner thick albumen or a function of this, such as the Haugh unit. In our research albumen height was not significantly affected by the strain of the hen. Similarly, Joubrane et al. (2019) showed that no significant differences in albumen height were observed between brown and white eggs. On the other hand, a ssignificant decrease in albumen height during storage was found in both white and brown shell eggs. Similar results were reported by Đukić Stojčić *et al.* (2017) who found that albumen height was approximately 3 mm higher in initial sampling than after 28 days of storage. These results agree with those of Scott and Silversides (2000) and Samli *et al.* (2005). These authors established significant decrease in albumen height during the prolonged storage of eggs.

Decrease in albumen height was also reflected in the decrease of Haugh units during storage. The primary cause of the decrease in Haugh units during storage is the loss of water and carbon-dioxide from the egg white during the storage period. Therefore, the egg mass loss and the decrease in the egg white quality took place (Samli *et al.*, 2005, Scott and Silverside, 2000; Akyurek and Okur, 2009).

In our research (Table 2) the yolk color was not significantly influenced by strain and storage time. On the contrary, Joubrane et al. (2019) found significant differences in yolk color between white and brown shell eggs. In their research yolk color score was higher in brown eggs, but authors emphasized that the primary determinant of yolk color is the xanthophyll (plant pigment) content of the diet consumed. Regarding the storage Perić *et al*. (2018) reported a significant reduction in yolk color during storage in both young and old flocks of laying hens. According to Santos *et al.* (2019) color changes in yolks are caused by the degradation of carotenoids by oxidative processes because of water diffusion from

albumen into yolks under conditions of longer storage periods and higher storage temperatures. Jin *et al.* (2011) determined significant changes in yolk color after only two days of storage under the temperature of 29$^{o}$ C. Carranco-Jauregui et al. (2006) also determined that the changes in yolk color occurred under high temperatures of storage (20$^{o}$C), but under lower temperatures (4$^{o}$C) no changes in yolk color occurred even after 30 days.

pH of albumen was not affected by treatments, but pH of yolk was significantly affected by storage ($P<0.01$). In research of Silversides and Scott (2001) the pH of the albumen was not different between white and brown strains, but it increased with time in storage. Samli *et al.* (2005) found significant increases in pH of albumen and yolk with increased storage time and temperature. Authors reported a rapid alkalinity increase in albumen, even after 2 days of storage time, regardless of temperature. It is interesting that the increase in pH observed in yolk was not as large as in albumen.

## CONCLUSIONS

According to the results of this trial it can be concluded that no significant differences in egg quality parameters between white and brown shell eggs were established, neither in fresh eggs nor after storage. However, the storage of eggs negatively affected egg quality in both strains. The albumen height and Haugh units which were significantly lower after 28 days of storage. pH values of albumen were not significantly affected by storage, but pH of yolk was significantly increased after 28 days of storage. The results confirmed that the color of the shell has no influence on the egg quality traits either in fresh eggs or after storage.

## ACKNOWLEDGEMENT

This work is supported and financed by the Ministry of Science and Technological Development, Republic of Serbia (Contract No. 451-03-9/2021-14/200117).

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**Original Scientific paper**
10.7251/AGRENG2103057I
UDC 631.563:633.49

# THE USE OF POTASSIUM SALTS UNIQUE PROPERTIES FOR SUPPRESSION OF POTATO PATHOGENS DURING STORAGE PERIOD

Alexey G. ISAEVICH, Alexander Y. MAKSIMOV, Konstantin N. KORLYAKOV*, Dmitriy A. POSPELOV, Lubov' G. TSEMA, Anna L. LATYPOVA, Nikita V. ZUBOV[1]

Perm Federal Research Center Ural Brunch Russian Academy of Sciences, Perm, Russian Federation
*Corresponding author: korlyakovkn@rambler.ru

## ABSTRACT

Natural sylvinite has the property to produce light air ions due to air molecules contact with potassium and sodium cations. Such environment has the ability to inhibit the growth of potato pathogens. In given report the results of different methods of medium saturation with light negative ions are presented. The studied treatments were following: the surface from bulk sylvinite; aerosol generator (blowing air through salt filters). The filters with lumpy sylvinite filling and special pills from pressed sylvinite were used. Various modes of airing regimes were evaluated – three and six hours a day. The safety of tubers for the storage period, as well as the qualitative set of potato pathogens were assessed. The least number of infected tubers was noted in treatments with "pill"salt filter with three hours ventilation regime and the surface from bulk sylvinite (0.56 and 0.57%, respectively), the largest number of diseased tubers - in the control variant - 1.18%. The smallest mass loss of tubers in the period from January 29 to April 14 was recorded in the treatment with pill salt filter with with three hours ventilation regime - 1.53%, the maximum loss - when using a surface from bulk sylvinite - 2.9%. After studies in typical storages equipped with standard ventilation systems, a technology for potato storage, based on the creation favorable air environment saturated with negative air ions and saline aerosol, will be developed.

**Key words:** *potato storage, sylvinite, air ions, salt aerosol, potato pathogens.*

## INTRODUCTION

Plant protection and preservation of the grown yield are urgent problems of agricultural production, as well as minimizing storage losses, which can reach 30-50 percent (Makarova et.al., 2017). A distinctive feature of the potato storage period is its long duration (7-8 months). During this time, complex biochemical processes take place in the tubers, pathogenic microorganisms often develop in the potato mound, tubers of some varieties begin to germinate already in the middle of

the main storage period. Application of different chemical preparations including pesticides, inhibitors etc., should be restricted. The development of systems and measures aimed to grow environmentally friendly products and to reduce the pesticide load on biocenoses has been intensified in recent years due to tightening environmental requirements for agricultural production. Some research works (Sharav'iev et al., 2014) showed the positive effect of ozonizer use on potato tubers safety during storage. The use of bactericidal properties of the air contacting with natural potassium salts is considered to be one of the most promising technologies in potato storage (Nakaryakov, 2017). One of the largest deposits of potassium, magnesium and sodium salts, located in Russia - Verhnekamskoe, contains a third of the world's reserves, which are mainly used for potassium fertilizers production. However, the unique physical and chemical properties of these salts, can significantly expand their use. Natural sylvinite has the property to produce light air ions due to air molecules contact with potassium and sodium cations. Such environment has the ability to inhibit the growth of potato pathogens (Krasnoshtein et.al., 2008). Preliminary studies fulfilled in Perm Federal Research Center showed that microorganisms colonies number in the air medium saturated with light negative ions and salt aerosol decreased by 13-25% compared with the control ( Shalimov, et al., 2019 ).

In this regard, the purpose of this research is to study the impact of these factors on the microclimate of the storage, the ability to inhibit the development of the main pathogens during potato storage, preservation of potato tubers during storage and to test different methods of medium saturation with salt aerosol and light negative ions.

## MATERIALS AND METHODS

In the fall of 2020, a batch of seed potato (variety Gornyak) was placed to typical storage area of 280 $m^2$. The storage was equipped with active ventilation system and thermal insulation (6 cm layer of polyurethane spraying). The experiment with potato storing was fulfilled in six isolated laboratory modules (six treatments) area of 12 $m^2$ each, installed in the potato storage.

Experiment scheme:

Treatment 1 - control (storage of seed potato, without potassium salts use);

Treatment 2 - ozonizer use ( airing regime: two times a week, three hours a day);

Treatment 3 - aerosol generator (blowing air through "pill"salt filter and the subsequent infiltration of the aerosol through the potato mass.), daily, six hours a day

Treatment 4 - aerosol generator - ( "pill" salt filter), daily, three hours a day

Treatment 5 - aerosol generator (filter with lumpy sylvinite filling), daily, six hours a day

Treatment 6 – the use of surface from bulk sylvinite.

16 nets with 30 kg potato tubers were stacked in 4 rows on pallets in every laboratory modules. According Standard GOST R 51 706-2001 and Sharav'iev et al., (2014), ozonizer use in storage facilities limited to two times a week. In

Treatment 6 bulk sylvinite was placed on special pallets around the pallet with potato. The microclimate regime in the storehouse (temperature, relative air humidity) was monitored with a "Testo" temperature and humidity meter, $CO_2$ concentration was determined by gas analyzer "Testo", the concentration of aeroions - by "Sapphire-3M" air ion register. The microclimate parameters were recorded daily in the "Microclimate control journal ".

Preservation capacity of potato during storage was evaluated on five-point scale according national standard of variety testing. The counting of the natural loss of tubers mass during the storage was carried out by weighing the entire batch of tubers in all treatments at the beginning and in the end of the storage. The tubers biochemical content was determined during storage, including: starch (by polarimetric method), dry matter (by weight method), nitrates (potentiometric according national standard GOST 13496.19-2015). Tubers for analyzes were sampled from the surface and from the depth of the stack - 10 pieces from every variant for each analysis. Sampling was carried out monthly. The following potato diseases were taken into account: *Phytophthora infestans, Phoma exiqua, Rhizoctonia solani, Pseudomonas solanacearum, Corynebacterium sepedonicum, Fusarium spp., Streptomyces spp.* The phytopathogens determination in the infected material was carried out according to morphological characteristics and microscopy observation data. Phytopathogen cultures were isolated by direct seeding on selective media (potato agar and Chapek's medium) from potato tubers with characteristic signs of diseases.

Biochemical analyzes were carried out in analytical laboratory of Perm Agricultural Research Institute, potato pathogens species – in the Institute of Ecology and Genetics of Microorganisms, both - the divisions of PFRC. Data processing included analysis of variance using the program SPSS (v.18).

## RESULTS AND DISCUSSION

The experiments on the effect of the air medium contacting with natural potassium salts on potato storing in special climatic chamber were started in 2017 ( Shalimov, et al., 2019 ) and continued in 2020 in typical storage. In particular, at this stage of research, the use of various methods of saturating the air medium with negative air ions and salt aerosol was studied.

The results of light air ions estimation in storage modules with different methods of medium saturation with light negative ions are presented on the Figure 1.

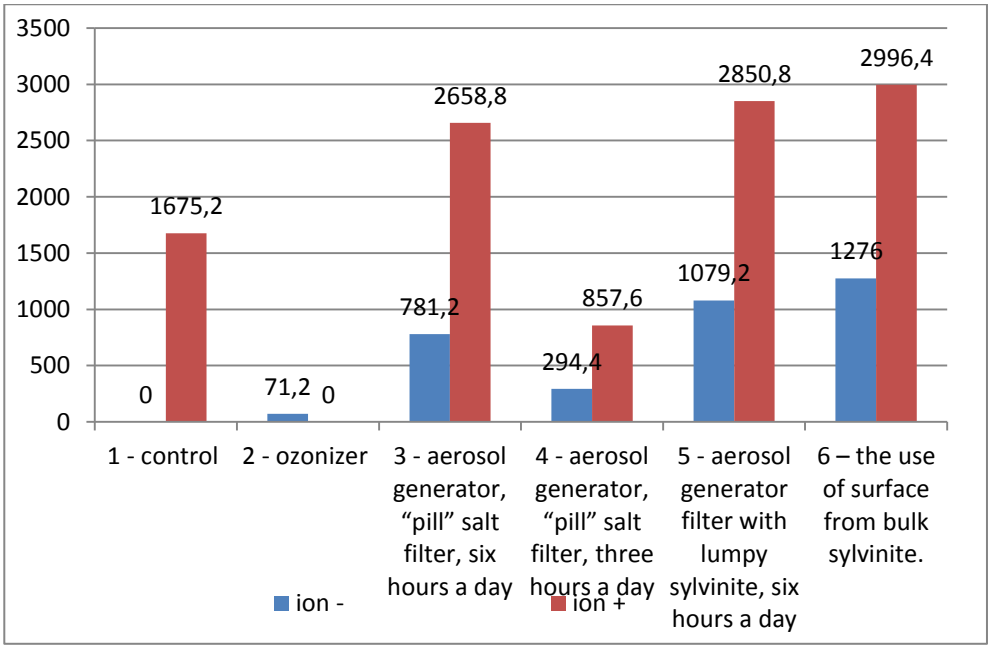


Figure 1. Results of light air ions estimation.

Comparing the measurement results, it can be noted that in all cases the number of positive air ions prevails over negative. Given fact needs an explanation. The process of ions formation is mainly due to the presence in the salts of the potassium isotope K-40, possessing radioactivity (beta and gamma radiation). Ionizing radiation, interacting with air molecules, "knocks out" electrons from them. A positive charge remains on the molecule, while the electron is captured by the electron-acceptor molecule and charges it negatively. $N_2$ is usually taken as the basis for the positive ion, and $O_2$ - for the negative ion. Taking into account the real gases content in the atmosphere ( nitrogen 78%, oxygen 21%) the greater probability of the meeting of an ionizing particle or a quantum of radiation with a nitrogen molecule take place. Faynburg et al., 2008). Herewith, the radiation influence of natural potassium salts to humans does not exceed the corresponding allowable limits regulated by national Sanitary Rules (SR 2.6.1.758-99) which is due to the fact that potassium isotope content in the total mass of potassium is about 0.012 percent. The maximum amount of negative air ions and ions total amount was recorded in storage with bulk surface - 4272 per $cm^3$, then in treatment with salt filter with lumpy sylvinite (with air supply for six hours) - 3930 ions per $cm^3$. The use of "pill" salt filter and air supply for six hours ensured the concentration 3440 e / $cm^3$. A similar filter, but with air supply for three hours, provided 1152 e / $cm^3$. The open sylvinite surface allows reaching the maximum values of light air ions in the atmosphere of the storage facility; however, from a practical point of view, it is more convenient to use salt filters. This is due to the fact that the relative air humidity in potato storages usually 90–95%, while the

critical air humidity (at which the dissolution of the mineral begins) for sylvinite is 70%. Thus, there is a risk that any sylvinite surface with a moisture content exceeding the critical value will be subject to partial dissolution.

Experiments with a sylvinite bulk surface showed that moisture accumulated on the floor in the test module, which was periodically removed. The use of salt filters avoids these risks. In addition, salt filters with compressed "pills" will allow changing the concentration of light air ions in the atmosphere by changing the pills composition, adding or reducing the amount of the component with K-40 presence.

All the necessary storage periods (treatment period, cooling, basic period, spring period) were maintained The microclimate formed in the experimental stands had insignificant differences among variants, with exception of relative air humidity (RAH).

The air temperature during the treatment period was 15°C, during the cooling - 11.4-10° C. In the basic period it changed by months, in October - 6.5C, in November - dropped from 6.0 to 2.7°C, in spring- increased from 4.3 to 18˚C. The minimum temperature was observed from December to March, inclusive, and amounted to 2.3-3.7°C. With outside temperature rise, the temperature rise inside the storage was noted. In April, the air temperature was 4.3-7.3 ˚C. In the first decades of May, the maximum temperature was 11-18 ˚C.

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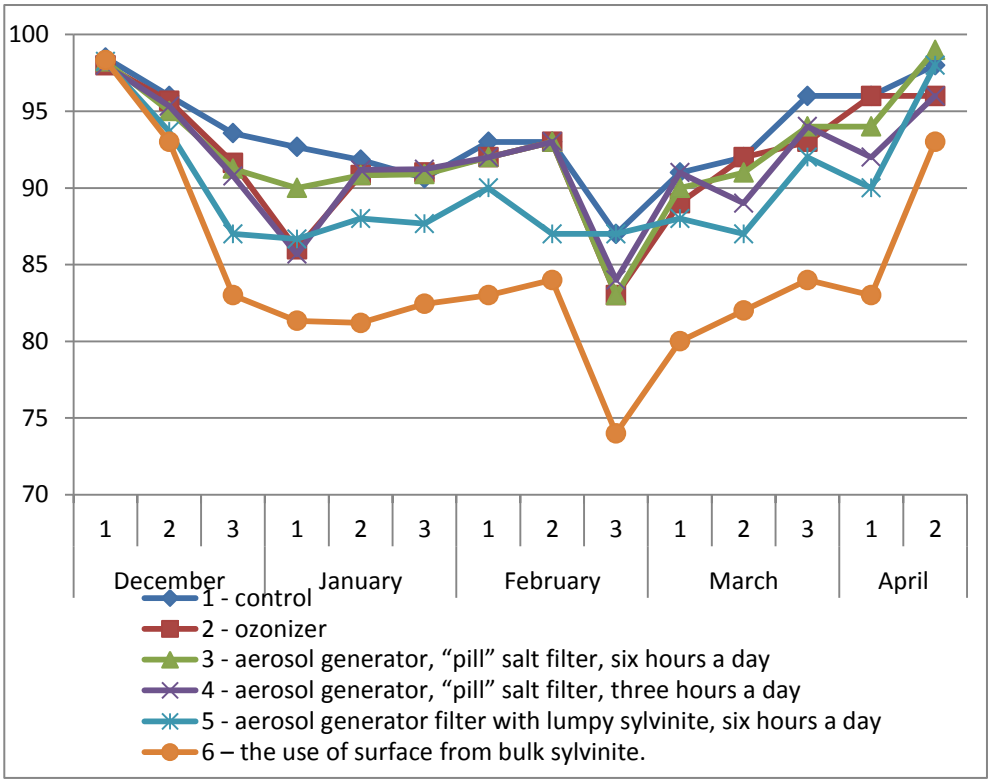


Figure 2. Dynamics of relative air humidity during the storage period.

RAH for the storage period from October to the first decade of December varied from 86 to 98 %, in the next period gradual decrease was noted. The lowest humidity was recorded in late February - early March: 74- 87%, depending on treatments. Maximum humidity (87-98%) during the entire storage period was noted at the control. RAH varied from 83 to 99% when using an ozonizer and "pills" salt filters. The lowest humidity was noted in the treatment with bulk surface of potassium salt 74-98% (Figure 2 ). The carbon dioxide content varied from 0.078 to 0.127%. The minimum concentration was recorded from February to the first ten days of April (the temperature in the storage facility during this period was minimal). With an increase in storage temperature, the concentration of $CO_2$ increased, in the middle of April it was 0.116 - 0.121%.

During the experiment, no regularities were revealed for the change in dry matter content depending on the treatments and storage period as well. The calculation of the natural loss of tubers mass to the end of storage period showed that the maximum mass decrease (2.07% from initial) was at the control, the least loss in mass was noted for treatment with "pills" salt filter, three hours - 1.53 %. Due to the fact that no significant decrease in dry matter content by the end of the storage period was observed, it can be assumed that the mass loss occurred on account of potato tubers respiration during storage period.

Various storage conditions have influenced the biochemical composition of the tubers. At the beginning of the experiment, the starch content was 12.15-13.05% (Table 1). During the entire storage period, its content in the control variant decreased to a minimum value of 8.98% in March, which can be explained by a air temperature decrease to minimum in the second decade of February (2.3°C). That was due to the fact that starch, when the temperature drops below + 4-5°C, transform into sugars. According data of Pshechenkov et al. (2007), it is possible to completely avoid the accumulation of sugars at a temperature of about 10°C.

Table 1. Starch content in potato tubers, %

| Treatments | | Analyses time | | | | |
|---|---|---|---|---|---|---|
| | 19.11.2020 | 28.12.2020. | 21.01.2021. | 02.03.2021. | 31.03.2021. | 28.04.2021. |
| Treatment 1 -control | 13,05 | 12,79 | 11,33 | 10,21 | 8,98 | 12,18 |
| Treatment 2 | 12,62 | **12,40** | 11,13 | **11,53** | **10,71** | 12,36 |
| Treatment 3 | 12,98 | 12,70 | **10,57** | **10,56** | **9,23** | 12,19 |
| Treatment 4 | 12,15 | **10,03** | **10,86** | **11,07** | **10,39** | **11,75** |
| Treatment 5 | 12,38 | **10,92** | 11,17 | **12,14** | **9,99** | **10,22** |
| Treatment 6 | 12,25 | **11,6** | **10,12** | **10,63** | **11,05** | **10,34** |
| $LSD_{05}$ | 0,3 | 0,3 | 0,29 | 0,14 | 0,19 | 0,2 |

With the temperature rise from March to April (from 3.1 to 7.3°C), the starch content began to grow to a maximum value of 12.8% ( sugars transform into starch at +5°C ) In the studied variants, the starch content varied from month to month. In December, the starch content was significantly lower in the treatments with ozonizer (12.4%), "pills" salt filter, three hours (10.03%), filter with lumpy filling (10.92%), bulk surface of potassium salt (11.6%) compared to the control (12.79 %).

