Veda mladých 2024

Science of Youth 2024

proceedings of papers from a scientific conference

May 30th - 31th, 2024

ISBN 978-80-552-2755-9

DOI: https://doi.org/10.15414/2024.9788055227559

SUA Slovak University of Agriculture in Nitra

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Veda mladých 2024 - Science of Youth 2024

PROCEEDINGS OF PAPERS FROM A SCIENTIFIC CONFERENCE

Nitra, Slovakia
30. – 31.05.2024

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ISBN 978-80-552-2755-9

DOI: https://doi.org/10.15414/2024.9788055227559

Conference is held under the project Environmental Center Climate-Landscape-Information of SUA in Nitra. Project is cofinanced by the EU.

Conference is organised in cooperation with Slovak academy of Agricultural Sciences.

CONTENT

POSTER SECTION

ARTICLE HISTORY:
Received 10.05.2024
Accepted 29.05.2024

SCALE MODELS AS A TOOL FOR PRESENTING ARCHITECTURE: A CASE STUDY OF THE VILLA K

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Abstract

Using the case study of the scale model of the Villa K, we are looking at the process of creating a scale model as well as the benefits of having one, when presenting a piece of architecture as well as specific concepts of the architectural and spatial design. The use of scale models has been shown to be an especially effective tool for sharing ideas and concepts especially in buildings that can't be experienced in the form they were initially designed. The Villa K and its restoration with the intent to preserve its architectural heritage values is a perfect example of having to make a choice between preserving conflicting values. In this scenario, a physical model proved to be a valuable addition to the path chosen for the heritage preservation, highlighting architectural values that were impossible to preserve.

Keywords: architecture, presentation, model, 3d printing, heritage preservation

1. Introduction

Physical scale-models have been a part of the architectural practice for a long time. Their creation is still being taught in universities, but they've become rare in the past decades for everything but the most high-profile projects. This case-study aims to show that not only is the scale model a relevant and highly efficient medium of sharing ideas, but modern technologies such as 3D printing and CNC routing make the creation of these models faster and easier. In this paper, we're going to discuss the choice of the architectural object to present, the design and production process as well as the current use and experiences with using the scale model.

2. Material and Methods

2.1. Scale models in general

2.1.1. Uses

While we may find some outliers to this classification, the four main uses for scale models in architecture are as follows:

1. Work / volume and shape verification
2. Simulation
3. Presentation
4. Education

Work models are usually just very coarse representations of an idea, most commonly boxes cut out of Styrofoam, cardboard or soft wood that help the architect zero in on the optimal shape and composition. This model is typically just part of the work process (usually the study) and doesn't leave the architect's office.

Further down the line, a scale model can be built to study the interactions of the building's shape with the spreading of daylight in and around the building. There are dedicated simulation labs, where a specific lighting situation for a concrete day and hour can be programmed into a moving light source representing the sun and the interactions between the light and the scale-model can be studied (Parsaee, 2020). Through the advent of computer simulation, this practice has become rare nowadays, but you can still come across very specific cases of unusual buildings being tested in wind tunnels for example.

Once the design is ready, a scale model can go a long way in convincing the investor or even the public of the quality or special design features, that would otherwise be difficult to explain through a 2D drawing or even drawn perspective. This presentation model is not just meant to introduce ideas about the design, but is typically meant as advertisement too, so a lot of care is given to the finish and details of this model to make it look presentable.

The last use case are architectural models purposely made for the purpose of education. While these share many similar characteristics with the previous group of presentation models. Typically, they represent germinal archetypes of buildings and are meant to illustrate their contribution to the evolution of typology or our understanding of space. It's useful if they can be produced in volume and compromises can be made to the level of detail or even a slight misrepresentation of shape can be acceptable in the name of abstraction to better get across the idea the model is meant to represent (Solčániová, 2023).

2.1.2. Technology

While there's a wide range of methods to create a scale model, ranging from fully artisanal to computer aided, For the purposes of this article, we're going to be focusing on 3D printing. 3D printers today can handle a wide range of materials ranging from concrete to chocolate, the most practical and common still are 3D printers that use thermoplastics or UV-reactive resins.

The most common technologies in this field are:

1. SLA - short for Stereolithography
2. SLS - Selective Laser Sintering
3. FDM - Fused Deposition Modelling (sometimes referred to as FFF - Fused Filament Fabrication)

SLA as a method relies on a liquid resin that bonds with Oxygen from the air, when exposed to UV light. This method was patented in 1984. (Hull, 1984) The model is sliced into layers a fraction of a Millimetre and with the help of a projector, an entire layer is created at the liquid's surface, where it can come into contact with Oxygen. This layer sticks to the previous layer until the model is finished. This method is relatively quick to print and creates unparalleled level of detail, these printers usually have a limited printing area, other specialised equipment for cleaning and curing the model and the resins are typically toxic and need special precautions not to damage the user's health.

SLS works on a completely different principle. Very fine thermoplastic powder is spread in a thin layer in the printer's chamber heated just a few degrees below the melting temperature of the thermoplastic (Deckard, 1986). Once the powder is in place, a laser draws the shape of the layer, adding just enough energy to the powder surface to melt and fuse with the rest of the model. This method also creates models with very fine detail, there are no limitations to the shapes the printer can create, but they can't create hollow models and the thermoplastic powder that wasn't used degrades with every exposure to heat, making it difficult to reuse the powder more than twice. Also, these machines are quite expensive and need a separate cleaning station too, to get rid of the powder that sticks to the model.

FDM is the most common method today. It relies on a thermoplastic filament being molten and extruded through a nozzle, where it hardens and sticks to previous layers (Crump, 1989). Once the patent became part of the public domain, this method of additive manufacturing became popular with the open-source community working on the self-replicating 3D printer REP-RAP and became even more popular with the DIY kits from Josef Prusa. While these printers might have limitations compared to the previous types when it comes to speed, shape-limitations or layer adhesion, they are cheap, safe to use, easy to maintain, come in a variety of sizes and can handle a wide range of materials.

2.2. Vila K

Figure 1 Street view of the Villa K (Vila K)

2.2.1. History

The Villa K is a modernist masterpiece of architecture designed by the architect Oskar Singer for Fridrich and Etela Kollmann. Finished in 1934 in rural Nitra, its modern shape must have looked out of place at the time and place of its completion. Architect Singer was a big admirer of the well-known French architect le Corbusier and designed the Villa K following le Corbusier's "Five points of modern architecture":

1. Pilotis - a concrete skeletal structure allows to free the design from the constraints of load-bearing walls
2. Free plan - also thanks to the skeletal structure, the designer gets more freedom to design the floor plan, which can be completely free of walls and very flexible
3. Free facade - also thanks to the skeletal structure, the facade can be fastened onto the load-bearing structure in the background and its design doesn't have to be constrained by the load-bearing structure
4. Ribbon windows - thanks to the facade losing its load-bearing function, it's possible to have long linear windows, even windows running around the corner of the building. This makes for sun-filled interiors.
5. Roof terrace - with the use of flat roofs, additional outside space can be gained. The plot of land taken over by the building can be recreated on the building's roof, including the greenery.

In 1937, the free space of the ground floor below the living area of the house was closed off by an addition designed by the original architect Oskar Singer (Novák, 2024). While the reason for this addition is still disputed, it makes Le Corbusier's Five points of modern architecture a bit more difficult to recognize in the design.

2.2.2. Present

Today, the villa has gained a National Monument status and has been thoroughly reconstructed in cooperation with the Municipal Heritage Protection Office. It serves as a cultural hub and a museum. One of the bigger questions during the planning of the reconstruction was whether to keep the addition from 1937, or whether to return the building to its original form right after completion. In the end, the decision was made that since the addition was designed by Oskar Singer and so shortly after the house's completion, it too has historical value.

2.3. Scale model

2.3.1. Choice of presentation medium

As already mentioned in the History section, there's two significant shapes of the house that have historical value, but only one can be experienced by the visitor. Since it was chosen to respect the addition during the reconstruction, the problem was presented as to how to present the application of Le Corbusier's Five points of modern architecture in the design of this building, as well as how to show the visitors the layout of rooms that they normally wouldn't have access to.

A scale model that can be taken apart floor by floor was chosen as the ideal medium. It has several advantages over digital media, in that it is a real 3-dimensional representation without the need for specialised equipment like VR-glasses but can also be viewed and experienced by multiple visitors at the same time. Discussions and explanations can be had over the model with just pointing at it with a finger. It's easily transported and doesn't need power. It also has the presence of an art-piece.

2.3.2. Preparation

The first step was to get as many resources as possible. Luckily, since the reconstruction was already underway, we had access to all the documentation from the architect and the Heritage Preservation Office. We were also provided with a digital 3D model in SketchUp .skp format.

When preparing to 3d print a model, the choice of scale is important. It is necessary to factor in the maximum printing volume of the available 3D printer, which was a Prusa i3 mk. II (an FDM printer) with a maximum printing volume of 250x210x200 mm. The scale of the model does not only govern the extents of the printed model, but also the level of detail of the finished model. It is possible to end up with a larger model than the volume of the printer, but in that case, it is necessary to put the model together from multiple parts, which leaves a visible line, where the parts connect. Choosing these lines strategically, so they are either hidden well or tie in with the design seamlessly is one way to go about seam placement and this strategy minimises the need for post-production.

The next step is to split up the model into manageable parts. As mentioned before, FDM printers have certain limitations as to the shapes they can print. These shapes include very thin vertical columns, overhangs and long bridges.

To make the model look more dynamic, underscoring the rounded corners of the house, it was chosen to recreate an elongated section of the model with part of the street in front of the house, since the street view was obviously the one with the most impressive facade facing it. To keep to a scale of 1:100, the ground plane of the model would have to be split up into two separate parts, since it wouldn't otherwise fit onto the printer's build plate. The resulting seam was hidden under the fence.

An important part of the model was a period appropriate car model. Architect Le Corbusier was known for posing in photographs with cars in front of his buildings, since cars were still this new, futuristic and exciting technology. Oskar Singer, as his admirer, has also later photos of himself posing in front of his buildings with a car, so adding the scale model of a period vehicle as another subtle hint to the connection with Le Corbusier seemed like the right decision.

To make the car look as good as possible, it needed to have a lot of detail, even more so than the architecture itself. This would be possible using a different layer height for the car, which would mean considerably longer printing times, but it wouldn't be a problem, since the car was only a very small part of the whole model. We ended up choosing a layer height of 0.1 mm for the car and a much more conventional 0.2 mm for the other parts of the model.

2.3.3. Digital model

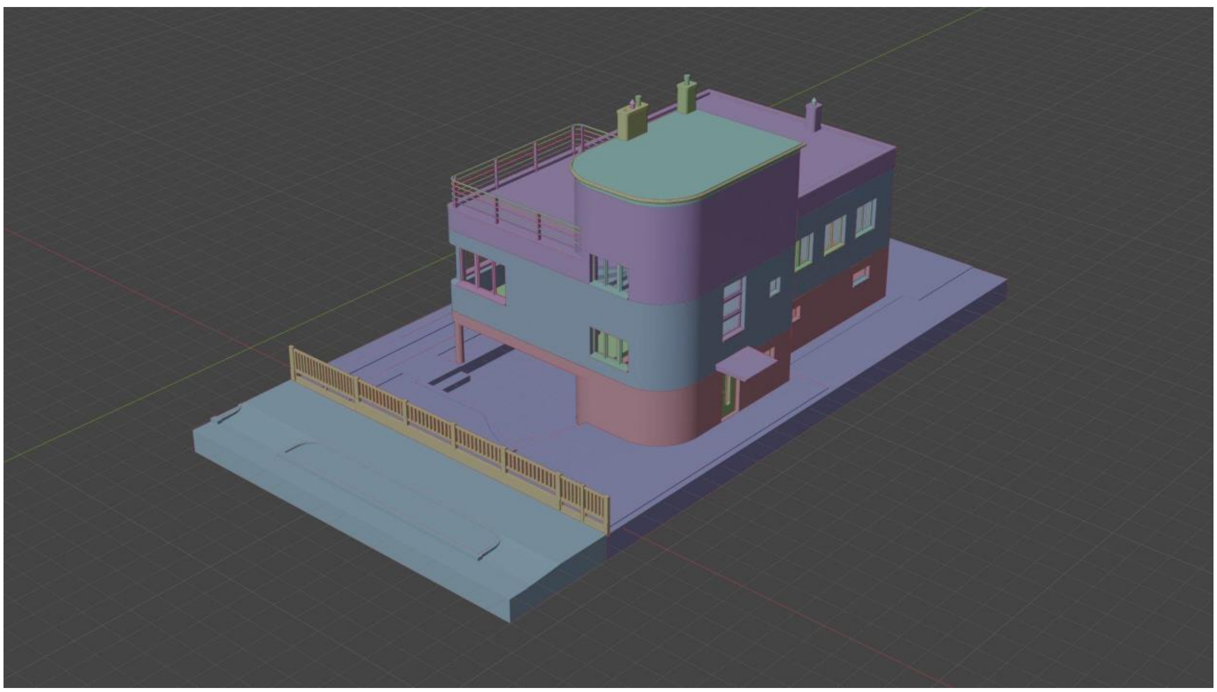

Figure 2 Finished digital model in Blender's 3D Viewport

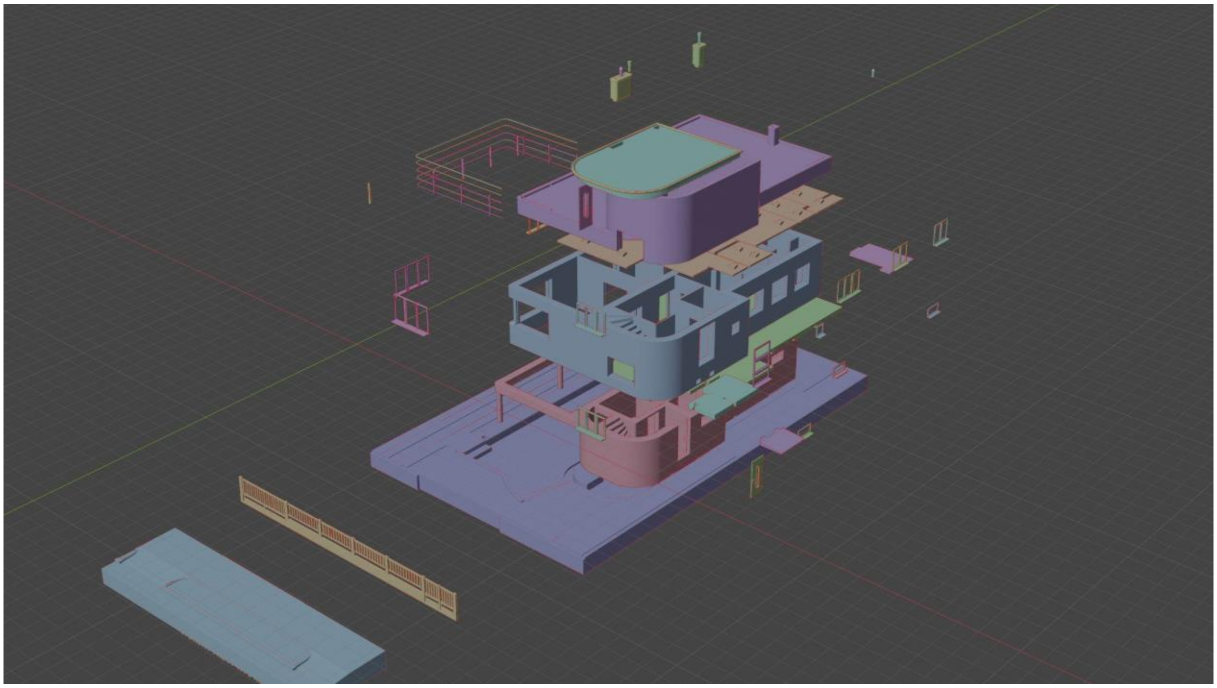

Figure 3 Exploded view of digital 3D model showing individual parts

As already mentioned, a SketchUp model made by the architect was provided by the owner. The problem with SketchUp is, that it creates double-sided geometry for visual purposes, but this is a problem when 3D printing, because the Slicer (program used to convert a digital 3D model into instructions for the 3D printer) need clean watertight geometry with normals that clearly define an inside and an outside. Also, modelling for 3D print needs a different approach to modelling for visual purposes. Apart from splitting up the model into manageable pieces that fit into the printer's build volume, it is necessary to model the pieces in a way, so that the printer can fabricate them without running into issues such as overhangs and long bridges.

For this, it might be advantageous to print some parts upside down or flipped to their side. The last thing to look out for is, that like in any industrial manufacturing process, with 3D printing we're also dealing with manufacturing tolerances. Every part, where two pieces are supposed to fit together must have a tiny tolerance accounting for extrusion inconsistencies and shrinkage a part experiences when cooling. Usually, 0.15 mm seems to be an overall good tolerance.

Rather than continuing work in SketchUp, it was chosen to move the modelling work into Blender, which is a very powerful open-source modelling software.

In the pictures above, you can see the finished model consisting of 43 individual parts and an exploded view of the same model. Some parts, like the railing spikes or certain windows were duplicates of each other and could be ignored during export, but their final number would have to be noted, so they could be copied the correct number of times in the slicer later.

Slicing was straightforward, since a lot of care was given to the design part of the model, so that it could be printed almost without supports (helping structures to mitigate the problems caused by overhangs and long bridges, that must be cut away or broken off after the print is finished).

2.3.4. Printing and post-production

The printing went smoothly with no failed pieces with the resulting parts requiring almost no post-production. This was partly due to the choice of material, PLA, which is a bioplastic derivative of cornstarch with very favourable properties. The trade-off to materials like ABS or PET-G is reduced strength, flexibility and heat resistance, but since this is a model that is not expected to have to withstand a lot of stress, a good surface finish and parts fitting together nicely without warping was the more important parameter. The model was glued together using off-the-shelf superglue. In later trials, welding pieces together using a soldering iron, or a 3D printing pen were also found to be very effective ways of connecting pieces. Some parts were designed to just snap into place, making the assembly, but also future repairs easier.

3. Results and discussion

The model was first exhibited inside the Villa K in May 2021. Since then, it has been a part of every exhibition and house-tour taking place. We get feedback from the owners, that the model is a centre of attention every single time, with people trying to either take it apart or put it back together, if the default position was taken apart and a lot of photos get taken with it. The owners try to reduce the physical interactions of visitors with the model, since careless manipulation by the visitors has already resulted in damage to multiple smaller and fragile parts prompting a need to take the model to the workshop for repairs. Photos of the model have also been featured in the book Vila K, The History and Present of the Kollmann Family House in Nitra.

Figure 4 Facebook post promoting the opening of the newly reconstructed Vila K and the launch of the book (Vila K)

Figure 5 Vila K as a stop during the commented architecture-themed bike tour during the Day of Architecture in July 2021 with the model in the centre (Vila K)

4. Conclusion

The physical model has proven to be a very effective medium for the presentation of architectural heritage sites that, like the Villa K, are often composed of multiple layers of valuable constructions and additions. This presents the conundrum of which of these layers to restore and which should be removed. The physical model has enabled us to present the other valuable layers and states of the building and its development that weren't possible to preserve.

Acknowledgment

Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00043 as well as the projects KEGA 004SPU-4/2023 KR:EK:IN a BIN SGS02_2021_013 ReImaGIne.

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ARTICLE HISTORY:
Received 10.05.2024
Received in revised form 30.05.2024
Accepted 30.05.2024

FUTURE DIRECTION OF THE MEAT MARKET: PERSPECTIVES OF MEAT SUBSTITUTES CONSUMPTION

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Abstract

In the coming years, the meat industry may face challenges related to meeting the growing demand for meat, as well as the risk of health problems for consumers, and negative environmental impacts resulting from excessive meat consumption, especially red meat. Therefore, it is desirable to incorporate meat substitutes into the diet of consumers and the aim of the paper was to investigate the consumer acceptance of individual meat substitutes, namely plant-based meat substitutes, edible insects and cultured meat. The research was based on a questionnaire survey conducted on a sample of 518 Slovak consumers up to 35 years. By implementing mathematical-statistical methods, consumer perception, reasons for consumption/non-consumption, as well as potential motives for supporting the consumption of individual meat substitutes were identified. Results showed that consumers have the highest awareness of plant-based meat substitutes. All meat substitutes are perceived as moral, ecological and healthy. Key motives for supporting the meat substitutes consumption in the future are higher consumer awareness and similarity of substitutes to meat. Results of study can be a suitable basis not only for the scientific sphere, but also for meat producers and other food companies who are interested in adapting to new trends in the meat market.

Keywords: consumer, plant-based meat substitutes, edible insects, cultured meat

1. Introduction

The future of the meat industry can be significantly determined by the increase in population, as the production of meat and meat products will not be able to cover the increasing demand. In addition, meat consumption is increasingly associated with a negative impact on consumer health and sustainable aspects (Font-i-Furnols, 2023; Modlinska et al., 2020). This is the reason why the latest directions come with substitutes for traditional meat and meat products in the form of production of animal products from stem cells, meat substitutes based on plant proteins, or consumption of edible insects. The mentioned alternatives are nutritionally beneficial, produced in a more environmentally friendly way and have aggressive marketing. The mentioned aspects are key to why "future foods" can compete with traditional meat and meat products. On the other hand, the taste and enjoyment that consumers have from consuming traditional meat and meat products is also an important factor. However, the current state of development of meat analogues is already at a very high level, which makes it possible to market products that will resemble meat in their character (structure, colour, texture, taste), but will be obtained in a different way than by slaughtering farm animals (Kameník and Král, 2020).

The first possibility is the consumption of plant-based meat substitutes as a more healthy and sustainable food (van den Berg et al., 2022; Apostolidis and McLeay, 2016; Szenderák et al., 2022; Bryant and Sanctorum, 2021). Meat analogues based on vegetable proteins are currently most consumed by the group of vegetarians and vegans (Kameník, 2017). The main sources of proteins of vegetable origin, which are used for the production of meat substitutes, are primarily soy, wheat, legumes, mushrooms and oilseeds. In connection with plant-based meat substitutes, it can be stated that they are preferred mainly because of the content of the necessary nutrients, including proteins, vitamins and minerals, and are a source of high-quality fibre, as well as a healthy variety of the usual diet (Kameník and Král, 2020). Ahmad et al. (2022) adds that plant substitutes are a good source of protein and have a similar nutritional profile to traditional meat.

Another possibility is the consumption of edible insects. Nowadays, it is becoming a real trend and is the only known source of food that could feed the growing population. In recent years, public and scientific interest in new foods from insects has been growing (Tan et al., 2015). This may be due to its nutritional and environmental benefits. The high nutritional values are due to the low content of saturated fatty acids, high digestibility and the presence of omega-3 fatty acids (Rumpold and Schlüter, 2013). Thus, edible insects have the potential to become a major source of human nutrition and can be produced more efficiently than livestock (Tabassum-Abbasi et al., 2016; van Huis, 2013). Insects are considered an important source of fats, proteins and micronutrients (Nowak et al., 2014, Rumpold and Schlüter, 2013) and can be produced with a lower level of greenhouse gas emissions and water consumption compared to conventional meat (van Huis, 2013).

In the future, the production of artificial meat may also affect the meat market (Ching et al., 2022; Hadi and Brightwell, 2021; Bryant and Barnett, 2020). Artificial meat, also called lab-grown, synthetic or cultured, is produced without the presence of the farm animal. Meat is produced by culturing animal stem cells in a medium containing nutrients and energy sources and producing muscle tissue in a laboratory manner (Bhat et al., 2015). Laboratory-grown meat tissues would be of the highest quality, healthy, unburdened by disease or artificial antibiotics (Alexander et al., 2017). However, the production of meat in laboratories is very expensive and unethical, as the production requires tissue from live animals, which is obtained alive from a farm animal. When it comes to producing cultured meat, it is also necessary to consider how consumers perceive this type of food product. (Hocquette, 2016). However, requirements are placed on laboratory meat in the form of the same taste, texture, appearance in comparison with classic meat. Products must meet given attributes in order to be accepted by consumers, which is currently very difficult to achieve (Moritz et al., 2015).

Based on the above, it is crucial for the future direction of the meat and meat products market to know consumer acceptance and consumers' willingness to consume individual substitutes for meat and meat products. For this reason, the aim of the paper was to identify consumers' awareness of plant substitutes, edible insects and cultured meat, as well as their expectations from consumption, willingness to consume these meat substitutes in the future, and to explore the key reasons for consumption and non-consumption of these alternative foods. In the context of the above, research questions were also determined:
RQ1: What is the current awareness of Slovak consumers about individual meat substitutes?
RQ2: Which of the meat substitutes are acceptable to consumers for consumption in the future and what are the motives for consumption?

2. Material and methods

The consumer study is based on a questionnaire survey that was carried out in 2021-2022 in the Slovak Republic on the young generation of consumers under 35 years of age. The mentioned generation of consumers was chosen because these consumers represent potential consumers of meat substitutes. The goal of the survey was to identify awareness and ideas about individual meat substitutes, such as plant-based meat substitutes, cultured meat and edible insects, as well as the possibility of their future consumption, pointing out the reasons and motives of consumer behaviour. The questionnaire survey was conducted on a sample of 518 respondents, who were divided into 6 categories according to gender, education, place of residence, number of members in the household, net monthly household income and economic status (Table 1).

Table 1 Socio-demographic profile of sample

		n	%
Gender	male	172	33.2
	female	346	66.8
Education	elementary	23	4.4
	secondary	177	34.2
	university	318	61.4
Members in household	1-2 members	115	22.2
	3 members	140	27.0
	4 members	185	35.7
	≥ 5 members	78	15.1
Place of residence	urban	243	46.9
	rural	275	53.1
Monthly income of household	≤ 1,000 €	60	11.6
	1,001-2,000 €	261	50.4
	2,001-3,000 €	135	26.1
	3,001-4,000 €	38	7.3
	≥4,001€	24	4.6
Economic status	employed, self-employed	180	34.7
	student	300	57.9
	other	38	7.4

Source: own research

The questionnaire survey was divided into three parts, while each part was focused on individual substitutes for meat and meat products, namely cultured meat, plant-based meat substitutes, edible insects. Consumers determined their knowledge of the existence of individual substitutes. Furthermore, they evaluated the degree of agreement with the statements regarding the ideas about individual substitutes on a scale from 1 to 5, with 1 representing complete disagreement and 5 complete agreement. The following claims were evaluated: healthy food, tasty food, nutritious food, safe food, affordable food, moral food, sustainable food, ecological food. Subsequently, consumers determined whether they would consume a specific meat substitute in the future. In the context of potential consumption/non-consumption, they also determined the key reasons on a scale from 1 to 5, with 1 representing a significant reason and 5 an insignificant reason. Consumers for whom consumption is acceptable evaluated the following reasons: environmental protection, sustainability, taste, health aspect, moral aspect, lifestyle, absence of food scandals. On the other hand, consumers for whom the consumption of meat substitutes is unacceptable evaluated the following possible reasons: distaste, lack of information about cultured meat, do not believe in the new alternative diet, production process of cultured meat, habit of classic meat, higher price. At the end of each part, consumers determined what would help to support the acceptance and future consumption of individual meat substitutes, while evaluating selected determinants: higher awareness of health effects, higher awareness of environmental impacts, taste like meat, higher awareness of production, affordable price, aroma like meat, appearance like meat, consistency like meat, colour like meat. Determinants were evaluated on a scale from 1 to 5, where 1 represented the importance of the determinant and 5 the unimportance of the determinant. Data were obtained by the questionnaire survey and evaluated by statistical software XLSTAT 2022.4.1. For the purposes of statistical testing, a significance level was set to 0.05.

3. Results and discussion

3.1. Consumption perspectives for plant-based meat substitutes

The first investigated meat analogy was plant-based meat substitutes. The research examined awareness of selected plant-based substitutes, namely soy, tempeh, tofu, quorn, corn, robi and seitan. The results of the consumer survey showed that Slovak consumers are most familiar with soy (98.6 %) and tofu (98.3 %), followed by tempeh (54.1 %), seitan (33.6 %) and klaso (31.5 %). It should also be emphasized that almost 75 % of young Slovak consumers do not know substitutes such as quorn and robi.

The conducted research also revealed the expectations and ideas about the consumption of plant-based meat substitutes by the young generation of consumers. Based on the results, it can be concluded that consumers consider the consumption of plant-based meat substitutes to be moral, ecological, safe and healthy. On the other hand, Slovak consumers think that plant-based meat substitutes will not be tasty and will not be affordable, especially in comparison with classic meat. Furthermore, by applying the Friedman test, we revealed statistically significant differences in the ideas of Slovak consumers about the consumption of plant-based meat substitutes from the point of view of different perspectives ($p<0.0001$). The Nemenyi method identified which aspects were evaluated equally and which differently (Table 2).

Table 2 Ideas about the consumption of plant-based meat substitutes

Sample	Frequency	Sum of ranks	Mean of ranks	Groups			
tasty food	518	1517.500	2.930	A			
affordable food	518	1698.500	3.279	A			
sustainable food	518	2375.000	4.585		B		
nutritious food	518	2397.500	4.628		B		
healthy food	518	2533.500	4.891		B	C	
safe food	518	2576.500	4.974		B	C	
ecological food	518	2655.000	5.125			C	D
moral food	518	2894.500	5.588				D

Source: own research

The research was further focused on whether Slovak consumers would consume plant-based meat substitutes as a substitute for traditional meat and meat products in the future. The results showed that almost 75% of young consumers would consume these substitutes in the future. The key reasons for their decision are the health aspect (mean = 1.70), environmental protection (mean = 1.81), taste (mean = 1.95), sustainability (mean = 2.01). On the other hand, the least significant reasons for consumption are the absence of food scandals (mean = 2.53), moral aspect (mean = 2.14) and lifestyle (mean = 2.12). The results showed that 25.1% of young consumers would not consume plant-based meat substitutes in the future, mainly for reasons of a strong habit of classic meat (mean = 1.56), fear of taste (mean = 1.75), distrust of alternative eating (mean = 2.38).

To support the consumption of plant-based meat substitutes in the future, we have identified the key motives for the acceptance of these substitutes. The key motive can be an increase in consumer awareness and information about plant-based meat substitutes, including health benefits, production, and environmental impacts. On the other hand, we have identified that for the future of the plant-based meat substitute market, similarity with classic meat is important, also in terms of appearance, texture, consistency, aroma, and price. By implementing the Friedman test, we revealed differences in the evaluation of consumption motives with a future perspective ($p<0.0001$). The Nemenyii method depicted using a Demsar graph showed the differences between motifs (Figure 1).

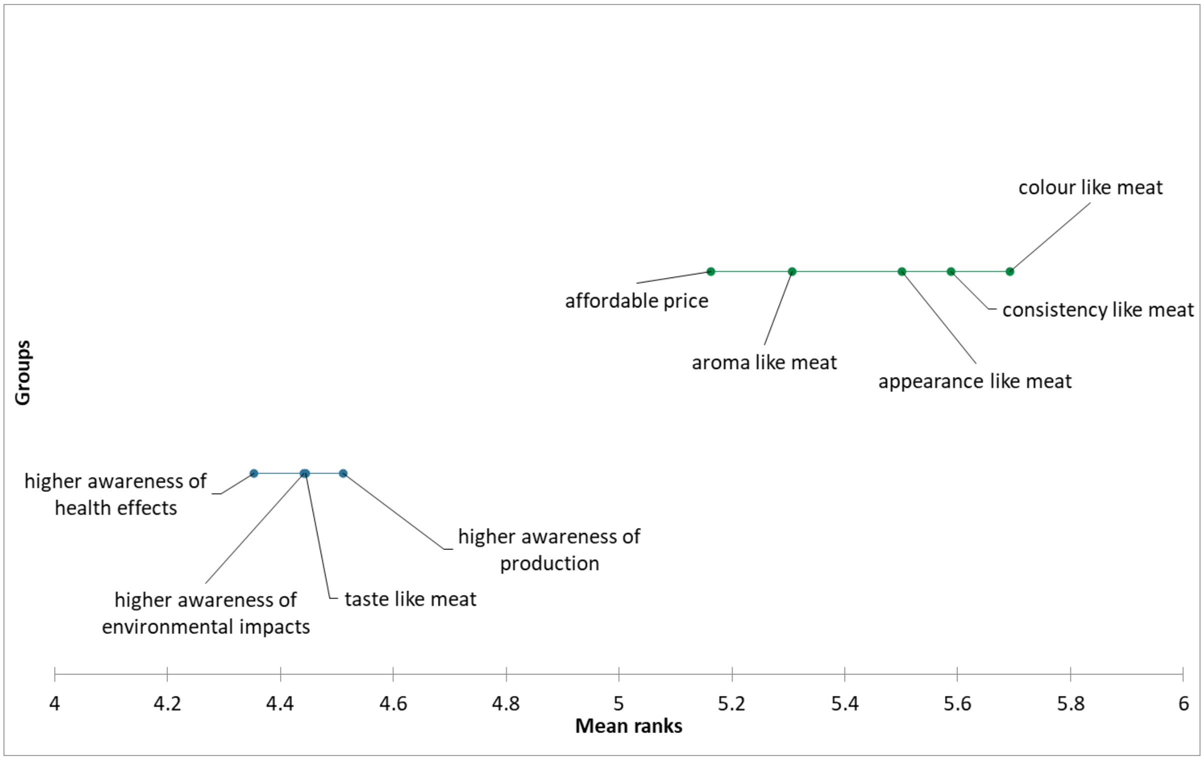

Figure 1 Motives for consumption of plant-based meat substitutes (Source: own research)

3.2. Consumption perspectives for edible insects

Another alternative to meat that was the subject of research was edible insects. The results of the survey showed that more than 75% of consumers under the age of 35 are aware of the existence of edible insects as a possible alternative to traditional meat in the future. The majority of young consumers consider edible insects to be nutritious food, ecological food, beneficial to health and advantageous from the point of view of sustainability. On the other hand, the biggest concern of consumers is about taste. The results of the Friedman test showed the existence of statistically significant differences in consumer perceptions in terms of various aspects and perspectives ($p<0.0001$). The Nemenyii method specified the differences between these characters (Table 3).

Table 3 Ideas about the consumption of edible insects

Sample	Frequency	Sum of ranks	Mean of ranks	Groups				
tasty food	518	1517.500	2.930	A				
moral food	518	2030.500	3.920		B			
safe food	518	2109.500	4.072		B			
affordable food	518	2235.500	4.316		B	C		
sustainable food	518	2465.500	4.760			C	D	
healthy food	518	2538.000	4.900				D	
ecological food	518	2781.500	5.370					E
nutritious food	518	2970.000	5.734					E

Source: own research

The results of the research further showed that edible insects would be acceptable for consumption in the future for only 30.1 % of young consumers. The main reasons that would encourage consumers to consume insects as an alternative to meat are taste (mean = 1.88), environmental protection (mean = 1.99), health aspect (mean = 2.06), sustainability (mean = 2.18). However, it is important to emphasize that the least significant reasons for consumption are the absence of food scandals (mean = 2.55), lifestyle (mean = 2.54) and moral aspect (mean = 2.54). On the other hand, the results showed that almost 70% of consumers would not consume edible insects in the future mainly for reasons of distaste (mean = 1.59), a strong habit of classic meat (mean = 1.86), or a lack of information (mean = 2.06).

In order to increase interest in edible insects as an alternative to meat and support the acceptability of their consumption, key motives were identified, which can be considered mainly the similarity with traditional meat related to taste and aroma, higher consumer awareness regarding production, health aspects and the environment, as well as the available price. The Friedman test identified statistically significant differences in the consumer evaluation of individual aspects that can support the consumption of insects ($p<0.0001$). The specifics of the differences were shown by the Nemenyi method, graphically represented by a Demsar plot (Figure 2).

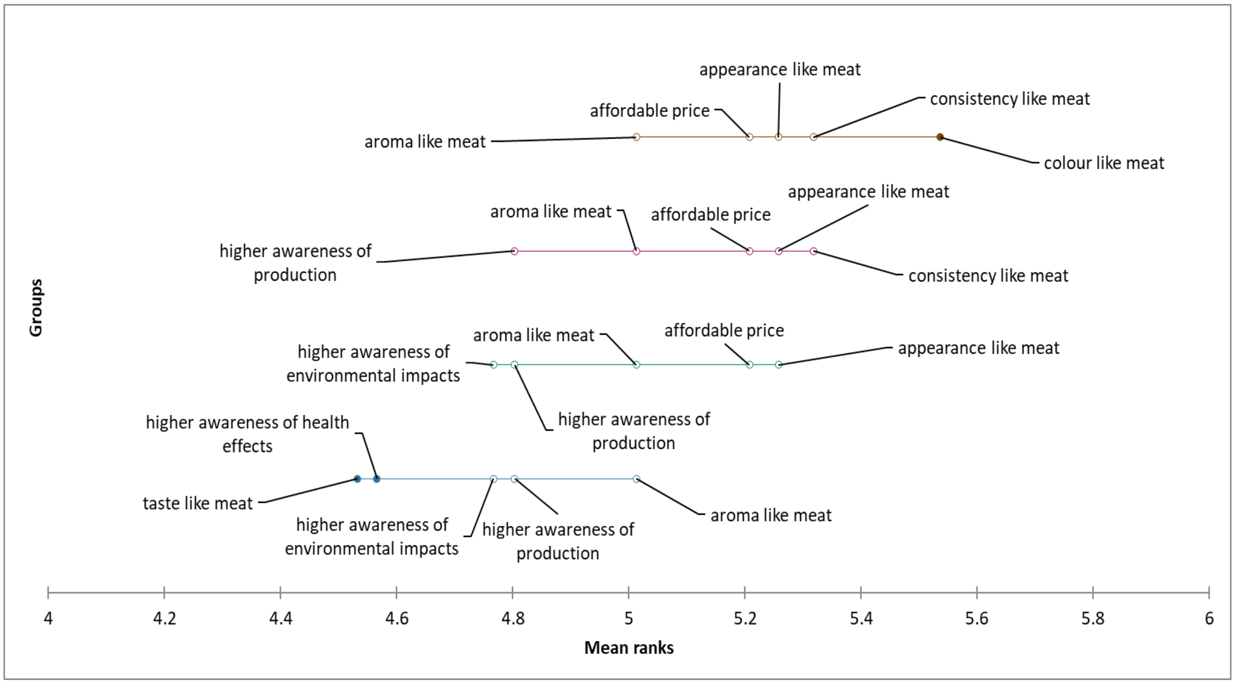

Figure 2 Motives for consumption of edible insects (Source: own research)

3.3. Consumption perspectives for cultured meat

The last meat alternative investigated was cultured meat. The results of the consumer survey showed that more than half of young Slovak consumers are aware of the existence of cultured meat, meat from laboratories. Young consumers have positive expectations for the consumption of cultured meat, especially from the point of view of ecology, sustainability, and the moral aspect. On the contrary, negative ideas about consumption are related to health, taste, and nutritional aspects. Statistically significant differences were identified between the evaluated aspects and ideas regarding consumption using the Friedman test ($p<0.0001$). The specification of differences was demonstrated by the Nemenyi method (Table 4).

Table 4 Ideas about the consumption of cultured meat

Sample	Frequency	Sum of ranks	Mean of ranks	Groups			
healthy food	518	1870.000	3.610	A			
tasty food	518	1982.000	3.826	A	B		
nutritious food	518	2077.000	4.010	A	B		
safe food	518	2117.500	4.088		B		
affordable food	518	2136.000	4.124		B		
moral food	518	2622.500	5.063			C	
sustainable food	518	2867.000	5.535				D
ecological food	518	2976.000	5.745				D

Source: own research

With a future perspective, up to 57.7 % of consumers can imagine consuming cultured meat as an alternative to traditional meat. Environmental protection (mean = 1.90), sustainability (mean = 2.12), taste (mean = 2.12) and health aspect (mean = 2.17) are perceived as the key reasons for consumption. Young consumers consider the absence of food scandals (mean = 2.64), lifestyle (mean = 2.61) and moral aspect (mean = 2.40) as less important reasons for consumption. However, the results showed that 42.3 % of consumers would not consume cultured meat in the future mainly because of distaste (mean=1.88), lack of information (mean=1.89) and distrust of the new alternative diet (mean=2.05).