At the end of the storage period (April 28), the starch content was significantly lower in the last three variants: with the use of "pills salt filter, three hours - (11.75%), filter with lumpy filling (10.22%) and sylvinite bulk surface (10.34%) compared with the first treatments.

During the entire storage period, seed potato tubers were analyzed for the presence of phytopathogens. The data are shown in the Table 2.

Table 2. Phytopathogens determination during storage of potato tubers.

| Treatments | Units | ***Pseudomonas ssp.*** | ***Streptomyces scabies*** | ***Phoma foveata*** | ***Fusarium ssp.*** | ***Phytophtora infestans*** |
|---|---|---|---|---|---|---|
| Treatment 1 - control | Infected tubers/ inspected tubers | 24/50 | 33/50 | 5/50 | 10/50 | 13/50 |
| | % | 48 | 66 | 10 | 20 | 26 |
| Treatment 2 | Infected tubers/ inspected tubers | 13/50 | 22/50 | 2/50 | 4/50 | 6/50 |
| | % | 26 | 44 | **4** | **8** | **12** |
| Treatment 3 | Infected tubers/ inspected tubers | 11/50 | 21/50 | 3/50 | 6/50 | 7/50 |
| | % | 22 | 42 | 6 | 12 | 14 |
| Treatment 4 | Infected tubers/ inspected tubers | 10/50 | 16/50 | 2/50 | 5/50 | 6/50 |
| | % | 20 | **32** | **4** | 10 | **12** |
| Treatment 5 | Infected tubers/ inspected tubers | 8/50 | 16/50 | 3/50 | 6/50 | 8/50 |
| | % | **16** | **32** | 6 | 12 | 16 |
| Treatment 6 | Infected tubers/ inspected tubers | 14/50 | 20/50 | 2/50 | 7/50 | 9/50 |
| | % | 28 | 40 | **4** | 14 | 18 |

*Pseudomonas ssp*., (48% of the infected tubers from the examined ones), *Streptomyces scabies* - 66%, *Phoma foveata* - 10%, *Fusarium ssp*.- 20%, *Phytophtora infestans* - 26%; were found at the control. That was the maximum number of infected tubers among all studied variants. The number of infected tubers was significantly less in all studied treatments.

The minimum percentage of tubers infected with *Pseudomonas ssp* was determined in treatment with aerosol generator and salt filter (lumpy filling ) - 16%. The share of tubers infected by *Streptomyces scabies* was the smallest in variants with "pills" salt filter ( three hours) and salt filter (lumpy filling ) - 32%. The smallest number of tubers infected by *Phoma foveata* was observed in treatments with the ozonizer, "pills" salt filter ( three hours) and the bulk surface of potassium salt - 4%.

The smallest number of tubers infected by *Phytophtora infestans* was noted for the treatment with the ozonizer and "pills" salt filter ( three hours) - 12%. Thus, during the storage period, the minimum tubers damage by major diseases was noted in different treatments. In general,

treatments with using aerosol generator and "pills" salt filter ( three hours) and salt filter (lumpy filling) appear to be the most promising with regard to suppression of potato pathogens during storage period.

## CONCLUSION

Storing in air medium, contacting with natural sylvinite, is one of the most promising technologies in potato storage. Such environment has the ability to inhibit the growth of potato pathogens due to formation of light negative and saturation of air medium by saline aerosol. The smallest number of infected tubers was noted in the treatment with the use of aerosol generator blowing air through "pill"salt filter, three hours a day and in the variant with sylvinite bulk surface, the largest share of infected tubers was determined at the control.

The maximum natural loss of tubers mass to the end of storage period noted for the control variant (mass decrease 2.07% from initial), the least loss in mass was noted for treatment with "pills" salt filter, three hours - 1.53 %. No significant decrease in dry matter content by the end of the storage period was observed, so natural loss of tubers mass occurred on account of potato tubers respiration during storage period.

After additional studies in typical storages equipped with standard ventilation systems, a technology for potato storage, based on the creation favorable air environment saturated with negative air ions and saline aerosol, will be developed.

## ACKNOWLEDGEMENTS

The research work was fulfilled and given paper was prepared with the financial support and as part of the scientific subprogramme "The development of potato selection and seed production in Russian Federation"

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**Original Scientific paper**
10.7251/AGRENG2103066K
UDC 663.2

# OENOLOGICAL CHARACTERISTICS AND VINIFICATION RESULTS OF THE YEAST OF MALAGOUSIA GRAPE ISOLATED IN GREECE

Aikaterini KARAMPATEA[1,2]*, Argirios TSAKIRIS[1], Ioannis KOURKOUTAS[3]

[1]Department of Wine, Vine and Beverage Sciences, University of West Attica, Aigaleo 12243 Athens, Greece
[2]Department of Agricultural Biotechnology and Oenology, International Hellenic University, 66100 Drama, Greece
[3]Department of Molecular Biology and Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece
*Corresponding author: katerina_karampatea@yahoo.gr

## ABSTRACT

Malagousia grapes, selected from 5 different PGI Greek zones, in 3 different growing seasons (2018-2019, 2019-2020, 2020-2021) were collected in the stage of technological maturity. Quantity of 25 Kg grapes fermented spontaneously in 30 l thermo regulated stain steel tanks, produced white wine. The indigenous yeast flora, isolated at three phases of the alcoholic fermentations, was studied. Different yeast species were isolated, purified and characterized. The restriction fragment length polymorphism of PCR-amplified fragments from the rDNA gene cluster (PCR RFLP of rDNA) has been used for the differentiation of yeast species. The standard identification procedure has been performed on representative strains that shared identical RFLP profiles showed great diversity of the yeast population. Including grape berries, must and fermented must, the following yeast species were identified: a number of Pichia and Candida species, Kloeckera apiculata, Cryptococcus curvatus, Metschnikowia pulcherrima, Kluyveromyces, as well as Saccharomyces cerevisiae and Saccharomyces stranieri. We performed microscopic, macroscopic and carbon assimilation tests by API 32C standardized system, biotechnological testing and hydrolase profiling obtained by the API-ZYM system. The most significant findings in population dynamics of yeasts in the spontaneous fermentations were bigger diversity of different species of Non-Saccharomyces in organic grapes and almost complete absence of non-Saccharomyces species, at least at grapes and at the beginning of the alcoholic fermentations at conventionally cultivated grapes, from all the examined PGI zones in all the three years of the study. The use and enhancement of indigenous yeasts is an increasing trend. Rapid identification of the yeast population is necessary for successful monitoring of the fermentation process and for obtaining a good wine quality as well as contributing to the optimization of Greek wine production.

**Keywords:** *Yeast strain selection, Spontaneous alcoholic fermentation, organic grapes.*

## INTRODUCTION

There are so many different factors contributing in the physicochemical and sensory profile of a wine. The variety, the terroir in general, and more specifically the microbial terroir can be a determining factor in regional variation among wine grapes (Alexandre 2020, Chalvantzi et al 2021). Vitivinicultural "terroir" is a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area (OIV 2010).

The deeper understanding of the microbiology of the wine-making process is a consequence of the employment of phenotypic and molecular techniques in wine yeast characterization. Monitoring of induced fermentations gave an understanding of the evolution of the entire microfora during this process, making clear that wine quality is a consequence of the dynamics and composition of the micro-organisms involved in its production (Querol et al., 1992; Schuetz et al., 1993). More detailed scientific researches on the geographical distribution of wine yeast strains in entire areas became possible, while phylogenetic affinities and evolutionary scenarios were explored (Pramateftaki et al., 1999; Versavaud et al., 1995; Nadal et al., 1996).

In order to ensure the microbiological control of the alcoholic fermentation process, a very common winemaking practice is the inoculation of grape juice with selected cultures of Saccharomyces cerevisiae. In this way there is a better management of the alcoholic fermentation (Barre et al., 1984; Bisson et al., 2010; Pretorius, 2000; Ranieri and Pretorius, 2000; Fleet 2008). It is believed that a selected and inoculated strain of Saccharomyces cerevisiae, once inoculated correctly in a given population, will dominate the fermentation process, suppressing any indigenous species, leading to a wine having fewer possibilities of organoleptic deviations. However, in addition to Saccharomyces, there are other genera of yeast in winemaking, present in different stages either pre-fermentative or at the early beginning of winemaking and alcoholic fermentation. These yeasts are known as non-Saccharomyces' (NS) and have long been considered undesirable and are usually classified into 15 different yeast genera including Dekkera/Brettanomyces, Candida, Cryptococcus, Debaryomyces, Hanseniaspora/Kloeckera, Kluyveromyces, Metschnikowia, Rhodotorula, Pichia, Saccharomycodes, Schizosaccharomyces, Zygosaccharomyces, and Saccharomyces (Kurtzman et al., 1998; Raspor et al., 2006).

The aim of this work was to study yeasts population diversity of indigenous yeasts isolated from grapes and must collected from 5 different viticulture regions of Greece, focusing on Malagousia grape, in order to study their oenological characteristics.

Malagousia has been characterized as the Cinderella of the Greek vineyard (Kourakou 2016). The variety is mentioned for the first time in the book Oenological (1888) by Othon Roussopoulos, with a cultivation area in the prefecture of Etoloakarnania. Most Greek varieties are considered local varieties as

they are associated with their place of origin. However, the same does not happen with Malagousia, because when the first PDO zones (1970) were legislated, there were no modern / organized wineries in its cultivation area. Malagousia is therefore not connected to a specific place. Malagousia is authorized to be cultivated in all 11 viticultural departments and more specifically is a recommended variety in 43 regional zones of Greece, while is only authorized in the remaining 21 of the total 64 regional zones(Official Gazette of the Hellenic Republic 3276 / B / 2017). Malagousia is an early-maturing variety, its harvest takes place the second fortnight of August. It is a vigorous growth variety with large, cylindrical, sometimes winged, dense grapes, with medium to large-sized rails, spherical to oval in shape and medium-thick rind, slender flowering and cricket green to golden yellow. The flesh is soft, sweet, moderately juicy and slightly aromatic. It is relatively drought resistant, very sensitive to mildew, downy mildew and botrytis.


Figure 1. Greek wine map, New wines of Greece, EDOAO Athens Greece

## MATERIALS AND METHODS

**2.1 Sampling sites:** In Greece there are 120 delimited zones with protected geographical indication. We collected Malagousia grapes in the following wine producing zones: Drama (PGI Drama), Kavala (PGI Pangeon), Chalkidiki (PGI Chalkidiki), Thessaloniki (PGI Thessaloniki), Fthiotida (PGI Atalanti Valley). The mode of culture was either conventional or biological and the configuration of the vineyards was linear.

**2.2. Grape sampling:** Grapes samples were collected in the stage of technological maturity in 3 different growing seasons (2018-2019, 2019-2020 and 2020-2021). 25 Kg of grape berries Malagousia variety were collected into sterile plastic bags. Samples were kept cold and transferred to the laboratory within 12 h.

**2.3. Microbial analysis of grapes and fermented musts:** Directly fresh grapes or defrosted were destemmed and crushed with hands. The grape mass obtained, quantity of 25 l for each batch, was fermented spontaneously at 25°C (constant temperature). Once placing the grape mass in the tank, diammonium phosphate (20gr/hl) was added, followed by good homogenization. All the experimental

spontaneous fermentations took place in 30l stain-steel thermo regulated tanks, exact copies of professional winemaking tanks. Before all the treatments hands, tanks and other equipment had been washed and disinfected with alcohol. The vinifications took place to the premises of Laboratory of Marketing and New Products Development of the Department of Agricultural Biotechnology and Oenology of the International Hellenic University in Drama.

Figure 2. Malagousia grape

Figure 3. Spontaneous fermentations in 30 l tanks

**2.4 Isolation of yeast strains from spontaneous fermentations:** The fermentations were carried out spontaneously for the whole grape mass, without separating the must from the marcs. The alcoholic fermentation was conducted at 25°C. The yeasts were isolated by taking wine samples from each tank during fermentation (beginning of the AF (12–14 Baumé), middle (6 Baumé) and end (<1 Baumé). Aliquots (0.1 mL each) of several decimal dilutions in 0.1% peptone-water were spread onto YPD Nutrient Agar (Sigma-Aldrich, USA) that had been treated with streptomycin sulfate (250 mg /l)(Fisher Scientific Belgium). Plates were incubated at 25°C for 5 days (Berber et al., 2017, Renouf et al., 2005, OIV 2017). Plates containing between 30 and 300 colonies were examined according to their macroscopic features to be re-isolated on YPD agar. A number of representative colonies were isolated and purified: 250 yeast isolates were stored at 4°C on YPD (glucose, peptone, yeast extract, chloramphenicol, biphenyl, agar-agar H2O qsp) and used for further analysis. Yeast isolates were preserved on YPD agar slants, stored at 4oC and subcultured every 2 months. The cultures were also kept at -20$^{o}$C with 20%v/v glycerol as a cryoprotectant agent (Monaco et al., 2014).

**2.5. Molecular analysis:** Genomic DNA was isolated from cultures using a commercial kit, Genomic DNA from tissue (Macherey-Nagel, USA), according to the manufacture protocol/ support protocol for yeast 01/2017, Rev.17. Finally, 50 μL of a mixture containing 5–30 ng/μL of genomic DNA was obtained. Internal transcribed spacers (ITS) (ITS1 and ITS2) and 5.8S rDNA gene regions were amplified using specific primers ITS1 (5_-TCCGTAGGTGAA CCTGCGG-3_)

and ITS4 (5_-TCCTCCGCTTATTG ATATGC-3_) (White et al., 1990). DNA amplification was carried out in the final volume of 25 µL containing 0.2 mM of dNTP (Invitek, Germany), 1.25 µL of each primer (100 pmol/µL) (KapaBiosystems, USA), PCR reaction buffer (1X) and 0.1 µmol/min of Taq DNA polymerase(KAPA2G Robus) (Kapa Biosystems, USA). PCR conditions were as follows: initial denaturation cycle at 95$^{o}$C for 5 min followed by 35 cycles of amplification, denaturation at 95$^{o}$C for 15 sec, annealing at 55$^{o}$C for 15 sec, and extension at 72$^{o}$C for 30 sec; final extension at 72$^{o}$C for 10 min. The amplification reaction was carried out in a PCR MiniOpticon (Biorad, France). PCR fragments were separated and detected by electrophoresis on agarose gel (1.5 mass %) in TBE buffer (1X) at 120 V for 2.5 h, Sub-Cell GT Agarose Gel Electrophoresis Systems (Biorad, France). The gel was stained with Gel Red (10 mg/L), visualized under UV light (Gel Doc EZ Imager, Biorad France) and documented by the Image Lab Software (Biorad, France). For RFLP, PCR products were purified by ethanol precipitation and digested by restriction endonucleases HaeIII, Hinf I, TaqI, AluI, MseI, HhaI, and HpaII (Takara, Japan) following the manufacturer's instructions. The restriction fragments were separated on agarose gel (1.5 mass %) under the same conditions as the amplified products. Representative samples, grouped by PCR-RFLP of ITS regions.

**2.6. Phenotypic identification:** Fermentative vigor without and with sulphites were measured according to Caridi et al., 2002: flasks containing 100 ml of sterile white must (20°Brix, pH 3.20, filtered by steri-cup vacuum filtration system, Millipore, Billerica, MA, USA), with and without $SO_2$ (100 mg/l) and covered with 10 ml of sterile liquid paraffin to prevent evaporation, were inoculated in triplicate with 5 ml of 48 h pre-cultures of each isolated yeast and incubated at 25°C. Fermentative vigor was measured as weight loss caused by $CO_2$ production (g $CO_2$ per 100 ml) after 2 and 7 days. Reducing sugars, ethanol, glycerol and volatile acidity were measured in must prepared as described above (without $SO_2$) using a Winematic apparatus (Gibertini, Italy) after 15 days of fermentation. We estimated hydrogen sulfide production on Biggy agar (Oxoid) recording the biomass color after 48 h at 25°C (Comitini et al., 2011). Beta-glucosidase production was assayed as in Strauss et al., 2001 onto selective medium containing 6,7 g /l Yeast Nitrogen Base (Difco, Detroit, MI, USA), 5 g/l arbutin (Sigma-Aldrich, Saint Louis, MO, USA), 0.2g/l ammonium ferric citrate and 20 g/l agar (pH 5.0).

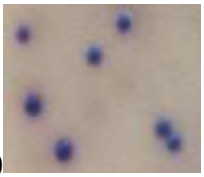
a)  b)  c)  d) 

Figure 4. Typical cultures of different yeasts in Chromagar culture medium. a)Candida albicans, b) Kluyveromyces thermotolerans c) Hanseniaspora/Kloeckera d)Saccharomyces cerevisiae

Chromagar (Chromagar France) is a chromogenic differential culture medium facilitates the isolation and presumptive identification of certain clinically important yeast species, especially when in the YPD medium the differentiation is not visible (Ainscough et al., 1998; Koehler et al., 1999; Romeo et al., 2011). The identification system ID 32C (Bio-Merieux, SA, Marcy-l'Etoile, France) was used for the carbon assimilation tests. ID 32 C is a standardized system for the identification of yeasts, which uses 32 miniaturized assimilation tests (dehydrated carbohydrate substrate) and a database.

## RESULTS AND DISCUSSION

**3.1. Yeast population:** The 'NS' isolates investigated were mostly Pichia and Candida. More specifically 31% Pichia, 17%Candida lusitanea, 10% Metschnikowia, 10% Cryptococcus curvatus, 7% Kloeckera apiculata, 7% Candida colliculosa, 7% Candida globosa and 7% Kluyveromyces thermotolerans. Candida species were mostly found in grapes and at the first day of the alcoholic fermentation, while Metschnikowia species were detected only at mi-fermentation and Pichia species were found at all the stages of the alcoholic fermentations. In organically grown grapes, Candida strains in a given area (PGI Pangeon) were isolated in the grapes of the same vineyard for three consecutive years. The same was observed for Pichia strains which seem to succeed Candida strains in fermentation, as for the same grapes they were isolated in sampling in the middle of the alcoholic fermentation also for three consecutive years. Metschnicowia strains, in another area (PGI Chalkidiki), were located in the middle of the alcoholic fermentation for two consecutive years at grape samples from two different vineyards of the same zone in proximity. In another area (PGI Thessaloniki) greater heterogeneity was observed as each year different strains were identified (Cryptococcus, Kloeckera apiculata, Pichia). In conventionally grown grapes, in grape samples of almost all zones, Saccharomyces strains were isolated during all years of observation. However, this picture is reversed as in the isolates at the middle of the fermentation there was identification of Metschnicowia strains (PGI Drama), Pichia two consecutive years (PGI Atalanti Valley) and Candida colliculosa two consecutive years (PGI Pangeon). At the end of the experimental spontaneous fermentations of conventionally cultivated grapes, in almost all five zones Saccharomyces strains were isolated in all three years of our study. Beside the impact of common oenological practices during prefermentation stage (clarification, temperature, sulphite and starter yeast addition) on the growth of 'NS' yeasts (Albertin et al., 2014), it seems that the culture mode is also influencing the 'NS' population composition. However, there also other factors: climate, viticultural practices that could determine the population dynamics differ from year to year. (Alexandre 2020, Chalvantzi et al 2021).

Table 1. Distribution of yeast species (%) during must fermentation and sampling stage (BF beginning of fermentation, MF middle of fermentation, EF end of fermentation

| **Yeast species** | **PGI Drama 2018** | | | **PGI Drama 2019** | | | **PGI Drama 2020** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** |
| Saccharomyces | 100 | 50 | 100 | 100 | 55 | 100 | 100 | 35 | 100 |
| Metschnicowia | | 50 | | | 45 | | | 65 | |
| **Yeast species** | **PGI Pangeon 2018** | | | **PGI Pangeon 2019** | | | **PGI Pangeon 2020** | | |
| | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** |
| Candida | 90 | 30 | | 95 | 35 | | 95 | 25 | |
| Pichia | 5 | 70 | 70 | | 60 | 65 | | 73 | 70 |
| **Yeast species** | **PGI Thessaloniki 2018** | | | **PGI Thessaloniki 2019** | | | **PGI Thessaloniki 2020** | | |
| | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** |
| Cryptococcus | | 25 | 30 | 20 | 35 | 45 | 35 | 10 | 15 |
| Kloeckera apiculata | 78 | 25 | 30 | 25 | 40 | 25 | 30 | 50 | 45 |
| Pichia | 15 | 50 | 35 | 50 | 20 | 15 | 35 | 40 | 40 |
| **Yeast species** | **PGI Chalkidiki 2018** | | | **PGI Chalkidiki 2019** | | | **PGI Chalkidiki 2020** | | |
| | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** |
| Metschnicowia | 10 | 40 | 20 | | 80 | 55 | 20 | 85 | 45 |
| Candida | 90 | 50 | 50 | 80 | 10 | 15 | 80 | 10 | 40 |
| **Yeast species** | **PGI Atalanti Valley 2018** | | | **PGI Atalanti Valley 2019** | | | **PGI Atalanti Valley 2020** | | |
| | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** | **BF** | **MF** | **EF** |
| Saccharomyces | 100 | 80 | 100 | 100 | 45 | 100 | 100 | 30 | 100 |
| Pichia | | 15 | | | 55 | | | 68 | |

### 3.2. Oenological characterization of the yeast species

Enzymatic profile: The enzymes α-fucosidase, esterase, esterase/lipase, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin aminopeptidase, phosphohydrolase, galactosidase were detected in all different strains. Both N-acetyl β-glucosaminidase and α-fucosidase activity were detected in all 'NS' yeasts except Pichia strains. Although each strain had a unique enzyme pattern, the mean enzyme activity was esterase, esterase / lipase, lipase with that of esterase / lipase slightly larger. The action of N-acetyl β-glucosaminidase shows maximum enzymatic activity in almost all strains except three in which does not appear at all or almost not at all. Low β-galactosidase activity is shown in all strains. Also β-glucosidase has generally low production with the exception of two Candida strains which show activity of 30 nmoles. The following enzymes were not detected in any of the tested strains: alkaline phosphatase, α-chymotrypsin, α-glucosidase (Canal-Llaubères, 1993).

*a)*

*b)*

Figure 5. a) API ZYM – Semiquantitation of enzymatic activities b) API ID 32 C Identification system for yeasts

Table 2. Number of Non Saccharomyces (NS) species isolates

| *PGI Region* | *Vintage* | *Nombre of Non Saccharomyces (NS) species isolates* | |
|---|---|---|---|
| | | *Conventional Grapes/Must* | *Organic Grapes/Must* |
| *Drama* | *2018* | *0/15* | |
| | *2019* | *5/10* | |
| | *2020* | *0/14* | |
| *Pangeon* | *2018* | *1/17* | *10/12* |
| | *2019* | *5/17* | *15/15* |
| | *2020* | *2/13* | *10/20* |
| *Thessaloniki* | *2018* | | *5/18* |
| | *2019* | | *2/10* |
| | *2020* | | *4/15* |
| *Chalkidiki* | *2018* | | *9/12* |
| | *2019* | | *11/14* |
| | *2020* | | *8/12* |
| *Atalanti Valley* | *2018* | *0/10* | |
| | *2019* | *2/12* | |
| | *2020* | *0/12* | |
| | | | |
| *Total* | | *89* | |
| *Total of yeast isolates* | | *248* | |

## CONCLUSION

To summarize this study provides more information regarding yeast communities on the conventionally cultivated and organic Malagousia grapes and musts from 5 different PGI Greek zones in 3 consecutive years. In the present study we applied modern molecular techniques that are suitable for rapid identification of Saccharomyces and Non Saccharomyces strains and further testing of various strains for their oenological properties. Several physiological characteristics of the yeasts used in this study are suitable for rapid selection of the different yeast strains that could be applied in the winemaking process. Inoculation or co-inoculation of

selected strains with suitable technological properties in the wine fermentation process could give in wines better organoleptic characteristics and enhance the quality of Malagousia wines.

## ACKNOWLEDGMENTS

All vine growers who allowed us to sample grapes in their vineyards for three consecutive years.

**Author Contributions:** A. Karampatea had the whole supervision of this work, making the analysis and writing sections of this paper, A. Tsakiris and I. Kourkoutas contributed in the interpretation of results and to writing.

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**Original Scientific paper**
10.7251/AGRENG2103077K
UDC 631.436:631.44 (497.2)

# EFFECT OF LAND USE AND SOIL PROPERTIES ON SOIL TEMPERATURE DISTRIBUTION OF CAMBISOLS IN MOUNTAIN REGIONS

Milena KERCHEVA[1*], Maria GLUSHKOVA[2], Katerina DONEVA[1], Stanimir STOINOV[1], Emiliya VELIZAROVA[3]

[1]Department of Physics, Erosion, Soil Biota, Institute of Soil Science, Agrotechnology and Plant Protection "N. Poushkarov", Sofia, Bulgaria
[2]Forest Research Institute, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Ministry of Environment and Water, Sofia, Bulgaria
*Corresponding author: mkercheva@abv.bg

## ABSTRACT

The aim of this study was to assess the effect of land use, location, and soil properties on the distribution and dynamics of the soil temperature, the accumulated heat and the apparent thermal diffusivity of Cambisols in three mountain regions in Bulgaria. Annual distribution of the soil temperature (Ts) of Cambisols was registered under different land use (grassland, bare soil, deciduous and coniferous vegetation) during period June 2018-May 2021. Every day measurements were realized at 0, 2, 5, 10, 20, and 50 cm depths in 11 plots within the territory of the experimental stations Gabra (~920 m.), Govedartzi (~1540 m.), and Igralishte (~850 m) of the Forest Research Institute, situated in the Lozen, Rila and Maleshevska Mountains, respectively, in the South-Western part of Bulgaria. The experimental stations differ in climatic conditions and soil physical and chemical properties. The heat accumulated in 0-50 cm soil layer was assessed by the sum of Ts for the period with Ts > 5$^{o}$C. The heat accumulated in bare soil was the highest (4067-3544$^{o}$C), followed by the sums under grassland (3849-3453 $^{o}$C) and forest (3096-3300 $^{o}$C) in Gabra and Igralishte. The relative decrease of the accumulated heat under woodland in comparison to grassland was 18-20% in Gabra and 4-7% in Igralishte. At the higher elevation in Govedartzi the heat accumulated under grassland was 10-12% less than under woodland. The apparent thermal diffusivities ($\alpha$) were estimated using annual amplitudes of Ts at depths 0.02 and 0.20 m. The data showed that the values of $\alpha$ under grass in Gabra 0.212 mm$^2$ s$^{-1}$ and in Igralishte 0.535 mm$^2$ s$^{-1}$ were higher than under forest vegetation 0.061-0.133 mm$^2$ s$^{-1}$ and bare soil 0.049-0.071 mm$^2$ s$^{-1}$. In Govedartzi the thermal diffusivity was higher 0.118 mm$^2$ s$^{-1}$ under Norway spruce than under grassland 0.070 mm$^2$ s$^{-1}$ and Scots pine 0.079 mm$^2$ s$^{-1}$.

**Keywords:** *Soil temperature distribution, Cambisols, Mountains, Land use, Bulgaria.*

## INTRODUCTION

Soil temperature is important pedoclimatic characteristic and influences a lot of physical, chemical, and biological processes in soils (Paul et al., 2004). Soil temperature is determined by the intercept of solar radiation and hence depends on the geographical location, vegetation cover and meteorological conditions. Soil temperatures are more affected by the presence woodland canopy than air temperatures (Morecroft et al., 1998). The canopy shading and the presence of litter layer lead to reduction of daily and annual soil temperature amplitudes (Morecroft et al., 1998; Andrade et al., 2010). The temperature distribution along the soil depth depends on the soil properties (texture, soil water and air content, soil organic carbon content, bulk density, etc.) and can be predicted by combining energy balance with heat flux equation or empirical relationships (Kang et al., 2000). The apparent thermal diffusivity which is a measure of the heat dissipation increases with the soil water content and bulk density (Ochsner et al., 2001), but it is less sensitive to water content in comparison to thermal conductivity and heat capacity (Morecroft et al., 1998). The information of soil temperature characteristics under adjacent woodland (coniferous, deciduous forest) and open site (bare soil/grassland) in the mountain regions was not found.

The aim of this study was to assess the effect of land cover, location and soil properties on the distribution and dynamics of soil temperature, the accumulated heat, and the apparent thermal diffusivity of Cambisols in three mountain regions in Bulgaria.

## MATERIALS AND METHODS

The soil temperature of Cambisols was measured under different land use in the experimental stations of the Forest Research Institute: Gabra in the Lozen Mountains (grassland, bare soil, deciduous and mixed forest); Govedartzi in the Rila Mountains (grassland, Scots pine, Norway spruce); Igralishte in the Maleshevska Mountains (grassland, bare soil, deciduous – oak forest, and Scots pine forest), all situated in South-Western Bulgaria. The geographic coordinates of the stations are presented in Table 1. The daily registrations of soil temperature were performed by mercury thermometers in the morning and at noon at 0, 2, 5, 10, 20, and 50 cm soil depths in 11 plots during the period June 2018 - May 2021. The daily sums of precipitation were measured at grassland plots in all stations and at forest plots in Govedartzi station.

The obtained soil temperature data for the studied period were used for determining the following characteristics: mean, maximum and minimum monthly temperatures, heat accumulation in 0-50 cm soil for the period with Ts > 5$^{o}$C; mean apparent thermal diffusivity ($\alpha$, mm$^2$ s$^{-1}$). The procedure for determination of the apparent thermal diffusivity using the annual soil temperature wave at two depths was described in detail by Marinova (1993) and applied by Doneva and Kercheva (2017) for Alluvial-meadow soil. In the current study the 3-years average daily temperature data were approximated with 5th-order polynomial curve to determine the position of the annual maximum and minimum and then the amplitudes

attenuation method was applied for determining of $\alpha$ (Horton et al., 1983). The term "apparent" means that both processes – conduction and convection – are performed in heat transfer in moist soil and in the presence of a temperature gradient.

## RESULTS AND DISCUSSION

Some of the main soil characteristics of the studied Cambisols are presented in Table 1. The highest contents of soil organic carbon (SOC) 4.2-6.8% were obtained for Govedratzi station, situated at the highest altitude – above 1500 m in the Rila Mountains. The soils in the other two stations were with lower SOC and coarser texture, especially in Igralishte where the soils were classified as loamy sand. The content of coarse fragments at 0-5 cm soil depth varied from 6 to 26% by mass in Gabra and Govedartzi, and from 1 to 18% by mass in Igralishte. The profiles are shallow and the content of rock fragments increased significantly below 25-30 cm soil depth. Additional information on the soil hydraulic properties of these profiles can be found in Kercheva et al. (2019).

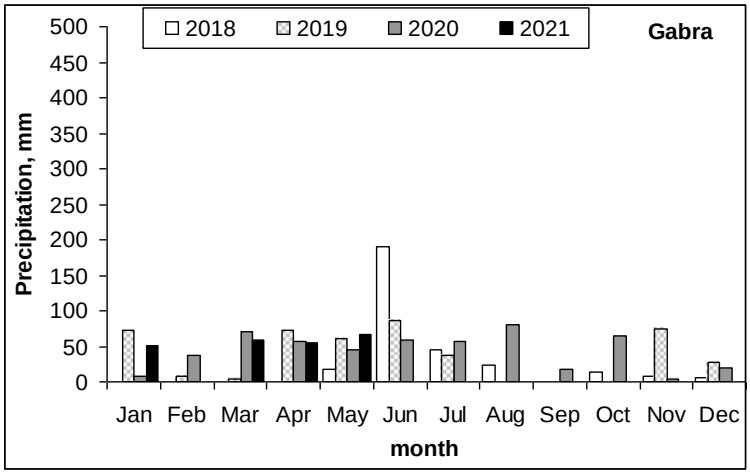
The sites differed according to the amount and distribution of precipitation during the studied period (Fig. 1). The southernmost experimental station Igralishte (Fig. 1b) was characterized with winter maximum of precipitation which is typical for the Mediterranean climate. During the studied period the monthly sums varied significantly in Govedartzi (Fig. 1c). The complete annual records obtained for 2019 and 2020 showed that the annual precipitation was higher in 2020. The annual sums of these years were 442 and 520 mm in Gabra (Fig. 1a), 446 and 505 mm in Igralishte (Fig. 1b), and 356 mm and 910 mm in Govedartzi (Fig. 1c). The highest monthly sums 346-435 mm were registered in June 2020 in Govedartzi.

The monthly average soil temperatures of the coldest month (January) and the hottest month (July or August) are presented in Table 2. The soil temperature slowly increased with depth in the coldest month which indicated the direction of heat flux towards the surface. The average winter temperatures were negative only at Govedartzi. The soil freezing and wetter climate at these high altitudes influence lot of soil properties through their effect on biological activity and physical disruption of soil aggregates. The monthly soil temperatures below 20 cm soil depth under forest were higher than under grass and bare soil. The lower temperatures under Norway spruce than under Scots pine and grassland at Govedartzi is resulting from the higher altitude (Table 1). The differences between the studied vegetation covers were well pronounced in the summer and at the lower altitudes (Gabra and Igralishte). These results coincide with finding of Morecroft et al. (1998) regarding soil temperature under woodland and open site registered at low altitude. The bare soil in our study was characterized with the highest surface temperature and this effect was preserved till 20 cm soil depth where the temperature equalized that under grass. The soil temperatures under woodland were lower than under grass by 2.2 to 3.6 $^{o}$C in Igralishte and by 4 to 5.5 $^{o}$C in Gabra. In Govedartzi such effect was less pronounced (1 to 2.2$^{o}$C) and was observed only in the surface 0-5 cm soil layer. The soil temperatures under grass in

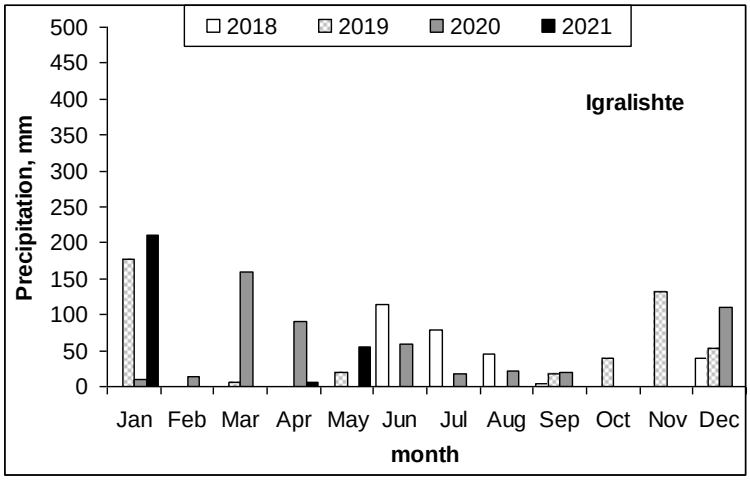
Govedartzi was 7-8 $^{o}$C lower than those in Igralishte and 8-9 $^{o}$C than in Gabra, which can be explained beside the difference in elevation with the dryer conditions in Igralishte.

Table 1. Location of soil profiles and basic properties: SOC – soil organic carbon content, pH, soil textural fractions and classification according to IUSS Working Group WRB (2015).

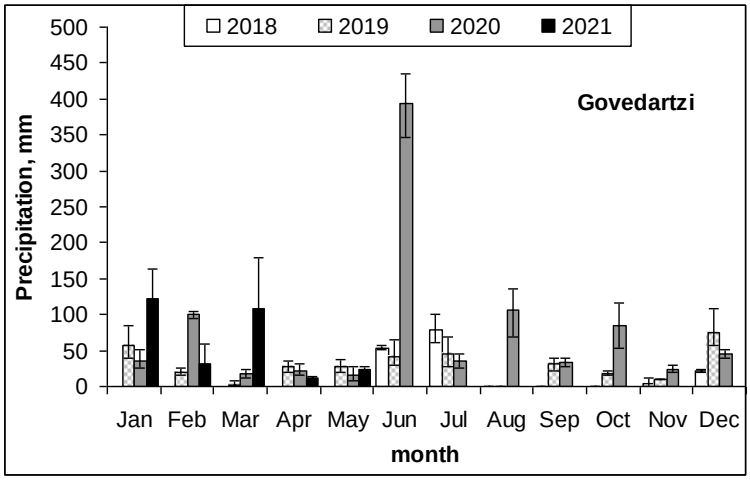
| Location | Land use | Depth (cm) | SOC (%) | pH ($H_2O$) | Sand (%) | Silt (%) | Clay (%) | Texture class |
|---|---|---|---|---|---|---|---|---|
| Gabra | Grassland | 0-5 | 1.5 | 5.2 | 32 | 47 | 21 | loam |
| 23.63E | Deciduous forest | 0-5 | 2.7 | 3.9 | 52 | 29 | 20 | sandy loam |
| 42.53N | | 15-20 | 0.9 | 3.8 | 52 | 31 | 17 | sandy loam |
| 916-937 m | Mixed forest | 0-5 | 1.5 | 4.0 | 54 | 32 | 14 | sandy loam |
| Govedartzi | Grassland | 0-5 | 5.9 | 4.1 | 46 | 34 | 20 | loam |
| 23.46-23.42E | Scots pine | 0-5 | 4.2 | 4.2 | 43 | 37 | 19 | loam |
| 42.22N | Norway spruce | 0-5 | 6.8 | 3.7 | 48 | 32 | 20 | loam |
| 1503-1579 m | | | | | | | | |
| Igralishte | Grassland | 0-5 | 2.9 | 4.6 | 72 | 21 | 7 | sandy loam |
| 23.13E | | 10-20 | 0.4 | 4.1 | 82 | 15 | 3 | loamy sand |
| 41.57N | Deciduous | 0-5 | 2.4 | 4.5 | 73 | 25 | 2 | loamy sand |
| 848-869 m | forest | 10-15 | 1.1 | 3.9 | 74 | 21 | 5 | loamy sand |
| | Scots pine | 0-5 | 0.3 | 4.5 | 82 | 14 | 4 | loamy sand |




a)




b)




**c)**

Fig. 1. Monthly sums of precipitation during the studied period (June 2018-May 2021) in Gabra (a), Igralishte (b) and Govedartzi (c)

Table 2. Average monthly soil temperature for the coldest (January) and hottest (July or August, the latter marked with "*") months.

a) Gabra

| Depth | Grassland | | Bare soil | | Deciduous | | Scots pine | |
|---|---|---|---|---|---|---|---|---|
| cm | Jan | Aug | Jan | Aug | Jan | Aug | Jan | Aug |
| 0 | 0.3 | 23.3 | 0.2 | 24.7 | 0.8 | 19.3 | 0.0 | 20.2 |
| 2 | 0.2 | 22.9 | 0.2 | 24.2 | 0.9 | 17.5 | 0.2 | 19.1 |
| 5 | 0.3 | 21.7 | 0.3 | 23.4 | 1.2 | 17.1 | 0.5 | 17.9 |
| 10 | 0.6 | 21.0 | 0.5 | 22.1 | 1.5 | 16.8 | 1.0 | 17.6 |
| 20 | 1.5 | 21.6 | 1.5 | 22.2 | 2.4 | 16.5 | 1.9 | 17.7 |
| 50 | 2.6 | 20.4 | 2.7 | 21.3 | 3.4 | 15.8 | 3.0 | 16.2 |

b) Igralishte

| Depth | Grassland | | Bare soil | | Deciduous | | Scots pine | |
|---|---|---|---|---|---|---|---|---|
| cm | Jan | Jul/Aug* | Jan | Jul/Aug* | Jan | Jul/Aug* | Jan | Aug |
| 0 | 0.0 | 23.2 | 0.1 | 25.3 | 0.3 | 21.0 | 0.8 | 19.7 |
| 2 | 0.4 | 20.9 | -0.1 | 25.4 | 0.4 | 20.2 | 1.0 | 18.3 |
| 5 | 0.2 | 20.9 | -0.2 | 24.1 | 0.6 | 18.2 | 1.1 | 18.1 |
| 10 | 0.3 | 20.0 | 0.3 | 22.3 | 1.2 | 17.8* | 1.5 | 17.6 |
| 20 | 0.8 | 19.7* | 0.6 | 19.5* | 2.0 | 17.4* | 2.7 | 17.2 |
| 50 | 2.5 | 18.7* | 2.5 | 19.0* | | | 3.5 | 16.4 |

c) Govedartzi

| Depth | Grassland | | Scots pine | | Norway spruce | |
|---|---|---|---|---|---|---|
| cm | Jan | Jul/Aug* | Jan | Aug | Jan | Aug |
| 0 | -1.7 | 15.3 | -2.0 | 13.2 | -2.2 | 13.1 |
| 2 | -1.4 | 14.2 | -1.9 | 12.8 | -1.8 | 12.7 |
| 5 | -1.2 | 13.4 | -1.6 | 12.3 | -1.5 | 12.0 |
| 10 | -0.9 | 12.7* | -1.2 | 12.6 | -1.1 | 12.2 |
| 20 | -0.6 | 12.2* | -0.6 | 12.0 | -0.6 | 11.7 |
| 50 | -0.4 | 11.1* | 0.6 | 10.9 | 0.5 | 10.8 |

The heat accumulated in 0-50 cm soil layers was expressed as sum of weighed temperatures along the whole layer for the period with Ts > 5$^{o}$C (Table 3). The mean sums under bare soil were the highest (4067-3544 $^{o}$C), followed by the sums under grassland (3849-3453 $^{o}$C) and forest (3096-3300 $^{o}$C) in Gabra and Igralishte. The decrease of the accumulated heat between woodland and grassland were best pronounced in Gabra by 18-20% and by 4-7% in Igralishte. The latter were in agreement with the reported decrease of 0-10% in woodland by Morecroft at al. (1998). At the higher elevation in Govedartzi the heat accumulated under grassland 1617 $^{o}$C was 10-12% less than under woodlands (Scots pine and Norway spruce) which was 1800 $^{o}$C in average.