The taste similarity to classic meat can contribute to increasing the acceptability of cultured meat as a meat alternative. Moreover, higher consumer awareness regarding production, health aspects, impacts on the environment, as well as the affordable price can also contribute to the support of consumption. Among the evaluated consumption motives, statistically significant differences were identified using the Friedman test ($p<0.0001$). Demsar plot indicated the results of the Nemenyi test and was used to graphically represent confirmation of differences in consumer evaluation of motives for higher acceptability and support for the consumption of cultured meat in the future (Figure 3).

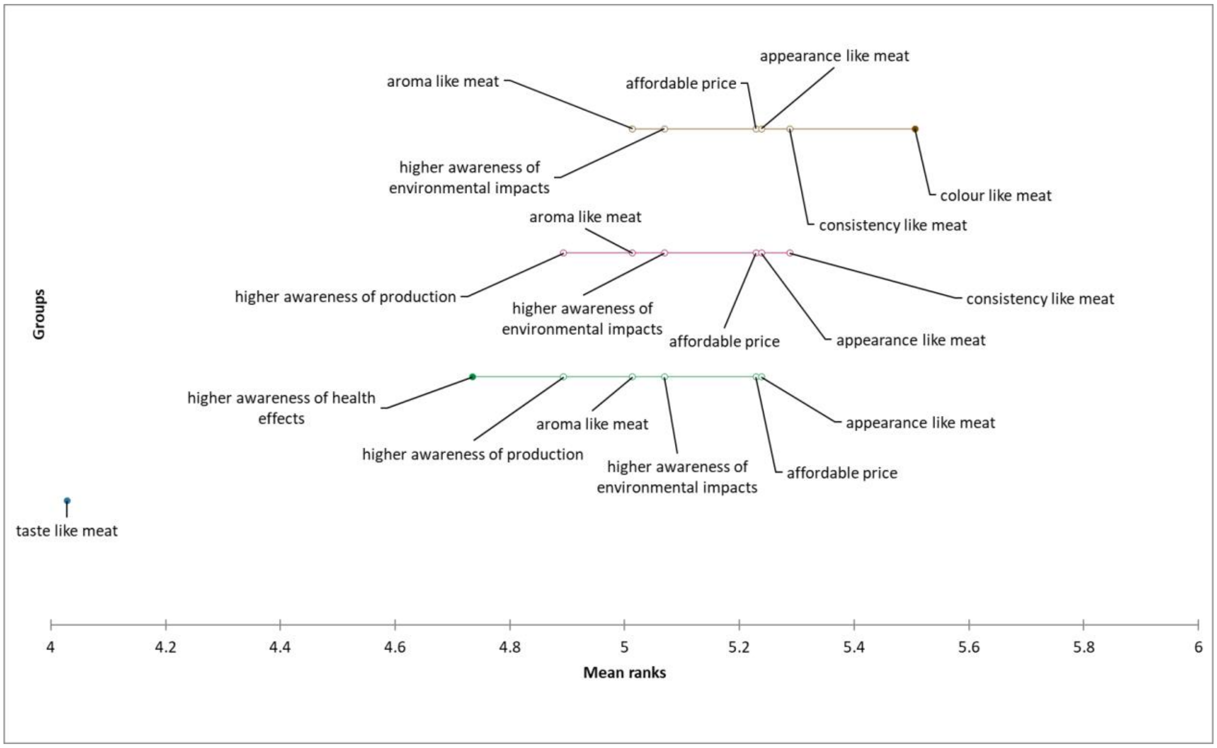

Figure 3 Motives for consumption of cultured meat (Source: own research)

The results of a survey conducted in Slovakia among the young generation of consumers focused on the acceptability of individual meat substitutes showed the highest consumer awareness regarding plant-based meat substitutes. Consumer ideas regarding the consumption of plant-based meat substitutes relate to morality, ecology, safety and health. Consumers' expectations of edible insects as food are linked to nutrition, ecology, health and sustainability. Cultured meat as an alternative is considered ecological, sustainable and moral for consumption by consumers. The results further showed that among all the alternatives, plant-based meat substitutes are the most acceptable for consumption, followed by cultured meat and finally edible insects. The key reasons for consumption are awareness of aspects of health, environmental protection, or sustainability. The biggest barriers to the consumption of meat alternatives are fear of taste and a strong habit of classic meat and meat products. Higher consumer awareness and the use of technological processes that bring the attributes of meat substitutes closer to traditional meat and meat products can positively contribute to the future of the meat substitutes market.

Other consumer research studies have also focused on the issues of meat substitute consumption with a future perspective. Estell et al. (2021) point to a growing demand for plant-based meat substitutes because consumers are beginning to realize that these foods are healthy and sustainable. The perception of these substitutes as better compared to meat in terms of health and sustainability was also identified by studies carried out by Vural et al. (2023) and Begho et al. (2022). Verbeke et al. (2015) found that cultured meat is perceived by consumers as safe, nutritious, ecological and ethical. Edible insects were the subject of a study by Bogusz et al. (2020) and emphasize that up to 60% of consumers agreed with the statement that edible insects have nutritional and environmental benefits.

A key motive for promoting the consumption of meat substitutes is to produce alternatives that are similar to meat, especially in terms of taste and texture (Michel et al., 2021). In the case of plant-based meat substitutes, it is important to fulfil the taste expectations of consumers (Graça et al., 2019). Bryant and Barnett (2020) identified taste and price as key determinants of the success of launching cultured meat in the meat substitutes market. According to Rolland et al. (2020) and Van Loo et al. (2020), higher consumer awareness can contribute to consumer acceptance of cultured meat. Higher consumer awareness, especially regarding health and environmental benefits, can positively affect the development of the market for edible insects (Sogari et al., 2019; Mancini et al., 2019). Burt et al. (2019) found that incorporating edible insects into food products and direct invisibility can contribute to acceptance of edible insects for consumers.

4. Conclusion

The paper reflects on the current situation on the meat market facing the growing demand for meat, but also on issues related to health risks resulting from excessive meat consumption, as well as sustainable aspects of meat production. The future of the meat market will be determined by the existence and consumption of meat substitutes, which are mainly plant-based meat substitutes, edible insects and cultured meat. The paper focused on the mentioned substitutes and tried to reveal consumer awareness, acceptance and future consumption of these foods among young consumers.

The survey in Slovakia among young consumers showed high awareness and acceptance of plant-based meat substitutes due to their moral, ecological, safety, and health benefits. Despite nutritional and ecological benefits, edible insects are the least acceptable to consumers. Cultured meat is seen positively for its ecological, sustainable, and moral benefits, making it more acceptable than insects but less than plant-based options. Key motives for choosing meat alternatives include health, environmental protection, and sustainability, while taste concerns and a preference for traditional meat are major barriers. To improve the acceptability of meat substitutes among young Slovak consumers, enhancing the taste and texture through research and development is crucial. Educational campaigns should highlight the health, environmental, and sustainability benefits to shift consumer preferences. Marketing efforts should focus on the ethical and ecological advantages of cultured meat and plant-based substitutes, while innovative culinary approaches can help increase the acceptance of edible insects.

The results of the study have significant implications for both science and practice. From a scientific point of view, the study contributes to the understanding of consumer perception of meat substitutes and potential consumer behaviour in the market of meat substitutes. These findings provide valuable insights for researchers investigating innovative foods and their consumption. In addition, the lack of consumer awareness of meat substitutes highlights the need for further research aimed at developing educational approaches. The findings of the study are also beneficial for enterprises in the meat industry, as well as other food companies that adapt to changing market demands and are interested in developing products in accordance with current trends. The study and its results may also be valuable for policy makers, who should focus on implementing educational campaigns aimed at addressing the lack of consumer awareness of meat substitutes and their consumption.

Acknowledgment
This publication was supported by the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

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HISTORY:
Received 22.03.2024
Received in revised form 26.04.2024
Accepted 30.04.2024

PRODUCTIVE POTENTIAL AND GRAIN QUALITY OF SORGHUM HYBRIDS GROWN ON BLACK SOIL AND TECHNOSOL AT THE LAND RECLAMATION STATION

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Abstract

The study was conducted at the Pokrov Research Land Restoration Station of the DSAEU based in area of manganese ore deposit exploration in the south of the Dnipropetrovsk region. The aim of the research was to compare the productive potential and grain quality of sorghum hybrids grown on black soil and technosol (long-term phytomeliorated loess-like loam). Sorghum hybrids obtained from Raelin Company including four sweet sorghum hybrids (SS506, Sioux, Mohawk, G1990), six grain sorghum hybrids (Kato, Ponki, Tzuni, Yuki, Milo W, Yutami) and two sorghum-sudanese hybrids (Koso, Ute BMR) showed a good potential for cultivation on black soil and technosol. Sweet sorghum hybrids and sorghum-sudanese hybrids can produce a yield of fresh biomass from 30 to 80 t/ha. The Sioux hybrid showed poor growth characteristics and cannot be recommended for obtaining this type of production on marginal lands. The grain yield of grain sorghum hybrids was high and even exceeded theoretically expected by 12-40 %. The most productive varieties were Ponki and Yutami. Sorghum grain grown on technosol was inferior to trial on black soil. The Yutami and Ponki hybrids had the highest protein content.

Keywords: Sorghum, hybrids, technosol, yield, protein

1. Introduction

Sorghum is a crop that is well-adapted to abiotic stresses including drought, salinity, heat, and cold stress (Behera et al., 2022). Sorghum can be classified into four main groups based on their production characteristics: grain sorghum, feed sorghum, industrial sorghum and sweet sorghum.

Sorghum grain contains 70-73 % starch, 12-15 % protein, 3.5-4.5 % fat. Grain and green mass are not inferior to corn in fodder properties. Groats from special sorghum hybrids are not inferior to rice and millet in terms of technological and organoleptic properties (Sorghum 2000). Sweet sorghum juice can be used to produce sugar, syrup and ethanol (Dweikat et al., 2012). Bagasse is also used as feed or a raw material for the paper industry (Taylor et al., 2006). The stems of sorghum crops contain readily available soluble carbohydrates. In this case, the enzymatic conversion of starch into sugar is not necessary. This gives sorghum an economic advantage over other starch-based crops. A short growth cycle (about four months), high water and nutrient efficiency utilization favors the use this crop for bioenergy applications (Reddy et al., 2005; Shoemaker et al., 2010; Rutto et al., 2013; Regassa and Wortmann, 2014). The unpretentiousness of sorghum to environmental conditions outlines the prospect of growing this plant on marginal lands. This avoids the conflict between food and biofuel production (Ameen et al., 2017; Mehmood et al., 2017; Yücel et al., 2022). Technosols are a new group of soils that are strongly influenced by technical human activity (Macia et al., 2014; Betancur-Corredor et al., 2020). The breeding of the most productive hybrids and their response to technosols has not yet been sufficiently studied. Today in Ukraine 8 million hectares are unproductive. The area of abandoned post-mining lands in Dnipropetrovsk province is about 200 thousand ha situated in several districts with deposits of manganese and non – ferrous ores (Kharytonov and Resio Espejo 2013). Land reclamation is conducted in one technological cycle after the process of ores mining. In doing so, soil mass is taken off, piled up and heaped onto the land after the rock has been replaced. Nowadays, the cultivation of sorghum in Ukraine takes place on zonal soils. This is mainly related to the production of grain as a component of the diet of broiler chickens and silage for anaerobic digestion in biogas complexes. Our intentions are related to the assessment of the prospects for growing hybrids of foreign selection on reclaimed land with the aim of complex utilization of biomass, including the production of bioethanol and pellets. So, such fast growing crop as sorghum can be first choice to grow in such areas, where large areas of reclaimed lands exist, a suitable climate is present for biomass growth and the harvested biomass can be transported to a regional energy market. The main objective of this study was to compare the productive potential and grain quality of sorghum hybrids grown on black soil and technosol.

2. Material and methods

Field experiments were conducted in Ukraine, in the South of Dnipropetrovsk province, at the Pokrov Research and Educational Station of the Dnipro State Agrarian and Economical University (47°39°N, 34°08°E), with an elevation of 60 m (Kharytonov et al., 2019; Havryushenko et al., 2022).

Sorghum plants presented by 12 hybrids of American selection obtained from Raelin Company were studied in the field experiments (Figure 1).

Figure 1 Field experiment with sorghum hybrids.

There are four sweet sorghum hybrids (SS506, Sioux, Mohawk, G1990), six grain sorghum hybrids (Kato, Ponki, Tzuni, Yuki, Milo W, Yutami) and two sorghum-sudanese hybrids (Koso, Ute BMR). The seeds were sown on two experimental plots with different types of substrates: technosol (long-term phytomeliorated loess-like loam) and black soil mass. The content of humus in the loess loam did not exceed 1.1 %, in black soil mass – 3.3 %. No fertilizers were added. Biometric indicators, yield of fresh biomass and grain were determined according to generally accepted methods. The following characteristics of sorghum have been studied: fresh, dry biomass, grain yield and protein content. Plant sampling was made two times. The first time, fresh biomass at the wax stage was taken from the 1 m^2 plot and weighed in three repetitions. Grain mass was taken at the full wax stage from the 1 m^2 plot and weighed in three repetitions as well. Fresh biomass was weighed. The dry matter level was determined by fresh biomass drying at 60 °C for 24 h. The yield of ripe grain was determined by weighing it after threshing with subsequent conversion to t/ha. Plant height was measured using a measuring ruler. Stem diameter was determined using a calliper by random sampling at a height of 15 cm from the soil surface. Protein content in grain samples determined using Kjeldahl method.

All obtained results were processed by statistical methods using the StatGraphics Plus5 soft at a probability level of 0.95 % ($P<0.05$).

3. Results and discussion

The height of all investigated sweet sorghum hybrids was below the potential average by 9-25 % on black soil, and by 22-37 % on phytomeliorated loess like loam. Growth indicators of the Sioux variety were the worst. Plants on both types of substrates did not exceed 95-105 cm with a potential height of 200-250 cm (Figure 2). Sorghum-Sudanese hybrids, on the contrary, showed good growth and had a height of 200 cm to 250 cm. This corresponds to the certified characteristics of these hybrids. Most of the grain sorghum hybrids can be rated as short with a height of 90-110 cm. The Kato variety is medium-sized with a height of 180-200 cm. The height of these hybrids on black soil was potentially average or exceeded it by 11-24 % (Yutami, Yuki, Tzuni).

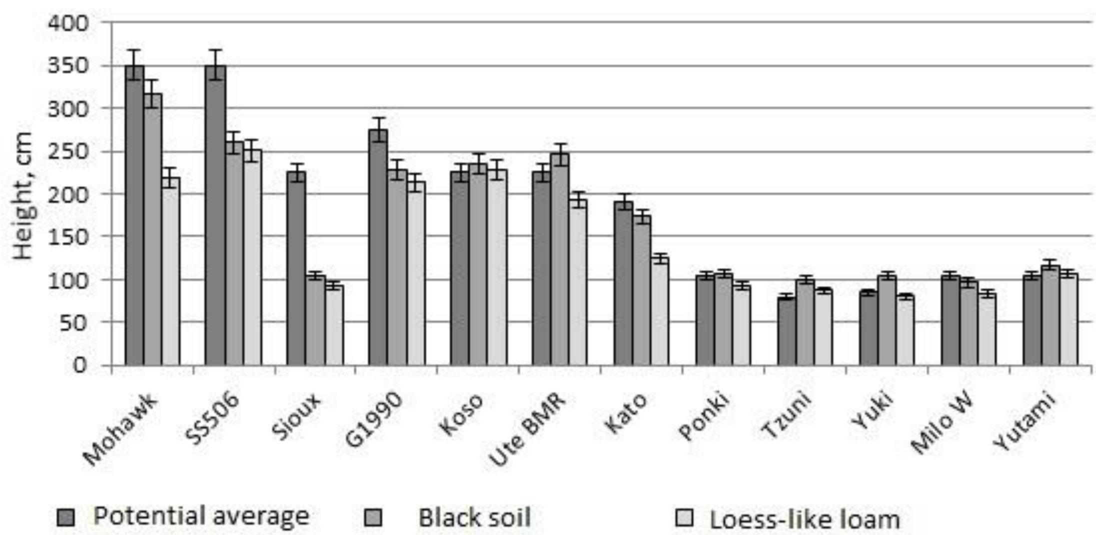

Figure 2 The sorghum hybrids height, (cm).

In the case of phytomeliorated loess-like loam, plants grew more slowly and were lower than on black soil by 5-34 %, with the exception of Yutami and Tzuni hybrids. Determination of the diameter of shoots showed that most hybrids have stronger stems on black soil than on phytomeliorated loess loam (Table 1). The difference is from 6.5 % to 27.3 %. Exceptions are Koso and G1990 hybrids. The diameter of their shoots on phytomeliorated loess-like loam was thicker by 14.4 and 28.1 %, respectively, than black soil.

Table 1 Diameter of shoots of sorghum hybrids, (mm).

Hybrid	Diameter of shoots, mm		Hybrid	Diameter of shoots, mm	
	Black soil	Loess Like Loam		Black soil	Loess Like Loam
SS506	17.22±0.30	19.11±0.38	Kato	16.11±0.35	14.56±0.25
Sioux	15.67±0.41	11.67±0.31	Ponki	17.11±0.29	16.67±0.28
Mohawk	18.78±0.44	16.33±0.41	Tzuni	13.56±0.21	12.67±0.33
G1990	15.01±0.36	19.22±0.47	Yuki	14.22±0.26	10.33±0.23
Koso	11.56±0.39	13.22±0.30	Milo W	13.78±0.23	12.44±0.24
Ute BMR	14.33±0.21	14.00±0.27	Yutami	16.67±0.32	15.33±0.35

Panicle length did not differ significantly on both substrates in cultivars G1990, Kato, Ponki, Tzuni and Yuki. Panicle length in hybrids SS506, Koso, Ute BMR, Milo W and Yutami was 14-21 % longer in plants growing on phytomeliorated loess-like loam, while in Mohawk and Sioux varieties, on the contrary, it was 10-12 % less. The yield of green mass was determined for hybrids of sweet sorghum, sorghum-sudanese hybrids and medium-growing grain sorghum Kato (Figure 3).

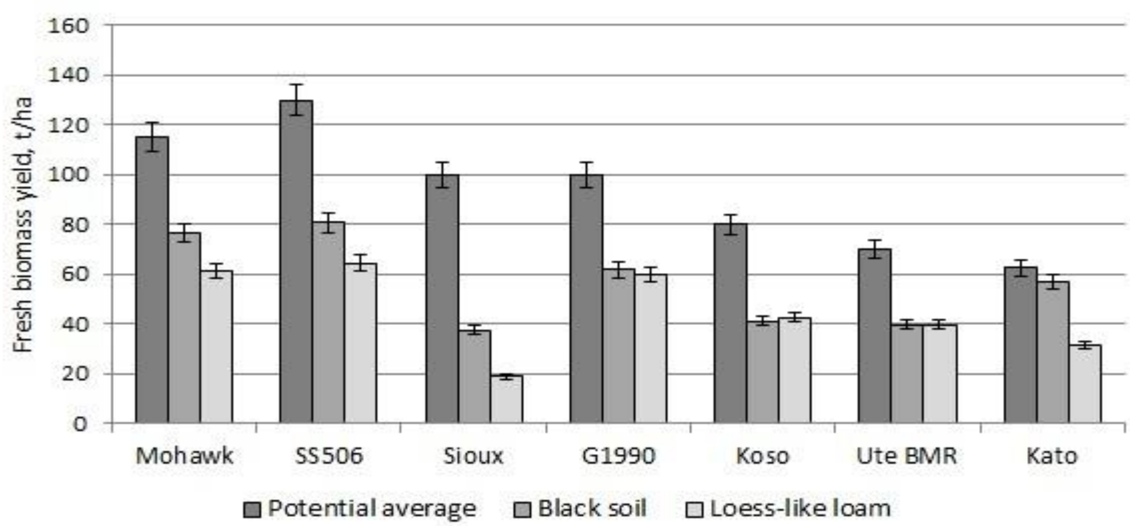

Figure 3 Fresh biomass of sorghum, (t/ha).

It was established that these hybrids are capable of producing biomass from 40 to 80 t/ha on black soil and 30-65 t/ha on phytomeliorated loess loam. Among sweet sorghum hybrids, only Sioux was poorly productive. Its yield was only 37.5 t/ha on black soil and 19 t/ha on phytomeliorated loess loam, or 35 % and 17 % of the potential yield. Sorghum-sudanese hybrids on both types of soil showed slightly more than 50 % of their potential. The yield of grain sorghum Kato on black soil was 91 % of the theoretically expected, and on loess loam – 50 %. Determination of grain yield in grain sorghum hybrids showed that the low fertility of phytomeliorated loess-like loam is not an obstacle to obtaining high yields in such conditions (Figure 4).

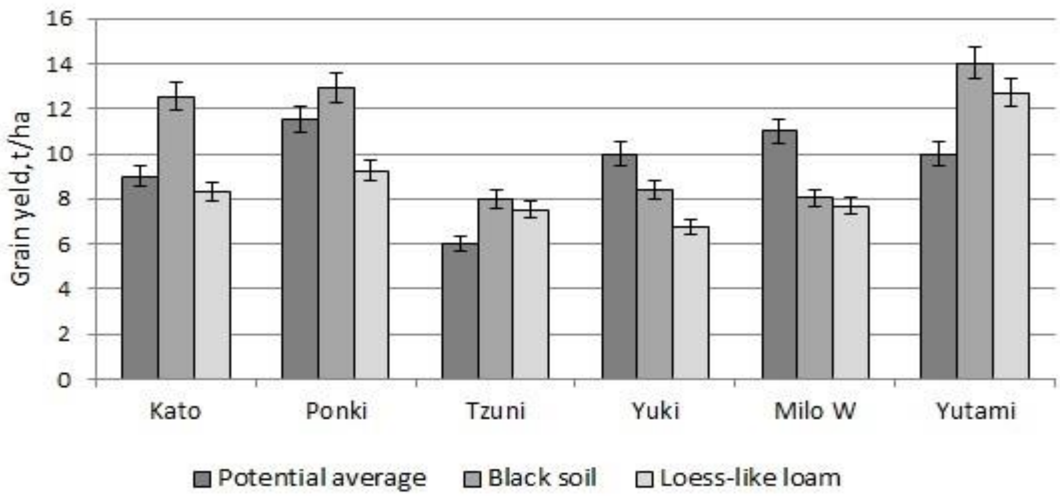

Figure 4 Grain yield of sorghum, (t/ha).

The grain yield of Kato, Ponki, Tzuni and Yutami hybrids on black soil exceeded theoretically expected by 12-40 %. The Ponki and Yutami varieties grown on phytomeliorated loess like loam were also highly productive with an excess of the potential average yield by 25 % and 27 %, respectively. A similar effect on low nitrogen levels in the soil was recorded in some other American lines of grain sorghum (Ketumile et al., 2021). The yield of other hybrids on this substrate was below the theoretical from 8 % (Kato) to 32 % (Yuki). The results of the analysis of the protein content in the grain of some sorghum hybrids are shown in Figure 5.

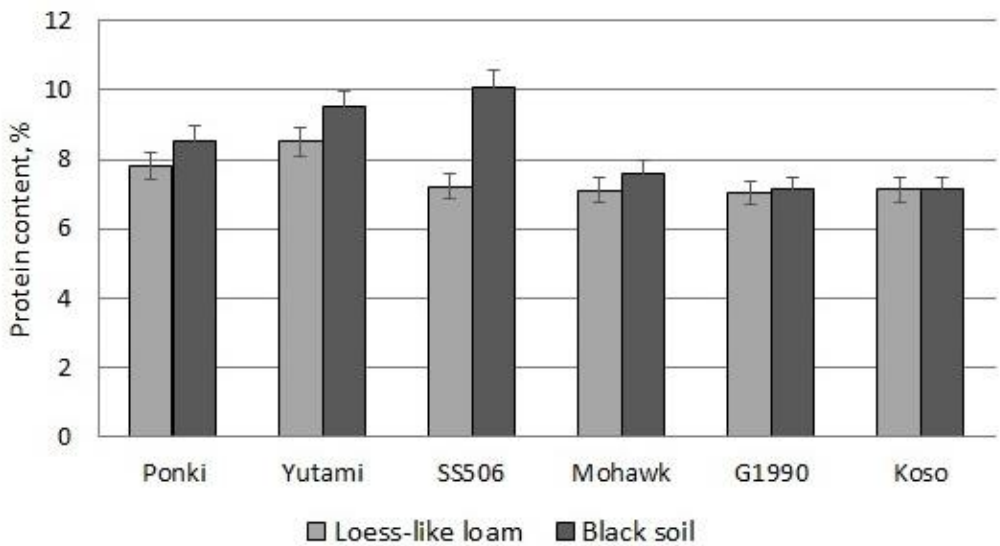

Figure 5 Protein content in sorghum grain, (%).

The grain of sorghum grown on phytomeliorated loess like loam was inferior to the trial on black soil. The hybrids Yutami and Ponki had the highest protein concentration.

4. Conclusion

The height of all investigated sweet sorghum hybrids was below the potential average by 9-25 % on black soil, and by 22-37 % on technosol. Sorghum-Sudanese hybrids, on the contrary, showed good growth and had a height of 200 cm to 250 cm. Most of the grain sorghum hybrids can be rated as short with a height of 90-110 cm. The fresh mass yield of sweet sorghum and sorghum-sudanese hybrids on the layer of black soil and loess-like loam reaches 30-80 t/ha, which is 60-90 % of the theoretical productivity. The grain yield of grain sorghum hybrids was high: 8-14 t/ha on black soil and 6.7-12.7 t/ha on loess-like loam. The most productive were Ponki and Yutami hybrids. The grain yield of sorghum grown on phytomeliorated loess-like loam was inferior to trials on black soil. The Yutami and Ponki hybrids had the highest protein concentration. The results obtained will be useful for growing the noted above promising sorghum hybrids on marginal lands for the integrated use of biofeedstock.

Acknowledgment

This work was supported by the grant from Ministry of Education and Science of Ukraine.

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ARTICLE HISTORY:
Received 28.03.2024
Received in revised form 27.04.2024
Accepted 30.04.2024

EFFICIENCY OF SWEET, SUDAN AND BROOM SORGHUM VARIETIES USING AS POLLINATORS FOR HIGH YIELD HYBRIDS BREEDING IN THE STEPPE ZONE OF UKRAINE

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Abstract

The main objective of this case study was to determine the effectiveness of using sweet (Silosne 42), sudanese (Strateya) and broom sorghum (Karlykove 45 and Krasen) as pollinators for obtaining high-yield hybrids in the conditions of the northern part of the steppe zone of Ukraine. Varieties Karlykove 45 and Krasen are representatives of broomcorn. 17 original kinds of grain and sweet sorghum were studied in the field experiment. Karlykove 45 variety turned out to be the most effective pollinator to ensure the highest yield (65.6-71.7 t/ha) in three newly created hybrids (Nyzkorosle 93s × Karlykove 45, Early 776s × Karlykove 45 and DN 71s × Karlykove45). The Sudanese sorghum variety Stratea showed effectiveness for obtaining the yield of fodder hybrids (54.6-65.5 t/ha) in combinations (DN 19s × Stratea and DN 17s × Stratea). Karlykove 45 variety can be recommended as a valuable pollinator for creating hybrids for solid biofuel. The best F1 hybrids can be placed in a line according to the decreasing in dry biomass: Nyzkorosle 93s × Karlykove 45 > Kafrske feed 186s × Krasen > Dn71s × Karlykove 45.

Keywords: sorghum, fertile lines, pollinators, yield

1. Introduction

Sorghum is grown in marginal areas where other crops grow poorly, by farmers who are among the world's poorest (Dalton and Zereyesus, 2013). African farmers grow several morphological forms or "races" of sorghum including caudatum (originating in eastern Africa), durra (with residual moisture) and Sorghum bicolor, which is broadly distributed (Smale et al., 2018). However, Sorghum bicolor is an important coarse grain crop in different countries (Tarumoto, 1974; Maves and Atkins, 1988; Murty, 1995, Bibi et al., 2010; Silva et al., 2022; Demarco et al., 2023). Genomic selection is expected to improve selection efficiency and genetic gain in breeding programs (Maulana et al., 2023). The results suggest that genomic prediction could become an effective tool for predicting the performance of sorghum hybrids based on parental genotypes. Ten sorghum hybrids were tested at two locations in Pakistan (Mumtaz et al., 2019). The following ranges were determined in the investigated traits for grain yield (28.6-52.7 t/ha) and fodder yield (28.7-45.7 t/ha). F1 hybrid forage sorghums take as advantageous for producing forage as F, grain sorghums are for producing grain (Tarumoto, 1974). It is possible to develop F, hybrids with high forage yield even when using the dwarf male-sterile lines of grain sorghum as seed parents. Testing of parent lines for general combining ability should be supplemented in hybrid breeding procedures, by evaluation of individual F1 hybrids for specific combining ability (Kenga et al., 2004). Sorghum has recently been identified as a promising bioenergy feedstock (Brenton et al., 2016; Mullet et al., 2014; Kharytonov et al., 2019). Heterosis is a key factor considered in hybrid breeding. The degree of heterosis was different among subpopulations even though the parental lines had similar genetic distances from the testers (Ishimori et al., 2020).These results suggest that heterosis groups of bioenergy sorghum should be considered for maximizing the performance of F1. It was shown that both general and specific combining ability effects are important but predominance of non-additive genetic variance indicate the presence of Sorghum heterozygosis in the population (Mulualem et al., 2020). Fixed male line exhibited highly significant positive general combining ability effect for total fresh and dry biomass weight (Wagaw and Tadesse, 2020). It could be used to develop dual purpose sorghum varieties. Last time sorghum hybrid breeding utilizes the cytoplasmic-nuclear male sterility (CMS) system for seed production and subsequently harnesses heterosis (Bohra et al., 2016). Since the cost of developing and evaluating inbred and hybrid lines in the CMS system is costly and time-consuming, genomic prediction of parental lines and hybrids is based on genetic data genotype (Sapkota et al., 2023). Success in developing promising hybrids depends on the choice of parents to hybridize and the amount and type of genetic variation present in the base population (Jadhav and Deshmukh, 2017). In Europe, broomcorns continue to be grown for a unique market (Berenji et al., 2011; Dahlberg et al., 2011). Most recently, various forage accessions have been evaluated for their potential for renewable fuel production. The main objective of this case study was to determine the effectiveness of using sweet, sudanese and broom sorghum as pollinators for obtaining high-yield hybrids in the conditions of the northern part of the steppe zone of Ukraine.

2. Material and methods

The research was conducted in 2021-2023 at the Sinelnykivo breeding research station of the Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine. This station is located in the Dnipropetrovsk region at the northern part of the Steppe zone of Ukraine.

17 original kinds of grain and sweet sorghum were studied in the competitive variety test field experiment (Figure 1).

Figure 1 Field experiment on sorghum hybrids breeding.

Four varieties were used for pollination. Karlykove 45 and Krasen varieties are representatives of broomcorn. Strateya is sudanese sorghum, and Silosne 42 is a sweet sorghum variety. Stratea is the parent component of the sorghum-sudanese hybrid. Krasen is the parent component of Vinnytsia sorghum. Karlykove 45 is the parent component of Vinnytsia sorghum. Silosne 42 is the parent component of sweet sorghum.

Dn5s - the maternal component, a sterile line of grain sorghum and its fixer Dn-5 f, belong to the Nubian subspecies of grain sorghum. Dn13s maternal component sterile line and its fixer Дп–13 f belong to grain Nubian sorghum. Dn 17s sterile line and its sterility fixer Дп–17 f refer to sweet sorghum. Dn 19s sterile line and its sterility fixer Dn–19 f refer to sweet sorghum. SK23s sterile line and its sterility fixer SK-2Z f, belong to the Nubian subspecies of grain sorghum. Dn 37s sterile line and its sterility fixer Dn –37 f refer to African sorghum. Dn 39s sterile line and its sterility fixer Dn–39 f refer to Kafrske grain sorghum. Medium-ripe, with a growing season of 100–117 days. Dn 71s sterile line and its sterility fixer Dn –71 f – grain sorghum. Niz81s sterile line and its sterility fixer Nizkorosle 81f refers to Kafrske sorghum. Niz93s sterile line and its sterility fixer. Low-growing 93 f refers to Kafrske sorghum. Kafrske early 2 s is a sterile line and its sterility fixer Kafrske forage 186 f refers to Kafrske sorghum. A 326 sterile line and its sterility fixer B326 refer to Kafrske grain sorghum.The area of plots in the nursery for the study of combinatorial ability was 7 m^2, the repetition in the experiment - three times. Harvesting was done manually with subsequent weighing. 3 weighing of 50 g were taken from two non-adjacent repetitions for further drying at a temperature of 100°C to a constant weight to determine the content of dry matter.

3. Results and discussion

The results of sorghum hybrides fresh biomass estimation for 2023 are shown in Table 1.

Table 1 Fresh biomass of the sorghum hybryds, t/ha

Hybryd	Krasen	Strateya	Karlykove 45	Silosne 42
Dn 5s	42.6	21.0	51.2	21.8
Dn 13s	26.5	31.0	31.7	32.6
Dn 17s	48.3	54.6	53.9	21.3
Dn 19s	41.0	65.5	52.8	33.3
Sk 23s	52.3	43.0	43.5	31.7
Dn 37s	50.2	32.7	41.4	25.9
Dn 39s	31.7	41.0	61.1	32.3
Dn 71s	41.6	42.0	65.6	41.5
Nyzkorosle 81s	33.7	35.0	67.5	24.4
Nyzkorosle 93s	31.3	33.3	71.7	22.5
A158	42.8	41.3	61.6	41.6
Kafrske feed. 186s	51.5	40.0	52.0	31.6
Rannye776s	52.4	32.0	66.4	22.5
A 326	23.4	31.5	22.8	21.3
Gaolyan 09-3094 s	52.6	48.6	51.0	22.8
Yeferemiv. byle 2s	31.8	41.1	61.0	31.0
Gos 11 s	41.4	31.0	55.3	33.5

The following combinations of parent and pollinating species stood out as the best hybryds: F1 (Nyzkorosle 93s × Karlykove 45) – 71.7 t/ha, F1 (Rannye 776s × Karlykove 45) – 66.4 t/ha; F1 (Dn 71s × Karlykove 45) – 65.6 t/ha, (Dn 19s × Strateya) – 65.5 and Dn 17s × Strateya – 54.6 t/ha. F1(Yefremivske bile 2s × Karlykove 45) – 61.0 t/ha.According to the average values, the following combinations F1(Nyzkorosle 81s × Krasen) – 33.7 t/ha, F1(Dn39s × Silosne 42) – 32.3 t/ha were selested. F1 (A 326 × Strateya) – 33.0 t/ha. It was established that the Karlykove 45 pollinator showed an average result of 53.6 t/ha. Pollinators Krasen and Strateya – 40.9 t/ha, 39.1 t/ha, respectively, Silosne 42 – 28.9 t/ha. The results of the dry biomass estimation for the sorghum hybrids are shown in Table 2.

Table 2 Dry biomass of the sorghum hybryds, t/ha

Hybryd	Krasen	Strateya	Karlykove 45	Silosne 42
Dn 5s	16.3	12.6	22.5	12.4
Dn 13s	11.3	11.2	12.7	11.9
Dn 17s	21.5	32.0	13.2	14.3
Dn 19s	18.8	18.6	25.8	15.9
Sk 23s	22.6	17.4	23.5	12.1
Dn 37s	22.9	16.5	18.5	11.7
Dn 39s	13.8	15.5	22.1	12.1
Dn 71s	18.6	13.7	33.3	24.5
Nyzkorosle 81s	9.0	13.6	45.0	13.3
Nyzkorosle 93s	18.7	12.1	28.9	13.3
A158	25.8	19.8	23.3	25.4
Kafrske feed. 186s	41.4	14.0	22.1	11.1
Rannye776s	31.8	13.8	31.3	13.6
A 326	17.6	9.9	13.2	10.3
Gaolyan 09-3094 s	27.4	13.9	12.5	13.2
Yeferemiv. byle 2s	22.9	21.2	21.1	14.1
Gos 11 s	22.2	13.2	17.3	17.0

In terms of dry matter yield of sorghum biomass, the best indicators were: F1 (Nyzkorosle 93s × Karlykove 45) – 45.0 t/ha, F1 (Kafrske feed. 186s × Krasen) – 41.4 t/ha, F1 (Dn71s × Karlykove 45) – 33.3 t/ha, F1 (Dn17s × Strateya) – 32.0 t/ha. The average yield of dry matter ranged from 17.6 t/ha F1(A 326 × Krasen) to 27.4 t/ha, F1(Gaolyan 09-3094c × Krasen). Pollinators Karlykove 45 (21.3 t/ha) and Krasen (23.1 t/ha) can be singled out by average values.

4. Conclusion

The following tasks were solved to achieve the goal: to evaluate self-pollinated lines and pollinators according to the combining ability of the yield of green mass, dry matter, to select self-pollinated sorghum lines depending on the directions of use. 17 original kinds of grain and sweet sorghum were studied in the field experiment. The highest yield of fresh biomass was obtained in the combinations: F1 (Nyzkorosle 93s × Karlykove 45) – 71.7 t/ha, F1 (Rannye 776s × Karlykove 45) – 66.4 t/ha; F1 (Dn 71s × Karlykove 45) – 65.6 t/haand (Dn 19s × Strateya) – 65.5, and Dn 17s × Strateya – 54.6 t/ha. The use of sorghum varieties for pollination made it possible to obtain dry biomass in a line by decreasing in the combinations: Nyzkorosle 81c × Karlikove 45 (45.0 t/ha), Kafrske feed. 186s × Krasen (41.4 t/ha), Dn 17s × Stratea (32.0 t/ha) and A158×Silosne 42 (25.4 t/ha).

Acknowledgment

This work was supported by the grant from National Academy of Agrarian Sciences of Ukraine.

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HISTORY:
Received 15.04.2024
Received in revised form 04.05.2024
Accepted 07.05.2024

THE PROTEIN BODIES DIFFERENTIATION OF TWO CORN LINES OF NORMAL AND OPAQUE 2 GENE KERNELS IN THE SOUTH OF UKRAINE

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Abstract

Corn lines Wf9 and R 168 at normal and their mutant forms with three doses of Opaque 2 gene saturation were studied. The main objective of this case study was to estimate the protein bodies' differentiation of two maize lines of normal and opaque 2 gene kernels. The protein bodies of corn lines Wf9 and R168 22 days after pollination are represented by two and three types differing in molecular weight, respectively. Protein bodies in the $Opaque_2$ (O2) mutant form are reduced 2-3 times compared to the original form. In terms of the number and ratio of amino acids, the isolated protein bodies bear the features of both zeins and glutelins. The amount of lysine and threonine in protein bodies increases almost 2 times when enriched with the O2 gene. At the same time, along with an increase in the content of essential amino acids, a decrease in the level of glutamic acid, proline and alanine was noted. The ratio of essential and non-essential amino acids in normal corn is 0.62, and in mutant corn – 0.7. This indicates an improvement in the synthesis of amino acids. A decrease in the leucine/isoleucine ratio from 5.6 in the normal form to 2.9 in the mutant form is an additional argument to characterize Opaque 2 genotype as quality protein corn.