Table 3. Soil heat accumulation (□Ts, °C) for 0-50 cm soil layer, period with $Ts_{0-50}$ > 5°C.

| Land use | Gabra | | Igralishte | | Govedartzi | |
|---|---|---|---|---|---|---|
| | ΣTs, °C | days | ΣTs, °C | days | ΣTs, °C | days |
| Grassland | 3849 | 268 | 3453 | 255 | 1617 | 160 |
| Bare soil | 4067 | 272 | 3544 | 250 | | |
| Deciduous | 3175 | 285 | 3227 | 249 | | |
| Scots pine | 3096 | 255 | 3300 | 286 | 1810 | 197 |
| Norway spruce | | | | | 1782 | 188 |

The annual soil temperature amplitudes at two depths 0.02 and 0.20 m are presented in Table 4. The reduction of the annual amplitude at 0.20 m under deciduous and coniferous vegetation in comparison to grassland was 6.3 °C and 4.6 °C in Gabra. In Igralishte these reductions were 3.8 and 5 °C correspondingly under deciduous and coniferous vegetation. The differences can be explained with thickness of litter layer and soil properties, creating different moisture conditions. Andrade et al. (2010) reported that removal of litter layer in Mediterranean conditions can lead to 2.5 °C higher annual amplitude at depth 0.16 m. The amplitudes at 0.02 m of bare soil were 3-4 °C higher than under grass (Table 4).
Based on the approximated annual soil temperature waves at 0.02 and 0.20 m depths the apparent thermal diffusivity was estimated. The relatively short-term period of observations and the variability of moisture conditions among the stations and years (Fig. 1) allowed to regard the obtained estimates for the apparent thermal diffusivity (Table 4) as preliminary. The highest values of $\alpha$ were obtained under grassland in Igralishte and Gabra, respectively 0.535 to 0.212 $mm^2 s^{-1}$ (Table 4). The values under bare soil were lower within the range 0.049 to 0.071 $mm^2 s^{-1}$, which can be attributed to the lower soil water content due to the higher evaporation rates. Most of the values of $\alpha$ under woodland were medium within the range 0.075 to 0.133 $mm^2 s^{-1}$. The exceptions of these ranges were found under grass in Govedartzi (0.070 $mm^2 s^{-1}$) and under deciduous forest in Igralishte (0.061 $mm^2 s^{-1}$).

Table 4. Annual amplitudes of soil temperature at 0.02 ($A_{0.02}$) and 0.20 m ($A_{0.20}$) soil depths and apparent thermal diffusivity ($\alpha$, $mm^2 s^{-1}$) of 0.02-0.20 m soil layer.

| Land use | Gabra | | | Igralishte | | | Govedartzi | | |
|---|---|---|---|---|---|---|---|---|---|
| | $A_{0.02}$ °C | $A_{0.20}$ °C | $\alpha$ $mm^2 s^{-1}$ | $A_{0.02}$ °C | $A_{0.20}$ °C | $\alpha$ $mm^2 s^{-1}$ | $A_{0.02}$ °C | $A_{0.20}$ °C | $\alpha$ $mm^2 s^{-1}$ |
| Grassland | 22.8 | 20.2 | 0.212 | 21.4 | 19.8 | 0.535 | 17.1 | 13.8 | 0.070 |
| Bare soil | 25.8 | 20.8 | 0.071 | 25.5 | 19.7 | 0.049 | | | |
| Deciduous | 16.9 | 13.9 | 0.085 | 20.1 | 16.0 | 0.061 | | | |
| Scots pine | 19.2 | 15.6 | 0.075 | 17.3 | 14.8 | 0.133 | 15.8 | 12.9 | 0.079 |
| Norway spruce | | | | | | | 16.0 | 13.5 | 0.118 |

## CONCLUSIONS

The obtained data and characteristics of the soil temperature in the studied mountain regions in Southwestern Bulgaria, allowed to evaluate the influence of vegetation cover, geographic position, and soil properties on the profile distribution and dynamics of Ts, the accumulated heat and the apparent thermal diffusivity of Cambisols. The information can serve as pedoclimatic characteristics, for predicting the heat exchange and soil temperature under bare soil, grassland, and forest and for describing the interrelations with other soil properties and processes.

## ACKNOWLEDGEMENTS

The authors acknowledge funding of research activities received from the National Science Fund under grant agreement DN16/11 (project "Thermal properties of soils at different land use and melioration").

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**Original Scientific paper**
10.7251/AGRENG2103085S
UDC 638.1

# TECHNICAL EFFICIENCY OF THE RIPARIAN BEEKEEPERS OF THE COMPLEX OF PROTECTED AREAS PÔ-NAZINGA-SISSILI IN BURKINA FASO

Soumaïla SAWADOGO[1, 3*]; Omer Souglimpo COMBARY [1]; Alexis KABORE [2]; Fabio BERTI [3]

[1]Economics and Management Department, Thomas Sankara University, Ouagadougou, Burkina Faso
[2]Sociology Department, Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso
[3]Economics and Rural Development Unit, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium
*Corresponding author: soumailasawadogo91@yahoo.fr

## ABSTRACT

This paper attempted to analyse determinants of the technical efficiency of beekeepers in villages impacted by the creation of elephant corridor called Corridor No. 1 of the Pô-Nazinga-Sissili protected area complex in southern Burkina Faso. The data used in this analysis were collected from a sample of 52 beekeepers in July and August 2018. A Cobb-Douglas type honey production function with inefficiency effects was estimated for this purpose. The results showed that 75% of the discrepancy between potential and actual honey production would be due to beekeepers' technical inefficiency and that the average score of beekeepers' inefficiency effects was 0.78. It should be noted that the location of the hives, the number of years of beekeeping training received as well as the possession of a beekeeping suit are the significant factors that increase the efficiency level of the beekeepers. On the other hand, membership to a beekeepers' association has a negative effect on honey production. The results highlight that locating hives within one kilometer of the elephant corridor may significantly improve the technical efficiency of the beekeepers. However, the survival of these pachyderms is threatened because of human reprisals against them following their possible overflow into the riparian villages. Thus, an effective and sustainable policy aiming at both the conservation of the forest and an improvement of the incomes of the riparian households could be implemented by encouraging beekeepers to locate their hives next to the protected forests. This should increase their yields and it could maintain elephants within forests.

**Keywords:** *Beekeeping, technical efficiency, protected areas, Burkina Faso.*

## INTRODUCTION

Like other sub-Saharan African countries, the fight against poverty is a concern for Burkina Faso. According to the National Institute of Statistics and Demography (INSD), the monetary poverty incidence in Burkina Faso stood at 47.50% in rural areas compared to 13.70% in urban areas in 2014 (INSD, 2020). Rural populations combine several livelihood sources to overcome their economic and structural problems. Exploitation of non-timber forest products (NTFPs) constitutes an opportunity to diversify their income and subsistence sources. According to the Food and Agriculture Organization of the United Nations (FAO), a NTFP is defined as any product of biological origin other than wood originating from forests, other wooded land and other trees outside forests (FAO, 1999). As stated by Angelsen et al. (2014), many NTFPs including honey also contribute to building rural households' resilience to food and monetary shocks.

According to the Technical Secretariat for Beekeeping (STA) of Burkina Faso, the country recorded an annual production of nearly 500 tonnes on average over the period 2011-2015 (STA, 2019). This production reached the maximum of 1000 tonnes in 2018 with a contribution of about 9% to the agricultural gross domestic product (INSD, 2020; STA, 2019). Nevertheless, the practice of modern beekeeping in the country seems to be at embryotic stage and occupies less than 1% of the national population. In 2019, only 16,261 beekeepers with 132,057 hives were identified, 82% of which were traditional against 18% modern, with a total production of 565.6 tonnes of raw honey. It was remarked that two traditional hives provide the same yield as one modern hive, and the yield per modern hive remains below 15 kg of raw honey (equivalent to 6 litres). This yield is much lower than what is observed in many other countries over the world, such as Madagascar, where the yield per hive goes to 50 litres (Lagarde et al., 2004).

Beekeeping yields seem to vary with vegetation cover in Burkina Faso. A study on honey production in villages bordering the Pô-Nazinga-Sissili Protected Areas Complex (PONASI-PAC) found that beekeeping yields are low compared to the national average. The quantity of honey produced per hive oscillates between 6 and 12 litres [Association Nature et Développement (NATUDEV, 2017)]. This is why an investigation into the causes of low beekeeping yields as technical efficiency of beekeepers is still of current interest for the economic development of the villages around the PONASI-PAC. From a theoretical point of view, a production unit is said to be efficient if it produces the maximum possible output from its basket of inputs or if it can produce a given quantity of output by using the minimum quantity of inputs (Atkinson and Cornwell, 1994). Hence, the question of the present research is: what are the determinants of the technical efficiency of beekeepers in the villages bordering the PONASI-PAC corridor No. 1? The objective of this research is to identify the determinants of the technical efficiency of beekeepers in the vicinity of the PONASI-PAC corridor No. 1. Our research hypothesis states that the location of hives near forests improves the technical efficiency of beekeepers.

## MATERIAL AND METHODS

Theoretical framework of technical efficiency analysis

Concept of technical efficiency was initiated in the mid-twentieth century by Debreu (1951) and Farrell (1957). At firm level, technical efficiency is estimated through either the cost frontier, the profit frontier or the production frontier. The present research is based on the production frontier of beekeepers. In economic theory, two categories of approaches are used to specify the production frontier, namely non-parametric approach and the so-called parametric approach (Kumbhakar et al., 2015).

Non-parametric approaches do not impose any particular specification on the production function. They have the advantage of being able to consider several outputs and inputs at the same time in a specific analysis (Ambapour, 2001). This category of approaches includes the Data Envelopment Analysis method and the Free Disposal Hull method. However, these approaches do not take into account the effect of measurement errors and random shocks in the evaluation of the technical inefficiency of producers.

As for parametric approaches, the focus is on econometric techniques for estimating production boundaries. They consist of an econometric estimation of the best practice frontier. These approaches can be stochastic (respectively deterministic) if there is presence (respectively absence) of a stochastic term capturing the hazards' effect not controllable by the producer in its production frontier. Regardless the limitations or advantages associated with each approach, the stochastic frontier approach is adopted in this research. This approach was introduced by Aigner et al. (1977) and Meeusen and Van Den Broeck (1977). These authors admit that the error term has two components: a component attributable to the producer noted $u_i$ and another component which captures the effect of hazards on the output noted $v_i$. It is the $u_i$ component that measures technical inefficiency. Thus, production frontier expression is given by equation (1).

$$y_i = f(x_i, \beta) e^{v_i - u_i} \quad (1)$$

where $\beta$ is a vector of unknown parameters; $f(x_i, \beta)$ is the production function; $x_i$ represents the inputs used by producer $i$. The interest of the analysis of technical efficiency by the stochastic production frontier approach is not only limited to the evaluation of efficiency levels. It allows also identification of the factors likely to influence this technical efficiency (Kumbhakar et al., 2015). For this purpose, authors such as Battese and Coelli (1995) and Abedullah et al. (2007) have formulated the technical inefficiency function as expressed in equation (2).

$$u_i = z_i \alpha + w_i \quad (2)$$

where $z_i$ is a vector of variables explaining the producer's inefficiency $u_i$; $\alpha$ is the vector of unknown parameters and $w_i$ is a random term representing the estimation errors of technical inefficiency. In equation (1), $f(x_i, \beta)$ can generally take several

forms, of which the Cobb-Douglas and translogarithmic forms are the most commonly used. Unlike the Cobb-Douglas function, the translogarithmic function allows to see the joint effect of the production factors taken two by two on the observed output. In practice, a test for the functional form choice of the production function and a test for the existence of technical inefficiency are usually performed in technical efficiency analyses. In our analysis, the results of these tests indicate that a Cobb-Douglas specification of the production frontier is appropriate and the assumption of no inefficiency in the model is rejected. This specification has been used by many authors such as Ahmed et al. (2014).

Data and Empirical Model

This research used cross-sectional primary data related to the 2017-2018 beekeeping season. They were collected in July and August 2018 using a structured questionnaire administered to the totality of 52 modern beekeepers registered. The study area consists of six villages (Bourou, Kollo, Oualem, Saro, Tiakané, and Yaro) impacted by the creation of the PONASI-PAC Corridor No. 1. This corridor permits elephants to move from the Pô National Park to the Nazinga Ranch and back. Table 1 summarises the definitions of the study variables, their expected effects on the explained variable (honey production or technical inefficiency), and the authors whose findings support directly or indirectly these effects.

Table 1. Description of the variables of the stochastic production frontier

| Variable | Unit | Definition | Expected sign | References |
|---|---|---|---|---|
| | | *PRODUCTION FRONTIER* | | |
| PRODUCT | Litre | Volume of honey produced by the beekeeper | | |
| RUCHE | Natural number | Number of hives used by the beekeeper | + | |
| MO | Hour | Time taken by the beekeeper to monitor his hives and harvest the honey | +/- | |
| | | *TECHNICAL INEFFICIENCY* | | |
| ALPHABE | Binary | 1=the beekeeper is literate and 0 otherwise | - | Abedullah et al. (2007) |
| FORMAAPIC | Natural number | Number of years the beekeeper has been trained in beekeeping | - | Becker (1964) |
| APGROUP | Binary | 1=the beekeeper is a member of a beekeeping association and 0 otherwise | - | Chebil et al. (2013) |
| POSCOMB | Binary | 1=the beekeeper has his own beekeeping suit and 0 otherwise | - | |
| FORET | Binary | 1= the beekeeper has hives within 1 km of PONASI-PAC forests and 0 otherwise | - | Nombre (2003) |

*Source: Authors' elaboration based on field survey data

The empirical model for investigating the technical efficiency of beekeepers is based on the specification of equations (1) and (2). In equation (1), we consider two production factors which are the number of modern hives possessed by each beekeeper noted RUCHE and the time that this beekeeper devotes to the follow-up of his hives and the harvest of honey noted MO with a Cobb-Douglas specification of $f(x_i, \beta)$. For equation (2), the inefficiency variables group the beekeeper's literate status noted ALPHABE, the number of years in which he considers he has benefited from beekeeping training noted FORMAAPIC, his membership in an association noted APGROUP, his own beekeeping suit endowment noted POSCOMB and the proximity of his hives to the components of PONASI-PAC noted FORET during the 2017-2018 beekeeping season. Thus, the empirical model combines equations (3) and (4).

$$ln\ \mathrm{PRODUCT}_i = \beta_0 + \beta_1\ ln\mathrm{RUCHE}_i + \beta_2\ ln\mathrm{MO}_i + v_i - u_i \quad (3)$$

$$u_i = \alpha_0 + \alpha_1\mathrm{ALPHABE}_i + \alpha_2\mathrm{FORMAAPIC}_i + \alpha_3\mathrm{APGROUP}_i + \alpha_4\mathrm{POSCOMB}_i + \alpha_5\mathrm{FORET}_i + w_i \quad (4)$$

Following Combary and Savadogo (2014) and N'Gbo (1994), the one-step maximum likelihood estimation approach is used in the present research. In practice, the *sfmodel* command developed by Kumbhakar et al (2015) with the Stata 16.0 package is applied.

## RESULTS AND DISCUSSION

Results show that the annual average quantity of honey produced in the six villages is about 36.5 litres per beekeeper. The least diligent beekeeper produced five litres while the most diligent beekeeper produced 270 litres per annum. The average number of hives per beekeeper is five. Along a year, the working hours for the monitoring and the harvest of honey are 50.93 on average per beekeeper. On average, the beekeepers have received at least one beekeeping training over three years. Results indicate also that, 76.9% of beekeepers are literate, and 76.9% are members of a beekeeping association. In addition, only 59.6% of beekeepers use their own beekeeping suits. Only 38.7% of the beekeepers have placed their hives within one kilometre of the PONASI-PAC.

Results from econometric analysis (Table 2) show that gamma (γ) equals to 0.751 and it is statistically significant. Thus, 75,10% of the difference between the potential yield of honey and the current yield would be attributable to technical inefficiency of the beekeepers. Only 24.90% of this difference would have been caused by factors that cannot be controlled by the beekeepers. Besides, the estimated coefficients of the honey production function are significantly different from zero. As these coefficients are directly interpreted in terms of elasticity, an increase of 1% in the number of hives leads to an increase of 1.238 % in the quantity of honey produced, *ceteris paribus*. This result was expected and conforms to the reality of the beekeeping activity. In the same way, an increase in working time of 1% leads to a decrease of 0.752% in the quantity of honey produced by the beekeepers, *ceteris paribus*. This is also aligned to the reality of

beekeeping activity. More working hours would expose the beekeeper to insect attacks and may provoke bee desertion from the hives. It may also increase bee mortality if the beekeeper tries to take a lot of time during visits and harvesting, as bees do not like to be disturbed. The beekeeper must therefore optimise the time he spends on his hives.

The Table 2 shows also results from econometric estimation of the beekeeper technical inefficiency model. The results show that four of the five socio-economic characteristics of the beekeepers are found to be the primary factors explaining significantly the beekeepers' technical inefficiency. As it was expected, the number of years of training received, the use of one's own beekeeping suit as well as the location of the hives near the components of the PONASI-PAC influence negatively the technical inefficiency. This implies that these factors affect positively the technical efficiency of the beekeepers and honey production. On the other hand, and in contrast to our expectations, the membership to a beekeepers' association influences positively the beekeeper's inefficiency and, consequently, it affects negatively technical efficiency and honey production. Our results point also to a positive but not significant effect of beekeeper's literate status on technical inefficiency and honey production.

Table 2. Estimation of the stochastic Cobb-Douglas production frontier of beekeepers

Notice that (i) standard errors are in parentheses and; (ii) *** p<0.01, ** p<0.05, * p<0.1

| VARIABLES | frontier | usigmas | vsigmas |
|---|---|---|---|
| ALPHABE | | 1.945 | |
| | | (1.195) | |
| FORMAAPIC | | -1.281*** | |
| | | (0.480) | |
| APGROUP | | 2.098* | |
| | | (1.186) | |
| POSCOMB | | -1.975** | |
| | | (0.914) | |
| FORET | | -1.907** | |
| | | (0.958) | |
| ln (RUCHE) | 1.238*** | | |
| | (0.221) | | |
| ln (MO) | -0.752*** | | |
| | (0.173) | | |
| Constant | 0.051 | -1.004 | -2.106*** |
| | (0,083) | (1.249) | (0.261) |
| Observations | 52 | Wald chi2(2) | 33.08*** |
| Log likelihood | -29.426 | Gamma (γ) | 0.751*** |
| Average efficiency score | 0.778 | | |

Source: Authors' elaboration based on field survey data

Following Battese et al. (1989), results from econometric analysis show that the average efficiency score is estimated to 0.778 in our study. This means that beekeepers produce on average 77.8% of the maximum (or potential) output. It implies that honey production can be increased by 22.2% by a better allocation of available inputs, without the need for additional amounts of inputs. In this study, the technical efficiency among beekeepers ranges from 19.3% to 100%.

## CONCLUSION

This paper analyses technical efficiency of beekeepers in rural Burkina Faso. A stochastic Cobb-Douglas production function was specified and estimated for identifying the determinants of honey production function and assessing the sources of technical inefficiency of beekeepers in the surroundings of PONASI-PAC Corridor No.1. Our research results highlighted that 75% of the difference between the potential yield of honey and the current yield would be due to technical inefficiency of the beekeepers. Also, an average efficiency score of 0.778 was estimated. The location of the hives next to the protected forests, the number of years of beekeeping training received and the possession of a beekeeping suit are the factors that increase the technical efficiency level of beekeepers. The results showed also that belonging to a beekeepers' association has a negative effect on the production of honey.

In view of the results obtained, the implications in terms of economic policies are the following: first, beekeepers should be encouraged to place their hives near forests. Secondly, it should be ensured that each beekeeper has a beekeeping suit. Finally, beekeepers should be trained or retrained annually.

## ACKNOWLEDGEMENT

We thank all the beekeepers and our teachers and colleagues who contributed to this study. The study was supported by the Research for Development Project on the Pô-Nazinga-Sissili ecological complex in Burkina Faso, funded by the Academy of Research and Higher Education (Belgium).

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**Original Scientific paper**
10.7251/AGRENG2103094P
UDC 631.11:338.43.01(497.11)

# ATTITUDES OF FARMERS FROM THE AREAS WITH NATURAL CONSTRAINTS TOWARD AGRICULTURAL AND RURAL DEVELOPMENT SUPPORT

Ružica PAPIĆ*, Natalija BOGDANOV

Institute of Agricultural Economics, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
*Corresponding author: papic.ruzica@agrif.bg.ac.rs

## ABSTRACT

Farmers and rural areas, especially those with natural constraints, face a number of challenges, such as lower yields, difficult access to markets, depopulation and devastation of the rural environment. Agricultural policy in Serbia does not provide a special measure for farmers in these areas which is opposite to the practice in the European Union. Nevertheless, farmers in Serbia can benefit from measures that have a specific treatment for farms located in the areas with natural constraints. The aim of this paper is to examine the attitudes of the farmers in the areas with natural constraints toward agricultural and rural development support in Serbia. Data collection was organized using a stratified simple random sampling and it included 371 farms. Face-to-face interviews were conducted during July-August 2018 in the mountainous area of East and South Serbia. The questionnaire contained information about socio-economic characteristics of the farms, attitudes on agricultural and rural policy and future plans. The data were analyzed using descriptive statistic method (measures of central tendency and variability). The results indicate that almost all farmers use direct payments and have enough information and experience to apply for this support. On the other hand, the research reveals a low level of application of rural development support especially for measures aimed at the improvement of the quality of life and diversification of the farm income, as well as measures for environmental improvement. Results provide information for policymakers that can be useful for creating more efficient rural development support aimed at farmers in the areas with natural constraints.

**Keywords:** *areas with natural constraints, subsidies, attitudes, Serbia.*

## INTRODUCTION

The idea of a support scheme for farmers in areas with natural constraints (ANC) in the European Union (EU) is to compensate the farmers for lower income they derive from agriculture production. ANC scheme is designed to maintain agriculture and population levels in rural areas, as well as preserve the environment. In Serbia, some policy instruments have elements that indicate

specific treatment of farms in areas with difficult working conditions in agriculture (ADWCA), but there is no particular measure for farmers in ADWCA. Farmers in ADWCA are supported by dairy premium (lower threshold of milk delivered to dairies) and payments for quality breeding sheep and goats (lower threshold for minimal number of animals on farm). Regarding rural development support, farmers in ADWCA are supported by a bigger share of grant in the total value of the investment (measures for improving competitiveness and measures for the improvement of quality of life and diversification of the farm income). Measures aimed at improving the environment and the countryside do not treat farmers in ADWCA in a specific way. Bogdanov (2014) and Vidojević et al. (2017) highlight that the set of support measures for farmers in ADWCA in Serbia is far from the practice in the EU in terms of policy objectives and instruments for its implementation. Namely, the existing measures are more oriented to economic and social issues, while the environmental ones are not in focus. Also, the delimitation of ADWCA is not aligned with the existing approach in the EU (European Commission, 2013). In Serbia, the criteria used for ADWCA delimitation include all settlements above 500 m a.m.s.l, villages within nature parks, and villages on the territory of municipalities with less than 100 employees/1,000 inhabitants (The Government of the Republic of Serbia, 2016). Since 2019, the third criterion has been changed and now covers the territory of devastated municipalities (The Government of the Republic of Serbia, 2018). However, large ratio of farms in Serbia could benefit from specific support to farms in ADWCA, since according to the ADWCA delimitation from 2016, 40% of the territory, 30% of the total population, 29% of farms, and 24% of the UAA is located in these areas (Statistical Office of the Republic of Serbia, 2012).

This paper aims to investigate farmers' attitudes toward agricultural and rural development support. Attitudes about certain phenomena are studied with the assumption that they can predict future behavior. According to Ajzen (2011), a positive attitude leads to an intention to implement certain behaviors. Therefore, it is expected that understanding the attitudes of farmers from ADWCA will provide some knowledge about motivational factors and barriers related to applying for available support. Also, these findings are important because in Serbia there is no evidence on the number of applications coming from ADWCA and how current support is adjusted to the specific needs of farmers in ADWCA.

## MATERIAL AND METHODS

The paper is focused on the results obtained in July-August 2018 through a face-to-face survey in the mountainous area of East and South Serbia (Papić, 2021). Several reasons influence the choice of the research area:

1. Delimitation of ADWCA in Serbia is aligned with the EU definition only in one criterion which refers to mountain areas (The Government of the Republic of Serbia, 2016);

2. Mountain areas occupy 89% of settlements, 74% of farms and 73% of the UAA of the total ANCs territory (Statistical Office of the Republic of Serbia, 2012); and
3. Previous research in Serbia found that mountain areas of East and South are rich in natural resources that are important for the development of the local rural economy and preservation of biodiversity (Bogdanov et al., 2008; Đorđević-Milošević and Milovanović, 2012).

Data collection was organized using a stratified simple random sampling. The sample included 371 farms. Sample selection criteria, that ensured the survey covers economically and demographically viable farms, were: 1. rural households had at least three members and 2. one member of households was younger than 50 years of age (Papić, 2021). Similar selection criteria were used in previous rural research in Serbia (Kotevska, 2015; Bogdanov, 2007). Therefore, the sample included farmers that would probably stay in the agricultural sector and who will be the beneficiaries of agricultural policy measures. The questionnaire contained information about socio-economic and structural characteristics of farms, attitudes on agricultural policy and future plans. Analysis was focused on questions about application and attitudes toward agricultural and rural development support. These attitudes were measured through a 5-point Likert scale (1 – strong disagreement and 5 – strong agreement). Also, the paper contained analyses of socio-economic characteristics of farms. The data were analyzed using descriptive statistics method: percentage response distributions; measures of central tendency – average value (hereinafter AV) and median (hereinafter Me); dispersion measures – standard deviation (hereinafter SD). The results are presented in the figures and tables.

## RESULTS AND DISCUSSION

*Socio-economic characteristics of surveyed farmers*

The average farm size in the sample is 12.7 ha, but high values of standard deviation (±9.9) indicate large variability in the data. The analysis of the sample showed that all farms have areas under permanent grasslands (meadows and pastures), as well as a high share of areas under arable land (97.6%). A quarter of the surveyed farms has areas under orchards and vineyards. On average, the farm in the study area owns 6.4 ha under arable land; 6.1 ha under meadows and pastures; and 0.8 ha under orchards and vineyards. High values of standard deviation are expressed in all land categories, especially in pastures (± 13.9) (Table 1). Cattle and sheep are the two main animal productions in ADWCA (Table 1). The average number of cattle is equal to the national average (6.3 Livestock unit – LSU/farm) and higher than the average of the Southern and Eastern Serbia region (4.2 LSU/farm) (Statistical Office of the Republic of Serbia, 2018). The situation is different with sheep production. The average number of sheep on farm is smaller than the national average (i.e., 3.2 LSU/farm in relation to 13.0 LSU/farm) as well as the average of the Southern and Eastern Serbia region (11 LSU/farm) (Statistical Office of the Republic of Serbia, 2018).

Table 1. Main characteristics of surveyed farms

| Indicators | AV | SD | Me | % farms in the sample that own listed resources |
|---|---|---|---|---|
| Utilized agricultural area UAA (ha) | 12.5 | ±9.9 | 10.0 | 100.0 |
| Arable land (ha) | 6.4 | ±5.2 | 5.0 | 97.6 |
| Permanent grassland (ha) | 6.1 | ±8.2 | 4.0 | 100.0 |
| Land under permanent crops (ha) | 0.8 | ±0.9 | 0.4 | 23.5 |
| Total Livestock Unit (LSU) | 8.5 | ±7.5 | 6.4 | 100.0 |
| Cattle (LSU) | 6.3 | ±5.7 | 4.7 | 87.8 |
| Sheep (LSU) | 3.2 | ±4.4 | 1.3 | 64.9 |
| Pigs (LSU) | 1.4 | ±2.0 | 1.1 | 43.2 |
| Goats (LSU) | 1.2 | ±1.7 | 0.5 | 14.6 |

Note: The total number of livestock units includes cattle, sheep, goats, pigs, poultry and horses.

*Source: Author's calculation based on the survey data; Papić, 2021

The majority of farm holders in ADWCA are men. However, the percentage of women holders is higher than the national average (19.9%) as well as the average of the Southern and Eastern Serbia region (21.9%) (Statistical Office of the Republic of Serbia, 2018). Previous research explains that a high percentage of women holders in this region is caused by the unfavorable age structure of holders, longer life expectancy of women and higher share of small farms whose holders are mostly women (Bogdanov and Babović, 2019). Farmers are with a low level of education, given that a quarter of the respondents has only primary education (Table 2), which is a great limitation for the expansion and penetration of new technologies into the agricultural sector.

Household income is an important determinant of livelihood diversification (Piennar and Traub, 2015). For about 60% of the holders (regardless of gender and age), agriculture is the most important household income. These results indicate that for farmers in ADWCA, agriculture is an important factor of social security and food supply. Salaries represent the most important income for around 20% of households, but it is important to note that 54% of households have members who have employment outside agriculture. This finding shows the importance of off-farm activities, especially for those households that cannot earn enough income from agriculture, as well as for those who want to reach a higher standard of living. Similar findings are confirmed in previous research in the Southern and Eastern Serbia region (Papić and Bogdanov, 2015). The most important income from agriculture is the sale of animal products (usually milk and cattle), while the sale of plant products as well as processed products have a modest share in responses (Table 2). According to Bogdanov (2007) farms, with significant sale of livestock and milk are those whose land does not provide sufficient competitiveness in farming, as well as farms with high hidden unemployment.

Table 2. Farmers profile and main characteristics of households

| **Indicators** | **Men holder** | **Women holder** | **Young holder (less than 40 years)** |
|---|---|---|---|
| Farm holder (%) | 74.1 | 25.9 | 11.1 |
| Age (AV±SD; Me) | 53.7±12.2; 53.0 | 56.7±1.3; 57.0 | 34.3±0.6; 35.0 |
| Primary education; Secondary school; College and University (%) | 21.2; 75.5; 3.3 | 36.5; 58.3; 5.2 | 4.3; 89.4; 6.4 |
| Main household income (%): | | | |
| Agriculture | 66.1 | 60.4 | 68.1 |
| Salaries | 24.8 | 24.0 | 23.4 |
| Pensions and social benefits | 9.1 | 15.6 | 8.5 |
| Main farm income (%): | | | |
| Sale of animal products | 80.3 | 82.3 | 87.2 |
| Sale of plant products | 9.1 | 7.3 | 6.4 |
| Sale of animal and plant processed products | 4.7 | 8.3 | 4.2 |
| Others | 5.8 | 2.1 | 2.2 |

Source: Author's calculation based on the survey data; Papić, 2021

*Attitudes toward agricultural and rural development support*

This survey shows that farmers from ADWCA predominantly use direct payments support. Of the total number of surveyed farmers, 90.3% use incentives for plant production (payments for field crops and permanent crops); 68.6% incentives for quality breeding dairy cows and 59.2% for dairy premium. All other types of incentives are used by less than 20.0%. The reason for the low applications for incentives for quality breeding sheep and goats is the existence of animals without registered pedigree (Figure 1). Only 12.9% of farmers from the sample applied and received rural development support. Of the total respondents, 9.2% used measures for improving competitiveness (mostly insurance premium subsidy and measures for improving physical assets). Incentives for environmental improvement (organic production and preservation of plant and animal genetic resources) were used by an extremely small percentage of farmers, located in the area of Stara Planina.

Incentives for income diversification and improvement of quality of life are rarely used in the research area (just one farmer applied for this support). Although income diversification and improvement of quality of life in rural areas is encouraged through various support schemes, the results show that in areas where infrastructure is not developed and farm holders do not have enough skills and resources, it is difficult to implement this group of measures. Therefore, the criteria and thresholds of this investment support must be reviewed.

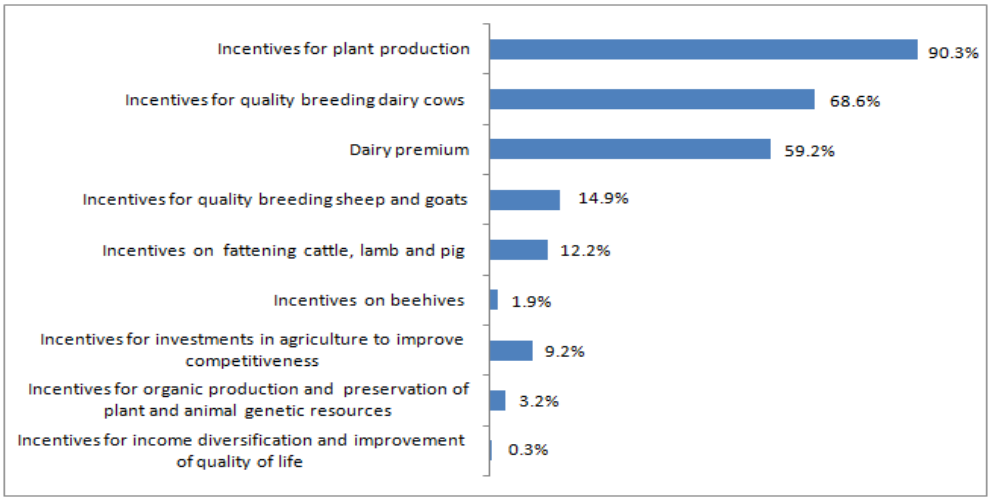


Figure 1. Subsidies used by farmers in ADWCA in 2018.

Note: Since farmers can use more than one measure, percentage of application exceeds 100%.

*Source: Author's calculation based on the survey data; Papić, 2021

A large percentage of surveyed farms are considered dependent on subsidies to maintain existing production (Figure 2). Previous research conducted by Kotevska et al. (2015) also indicated a high dependence of farmers in Serbia on subsidies (26.3%), but not to the extent that was found in this research (61.0%). Subsidies help farmers maintain current agricultural production, but do not encourage investment and structural change in rural areas. Irazioz et al. (2007) argued that high levels of direct payments dampen pressures for restructuring rather than stimulating improvements in productivity.

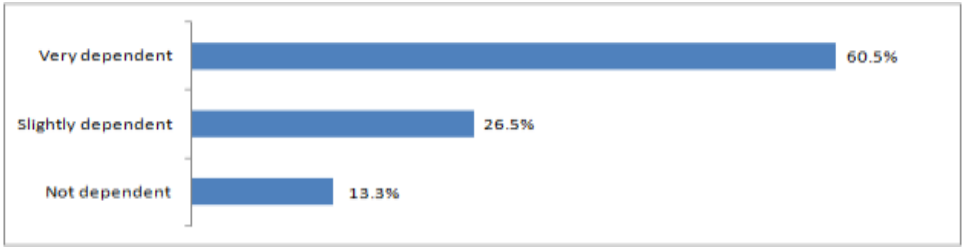


Figure 2. Dependency on subsidy

*Source: Author's calculation based on the survey data; Papić, 2021

Understanding the attitudes of farmers from ADWCA provides information about their knowledge and barriers related to the application for available support. In ADWCA, the level of familiarity with direct support measures is higher (mean 3.4), than familiarity with rural development support (mean 2.8). This finding is not surprising given that a very small percentage of farmers use rural development support. In addition, farmers do not have enough means to co-finance rural

development investments (Table 3). Results show the need for mechanisms that will help farmers to overcome problems with the preparation and administration of rural development support and access to finances.

Table 3. Attitudes of farm holders towards subsidies

| **Attitudes** | **AV** | **SD** | **Me** |
|---|---|---|---|
| *Direct payments support* | | | |
| My knowledge and experience is enough to independently prepare the application | 3.4 | ±1.4 | 4.0 |
| *Rural development support* | | | |
| My knowledge and experience is enough to independently prepare the application | 2.8 | ±1.4 | 3.0 |
| I have enough own means to co-finance a rural development investment | 2.9 | ±1.4 | 3.0 |
| I am able to get bank credit to co-finance a rural development investment | 3.1 | ±1.3 | 3.0 |

Source: Author's calculation based on the survey data; Papić, 2021

It is highly likely that the farmers from the sample will stay in agriculture, though most do not know who will take over their farm and continue the agriculture production (Table 4). However, their intention to apply to rural development support and get credit to co-finance rural development investment is weak (mean 2.8). The intention to invest on the farm in the next 3–5 years is strong (mean 4.0). The planned investments are low-risk mainly in the purchase of equipment and the extension of the current production.

Table 4. Attitudes of farm holders towards future plans

| **Attitudes** | **AV** | **SD** | **Me** |
|---|---|---|---|
| Identified successor | 3.8 | 1.4 | 4.0 |
| Intention to keep agricultural production next 3-5 years | 4.5 | 0.9 | 5.0 |
| Plan to invest in the next 3-5 years | 4.0 | 1.2 | 4.0 |
| Intention to apply for direct payments support in next 3-5 years | 4.5 | 1.0 | 5.0 |
| Intention to apply for rural development support in next 3-5 years | 3.5 | 1.3 | 3.0 |
| Intention to get credit to co-finance rural development investment | 2.8 | 1.3 | 3.0 |

*Source: Author's calculation based on the survey data; Papić, 2021

## CONCLUSION

The research confirms the general attitudes of farmers toward direct payments as positive. There are no difficulties when it comes to collecting documentation and information, but delays in payments make the production process more difficult, especially for those farms for which agriculture is the only source of income. Considering that farms in ADWCA plan to use this type of payment in the future, it is desirable to introduce mandatory conditions related to the preservation of natural landscapes for developing other functions of rural areas which is a practice in EU.
Farmers in ADWCA are not sufficiently informed about rural development measures, and complicated procedures discourage them from applying for support.

Also, farmers are not sure whether they have enough funds to co-finance the investment themselves and they are not ready to take a loan. Research findings show that the existing rural development measures (that provide benefits for farmers in the ADWCA) are not adjusted in accordance with their specific needs. For example, support for income diversification that could help farmers to start new productions and create new activities on their farm, in the way in which it is now being implemented, is not available to farmers from the researched area. Therefore, it is imperative to develop mechanisms and support farmers in overcoming these barriers. Desired action can be the simplification of the application procedures, providing technical assistance and facilitating access to finance.