Keywords: maize, corn endosperm, mutant forms, particulates, protein quality.

1. Introduction

Maize, often called as corn serves as an important source of food among various cereals. Together with rice and wheat it provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al., 2011). Traditional corn contains 8-10 % protein in the endosperm, but it's deficient in two essential amino acids such as lysine and tryptophan, whose amount is less than one-half of the concentration recommended for human nutrition (Gupta et al., 2015). Several mutants with increased lysine content, opaque2 (o2) and floury2 were identified in the research groups of Mertz and Nelson at Purdue University (Nelson et al., 1965). The discovery of nutritional value of the opaque2 (o2) mutation in maize was a significant breakthrough, as it alters the amino acid composition of the endosperm protein, resulting in nearly two-folds increase in lysine and tryptophan contents of wild-type kernels (Mertz et al., 1964). However, the agronomic problems associated with the soft endosperm of this mutant limited its use (Vega-Álvarez et al., 2022). There are several publications devoted to the effects of different o2 on accumulation of lysine and tryptophan in the endosperm (Hunter et al., 2002; Gutierrez-Rojas et al., 2008; Pandey, 2018). Over the past half century, it has been established that the glassiness of corn kernels is affected by formation of zein protein bodies (Holding and Larkins, 2006). Actually, high-lysine corn has been reported in which the transcriptional activator of the 22-kDa α-zein gene Opaque 2 (O2) is mutated and non-zein proteins are proportionally increased (Schmidt et al., 1992). However, this o2 mutant has several draw-backs, such as reduced grain yield, soft chalky endosperm, greater susceptibility to pests and diseases, and higher moisture content at harvest time, which precluded its direct commercialization. Fortunately, there are quantitative trait loci, referred to as o2 modifiers (Mo2s), that convert soft into hard endosperm without losing the high lysine trait (Wu and Messing, 2011). This combination is called quality protein maize or QPM (Vasal et al., 1980). Despite these advances in developing maize lines with higher nutritional value, the underlying physiological and molecular mechanisms that cause soft kernels is still not well understood. Several studies have investigated the changes in transcriptional patterns caused by the o2 mutation, including identification and characterization of lysine-rich proteins and starch biosynthesis genes in the opaque2 mutant (Jia et al., 2007; Hartings et al., 2011; Jia et al., 2013). Some studies have demonstrated an essential role for the 27-kDa γ-zein in endosperm modification (Wu et al., 2010; Liu et al., 2016). The enhanced accumulation of 27-kDa γ-zein favours the formation of protein bodies and their compaction between starch granules.

The main objective of this case study was to estimate the protein bodies' differentiation of two maize lines of normal and opaque 2 gene kernels.

2. Material and methods

Embryos were harvested from cross-pollinated F1 hybrids, self-pollinated inbred lines and germplasm stocks in the research field of the Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine (48°22’35”N35°01’06”E) with an elevation of 115 m. Corn lines Wf9 and R 168 at normal and their mutant forms with three doses of Opaque 2 gene saturation were studied. Seeds were obtained from the Maize Genetics Cooperative, Department of Agronomy, University of Illinois, Urbana, USA. Maize protein quality is determined by the composition of its endosperm proteins, which are classified as two groups: nutritionally poor zeins (prolamin and prolamin-like) and nutritionally rich non-zeins including albumin, globulin, glutelin-like, and glutelin (Sethi et al., 2021). Studies on the rate of protein synthesis in corn endosperms have shown that protein synthesis is most active between 20 and 28 days post pollination (Murphy and Dalby, 1971). That is why we have checked corn protein bodies’ formation on 22 day post pollination. Isolation of protein bodies was carried out in an ultracentrifuge VAC 601 (Janetzki company, Germany) according to Christianson et al. (1977). Corn grains separated from the pericarp and embryo were homogenized with a Teflon pestle in 5 volumes of buffer (0.1 M KH_2PO_4-0.006 M $MgCl_2$ with pH 7.5), filtered and centrifuged in a centrifuge K24 (Janetzki company, Germany) for 5 min at 400 g. 3 ml of supernatant was layered onto a preformed sucrose gradient (15-70 %) in buffer. The supernatant was separated into fractions in a centrifuge at a speed of 22,000 rpm. The supernatant containing membrane-bound ribosomes was decanted. The sediment containing protein bodies was suspended in phosphate buffer and layered on a linear sucrose gradient of 40-70 % and centrifuged for 2.5 hours at 20,000 rpm. Subsequently, the protein bodies were combined taking into account their distribution in the sucrose gradient, diluted with 0.1 M phosphate buffer pH 7.3 and centrifuged at 10,000 g to sediment them. The contents of the tubes were taken with a peristaltic pump and dispensed into the tubes using an automatic fraction collector. The protein concentration in the samples was determined following the recommendations made by Lu et al. (2010). Zeins and glutelins were extracted to get five fractions including alcohol and alkali soluble proteins (Sethi et al., 2021). Analysis of the amino acids content in the protein bodies, endosperm zeins and glutelins was performed using an amino acid analyzer as described by Scott et al. (2004).

3. Results and discussion

Representative large scale separations of homogenate dispersions of normal and O2 Wf9 and R168 endosperms at 22 day development are illustrated in Figure 1 and 2.

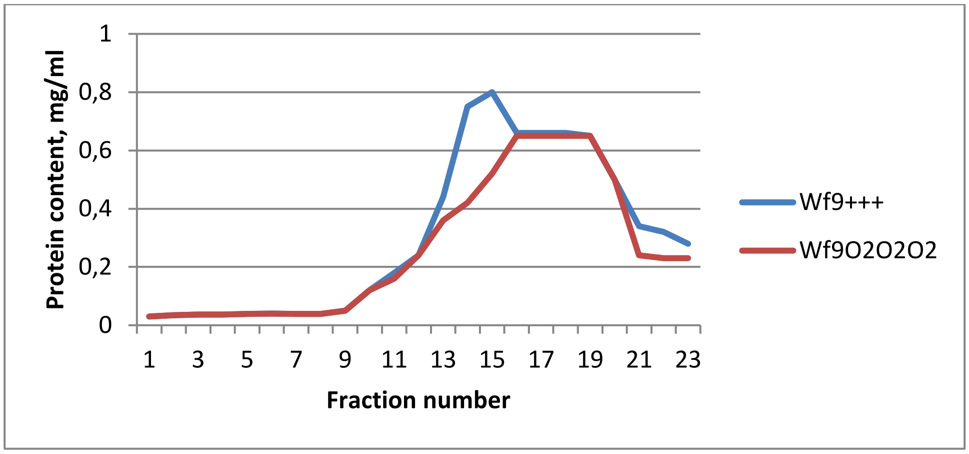

Figure 1 Relative amounts of particulate proteins in normal and O2 Wf9 endosperm dispersions at 22Day of development.

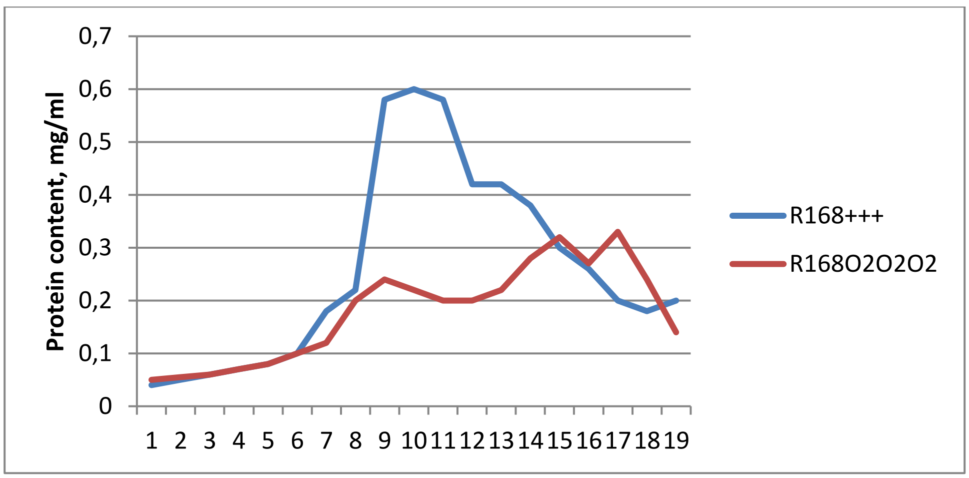

Figure 2 Relative amounts of particulate proteins in normal and O2 R168 endosperm dispersions at 22Day of development.

Differences in sedimentation rates of protein bodies from normal and O2corn endosperms are attributable to variations in size and densities of protein bodies. Due to the fact that as the endosperm matures, starch and protein granules are firmly bound into particles - agglomerates - it is quite difficult to destroy this protein-starch complex in mature grain (Christianson et al., 1969). Therefore, further study of the formation of protein bodies depending on the period of formation of the corn endosperm is necessary.

Normal and Opaque 2 WF9 and R168 kernel forms on the 22nd day are represented by two and three types of protein bodies.

The results of the amino acid composition assessment in the protein bodies of the Wf9 line are presented in Table 1.

Table 1 Amino acid composition in protein bodies of line Wf9

Amino acid	Protein bodies	
	Wf9+++	Wf9 O_2 O_2 O_2
Lyzine	2.09	3.68
Gistidine	3.46	2.62
Arginine	4.19	5.38
Cytozyne	-	-
Asparagine	trace	9.0
Threonine	2.10	4.86
Serine	4.63	5.4
Glutamine	24.97	21.8
Proline	12.9	10.29
Glicin	2.85	4.45
Alanine	8.7	2.76
Cysteine	trace	trace
Valine	2.72	5.48
Methionine	1.59	1.48
Isoleucine	3.08	4.27
Leucine	17.29	12.4
Tyrosine	4.31	3.08
Phenylalanine	1.31	4.88

The amount of lysine and threonine in protein bodies increases almost 2 times in case of saturation with the Opaque 2 gene. The content of such essential amino acids as arginine and isoleucine increased less significantly. At the same time, along with an increase in the content of essential amino acids, a decrease in the level of glutamic acid, proline and alanine was noted. The ratio of essential and non-essential amino acids in normal corn is 0.62, and in mutant corn – 0.7. This indicates an improvement in the synthesis of amino acids. The leucine/isoleucine ratio in the normal form of Wf9 line 9 is 5.59, and in the mutant form - 2.9. Isoleucine has been identified as the fourth or fifth limiting amino acid in the diet, depending on the feedstuffs used (Kidd et al., 2000). Considering that to ensure a balanced diet for broilers (Gutierrez-Rojas et al., 2008) it is necessary to introduce an additional amount of isoleucine, the genotype mutant form gives more reason to be called as quality protein maize.

The results of determining the amino acid composition in zein and glutelin of two Wf9 lines isolated from corn endosperm are presented in Table 2.

Table 2 Amino acid composition of zein and glutelin of two Wf9 lines isolated from maize endosperm

Aminoacid	Zeins		Glutelins	
	Wf9+++	Wf9 O_2 O_2 O_2	Wf9+++	Wf9 O_2 O_2 O_2
Lyzine	trace	0.53	1.13	2.35
Gistidine	0.95	1.1	3.31	2.92
Arginine	1.33	1.57	2.52	3.32
Cytozyne	5.0	5.4	-	-
Asparagine	-	-	3.78	5.32
Threonine	3.25	3.31	3.98	4.24
Serine	6.67	6.46	5.85	5.78
Glutamine	19.2	19.0	18.38	16.8
Proline	10.63	10.44	14.92	12.18
Glicine	1.99	2.68	6.8	7.48
Alanine	13.2	12.7	9.62	9.92
Cysteine	trace	1.48	2.11	2.93
Valine	3.98	3.93	5.51	5.64
Methionine	0.63	0.37	1.86	2.07
Isoleucine	3.95	4.22	2.52	3.2
Leucine	17.19	17.89	11.68	10.12
Tyrosine	3.59	3.67	3.51	3.3
Phenylalanine	5.38	5.5	2.7	3.5

In terms of the number and ratio of amino acids, the isolated protein bodies bear the features of both zeins and glutelins.

4. Conclusion

Protein bodies of maize lines Wf9 and R168 22 days after pollination are represented by two and three types, differing in molecular weight respectively. Protein bodies in the Opaque 2 mutant form are reduced by 2-3 times compared to the original form. In terms of the number and ratio of amino acids, the isolated protein bodies bear the features of both zeins and glutelins. The amount of lysine and threonine in protein bodies increases almost 2 times when saturated with the Opaque 2 gene. A decrease in the level of glutamic acid, proline and alanine was established along with an increase in the content of essential amino acids. The content of such essential amino acids as arginine and isoleucine increases less significantly. Along with an increase in essential amino acids, a decrease in the content of glutamic acid, proline and arginine in the opaque form of corn was noted. A decrease in the leucine/isoleucine ratio from 5.6 in the normal form to 2.9 in the mutant form is an additional argument to characterize Opaque 2 genotype as quality protein corn.

It was difficult to destroy protein-starch complex in mature grain. Therefore, further study of the formation of protein bodies depending on the period of formation of the corn endosperm will be studied.

Acknowledgment

This work was supported by the Ministry of Education and Sciences of Ukraine.

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HISTORY:
Received 12.05.2024
Accepted 28.05.2024

THE LEAVES' NUTRITION STATUS ASSESSMENT DEPENDING ON THE SEWAGE SLUDGE IMPACT ON THE CONDITION OF PAULOWNIA PLANTED ON TECHNOSOL

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Abstract

The field experiment with Paulownia tomentosa (Thunb.) Steud was established on phyto-meliorated loess-like loam as technosol (Maacia et al, 2014) at the Pokrov Research and Educational Station of Dnipro State Agrarian and Economic University. Experimental scheme included four treatments as following: control and two sewage sludge treatments (SS40 and 80 t/ha). The trial SS80 t/ha + Biochar (B)5 t/ha was made to mitigate high level of heavy metals. The concentration of manganese, zinc and copper in paulownia leaves increases with increasing dose of sewage sludge. The addition of biochar had a mitigation effect and led to a decrease in the content of heavy metals in the biomass, especially zinc and copper. The decomposition of the lignocellulose, cellulose and lignin proceeded through exothermic reactions of paulownia leaves thermal decomposition. There was a tendency for thermal effects to increase with increasing sewage sludge dose. The greatest differences between the control and experimental indicators were noted for the 80t/haSS+biochar treatment. Lowest reflectance in visible and Red-Edge ranges talk about highest leaves chlorophyll adsorption and nutrient contents at the 80SS and Biochar treatment. Lowest leaves spectral reflectance in visible range at the 80SS and biochar treatment talk about highest chlorophyll adsorption. Nutrients content affected reflectance in the Red-Edge range according highest sewage sludge dose with biochar as well. The data obtained are in relation

with leaves component thermal decomposition analyze and confirm that spectral reflectance in the SWIR range vary depending on changes in cellulose and lignin.

Keywords: biomass, loess like loam, amendments, heavy metals, spectral indication

1. Introduction

Paulownia is a genus of fast-growing and multipurpose tree species grown for their valuable wood and for ornamental purposes (Yadav et al., 2013; Li et al., 2015; Sinchenko et al., 2020; Palma et al., 2021). Meantime they yield large amounts of leafy biomass rich in nitrogen and antioxidant substances (Al-Sagheer et al., 2019; Uğuz and Kara, 2019, Sakr et al., 2022). Leaves of Paulownia are an excellent source of fats, sugars and proteins for cattle nourishment (Steier et al., 2022). They have the same nutritious values as alfalfa (Marshay Stewart et al., 2018). The fallen leaves of Paulownia improve soil quality by increasing organic matter as green manure (Woźniak et al., 2018). Three kind of composts improved the biomass yield of these fast-growing trees (*Populus nigra, Eucalyptus globulus and Paulownia fortunei*), especially in the case of Eucalyptus (Madejón et al., 2016). The compost sewage sludge application of 15 t/ha per year resulted in the largest average increase in diameter at breast height of 11.1 % (Xu et al., 2019). The addition of biochar at 5 % (w/w) supported the adaptation of Paulownia seedlings growing on mining sludge (Drzewiecka et al., 2021). However, an optimal addition of waste was necessary in order to limit unfavorable changes in the plant. Growing Paulownia on soil collected from a field contaminated with Cd near the waste dump of the Kremikovica iron and steel plant near Sofia was reflected in an increase in the fresh weight/dry weight ratio, but decreased the stem length, number of leaves and total leaf area (Tzvetkova et al., 2015). High concentrations of heavy metals (Pb, Zn, As, Sd) in the vegetative and generative organs of the plant *P. tomentosa (Thunb.) Steud., 1841* were found in areas of Samarkand with industrial production and along transport routes (Umrzokovich et al., 2021). Based on heavy metals research suggestion was made to recommend Paulownia elongata S.Y.hu for growing in tree alleys and wind protection zones along urban and regional traffic lines (Stankovic et al., 2009). Paulownia tomentosa could survive in the presence of heavy metals pollution by developing adaptive tolerance mechanisms for protection of toxicity (Wang et al., 2010; Ben Bahri et al., 2015). Proline and MDA biosynthesis under joint stress of Zn, Pb and Cd is considered as one of the characters of paulownia tolerance to heavy metals. The pot experiment results when Zn and Cd were added to the substrates of culture at various concentrations proved the ability of Paulownia tomentosa to maintain its photosystem activity even on Zn and Cd contaminated sites, despite the restrictive effect of Zn on the biosynthesis of photosynthetic pigments when its concentration exceeds 500 µM (Ben Bahri et al., 2014). P. tomentosa plants are able to limit Zn induced damages by activating effective mechanisms of Zn sequestration and accumulation of excess Zn in dedicated structures, such as petiole cell walls and root hairs, or by excluding part of the Zn in exudates located on the petiole surface (Azzarello et al., 2012). Last time the leaves biomass from some fast growing trees is considering as potential biomass that can be used as an alternative energy source from any points of view (Ram et al., 2014; Winaya et al., 2019).

The main objective of this study was estimate condition of two years old Paulownia trees growth on technosol depending on rate of sewage sludge.

2. Material and methods

The field experiment with *Paulownia tomentosa (Thunb.) Steud* was established on phyto-meliorated loess-like loam as technosol (Maacia et al., 2014) at the Pokrov Research and Educational Station of Dnipro State Agrarian and Economic University at 47°39' N, 34°08' E, at an elevation of 60 m (Kharytonov et al., 2023). Experimental scheme included four treatments as following: control, flocculated sewage sludge SS40 t/ha, SS80 t/ha, SS80 t/ha + Biochar (B)5 t/ha. Biochar was obtained after acacia wood pyrolysis at the temperature 600^{0}C. The motor drill was used to prepare soil hole to incorporate sewage sludge and biochar (Figure 1).

Figure 1 Preparation of soil hole for introduction of amendment

Two-year-old *Paulownia tomentosa (Thunb.) Steud* plantation photo is shown in the Figure 2.

Figure 2 Two-years-old *Paulownia tomentosa (Thunb.) Steud* plantation

The samples of biomass were dried in open air, then at 105 °C, cooled in a desiccator for 1 h. Dry leave plant material, ground to a fine powder, was used for microwave digestion with nitric acid and hydrofluoric acid (Markert, 1989). Standard procedures for determination of heavy metal concentrations in the plant samples were used (Hassan et al., 2007, Masson et al., 2010). Thermal analysis of dry paulownia leaves biomass was performed with a MOM Q-1500D *Derivatograph* -C (MOM, Budapest) following Paulik and others. Differential mass loss and heating effects were recorded and the results were processed with the software package supplied with the device. Samples of biomass were analysed dynamically at a heating rate of 10 °C/min in air.

The spectroradiometer 'FieldSpec 3.1' was used as the prime hardware tool to gather spectral data from paulownia leaf blades. The spectroradiometer was calibrated with a standard white reference panel (Analytical Spectral Devices Inc., Boulder, CO, USA) to determine the reflectance reference standard. The collected spectral observations were grouped into four main distinctive wavelength ranges: (i) visible (400 to 700 nm), (ii) near-infrared (NIR: 700 to 1300 nm), (iii) shortwave infrared 1 (SWIR- 1: 1300 to 1900 nm) and (iv) shortwave infrared 2 (SWIR-2:1900 to 2300 nm), in addition to the Red-Edge (680-780 nm) component (Tola et al., 2023).

3. Results and discussion

The data on heavy metals content in the leaves biomass are shown in the Figure 3 and 4.

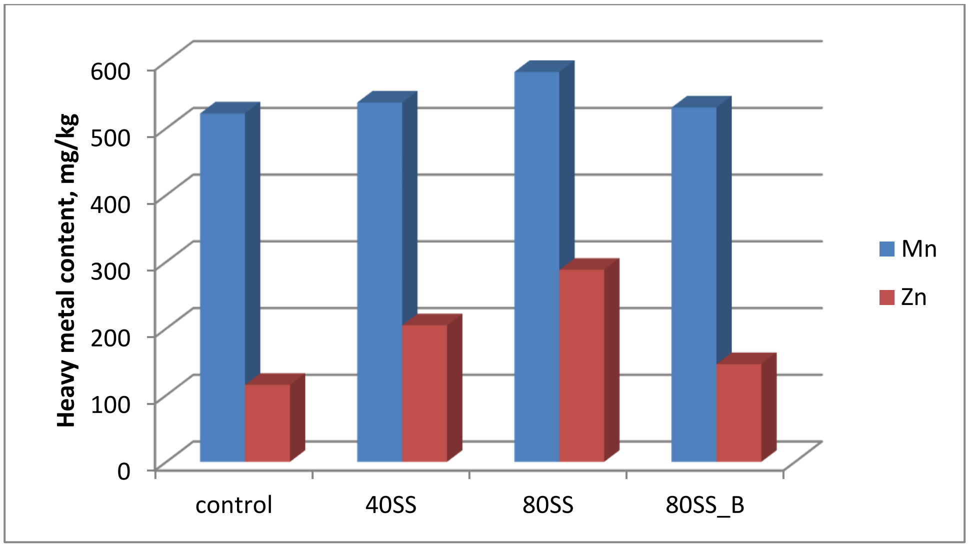

Figure 3 Manganese and zinc content in the Paulownia leaves

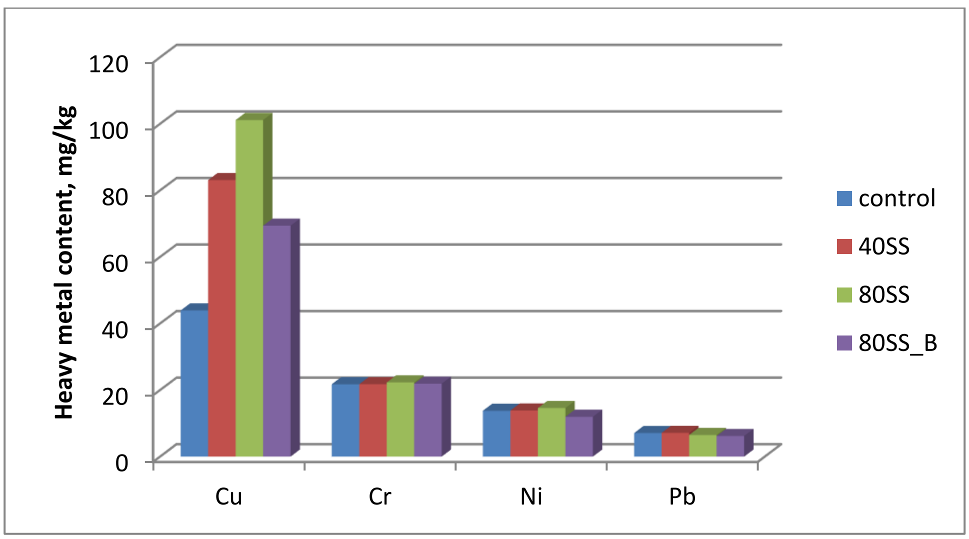

Figure 4 Heavy metals content in the Paulownia leaves

The concentration of manganese, zinc and copper in leaves increases with increasing dose of sewage sludge. The addition of biochar had a mitigation effect and led to a decrease in the content of heavy metals in the biomass, especially zinc and copper.

Thermogravimetric analysis of the thermal decomposition of paulownia leaves showed that this process is divided into three clearly defined stages. The first stage of decomposition of highly volatile components takes place in the temperature range of 30-210 °C (Table 1).

Table 1 Parameters of thermal decomposition of paulownia Leaves

Treatments	Stage	Interval, °C	Peak, °C	Max. rate, %/min	Mass lost, %	Residual mass fraction, %
Control	I	30-210	91	11.2	13.52	
	II	211-430	310-320	10.2	30.68	
	III	431-668	480	14.1	30.68	25.12
40t/haSS	I	30-180	102-111	11.1	12.69	
	II	181-420	301-310	11.2	33.59	
	III	421-600	481	16.2	30.47	23.25
80t/haSS	I	40-200	110	8.2	9.67	
	II	201-420	300	8.1	32.66	
	III	421-630	490	9.2	27.66	30.01
80t/haSS+biochar	I	50-130	101	6.9	5.2	
	II	132-400	290	7.2	29.22	
			320	6.9		
	I	401-600	480	9.1	28.4	37.18

The process speeds are low, in the range of 5.15-10.2 %/min. One peak was observed at this stage of destruction at a temperature of 91 °C. The weight loss was 13.5 %. In treatments with sewage sludge, some changes in the thermal behavior of the biomass at this stage were observed. The duration of the stage was shorter. This is especially noticeable in the 80 t/ha SS+biochar option. In addition, the peaks of destruction were shifted to higher temperatures, the speed of the stage and weight loss were lower than in the control (Figure 5).

The end of the second stage was recorded in the control sample at a temperature of 430 °C, in the test samples a little earlier - at temperatures of 400-420 °C. In the control and experiments with sewage sludge in this area, one peak of destruction was observed at a temperature of 300-320 °C, and in the 80 t/ha SS+biochar option - two peaks at a temperature of 290 °C and 320 °C. The average and maximum rates of biomass decomposition at this stage in all test samples were lower than in the control. The weight loss in the 40 t/ha SS and 80 t/ha SS trials was slightly greater, and in the 80 t/ha SS+biochar treatment slightly less than in the control. The third stage of lignin decomposition and the formation of a non-combustible residue occurred in the temperature range of 430-670 °C. One peak of destruction was observed at a temperature of 480-490 °C.

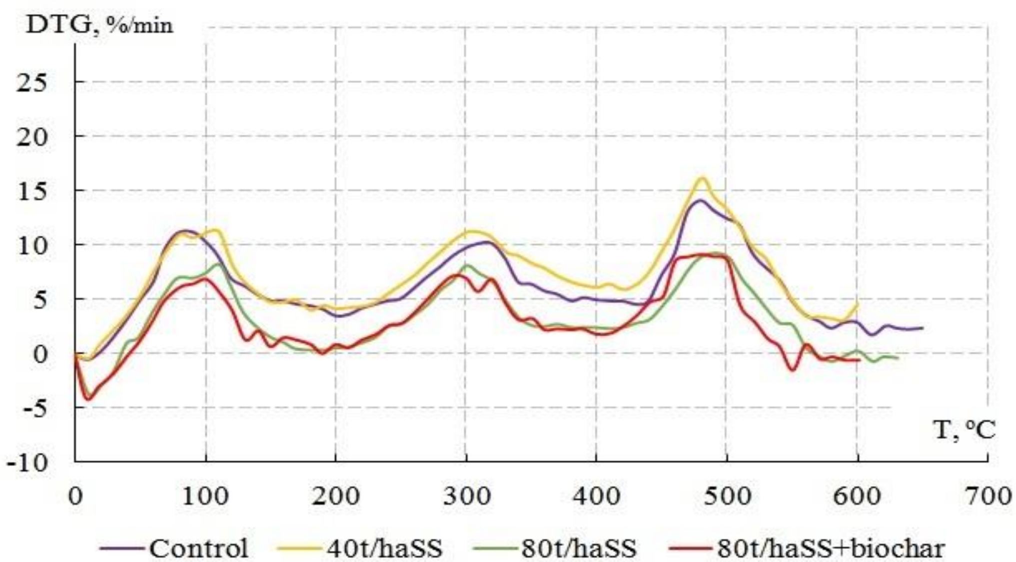

Figure 5 Comparison of DTG curves in control and sewage sludge treatments

The average and maximum decomposition rates were slightly higher than at previous stages, which is especially noticeable in the control and 40 t/ha SS treatments. In all test samples, thermolysis ended earlier than in the control. The weight loss ranged from 27.5-28.5 % (80 t/ha SS and 80 t/ha SS+biochar) to 30.5-30.7 % (control and 40 t/ha SS).

The onset of thermal decomposition of leaves was characterized by endothermic reactions (Figure 6). The decomposition of the lignocellulose, cellulose and lignine proceeded through exothermic reactions. Small peaks of exothermic effects were noted in the temperature range of 280-320 °C, and the most pronounced peaks in the temperature range of 480-510 °C.

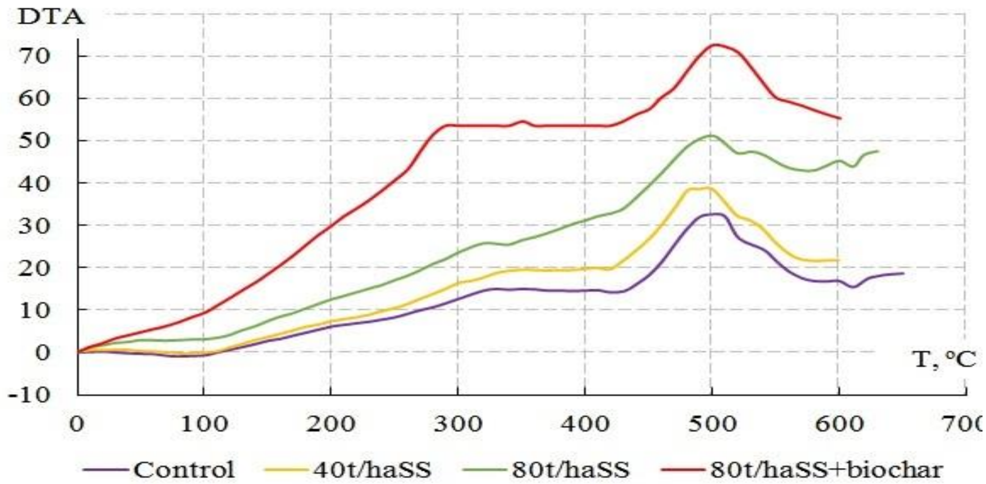

Figure 6 Comparison of DTA curves in control and sewage sludge treatments.

The combustion process was not complete due to the high ash content of the leaves. The proportion of residual mass in the control sample was 25.12 %. The introduction of a large dose of sewage sludge, as well as its combination with biochar, contributed to an increase in the fireproof residue to 37.2 %.

Spectral signatures of paulownia leaves at whole and grouped into four main distinctive wavelength ranges are shown in the Figure 7 and 8.

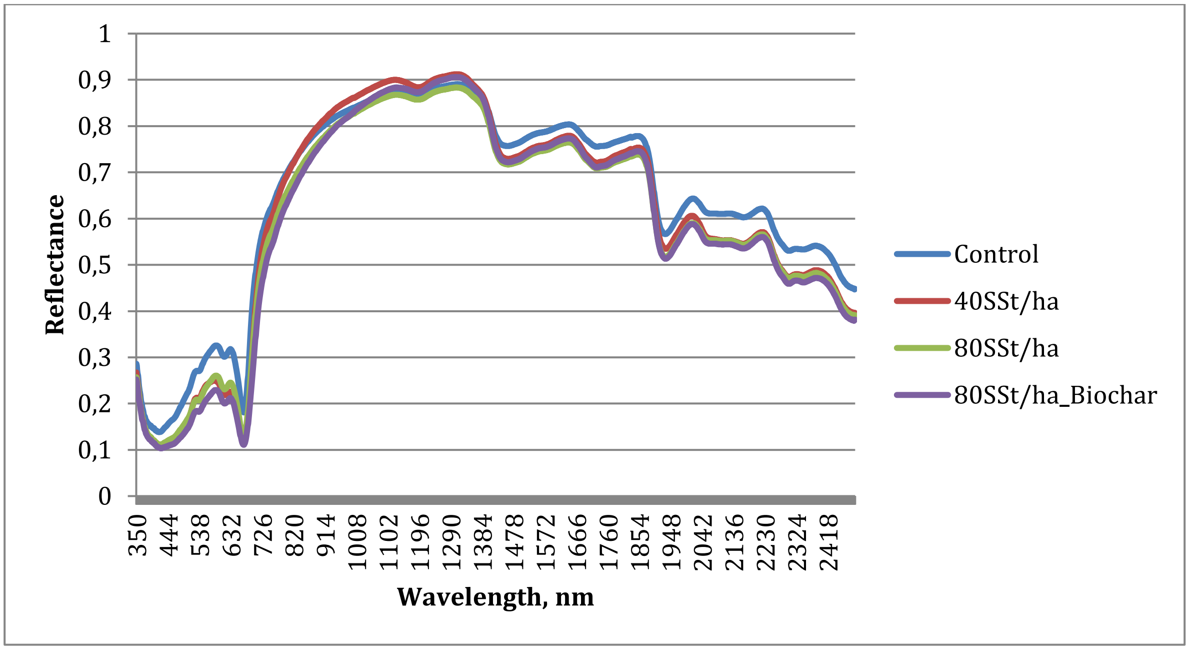

Figure 7 Spectral reflectance curve of paulownia leaves

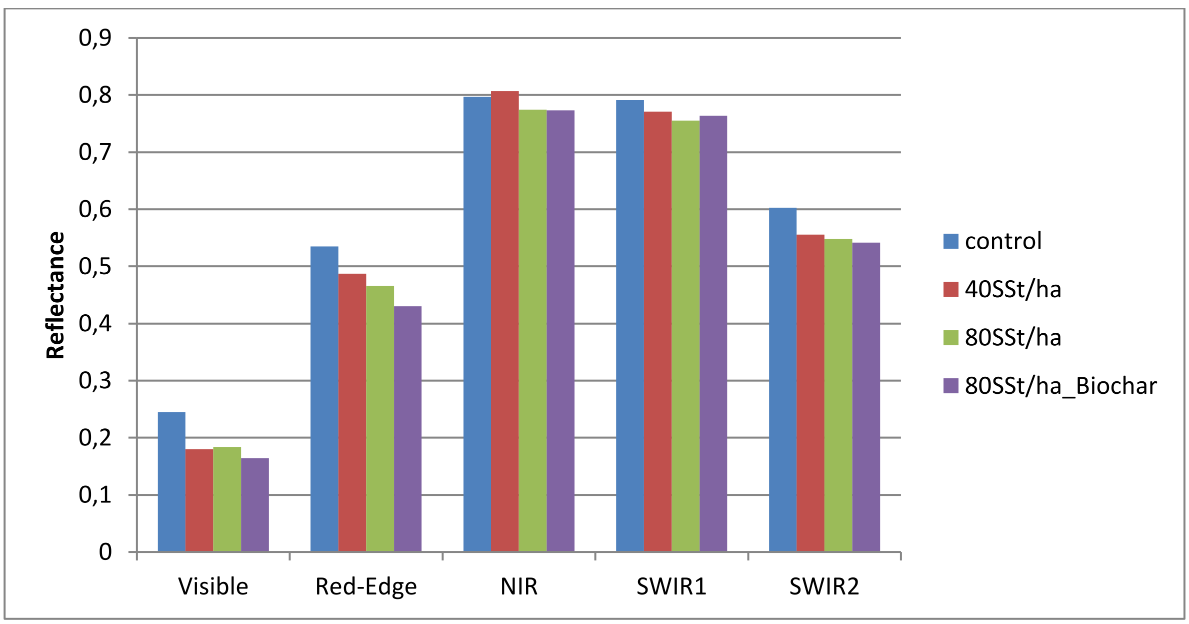

Figure 8 Spectral response of paulownia on different rates of sewage sludge

Lowest reflectance in visible range at the 80SS and Biochar treatment talk about highest chlorophyll adsorption. Nutrient contents affected reflectance in the Red-Edge range according sewage sludge dose. NIR is connected with reflectance because of spongy mesophyll (Tayade et al., 2022). Data obtained in the SWIR 1 and SWIR 2 regions confirm own obtained information and confirm that spectral reflectance in the SWIR range vary depending on changes in cellulose, lignin and water content in leaves (Buitrago et al., 2018).

4. Conclusion

The concentration of manganese, zinc and copper in paulownia leaves increases with increasing dose of sewage sludge. The addition of biochar led to a decrease in the content of heavy metals in the leaves biomass, especially zinc and copper.

Thermogravimetric analysis of the thermal decomposition of paulownia leaves showed that this process is divided into three clearly defined stages. The first stage of decomposition of highly volatile components takes place in the temperature range of 30-210 °C. The end of the second stage was recorded in the control sample at a temperature of 430 °C. The third stage of lignin decomposition and the formation of a non-combustible residue occurred in the temperature range of 430-670 °C. In all test samples, thermolysis ended earlier than in the control. The weight loss ranged from 27.5-28.5 % (80 t/ha SS and 80 t/ha SS+biochar) to 30.5-30.7 % (control and 40 t/ha SS). The introduction of a large dose of sewage sludge, as well as its combination with biochar, contributed to an increase in the fireproof residue to 37.2 %.

Lowest reflectance in visible range at the 80SS and Biochar treatment talk about highest chlorophyll adsorption. Nutrients content affected reflectance in the Red-Edge range according sewage sludge dose. Data obtained in the SWIR 1 and SWIR 2 regions confirm that spectral reflectance in the SWIR range vary depending on changes in cellulose and lignin content in paulownia leaves.

Acknowledgment

This study was funded by the NDR0122U001066 research project coordinated with the Ministry of Education and Science of Ukraine and by the research grant application D.3 "Stays for distinguishhed researchers at the University of Malaga" within the framework of UMA Research Plan for the invitation of Ukrainian Professors and Researchers.

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HISTORY:
Received 29.04.2024
Accepted 03.05.2024

ANTIOXIDANT PROPERTIES OF THREE LESSER-KNOWN MEDICINAL PLANTS CULTIVATED IN CONDITIONS OF THE SLOVAK REPUBLIC

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Abstract

Medicinal plants are known as a rich source of bioactive compounds with different effects on human health. Some of these plants exhibit effects on the male reproductive system. Among these plants are three lesser-known species, *Tribulus terrestris*, *Satureja hortensis* and *Trigonella foenum-graecum*. The aim of this study was to verify the possibility of cultivating these species in the climatic conditions of Slovak Republic and to determine their antioxidant properties. Antioxidant activity was tested using the DPPH and ABTS methods, and the content of phenolic compounds was determined using HPLC-DAD method. Our results suggest that *S. hortensis* has the highest and *T. foenum-graecum* has the lowest antioxidant activity. Phenolic compounds identified in all three medicinal plants were rutin and sinapic acid with highest levels present in *T. terrestris*. Myricetin and quercetin were identified and quantified in *T. terrestris* and *S. hortensis*. Gallic acid was present only in *T. foenum-graecum*. Cinnamic and caffeic acids were identified in *S. hortensis* and coumaric acid was present in similar amount in both *T. terrestris* and *S. hortensis*. All three species were cultivated successfully in conditions of Slovakia. Our results create a foundation for further scientific and practical studies of these plants cultivated in specific conditions.

Key words: medicinal plants, antioxidant activity, flavonoids, phenolic acids

1. Introduction

Medicinal plants and their bioactive compounds have been utilized for thousands of years in traditional medicine and nowadays, continuous efforts are being made to study them and use them in pharmacology. Interestingly, nearly 50 % of used medicine on the market nowadays is made from natural plant materials and 60 % of antitumor drugs are derived from plant natural products. In some cases, the active ingredient cannot be even synthesized through artificial process yet (Rasool et al., 2020).