## ACKNOWLEDGMENT

This paper is a result of the research funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia based on the agreement between the Ministry and the Faculty of Agriculture, University of Belgrade (Contract No.451-03-9 /2021-14/ 200116) on the realization and financing of scientific research in 2021.

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**Original Scientific paper**
10.7251/AGRENG2103103G
UDC 634.11:664.1.03

# EVALUATION OF POLYPHENOL EXTRACTION FROM APPLE POMACE

Faraja Deo GONELIMALI[1, 2*], Beatrix SZABÓ-NÓTIN[1], Lilla SZALÓKI-DORKÓ[1], Ákos RIBÁRSZKI[1], Mónika MÁTÉ[1]

[1]Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology, Department of Vegetable and Fruit Processing Technology, Hungary
[2]University of Dar es Salaam, College of Agricultural Sciences and Food Technology, Department of Food Science and Technology, Dar es Salaam, Tanzania
*Corresponding author: fgonelimali@gmail.com

## ABSTRACT

Food industry is searching for natural additives because consumers demand natural, safe and environmentally sound food additives. The use of different plant extracts such as grape pomace and cranberry extracts which have antimicrobial effect in food preservation has gained an increasing interest. In the latest years many researches deal with investigation of the antimicrobial effect of apple pomace extracts. Apple pomace, a residue from apple juice production, contains high amount of polyphenols which are known to have antioxidant effect. Apple pomace is a by-product of the apple juice and cider processing industry and represents about 20–35% of the original fruits. It can be used for value-added products, because it is rich in pectin, fibre, macro and micro elements and antioxidants mainly, polyphenols. Previous studies indicate that the apple pomace has high phenolic content and antioxidant activity and thus can be regarded as a valuable source of antioxidants and bioactive compounds. The contents of phenolic compounds vary greatly among different varieties of apples. Moreover, apple peels contain higher concentration of phenolic compounds compared to flesh. Conventional production of apple juice results in a juice with poor phenolic content and with only 3–10% of the antioxidant activity of the fruits that they are produced from. Thus, leaving huge amount of the phenolic compounds in the pomace. Because of this, studying extraction method for extracting high amount of polyphenol from the pomace is important. In this paper apple pomace was dried with different drying method (atmospheric and vacuum ovens, 80° C and 60°C). Extraction was performed using distilled water as solvent, and the extracts were evaluated by the colour, water soluble sugars, total polyphenol content (TPC) and antioxidant capacity.

**Keywords**: *Food waste, Apple pomace, Drying, Extraction, Antioxidant activity.*

## INTRODUCTION

Apples (*Malus domestica* Borkh) can be processed into numerous commercial products, such as apple juice, apple compote, jams and jellies. Apple juice is loved by many people all over the word. Apples contains a lot of polyphenols and they are retained in the pomace during juice processing (Van Der Sluis *et al*., 2002; Van Der Sluis *et al.*, 2004).

Apple pomace is the solid residue that remains after milling and pressing apples for juice (Givens *et al*., 1987; Kafilzadeh *et al.*, 2008). It contains flesh and skin (94.5%), seeds (4.1%) and stems (1.1%) (Linskens & Jackson, 1999, Sudha *et al.*, 2007) and represents 20–35% of the fresh weight of the apples (Rabetafika *et al*., 2014). According to the amount of raw apple processed, 3.3 million tons of waste are produced per year.

Apple pomace has a variety of utilization, such as production of ethanol, methane, pectin, citric acid, fibres, direct burning, food additives (pomace in jams, sauces, confectionery products, toffees and bakery products), and livestock feed (Shalini *et al*., 2010; Wang & Thomas, 2008).

Fresh apple pomace spoils quickly, since they contain large amount of water and because of this, drying is necessary. Drying of the pomace is an important technical step required during the extraction process where most of the bioactive compounds are extracted better on dried pomace rather than fresh (Jung *et al*., 2015).

In recent years, interests in extracting phenolic compounds from apple pomace has increased. The pomace can be used as cheap alternative source of polyphenols (Bhushan *et al*., 2008; Cetkovic *et al*., 2008; McCann *et al.*, 2007; Soler *et al.*, 2009). Several studies have shown that the phenolic compounds from the pomace are extractable using organic solvents, such as methanol, acetone and ethanol (Ajila *et al.*, 2011; Hayat *et al*., 2010 Reis *et al.*, 2012; Suárez *et al*., 2010; Van Der Sluis *et al*., 2004). However, water can also be a suitable solvent in extracting phenolic compounds from the apple pomace (Çam & Aaby, 2010; Reis *et al*., 2012, Vincent *et al.*, 2015). It has a lot of advantages: cheap, non-toxic, easily obtained and there is no aversion from the consumers.

The objective of the present paper is to evaluate the effect of drying method on polyphenol extraction from the apple pomace.

## MATERIAL AND METHODS

Apple pomace from industrial juice production were obtained from Agrana Juice Ltd (Hungary).

Drying was done using the conventional oven (LP 232/1, Hungary), 200g of the pomace were spread in a drying tray; 0.5 cm pomace depth layer. Trays were then taken to the oven and dried at 60°C and 80°C and the moisture content was being monitored every hour. For a vacuum oven drying, 200g of the samples were first dried using a conventional oven to almost 10 percent moisture content at 80°C and 60°C. Thereafter, samples were dried using vacuum dryer at 60°C and a pressure of 65 mbar, moisture content was monitored every hour until reached 3 – 4 %. Dried samples were ground into fine powder using a "PRINCESS" multi chopper and

grinder and thereafter they were vacuum packaged until the day of extraction. Determination of moisture content was performed by using a MAC-50 rapid moisture analyzer (Radwag Waagen GMBH, Hilden, Germany). To determine the water activity, Novasina, LabMaster-aw equipment was used.

Pomace extracts were obtained by adding 15 g of apple pomace into deionised water (450 ml distilled water (1:30 w/v)) at room temperature. The mixtures were placed into a sonication bath (Bandelin, RK 52), at 35 kHz for 1 hour. Obtained solution was filtered by Whatman filter paper No.1, using vacuum pump. Solvent from the obtained filtrate was removed using rotary evaporator (IKA, Model: RW 10C S99) and further removed on circulating air oven (60°C) in a petri dishes. Weight of the obtained extracts was determined and diluted accordingly with distilled water to obtain a final extract solution with a concentration of 200mg $mL^{-1}$.

Total phenolic contents were determined using the Folin–Ciocalteu colorimetric method as described by Singleton & Rossi (1965). Briefly, 1250µL of Folin reagent (1:10 v/v Folin; distilled water) was added in the test tube followed by 150µL of methanol (4:1 v/v methanol; distilled water). Then, 100µL of the sample was added and allowed to stand for 1 minute, followed by the addition of 1000µL of sodium carbonate. The results were expressed in gallic acid equivalents (GAE, µg/mL pomace extract).

Antioxidant capacity was determined using Ferric Reducing Ability of Plasma (FRAP) assay (Benzie & Strain, 1996). FRAP reagent was prepared by using acetate buffer (pH 3.6), 2, 4, 6-tripyridyl-s-triazine (TPTZ), and $FeCl3 \times 6\ H2O$. The reagent and samples were mixed thoroughly in the tube. The absorbance was taken at 593 nm after 5 min. Results were expressed as ascorbic acid equivalent (mg ascorbic acid/mL extract) using an ascorbic acid standard calibration curve. Colour was determined according to C.I.E.LAB system using a tristimulus colorimeter (Konica Minolta CR 410, Minolta Canada Inc.).

Statistical analysis was performed using one factor complete randomized ANOVA using IBM SPSS version 20.

## RESULTS AND DISCUSSION

The data of drying apple pomace using atmospheric oven at different temperatures and atmospheric oven combined with vacuum oven is shown in Fig.1. Depending on operational conditions (atmospheric or vacuum), air velocity (1–2 m/s), temperature (60–105 °C), and amount of sample, apple pomace drying takes about 3–10 h (Wang et al., 2007). Raw apple pomace had an initial moisture content of 69 %, and drying procedure was continued until the final moisture content of the sample was reached (2.94 - 4.28%; 3 h and 6 h) (Table 1.). In case of atmospheric drying at 80°C during the first 2 h (to 11.67-17.17%) the moisture content of apple pomace decreased quickly. Thereafter, moisture content decreased slightly.

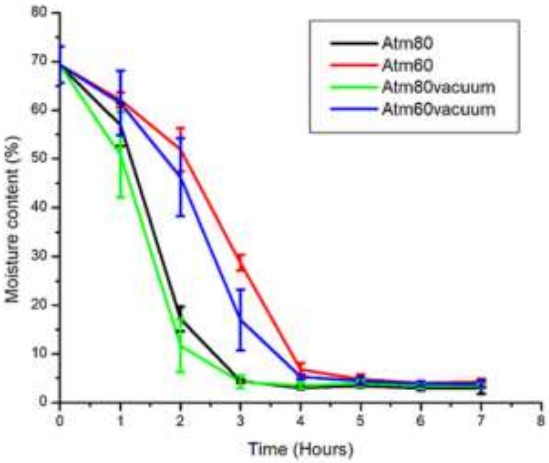


Figure 1. Decrease of moisture content during drying of the apple pomace (Gonelimali et al., 2021).

Atm80 and Atm60 stand for drying the pomace at 80 and 60 using conventional atmospheric oven respectively. Atm80vacuum and Atm60vacuum represent drying the pomace at 80°C conventional oven plus vacuum drying and 60°C conventional oven plus vacuum drying respectively.

Table 1. Shows time, final weight and percentage recovery after drying the pomace using a conventional oven and a combination of a conventional oven with vacuum drying to reach a moisture content of about 4%. The final weight was the highest in case of using 60°C atmospheric drying, and the smallest weight was obtained in case of 80°C atm+ Vacuum drying method, however, there was no significant difference. The recovery shows similar tendency. The highest recovery % was achieved using 60°C atmospheric drying, and the smallest recovery was in case of 80°C atm+ Vacuum drying method.

Table 1. Drying time, final moisture content, final weight and percentage recovery of the pomace using different drying method (Gonelimali et al., 2021)

| **Drying method** | **80°C atm.** | **60°C atm.** | **80°C atm. + Vacuum** | **60°C atm. + Vacuum** |
|---|---|---|---|---|
| **Time (hours)** | 3 | 6 | 3 | 6 |
| **Final Moisture content** | 2.94[a] | 4.28[a] | 3.42[a] | 3.82[a] |
| **Final weight (g)** | 41.92 ± 2.42 | 45.25 ± 1.26 | 39.97 ± 2.84 | 40.98 ± 2.34 |
| **Recovery (%)** | 20.96[a] | 22.63[a] | 19.99[a] | 20.49[a] |

Superscript letters, a: no different groups based on the statistical analysis by drying methods

The water soluble sugar content of the extracts were between 6.2-6.9%. According to the ANOVA results, the drying method had not significant effect on the water soluble sugar content (p=0.056).

Table 2 shows colour values of the extracts. L* defines the lightness of the pomace extracts. In case of Atm 80°C+vac drying method, the L* value is smaller than in the two other cases. In the case of a* values (green–red opponent colors, with negative values toward green and positive values toward red), values were in range of 2.51- 6.73.

The b* values (blue–yellow opponents, with negative numbers toward blue and positive toward yellow) were in the range of 5.75 - 14.83. For both values a* and b*, there is similar tendency as in the case of L*values. The effect of drying method on the colour values of the extracts was evaluated by one-way analysis of variance (ANOVA). The drying method had significant effect on colour values of the extracts (p=0.000).

Table 2. Color values of the extracts

| | **Atm_80** | **Atm_60** | **Atm_80_vac** | **Atm_60_vac** |
|---|---|---|---|---|
| **L*±SD** | $27.78\pm0.94^{c}$ | $32.00\pm1.19^{a}$ | $28.92\pm0.0^{c}$ | $30.47\pm0.21^{b}$ |
| **a*± SD** | $2.51\pm0.60^{c}$ | $6.14\pm0.07^{a}$ | $3.58\pm0.11^{c}$ | $6.73\pm0.10^{b}$ |
| **b*± SD** | $5.75\pm0.72^{a}$ | $14.55\pm2.17^{b}$ | $6.11\pm0.44^{a}$ | $14.83\pm1.07^{b}$ |

Superscript letters, a, b, c: indicate significance difference by drying methods

Table 3 contains the total phenolic content (TPC) of the apple pomace extracts. The TPC of the final apple pomace extract was the highest (479 μg $mL^{-1}$) in case of atmospheric drying at 80°C + vacuum drying, and was lowest when atmospheric 60°C was used (396 μg $mL^{-1}$). The effect of drying method and the TPC content of the extracts was evaluated by one-way analysis of variance (ANOVA). The drying method had no significant effect on TPC content of the extracts (p value at 95% confidence: 0.074).

Table 3. TPC content of the extract (μg $mL^{-1}$ extract)

| | **Atm_80** | **Atm_60** | **Atm_80_vac** | **Atm_60_vac** |
|---|---|---|---|---|
| **TPC±SD** | $445\pm107^{a}$ | $396\pm51^{a}$ | $479\pm16^{a}$ | $410\pm7^{a}$ |

Superscript letters, a: no different groups based on the statistical analysis by drying methods

Table 4 shows the antioxidant capacity of the apple pomace extract expressed in ascorbic acid equivalent (μg ascorbic acid/mL extract). The highest antioxidant capacity was obtained when the sample was dried at 80°C using a combined atmospheric and vacuum oven. Lowest value was determined in samples dried at

80°C atmospheric oven. According to the statistical analysis, drying method has significant effect on the antioxidant capacity (p values at 95% confidence: 0.000). This shows the importance of optimizing the drying method since there was no significant difference on recovery of the apple pomace as well as total phenolic content when dried using different methods. However, there was a significant difference on antioxidant activity which is an important properties of the extracts.

Table 4. FRAP values of the extract (μg AS $mL^{-1}$ extract)

| | **Atm_80** | **Atm_60** | **Atm_80_vac** | **Atm_60_vac** |
|---|---|---|---|---|
| **FRAP value ±SD** | $496±36^{b,c}$ | $426±86^{a,b}$ | $521±166^{c}$ | $374±79^{b}$ |

Superscript letters, a, b, c: different groups based on the statistical analysis by drying methods

## CONCLUSIONS

This work is an important starting point to valorize apple pomace, a very cheap and common by-product that is obtained in tons during processing of the apples to produce juice. It has been demonstrated that different drying method has an effect on antioxidant activity of the extracts from the apple pomace. Drying the pomace at 80°C using conventional oven in combination with vacuum dryer or without, results in polyphenols with higher amount of antioxidant activity when water is used as an extraction solvent. Apple pomace could be considered as a valuable type of by-products, a source of polyphenols, which could be suitable for the functional foods. Comparison of water as extraction solvent with other organic solvents, further purification of the apple pomace, identification and stability examination of the phenolic fractions are necessary to be performed in further studies. Apple pomace should be regarded as a valuable product and has potential as a value-added ingredient for functional foods as natural antioxidants and functional food ingredients (jams, jellies, juices and biscuits).

## ACKNOWLEDGMENT

This work was supported by EFOP-3.6.3-VEKOP-16-2017-00005.
The authors acknowledge the Hungarian University of Agriculture and Life Science's Doctoral School of Food Science for the support in this study.

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**Original Scientific paper**
10.7251/AGRENG2103111G
UDC 636.085.2 :[635.65:633.1]

# SILAGE YIELD AND PROTEIN CONTENT OF FORAGE LEGUMES INTERCROPPING WITH CEREALS IN TWO SPATIAL ARRANGEMENTS

Konstantinos GENNATOS, Theano B. LAZARIDOU*

University of Western Macedonia - Florina, School of Agricultural Sciences 53100 Florina, Greece
*Corresponding author: thelazaridou@yahoo.gr, tlazaridou@uowm.gr

## ABSTRACT

Intercropping of most annual legumes with winter cereals is a very common practice for forage production in many countries. The aim of this study was to determine the effect of different spatial arrangements of intercropping cereals with forage legumes on silage yield and protein content. The completely randomized design was applied with three replications. Particularly, common vetch and forage pea were used as forage legumes, and barley, and triticale were used as cereals, which were grown individually as well as intercropped with each other in mixed rows in a sowing ratio 65:35 or in alternate rows. The plants were harvested when the legumes were at the end of the flowering period and were separated by hand to determine the weight of fresh matter for each species. Samples of 100 g of hay from each experimental plot were used to calculate the dry matter and to determine the total N and subsequently the total protein content using the Kjeldahl method. In most cases differences were found between the treatments concerning the dry matter and the protein content. Regarding the fresh weight the mixtures triticale+ common vetch (alternate rows), barley + common vetch (alternate rows) and barley+ forage pea (alternate rows) showed the higher yield. The forage pea gave the higher yield among the nonocrops. Regarding the dry weight the mixtures barley+ forage pea (alternate rows) and triticale+ common vetch (alternate rows) showed the higher yield. Additionally, significant differences were recorded between the examined genotypes in grain yield. The barley intercropped with forage pea (mixed rows) gave the higher yield. Regarding the grain yield of legumes, common vetch intercropped with barley (alternate rows) and forage pea intercropped with barley (both cases) gave the higher yield. In all cases the forage yield (weight of dry matter) was higher in separated lines compared to mixed lines. However, concerning the grain yield the mixed rows were probably more productive.

**Key words:** *intercropping, dry matter, protein content, spatial arrangement.*

## INTRODUCTION

Intercropping is the cultivation of two or more species simultaneously in the same field during the same growing season and depending on the design, there are mixed intercropping, row intercropping, strip intercropping and relay intercropping. Intercropping of most annual legumes with winter cereals is a very common practice for forage production in many countries. Intercropping of cereals and legumes can provide high biological value protein animal feed and additionally it is suitable for agricultural systems with low inputs (Lithourgidis et al., 2008, Lithourgidis and Dordas 2010, Dordas et al., 2012, Tang et al., 2021). This may be due to some of the potential benefits for intercropping systems such as high productivity, improvement of soil fertility, reduction of soil erosion, most effective competition with weeds, reduction of effects from enemies and diseases and improvement of forage quality (Banik et al., 2006, Vasilakoglou et al., 2008, Malezieux et al., 2009). The ecological advantages of intercropping are also under consideration (Mohammed et al., 2008) because according to Jensen et al. (2020) the intercropping of legumes and cereals can reduce the nitrogen fertilization by 26%. However, there are some disadvantages of intercropping such as the competition for light, water and nutrients that can reduce yields compared to monoculture (Lithourgidis et al., 2008, Lithourgidis and Dordas 2010, Menbere et al., 2015, Iqbal et al., 2019). So, careful considerations are needed in order to optimize spatial arrangements in intercropping systems to achieve maximum productivity.

The objective of this study was to determine the effect of intercropping cereals with forage legumes on silage yield and protein content in two spatial arrangements (1:1 alternate rows, and mixed forage legume and cereal in the same row) in the special climatic conditions of the Florina area. The forage yield and the quality characteristics of the hay and the yield of produced seed were studied as well.

## MATERIALS AND METHODS

The experiment was carried out in the farm of the University of Western Macedonia in Florina, during the vegetation period 2020-2021. The cultivars were sown in early November 2020 in a field at the University of Western Macedonia Farm in Florina Greece (40°46′ N, 21°22′E, 707 m asl), in a sandy loam soil with pH 6.3, organic matter content14.0 g $kg^{-1}$, N-NO3 100 mg $kg^{-1}$, P (Olsen) 50.3 mg $kg^{-1}$ and K 308 mg $kg^{-1}$ and water holding capacity 21.8% (0 to 30 cm depth). Greek winter varieties of forage legumes and cereals were intercropped in two different ways: in mixed rows in a sowing ratio 65: 35 (cereals: legumes) and in alternate rows. Particularly, common vetch (cv. Leonidas), and forage pea (cv. Olymbos) were used as forage legumes, and barley (cv. Triptolemos) and triticale (cv. Niovi), were used as cereals, which were grown individually as well as intercropped with each other. Thus 10 different treatments were created (4 treatments for monocrops and 6 treatments for intercrops = 2 spatial arrangements x 3 mixtures). The plots consisted of six rows five meters long of which the four inner were harvested. The distances between rows were 0.25m. The completely

randomized design with three replications was used. The field was fertilized only with basic fertilization and all the cultivation practices used by farmers were applied. The crop was kept free of weeds by hand hoeing when necessary. The fresh biomass of the two inner rows was harvested at the end of the legume flowering period (early June) and dried naturally to form hay. The plants were separated by hand to determine the fresh weight of each species. The other two rows were harvested when the seeds were mature and the yield was calculated. Samples of 100 g of hay from each experimental plot were placed at 65° C for 96h to calculate the dry matter, and in addition to determine the total N using the Kjeldahl method and subsequently the total protein content. Data were evaluated by analysis of variance (ANOVA) and the means were compared according to LSD test at $p<0.05$.

## RESULTS AND DISCUSSION

Significant differences were recorded between the examined cultivars in fresh and dry weight (significant differences at p=5%, Table 1). Fresh weight ranged from 6400 kg/ha in barley (monocropping) to 31190Kg/ha in mixture triticale+ common vetch (alternate rows) (Table 1).The mixtures barley + common vetch (alternate rows) and barley+ forage pea (alternate rows) showed high yield as well. The forage pea gave the higher yield among the nonocrops. Additionally, the superiority of the sowing in different and distinct rows compared to mixed ones, emerged from the data. The same was reported by Cheriere et al (2020) who intercropped soybean with buckwheat, lentil, sorghum and sunflower and found that alternate-row intercropping helped to increase soybean production. Spatial arrangement did not significantly affect the growth of maize plants in a maize-soybean intercropping system, whereas, soybean growth was greatly affected by spatial arrangement (Addo-Quaye et al., 2011). Dry weight ranged from 3500 kg/ha in common vetch (monocropping) to137578 Kg/ha in mixture barley+ forage pea. So concerning the dry matter, the mixture barley+ forage pea (alternate rows) is still more productive. The above results suggest that the intercrops may have higher dry matter yield than the respective monocrops. The same was reported by Galanopoulou et al. (2019), when they studied the effect of various spatial arrangements in faba bean and barley intercropping systems on the growth rates of the two species. In an experiment of intercropping barley with vetch Lithourgidis et al., (2007) found that, in two different sowing ratios (55% / 45% and 65% / 35%), biomass production was increased by 29.9% and 13.3% respectively compared to vetch monocrop, but reduced by 12.2% and 23.4% respectively compared to barley monocrop.

Table 1. The fresh weight, the weight of dry matter, the grain yield of intercropping crops and monocropping

| Genotype | Fresh Weight Kg/ha | Dry Weight Kg/ha | Grain yield Kg/ ha (for cereals) | Grain yield Kg/ha (for legumes) |
|---|---|---|---|---|
| Triticale | 10130de | 5117d | 1068.2c | |
| Common vetch | 9100de | 3500e | | 257.7c |
| Forage pea | 13860d | 3960e | | 326.3c |
| Barley | 6400e | 3879e | 1658bc | |
| Triticale +Vetch mixture | 20133c | 8346c | 1376 bc | 328.6c |
| Triticale+Vetch alt. rows | 31190a | 12475a | 1056 c | 340.9c |
| Barley+ Vetch mixture | 23467bc | 11174b | 1852.9b | 294.4c |
| Barley+Vetch alt. rows | 28533ab | 11413b | 1297.4bc | 866.9a |
| Barley+forage pea mixture | 21867c | 7289c | 2961.5a | 607.3b |
| Barley+forage pea alt.rows | 28800a | 13758a | 1529.2bc | 572.6b |

Means in columns followed by different letters are significantly different at $p<0.05$ by LSD test.

Intercropping had significant effects on grain yield. Specifically, significant differences were recorded between the examined genotypes in yield (significant differences at $p<5\%$, Table 1). Yield ranged from 1056 Kg/ ha in triticale intercropped with vetch (alternate rows) to 2961.5 Kg/ha in barley intercropped with forage pea (mixed rows). The barley intercropped with forage pea (mixed rows) gave the higher yield. These results indicated that the intercropping increased the yield of barley intercropped with forage pea only in mixed rows. Regarding the yield of legumes, common vetch intercropped with barley (alternate rows) and forage pea intercropped with barley (both cases) performed the higher yield. These results were in disagreement with those of other researchers who reported that the yield of sole crops was higher compared to their intercrops (Duşa and Stan 2013).

The above results suggest that there was a positive effect of intercropping concerning the silage yield and it seemed that the species involved in the intercropping system had a better behavior when they were sowing in different rows. However, this was not the case concerning the grain yield. In this case the mixed rows were probably more productive.

Preliminary data of this study showed that the mixtures had higher protein content than the monocrops (data not presented).

## CONCLUSION

It was concluded that concerning the dry matter the mixture triticale + common vetch (alternate rows) and barley+ common vetch (alternate rows) are more productive followed by the mixtures of barley intercropped with common vetch

(different rows and mixed rows). Barley intercropped with forage pea in mixed rows have higher grain yield compared to other monocrops and the mixtures. Among the legumes common vetch intercropped with barley (alternate rows) and forage pea intercropped with barley (both spatial arrangements) gave the higher grain yield. A first estimate of these results leads to the conclusion that the sowing in alternate rows results in promising mixtures for production of high quality forage. However further research, including several seeding ratio and different cultivars, is needed to confirm the results of the present study.

## ACKNOWLEDGEMENTS

Financial support of this project (project code 70313) by the Special Account for Research Grants of University of Western Macedonia, Greece, is gratefully acknowledged**.**

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**Original Scientific paper**
10.7251/AGRENG2103117B
UDC 614.2:316.334.55(47)

# ACCESS TO PUBLIC HEALTH SERVICES FOR DIFFERENT GROUPS OF RURAL POPULATION IN RUSSIA

Tatiana BLINOVA[1], Anna VYALSHINA[1]*, Nikita PETROV[2]

[1]Laboratory of the social development agrarian complex and rural territory, Institute of Agrarian Problems of the Russian Academy of Sciences, Saratov, Russia
[2] Department of Information, Analytical, Organizational, Legal and Personnel Work of the Ministry of Digital Development and Communications of Saratov Region, Saratov, Russia
*Corresponding author: anvyal@mail.ru

## ABSTRACT

During the COVID-19 pandemic, the problems of accessibility of primary health care and quality health services for rural residents are exacerbated. The purpose of the article is to conduct comparative studies of the access to public health services of rural medical posts (RMPs), polyclinics, specialized medical facilities for different groups of the rural population of Russia. The results of the studies showed that the majority of rural residents living in small remote settlements request medical assistance at the RMPs. Among those who requested medical assistance, 70.7% were over 45 years old, and 43.9% were pensioners. Most of them have low requirements for medical assistance. They are primarily interested in the services provided for free. Younger rural residents (35.2% under the age of 45 years) request medical assistance at polyclinics, 47.8% of them are self-employed, and about 71.1% are low-income groups. The main reasons for applying for paid medical care, they call the lack of specialists in public institutions. Visitors to specialized centers have the highest requirements for the quality of medical services received. The maximum share belongs to young people (24.4% of people aged 16-30 and 20.3% aged 31-45), about 60.5% have jobs, 40.6% have incomes higher than the subsistence level. They are more likely than others to seek paid medical services. The main reason for choosing commercial institutions is the belief that paid medical services are of better quality. The construction of multidimensional distributions was performed using the statistical processing package STATISTICA Advanced for Windows 10.0.

**Keywords:** *public health services, access, rural population, rural development, Russia.*

## INTRODUCTION

The main social impacts of the COVID-19 pandemic are increased risks of mortality, morbidity, poverty and vulnerability. Both the growth of social inequality and the deterioration of the position of socially vulnerable groups (the elderly, youth, children, women) are inevitable. During the COVID-19 pandemic, the rural population's access to quality health care and social support was weak. In

rural areas of the Russian Federation, the concentration of older age categories of citizens is higher, which makes the rural health care system more vulnerable to new challenges. Villagers with chronic illnesses are at higher risk of contracting the virus, which will have serious health consequences. Most district hospitals are not prepared to deal with COVID-19 (there are no intensive care units, there are not enough doctors). Increasing the availability of hospitals, testing centers, consultative and diagnostic centers is of great importance for rural residents.

A lot of work is devoted to the problems of developing public health and increasing the accessibility of medical services for the rural population in various countries and regions (Footman et al, 2013; Tashobya, et al 2014; Chaudary, 2016; Zhai et al, 2017). In Russia, improving the accessibility and quality of health services is a priority for the state's social policy (Ministry of Health Care of the Russian Federation, 2018) and an important factor in increasing the life expectancy of the rural population (Blinova et al, 2020). At the same time, rural health care is faced with many problems, and rural residents have difficulties not only with medicine provision and high-tech medical services, but also with primary medical care. According to scientists, the villagers are constantly faced with a shortage of medical institutions, doctors and other health workers, medical materials, equipment, and medicines (Kozyreva, Smirnov, 2018, p. 34). One of the explanatory reasons is the territorial remoteness of many rural settlements from regional centers. If in the EU countries there is a more uniform distribution of medical personnel, in Russia the number of doctors in the city is much higher than in the village (Panova, 2019, p.177).

Russia ranks first in the world in terms of an area that reaches 17.1 million $km^2$, the rural population is 37.3 million people, or 25% of the country's population, and the number of rural settlements exceeds 17 thousand (17380). Many of them are poorly populated and remote, which exacerbates the problem of access to medical care. The main barriers to the effective organization of rural health care are the peripheral and transport remoteness from the service center, the large number of rural settlements, the dispersive nature of settlement, the low provision of rural people with doctors, and the poor development of road transport infrastructure (Kozyreva, Smirnov, 2018; Belova, 2019).

The relevance of the study of the problems of access to medical services for rural residents is due to the low quality of rural medicine and the difficult living conditions, especially in remote and inaccessible settlements. The purpose of the article is to conduct a sociological analysis of the degree of accessibility of free and paid medical services in assessments of different groups of the rural population.

## MATERIAL AND METHOD

The research information base was made up of data from a Selective Surveillance of the Quality and Accessibility of Services in the Spheres of Education, Health and Social Services, Promotion of Employment, conducted by Rosstat in Russia. The construction of multidimensional distributions was performed using the statistical processing package STATISTICA Advanced for Windows 10.0.

The information base of the study is the results of a sample observation of the quality and accessibility of services in the fields of education, healthcare and social services, employment promotion conducted by Rosstat in 2017 (Rosstat, 2017). The sample consisted of 8,898 rural residents over 16 years old, including 3,832 men (43.1%) and 5,066 women (56.9%). Of these, 5,051 people (56.8%) requested medical service in the last 12 months. Thus, an in-depth analysis was conducted based on an analysis of the responses of 5,051 people.

To provide treatment and preventive care to the rural population, there is a complex step system, including both rural and urban health facilities. However, if there are health problems, the villagers at the first stage apply to rural medical posts (RMPs), rural medical outpatient polyclinics, district hospitals, etc. RMPs for rural residents are the first medical institutions where they receive first aid. Patronage of children and pregnant women is organized there, as well as sanitary and hygienic and other measures are carried out. A popular form for small remote settlements is "mobile medicine", when medical care is provided by mobile medical complexes and mobile medical teams. It should also be noted that some rural residents, regardless of the size of the settlement, do not go to doctors and are self-medicating. So, according to researchers, "among the surveyed rural residents who have had any health problems during the last month", 68.4% were self-medicating (Kozyreva, Smirnov, 2018, p. 40). In RMPs, rural residents receive primary treatment and preventive care. To receive specialized care, rural residents are sent to polyclinics (at district hospitals, central district hospitals) or to other specialized medical organizations, using doctor's referral or on a paid basis.

## RESULTS AND DISCUSSION

### Demographic and socioeconomic profile of rural residents requesting medical assistance

Age structure of rural residents requesting medical assistance. Young people and middle-aged people (up to 45 years), who, as a rule, are in good health, are less likely to request medical help, health problems do not limit their livelihoods. In case of health problems, they contact specialized medical institutions and are ready to bear certain costs (time, money) to maintain their health. People who are 46-60 years old make up a little more than a third of those who request medical help, regardless of the type of medical facility. At this age, there are major health problems that determine the future line of life. Persons over 60 years of age account for about 36.0% of those who applied for medical care to RMPs and 29.7% of those who went to polyclinics. Among those who contact other medical institutions, persons over 60 make up only 17.5%.

Gender features. The visiting rate of rural people seeking medical care has gender differences. Women request medical assistance more often than men, despite the fact that their life expectancy is higher. One explanation is gender differences in self-esteem of one's state of health. Among rural men, about 45.1% rate their health as good and 4.0% as very good, among women this share is 36.4 and 2.8%, respectively. More than half of women (54.3%) rate their health as satisfactory, this

share among men is lower (46.8%). In the past 12 months, 57.9% of women and 44.8% of men requested medical assistance. Women visit polyclinics and RMPs more often, and men attend highly specialized medical facilities. The differences remain in models of self-preserving behavior.

Level of education and employment status. An equally significant impact on the structure of rural residents who request medical assistance is provided by the level of education. The higher it is, the less likely it is to contact the RMP. So, among those who request medical assistance at RMPs, only 9.7% of people with higher education, at polyclinics - 16.4%, and specialized medical institutions - 23.3%. Persons with secondary vocational education make up 48.7% of RMP visitors, 51.4% of polyclinics and 47.3% of other medical facilities. Persons who do not have a vocational education are much more likely to request medical assistance at RMPs, polyclinics, and much less often at other medical facilities. Among those who have basic general education, about 20.1% have requested assistance at RMPs over the past year, while only 10.7% have requested at other medical institutions. Empirical studies show the high importance of employment status and rural income. Thus, the majority of non-working rural residents requested medical assistance at RMPs and polyclinics, while the majority of workers took treatment at specialized medical facilities.

Most often, pensioners (41.2%), unemployed citizens (38.8%) and people who do not work and study anywhere (37.5%) request medical assistance at RMPs. Outpatient services are preferred by entrepreneurs (83.3%), persons with disabilities (73.7%), as well as housewives and people with household plots and engaged in the production and sale of agricultural products (65.2%). A large part of rural residents with average per capita cash incomes less than the subsistence minimum (70.1 and 71.1%, respectively) request medical assistance at RMPs and polyclinics.

Health status. The analysis allows us to conclude that RMPs and polyclinics often turn to those whose health according to their subjective assessment is worse. People who assess their health as good and very good often request medical assistance at other health facilities. About 58.3% of RMP visitors consider their health status to be satisfactory, and 7.2% - poor, among people who went to polyclinics this share is 59.9% and 9.3%, respectively. Among those who requested medical assistance at highly specialized medical institutions, about 47.7% consider their health status good and another 6.8% consider it very good.

Thus, RMPs and polyclinics are the main medical facilities that provide primary medical care to older people, pensioners, and housewives, as well as self-employed persons, that is, rural residents with low incomes. Residents of the village who have paid employment, a high level of education and income, prefer to go to specialized medical facilities located in other settlements or cities, depending on their health problems.

**Paid and free medical assistance**

Rural residents appeal to the RMPs (37.1%) and polyclinics (53.1%) for primary medical care. Every tenth villager (9.9%) appeals to specialized medical facilities (dispensaries, diagnostic centers, dental clinics). Polyclinic visitors are better aware of the list of free medical services under the compulsory health insurance policy, 74.5% are more or less familiar with this list, 70.9% visitors of RMPs are knowledgeable. Visitors to other medical facilities are least aware of this fact (68.7%). About 29.2% of the villagers appealed to the polyclinics indicated that they used their right to choose a local doctor, but about a third of the visitors said they did not know about this possibility (31.0%). Rural residents who requested medical assistance at the RMP more often than others indicate a convenient work schedule (72.5%). In polyclinics, about a third of applicants indicated that the doctors 'work schedule was not fully convenient (32.7%), or completely inconvenient (4.7%). About 65.2% of visitors to other medical facilities are completely satisfied with the doctors' work schedule, 30.1% - partially, and 4.7% are not satisfied at all. Visitors to polyclinics indicate a lack of admission in the afternoon (42.4%) and a lack of necessary specialists (43.2%) as the main reasons for the inconvenience of the schedule. The respondents indicated difficulties with visiting a doctor during working hours (31.0%), lack of appointments on weekends (24.6%) and after work (7.0%) as the main reasons for the inconvenience of the work schedule of doctors of specialized medical facilities.

Over the past 12 months, the vast majority of respondents received medical assistance on an outpatient basis for free - according to the compulsory medical insurance policy. This was indicated by 99.3% of RMP visitors and by 98.6% of polyclinics' visitors. Among those requested at other medical facilities, 10.4% received paid medical assistance. Rural residents, who requested at polyclinics on a paid basis, more often than others indicated that the reason for applying for money was the lack of time to visit medical facilities that provide assistance free of charge (14.3%). Villagers who requested paid medical assistance at other organizations, indicated queues in medical organizations providing free aid or an uncomfortable work schedule (39.2%).

Thus, the main reasons for paid treatment are the lack of specialists of the required profile and the low quality of the free services provided. Visitors to specialized medical facilities, as a rule, more often than others consider the received medical care effective, and the work of doctors is of high quality. In order to seek medical advice from a specialist physician, rural residents most often appeal to polyclinics, or specialized medical facilities, usually located in the city. Most of the medical organizations that rural residents applied to belong to the state (municipal) form of ownership, however, the number of private ownership organizations is growing. Most of those who applied received consultations of medical specialists on a free basis (according to the compulsory medical insurance policy); however, 16.9% of visitors to polyclinics and 19.5% to specialized medical facilities applied for paid consultations. Visitors to polyclinics more often indicate the lack of specialists in state, municipal or departmental medical organizations located nearby (35.7%) as

the reason of paid medical assistance. Rural residents requesting medical assistance at specialized medical facilities consider that paid treatment is better and more reliable (40.4%), Table 1.

Table 1. Reasons for the appeal of rural residents to commercial medical facilities on a paid basis, % of requested persons

| Reason | Polyclinic | Other medical facility |
|---|---|---|
| Registered to this medical organization | 0.3 | 0.0 |
| Paid consultation (medical examination) was offered by a doctor of a state (municipal) medical organization | 21.6 | 1.8 |
| There are no such specialists in state (municipal, departmental) medical organizations located nearby | 35.7 | 24.6 |
| Do not have the opportunity to check into the state (municipal) medical organization due to lack of time, long queue, the need for pre-appointment | 17.2 | 29.8 |
| Consider consultations (medical examinations) in paid medical organizations as better and more reliable | 20.7 | 40.4 |
| Another reason besides those listed | 4.4 | 3.5 |

Source: Own calculation based on Rosstat data (Rosstat, 2017).

Thus, it can be concluded that the villagers, who consulted specialist doctors in private clinics, are convinced that paid medical services are better and more reliable.

**Medical specialists that the villagers could not get to**

Results of the research made it possible to determine that 42.4% of applicants to a medical facility were not able to get consultations with a specialist, including 17.2% of clinic visitors and 11.4% of applicants to specialized medical facilities. The main reason is the remoteness of medical organizations, where one can get consultation or undergo examination. This was indicated by 34.5-36.8% of rural residents, regardless of where they requested medical assistance. For visitors to specialized medical facilities, the reason is the high cost of the offered paid services, for which the villagers have no funds (12.3%). Clinic visitors more often than others mention such reasons as lack of time (17.1%) or other reasons (20.0%). Based on a survey of rural residents, it was compiled a ranking of medical specialists, which rural residents could not get to. Authors analyzed the answers to the question: "What medical specialists could not you get to or did you have to postpone a visit to them for any reason if you needed a consultation?". Based on the calculation of the answers of the villagers, the share of rural residents who could not get to consultations was assessed. Most of the rural residents who requested medical assistance at polyclinics could not get to consultations with such medical specialists as cardiologists (6.1%), neurologists and endocrinologists (5.7%), and rheumatologists (4.8%). For various reasons, visitors to other medical facilities could not get to otolaryngologists (6.2%), neurologists (4.6%), and

cardiologists (4.3%). Rural residents often attribute the inability to get to consultations with medical specialists to a lack of financial resources. Clinic visitors request medical assistance at both state (municipal) facilities and private clinics. The reasons for the paid treatment are the lack of necessary specialists in state (municipal, departmental) medical organizations located nearby.