Medicinal plants play a crucial role globally, whether used individually or as supplements with conventional treatments. Humans have relied on medicinal plants for sustenance, flavoring, and healing for centuries. The different parts of medicinal plants—seeds, leaves, flowers, fruits, stems, and roots—contain abundant bioactive compounds, offering valuable therapeutic properties (Sun and Shahrajabian, 2023). Aromatic plants are used to enhance the flavour and aroma of food, for preservation, but they also exhibit medicinal properties (Gonçalves et al., 2020). Aromatic plants are a rich source of essential oils and phenolic compounds as well as fibre, vitamins, and minerals (Fierascu et al., 2021).

Phenolic compounds are a large group of phytochemicals, which include phenolic acids, chalcones and coumarins as precursors for other compounds. Example of phenolic acids are p-coumaric, caffeic, cinnamic, benzoic or gallic acid. Phenolic acids can be precursors in the synthesis of lignans. Chalcones are aromatic ketones, that act as precursors of polyphenols, mainly flavonoids which can be subdivided into flavonols, dihydroflavonols, isoflavones, and flavanols. Few of the most important flavonoids are quercetin and kaempferol. The third group are coumarins, that often occur in spices and herbs like mint. Both essential oils and phenolic compounds have biological effect on human organism including antimicrobial, anti-inflammatory or antitumor activity. Some of them exhibit effect on neurological, immune, or cardiovascular systems (Delgado et al., 2023). Of the various properties associated with medicinal plants, their antioxidant activity is particularly significant in human health promotion, because most of chronic diseases are related to oxidative stress (Gonçalves et al., 2020).

Oxidation is a vital process in the aerobic cell metabolism and uncontrolled metabolic pathways cause the formation of reactive oxygen species (ROS). These free radicals contribute to oxidative stress which is the result of imbalance between production an elimination of ROS by the antioxidant defence mechanisms. This imbalance is linked to several chronic human illnesses like cancer, cardiovascular and neurodegenerative diseases, diabetes, and infertility (Klotoé et al., 2021).

Male infertility can stem from various factors such as testicular underdevelopment, reproductive diseases, elevated scrotal temperature, immune issues, and hormonal imbalances, all of which negatively impact sperm quality. Oxidative stress, hormonal dysregulation, and genetic defects have been identified as key contributors to infertility. While traditional medicine has several treatments for this diagnosis, there is a growing interest in natural antioxidants from herbs as alternatives to synthetic drugs. Medicinal plants have been explored for their fertility-enhancing or spermicidal properties, with some influencing hormone levels and sperm characteristics (Boroujeni et al., 2022).

Among the medicinal plants with proven effect on spermatogenesis, sperm count and motility or increase in serum testosterone are *Zingiber officinale*, *Mucuna pruriens*, *Withania somnifera*, *Nigella sativa*, *Alpinia officinarum*, *Crocus sativus*, *Panax ginseng* and others (Gamit et al., 2022).

Puncture vine (*Tribulus terrestris*) is a well-researched medicinal plant from *Zygophyllaceae* family with potential effects on male fertility. This plant is native to India, China, the United States, and Bulgaria and it thrives in Mediterranean, subtropical, and desert climates. It is a low-growing shrub, reaching heights of 10–60 cm, with opposite, irregularly shaped leaves and yellow flowers. Its distinctive carpel fruits have a round shape with sharp spikes and they contain oily-textured seeds (Ahmed et al., 2023). Puncture vine contains significant secondary compounds including saponins, polyphenols, and alkaloids, such as kaempferol, tribuloside, rutin, quercetin, and harmane (Ștefănescu et al., 2020). It is known for its traditional use as a remedy for enhancing reproductive health, including increasing sperm count and improving sperm motility and quality (Aleksandrov et al., 2020; Sirotkin and Kolesárová, 2021), levels of serum testosterone and erectile dysfunction (Russo et al., 2016).

Summer savory (*Satureja hortensis* L.) as an annual aromatic herb belonging to the *Lamiaceae* family. The plant species originates from southern Europe and Turkey, but it is grown in other regions for its culinary uses. The plant typically grows to around 10-25 cm tall, with a heavily branched stem covered in short white hairs. Its leaves are long and narrow, with smooth edges and a slightly thick texture. The flowers are clustered together in groups of 5-15, with bracts that extend beyond the clusters. The petals are usually purple, pink or white (Hassanzadeh et al., 2016). This plant contains thymol, methyl carvacrol, chlorogenic acid, rosmarinic acid and flavonoid compounds. Essential oil contains a range of phenolic compounds, and the leaves contain tannins. Thanks to these phytochemicals, summer savory has antifungal, antimicrobial, anti-inflammatory, anti-drug resistance bacteria and mainly antioxidant activities (Abou Baker et al., 2020; Khalighi et al., 2021).

Fenugreek (*Trigonella foenum-graecum L.*) belongs to the *Fabaceae* family and originates from Greece. It is a fragrant herb widely used as spice. This plant is erect, with sparse branching and a height of under 1 meter. It has light green trifoliate leaves and petite white flowers. The elongated pods, reaching lengths of up to 15 cm are curved with a pointed tip, enclosing yellow-brown seeds measuring less than 0.5 cm in length (Britannica, 2024). Due to its abundance of various phytochemicals, including alkaloids (trigonelline and choline), polyphenols, saponins, and minerals, fenugreek finds application in many therapeutic contexts. There is some evidence, that fenugreek can be effective against cardiovascular diseases and hypercholesterolemia, but also sexual disorders like testosterone deficiency syndrome. It also possesses anticarcinogenic and antioxidant activity (Syed et al., 2020).

Understanding how the environment influences the composition of bioactive compounds in medicinal plants is crucial for ensuring their efficacy and safety in therapeutic applications. Environmental factors such as soil composition, climate, altitude, and sunlight exposure can significantly impact the synthesis and accumulation of bioactive compounds in plants. Plant species used in this study originate from warmer climates and climatic condition of Slovak Republic are not optimal for their cultivation. In this study, the focus was on cultivation of these species while monitoring the climatic conditions at the cultivation site and analysing antioxidant activity and the content of bioactive phytochemicals in these medicinal plans.

1. Materials and methods

1.1. Plant material

Three species of medicinal plants used in this study were: puncture vine (*Tribulus terrestris* L.), summer savory (*Satureja hortensis* L.) and fenugreek (*Trigonella foenum-graecum* L.). The seeds of summer savory were purchased from online shop moravoseed.sk. Puncture vine and fenugreek seeds were purchased from the online store osiva-semena.sk.

Figure 1 Cultivated medicinal plants, A- *Tribulus terrestris*, B- *Satureja hortensis*, C- *Trigonella foenum-graecum* (Brahmbhatt, 2022; MyseedsCo, 2021; Rupasinghe, 2024)

2.2. Soil nutrition, fertilization and climatic conditions

The plants were cultivated in year 2023 in demonstration garden of Institute of Horticulture in Nitra. Before planting outdoors, the soil on the plot was cleared of weeds and aerated. Soil analysis was also conducted in the spring (Table 1). This analysis was provided by Institute of Agronomic Sciences, Faculty of Agrobiology and Food Resources. Cultivation site is located in the cadastral area of Chrenová in the city of Nitra (BZ SPU 2023). The weather is mild and temperate, with yearly average temperatures fluctuating between 8.28 °C and 10.05 °C. The average yearly precipitation in this area varies from 529 mm to 895 mm (Kočický et al., 2019). The specific data of climatic conditions at the cultivation site for 2023 were obtained from the meteorological station located in the Botanical Garden provided by the Institute of Landscape Engineering of Slovak University of Agriculture in Nitra. This data is shown in Table 2.

Table 1 Soil analysis of the experimental area

Year	pH	Nan	Nutrient content ($mg.kg^{-1}$) (Mehl.III)				Cox (hummus)
		$mg.kg^{-1}$	P	K	S	Mg	%
2023	7.45	8.9	232.5	600	85.0	729	4.38

Table 2 Climatic conditions (precipitation and temperature) at experimental site

Month	Temperature [ºC]			Precipitation [mm]		
	Normal 1991-2020	t	Characteristic	Normal 1991-2020	Precipitation	Characteristic
IV.	11.4	4.1	extremely cold	36	40	normal
V.	16.0	9.9	extremely cold	59	111	extremely wet
VI.	19.6	13.3	extremely cold	59	83	wet
VII.	21.7	17.4	extremely cold	65	8	extremely dry
VIII.	21.1	17.9	extremely cold	55	46	normal

2.3. Plant cultivation, harvest, and postharvest processing

Medicinal plants were cultivated in 2023 in demonstration garden of Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra. Fenugreek was sown directly into the soil on the field plot in rows spaced 0.4 × 0.10 m, with 5 rows sown in length of 4.5 m. Summer savory was sown into plastic seedling trays and later transplanted into rows spaced 0.5 × 0.5 m in the field. Puncture vine was also sown into plastic seedling trays, followed by transplantation into the field plot in 5 rows with 9 plants spaced 0.5 × 0.5 m. The dates of sowing, transplanting and harvest are depicted in Table 3. The crops were not treated with any pesticides. Additional drip irrigation was applied as needed during the growing season. Weed control was carried out by hand hoeing.

Table 3 Course of plant cultivation and harvesting

Species	Sowing	Planting	1st Harvest	2nd Harvest
puncture vine	28.4.	30.5.	27.6.	13.7.
summer savory	23.3.	10.5.	23.6.	24.7.
fenugreek	21.4.		24.7.	

In the case of puncture vine and summer savory, two manual harvests were conducted during the flowering period. During harvesting, the stems were cut using disinfected scissors. After harvest, the plant material was evenly distributed on sieves and dried at 25-30 °C in a well-ventilated room. For fenugreek, entire plants were harvested at the mature fruit stage, then dried. Subsequently, seeds were extracted from the pods. The dried biomass and seeds of summer savory, fenugreek and puncture vine were stored in plastic containers in darkness at room temperature, prepared for further analyses.

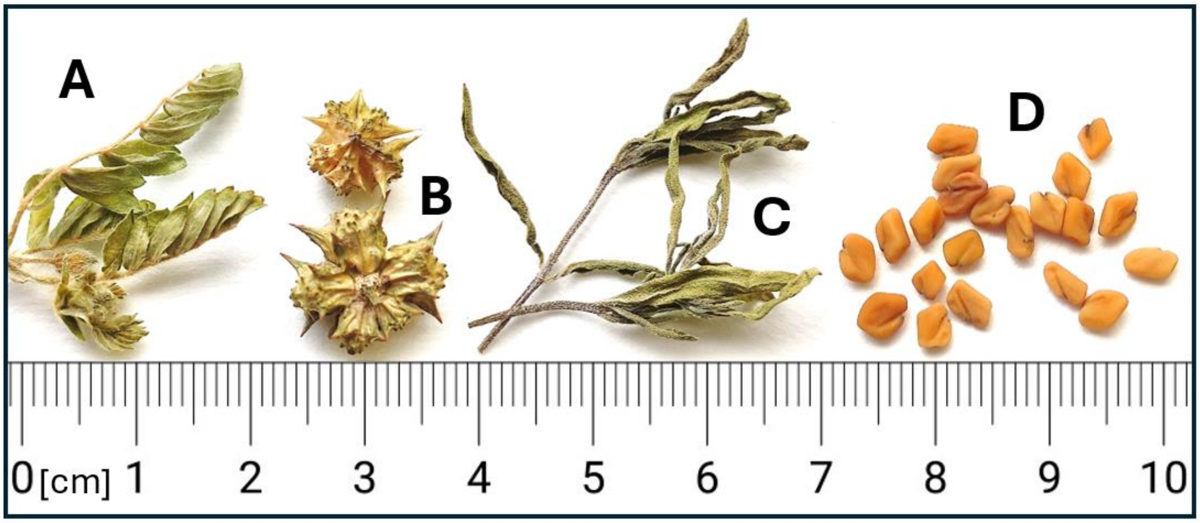

Figure 2 Harvested and dried plant parts used for experiments, A- herb of *T. terrestris*, B- fruits of *T. terrestris*, C- herb of *S. hortensis*, D- seeds of *T. foenum-graecum* (Kollárová, 2024)

2.4. Sample preparation

Dried plant material or seeds of each species were homogenized into powder and 1 g was combined with 5 mL of 80 % methanol in test tube. This mixture was then placed on orbital shaker (Biosan, PSU-10i) and extraction process started. Three repetitions from every species were prepared, so nine extracts in total were analysed. After 48 hours, mixture was filtered, and the extract was stored at 4 °C for further analyses. Before antioxidant activity evaluation, extracts were centrifuged for 1 min at 2500 rpm to ensure any impurities collect on the bottom of the test tube.

2.5. Evaluation of antioxidant activity

Antioxidant activity of selected medicinal plants was evaluated in laboratories of research centre Agrobiotech in Nitra.

2.5.1. DPPH method

The DPPH method is based on the scavenging of the stable free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH). Trolox (Sigma Aldrich, Schnelldorf, Germany) is used as a standard for calibration curve and calculating antioxidant activity. A stock solution of DPPH is prepared by dissolving 0.025 g of DPPH (Sigma Aldrich, Schnelldorf, Germany) in methanol (99.8 %) and stored at 4°C in the refrigerator. Before analysis, a working solution of DPPH is prepared from the stock solution by mixing with methanol (1:10). The analysis is performed as follows: the absorbance of the working solution of DPPH is measured at a wavelength of 515 nm using Glomax spectrophotometer (Promega Inc., Madison, WI, USA). In this experiment, this method was performed using 96-well microplate and 195 µL of DPPH was mixed with 5 µL of prepared sample and shaken on horizontal shaker (IKA Inc., Staufen im Breisgau, Germany) for 30 minutes at 200 rpm in the dark at laboratory temperature. Then, the change in absorbance is measured at 515 nm. After calculations based on calibration curve, the antioxidant activity of the samples is expressed as the Trolox equivalent antioxidant activity converted to the TEAC value per 1 mL of the sample. (TEAC- Trolox Equivalent Antioxidant Capacity)

2.5.2. ABTS method

The ABTS method (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) is based on the scavenging of the synthetic cation radical ABTS+, which is prepared by reacting ABTS and potassium persulfate solutions to achieve an absorbance of 0.700 at a wavelength of 750 nm using Glomax spectrophotometer (Promega Inc., Madison, WI, USA). As well as previous method, this analysis was performed using 96-well microplate and 195 µL of ABTS was mixed with 5 µL of prepared sample and shaken on horizontal shaker for 30 minutes in the dark at laboratory temperature. After the specified time the change in absorbance at a wavelength of 750 nm is measured. After calculations based on calibration curve, the antioxidant activity of the samples is expressed as the Trolox equivalent antioxidant activity converted to the TEAC value per 1 mL of sample.

2.6. Evaluation of phenolic compounds by HPLC-DAD

HPLC-DAD method (high-performance liquid chromatography with diode-array detection) was executed in laboratory of Institute of Food Sciences, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra.

The dried plant samples were homogenized, and 1 g of plant material was extracted with 20 mL of 80% ethanol at laboratory temperature for 8 h using a horizontal shaker Unimax 2010 (Heidolph

Instruments, GmbH, Germany) 250 rpm. The extract was filtered through Munktell No 390 paper (Munktell & Filtrak GmbH, Bärenstein, Germany). Prior to HPLC analysis, the extract was filtered through the syringe filter QMax (0.22 µm, 25 mm, PVDF) (Frisenette ApS, Knebel, Denmark). For the analysis, HPLC-quality standards are used (Sigma-Aldrich, St. Louis, MO, USA). All compounds were determined using an Agilent 1260 Infinity II HPLC (Agilent Technologies GmbH, Wäldbronn, Germany) with diode-array detector (G1315C). All HPLC separations were performed on a Purosphere® reverse phase C18 column (Waters Inc., CA, USA). The mobile phase consisted of gradient acetonitrile (D) and 0.1 % phosphoric acid in ddH_2O (ultra-pure and sterile water) (C). The gradient elution was as follows: 0–1 min isocratic elution (90 % C and 10 % D), 1–6 min linear gradient elution (85 % C and 15 % D), 6-12 min (80 % C and 20 % D), 12–20 min (30 % C and 70 % D) and 20-25 min (30 % C and 70 % D). The initial flow rate was 1 mL per min, and the injection volume was 5 µL. The column thermostat was set up to 30°C, and the samples were kept at 4 °C in the sampler manager. Data were collected and processed using software AgilentOpenLab ChemStation for LC 3D Systems.

2.7. Statistical analysis

The statistical analysis was conducted using the Statgraphics Centurion XVII software (StatPoint Inc., The Plains, VA, USA). The obtained results were evaluated through analysis of variance (ANOVA), and the mean values were tested using the Least Significant Difference (LSD) test performed at a significance level of 95 % ($P \leq 0.05$).

3. Results and Discussion

3.1. Antioxidant activity

In this study, we evaluated the antioxidant capacity of medicinal plant extracts using DPPH and ABTS methods. The highest values of antioxidant capacity were detected using both methods in summer savory compared to the other plants. The lowest levels of antioxidant capacity were detected in fenugreek. Used methods resulted in different values depicted in Table 4, and the DPPH method seems to be more sensitive than ABTS method. DPPH and ABTS assays measure antioxidant capacity but may respond differently to various antioxidants present in the tested samples. Some antioxidants may be more effective at scavenging DPPH radicals, while others may be more effective at scavenging ABTS radicals. This also highlights the importance of using multiple methods for antioxidant evaluation.

Table 4 Evaluation of antioxidant activity (TEAC/mL)

Species	Method	
	DPPH	ABTS
puncture vine	1021,63 ± 78,53b	238,79 ± 4,79b
summer savory	1398,26 ± 14,62c	242,68 ± 10,34b
fenugreek	308,44 ± 37,60a	222,41 ± 1,29a

n = 9; a,b,c - statistically significant differences at P ≤ 0.05 by LSD in ANOVA

When analysing puncture vine in a study conducted by Zuščíková et al. (2022) the antioxidant activity of leaves extract was measured using DPPH assay. It was determined at 92.99 ± 2.99 mgTEAC.g^{-1} DW. A different study from 2023 conducted by Uysal et al., was comparing different types of *T. terrestris* extracts and their antioxidant activity using both DPPH and ABTS methods. The water extract seems to have the highest antioxidant activity compared to methanol and ethyl acetate extracts. The values for DPPH analysis are: 15.48 ± 0.19 mgTE/gin ethyl acetate extract < 23.91 ± 0.23 mgTE/gin methanol extract < 30.50 ± 0.95 mgTE/gin water extract. Results of ABTS method have a similar tendency: 26.85 ± 0.82 mgTE/gin ethyl acetate extract < 37.75 ± 0.74 mgTE/gin methanol extract < 44.49 ± 2.92 mgTE/gin water extract.

In a study of Wojdyło et al. (2007), they evaluated the antioxidant activity of 32 selected herbs, with fenugreek being one of them. Their results show that using DPPH method antioxidant activity was 364±7.02 TEAC (µM Trolox/100 g DW). Using ABTS method, the antioxidant activity was determined at 6.74±1.01 TEAC (µM Trolox/100 g DW).

Extracts from summer savory prepared using different types of extraction technique including Soxhlet extraction, maceration, ultrasound-assisted, microwave-assisted extraction, and subcritical water extraction, were analysed for their antioxidant activity using DPPH method. Results suggest that the highest level of antioxidant activity was measured in subcritical water extract (23.33 ± 0.48 IC_{50} µg/mL) followed by microwave-assisted extract (32.87 ± 0.60 IC_{50} µg/mL), ultrasound-assisted extract (38.54 ± 0.33 IC_{50} µg/mL), macerate (45.13 ± 0.95 IC_{50} µg/mL) and Soxhlet extract (51.79 ± 0.47 IC_{50} µg,mL^{-1}) (Mašković et al., 2017). The IC_{50} value represents the concentration of an antioxidant substance required to inhibit 50 % of the DPPH in analysed samples. The lower the IC_{50} value, the higher antioxidant activity (Olugbami et al., 2014).

3.2. Phenolic compounds

In this study, the content of phenolic compounds in medicinal plants was evaluated using HPLC analysis. Results show that puncture vine contains more myricetin than summer savory and has the highest amount of rutin compared with the two other plants. Summer savory, on the other hand, contains more quercetin than puncture vine. Kaempferol was not detected in puncture vine nor fenugreek, but it was present in summer savory (40.50 ± 0.23 mg/kg DW). The only flavonoid identified in fenugreek using available standards in our laboratory was rutin (1819.039 ± 14.37 mg/kg DW). All values of flavonoid content in medicinal plants are shown in Table 5.

From phenolic acids, gallic acid was detected only in fenugreek (20.38 ± 0.49 mg/kg DW). Cinnamic acid and caffeic acid were only detected in summer savory (8.72 ± 0.15 and 126.49 ± 0.4 mg/kg DW). Puncture vine contained more coumaric acid than summer savory and it was not detected in fenugreek. Sinapic acid was detected in all tested medicinal plants and the highest value was measured in puncture vine (62.25 ± 1.07 mg/kg DW) followed by fenugreek and summer savory. Specific values of phenolic acid content are shown in Table 6.

Table 5 Evaluation of flavonoid content (mg/kg DW)

Species	rutin	myricetin	quercetin	kaempferol
puncture vine	4600.1 ± 17.71c	14.54 ± 0.25b	5.11 ± 0.46a	-
summer savory	110.69 ± 2.03a	6.08 ± 0.09a	6.35 ± 0.32b	40.50 ± 0.23
fenugreek	1819.039 ± 14.37b	-	-	-

n = 9; a,b,c - statistically significant differences at P ≤ 0.05 by LSD in ANOVA

Table 6 Evaluation of phenolic acid content (mg.kg^{-1} DW)

Species	gallic acid	cinnamic acid	caffeic acid	coumaric acid	sinapic acid
puncture vine	-	-	-	13.71 ± 0.48a	62.25 ± 1.07c
summer savory	-	8.72 ± 0.15	126.49 ±0.4	12.33 ± 0.85a	15.21 ± 0.15a
fenugreek	20.38 ± 0.49	-	-	-	20.1 ± 0.12b

n = 9; a,b,c - statistically significant differences at P ≤ 0.05 by LSD in ANOVA

The content of bioactive compounds in *T. terrestris* is well-researched, but the focus is mainly on its fruit. In a study by Mubarik et al. (2022) the identification of phenolic compounds in *T. terrestris* fruits was conducted using RP-HPLC method. In addition to the flavonoids we identified during our analysis, in their experiment few others were present including naringin, hesperidin, narenginin and quercetrin. Compared to our results, kaempferol was also detected at concentration of 388.42 mg/100g dried extract, indicating, that this flavonoid is present in higher concentrations in puncture vine fruit. From phenolic acids, similar to our results coumaric acid (92.81 mg/100g dried extract) was present. Even though these were not identified in our case, 19.46 mg/100g dried extract of caffeic acid, 39.28 mg/100g dried extract of cinnamic acid, and 137.88 mg/100g dried extract of gallic acid were identified, supporting a theory of richer phenolic compound content in fruits. In a study conducted by Abbas et al. (2022) they analysed bioactive compounds present in ethyl acetate fraction of *T. terrestris* leaf and fruit methanol extract using RP-HPLC analysis. The result show, that 2.19 µg/mg of rutin and 4.2 µg/mg myricetin was detected. Rutin (1090.25 mg/100g dried extract), quercetin (255.34 mg/100g dried extract) and kaempferol (384.87 mg/100g dried extract) were also identified using HPLC-DAD in 2015 study by Hifnawy et al. and additionally apigenin and hespertin were present in fruits of puncture vine. Gallic, coumaric, caffeic and cinnamic acids were identified at concentrations of 130.552 mg/100g, 83.89 mg/100g, 24.93 mg/100g and 32.17 mg/100g dried extract, respectively. Other phenolic acids like syringic, chlorogenic, ferulic, vanillic and salicylic acids were also present.

Scientific research is more focused on *Satureja montana* compared to *Satureja hortensis*, but there are some results of its bioactive content. For example, aforementioned Mašković et al. (2017), conducted a study addressing the content of phenolic compounds in summer savory extracts using HPLC-PDA method. It was determined, that in extract prepared by maceration among other compounds, rutin, quercetin and kaempferol were identified at concentrations 10.75 µg/g, 1.77 µg/g and 11.75 µg/g, respectively. Compared to our results, caffeic acid was not detected, but sinapic acid was present at concentration 1.472 µg/g. The phenolic acid with highest concentration present in summer savory was rosmarinic acid (287.59 µg/g).

Lastly, fenugreek was also analysed for its bioactive compounds. Fatima et al. (2022) used HPLC-DAD assay to determine phenolic content in fenugreek seed extract. They identified 14 compounds including gallic acid (117.6 ± 1.5 mg/100g DW), ferulic acid (168.4 ± 1.8 mg/100g DW) and p-coumaric acid (256.7 ± 6.8 mg/100g DW). In a study by Hafeez et al. (2023), using HPLC, they identified quercetin (4.12 ppm), gallic acid (0.43 ppm), caffeic acid (0.57 ppm), vanillic acid (0.61 ppm), syringic acid (0.15 ppm) and m. coumaric acid (0.14 ppm).

4. Conclusion

In conclusion, these three species of medicinal plants can be cultivated successfully in the conditions of Slovak Republic. The antioxidant activity of these plants varied significantly ($P \leq 0.05$) depending on the species and used analytical method. Summer savory demonstrated the highest antioxidant capacity among the tested medicinal plants, while fenugreek exhibited the lowest levels. Furthermore, the content of phenolic compounds also varied across different medicinal plants and in the available literature. These findings underscore the importance of understanding the effect of environment and climatic conditions on phytochemical composition of medicinal plants for optimizing their therapeutic potential.

Future research should continue to explore the influence of environmental factors and extraction methods on the composition of bioactive compounds, ultimately contributing to the development of effective herbal remedies for various health conditions including male infertility.

Acnowledgements

This research was funded by KEGA, grant number KEGA 004SPU-4/2022, "Interactive Classroom for Horticulture study program in the Context of Innovation of the Current Student's Teaching Process".

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ARTICLE HISTORY:
Received 29.04.2024
Accepted 22.05.2024

EVALUATION OF THE POTENTIAL OF VINEYARD PLANTING IN THE CADASTRE OF THE WINE-GROWING VILLAGE OF KAMENNÝ MOST – CULTIVATION AND ECONOMIC MODEL

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Abstract

The aim of the study was to develop a vineyard planting project in the cadastre of the wine-growing village of Kamenný Most, located in the South Slovakian wine-growing region, on a plot of 1.7 ha. In the project, we consider the quality of the soil and climatic conditions of the selected location. Based on the climatic conditions and soil type, we selected white wine grape varieties (*Vitis vinifera*), namely Medina and Aromína. The output of the cultivation model is the method of planting. In the economic model, we calculated a budget for fencing, planting grafted grapevines, and the construction of a trellis system. In the summary, we evaluated an economic efficiency and the return on investment. We quantify the total costs of the investment at 65,052.11 €. The annual profit from grape production is 8392.02 €. Based on our calculations, the investment efficiency coefficient was positive, with a value of 12.90 %. The production of grapes for the purpose of selling is profit-making, and to increase the profit is necessary to produce wine.

Key words: vineyard, Kamenný Most, grapevine, vineyard project

1. Introduction

One alternative approach that has gained traction in recent years is sustainable viticulture. Sustainable viticulture aims to minimize the environmental impact of grape cultivation by promoting biodiversity, reducing the use of synthetic chemicals, and conserving water resources. By implementing sustainable practices such as cover cropping, integrated pest management, and organic fertilization, grape growers can cultivate grapes in a more environmentally friendly manner. These practices not only help preserve soil and water quality but also contribute to the overall health of the vineyard ecosystem (Ferreira, Carla, SS, et al,. 2020).

Soil fertility, encompassing chemical, physical, and biological factors, plays a significant role in supporting plant growth and overall soil health. Understanding and managing these components is essential for cultivating healthy crops and maintaining sustainable agricultural systems. In modern oenology, the science of wine production, the emphasis is on producing wines of higher designation protected of origin that appeal to a discerning consumer. Soil properties, in conjunction with climate conditions, directly influence the development of grapevines, the composition of berries, and the potential quality of wine produced (Serrano et al., 2017).

The greening mixture used should contain at least 3 species from different genera. It is not suitable for grass species to be dominant and therefore should make up less than 50 percent of the plants. Plants from the Fabaceae family should make up at least one third of the mixture. There should be 1-2 deep-rooting plant species in the mixture. It is desirable that we also leave room for the development of natural flora. In that case, we will use a lower sowing amount of greening mixture (Pavloušek, 2013).

Permanent greening can be used on deeper soils with a high humus content, with a high annual rainfall and its uniform distribution during the vegetation. It is most suitable to use it only in the inter-row and to keep black eel in the root zone. Full-scale permanent greening requires 25 % more water compared to black eel. Other variants are the permanent greening of every other inter-row (Masníková, 2014).

Among the measures that are important to implement before planting a vineyard is the restoration of soil biological activity. This is done by incorporating higher doses of organic matter into the soil. Farmyard manure, green manure and compost are mainly used for these purposes. Other important actions are the adjustment of nutrient reserves and the adjustment of the soil reaction. Due to the better development of the root system, deep processing of the soil before planting is also used. All these measures taken before the construction of the vineyard will ultimately affect its condition and lifespan (Burg et al., 2010).

2. Material and methods

The aim of the work is to propose a vineyard planting project in the South Slovak wine-growing region, in the village of Kamenný most, to create an itemized summary of the material, to select suitable varieties of grape vines (*Vitis vinifera*), and to evaluate the efficiency of the investment.

2.1. Characteristics of the selected locality

The cultivation site is in the cadastral territory of Kamenný Most wine-growing village, Štúrovo wine-growing district, South Slovakian wine-growing region. The village of Kamenný Most is in the southwest of Slovakia. The information provided about the location near the Mužla vineyards and falling under the cadastre of Kamenný Most, along with the detailed soil characteristics based on the Soil Maps of 2024, offers valuable insights into the agricultural potential of the area for grape cultivation. The description of the climatic region as "very warm, very dry lowland" suggests specific environmental conditions that can influence grapevine growth and grape quality. Such climatic conditions may impact factors such as grape ripening, sugar accumulation, and overall vine health. The mention of "degraded black earth" as the main soil unit indicates the predominant soil type in the area. Black earth soils are typically fertile and well-suited for agriculture. However, the term "degraded" suggests that there may be some issues or challenges with soil quality that could affect grape production. The details provided about soil depth, soil grain, and the absence of skeletal content give further insights into the soil structure and composition of the area. Deep soil with medium-heavy grain can influence water retention, root development, and nutrient availability for the grapevines. The production potential point value of 63 indicates an assessment of the area's ability to support agricultural production, specifically in terms of grape cultivation. This value serves as a reference point for evaluating the productivity and suitability of the land for vineyard establishment and management. Overall, the combination of climatic conditions, soil characteristics, and production potential point value provides important information for vineyard planning and management in the specified location, guiding decisions related to grape variety selection, vineyard practices, and overall vineyard sustainability.

According to SHMÚ statistics, the average annual temperature was at 12.5 °C in 2023. The soil temperature is below the level of 5 °C in the months of December, January, February, and partly in March. The temperature rarely drops below zero. In 2023, the total monthly precipitation in seven of the twelve months was above the level of 80 mm per month per 1 m^2. The number of hours of sunshine is 8.5 hours on average. The altitude of the plot is approximately 200 to 240 meters above sea level.

2.2. Rootstocks selection

From the group of well-known rootstocks, we evaluated the following 2 rootstocks as the best option: SO 4 (*Vitis berlandieri* x *Vitis riparia*) syn. (*Berlandieri* x *Riparia Oppenheim*) – The origin of the rootstock dates back to 1956, when it was entered in the List of Permitted Varieties in Germany. As part of the selection of virus-free material, three clones were selected - 4/31, 7/14 and 4/16. In Slovakia in 2005, its spread in vineyards was approximately 10%, which ranks it in fifth place among other rootstocks. One-year wood is dark brown, winter buds are small to medium in size. It has a relatively short growing season; the wood matures early. It buds relatively early, so it can be damaged by spring frosts. Medium to good soils suit it. It is especially suitable for calcareous soils. From the point of view of affinity, it is suitable for growing well-known noble varieties such as Riesling, Pinot Gris. Compared to the Kober 5 BB rootstock, it provides higher yields, mainly because it protects against rain. It is more suitable for quality varieties, but especially those that are prone to rain. It is characterized by resistance to nematodes and vine *Phylloxera* (Pospíšilová, et al., 2005) (Gaser, 2007). We propose to use the SO4 rootstock for the Medina variety.

Craciunel 2 (*Vitis berlandieri* x *Vitis riparia*) syn. (*Berlandieri* x *Riparia Cr 2*) – It is a Romanian selection from the *Vitis berlandieri* x *Vitis riparia* Kober 5 BB population, created in the Craciunel-Blaj viticultural research station. Single-horned wood is dark brown, grooved. Winter buds are small. It is a medium-late rootstock, the power of growth is great. It has deep rooting; therefore, it is suitable for large forms of a truss on a high line. It has higher resistance to drought and calcium content in the soil. We can use it for all varieties grown in our country. Its location is most suitable in the southern parts of Slovakia because it partly extends the growing season. It is less suitable in northern regions, because the lush growth can reduce resistance to winter frosts (Pavloušek, 2011; Pospíšilová et al., 2005). We propose to use the Craciunel 2 rootstock for the Aromína variety.

2.3. Selection of grapevine varieties (Vitis vinifera)

Medina (syn. ECS, Egri csillagok 7) - It is a Hungarian variety that was bred by J. Csizmazia and L. Bereznai in the village of Eger in 1949. It is a variety that was created by crossing the varieties Seyve Villard 12 286 x Medoc Noir. It has medium strong growth. It sprouts early, flowers relatively early. Resistance to fungal diseases is moderate. Resistance against gray mold is high. It is not too demanding on the site. The wines are full, darker red with a higher content of tannins, the taste is fruity reminiscent of blackberries and cranberries (Sotolář, 2023).

Aromína - It was created by crossing varieties (Tramín red x Veltlín red, white/c) x Iršai Oliver 23/26. It is an early variety with an early onset of phenological phases. It is moderately resistant to fungal diseases and to animal pests. It is relatively demanding on location and soil. It belongs to frost-free locations. It achieves high fertility and good growth power. The berries are yellow in appearance and taste very good. The wines are lighter and aromatic (Pospíšilová et al., 1998).

2.4. Dimensions and installation

The division of the vineyard area is 19,000 m^2. Area planted with vines: 17,000 m^2 Roads are registered as other areas. Planting clip: 2.4 m x 0.8 m. Nutrient area of the grape: 1.92 m^2. The number of rows, when viewed from the west side of the planting, is 87. The number of individuals per 1 hectare is 4100, on an area of 1.7 hectares it is 6970 trees. The plot will be fenced on all sides. 755 meters of mesh are needed to fence the plots.

2.5. Timetable

The area of interest, on which the planting will be done, is registered as a vineyard in the Real Estate Registry. The vineyard hunt on which the vineyard will be reconstructed is part of the vineyard complex. A paved access road has been built to the plot. The construction does not require costs for the construction of economic buildings. The plot on which the vineyard will be built does not have energy, water, connection, or municipal networks and is not the subject of archaeological research. The construction will not endanger the environment. The workforce will be provided from the local population. The construction of the vineyard will not endanger the local environment. There are no buildings with social facilities for workers on the plot - they will be handled by a transportable, heated cell. The construction is planned and technically divided into two components. They are Liquidation - removal of the old vineyard - was carried out and construction of a new vineyard; restoration of soil fertility, construction of a supporting structure and maintenance of a young vineyard/orchard until the end of the 4th year after the planting of vine seedlings. The sequence of works and the prices of the works for each stage of the construction, as well as the amount and prices of the materials used, are given in part B - Summary technical report and economic evaluation of the building. Work related to the cultivation and fertilization of the soil, the planting of vine seedlings and the care of plants must be done depending on the agro-climatic conditions and the weather of the growing place in agrotechnical terms. The number of agrotechnical operations in the project is planned for an average vineyard year and can be adjusted according to the climatic conditions of the growing place, the condition of the soil and plants. In the 4th year after planting, the vineyard will be reclassified to the category of material investment means as a producing vineyard. At this time, the vineyard must be 100 % involved, which we achieve by repotting dead or damaged young plants every year. When growing a fruiting vineyard, it will be necessary to apply varietal agrotechnology in cutting, treating, and feeding the grapevines. Until the 12^{th} year of the vineyard's age, the completeness of the stand must be maintained by annual replanting of dead or damaged vine plants with the same variety. Incomplete growth reduces the grape harvest. Later, fill the space after the missing steps by extending their arms and by increasing the number of remaining stems with fertile buds.

2.6. Cultivation shape and planting clip

The planting of the vineyard will be organized in the middle trellis system from the point of view of height. Among the well-known types of wiring, we will select two types of cutting and wiring in particular - the Rhine-Hessian wiring with one native pull and the cordon wiring.

Rhineland - Hessian management - this is the most common type of management in vineyards in Slovakia. It is mainly used for varieties that require a long cut. The height of the trunk is approximately 0.7 meters. The principle of cutting consists in removing the dead wood and replacing it with a new fertile stem created from the stock 2-bud cone, which was left last year and forms the basis of the fertile wood, while this process is repeated every year (Bernáth, 2017).

Cordon line - they are typical in that the end of the trunk is formed by one or two arms, which can be horizontal, oblique, or vertical. On the remaining shoulders, we leave different length cones, which are usually shorter than longer (Bernáth, 2017).

In planting, we recommend growing grapevine varieties in the cultivation form of grapevine in a clip of 2.4 x 0.8 meters using a support system in the form of a wire frame with 3 wires at a height of 0.6 m, 1.2 m and 1.8 m fixed to row posts - U profile metal posts.

2.7. Calculation of efficiency and profitability

Annual profit represents annual production less depreciation and annual costs. The formula for calculating the annual profit is therefore:

Annual profit = (annual production - depreciation) - (annual costs - integrated support) (results in €)

The calculation of the efficiency of the investment is based on the calculation of the annual profit and we will use this formula.

Investment efficiency coefficient = (profit x 100) / investment costs (results in %)

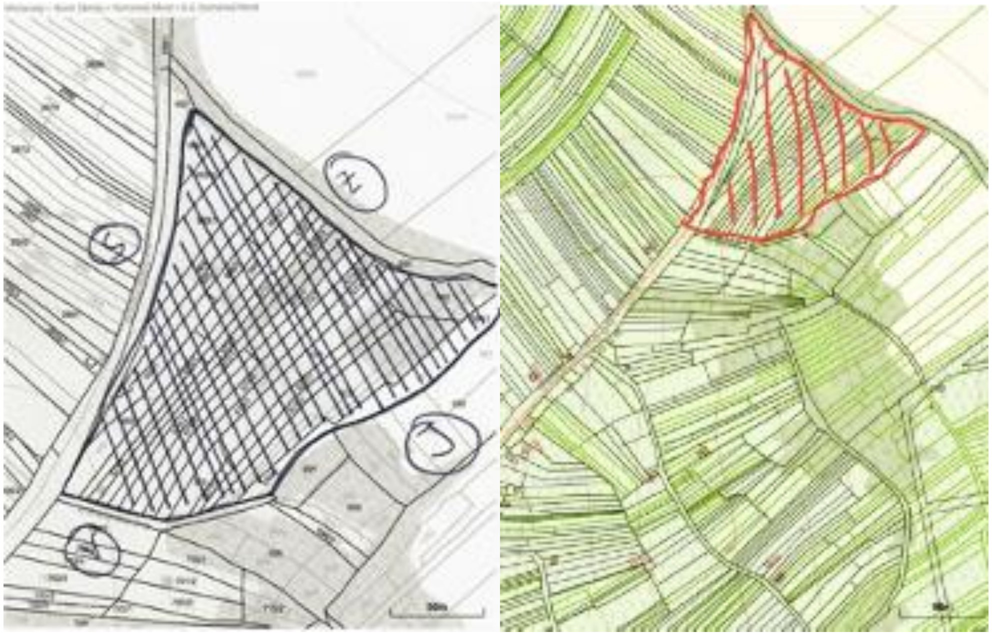

Figure 1 Geodetic images of the plot. The parcel in question is highlighted - the image on the right shows the location within the compass (J - south, Z - east, V - west, S - north) (ZBGIS, 2024)

3. Results

3.1. Fertilization costs

Table 1 – Fertilization costs – material part

Material	UoM	P (€)	UoMt	Pt (€)
Green manure				
Pisum sativum	t	539.00	0.2	107.8
Trifolium incarnatum	25 kg	56.75	2	113.5
Hordeum distichon MALZ	t	400.00	1	400.00
Brassicaceae	25 kg	53.50	1	53.50
Manure				
Manure	t	25	85	2125.00
Fertilizers				
Triple superphosphate	t	318.05	2	636.1
Potassium chloride	t	331.36	1	331.36
Kieserit	t	277.37	8	2218.96
Sum				**5986.22**

UoM – Unit of Measure, P- price, UoMt – Unit of Measure total, Pt – Price Total

Table 2 – Fertilization costs – price for work

Operation	UoM	P (€)	UoMt	Pt (€)
Landscaping	h	153.00	2	306.00
Delivery of manure	km	2.00	80	160.00
Loading of manure	h	35.00	4	140.00
Fertilization of manure	ha	87.50	1.7	148.75
Delivery of fertilizers	km	2.00	80	160.00
Loading of fertilizers	h	35.00	4	140.00
Fertilization 2x	ha	18.50	3.4	62.90
Deep plowing 1x	ha	70.00	1.7	119.00
Land development 3x	ha	40.00	5.1	204.00
Shallow plowing 2x	ha	39.00	3.4	132.60
Sowing of plants 3x	ha	53.00	5.1	270.30
Disking of green matter 3x	ha	40.00	5.1	204.00
Medium plowing 2x	ha	60.00	3.4	204.00
Rigolation	ha	730.00	1.7	1241.00
Soil analyses (P, K, Mg, Ca) according to Mehlicha 3, pH in soil with protocol	pc	20.50	3	61.50
Sum				**3554.05**

UoM – Unit of Measure, P- price, UoMt – Unit of Measure total, Pt – Price Total

3.2. Costs for trellis system and fencing

The trellis system of the high line of the vineyard, type Rhine-Hessian line, will be made up of columns 240 cm high. One row of columns will support six trusses of vines. The need for row columns is therefore 1,162 pieces (6970:6). For every 10 row columns, there is one row column as a support, which is 117 columns. The need for columns that will carry the wire at their top is a total of 1,045 pieces. The need for supporting metal rods with a diameter of 8 mm and a height of 170 cm is 6,970 pieces. For the trellis system, 2 x 0.80 m wire is needed per bush to carry the arms and 3 x 0.20 m to tie the trunks to the support bar, which is a total of 2.20 m. 19,360 m (2.2 m x 8800 roofs) of wire is needed for the entire vineyard.

Table 3 – The cost of fencing – material part

Material	UoM	P (€)	UoMt	Pt (€)
Mesh for fences 25 m	25 m	82.00	30	2460
Fence post PVC 48/2800 mm	pc	10.20	258	2631.6
Side strut PVC 38/2800 mm	pc	13.08	26	340.08
Screws 60x1,70x550 mm	pc	6.37	284	1809.08
Screws M8x40 mm Zn	pc	1.00	284	284
Wire PVC 3,50 mm/78 m	78 m	9.03	10	90.3
Wire holder PVC zelená	pc	0.20	775	155
Wire PVC 2,00 mm/50 m	50 m	3.00	10	30
Barbed wire PVC 100 m	100 m	19.71	8	157.68
Clasp Rapid PVC	1600 pc	26.90	2	53.8
Wire tensioner PVC	pc	0.53	258	136.74
Belt for tensioner Ø 48 mm PVC	pc	0.35	258	90.3
Eye for tensioner PVC	pc	0.51	258	131.58
Gate BD Solid HNZ+PVC	m^2	174.00	6	1044
Sum				**9 414.16**

UoM – Unit of Measure, P- price, UoMt – Unit of Measure total, Pt – Price Total

Table 4 – The cost of fencing – price for work

Operation	UoM	P (€)	UoMt	Pt (€)
Installation of columns	pc	8.00	258	2064.00
Stretching of wires	m (3x)	3.00	775	2325.00
Mesh placement	m	3.00	775	2325.00
Barbed wire stretching	m	2.00	775	1550.00
Installation of gate	pc	500.00	1	500.00
Sum				**8764.00**

UoM – Unit of Measure, P- price, UoMt – Unit of Measure total, Pt – Price Total

Total costs for the construction of the fence: € 9,414.16 + € 8,764.00 = € 18,178.16.

Table 5 – The cost of the trellis system– material part

Material	UoM	P (€)	UoMt	Pt (€)
Vineyard column 2,5 m(I-30)	pc	8.63	1162	10027.44
Vineyard end column 2,7 m(E-50)	pc	13.56	117	1587.6
Wire CRAPAL 2TOP s ø 2,5 mm	25 kg	60	18	1080
Ground anchor ø 120 mm	pc	4.80	174	835.2
Anchor rope GPAK 4, GP Large, length 4,5 m	pc	7.20	174	1252.8
Tensioner and clutch Gripple Medium	pc	1.08	348	375.84
Tension chain 225 mm	pc	0.54	348	187.92
Steel buckle ø 2,8 mm	pc	0.48	186	89.28
Supporting rod	1000 pc	360.00	9	3240
Fixing clip ø 6 - 8 mm	pc	0.08	7000	528
Pipe for tying	125 m	3.30	22	72.6
Sum				**19 276.68**

UoM – Unit of Measure, P- price, UoMt – Unit of Measure total, Pt – Price Total

Table 6 – The cost of trellis system – price for work

Work	UoM	P(€)	UoMt	Pt (€)
Transport material	hour	10	26	260
Installation of raw columns	ha	1750	1.7	2975
Installation of end columns	ha	550	1.7	935
Installation of wires	ha	450	1.7	765
Instalation of double wire	ha	950	1.7	1615
Sum				**4935. 00**

UoM – Unit of Measure, P – price, UoMt – Unit of Measure total, Pt – Price total

Total costs for the construction of the support structure: € 19,276.68 + € 4,935 = € 24,211.68

3.3. Planting costs

Vine scions will be planted on an area of 1.7 ha. The remaining acreage is for mechanization handling lines. In the vineyard, 2 grape varieties will be planted on approximately equal areas. The vines will be planted in a spacing of 1.0 x 2.4 m on the Rhine-Hessen line.

Table 7 - Planting cost – material and labour

Material	UoM	P (€)	UoMt	Pt (€)
Vine grafts	pc	1.49	6970	10354.5
Mycorrhizal fungi	20 kg	180.00	8	1440
Labor				
Machine planting	pc	0.19	6970	1327.5
		Sum		**13122.00**

UoM – Unit of Measure, P- Price, UoMt – Unit of measure total, Pt- price total

Total costs for planting € 13,122.00.

3.4. Recapitulation of costs

Table 8 - Summary of the establishing costs of a vineyard

Operation	Material costs (€)	Labor costs (€)	Sum (€)
Fencing contruction	9414.16	8764.00	18178.16
Pre-planting soil preparation	5986.22	3554.05	9540.27
Planting grafts	11794.5	1327.50	13122.0
Trellis system	19276.68	4935.00	24211.68
1 ha			**38265.95**
Sum together			**65052.11**

Table 9 - The deprecition of the vineyard

Harvest year	Costs (€)	DOC (%)	AOD (€)	VAD (€)
1.year	65052.11	3.40%	2211.77	62840.34
2.year	62840.34	6.90%	4335.98	58504.35
3.year	58504.35	6.90%	4335.98	54168.37
4.year	54168.37	6.90%	4335.98	49832.39
5.year	49832.39	6.90%	4335.98	45496.40
6.year	45496.40	6.90%	4335.98	41160.42
7.year	41160.42	6.90%	4335.98	36824.44
8.year	36824.44	6.90%	4335.98	32488.45
9.year	32488.45	6.90%	4335.98	28152.47
10.year	28152,47	6.90%	4335.98	23816.49
11.year	23816.49	6.90%	4335.98	19480.50
12.year	19480.50	6.90%	4335.98	15144.52
13.year	15144.52	6.90%	4335.98	10808.54
14.year	10808.54	6.90%	4335.98	6472.55
15.year	6472.55	6.90%	4335.98	2136.57
16.year	2136.57	6.90%	4335.98	

DOC – Depreciation of costs, **AOD** – Annual of depreciation, **VAD –** Value after dpreciation

3.5. Grape production

We expect a harvest in the third year of planting, they reach full fertility in the fourth year after planting. The number of grapevines with a clip of 2.4 x 1 m is 6,970 on the mentioned area of 1.7 ha. With an average yield of 2 kg per grapevine, the total yield is 13,940 kg. At the price of grapes EUR 1.2 without VAT, the annual production is EUR 16,728 per year.

Table 10 Annual production les depreciation

	GP (€)	AD (€)	APADD (€)
In first year	16728	2211,77	14008,82
Other years	16728	4335,98	11535,67

3.6. Annual profit

Annual profit = (annual production - depreciation) - annual costs

Annual profit = (16728 - 4335.98) - 4000

Annual profit = 8392.02 €

3.7. Efficiency of agricultural investment

Investment efficiency coefficient

IEC = (profit x 100) / investment costs

IEC = (8392.02 x 100) / 65052.11

IEC = 12.9 %

3.8. Return on investment

ROI = investment costs / (annual profit + annual depreciation)

ROI = 65052.11/ (8392.02 + 4335.98)

ROI = 5.11 years (from full fertility)

4. Conclusion

We expect annual costs of 4,000 € per ha in the fruiting orchard. Integrated support, according to the Ministry of Agriculture and Rural Development of the Slovak Republic, from the EU amounts to 601 € per ha. Annual profit represents annual production less depreciation and annual costs. The annual profit is 8392.02 €. It is clear from the results that the vineyard is profit-making, at least for the duration of the depreciation. The results also show the efficiency coefficient of the investment, which is positive with a value of 12.90 %, so the investment is effective, at least for the duration of depreciation. To keep the efficiency of the investment, it is necessary to retain the purchase prices of grapes and ensure the stability of the profitability of grape vine cultivation (Vitis vinifera).

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ARTICLE HISTORY:
Received 29.04.2024
Accepted 20.05.2024

EVALUATION OF INCIDENCE OF ESCA FOLIAR SYMPTOMS IN CENTRAL SLOVAKIAN WINE-GROWING REGION

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Abstract

ESCA syndrome is a critical threat to viticulture in all regions of the world. The disease is caused by a complex of wood destroying fungi leading to grapevine dieback. The study's goal was to monitor the occurrence of ESCA foliar symptoms in the 5 vineyards in Central Slovakian wine-growing region. During the monitoring, we employed visual observations of foliar symptoms to evaluate the degree of grapevine damage based on vineyard age structure, varietal composition, and rootstock used. For monitoring purposes, we employed a 1–5 rating scale. We investigated the methods used to prevent and protect against ESCA syndrome in monitored vineyards. The highest incidence of ESCA syndrome was found in the locality 'Modrokamenský II', more than 10%. The lowest incidence was found in the locality 'Tornaľský', less than 3 %. Control of ESCA should be comprehensive and include a combination of biological and chemical approaches as well as preventive methods to eliminate the spread of wood-destroying fungi. It is essential to focus on innovative diagnostic tests and technologies, grapevine defence mechanisms and management strategies to eliminate damage caused by grapevine trunk diseases. Strict international monitoring of propagating material and its traceability is essential to eliminate the spread of wood-destroying fungi.

Keywords: ESCA, wood-destroying fungi, foliar symptoms, disease incidence, monitoring

1. Introduction

Grapevine trunk diseases (GTD, ESCA syndrome) currently represent one of the greatest challenges for world viticulture (Fontaine et al., 2016). A few decades ago, GTD symptoms appeared only in old vineyards. Replacing infected grapevines with young seedlings was a common and effective practice to prevent further spread of grapevine trunk diseases. Nowadays, GTD symptoms are increasingly occurring on young grapevine plants. Often, disease symptoms appear in the first few years after vineyard planting (Díaz and LaTorre, 2013; Bruno and Sparapano, 2007). The manifestations of GTD are seasonally variable and depend on many factors, which mainly include the weather patterns during the vintages and the genetically determined resistance of a particular cultivar (Calzarano et al., 2014; Di Marco, 2011).

Microscopic fungi associated with ESCA include *Phaemoniella chlamydospora*, *Phaeoacremonium* spp., *Fomitiporia* spp., and *Stereum hirsutum*. These microscopic fungi mainly infect older vineyards, but their occurrence has also been confirmed in newly planted vineyards. Fungi associated with GTD exhibit different wood colonising capabilities, which can be divided into the ability to decompose wood, the ability to produce defensive compounds, or the ability to counteract the production of vine metabolites. GTD fungi have different enzymatic capabilities to degrade lignin, pectin, or starch (Claverie et al., 2020). From the time of the fungal infection to the appearance of the first symptoms, GTDs generally develop slowly in the plant. After several years from the primary infection, the wood-destroying fungi start to behave invasively, which manifests in the form of specific symptoms (Verhoeff, 1974). The foliar symptoms of GTD do not appear every growing season. Even asymptomatic grapevines can be heavily infected by wood-destroying fungi.

Wood-destroying fungi are a natural part of the environment and are involved in the decomposition of organic matter. There are several hypotheses related to the causes of the change of the endophytic phase to the pathogenic phase of wood-destroying fungi. The triggers for this change are considered to be the exposure of the plant to biotic or abiotic stresses such as inadequate nutrition, prolonged drought, mechanical injury to the plant, and salinization of the soil in a vineyard (Hrycan et al., 2020; Petrini, 1991; Stone et al., 2000). There are other factors that significantly influence the incidence of GTD, namely the varietal composition of the vineyard, the rootstock used, and the agrotechnology used to cultivate the grapevines. The tools used during vine pruning can also be a vector of disease transmission (Agustí-Brisach et al., 2015).

On symptomatic grapevines, the disease reduces the quality of both wine and table grapevine varieties (Rolshausen and Kiyomoto, 2007; Bruno and Sparapano, 2007; Cloete et al., 2014). The chronic form of ESCA causes typical foliar symptoms, which are referred to as "tiger stripes" or "tiger spot". The apoplectic form is characterized by the sudden death of part or the whole grapevine. Lesions and wood decay appear on the cross-section of symptomatic trunks (Gramaje et al., 2008; Choueiri et al., 2014; Maher et al., 2012). Soft rot appears in the wood of older grapevines and spreads over time (Larignon and Dubos, 1997; Chiarappa, 1997; Fischer, 2002; Fischer and Kassemeyer, 2003).

2. Material and methods

2.1 Characteristics of the monitored localities

As part of ESCA syndrome monitoring, we monitored disease incidence in 5 vineyards. The monitored vineyards are located in the 'Modrokamenský', 'Hontiansky', and 'Tornaľský' wine-growing districts, which are part of the Central Slovakian wine-growing region. The Central Slovakian wine-growing region consists of 7 wine-growing districts with 107 wine-growing villages. The vineyards are situated on the southern slopes of the Krupina Hills and the Ipeľ Lowland. The average altitude of the vineyards is 180 m, with predominantly medium heavy soils (Jobbágy et al., 2022).

2.2 Damage assessment of the vineyards

For vineyard damage assessment, we developed a rating scale of 1 to 5 (Table 1). It determines the percentage of symptomatic grapevines with typical foliar symptoms (Figure 1), visually, twice, in the months of July and August, according to Ailer (2022). We took into account the age structure of the vineyards, varietal composition, and rootstock used.

Table 1 Method for assessing the level of GTD symptoms in vineyards

Symptomatic grapevines up to 3 % of the area – damage degree 1
Symptomatic grapevines up to 5 % of the area – damage degree 2
Symptomatic grapevines up to 7 % of the area – damage degree 3
Symptomatic grapevines up to 9 % of the area – damage degree 4
Symptomatic grapevines up to 10 % of the area – damage degree 5

(Ailer, 2022)

Figure 1 ESCA foliar symptoms – tiger stripes (Janás, 2023)

We calculated the disease incidence rate by dividing the sum of the area of symptomatic grapevines by the total area of the monitored vineyard. We expressed the resulting value as a percentage. Based on the percentage of damaged vineyard area, we classified the vineyards into damage degrees 1–5 (Table 1).

3. Results and discussion

3.1 Monitoring the incidence of ESCA foliar symptoms

In the wine-growing village of Veľký Krtíš (Modrokamenský I.), we monitored the white wine grape varieties of MOPr, Grüner Veltliner, Irsai Oliver, Pinot grigio, Gewürztraminer and red wine grape variety of Cabernet Sauvignon (Table 2). The line in the vineyard is Rhine-Hessian. Every second row is grassed. The grapevines are grafted on the SO4 rootstock. The parts of vineyard have a different age, which vary from 5 to 23 years. The grower solves the problem by removing the symptomatic grapevines.

In the wine-growing village of Veľký Krtíš (Modranský II.), we observed the varieties Pinot grigio, Gewürztraminer, Sauvignon blanc, and Slovak red wine grape variety Hron on the rootstock SO4 (Table 2). The age of the vineyard is 15 years. Every second row in the vineyard is grassed. The line in the vineyard is Rhine-Hessian. The degree of vineyard damage is 5.

In the wine-growing village of Rykynčice (Hontiansky I.), we observed the varieties MOPr, Müller Thurgau, Feteasca regala, Gewürztraminer, St. Laurent (Table 2). The line in the vineyard is Rhine-Hessian. Every second row in the vineyard is grassed. The age of the vineyard is 48-50 years. We found a vineyard damage degree of 3. The grower prevents the spreading of ESCA by disinfecting of tools used in pruning.

In the wine-growing village of Ladzany (Hontiansky II.), we observed the varieties Grüner Veltliner, Irsai Oliver, St. Laurent, Chardonnay, Feteasca regala, Pálava, Mädchentraube (Table 2). The age of the vineyard is 42 years. The line in the vineyard is Rhine-Hessian. The degree of vineyard damage is 3. The grower solves the disease by adjusting nutrition, revitalizing the vineyard, and planting new trunks.

In the wine-growing village of Valice (Tornaľský), we observed the white wine grape varieties Grüner Veltliner, Pálava, Pinot blanc, Chardonnay, Noria, Riesling, Gewürztraminer and blue wine grape varieties Cabernet Sauvignon, Cabernet Franc, Lemberger, St. Laurent, Merlot, Alibernet, Dunaj (Table 2). The line in the vineyard is Rhine-Hessian. Vineyard has a 2 parts that are 42 and 7 years old. We found a low vineyard damage degree of 1. The grower is solving the problem by removing the symptomatic grapevines.

The prevalence of GTD in France has been increasing since 2000. The average annual incidence of GTD is 13 % (Claverie, 2020; Bruez et al., 2013). Based on our monitoring of selected vineyards in the Central Slovakian wine-growing region, we found an average incidence of tiger stripes below 10 %. This value indicates a lower incidence of ESCA foliar symptoms in the Central Slovakian wine-growing region compared to France. In Spain, the incidence of GTD ranges from 1.8 to 10.5 %, and in Italy, it ranges from 8 to 19 %. The incidence of these diseases has reached 60-80 % in some old vineyards in Tuscany, Puglia, and Sicily (De La Fuente et al., 2016). In our case, we did not find a higher incidence of foliar ESCA symptoms in old vineyards compared to young plantings.

Table 2 Monitoring of ESCA foliar symptoms

Wine-growing district	Variety	Origin of grafted young	Rootstock	Age of vineyard	Evaluated area	Damage degree
Modrokamenský I.	MOPr, Grüner Veltliner, Irsai Oliver, Pinot grigio, Gewürztraminer, Cabernet Sauvignon	Unknown	SO4	23, 10, 9, 6, 5	28,5 ha	4
Modrokamenský II.	Pinot grigio, Gewürztraminer, Sauvignon blanc, Hron	Unknown	SO4	15	10 ha	5
Hontiansky I.	MOPr, Müller Thurgau, Feteasca regala, Gewürztraminer, St. Laurent	Unknown	Unknown	48-50	5 ha	3
Hontiansky II.	Grüner Veltliner, Irsai Oliver, St. Laurent, Chardonnay, Feteasca regala, Pálava,	Unknown	Unknown	42	20 ha	3
Tornaľský	Grüner Veltliner, Pálava, Pinot blanc, Chardonnay, Noria, Cabernet Sauvignon, Riesling, Gewürztraminer, Cabernet Franc	Unknown	Kober 5 BB	42 and 7	30 ha	1

4. Conclusion

Wood-destroying fungi are controlled primarily by prevention, effective agrotechnology, and the reduction of cultivation stress. The key to controlling grapevine trunk diseases is precise international monitoring of biological propagation material. Grapevine's natural defence is the flow of sap, which appears as drops of exudate on the surface of cut wounds. Pruning in early spring increases the grapevine's resistance to infection due to tearing which reduces pathogen entrance into the cut wound. Tools used for pruning and green work should be disinfected on a regular basis. Beyond that, we can disinfect the cutting wounds.

There are two methods of wound protection: spraying fungicides and using products such as waxes or balms. In the case of larger wounds, applying acrylic paint on the surface of the pruning wound has proven effective. During the growth season, we aim to reduce the number of wounds created by mechanisation, which are a source of infection. The fight against ESCA in vineyards is very difficult, inefficient, and expensive. If we find foliar symptoms of grapevine infection in a fruiting vineyard, one approach is to remove the affected part of the grapevine then plant a new, healthy trunk.

Acknowledgment

The work was carried out with the support of the project "Support of research activities in VC ABT" No. 313011T465".

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HISTORY:
Received 24.04.2024
Received in revised form 28.05.2024
Accepted 29.05.2024

SURVEY OF THE POSSIBILITIES OF THE THEMATIC PRESENTATION OF RED WINE IN EXPERIENTIAL ENOGASTRONOMY

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Abstract

Experiential enogastronomy must be adjusted to accommodate proper management of both the intake of calories and ethanol and their subsequent burning and degradation. When compiling a multi-course menu, it is also necessary to ensure adequately sized portions and account for the energy value of each meal. The purpose of this research was to design realistic models of experiential enogastronomy thematically with red wines and to propose the management of calorie and ethanol degradation. The product of the study is the concrete results of pairing food and red wine, with exact calculations of the intake of calories during the consumption of multi-course menus. We implemented the study in the form of a menu proposal and a questionnaire. The survey shows that out of the total number of respondents (51), the term experiential enogastronomy is known to 39 respondents (76 %). 58 % of respondents are willing to pay an average of EUR 37.50 for a moderated gastronomic event combining food and wine. The monothematic pairing of red wines and food is feasible in our conditions, in many variants.

Keywords: red wine, grape variety, enogastronomy, experiential wine tourism

1. Introduction

Nowadays, enogastronomy is a modern phenomenon and is proving to be a promising field. Consumer interest in wine and wine-related experiences is on the rise, but it is necessary to offer impeccable quality, including infrastructure (Šenková, 2010). The concern about passive lifestyles and the associated diseases of civilization is becoming more and more relevant in society. Therefore, excessive caloric intake should be avoided when designing menus. The aim of this thesis was to propose realistic models of experiential enogastronomy, themed around red wines, and to propose the management of ethanol and calorie removal.

The consumption of red wines worldwide is much higher than in Slovakia. The reason is that our climatic conditions are more favorable to produce wine from white grapes and rosé from red grapes. The increased interest and consumption of red wine was also caused by the latest results of medical studies on the health benefits of moderate consumption of red wine (URL2). Propagation of cultural consumption of red wine may be one of the ways to improve the stagnating viticulture and the low per capita wine consumption in Slovakia, which is 13.5 l/year (Trubačová and Repka, 2023). On the total area of SR vineyards, the share of red wine grape varieties makes just for approximately 25 % (Figure 1). Nowadays, however, the cultivation areas of red wine grape varieties are gradually expanding. The varieties are that are planted achieve higher sugar content and quality in the northern peripheral areas. The red wine grape variety composition in Slovakia is shown in Figure 2.

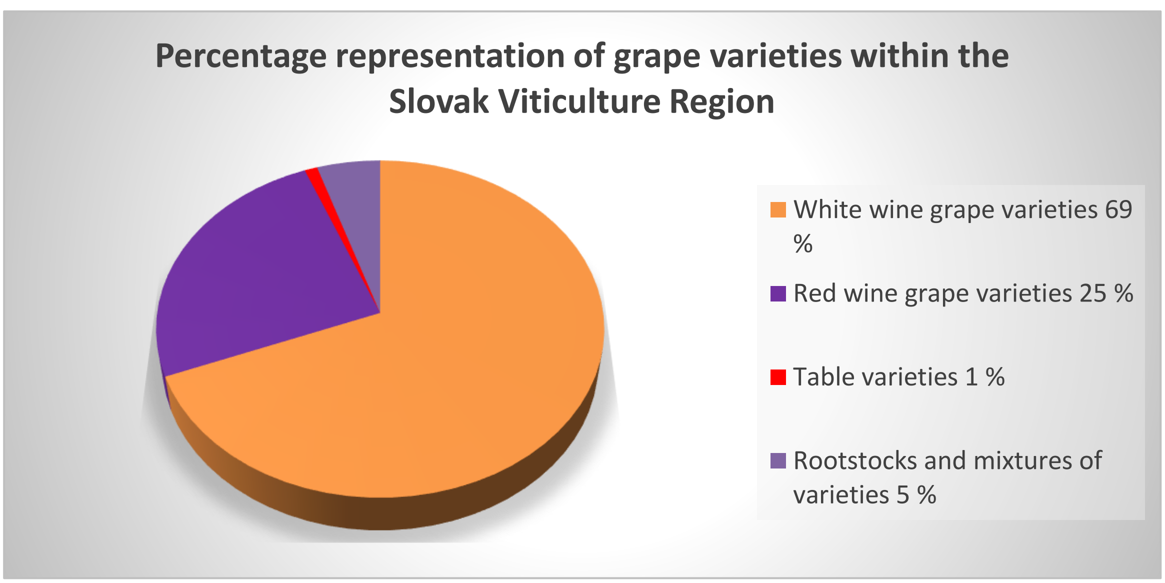

Figure 1 Percentage representation of varieties according to the purpose of use in Slovakia (source: ÚKSÚP, 2021 (URL 1))

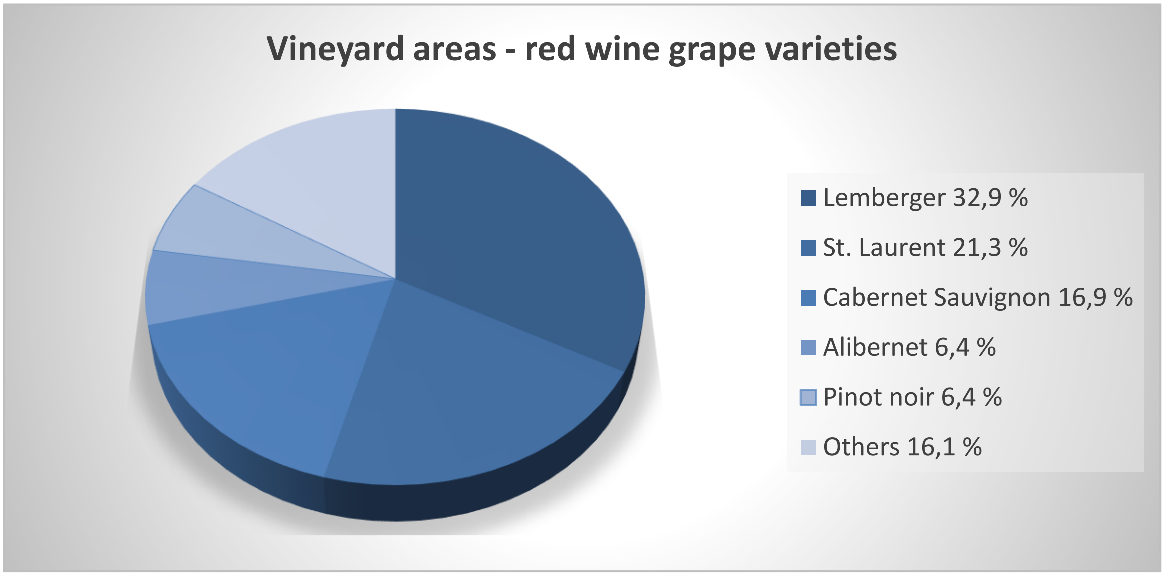

Figure 2 Red wine grape varieties – variety composition in Slovakia (source: ÚKSÚP 2021 (URL 1))

Gastronomic trends follow the lifestyle of the inhabitants and their needs as consumers reflect on the evolution of menus (Kompasová, 2021). At present, the quality and freshness of ingredients are the cornerstone of dish preparation - the use of additives is reduced (Habánová and Habán, 2021).

2. Material and methods

The survey was aimed at different segments and age categories (from students to families with children, singles and childless, to seniors). We assumed that the respondents had encountered the term experiential enogastronomy at least once in their lives. The survey was created using Google Docs and shared via the Facebook platform.

Procedure of survey:

1. Creating an online form.

2. Sharing the form with the target group.

Respondents answered questions that focused on experiential gastronomy and physical activity. 51 respondents participated in the survey in electronic form. The greatest advantages of electronic inquiries are considered to be low financial and time requirements, as well as its addressability.

2.1. Energy contained in wine

Wine contains three components that have a significant caloric value:

- ethanol,
- sugar,
- sugar-free extract.

The classic formula for calculating the number of calories (Q) can be expressed by the following equation:

$$Q = 5.5 \times AC + 0.4 \times SU + 0.24 \times EX$$

where:

Q = number of calories per 100 mL of wine

5.5 = significance coefficient of ethanol content

AC = ethanol concentration (vol.% = v/v = ABV = alcohol by volume)

0.4 = significance coefficient of residual sugar content

SU = sugar concentration (g/l)

0.24 = significance coefficient of sugar-free extract content

EX = sugar-free extract (g/l)

Caloric value of wine - calculation

- Example for red wine:

Based on a qualified estimate, in the case of high-quality red wines, can be assumed a sugar-free extract content of 25 g/l, equivalent to 6 calories. The usual sugar content is 2 g/l and the ethanol content is 13 vol.%.

Every 100 ml of red wine with a sugar content of 2 g/l, ethanol 13 vol. % and sugar-free extract 25 g/l has:

$$Q = 5.5 \times 13 + 0.4 \times 2 + 0.24 \times 25 = 71.5 + 0.8 + 6 = 78.3 \text{ calories}$$

Result: Most dry wines contain 70 to 90 calories kcal per 100 ml (Ailer, 2016).

2.2. MET

The number of calories burned during physical activity depends on several factors, such as body weight, pace, terrain, etc. It is calculated, for example, by the MET (metabolic equivalent of task) method. This unit expresses an estimate of the amount of energy our body needs for a certain activity. 1 MET is defined as the amount of oxygen consumed while sitting at rest. Its value is 3.5 ml of oxygen per kilogram of weight per minute (ml/kg/min.).

3. Results and discussion

Table 1 Composition and caloric values of one of the proposed menus

Menu	***Name***	***Amount (l, g)***	***Energy (kJ)***	***Calories (kcal)***
Aperitif	Sparkling wine Alibernet, dry, red, year 2017	0,05 l	165,95	39,7
Appetizer	Game pheasant pâté	150 g	1636,47	391,5
	Cabernet Sauvignon, dry, red, year 2019	0,05 l	169,42	40,53
Soup	Halászlé	250 g	344,85	82,5
	Young Blauer Portugieser, dry, red, year 2023	0,05 l	163,65	39,15
Main course	Roast beef with champignons, baked potatoes	350 g	1475,54	353
	Lemberger, red, dry, year 2019	0,05 l	165,74	39,65
Dessert	Chocolate souffle with thick raspberry sauce	60 g	283,82	67,9
	Merlot barrique, dry, red, year 2018	0,05 l	175,14	41,9
Total			4580,58	1095,83

Table 2 Recommended calorie intake (kcal/day) for woman and man of different ages

		Woman		**Man**	
AGE		**19 – 30**	**31 – 50**	**19 – 30**	**31 – 50**
Load	low	2000	1800	2400	2200
	medium	2200	2000	2600	2500
	high	2400	2200	3000	2900

Source: Kajaba et al., 2015

3.1. Examples of MET unit calculation

The consumption of oxygen and energy is directly proportional to the intensity of the given physical activity. Such metabolic calculations are accessible on many reputable online sites. Example: a woman with a body weight of 60 kg who walks at a speed of 6.4 km/h burns 89 calories during a 60-minute walk.

Table 3 MET values at different walking speeds

Walking speed	MET
less than 3,2 km/h	2
3,2 km/h	2,8
4 km/h	3
4,8 km/h	3,5
5,6 km/h	4,3
6,4 km/h	5
7,2 km/h	7
8 km/h	8,3

Source: URL 2

3.2. Questionnaire about experiential gastronomy and physical activity

The aim of the questionnaire was to survey the respondents' knowledge regarding experiential gastronomy associated with wine tasting and physical activity. The questionnaire was created via Google and then shared via Facebook. The survey was completed by 51 respondents from a circle of friends, acquaintances, coworkers, and family. The questionnaire was filled out by respondents of different age categories.

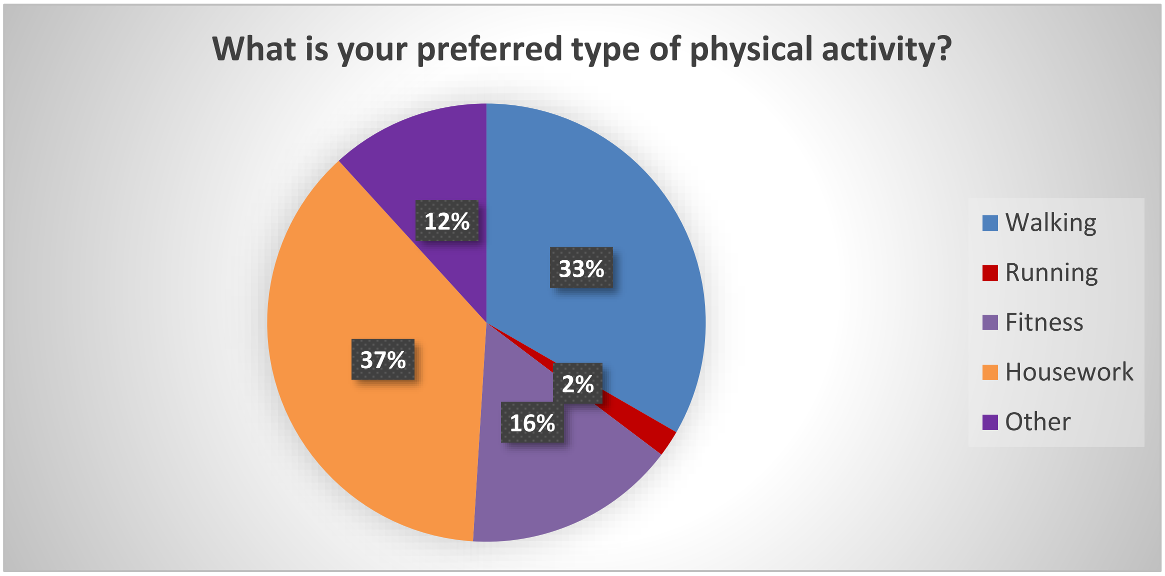

Figure 3 Question: What is your preferred type of physical activity?

Survey result: the largest sample of respondents – 37 % prefer housework. Ailer et al. (2019) found in a physical activity preference survey that 60 % of respondents were willing to perform physical activity after experiential enogastronomy.

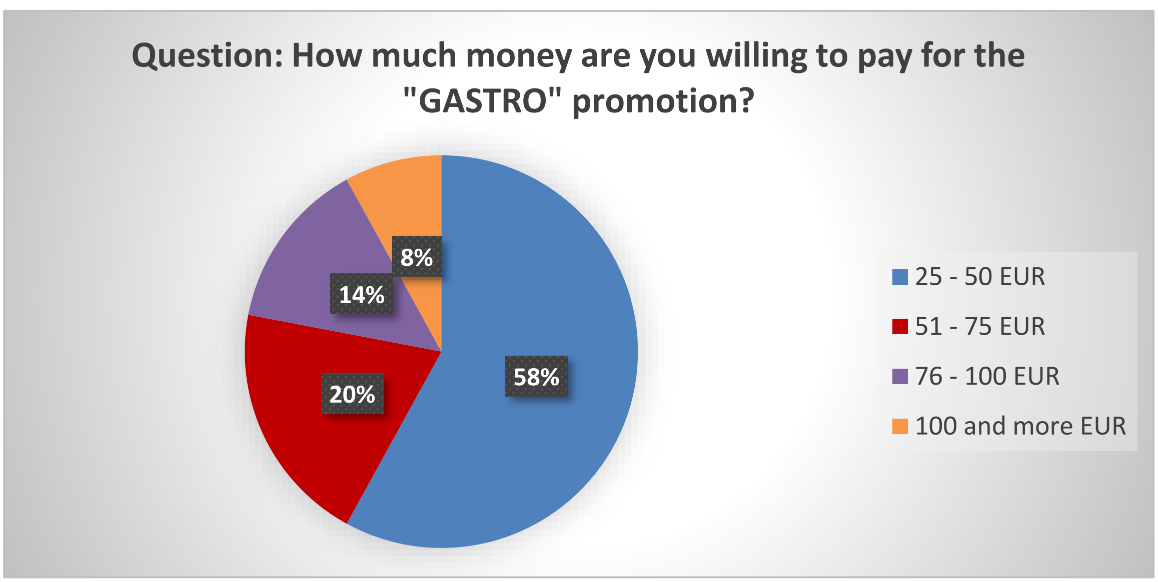

Figure 4 Question: How much money are you willing to pay for the "GASTRO" promotion?

Survey result: The largest number of respondents (58 %) would be willing to pay from 25 to 50 EUR for such an event. The results regarding the willingness to spend money on an enogastronomic experience correspond with the work of Šípeková et al. (2021), who conducted a survey of experiential gastronomy with white wine in Western Slovakia.

4. Conclusion

In the study, we wanted to highlight the fact that an unforgettable and inimitable experience is a commodity that must be paid for. If the consumer is satisfied with it, he will return to it repeatedly and will be glad to spend an adequate amount of money on it. The monothematic pairing of red wines and food is feasible in our conditions in many variants, thanks to the rich grape variety present. Consumers expressed interest in participating in a moderated gastronomic experience. The majority-chosen price range of the event, an average of EUR 37.50/person, would, from an economic point of view, be on the verge of profitability for catering establishments. In today's hectic world, visiting a good restaurant and enjoying freshly prepared food and a quality drink in the company of friends is what brings many people well-deserved moments of respite. However, the development of gastronomic tourism requires constant conceptual work on promotion, wine tourism, infrastructure, education, and strengthening customer loyalty.

Regarding the management of caloric expenditure during experiential enogastronomy, we do not recommend strenuous physical activity and excessive exertion after consuming alcoholic beverages, but e.g., a brisk walk in the field, during which we burn up to 70 kcal in 10 minutes. Such walking is usual for the implementation of wine tourism during transfers within the event.

Acknowledgment

This publication has been produced thanks to the support of the international research-educational project Initiative project funding AT/SK No. 2023-05-15-004: "Evaluation of the status of the implementation of interspecific grape varieties in cultivation systems, from the point of view of the application of nature-friendly agrotechnical processes".