## CONCLUSION

The results of the studies showed that the majority of rural residents living in small remote settlements attend RMPs for primary medical assistance. Among requesting persons, 70.7% were over 45 years old, the majority had no work, more than 41.6% did not have a vocational education, and 43.9% were pensioners. All of them have low requirements for the medical services provided, they are more interested in a convenient visit schedule and a list of services provided for free. Polyclinics are more often visited by younger rural residents (35.2% under the age of 45 years), among them 16.4% have higher education and 51.4% have secondary vocational education, 47.8% of them work for hire; about 71.1 % have low incomes. The main reasons for attending commercial medical facilities, they call the lack of specialists in state ones. Visitors to highly specialized medical facilities have the highest requirements for the quality and reliability of medical assistance. These facilities are mainly attended by young people (24.4% of people aged 16-30 and another 20.3% aged 31 to 45), about 60.5% of the visitors have official employment, 40.6% have incomes above the subsistence level, the majority of them has higher education (23.3%). They most often requested paid medical assistance at the city. About 10.4% of them paid for primary medical assistance, 19.5% – for consultations of medical specialists, 11.7% – for medical research. The main reason for choosing commercial facilities is the belief that paid medical assistance is of better quality, 45.0% of young people indicate the effectiveness of medical assistance. Thus, the sources of inequality and differences in the accessibility of medical assistance are not only the territorial remoteness of rural settlements and the poor development of transport infrastructure, but also the incomes of the population. Financial barriers persist for low-income rural population. It limits the accessibility of medical assistance and high-tech medical services. Reducing inequalities in access to health services will increase the life expectancy of the rural population.

## ACKNOWLEDGEMENTS

This research received support from the Russian Foundation for Basic Research (RFBR), project No 19-010-00229A

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**Original Scientific paper**
10.7251/AGRENG2103125Z
UDC 631.41:631.152.33

# HUMIC ACIDS COMPOSITION OF ARABLE SOD-PODZOLIC SOIL AFTER LONG-TERM APPLICATION OF TRADITIONAL AND UNCONVENTIONAL ORGANIC FERTILIZERS

Nina E. ZAVYALOVA, Marina T. VASBIEVA*

Perm Federal Research Center Ural Brunch Russian Academy of Sciences, Perm, Russian Federation
*Corresponding author: vasbieva@mail.ru

## ABSTRACT

The elemental composition and structure of humic acids (HA) of arable sod-podzolic soil (Eutric Albic Retisols (Abruptic, Loamic, Cutanic) was studied in Perm Agricultural Research Institute – division of PFRC. Application of traditional and unconventional organic fertilizers was fulfilled in long-term stationary experiment. The carbon content in HA of sod-podzolic soil varied from 30.7 to 34.6; hydrogen – 28.9-35.5, oxygen – 21.1-27.9, nitrogen – 1.9-2.2%. The H/C ratio for all treatments was >1, the structure of the supramolecular associations of humic acids is predominantly aliphatic. Long-term use of manure, sewage sludge (SS) and their combination with mineral fertilizers led to the enrichment of humic acids with nitrogen. The maximum degree of HA oxidation was observed with the use of cattle manure. The FTIR spectra of humic acids had absorption bands of carboxyl, hydroxyl, methyl, methylene, methoxyl and other groups in a wide wavelength range. At 1720 $cm^{-1}$, an absorption band was recorded, which had a high intensity in the control variant and was due to oscillations of the >C=O group of carboxylic acids. With an increase in the load of the anthropogenic factor on the soil (application of organic and mineral fertilizers), a decrease in its intensity is observed. The structure of supramolecular HA aggregates of the control variant, with the introduction of NPK and unconventional organic fertilizer – SS, is characterized by a higher content of aromatic fragments, as evidenced by a clear existence of the absorption band at 1628 $cm^{-1}$. Cattle manure application promoted the formation of humic acids with a branched aliphatic structure.

**Keywords:** *element analysis, atomic ratios, humic acids oxidation degree, IR spectroscopy, fertilizers.*

## INTRODUCTION

Soil organic matter undergoes continuous destruction, creating a continuum of more or less decomposed materials of varying size, composition, and structure (Lehmann, 2015; Mohinuzzaman, 2020). According to the International Humic Substances Society (IHSS) and the American Soil Science Society, the main criterion for determining humus substances today is still alkali solubility (Kleber,

2019). The different solubility of humus substances in acid-base media is the basis for their division into humic acids, fulvic acids, and non-extractable residue (humin).

The study of the transformation processes of humic acids (HAs) as the most important fraction of organic matter under long-term anthropogenic impact on the soil is of great scientific interest, since HAs exhibit high functional activity and determine the specificity of the water, physical, chemical and thermal properties of the soil. Their composition and structure depend on the conditions of soil formation and change under anthropogenic impact on the soil (Orlov, 1990; Stepanov, 2008; Kholodov, 2011; Hasanova, 2018).

Modern methods of spectroscopic analysis, in particular, the method of nuclear magnetic resonance, made it possible to characterize in more detail the chemical composition of humus substances in soils. Scientists concluded that humus substances and humic acids in particular are not heteropolymers, but are "supramolecular associations of self-assembled heterogeneous and relatively small molecules formed as a result of degradation and decomposition of dead biological material." The formation of supramolecular aggregates is provided by non-valent interactions (aromatic π-π and hydrophobic interactions, van der Waals forces, electrostatic and hydrogen bonds) (Piccolo, 2002; Semenov, 2013; Ivanov, 2017; Baveye, 2019; Olk, 2019; Kholodov, 2020).

The aim of the research is to reveal the effect of long-term use of traditional and non-traditional fertilizers on the elemental composition and structure of humic acids in arable soil.

## MATERIAL AND METHODS

The object of the study was sod-podzolic heavy loamy soil (Eutric Albic Retisols (Abruptic, Loamic, Cutanic)) of a stationary field experiment founded in 1976 on the experimental field of Perm Agricultural Research Institute – division of Perm Federal Research Center Ural Brunch Russian Academy of Sciences. In a stationary experiment, the following treatments were studied: control (without fertilizers); manure 40 t/ha, sewage sludge (SS) 40 t/ha, NPK – background, background + manure 40 t/ha, background + SS 40 t/ha.

Organic fertilizers (SS, cattle manure) at 40 t/ha of natural moisture were applicated in the fallow one time per crop rotation. SS was applied in rotations I – VI, manure in III – VI rotations of the crop. Mineral fertilizers (rotation I – $N_{120}P_{120}K_{120}$, rotation II – $N_{90}P_{90}K_{90}$, III-VI rotation – $N_{60}P_{60}K_{60}$) were applied to all grain crops of the crop rotation before pre-sowing cultivation in the form of ammonium nitrate or urea, simple superphosphate and potassium chloride. The aftereffect of mineral fertilizers application was studied on red clover.

The soil of The experimental plots were located on sod-podzolic heavy loamy soil. Agrochemical characteristics of the soil at the time of the experiment: Corg – 1.28%, pHKCl – 4.8, Ng – 3.7 and S – 18.1 cmol (equiv) / kg, mobile $P_2O_5$ and exchange $K_2O$ (according to Kirsanov) – 154 and 170 mg/kg, the content of physical clay according to the Kachinsky method (particles with a size of <0.01

mm) was more than 40%. The observations were carried out in a seven-course field crop rotation with following rotation scheme: bare (full) fallow – winter rye – spring wheat as cover crop for red clover – first year clover – second year clover – spring barley – oat. Treatment placing is systematic, each treatment has three replications on field area. Total area of each plot – 47.5 $m^2$, the accounting area is 24.0 $m^2$. $m^2$. SS and manure agrochemical characteristics are presented in the Table 1.

Table 1. Agrochemical characteristics of SS and manure (averaged data)

| Fertilizer | Humidity, % | $pH_{KCl}$ | Composition, % for absolutely dry matter | | | |
|---|---|---|---|---|---|---|
| | | | Organic matter | $N_{total}$ | $P_{total}$ | $K_{total}$ |
| SS | 68 | 6.5 | 66 | 1.5 | 3.1 | 0.5 |
| Manure | 75 | 7.3 | 75 | 1.7 | 2.1 | 1.0 |

Wastewater sludge from biological treatment facilities in the city of Perm were used in given experiment The SS used in the experiment in the V-VI rotations of the crop, in terms of agrochemical indicators and heavy metals content (HM) corresponded to the requirements of national standard GOST R. 17.4.3.07-2001. SS samples were used after holding on sludge sites for at least three years according to SanPiN 2.1.7.573-96; as a result, they are disinfected and they correspond to the required microbiological and parasitological indicators.

The soil samples were taken at the end of the sixth rotation in the 0-20 cm layer from two non-adjacent replication at five points on each plot. Preparations of humic acids were extracted according to the classical method of the Russian School of Soil Science, which differs from the recommendations of the International Humic Society (IHSS) (Swift, 1996): specifically, the soil was extracted with alkali at least three times in ordinary air medium, then combined extract was analyzed. The elemental composition of humic acids was determined on a CHN – elemental analyzer from Perkin – Elmer (USA), the amount of oxygen was calculated by difference (all calculations are given for ash free preparations); IR absorption spectra were recorded on a VERTEX-80v Fourier spectrometer (Bruker, Germany) in the range 4000–400 $cm^{-1}$ at a spectral resolution of 2 $cm^{-1}$. The spectra were processed using the OPUS software package. Data processing included analysis of variance.

The studies were carried out in IV agroclimatic district of Perm Region. Regarding the physical and geographical conditions, the region is located in the subzone of the southern taiga and coniferous-deciduous forests. Perm Region belongs to the Vyatka-Kama soil province according with soil-ecological zoning (Eremchenko, 2016). The climate is temperate continental with cold, long, snowy winters and warm short summers. The sum of the average daily temperatures above 10°C is 1700-1900. The duration of the active growing season with temperatures above 10°C is 115 days average, with temperatures above – 15°C -60 days. The region belongs to the zone of sufficient moisture: annual precipitation is 470-500 mm,

during the growing period is about 320 mm, evaporation from the soil surface is about 340 mm, hydrothermal coefficient 1,4. The number of days with snow cover averages 176 (Korotaev, 1962; Agroclimatic resources, 1979).

## RESULTS AND DISCUSSION

The analysis of humic acids elemental composition in sod-podzolic soil showed that the carbon (C) content varied from 28.9 to 35.5 at. %, hydrogen (H) – from 38.5 to 43.2 at. %, oxygen (O) – from 21.1 to 27.9 at. %, nitrogen (N) – from 1.9 to 2.2 at. % depending on the experiment treatments (table 2). The H/C ratio for all treatments was >1, the structure of the supramolecular aggregates of humic acids was predominantly aliphatic. Long-term use of manure, SS and their combination with mineral fertilizers led to the enrichment of humic acids with nitrogen, the C/N ratio was 14.96-15.64 versus 16.18 in the control variant. The use of fertilizers increased the degree of oxidation (from 0.03 in the control variant to 0.28-0.52). When SS was applied on mineral fertilizers background, the degree of oxidation remained at the control level. The maximum degree of oxidation and the enrichment of humic acids with nitrogen was observed after cattle manure application - traditional organic fertilizer.

An increase in aromatic fragments in the supramolecular associations composition of humic acids (H/C = 1.13) was noted in the variants "NPK – background" and "SS 40 t/ha". The use of traditional organic fertilizers (cattle manure) led to an increase in aliphatic groups in the HA structure, as evidenced by a wider H/C ratio of 1.44 (Figure 1). When organomineral fertilization system was used, the HA structure was formed, apparently consisting of a wide variety of aliphatic and aromatic fragments with various functional abilities. This structure is optimal from an agronomic point of view.

According to FTIR spectroscopy, humic acids of all variants have a wide absorption band at 3466-3423 $cm^{-1}$, caused by stretching vibrations of OH groups linked by intermolecular hydrogen bonds (Figure 2). In the range of C-H valence oscillations of methyl and methylene groups, the HA spectra show up to two absorption bands at 2927-2925 and 2863-2862 $cm^{-1}$. The absorption bands at 2925 and 2862 $cm^{-1}$ (valence oscillations of C-H methylene groups) are most intensive in the control and NPK variants, which indicates the presence of branched aliphatic chains with terminal methyl groups in these HAs (absorption band at 2925 $cm^{-1}$). In other cases, the intensity of these absorption bands decreases.

Table 2. Elemental composition of humic acids in long-term experiment with traditional and non-traditional fertilizers.

| Variant | Composition, % | | | | Atomic ratios | | | Oxidation degree (*W*) |
|---|---|---|---|---|---|---|---|---|
| | C | H | O | N | H/C | O/C | C/N | |
| Control | 38.16<br>35.5 | 3.67<br>40.9 | 30.21<br>21.1 | 2.75<br>2.2 | 1.15 | 0.59 | 16.18 | 0.03 |
| Manure 40 t/ha | 26.30<br>28.9 | 3.09<br>40.9 | 33.85<br>27.9 | 2.05<br>1.9 | 1.41 | 0.97 | 14.96 | 0.52 |
| SS 40 t/ha | 37.95<br>34.2 | 3.56<br>38.5 | 36.75<br>24.8 | 2.83<br>2.2 | 1.13 | 0.73 | 15.64 | 0.33 |
| $N_{60}P_{60}K_{60}$ – background | 34.05<br>34.4 | 3.21<br>38.9 | 32.03<br>24.8 | 2.44<br>2.1 | 1.13 | 0.71 | 16.27 | 0.28 |
| Background + manure 40 t/ha | 32.54<br>31.8 | 3.49<br>40.9 | 33.96<br>24.9 | 2.48<br>2.1 | 1.29 | 0.78 | 15.30 | 0.28 |
| Background + SS 40 t/ha | 31.04<br>31.8 | 3.51<br>43.2 | 29.39<br>22.6 | 2.41<br>2.1 | 1.36 | 0.71 | 15.02 | 0.06 |
| $LSD_{05}$ | 2.01 | 0.1 | 1.1 | 0.3 | - | - | - | - |

Note: above the line — mass fraction, below the line – atomic fraction (all calculations are given for ash-free preparations). $HCP_{05}$ is presented for mass fraction.

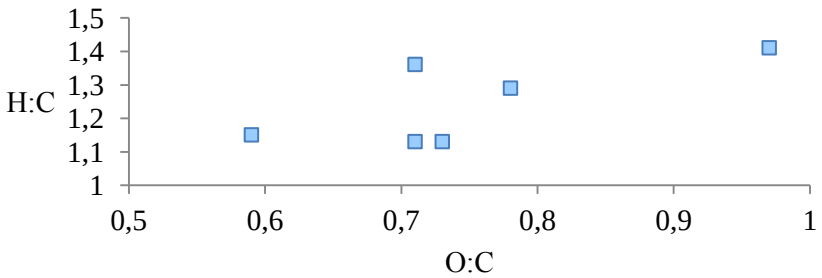


Figure 1. Diagram of atomic ratios of HA in sod-podzolic soil in long-term experiment:
1 – without fertilizers, 2 – manure 40 t/ha, 3 – SS 40 t/ha, 4 – NPK (background), 5 – background + manure 40 t/ha, 6 – background + SS 40 t/ha.

In the range of wavenumbers 1800-1300 $cm^{-1}$ in the HA spectrum of the control variant, the absorption band at 1720 $cm^{-1}$ of high intensity was recorded, which was caused by oscillations of the >C=O group of carboxylic acids. The increase of the anthropogenic impact on the soil (application of organic and mineral fertilizers) led to decrease in its intensity.

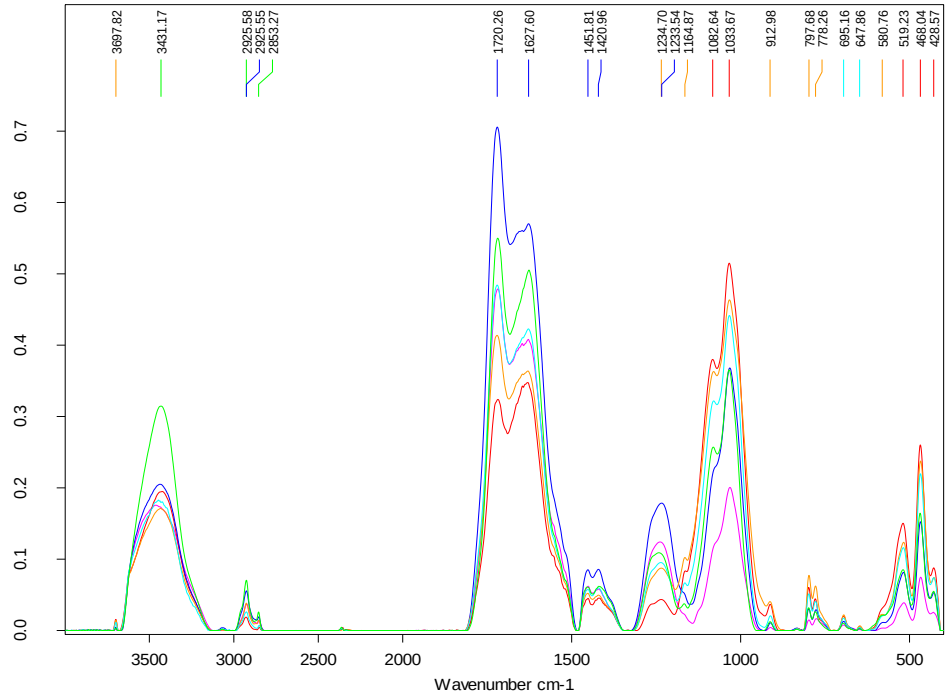


Figure 2. IR spectra of HA of sod-podzolic soil in long-term experiment: control (dark blue), manure 40 t/ha (red), SS 40 t/ha (pink), NPK – background (green), background + manure 40 t/ha (light blue), background + SS 40 t/ha (brown).

In the HA spectrum of the control treatment, a clear presence of the absorption band at 1628 $cm^{-1}$ was observed. This absorption band was quite intense for the NPK variant. Absorption in this area indicates the presence of aromatic rings in the supramolecular associations of HA due to valence oscillations of double bonds of carbon atoms. The use of organic fertilizers (manure and SS) caused the least impact on the intensity of this absorption band.

The absorption bands (1440–1430 $cm^{-1}$ and 1405–1400 $cm^{-1}$) can be caused by deformation vibrations of C–H methyl and methylene groups, carboxylic acids, carboxylate ion, and the intensity of these bands in the HA spectra of the control variant is significantly higher compared with other experiment variations.

Spectroscopic study of humic acids preparations made it possible to reveal some differences in the set of absorption bands and in the magnitude of their intensity, as well as the generality of their structure. That was especially true for the IR spectra of humic acids in the soil of the control variant: clear absorption bands in the range of 1720 $cm^{-1}$ (C=O carbonyl group) and at 1628 $cm^{-1}$ (C=C aromatic rings) characterize a greater number of aromatic structures in the composition of HAs than in other experiments. Mineral fertilization system ($N_{60}P_{60}K_{60}$ – background) also contributed to the enrichment of supramolecular aggregates with aromatic fragments. The application of cattle manure and SS together with NPK led to increase of aliphatic groups in humic acids composition.

## CONCLUSION

The humic acids preparations extacted from sod-podzolic heavy loamy soil in long-term experiment correspond to humic acids of natural sod-podzolic soils according to the data of elemental analysis and IR spectroscopy. They have a common structural composition, but are characterized by an individual set of absorption bands and their different intensities depending on the degree of anthropogenic load on the soil. Extensive (without fertilizers) cultivation of agricultural crops and the use of only mineral fertilizers led to an increase in aromatic functional groups in the structure of HA. Such composition of supramolecular aggregates is resistant to microbiological and thermal decay. The increase in the number of functional groups in humic acids composition was observed after long-term use of organic fertilizers (manure, sewage sludge), separately and together with NPK. The process of transformation of their structure is moving towards the strengthening the aliphatic nature of HA. Humic acids with such structure are characterized by high functional activity.

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Results and Discussion should be combined into a single section.

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

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

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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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