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ARTICLE HISTORY:
Received 07.05.2024
Accepted 30.05.2024

AN INDICATION OF THE PHYSIOLOGICAL REACTIONS OF THE AVOCADO ROOTSTOCK LEAVES DEPENDING ON THREE KINDS OF IRRIGATION WITH DIFFERENT WATER SALINITY LEVEL

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Abstract

A pot experiment with Avocado rootstocks was equipped with lysimeters in the facilites of the «La Mayora Experimental Field site» of the Higher Council for Scientific Research in the Malaga province (Spain). An evaluation of physiological reactions of the avocado rootstock leaves was made after 3.5 month of irrigation with three kinds of irrigation with different water salinity level including groundwater (GW), regenerated wastewater (RWW) and a mix of ground and regenerated municipal wastewater (GW/RWW-1:1). All substrata were taken in local alluvial deposits. Technosol texture is characterized mainly as silt. The ground water salinity level is 2 times less comparative with GW/RWW-1:1 and RWW treatments. The avocado leaf blade area decreased by the end of observations in the GW/RWW-1:1 and RWW treatments by 1.2 and 1.6 times compared to the groundwater option. Cell membrane Injury Ratio in the GW/RWW-1:1 and RWW treatments are 27 and 43 % correspondingly. Lowest level of chlorophyll a, chlorophyll b and carotioids were in the RWW treatment. 50 % level using of mix consisted of ground and regenerated water (1:1) took intermediate indexes. Increase in salinity level of GWW/RWW and RWW treatments was connected with their high the spectral reflectance in the visible region.

Similar situation was fixed for RWW option in Red-Edge and NIR wavelength ranges. Highest reflectance level was noted in ground water option in SWIR-1 and SWIR-2 regions.

Key words: avocado, technosol, water; salinity, pigments, vegetation indices

1. Introduction

Andalusia, in the south of Spain, represents the 90 % of the Spanish production, concentrated in the so-called subtropical coast of Malaga and Granada. Over the 80 % of the avocado fruits harvested in Andalusian are exported to the European Union. Meantime, water supplies 40 % and 60 % below FAO's needs caused significant water affects numerous functions of the plant physiology, such as photosynthetic capacity, vegetative growth and productivity (Moreno-Ortega et al., 2019; Cárceles Rodríguez et al., 2023). Avocado (*Persea americana Mill*.) is a member of the Lauraceae family, which includes 50 genera and about 2500–3000 species, with three main horticultural breeds (Mexican, Guatemalan, and West Indian) (Chanderbali et al., 2008; Ibarra et al., 2015). Salinity/Salt stress is one of the most significant environmental challenges limiting avocado productivity, particularly in arid and semi-arid climates (Bernstein et al., 2003; Berkessa, 2020). It is also one of the major factors reducing plant growth and productivity (Musyimi, 2007). Three major hazards associated with salinity are: osmotic stress, ion toxicity and mineral deficiencies (Hasegawa et al., 2000). Avocado is known also to be the most salt-sensitive of the cultivated fruit trees (Maas, 1986). Even low levels of salt inhibit the vegetative growth of the avocado shoot (Bernstein et al., 2001; Mickelbart and Arpaia, 2002).

Salt damage in avocado orchards has been reported in Spain (Moreno-Ortega et al., 2019), California (Branson and Gustafson, 1972), Australia (Downton, 1978), and Israel (Ben-Ya'acov et al., 2009). Strategies for handling salinity usually aim at preventing the build-up of salts in the root zone, while studies on the response of avocado trees to saline water have shown that it is highly influenced by rootstock selection (Mickelbart and Arpaia, 2002; Kourgialas and Dokou, 2021; Lazare al., 2021). The maximum yields are not achievable when irrigated with waters that have an average annual salinity greater than 0.6 dSm^{-1} because of the unusually high sensitivity of 'Hass' avocado on Mexican seedling rootstocks to salinity (Oster et al., 2007). The data obtained show the combined effects of salinity and low photosynthetic active radiation (PAR) irradiance may contribute to reduced photosynthetic rate (Musyimi et al., 2007). Differences in sensitivity to salinity among cultivars were reflected in different growth reductions and leaf necroses among cultivars in response to elevated salt concentrations in the rhizosphere rather than by photosynthetic responses (Schaffer and Whiley, 2003). Salinity may interfere with mineral nutrition acquisition by plants in the total ionic strength of the soil solution and uptake competition with specific ions such as sodium and chloride can inhibit of NO_3 uptake (Xu et al., 2000). Chloride is actively taken up and accumulated to macronutrient levels in higher plants, leading to adaptive functions that improve growth and water relations, acting as a beneficial macronutrient (Franco-Navarro et al., 2016). Introduction of nitrate to the irrigation water reduced chloride accumulation in avocado plant and alleviated its adverse effects (Salvo and Lovatt, 2016). Availability of good quality water for irrigated agriculture is significantly affected by climate change and increased urban and agricultural demands on fresh water supplies (Boretti and Rosa, 2019). Extended periods of drought further exacerbate the already dwindling stocks and flows of existing ground and surface water (Clifton et al., 2010). Farmers can respond to decreased water availability and quality by adopting various technologies and water conservation management practices that have short- and long-term impacts, changing irrigation patterns, using various monitoring and irrigation planning programs (Reints et al., 2020; Beya-Marshall et al., 2022). The quality of the water derived from reclaimed wastewater for irrigated agriculture depends on the initial water source, compounds that reach the water treatment plant from domestic and industrial users, treatment grade and technology and post-treatment dilution (Pedrero et al., 2009). It is necessary to analyze its viability including the presence of salts and other compounds in order to optimize the management of reclaimed wastewater for long-term irrigation that can affect both the structure of the soil and plants (Assouline and Narkis, 2013; Assouline et al., 2016). In the case of fruit tree crops, most of the work in Spain has been performed in citrus (Cerezo et al., 2000).

The objective of the present study was to investigate the physiological changes depending on three kinds of irrigation to determine the capabilities of avocado rootstock to tolerate salt stress.

2. Materials and Methods

A pot experiment with Avocado rootstocks was equipped with lysimeters in the facilites of the «La Mayora Experimental Field site» of the Higher Council for Scientific Research in the Malaga province (Spain). An evaluation of physiological reactions of the avocado rootstock leaves was made after 3.5 month of irrigation with three kinds of irrigation with different water salinity level. The scheme of the pot experiment included three sources of irrigation: a) groundwater (GW); b) regenerated wastewater (RWW); c) a mix of ground and regenerated municipal wastewater (GW/RWW-1:1). All substrata were taken in local alluvial deposits. Technosol texture is characterized mainly as silt. Each trial includes 16 vessels. The total number of vessels – 48. The volume of the vessel was 50 liters. The irrigation frequency was two times per a week.

The pH of both sources was estimated as doubtful for irrigation. The electric conductivity (EC) of regenerated wastewater is 2.3 times more than groundwater (Gonzalez et al., 2004). There is an excess of sodium and chloride in wastewater compared to groundwater at 6.5 and 7.4 times respectively. Leaf burn symptoms caused with high chloride level in regenerated waste water were estimated following four grades: none, slight, moderate and severe (Bingham and Fenn, 1966; Acosta-Rangel et al, 2019). Electrolyte leakage and cell membrane stability were determined in order to determine the injury due to water stress at a cellular level on the leaves of the plant (Kamiloğlu et al., 2014). The yield of electrolytes (P) was calculated as a percentage of the total yield using the formula:

$$P = \frac{EC_1}{EC_2} \cdot 100\%,$$

where: EC – electrical conductivity after infusion, *mcCm*.

Electrolyte leakage on leaves and roots was calculated as EC_1 / EC_2 and stated as %, where: EC – electrical conductivity after infusion, mcCm.

Cell membrane stability was calculated according to the following formula using the same data and state as cell membrane injury:

Cell Membrane Injury Ratio (%) = $[1-(1-EC_1/EC_2)/(1-EC^*_1/EC^*_2)*100]$,

where EC^* is electrical conductivity of control samples.

The leaf area was measured using three randomly selected leaves from each plant (Gonzalez et al., 2023). The concentrations of chlorophyll a, chlorophyll b and carotenoids were measured at the spectrophotometer (Brunold et al., 1996). Extinction was measured at 663, 646 and 470 nm (E663, E646, E470) of electromagnetic spectrum using the spectrophotometer V1100D (China). The spectroradiometer 'FieldSpec 3.1' was used as the prime hardware tool to gather spectral data from avocado leaf blades. The spectroradiometer was calibrated with a standard white reference panel (Analytical Spectral Devices Inc., Boulder, CO, USA) to determine the reflectance reference standard. The collected spectral observations were grouped into four main distinctive wavelength ranges: (i) visible (400 to 700 nm), (ii) near-infrared (NIR: 700 to 1300 nm), (iii) shortwave infrared 1 (SWIR-1: 1300 to 1900 nm) and (iv) shortwave infrared 2 (SWIR-2:1900 to 2300 nm), in addition to the Red-Edge (680-780 nm) component (Tola et al, 2023). The statistic assessment of the pot experiment data was made using Statistica 6 soft.

3. Results

The data on pH and electric conductivity of the two kinds of irrigation water are shown in the Table 1.

Table 1 pH and electric conductivity of leachates

Source	pH	EC, ds/m
GW	8.99±0.18	3.39±0.99
GW/RWW 1:1	8.4±0.38	7.22±2.59
RWW	8.75±0.26	7.75±2.2

The ground water salinity level is more than 2 times low comparative with GW/RWW 1:1 and RWW treatments. It was established that electric conductivity of waste water 2.3 times more than ground water.

The data on leaf burn injury depending on irrigation treatment of avocado in pot experiment are shown in the Figure 1.

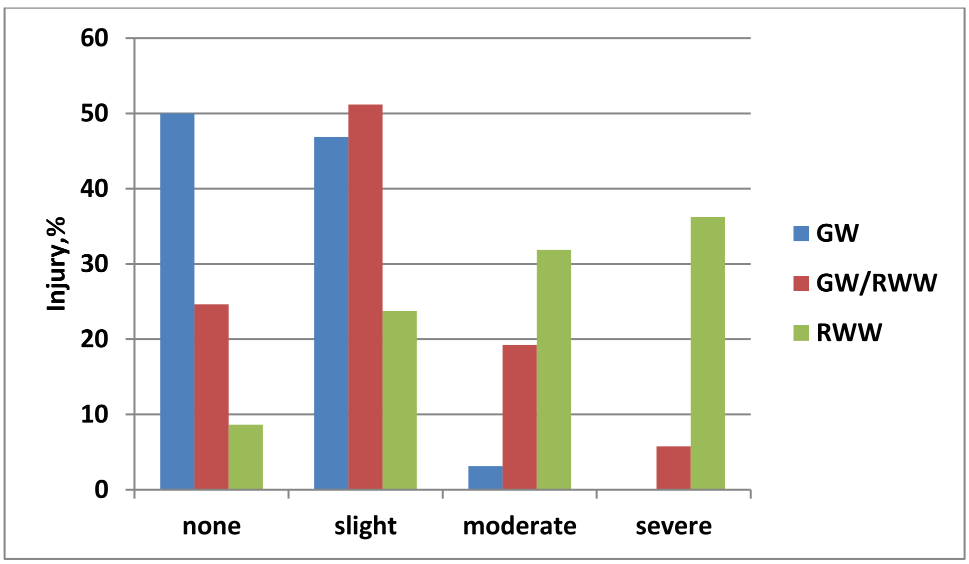

Figure 1 Leaf burn injury estimation of avocado in pot experiment

50 % of regenerated water with ground water dissolution led to decreasing of leaf burn injury to slight level to 52 % cases comparative with 100% regenerated water treatment.

The data on leaf square in pot experiment with avocado rootstock are shown at the Figure 2.

Figure 2 Leaf square in pot experiment with avocado

The data obtained tell us that avocado leaves growth depression fixed most of all in the 100% treatment. The data on electrolyte leakage and cell membrane stability were determined in order to determine the injury due to water stress at a cellular level on the leaves of the avocado seedlings (Figure 3 and 4).

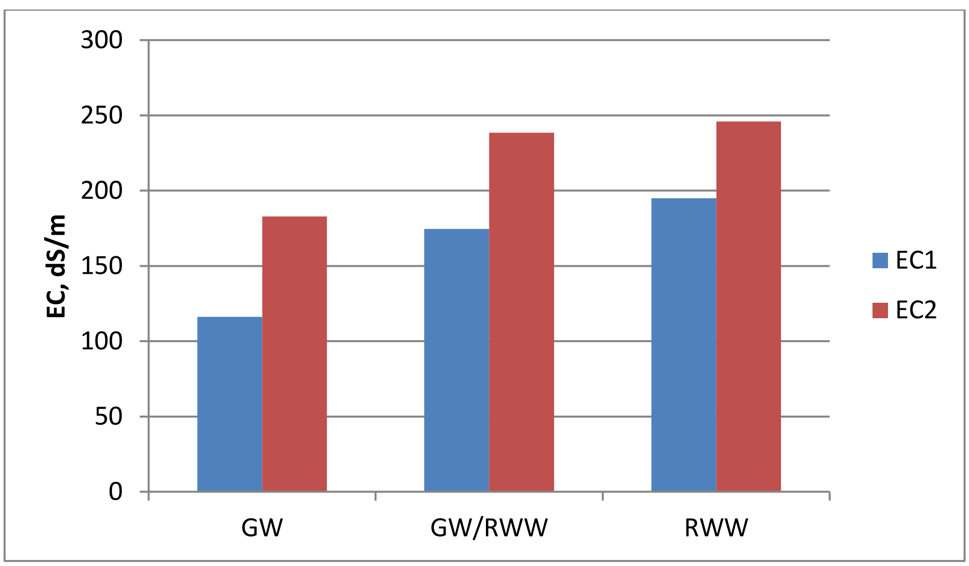

Figure 3 Electrical conductivity of leaves leakage, dS/m

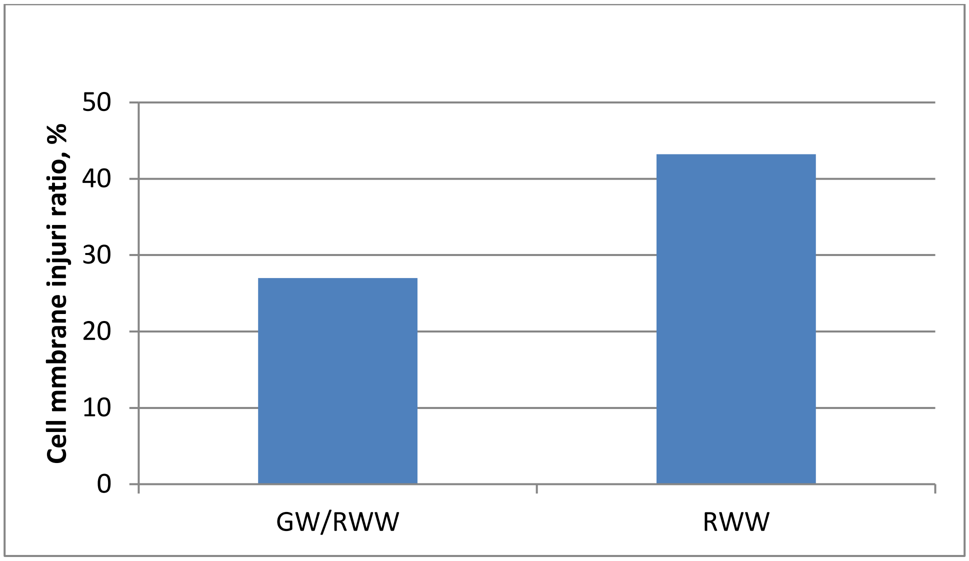

Figure 4 Cell Membrane Injury Ratio

The data on pigments (chlorophyll a, chlorophyll b and carotenoids) content in the leaves tissue are shown in Figure 5.

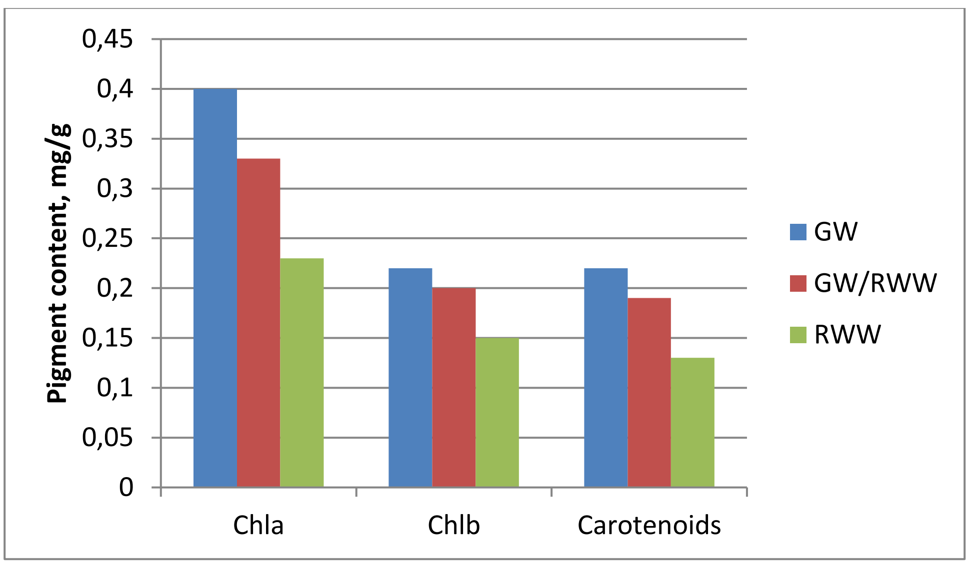

Figure 5 Pigments content in the Avocado leaves

It is obvious that lowest level of three pigments is in the RWW treatment with 100 % regenerated water. 50 % level using of mix consisted of ground and regenerated water (1:1) take intermediate indexes.

Spectral signatures of avocado leaves at whole and grouped into four main distinctive wavelength ranges are shown in the Figure 6 and 7.

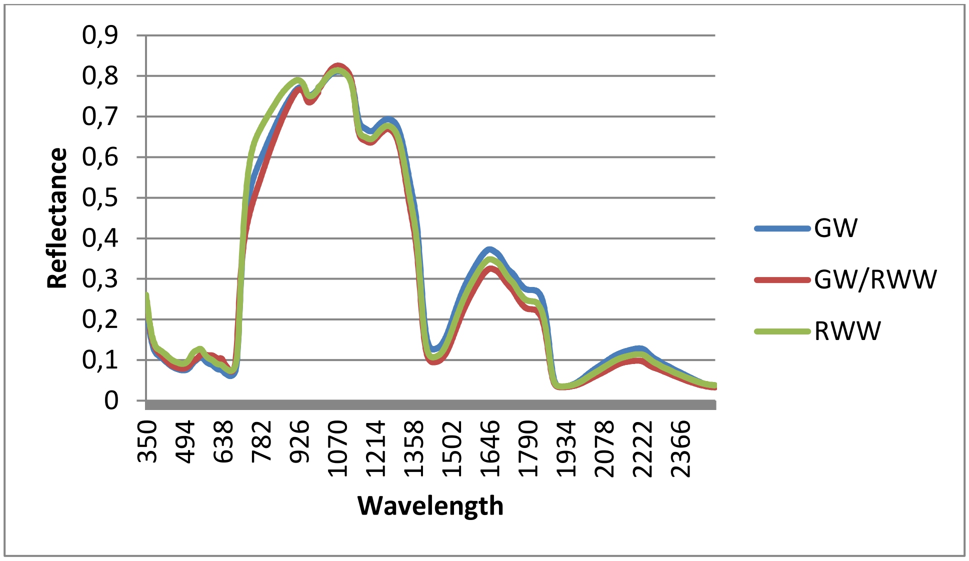

Figure 6 Spectral reflectance curve

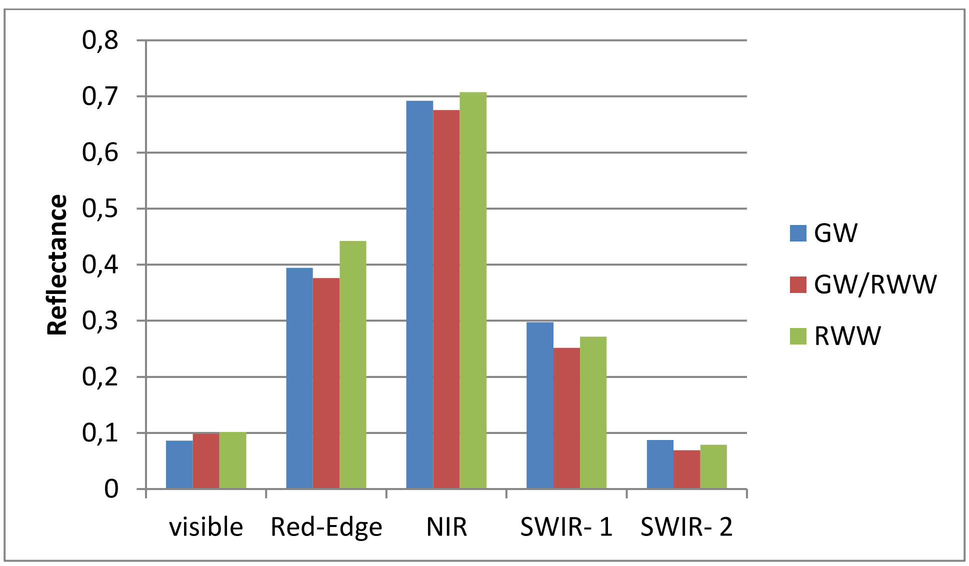

Figure7 Spectral response of avocado rootstocks on salinity

Visible range is connected with chlorophyll adsorption, NIR – with reflectance because of spongy mesophyll, shortwave NIR (SWIR-1 and SWIR-2) – with moister absorption (Tayade et al., 2022). Water and nutrient contents affect reflectance in the Red-Edge range (Katsoulas et al., 2016). Increase in salinity level of GWW/RWW and RWW treatments is connected with their high the spectral reflectance in the Visible region. Similar situation was fixed for RWW option in Red-Edge and NIR wavelength ranges. Highest reflectance level was noted in ground water option in SWIR-1 and SWIR-2 regions.

4. Conclusion

The ground water salinity level is 2 times less comparative with GW/RWW 1:1 and RWW treatments. It was established that electric conductivity of waste water 2.3 times comparative with ground water. 50 % of regenerated water with ground water dissolution led to decreasing of leaf burn injury to slight level to 52 % cases comparative with 100 % regenerated water treatment. The avocado leaf blade area decreased by the end of observations in the GW/RWW-1:1 and RWW treatments by 1.2 and 1.6 times compared to the groundwater option. Cell Membrane Injury Ratio in the GW/RWW-1:1 and RWW treatments are 27 and 43% correspondingly. Lowest level of chlorophyll a, chlorophyll b and carotioids were in the RWW treatment. Increase in salinity level of GWW/RWW and RWW treatments is connected with their high the spectral reflectance in the visible region connected with chlorophyll absorption. Similar situation was fixed for RWW option in Red-Edge and NIR wavelength ranges because of spongy mesophyll. Highest reflectance level was noted in ground water option in SWIR-1 and SWIR-2 regions connected with moister absorption. We will conduct soil leachate control combined with phytomonitoring during next months. It would be interesting also to compare mitigation effect in three experiment options from several alternatives in the future: a) an additional proportional injection of ammonium and nitrates forms and b) using salt-tolerant avocado rootstocks.

Acknowledgment

This study was funded by the TED - 2021-129664B - C22 research project coordinated with the Mayora - CSIC (Ministerio de Ciencia e Innovación, Spain), the research grants applications D.3 "Stays for distinguishhed researchers at the University of Malaga" within the framework of UMA Research Plan for the invitation of Ukrainian Professors and Researchers.

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ARTICLE HISTORY:
Received 10.05.2024
Accepted 30.05.2024

PHYSIOLOGICAL RESPONSES OF MAPLE AND ROBINIA TREE SEEDS UNDER TECHNOGENIC STRESS

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Abstract

The changes in physiological and biochemical parameters of ripening and mature seeds of Black locust and Norman maple in the technogenically polluted environment were analysed. Changes in the quantitative content and qualitative composition of easily soluble proteins were established. Peroxidase activity of seeds of woody plants under conditions of motor transport emissions was investigated

Keywords: Motor transport emissions, easily soluble seed proteins, peroxidase, *Acer platanoides, Robinia pseudoacacia*.

1. Introduction

In recent decades, a new hazard has been added to the many threats to the functioning of green spaces - industrial pollution. The most widespread anthropogenic pollutants include gases such as sulphur, nitrogen, fluorine, etc., as well as solid and liquid aerosols of various origins, including products of incomplete combustion of fuel, waste from ferrous and non-ferrous metal smelting, cement plant emissions, and products of transformation of primary pollutants.

Vehicles are one of the most visible and widespread components of human life, which can seriously affect ecosystems (Patrick et al., 2024). In the Dnipro region, the length of paved roads is more than 8300 km, and vehicle emissions into the atmosphere exceed 60,500 tonnes of harmful substances (Shupranova et al., 2019). In the conditions of urban ecosystems, certain chemical elements accumulate, which disrupts the interrelations and natural biogeochemical cycle of substances. As a result of these processes, urban ecosystems are characterised by reduced biodiversity, disrupted connections between components, and deterioration of the functional state of the biota (Lovynska et al., 2022).

Woody plants are involved in the accumulation and migration of chemical elements and also serve as a biological barrier to their distribution in the biosphere (Robinson et al., 2005; Nong et al., 2023). Environmental pollution has a negative impact on the vital activity and biological productivity of all living organisms, including woody plants, which allows them to be used as bioindicators of the environment. Many researchers have obtained a significant number of experimental data on the impact of vehicle emissions and certain environmental pollutants on the vegetative organs of various plant species (Leisner et al., 2023; Lobo et al., 2022). However, there is virtually no research on the impact of pollutants on the reproductive organs of plants, in particular, seeds. Successful renewal of green spaces is possible only with the use of high-quality seeds with high economically valuable hereditary properties, and therefore the study of changes in the physiological and biochemical parameters of generative organs is one of the most pressing issues of our time.

Proteins are synthesised during the development of plants, their heterogeneous composition does not depend on the conditions of plant cultivation and is determined by the genotype and is genetically fixed in a number of generations (Murphy, Dalby, 1971). This makes the use of protein markers a convenient object in the informational and practical use of protein markers. Simplicity and availability of electrophoretic separation of proteins and detection of protein, including native enzymes, provide researchers here with a modern and accurate tool.

The main objective of this case study was the study of changes in protein metabolism and antioxidant system of seeds of *Acer platanoides* and *Robinia pseudoacacia* species growing in conditions of technogenically polluted areas.

2. Material and methods

The study area is located in the temperate, continental climate zone of Central Europe, within Steppe zone of the Ukraine and is characterised by warm, often hot summers and cold, wet winters. The average annual precipitation in the region is approximately 480 mm, and the average annual air temperature is +9 °C. The growing season for plants is about 220 days. The objects of research were seeds of *Acer platanoides* L. (Norway maple, native species) and *Robinia* *pseudoacacia* L. (Black locust, introduced) collected at monitoring plots in Petrykivka village (conditionally clean zone – control) and at certain points of the motorway of the main street of Dnipro city (D. Yavornytskyi Avenue – experiment). Plant seed samples were collected by random selection from 5-10 specimens of *R. pseudoacacia* and *A. platanoides* trees. To study the changes in physiological and biochemical parameters of the experimental objects in the process of maturation, seeds were collected in August. In October, ripe seeds were collected for the study.

The protein content in seeds was determined by the Bradford method (Bradford et al., 1976), which is based on the formation of coloured protein complexes with a solution of Coomassie G-250 solution. The protein concentration in the samples was determined with using method described by some authors (Laemmli et al., 1970). Peroxidase activity was determined by the Boyarkin method (Asada, 1992). The content of heavy metals was determined by the atomic absorption method at the Department of Product Quality and Radiological and Toxicological Research of the State Research and Development Centre 'Oblderzhrodyuchist'.

3. Results and discussion

Proteins are one of the key components that maintain cellular homeostasis under stressful conditions and ensure the formation of a plant resistance system to various environmental factors (Wang et al., 2004), so it was advisable to study changes in protein accumulation by cotyledons of plant seeds under the influence of vehicle emissions. The results of the assessment of protein content in woody plant seeds are presented in Table 1.

Table 1 Soluble protein content of *A. platanoides* and *R. pseudoacacia* seeds under the influence of vehicle emissions

Type of seed	Protein content, mg/g of dry matter	Month of the selection	Protein content, mg/g of dry matter	% to control	t-test
A. platanoides					
Control		Experiment			
ripening	2.5 ± 0.05*	ripening	2.1 ± 0.04*	84	6.84
mature	3.2 ± 0.07*	mature	2.6 ± 0.08*	81	5.64
R. pseudoacacia					
ripening	1.9 ± 0.05*	ripening	1.6 ± 0.05*	82	2.91
mature	2.9 ± 0.02*	mature	2.8 ± 0.01*	98	4.47

*statistically significantly different at α = 5%

As can be seen from the presented data, both ripening and ripe seeds of *A. platanoides* and *R. pseudoacacia* show a decrease in the total protein content by a maximum of 19 % (ripe seeds of *A. platanoides*). The decrease in the total content of easily soluble proteins in plant seeds under stress may be due to increased protease activity (Hellinger, Gruber, 2019; Vorster et al., 2023).

Electrophoresis of easily soluble seed proteins revealed changes in the qualitative composition of the studied plants under anthropogenic stress. When comparing the results of the analyses, there were clear differences in the sets of protein components of seeds collected from *A. platanoides* and *R. pseudoacacia* plants in August and October (Table 2). In the ripening seeds of *A. platanoides*, the presence of polypeptides in the range of molecular mass (Mr) values from 14.5 to 47.9 kD (22 components in total) was recorded, while for ripe seeds a much wider range of individual proteins was determined from 12.6 to 60.3 kD (32 components).

Table 2 Fractional composition of soluble proteins of *A. platanoides* seeds under the influence of vehicle emissions

Value of M_r	Ripening seeds		Value of M_r	Mature seeds	
	control	experiment		control	experiment
47.9	10.83	3.58	60.3	52.17	–
46.8	0.42	0.31	58.9	44.66	–
45.8	11.37	0.82	57.6	–	44.37
44.7	2.22	–	53.7	0.25	41.89
43.7	10.16	1.56	52.5	26.39	27.78
42.7	3.03	–	50.2	1.98	–
41.7	–	1.26	49.0	36.95	28.56
40.8	16.94	3.69	47.9	6.40	7.82
39.9	12.42	–	46.8	–	28.21
38.1	9.69	1.97	44.7	0.42	10.16
37.2	–	9.43	43.7	40.19	–
36.4	–	6.69	42.7	22.84	–
35.5	14.54	5.67	41.7	6.88	0.43
34.7	3.50	–	38.1	23.68	–
33.2	6.39	–	36.4	19.18	–
31.7	9.54	–	35.5	0.93	–
29.6	12.45	10.25	34.7	–	1.10
28.2	21.61	0.98	33.9	0.82	1.39
23.5	0.08	27.05	32.4	6.01	–

22.4	29.72	14.54	31.7	–	1.09
15.2	–	2.58	28.2	0.09	2.19
14.5	6.94	40.36	27.6	1.22	3.49
			27.0	1.47	2.55
			25.2	0.60	–
			22.4	1.30	–
			21.9	2.13	–
			21.4	–	5.06
			20.9	0.11	–
			20.5	–	3.58
			13.9	5.02	–
			13.2	–	3.98
			12.6	–	3.41

The reaction of *A. platanoides* to the polluted environment was demonstrated by the disappearance of six protein components with Mr 44.7, 42.7, 39.9, 34.7, 33.2, 31.7 kD in the ripening seeds of the experimental variant, accompanied by the simultaneous appearance of four fractions with Mr 41.7, 37.2, 36.4 and 15.2 kD. In ripe seeds, a significant reduction in the fractional composition of proteins (by 14 components) was recorded with a subsequent accumulation of mainly low-molecular mass protein compounds (8 components in total), which may reflect the process of intensive decomposition of polypeptides with a higher Mr. A decrease in the amount of 47.9, 45.8, 43.7, 40.8, 38.1, 35.5, 28.2 and 22.4 kD proteins of ripening seeds was also found, while in ripe seeds, mainly the accumulation of protein components was observed - 53.7, 52.7, 47.9, 44.7, 33.9, 28.2, 27.6 and 27.0 kD compared to the control variant.

The data obtained from the study of protein metabolism in *R. pseudoacacia* seeds indicate that the identified protein components of ripening seeds are represented in 29 fractions (10.1-32.4 kD), and ripe seeds - in 26 (from 14.6 to 41.7 kD) (Table 3).

Table 3 Fractional composition of soluble proteins of *R. pseudoacacia* seeds under the influence of vehicle emissions

Value of M_r	Ripening seeds		Value of M_r	Mature seeds	
	control	experiment		контроль	control
32.4	10.86	–	41.7	–	0.75
31.7	0.74	3.26	39.9	–	2.75
23.5	–	10.01	38.1	8.07	27.58
22.4	–	2.85	37.2	8.17	–
21.4	–	3.50	35.5	12.67	–
20.9	2.73	–	33.9	12.59	–
20.0	–	2.87	33.2	–	36.79
19.5	–	25.45	30.9	2.31	0.56
18.7	7.55	–	29.6	–	9.49
18.2	–	9.25	28.2	–	0.54
17.8	6.39	–	27.6	3.15	–
17.4	16.02	–	25.2	–	2.19
17.0	–	13.57	24.0	0.20	6.05
16.6	–	5.81	23.5	2.21	1.15
16.3	–	5.50	22.4	5.54	4.77
15.5	18.93	–	20.0	0.34	–
15.3	3.82	–	19.5	3.59	19.07
14.8	–	3.06	19.1	–	3.84
14.5	7.01	3.80	18.7	21.80	3.53
13.9	17.03	–	18.2	–	2.41
13.5	1.42	–	17.4	–	0.81
13.2	5.59	–	17.0	9.66	–
12.9	2.27	2.78	16.6	3.45	7.73
12.1	45.07	–	16.3	3.60	–
11.8	2.41	2.33	14.6	–	0.15
11.0	2.27	3.11	14.5	4.05	1.18
10.5	099	–			
10.2	–	7.52			
10.1	–	14.39			

It should be noted that in the ripening seeds of *R. pseudoacacia*, the effect of stress factors led to the appearance/disappearance of new protein components in the same amount [12]. A significant redistribution of protein fractions was also characteristic of ripe *R. pseudoacacia* seeds with the formation of 10 and disappearance of 7 components compared to the control variant.

Apparently, intensive changes in the protein spectra of *R. pseudoacacia* indicate the formation of rapid adaptive changes that will increase the survival and life cycle of these plants in unfavourable conditions of technogenically polluted environment.

In general, the results of our studies have shown that changes in the quantitative and qualitative composition of proteins of *A. platanoides* and *R. pseudoacacia* seeds may indicate the participation of polypeptide components in the formation of adaptive changes in macromolecules in response to the stress factor (Van Damme et al., 1995).

One of the main intracellular inhibitors of free radical processes is the enzyme peroxidase, which plays a significant role in maintaining the plant organism in the restored state necessary for life. This enzyme reacts to a variety of factors, changing the set of isozymes or increasing the activity of existing molecular forms (Ali et al., 2021). Therefore, one of our tasks was to determine the enzymatic activity of peroxidase in the seeds of the plants under study (Figure 1).

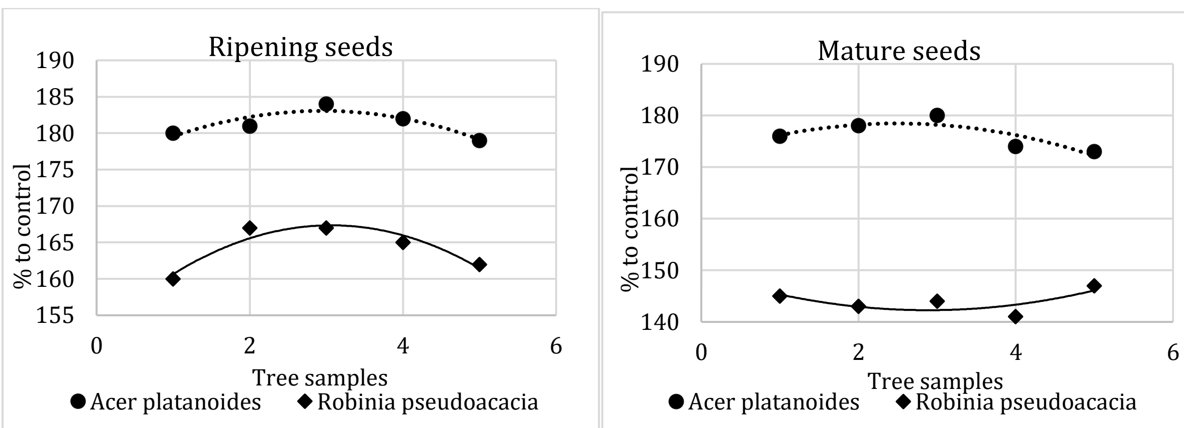

Figure 1 Peroxidase activity in ripening and mature seeds of separate samples of *A. platanoides* and *R. pseudoacacia* under the influence of vehicle emissions, % to control

As can be seen from the data presented here, due to the influence of vehicle emissions, there is a significant intensification of metabolic processes with an increase in peroxidase activity in *A. platanoides* by 87 % (ripening seeds) and 86 % (mature seeds) and in *R. pseudoacacia* by 48 % and 68 %, respectively.

This fact may indicate the inclusion of plant adaptogenesis mechanisms to the technogenically polluted environment. The detected changes in the activity of peroxidases in both species of plants under the influence of vehicle emissions are one of the nonspecific responses of plants to a stress factor (Caverzan et al., 2012).

The results of our research indicate species-specific features of the response of seeds of the studied plants to anthropogenic load and can serve as a basis for further study of the development of adaptive mechanisms of plants of the genus *Acer* and *Robinia* to the influence of other stress factors.

4. Conclusion

Under conditions of anthropogenic stress, the content of easily soluble protein of seeds of both analysed plant species decreased, more intensively in *A. platanoides* plants. Electrophoretic analysis of proteins of *A. platanoides* and *R. pseudoacacia* seeds under the influence of stress factors revealed changes in the content and ratio of protein fractions, which were manifested in the appearance/disappearance of individual polypeptide components. Under the influence of vehicle emission ingredients, the increase in the level of peroxidase activity in the studied plants depends mainly on the plant species and is more intense in the seeds of *A. platanoides*.

The plants of *R. pseudoacacia* were more resistant to the influence of stress factors in terms of changes in protein metabolism and activity of the antioxidant enzyme peroxidase.

Acknowledgment

This work was supported by the Ministry of Education and Sciences of Ukraine.

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ARTICLE HISTORY:
Received 17.05.2024
Accepted 26.05.2024

MONITORING PLANT NITROGEN STATUS USING THE NORMALIZED DIFFERENCE GREENNESS INDEX

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Abstract

Efficient nitrogen management is essential for sustainable agriculture, minimizing environmental impacts while maximizing crop productivity. This study investigates the use of the N-Pen device, which utilizes the Normalized Difference Greenness Index (NDGI) to monitor plant nitrogen status in real-time. Capsicum annuum RADMILA F1 plants were subjected to varying nitrogen fertilization levels in a controlled indoor environment. NDGI values and plant weight were recorded before and after fertilization. Results indicated increase in NDGI values for fertilized plants, with Plant 1 (49.8 g NPK) and Plant 2 (24.9 g NPK) showing 31.6 % and 26.7 % improvements, respectively. Unfertilized plants displayed smaller NDGI increments. While weight changes were observed, they did not consistently correlate with NDGI variations. The findings underscore the potential of the N-Pen device for effective nitrogen management, promoting optimal fertilizer usage and environmental sustainability. Future research should expand sample sizes and parameters to enhance the precision and applicability of these results in diverse agricultural settings.

Keywords: Nitrogen, NDGI, Irrigation

1. Introduction

Nitrogen, a pivotal macronutrient, is crucial for plant growth and productivity, influencing processes such as photosynthesis, energy transfer, and biomass production (Zhang et al., 2020). Plants predominantly absorb nitrogen in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), which are often scarce in both agricultural and natural ecosystems. This scarcity drives the extensive application of nitrogen fertilizers, which, while boosting plant growth, often leads to inefficiencies in usage with significant environmental repercussions, such as soil acidification and water eutrophication. Additionally, nitrogen mismanagement can lead to substantial leaching, where a significant portion of nitrogen ends up contaminating groundwater, thus affecting ecosystem health and water quality. The genus *Capsicum*, known for its significant agricultural and economic role, showcases a wide range of fruit characteristics influenced by nitrogen availability. Given the critical role of nitrogen and the challenges associated with its management, there is a pressing need for tools that can efficiently monitor and manage plant nitrogen levels to optimize fertilizer use and minimize environmental impact. The N-Pen device, employing the Normalized Difference Greenness Index (NDGI), represents a technological advancement in this area. It facilitates non-destructive, real-time monitoring of nitrogen status in plants, offering a promising solution for sustainable agricultural practices. This study aims to evaluate the efficacy of the N-Pen in capturing changes in NDGI values in response to nitrogen fertilization and assess its potential to improve nitrogen management in controlled irrigation settings, using *Capsicum annuum RADMILA F1* as a model system.

1.1. Capsicum annuum

Capsicum annuum, encompassing a diverse array of flavors and varieties, retains substantial consumption rates and economic importance. It is extensively cultivated globally, primarily as a culinary spice and a key component in numerous international dishes. Additionally, *Capsicum annuum* is utilized in various pharmaceuticals, cosmetics, natural dyes, and ornamental applications (Kim et al., 2014). *Capsicum*, originally indigenous to Mexico, was first domesticated around 8000 BC. Currently, there are more than 30 varieties of *Capsicum*, which exhibit a wide range of fruit sizes, shapes, colors, and flavors (Mendes et al., 2020). *C. annuum* has evolved to become a critically important spice and vegetable crop worldwide (Hill et al., 2013). The genus *Capsicum*, part of the *Solanaceae* family, is native to regions spanning southern North America to northern South America. This family also comprises significant crops such as *Solanum tuberosum* (potato), *Solanum lycopersicum* (tomato), *Nicotiana tabacum* (tobacco), *Solanum melongena* (eggplant), and ornamental species like *Petunia sp.* The number of species within the *Capsicum* genus varies by source, ranging from 25 to 36. Of these, only five species have been domesticated: *C. annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens*, each native to distinct geographic regions in the Americas (Perry, 2007; Hayano, 2016). Fruits from different *Capsicum annuum* cultivars exhibit considerable morphological diversity in terms of shape, size, and pungency, which ranges from sweet to intensely hot (Melendez, 2009). These peppers are rich in beneficial compounds such as carotenoids, which contribute to their vibrant colors, as well as vitamins E, C, B complex, and provitamin A, which have health-promoting properties. Additionally, they contain polyphenols like flavonoids and cinnamic acid derivatives, which possess anticancer activities, along with essential minerals and capsaicinoids, the alkaloid compounds responsible for their spiciness (Wahyuni et al., 2013).

1.2. Nitrogen in plants production

Nitrogen, a critical macronutrient, plays a pivotal role in plant productivity (Chen et al., 2020). Plants primarily absorb nitrogen in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), though both are often limited in supply within agricultural and natural ecosystems. To meet global food requirements, over 110 teragrams of nitrogen fertilizer are applied to crops annually, leading to increasing demand for agricultural nitrogen (Schroeder et al., 2013). However, the overuse and mismanagement of nitrogen fertilizers result in only 30-50 % utilization by crops, with the remainder contributing to environmental issues such as soil acidification and water eutrophication (Ye, 2022; Kissel, 2020). Nitrogen is an essential nutrient for plants, integral to processes like photosynthesis, energy transfer, structural development, and biomass production (Xu et al., 2012). Nitrogen is uptaken from the soil by plants, where it circulates through the vascular system, integrates into organic compounds, and is then cycled back to adjust nutrient balances (Xu et al., 2012). This process is controlled by a complex gene network influencing key physiological pathways, including carbon metabolism. Remote sensing technology, a non-invasive method, is highly effective in assessing Nitrogen Use Efficiency by capturing spectral data that reflects plant interactions with solar radiation (Thenkabail et al., 2011). Innovations in optical technologies and devices like the SPAD-502 chlorophyll meter and the N-pen nitrogen meter facilitate accurate measurement of leaf pigment levels through reflectance data.

1.3. Nitrogen Leaching

Nitrate leaching is a primary contributor to the increasing nitrate levels in groundwater, directly affecting ecosystem eutrophication and water quality degradation. The soil acts as the primary source of nitrate leaching, with total nitrogen input serving as a strong predictor of leaching intensity. The concept of a fertilizer-induced emission factor has been widely adopted by entities including the Intergovernmental Panel on Climate Change to estimate nitrate losses from fertilized fields, typically assuming that 30 % of applied nitrogen is lost through leaching. Recent statistical modeling and integrated data analysis support the consistency of this global EF (Wang et al., 2019). Nitrate leaching from agricultural soils can result in significant losses of fertilizer nitrogen, although reports indicate considerable variability in these losses (Riley et al., 2001).

1.4. Imaging spectroscopy

The application of imaging spectroscopy to plant phenotyping stems from research aimed at non-invasive and non-destructive observation of vegetation. Plant imaging spectroscopy exploits the interactions of solar radiation produced by the plant. In the visible spectrum (400-700 nm), the reflectance of an ordinary leaf or even entire stands is very low. This low reflectance is due to the absorptivity of leaf pigments, primarily chlorophyll which has a characteristic peak reflectance in the green spectrum (approximately 550 nm). When moving from visible to infrared wavelengths, increased reflectance values can be observed. In the infrared spectrum (700-1200), much of the radiation 42 is reflected by the leaf mesophyll and can pass through the upper leaves of the stand to the lower leaves, allowing the photons to be reflected into the upper parts of the stand. As a result of this phenomenon, leaf structure, distribution, leaf thickness and growth manifestations are the main determinants of reflectance in this part of the spectrum. By increasing wavelengths up to 2500nm, reflectance decreases due to increased absorption by water present in the leaves (Berger, 2010). The spectral reflectance information from leaves or stands is further used in the construction of vegetation indices, which are simple operations on reflectance data at specific wavelengths. These operations led to the discovery of the normalized differential vegetation index and a wide range of other related indices that are linked to certain plant characteristics such as photosynthetic active biomass, pigment content, or plant water status (Li, 2014). Infrared spectroscopy and spectral reflectance are thus dependent on the development of calibration models that are related to the spectral information and reference data of the traits in question. Most often, a subset of the complete dataset representing the population (in terms of the range of spectral variation) is selected and used to calibrate the algorithms in the development of predictive models. Once completed, the calibration models are used to predict phenotypic values from external data obtained by spectral scanning. The measurements are performed using multispectral or hyperspectral cameras that can image the wavelengths of interest to us, at high resolution (Li, 2014). In plant phenotyping, hyperspectral reflectance indices are used in the rapid and non-destructive measurement of biomass, chlorophyll content of the stand, senescence of the leaves of the stand and water status of the plant (Mistele, 2008).

1.5. Normalized Difference Greenness Index

To identify the optimal wavelength for predicting nitrogen content, various experimental plant groups with differing nitrogen nutrition levels were analyzed using spectral reflectance measurements. Reflectance values across the spectrum were analyzed to correlate with nitrogen content in experimental plant groups, utilizing Pearson's correlation coefficient for statistical validation. The strongest correlations were observed in the wavelength ranges of 530-630 nm and

700-720 nm (negative correlation), and in the near-infrared (NIR) region of 750-900 nm (positive correlation). Notably, indices derived from the green region (around 560 nm) demonstrated higher sensitivity to nitrogen and chlorophyll content compared to traditional indices like NDVI. The Normalized Difference Greenness Index (NDGI), calculated using leaf reflectance at 565 nm and 760 nm, showed a promising correlation with nitrogen content in barley at specific growth stages, suggesting its utility for assessing nitrogen status in crops (Photon System Instruments, 2021).

2. Material and methods

2.1. N-Pen

The PlantPen/N-Pen N 110 is a portable, battery-operated instrument that utilizes reflectance to assess plant nitrogen levels throughout their growth period. It links chlorophyll content closely with nitrogen levels for accurate measurements. The N-Pen includes a GPS module and is water-resistant. It features non-invasive, rapid testing capabilities with data stored internally and transferable via USB or Bluetooth. The accompanying FluorPen 1.1 software enhances data management through various analytical tools. The N-Pen N 110 measures leaf reflectance using dual LED sources at 565 nm and 760 nm to calculate the NDGI index and estimate nitrogen content in plant dry matter or postharvest grain. (Photon System Instruments, 2021).

2.2. Experiment design

The plants were grown indoors at a controlled temperature of between 20 and 25 °C and humidity of approximately 60 %. During cultivation, we used LED lighting from MARS HYDRO to simulate daylight. The lighting was set for a 13-h photoperiod. The weight of the experimental pots together with the soil was measured separately for each plant before starting the measurements. A kern PCB10000 analytical balance was used to monitor weight changes. We used PSI's N-pen device to capture NDGI values. Continuous data collection was conducted at daily intervals from April 10 2024, to April 26, 2024. Measurements were taken in two phases namely at 9:00 a.m. and 12:00 p.m. Irrigation was done manually at the rate of 400 ml on 10.4, and at the rate of 50 ml during the days 12.4, 16.4, 17.4, 19.4, 25.4 and 26.4. Measurements were carried out on 4 plants to which we delivered different doses of fertilizer on 22.4. The 1st plant received a full dose of 49.8 g of NPK fertilizer (7-4-6), the 2nd plant received ½ of the dose of the first plant, the 3rd and 4th plants were left without a dose of fertilizer.

3. Results and discussion

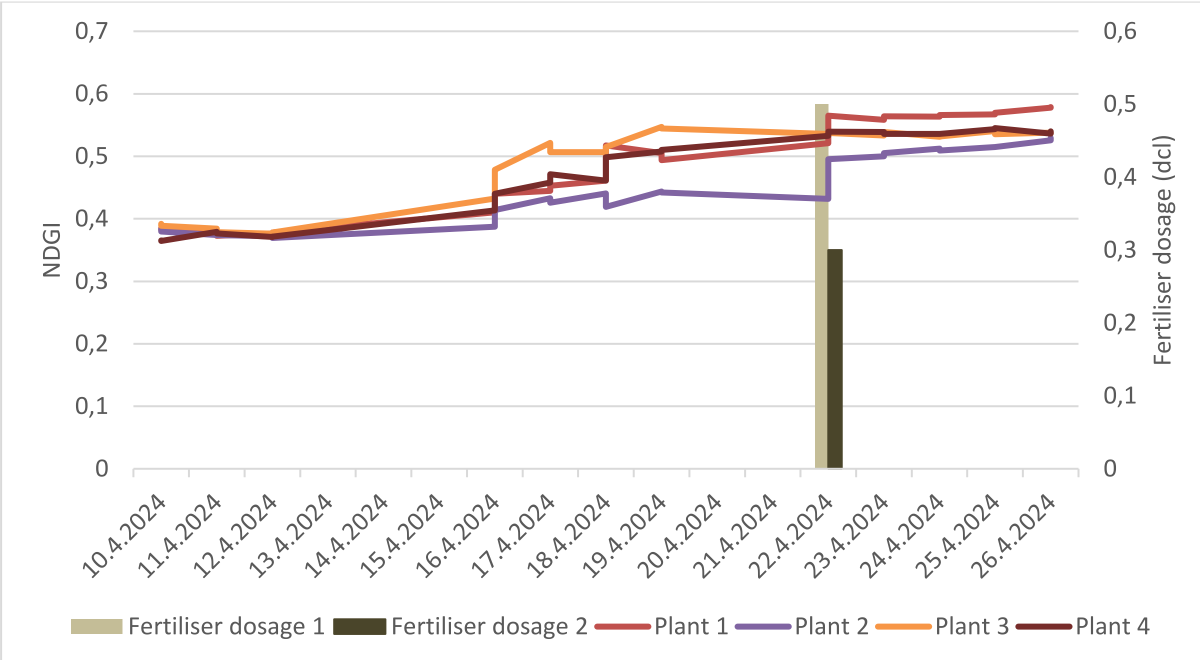

Figure 1 Graph of measured NDGI values compared with fertiliser dosage.

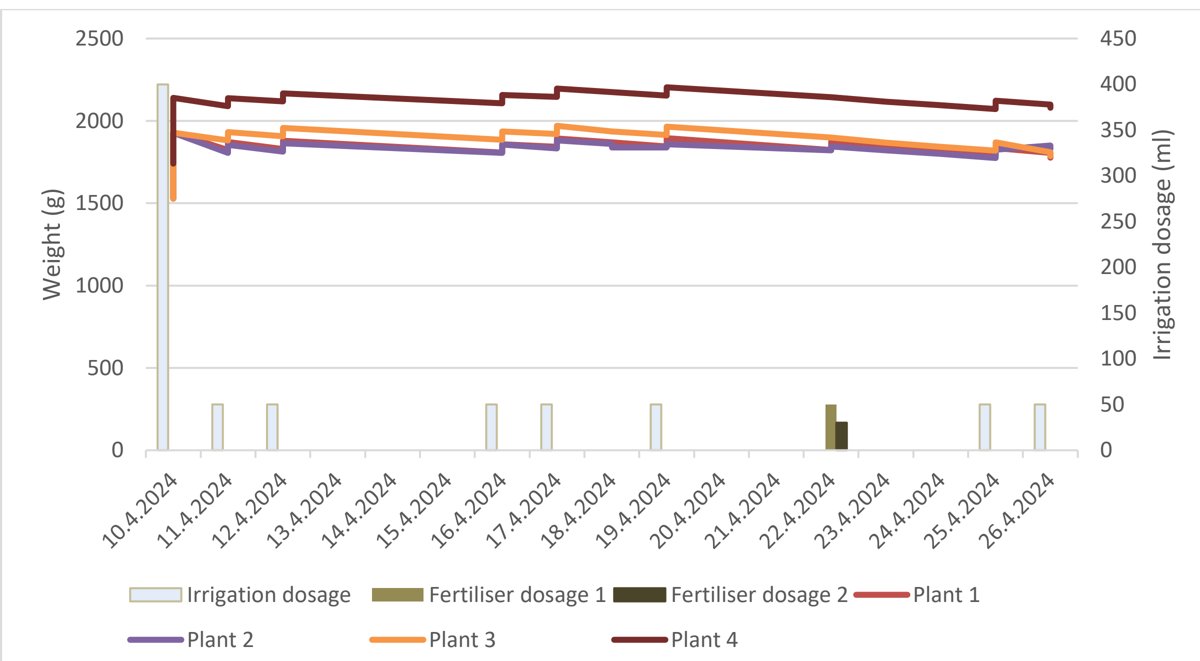

Figure 2 results of plant weight measurements, compared to irrigation and fertiliser rates.

The analysis of NDGI values before and after the application of NPK fertilizer revealed marked enhancements in nitrogen content and overall plant health in fertilized plants. Specifically, Plant 1, which received 49.8 g of NPK fertilizer, demonstrated an increase in its average NDGI value from 0.4317 to 0.5681, representing a 31.6 % improvement. Plant 2, which received 24.9 g of NPK fertilizer, showed an increase from 0.4057 to 0.5139, equating to a 26.7% enhancement. In contrast, the NDGI values of the unfertilized Plants 3 and 4 also increased during the same period but to a lesser extent, with increments of 16.9 % and 24.6 %, respectively. These comparative data may illustrate the impact of fertilizer application on NDGI values, indicating improvements in plant vigor attributable to fertilization. The analysis of plant weights in response to differential NPK fertilizer treatments demonstrated noticable growth enhancements in the fertilized plants. Specifically, Plant 1, receiving a 49.8 g dose, and Plant 2, receiving a 24.9 g dose of NPK fertilizer, exhibited noticeable increases in weight post-fertilization. Quantitatively, Plant 1 showed 16,09 % increase in weight, while Plant 2 exhibited a 19,69% increase. Plant 3's weight increased by 16.9 %, and Plant 4 saw a 19,49% increase over the same period.

4. Conclusion

This study evaluated the use of the N-Pen device to monitor nitrogen status in Capsicum annuum plants through the Normalized Difference Greenness Index (NDGI). The results showed increases in NDGI values following the application of NPK fertilizer. Plant 1, which received 49.8 g of NPK fertilizer, had an NDGI increase from 0.4317 to 0.5681 (31.6 %). Plant 2, which received 24.9 g of NPK fertilizer, showed an NDGI increase from 0.4057 to 0.5139 (26.7 %). In contrast, the unfertilized plants showed smaller NDGI increases of 16.9 % and 24.6 %. However, the weight measurements did not show a clear correlation with fertilization. Plant 1 and Plant 2 showed increases in weight by 16,09 % and 19,69 % respectively, while the unfertilized plants showed increases of 16.9 % and 19,49 %. This suggests that while NDGI could be a reliable indicator of nitrogen content, weight differences between fertilized and unfertilized groups were not as pronounced. The N-Pen device proved effective in capturing changes in NDGI related to nitrogen fertilization, indicating its potential for improving nitrogen management. Future studies should include a larger sample size and additional parameters to enhance the accuracy and applicability of these findings in agricultural practices.

Acknowledgment

This work was supported by the project of the Cultural and Educational Grant Agency VEGA-1/0300/22, Agency for Research and Development APVV-15-0562 and APVV-20-0071.

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ARTICLE HISTORY:
Received 24.05.2024
Received in revised form 29.05.2024
Accepted 30.5.2024

BIOCHAR APPLICATION AND NITROGEN FERTILIZATION: IMPACTS ON SOIL PROPERTIES AND SOIL CO_2 EMISSIONS

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Abstract

Increasing soil organic matter and improving soil quality are essential strategies for enhancing agronomical fertility and reducing greenhouse gas emissions, with biochar conversion of organic waste emerging as a promising environmentally friendly approach. This study examines the effects of initially applying biochar at rates of 0 $t.ha^{-1}$, 10 $t.ha^{-1}$, and 20 $t.ha^{-1}$, and reapplying biochar at rates of 0, 10, and 20 $t.ha^{-1}$, particularly when combined with varying amounts of nitrogen fertilizer (0, 40, and 80 $kg.ha^{-1}$). Conducted in the temperate climatic zone of Slovakia, the study focuses on soil characteristics and CO_2 emissions from Haplic Luvisol. Biochar had a positive impact on increasing soil temperature, water content, and soil organic carbon. A statistically significant ($p<0.05$) increase in soil pH was found in all N-fertilized treatments compared to the relevant controls. The content of NH_4^+ and NO_3^- generally increased in treatments without N-fertilization and decreased in treatments with the first and second level of N-fertilization compared to the relevant controls. Generally, the impact of biochar application on average daily CO_2 emissions decreased in treatments with the first and second levels of N-fertilization compared to the control variants. Integrating biochar into soil is crucial for agriculture and climate change mitigation.

Keywords: biochar, emissions of CO_2, N-fertilization, soil properties

1. Introduction

The use of fossil fuels in a variety of industries, including as transportation, agriculture, and electricity generation, is the main cause of greenhouse gas emissions (Waheed et al., 2018).

Significant carbon dioxide (CO_2) emissions resulted from this in basically every part of the world (Ben Jebli and Ben Youssef, 2017). Global CO_2 concentrations rose from 283 to 419 parts per million (ppm) over the past 220 years (NOAA, 2022). At 24 % of global GHG emissions, agriculture, forestry, and other land use (AFOLU) is the second-largest emitter (Waheed et al., 2018). Because to the rising concentrations of greenhouse gases (GHGs) in the atmosphere, the global temperature is getting closer to a point where it will have irreversible effects on Earth's future (IPCC, 2021). Due to the significant increase in greenhouse gas concentrations in the atmosphere, which is the cause of the ongoing global trend of climate change, significant investments in technology targeted at lowering GHG emissions and improving carbon sinks are required. To achieve the challenging targets for climate mitigation set forth in the Paris Agreement, this investment is essential (Bovsun et al., 2021). The agricultural sector is highly dependent on favorable climate conditions, such as temperature, rainfall, and the absence of floods. These climate factors significantly affect agricultural productivity, food supply, commodity prices, and overall economic stability (Waheed et al., 2018). The manufacture and application of biochar to fields is one of the most recent technologies studied in the agroforestry sector to promote sequestration of carbon in soil and plant biomass. In addition to improving carbon sequestration, biochar is a soil additive that frequently lowers greenhouse gas emissions from the soil (Bovsun et al., 2021). The pyrolysis process of biomass involves thermal decomposition at temperatures typically between 300 and 1000 °C under low oxygen concentrations. This thermal breakdown results in an organic substance known as biochar, which is rich in carbon (Domene et al., 2014). When applied as a soil amendment, biochar can help manage waste, enhance C sequestration, restore soil fertility, and immobilize contaminants (Jeffery et al., 2017). Biochar contains a high content of organic materials and a high surface area. Soil micro- and macroorganisms find it to be a suitable environment. Thus, by enhancing soil quality, it can raise soil biota (Lehmann et al., 2011). Biochar also influences the long-term impact of GHG emissions from the pedosphere and contributes to mitigating global warming (Mensah and Frimpong, 2018). Furthermore, biochar-amended soil aids nutrient uptake for adequate plant growth by supporting root growth, biomass, and the root-to-shoot ratio (Zhu et al., 2018). The purpose of this study was to evaluate the effects of applied and reapplied biochar on soil CO_2 emissions and soil physical (SWC, soil T) and chemical (pH, NH4+, NO3-, SOC) parameters in 2023, either alone or in conjunction with an N-fertilizer. Our specific goal was to look into the hypothesis (H1) that says that biochar enhances the chemical and physical characteristics of soil while reducing CO_2 emissions from arable soils.

2. Material and methods

2.1. Field site

The field study was set up in 2014 at the experimental site of the Slovak University of Agriculture in Dolná Malanta, near Nitra, Slovakia (48°19´N, 18°09´W). This location falls within the temperate climate zone, characterized by an average annual air temperature of 9.8°C and average annual rainfall of 539 mm (based on a 30-year climate normal from 1961 to 1990). In 2023, the mean air temperature and rainfall measured 8.7°C and 767 mm, respectively, for the year under study. Prior to the implementation of the field experiment, the agricultural practices at this site followed conventional methods for several decades. The soil classification, based on soil taxonomy, identified it as a silt loam Haplic Luvisol, with sand, silt, and clay content percentages of 15.2%, 59.9%, and 24.9% respectively. The average soil pH was 5.71, with a soil organic carbon (SOC) content of 9.13 $g.kg^{-1}$.

2.2. Experimetal design

The field experiment started in March 2014, during which biochar was applied at rates of 0, 10, and 20 $t.ha^{-1}$ (B0, B10, B20). This was followed by three levels of calcium ammonium nitrate (LAD 27) applied at different doses as mineral nitrogen fertilizer, designated as N0, N1, and N2. At the N0 level, no nitrogen fertilizer was applied. The N1 level was determined based on the crop's requirements using a balancing method. The N2 level consisted of 100% more nitrogen than the N1 level. Spring barley (*Hordeum vulgare* L.) variety LG OVERTURE was planted in March 2023 and harvested in July of the same year. In 2023, nitrogen fertilizer was applied at rates of 0, 40, and 80 kg $N.ha^{-1}$. In 2018, the original plots previously treated with biochar were divided into two sections (two 4 x 3 m subplots), and biochar was reapplied at the same rates as in 2014. Consequently, there were a total of 15 treatments with three replicates during the 2018 study period (27 previous plots plus 18 reapplication subplots, totaling 45 plots), as illustrated in Figure 1. The field experiment comprised 15 treatments replicated three times, as outlined in Table 1.

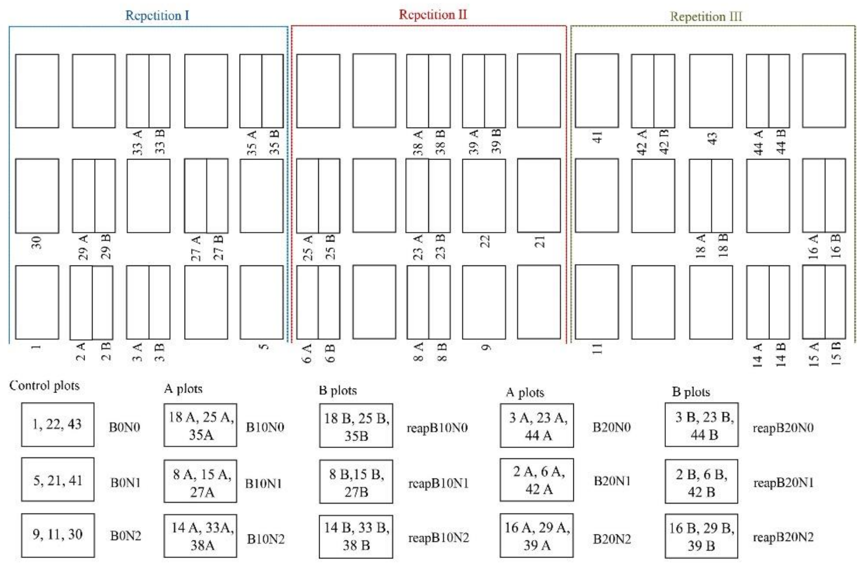

Figure 1 Schematic arrangement of the experimental field

Table 1 An overview of the field experiment's treatments, including the precise amounts of biochar and inorganic mineral nitrogen fertilizer used

Treatments	Biochar application in 2014 (t.ha^{-1})	Biochar reapplication in 2018 (t.ha^{-1})	N-fertilization application in 2023 (kg N.ha^{-1})
N0 level – unfertilized treatments			
B0N0	0	0	0
B10N0	10	0	0
B20N0	20	0	0
reapB10N0	10	10	0
reapB20N0	20	20	0
N1 level – fertilized treatments			
B0N1	0	0	40
B10N1	10	0	40
B20N1	20	0	40
reapB10N1	10	10	40
reapB20N1	20	20	40
N2 level – fertilized treatments			
B0N2	0	0	80
B10N2	10	0	80
B20N2	20	0	80
reapB10N2	10	10	80
reapB20N2	20	20	80

The 27 experimental plots, each measuring 4 meters by 6 meters, were arranged in a random layout, with 1.2-meter-wide access paths between the rows and 0.5-meter-wide protective strips separating them. The entire field underwent ploughing before the start of this experiment. Following this, the plots with different treatments were then localized, and biochar was applied and immediately incorporated into the soil at a depth of 0 to 0.1 meters. To produce the biochar used in the experiment, a mixture of paper fiber sludge and grain husks (in a 1:1 ratio, sourced from Sonnenerde, Austria) underwent pyrolysis at 550°C for 30 minutes in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). The biochar exhibited the following fundamental physical and chemical properties: pH of 8.8, bulk density of 0.21 $g.cm^{-3}$, surface area of 21.7 $m^2.g^{-1}$, ash content of 38.3 %, and carbon content of 53.1%.

2.3. Measurement of CO_2 emission from soil

Air samples were gathered from March to October in 2023 utilizing the closed chamber method (Elder & Lal, 2008). Within each treatment plot, a metal collar frame made of galvanized sheet was inserted 0.1 meters deep into the soil and remained undisturbed during soil management activities. These collars were removed during management operations and then replaced in their original positions. Removable PVC chambers, measuring 0.25 meters in height and 0.3 meters in diameter, were securely attached to the bottom collars during each sampling event, forming a water-tight seal. Gas samples were collected at two-week intervals using a 60 mL plastic syringe from 20 mL tube fittings sealed with a septum at 0, 30, and 60 minutes after chamber deployment. The collected gas was then transferred into 10 mL glass vials (Agilent). Gas samples were analyzed for CO_2 using a gas chromatograph (GC-8890 Agilent) equipped with a thermal conductivity detector (TCD). CO_2 emissions were calculated on a daily average basis and expressed in $kg.ha^{-1}.day^{-1}$. Cumulative CO_2 fluxes between each sampling day were interpolated to provide the total CO_2 fluxes from March to October in 2023, expressed in $t.ha^{-1}$.

2.4. Measurements of the chemical and physical soil properties

Soil samples were collected every two weeks from March to October 2023 to assess soil pH (determined potentiometrically in 1 M KCl at a KCl:soil ratio of 1:2.5 w/v) and the levels of inorganic nitrogen forms ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$). Soil $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ quantities in soil filtrates were measured using a spectrometer (WTW SPECTROFLEX 6100, Weilheim, Germany) with a calorimetric approach (Yuen and Pollard, 1954). Three disturbed soil samples were taken randomly from each plot at a depth of 0–0.1 m monthly from March to October. After mixing, representative soil samples were created for each plot. C_{org} content in samples collected in March and October was determined using the Tyurin wet oxidation method (Dziadowiec and Gonet, 1999). Disturbed soil samples were collected at a depth of 0–0.1 m during gas sampling events to estimate soil water content (SWC) using the gravimetric method. Soil temperature at a depth of 0.05 m was measured twice a month using a Volcraft DET3R thermometer.

2.5. Statistical analyses

The study evaluated the effects of biochar to CO_2 emissions, and certain chemical and physical soil properties by a one-way analysis of variance (ANOVA) using the Statgraphics Centurion program (XV v. 15.1.2) and the LSD test ($p<0.05$).

3. Results and discussion

3.1. Soil physical and chemical properties

Table 2 contains the results of soil physical and chemical properties, which varied according to the dose of added biochar and different levels of N-fertilization. Soil water content (SWC) in variants without N-fertilization increased from 1.12% to 3.61% compared to the control (B0N0) variant. Generally, the application of biochar in treatments with the first and second levels of N-fertilization increased SWC compared to the relevant control variants (B0N1, B0N2), respectively. In the first level of N-fertilization, SWC increased from 3.42% to 8.55% compared to the control (B0N1). In the second level of N-fertilization, SWC increased from 1.47% to 9.32% compared to the control (B0N2). In general, soil temperature increased in all treatments with or without N-fertilization compared to the relevant control variants (B0N0, B0N1 and B0N2). A study by Ali et al. (2020) showed that after the application of biochar at a rate of 60 $t.ha^{-1}$ in combination with N-fertilizer at 270 $kgN.ha^{-1}$, there was an average increase of 63.33% in soil water content. Soil temperature (Soil T) in treatments without N-fertilizer increased from 0.75% to 5.50% compared to the control (B0N0). In the first level of N-fertilization, the temperature increased from 0.71% to 4.17% compared to the control (B0N1). In the second level of N-fertilization, temperature increased from 7.65% to 12.65% compared to the control (B0N2). According to Wang et al. (2021), the application of 30 $t.ha^{-1}$ biochar and N-fertilization increased soil temperature by 91.25% at a depth of 0.1 m compared to the control.

Table 2 Soil physical and chemical properties

Treatments	SWC (% mass)	Soil T (°C)	$pH_{(KCl)}$	NH_4^+ ($mg.kg^{-1}$)	NO_3^- ($mg.kg^{-1}$)	C_{org} (Spring) ($g.kg^{-1}$)	C_{org} (Autumn) ($g.kg^{-1}$)
	n = 3	n =3	n = 3	n = 3	n = 3	n = 3	n = 3
			N0 level – unfertilized treatments				
B0N0	15.22 ± 0.86a	18.55 ± 0.99a	5.37 ± 0.13a	13.52 ± 2.42a	6.71 ± 1.23a	8.65 ± 2.92a	10.20 ± 1.39a
B10N0	15.05 ± 0.83a	18.69 ± 0.57a	5.43 ± 0.11a	15.39 ± 1.12ab	7.02 ± 0.65a	9.39 ± 0.70a	8.82 ± 1.29a
B20N0	15.62 ± 0.86a	18.69 ± 1.18a	5.65 ± 0.10c	14.96 ± 1.68ab	6.79 ± 1.03a	9.40 ± 2.87a	11.68 ± 0.44a
reapB10N0	15.05 ± 0.77a	19.57 ± 0.69a	5.53 ± 0.09b	16.86 ± 2.75b	7.24 ± 1.05a	10.01 ± 0.93a	9.06 ± 0.73a
reapB20N0	15.77 ± 0.67a	19.04 ± 1.04a	5.65 ± 0.09c	14.94 ± 1.93ab	6.85 ± 0.71a	11.65 ± 1.45a	11.35 ± 0.57a
			N1 level – fertilized treatments				
B0N1	15.21 ± 0.88a	18.21 ± 1.00a	4.82 ± 0.17a	17.32 ± 2.84a	9.35 ± 1.98a	7.28 ± 2.08a	11.32 ± 1.47ab
B10N1	15.74 ± 0.89a	17.46 ± 0.44a	5.52 ± 0.59b	17.89 ± 4.12a	9.18 ± 1.45a	8.34 ± 1.10a	11.45 ± 2.21ab
B20N1	16.51 ± 0.89a	17.45 ± 1.20a	5.25 ± 0.17b	16.63 ± 3.69a	8.40 ± 1.86a	11.25 ± 2.91a	13.77 ± 0.74b
reapB10N1	15.73 ± 0.54a	18.08 ± 0.69a	5.54 ± 0.67b	14.92 ± 1.61a	8.34 ± 0.73a	9.18 ± 1.29a	9.04 ± 1.52a
reapB20N1	15.81 ± 0.98a	17.89 ± 1.33a	5.40 ± 0.12b	15.11 ± 2.50a	9.25 ± 1.31a	10.91 ± 3.18a	12.63 ± 0.89ab
			N2 level – fertilized treatments				
B0N2	14.92 ± 0.69a	16.99 ± 0.39a	4.37 ± 0.12a	28.31 ± 6.44b	11.78 ± 1.94a	9.24 ± 3.66a	12.44 ± 2.45a
B10N2	15.14 ± 0.81a	18.77 ± 0.84a	4.97 ± 0.42b	23.91 ± 6.82ab	13.55 ± 3.51a	11.19 ± 0.70a	10.59 ± 2.53a
B20N2	15.97 ± 0.83a	18.29 ± 1.04a	4.98 ± 0.30b	21.16 ± 4.80a	12.26 ± 2.13a	12.02 ± 0.85a	11.80 ± 1.28a
reapB10N2	15.15 ± 0.74a	19.14 ± 0.84a	5.29 ± 0.52c	18.86 ± 2.89a	12.92 ± 2.96a	10.83 ± 1.71a	12.45 ± 2.47a
reapB20N2	16.31 ± 0.74a	18.84 ± 0.79a	5.28 ± 0.24c	19.45 ± 4.27a	11.25 ± 2.38a	9.74 ± 1.88a	11.58 ± 1.47a

Biochar applied in all treatments, with or without N-fertilization, increased soil pH compared to the relevant control variants (B0N0, B0N1, B0N2). A statistically significant ($p<0.05$) increase was found in all N-fertilized treatments compared to the relevant controls (B0N1, B0N2). In treatments without N-fertilizer, soil pH increased from 1.12% to 5.21% compared to the control variant (B0N0). A statistically significant ($p<0.05$) difference was found in variants B20N0, reapB10N0, and reapB20N0 compared to the control variant (B0N0). In the first level of N-fertilization, soil pH increased from 8.92 % to 14.94 % compared to the control (B0N1). Statistically significant ($p<0.05$) differences were found in all variants at the first level of N-fertilization compared to the control variant (B0N1). At the second level of N-fertilization, soil pH increased from 13.73 % to 21.05 % compared to the control (B0N2). Statistically significant ($p<0.05$) differences were found in all variants at the second level of N-fertilization compared to the control (B0N2). According to a study by Ali et al. (2020), there was an average increase of 14.85% in soil pH following the application of biochar at a rate of 60 $t.ha^{-1}$ in conjunction with N-fertilizer at 270 $kgN.ha^{-1}$. In the study by Angst et al. (2013), an increase of 14.29 % in soil pH was shown in the treatment with biochar and N-fertilization. The content of NH_4^+ generally increased in treatments without N-fertilization and decreased in treatments with the first and second level of N-fertilization compared to the relevant controls (B0N0, B0N1, B0N2), respectively. NH4$^+$ increased in treatments without N-fertilization from 10.50% to 24.70% compared to the control (B0N0). A statistically significant ($p<0.05$) difference was found in variant reapB10N0 compared to the control variant B0N0, which showed the highest increase in NH_4^+ content (24.70 %). In the first level of N-fertilization, the content of NH_4^+ increased in variant B10N1 by about 3.29 % and decreased in variants B20N1, reapB10N1, and reapB20N1 from 3.98 % to 13.86 % compared to the control (B0N1) without statistically significant ($p<0.05$) differences. In treatments with the second level of N-fertilization, the content of NH_4^+ decreased from 15.54 % to 33.38 % compared to the control variant B0N2. Statistically significant ($p<0.05$) differences in NH_4^+ at the second level of N-fertilization were found in the control variant (B0N2) compared to variants B20N2, reapB10N2, and reapB20N2. In the study by Angst et al. (2013), a decrease of 90.91% in the content of NH_4^+ was shown in the treatment with biochar and N-fertilization compared to the content at the start of the experiment. The content of NO_3^- in treatments without N-fertilization increased from 1.1 9% to 7.90 % compared to the control (B0N0). At the first level of N-fertilization, the content of NO_3^- decreased from 1.98 % to 10.80 % compared to the control (B0N1). At the second level of N-fertilization, the content of NO_3^- decreased in variant reapB20N2 by 4.50 % compared to the control (B0N2) and increased in variants B10N2, B20N2, and reapB10N2 by 15.03 %, 4.07 %, and 9.68 %, respectively, compared to the control (B0N2). In the study by Angst et al. (2013), a decrease of 96.70 % in the content of NO_3^- was shown in the treatment with biochar and N-fertilization compared to the content at the start of the experiment. Generally, the content of organic carbon (C_{org}) in spring samples increased with the application of biochar in all treatments with and without N-fertilizer compared to the relevant control variants (B0N0, B0N1, B0N2). In treatments without N-fertilization, the content of C_{org} increased from 8.55% to 34.68% compared to the control (B0N0). In treatments with the first level of N-fertilization, the content of C_{org} increased from 14.56 % to 54.53 % compared to the control (B0N1). At the second level of N-fertilization, the content of C_{org} increased from 5.41 % to 30.09 % compared to the control (B0N2). The content of C_{org} in autumn samples varied across all treatments with or without N-fertilization. The variant B20N1 at the second level of N-fertilization was found to be statistically significant ($p<0.05$) compared to variant reapB10N1 at the same level of N-fertilization. According to a study by Ali et al. (2020), there was an average increase of 58.00 % in organic carbon following the application of biochar at a rate of 60 $t.ha^{-1}$ when combined with N-fertilizer at 270 $kgN.ha^{-1}$.

3.2. Carbon dioxide emissions

Generally, the impact of biochar application on average daily emissions of CO_2 decreased in treatments with the first and second levels of N-fertilization compared to the control variants (B0N1, B0N2). In the treatments without N-fertilization (Figure 2), the average daily emissions of CO_2 increased in all treatments from 5.52 % to 23.24 % compared to the control (B0N0). In the first level of N-fertilization (Figure 3), average daily emissions of CO_2 decreased from 1.85 % to 16.47 % in variants B10N1, B20N1, and reapB10N1 compared to the control (B0N1) and increased by 4.58 % in variant reapB20N2 compared to the control variant (B0N1). At the second level of N-fertilization (Figure 4), average daily emissions of CO_2 decreased from 19.99 % to 46.71 % in variants B10N2, B20N2, and reapB10N2 compared to the control (B0N2) and increased by 6.30% in variant reapB20N2 compared to the control variant (B0N2). Statistically significant ($p<0.05$) differences were found at the second level of N-fertilization in treatment B10N2 compared to the control variant (B0N2), with a decrease of about 46.71 %.

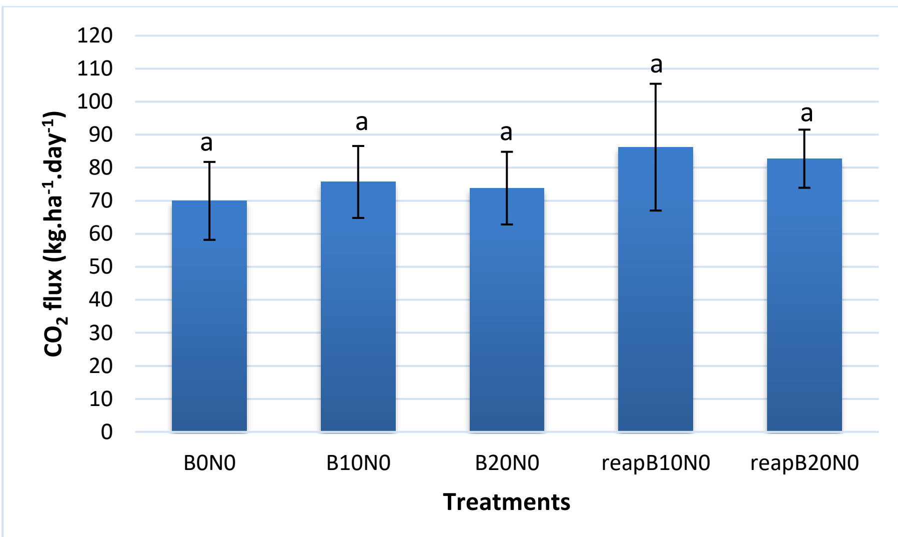

Figure 2 Average daily emissions of CO_2 in treatments without N-fertilization

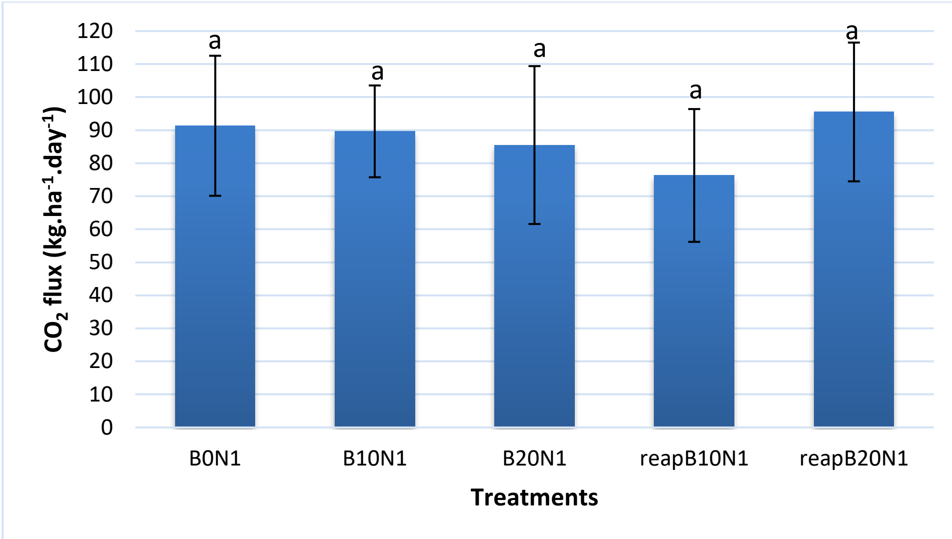

Figure 3 Average daily emissions of CO_2 in treatments with the first level of N-fertilization

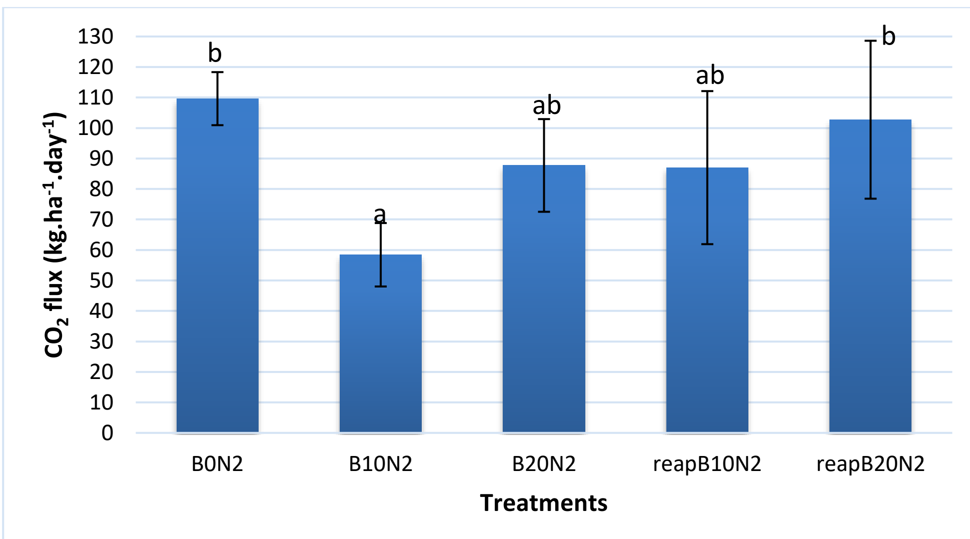

Figure 4 Average daily emissions of CO2 in treatments with the second level of N-fertilization

According to Yeboah et al. (2018), the flux of CO_2 emissions was 12-16% higher ($p<0.05$) in treatments with the application of 10 and 20 t.ha^{-1} of biochar compared to the variant without biochar application. The biochar application of 40 t.ha^{-1} significantly decreased CO_2 flux by 24 %, with 50 t.ha^{-1} by 13 %, and 30 t.ha^{-1} by 11 % compared the treatment without biochar application. In the study by Wang et al. (2020), generally, in treatments where different doses of biochar were applied in combination with N-fertilizer at a rate of 50 mgN.kg^{-1}, CO_2 emissions increased by 51.1 %-57.1 %. In treatments with 150 mgN.kg^{-1} and biochar application, the CO_2 emissions increased in the soil by 5.3 %-9.5 %. These results can vary depending on soil types, biochar producing process and climate conditions.

4. Conclusion

This study investigated the effects of biochar on soil properties and CO_2 emissions. Biochar positively influenced soil water content, temperature, pH, NH_4^+ and NO_3^- concentrations, and organic carbon content. Generally, it significantly increased soil pH in the first and second levels of N-fertilization compared to the relevant controls. NH_4^+ content generally decreased with biochar application, while NO_3^- varied across treatments. Organic carbon content notably increased, indicating biochar's potential for carbon sequestration. CO_2 emissions increased in treatments without N-fertilization but decreased with N-fertilization, notably by up to 46.71 % in some cases compared to the relevant control variants. These results support biochar's role in improving soil quality, carbon sequestration, and reducing CO_2 emissions, aligning with previous studies. Integrating biochar into soil management practices is crucial for sustainable agriculture and climate change mitigation.

Acknowledgment

This study was supported by the Scientific Grant Agency, grant number VEGA 1/0116/21 and by the Slovak Research and Development Agency under the contract No. APVV-21-0089.

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ARTICLE HISTORY:
Received 29.04.2024
Accepted 30.05.2024

GREEN MANURING AND ITS IMPORTANCE FOR HORTICULTURAL PRODUCTION: A REVIEW

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Abstract

The green manuring is process by which green plant mass (biomass) returns to the soil to increase of organic matter ratio, nutrient intake and their keeping in the soil. The improvement of soil microbiome, soil properties or health plant status belong to other important advantages of green manuring. Various vegetable species (mostly *Brassica* or fruit vegetables) are characterized by relatively high water and nutrient requirements. In this view, using of green manure species significantly improves growing environment conditions by soil enrichment about nutrients and increase of water holding capacity. Result of this process is sequential increase of yield and quality of vegetable production. This review study is focused on description of idea, advantages and effects of green manuring for sector of horticulture, especially vegetable sector.

Keywords: green manure, ecological production, horticulture, soil, environment, yield

1. Introduction

Under pressure of increasing human population, horticultural or generally agricultural sector needs to find way how to optimize or increase production of grown crops in connection with human health and environment. However, it is partially complicated to find suitable and balanced compromise combination of sufficient crop yield and more ecological production.

The use of conventional industrial fertilizers helps to optimize yield of vegetable crops; on the other side, their use can lead to negative effect on environment or human health (Kulkarni and Goswami, 2019). Industrial fertilizers used in conventional vegetable production system contain phosphates, nitrates, ammonium or potassium salts which are considered as a potential source of natural radionuclides and heavy metals (Savci, 2012). The excessive use of these fertilizers can result in soil, water and atmosphere contamination. Sequentially, mentioned undesirable substances are accumulated in vegetable plants and their edible parts. From this reason, they represent potential risk for food chain (Begho et al., 2022; Khan et al., 2022). Next view for using of industrial fertilizers are financial costs. The use of industrial fertilizers is probably the fastest and shortest way for boosting of crop production. On the other hand, fossil fuel resources are shrinking with continuously escalating costs and production of inorganic fertilizers are becoming more expensive. Thus, this is second important aspect for using of alternative nutrient sources within horticulture growing (De Keyser et al., 2023; Brunelle et al., 2015).

In recent period, several alternatives have been proposed to conventional farming system to increase sustainability of horticultural production and reduction in using of synthetic agrochemicals, e. g. pesticides or industrial fertilizers. In last decade, use of natural plant biostimulants has been propagated as a promising environmentally friendly innovation for horticultural production (Muhie, 2023; Sible et al., 2021; Colla and Rouphael, 2015).

Sufficient intake of organic matter to the soil is another way how to keep a good soil quality and fertility without using of high doses of industrial fertilizers. This aspect is one of main essential requirements for growing of nutrient-demanding crops, including vegetable or fruit species, within sustainable horticulture (Asghar et al., 2022). In Slovakia, similarly to several other European countries, the animal production was significantly decreased within last 2-3 decades. It resulted in lower availability of farmyard manure in horticulture, or agriculture generally.

Suitable alternative source of organic matter for keeping of soil quality is a green manuring. According to Alam et al. (2022), green manuring is defined as an environmentally friendly way to provide nutrients to plants. It is a process of turning or ploughing disintegrating green plant tissues into the soil to improve soil fertility (Meena, 2019) and support soil microbiome (Walker et al., 2022), health plant status (Tanveer et al., 2019), yield and quality of grown crops (Grazziotti et al., 2023; Martirosyan et al., 2023; Appiah et al., 2017). Green manure can be also described as a potential alternative for ecological restoration of ecosystems, providing numerous physical, chemical, and biological benefits in soil properties (Fonseca et al., 2023) and its contribution to climate change mitigation through carbon sequestration (Delgado et al., 2021). Tanveer et al. (2019) defined green manuring as a low cost and effective technology in minimizing cost of inorganic fertilizers and safeguarding soil productivity.

Goals of green manuring

As it was mentioned, a green manuring has high potential for using in horticultural/agricultural practice. Main goals for its realisation are following (Verma et al., 2023; Ramanjaneyulu et al., 2021; Meena, 2019; Tanveer et al., 2019):

- addition/sustaining of organic matter in the soil,
- crops with nitrogen-fixing bacteria (reduction of financial costs on nitrogen fertilization),
- soil covering as a prevention against soil erosion,
- increase of soil water-keeping capacity and cation exchange capacity,
- reduction of soil acidity,
- enhancement of soil microbial activity,
- cutting of green fodder for cattle in early plant stage,
- improvement of water permeability and soil aeration,
- keeping of loose and porous soil,
- reduction of mineral nutrient leaching,
- increase of nutrient availability (P, K or Ca) for plants,
- space for sufficient time distance within crop rotation,
- decrease of soil bulk density,
- improvement of soil physical condition (soil structure),
- suppression of soil pests, diseases and weeds,
- support of biodiversity, especially plant growth promoting rhizobacteria (PGPR) and microorganisms antagonistic to pathogens,
- increase of crop yield,
- improves/changes nutritional value of vegetables.

Necessary characteristics of plant species suitable for green manuring

Basic factors determining suitability of plants for using as a green manure are seed cost, land, irrigation and species fitness for cropping system (Tanveer et al., 2019). Main properties of species suitable for green manuring are (Verma et al., 2023; Alam et al., 2022; Ramanjaneyulu et al., 2021; Meena, 2019; Tanveer et al., 2019):

- most suitable - species from legume (*Fabaceae*) family (nitrogen fixation),
- adaptability to extraordinary climate (shade, drought, floods, high temperature etc.), soil and cropping system,

- ability for normal growth in all agro-climatic zones,
- lower water requirements,
- photoperiod insensitivity,
- low production costs,
- low management requirements,
- easy availability and affordability,
- fast germination (ability to compete with weeds),
- quick rotting,
- fast growing,
- short growing period,
- high ability of nutrient accumulation,
- high nitrogen sink in underground plant parts,
- low C:N ratio and lignin content,
- timely release of nutrients,
- high production and viability of seeds,
- easy incorporation to the soil and biomass decomposition,
- multipurpose using,
- pest and diseases resistance (preferred are species with potential to reduce pest population),
- without close relation with following crop (risk of pest/disease attraction to following crop),
- with a fumigation effect of plant biomass against soil pathogens or pests.

Green manure crop types

Long term green manure species

These crops are established for two or three years. Its culture is a basic and very important part of many organic field vegetable and arable rotations. If animals are present on the farm, crops are usually grazed or cut for silage. If farm is without animals, plants are normally cut monthly during the summer period and mown biomass remains on the surface as a mulch. In this group, pure clover (priority to nitrogen fixation), its mixture with grass (priority of biomass amount) or ryegrass are often used for green manuring (Rosenfeld and Rayns, 2024; Knight, 2024; Rayns and Fosenfeld, 2010).

Winter green manure species

Winter green manure crops are usually sown in the autumn and incorporating to the soil in the following spring. It is a good way for support fertility of following crop and utilisation of soil which would be normally bare. On the other hand, slight disadvantage is more difficult planning of crop rotation. From some reason (e. g. rainy period), there could be problem to do harvest of green manure crop early enough and establishment of following crop is sequentially delayed. The most reason for use of these crops is to minimalize nitrogen leaching from soil, thus they are often called as winter covering crops. From this group, several species of legumes can be used for green manuring process, e. g. vetch (Rosenfeld and Rayns, 2024; Knight, 2024; Rayns and Fosenfeld, 2010).

Summer green manure species

Legumes are usually grown as summer green manure species to provide a boost of nitrogen in mid rotation. These species may be grown for a whole season (spring-autumn) or just short period between two main "cash" crops. As a shorter green manure species, some non-legume species can be used, e.g. mustard or phacelia (Rosenfeld and Rayns, 2024; Knight, 2024; Rayns and Fosenfeld, 2010).

Undersown green manure species ("intercropping")

Some green manure species may be also used by their undersowing with some horticultural crops (or cereal crops). Advantage of this system is help for weed control or pest control measure. On the other hand, there can be also a strong competition among green manure crop and grown "cash" crop in case of worse planned growing system (Rosenfeld and Rayns, 2024; Knight, 2024; Rayns and Fosenfeld, 2010).

Table 1 List of some species suitable for green manuring (Rayns and Fosenfeld, 2010)

Common name	Latin name	Family	Nitrogen fixer
	Long-term species		
white clover	*Trifolium repens* l.	*Fabaceae*	yes
red clover	*Trifolium pratense* L.	*Fabaceae*	yes
alfalfa / lucerne	*Medicago sativa* L.	*Fabaceae*	yes
perennial ryegrass	*Lolium perenne* L.	*Poaceae*	no
cocksfoot	*Dactylis glomerata* L.	*Poaceae*	no
chicory	*Cichorium intybus* L.	*Cichoriaceae*	no
	Winter green species		
common vetch	*Vicia sativa* L.	*Fabaceae*	yes
fava bean / broad bean	*Vicia faba* L.	*Fabaceae*	yes
grazing rye	*Secale cereale* L.	*Poaceae*	no
ryegrass	*Lolium multiflorum* Lamk.	*Poaceae*	no
Brassica species	various *Brassica/Raphanus* species	*Brasssicaceae*	no
	Summer green species		
crimson clover	*Trifolium incarnatum* L.	*Fabaceae*	yes
fenugreek	*Trigonella foenum-graecum* L.	*Fabaceae*	yes
lupins	*Lupinus* spp.	*Fabaceae*	yes
sweet clover	*Melilotus officinalis* (L.) Pallas	*Fabaceae*	yes
phacelia	*Phacelia tanacetifolia* Benth.	*Boraginaceae*	no
buckwheat	*Fagopyrum esculentum* Moench.	*Polygonaceae*	no
white mustard	*Sinapis alba* L.	*Brassicaceae*	no

Description of benefit examples by using of green manuring

Nitrogen leaching

De Notaris et al. (2018) analysed the change rate of nitrogen leaching within four-year crop rotations by using of green manure in the conventional and organic growing system. Tested crops used in the field experiment were spring barley (*Hordeum vulgare* L.), hemp (*Cannabis sativa* L.), garden pea (*Pisum sativum* L.) as intercrop, spring wheat (*Triticum aestivum* L.) and potatoes (*Solanum tuberosum* L.). Alfalfa (Medicago sativa L.) and mixture of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) were used as a green manure species. In both tested growing system, using of green manure was expressed by decrease of nitrogen leaching about 23 kg $N.ha^{-1}.y^{-1}$ (60 %) across the four-year crop rotation.

Soil properties

The biomass of leguminous green manure species (vetch, clovers, alfalfa etc.) is rapidly decomposed. It has a relatively little effect on long-term soil organic matter; however, it stimulates a microbial activity in first few months after biomass incorporation to the soil. On the other hand, biomass of non-leguminous green manure species (buckwheat, chicory, radish, mustard, grazing rye, phacelia etc.) is decomposed relatively slowly and its residues providing a physical effect for soil longer time. The improvement of soil structure by green manuring can be done by various ways:

- implication of extensive, fine roots in the soil helping to stabilise its aggregates and increase soil pore size,
- breaking up of compacted soil by formation of deep main (tap) roots (alfalfa, chicory, red clover etc.),
- providing of food for microorganisms by production of root exudates and sequential production of polysaccharide gums ("mucilage") with ability to keep soil aggregates together,
- maintenance of soil mycorrhiza population between growing of "cash" crops; it is important e.g. for phosphorus nutrition; *Brassica* species and lupins are non-mycorrhizal,
- binding effect of root system to the soil - reduction of water soil erosion,
- increase of surface roughness - wind speed reduction close to the soil and minimisation of wind soil erosion,
- increase of soil organic matter ratio - improvement of soil texture, structure, infiltration rate, bulk density and water-holding capacity (Rosenfeld and Rayns, 2024; Knight, 2024; Verma et al., 2023; Shirale et al., 2018; Dubey et al., 2015; Rayns and Rosenfeld, 2010).

Crop yield and quality

Stein et al. (2023) tested the effect of leguminous green manure crops on the yield of the subsequent cash crop - white **cabbage** (*Brassica oleracea* convar. *capitata* var. *alba*) in Germany. Winter cover crop treatments were following: winter Hungarian vetch (*Vicia pannonica* Crantz, family *Fabaceae*), winter pea (*Pisum sativum* L., family *Fabaceae*), and winter faba bean (*Vicia faba* L., family *Fabaceae*) and bare soil as a control. Using of green manure were showed by significant yield increase of marketable cabbage heads (weight > 1.0 kg) in range from +18.7 % (vetch) to 35.0% (faba bean). The results of this study showed that leguminous winter cover crops do not reduce the soil nitrogen availability for a succeeding high nitrogen-demanding crops like cabbage.

Martirosyan et al. (2023) tested effect of green manuring by using of bean (*Phaseolus vulgaris* L., family *Fabaceae*), green pea (*Pisum sativum* L., family *Fabaceae*) and soybean (*Glycine max* (L.) Merr., family *Fabaceae*) within field experiment with **eggplant** (*Solanum melongena* L.) in Armenia. The eggplant yield was higher in all treatments of green manuring compared to the control (bare soil). Its increase was ranged from 14.5 % (pea) to 24.5 % (soybean). In addition, green manuring has also positively effect to the content of vitamin C and total sugars in eggplant fruits. Vitamin C content in eggplant fruits was increasing in the following treatment order: control (3.10 mg%) < pea (3.65 mg%) < bean (3.95 mg%) < soybean (5.65 mg%). Total sugar content in eggplant was increasing in the following treatment order: control (2.8 %) < pea (3.0 %) < bean (3.2 %) < soybean (3.6 %). From this aspect, soybean using was evaluated as the most suitable treatment of green manuring.

Kim et al. (2023) analysed yield of **pepper** (*Capsicum annuum* L.) fruits after green manuring by spinach (*Spinacia oleracea* L.), red and green mustard (*Brassica juncea* (L.) Czern.) in the pot experiment in South Korea. Compared to the control treatment, authors found significantly higher pepper yield in all tested green manure treatments (spinach +159 %; red mustard +30 %; +28 %).

Within experiment realised by Grazziotti et al. (2023) in Brasilia, yield and quality of **carrot** (*Daucus carota* L.) and **lettuce** (*Lactuca sativa* L.) was tested in dependency on the green manuring by sunn hemp (*Crotalaria juncea* L.) and lablab (*Dolichos lablab* L.). In both tested vegetable species, positively effect of green manuring to the yield quantity was found. The statistically significant increase of lettuce yield was found, concretely from 14 $t.ha^{-1}$ (control) to 26 $t.ha^{-1}$ (sunn hemp) or 38 $t.ha^{-1}$ (lablab). Regarding to carrot roots, marketable yield was also significantly higher than in the control treatment.

Javanmard et al. (2022) tested effect of green manuring by using of barley (*Hordeum vulgare* L., family *Poaceae*) and hairy vetch (*Vicia villosa* L., family *Fabaceae*) and their various combination to the yield and quality of **peppermint** (*Mentha piperita* L.) in Iran. The yield of peppermint dry matter was significantly higher in all treatments with green manuring compared to the control (bare soil) and its values were increasing in following treatment order: control (194.4 $g.m^{-2}$) < barley monoculture (203.3 $g.m^{-2}$) < vetch monoculture (217.7 $g.m^{-2}$) < 75 % vetch + 25 % barley (240.8 $g.m^{-2}$) < 25% vetch + 75% barley (249.3 $g.m^{-2}$) < 50 % vetch + 50 % barley (266.7 $g.m^{-2}$). On the other side, green manuring was showed by variable effect of tested treatments to the content of essential oils in peppermint which are the most important qualitative of peppermint or aromatic and medicinal plant generally. The using of barley (94.53 %) and hairy vetch (95.56 %) monoculture or combination 25 % vetch + 75 % barley (95.22 %) resulted in slight decrease of essential oil content compared to the control (95.62 %). However, treatments with combination 50 % vetch + 50 % barley (96.93 %) and 75 % vetch + 25 % barley (97.34 %) were expressed by increase of essential oil content in comparison with control - bare soil. Generally, effect of used green manuring to the essential oil content could be evaluated as a less important.

The quality of vegetable crops is important aspect of its production. Within experiment in Ghana, Appiah et al. (2017) evaluated the quality of **carrot** (*Daucus carota* L.) roots in dependency on the using of three various green manure species: velvet bean (*Mucuna pruriens*, family *Fabaceae*), Siam weed (*Chromolaena odorata*, family *Asteraceae*; traditional medicinal plant in some countries, but it is invasive species), gliricidia (*Gliricidia sepium*, family *Fabaceae*) on the quality of carrot roots (vitamin C, total sugars, mineral elements). Total sugar content (important parameter for taste) was variously influenced by used green manuring. The using of Siam weed (86,5 $g.kg^{-1}$) resulted in increase of sugar content in carrot compared to the control (74.0 $g.kg^{-1}$). On the contrary, application of velvet bean (55.6 $g.kg^{-1}$) and gliricidia (49.3 $g.kg^{-1}$) was shown by decrease of carrot sugar content in comparison with control. Vitamin C content in carrot was positively, significantly effected by using of green manure in the following treatment order: control (4.2 $mg.kg^{-1}$) < gliricidia (12.5 $mg.kg^{-1}$) < velvet bean (22.5 $mg.kg^{-1}$) < Siam weed (35.3 $mg.kg^{-1}$). These results indicate that selection of suitable green manure species is one of conditions for quality keeping of grown carrot or vegetables generally.

Pest and diseases

According to Larkin et al. (2013), using of green manure crops from *Brassica* genus or related crops has been emerged as the most effective way for management of multiple plant diseases due to their biofumigation potential referred to the suppression of pathogens and disease through the release of volatile toxic breakdown products, as well as other unique effects on soil microbial ecology. *Brassica* crops and some of other green manure species have been used to control a variety of various plant pathogens, including important and dangerous species of *Rhizoctonia*, *Verticillium*, *Sclerotinia*, *Phythophthora*, *Pythium*, *Aphanomyces* or *Macrophomina* in various crop production systems, e. g. management of multiple soilborne diseases of potato, including black scurf, common scab and Verticillium wilt. Authors emphasize that disease reduction by using of green manures is variable on the crop and its cultivar and it provides just partial control against diseases, not complete protection. It is a versatile and readily implementable additional management strategy that should be used as an important component within a larger integrated disease management program to provide improved sustainable production systems.

Rayns and Rosenfeld (2010) indicate that using of green manure species can form a natural environment for general predators of various pests, e.g. summer flowering plants will support populations of hoverflies, lacewings and parasitic wasps. Cabbage root fly and other important pests of *Brassica* species can be disrupted by greater diversity in the field. The using of high-glucosinolate mustard cultivars offers an effective control against soil pests after its fast production of large biomass amount and its rapid incorporation to the sufficiently damp soil. The abundance of glucosinolates in *Brassica* green manure species has been also used in disease control, e. g. against *Verticillium* wilt of strawberries or *Rhizoctonia* occurrence in various vegetable species. Various green manure crops support microbial communities of non-pathogenic bacteria and fungi which will antagonise pathogenic organisms. On the other hand, un-sufficient time distance after using of *Brassica* green manure crops can lead to increased risk and danger of some diseases within growing of cabbage and other *Brassica* vegetables, e.g. clubroot.

The using of green manure crops as intercrops is one of the methods for control and regulation of several nematode species. The radish (*Raphanus sativus* L.) and white mustard (*Sinapsis alba* L.) can reduce population of beet cyst nematode (*Heterodera schachtii*), pest of various vegetable (e. g. spinach) or field crops, by interruption of sexual differentiation in nematode life cycle (Wright et al., 2019). Various tagetes species (*Tagetes* spp.) have a suppressive effect on several nematode species, e. g. root lesion nematode (*Pratylenchus penetrans*) - important pest for onion and other vegetable species (Marahatta et al., 2012). Some radish species can interrupt the transmission of several virus diseases (tobacco mosaic, early pea browning etc.) by elimination of nematodes from genus *Trichodorus*. Using of several radish cultivars can help to inhibit life cycle of nematodes from *Meloidogyne* genus and reduce its occurrence in growing of many horticultural crops (Hamidi and Hajohassani, 2020).

Weeds

The ability to suppress weeds belongs to one of the major benefits of green manure crop using. The using of green manure species to the crop rotation supports diversity and reduces opportunities for weed spreading. Mowing and grazing as management steps associated with using of green manure also supports the suppression of weeds. The green manure growing reduces of seed viability by shortening of ley period. Rapidly growing crops, e. g. mustard, are the most effective in competition with weeds for light, water and nutrients and it contributes to significant suppression of weed spreading. Some green manure species (mainly clovers) are known for allelopathic effect by secretion of specific chemicals and sequentially inhibition of weed seed germination. On the other hand, there can be also inhibition risk of subsequent crops, mainly sown species with small seeds, and seed delayed germination because “suppressive” effect can last several weeks. Some green manure species, e. g. phacelia, have ability to self-seed proliferation and may become a weed in subsequently grown crops (Rayns and Rosenfeld, 2010).

Kim et al. (2023) evaluated the effect of three green manure species (spinach, red mustard, green mustard) on the emergence of monocotyledonous and dicotyledonous weeds in pot experiment. Use of all green manure species resulted in significant decrease of weed emergence compared to the control treatment. The number of monocotyledonous weeds were decreased in following treatment order: control (15.5 pieces/pot) > red mustard (7.2 pcs/pot) > green mustard (3.3 pcs/pot) > spinach (1.5 pcs/pot). The number of dicotyledonous weeds were decreased in treatment order: control (15.8 pieces/pot) > red mustard (10.9 pcs/pot) > green mustard (8.7 pcs/pot) > spinach (2.8 pcs/pot).

2. Conclusion

The review study concludes description of green manuring, its advantages and benefits for using in more ecological and sustainable horticulture production. The keeping of high soil quality is one of basic conditions for successful growing of horticultural or agricultural crops generally. As it is described in this study, selection of suitable species and their cultivars is the basic and very important factor for effective process of green manuring. The green manuring is one of the effective ways how to decrease the intake of industrial fertilizers or chemicals for plant protection and contribute to more economically and environmentally friendly horticulture (agriculture) production.

Acknowledgment

This work was solved within multilateral call of the Slovak Research and Development Agency with no. DS-FR-22-0014: Green manuring as a tool for improvement of soil microbiome and quality of vegetables in sustainable agriculture.

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ARTICLE HISTORY:
Received 10.05.2024
Accepted 23.05.2024

THE EFFECT OF TEMPERATURE DURING THE WINTER MONTHS ON THE GROWTH OF WINTER CROPS AND THE EARLY ONSET OF FLOWERING OF FRUIT SPECIES

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Abstract

For the comparison of temperature changes in winter periods we chose the period 1961 - 2020 for our work. Data on average monthly temperatures in December, January and February were provided by the Slovak Hydrometeorological Institute in Bratislava. For the sake of illustration and representativeness we have chosen five stations in Slovakia. The selection was based mainly on location parameters. These stations are Hurbanovo, Holíč, Oravský Podzámok, Sliač and Prešov. Winter temperatures mainly affect the phenological phase of stemming, flowering, and ripening, which in most of Slovakia takes place mainly in the spring period.

From the graphical comparison, over the 60-year period under study, the average monthly temperatures in the winter months had approximately the same pattern at all stations monitored. Periods of increase were interspersed with periods of decrease. In 2020, all mean temperatures are in the positive range, with an increase of 2.0 to 4.0 °C compared to 1960. In the context of winter cereal branching, although a direct effect of temperature on this phenophase has not been demonstrated, if the higher temperatures have an earlier onset, the earlier the stalking phenophase begins. The latter is already threatened by late frosts. The situation is similar for fruit trees. Higher average temperatures during the winter months can delay the onset of the first flowering phenophase. Apricots and cherries need temperatures of at least 7,0 °C to flower. If the average temperature gradually approaches this value, the flowering trees will be very vulnerable to late frosts. A delay of 7-10 days in the onset of first flowering is observed at all stations. According to the Slovak Hydrometeorological Institute in Bratislava, the first flowering has been recorded on some varieties of fruit trees in March 2023. According to the long-term average, this flowering is not expected to occur until the first half of April.

Key words: climate change, first flowering, winter plants, development of temperature

1. Introduction

All of us are feeling the effects of ongoing climate change. One of the manifestations of climate change that is easily observable is that the winter months are not as cold and hard as they used to be in the past. This is linked to the increasing average air temperature. Higher temperatures also affect the height and amount of snow cover. In addition, the frequency of snowfall is also affected. It is logical that warmer temperatures do not create suitable conditions for snowfall. Warm winters with a lack of snow increase the damage caused by wildlife. Winter crops, which would be protected from biting by a layer of snow, are most at risk. As warmer temperatures encourage the growth of the above-ground part of winter crops, game seeks out such fields as a source of fresh food. In some cases, grazing by animals can destroy up to half of the sown plants. Crops that have overwintered in good condition are threatened by the onset of diseases and pests. Their occurrence is encouraged by wet and warm weather. Snow mould, despite the absence of snow, is the first disease to appear on the plants.

Various root, stem and leaf diseases follow. Finally, powdery mildew, which young plants may not be able to cope with. Mild and dry winters do not only have negative effects on agricultural crops. On the positive side, it has created suitable conditions for winter crops that were sown late because of late agronomic interventions. Thanks to the warmer temperatures, such plants will catch up with the development of those sown earlier and will therefore not need as much care as they would have needed in a long winter with a long duration of snow cover (session Farmárska revue, 2024).

The development of agricultural crops is influenced by the external environment, which is mainly represented by meteorological factors. The factors with the greatest influence are light and heat conditions and the air and water regime, in a wide variety of interactions. Their importance is all the greater because all developmental stages, stages of organogenesis, growth phases, i.e. the course of all plant life is directly linked to certain optimum values of the meteorological elements. The proportion of factors that directly influence yield can be expressed as a percentage. Research to date has shown that weather conditions account for 15-20 % of the total and are immediately followed by the influence of natural soil fertility (Kisty F., 1979).

2. Material and Methods

Meteorological data for the period 1961 - 2020 were used as a basis for comparison. These were provided by the Slovak Hydrometeorological Institute in Bratislava. The climatic normal, which is commonly used in the assessment of long-term weather manifestations, usually lasts for 30 years. This was determined based on the World Meteorological Organization (WMO). Our period is covered by up to two consecutives climatic normals. This gives us enough room to assess the longer-term trend of temperatures in the winter months.

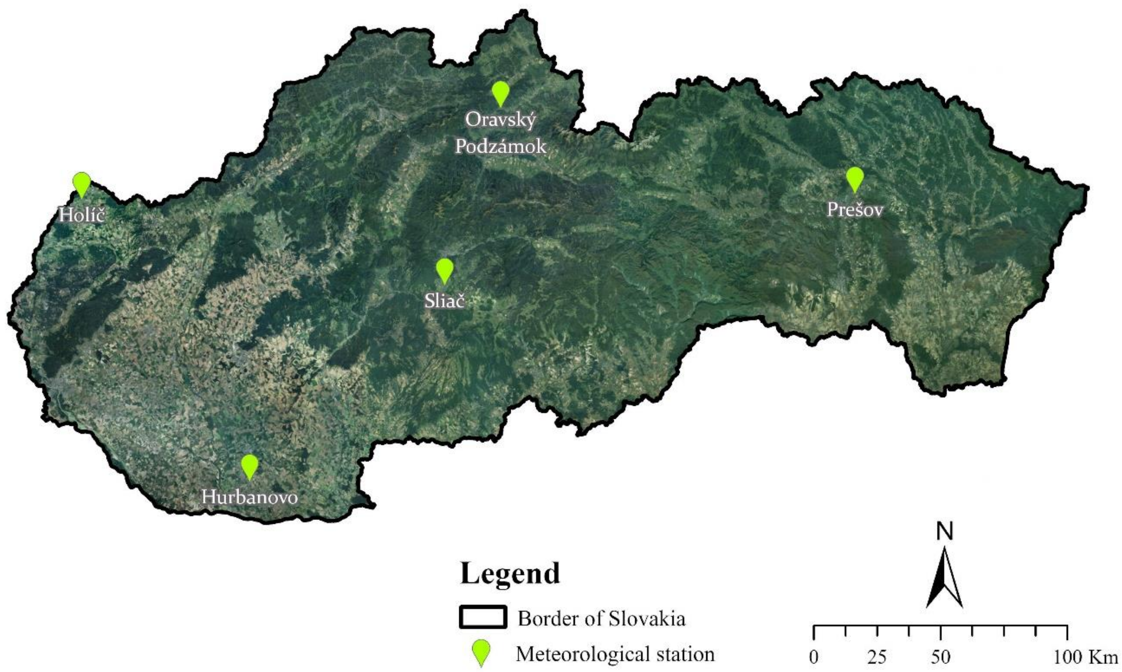

Figure 1 Location of selected meteorological stations (Minárik M., 2024)

For the sake of comparison, we have chosen five stations from the territory of Slovakia. Specifically, the stations are Hurbanovo (south of Slovakia), Holíč (west of Slovakia), Oravský Podzámok (north of Slovakia), Sliač (centre of Slovakia) and Prešov (east of Slovakia). Each of the stations was selected mainly based on location parameters to represent the area as much as possible. The Hurbanovo station was especially chosen because continuous meteorological measurements have been made at this station since the second half of the 19th century. The division was necessary due to the significant ruggedness of the territory of Slovakia. However, we are aware that the selected stations do not represent a universal evaluation criterion for individual areas, but they are sufficient for the purposes of this article. The location of each station is shown in the map in Figure 1.

As we needed temperature data for the winter months to process this comparison, we selected the months of December, January, and February from the whole set of data provided. Since we were provided with average daily data for each station, it was first necessary to make monthly averages from these daily averages. We did this for all selected stations throughout the study period. This whole process was done in MS Office Excel environment. The monthly averages thus arranged for each winter month and station were exported to separate tables for greater clarity. Subsequently, the data were used to create graphical dependencies for each month separately, showing the dependency of the monthly mean temperature height in each month over the years of the study period.

Another task was to obtain data on the individual growth phenophases of the most cultivated winter crops and the most threatened fruit trees. Among winter crops we selected winter wheat, which is grown on the largest area in Slovakia. Next were winter rye and winter oilseed rape. From fruit species we selected apricot, cherry, and currant. These fruit trees are the first to flower of all the others and are therefore most affected by temperatures during the winter months. In collecting the data, we focused mainly on the temperature needs of each species. The observed temperature data can be seen in Table 1.

Table 1 Minimum and average temperatures for the observed phenological phases

Phenological phase: First flowering	**Temperature [°C]**	
	minimum	**average**
apricot (*Prunus armeniacum*)	6,6	9,3
cherry (*Prunus avium*)	8,0	10,3
red currant (*Ribes rubum*)	7,1	9,8

Phenological phase: Stem extencion	**Temperature [°C]**	
	minimum	**average**
winter wheat (*Triticum aestivum*)	5,0	7,5
winter rye (*Secale cereale*)		
rapeseed (*Brassica napus L. ssp. Oleifera*)		

(Kurpelová M., 1958, own processing)

3. Results

As can be seen from the accompanying graphs in Figures 2 to 4, the temperature pattern during the 1961 to 2020 study period was neither uniform nor linear. There have always been alternating periods of higher average monthly temperatures with periods of lower ones. The period 1960-1970 is generally regarded as a cold period. This can be clearly seen in the graphs, where all three months have the lowest temperatures ever recorded. This decade is also the coldest of all the decades compared. In subsequent years, the temperature trend has been about the same in all months. Warmer and cooler periods have alternated. The period 1980-1990 saw a further major cooling. As can be seen, the lowest average temperatures in this period for the month of December are in the early 1980s. In January and February, this low moves into the mid-80s.

The next major cooling came in 2000-2010. December average temperatures showed this decline again at the beginning of the decade. For January and February, the decline was not apparent until the middle of the decade. The last decade has not been characterized by such large extremes.

If we look at the average temperature at the beginning and at the end of the period, we can see that it has increased by an average of 2.0 to 4.0 degrees at each station. Such an increase in average temperature over a period of 60 years can be described as significant. Also, if we overlay the individual curves with a linear trend line, we see that for all stations the trend is upwards. In practice, this means that further increases in average monthly temperatures in the winter months can be expected in the future. Interestingly, temperatures do not change that extremely during December. More extreme changes during temperatures are seen in January, less so in February. Further, December temperatures are likely to decrease slightly over the next few years but are expected to increase even more in January and February.

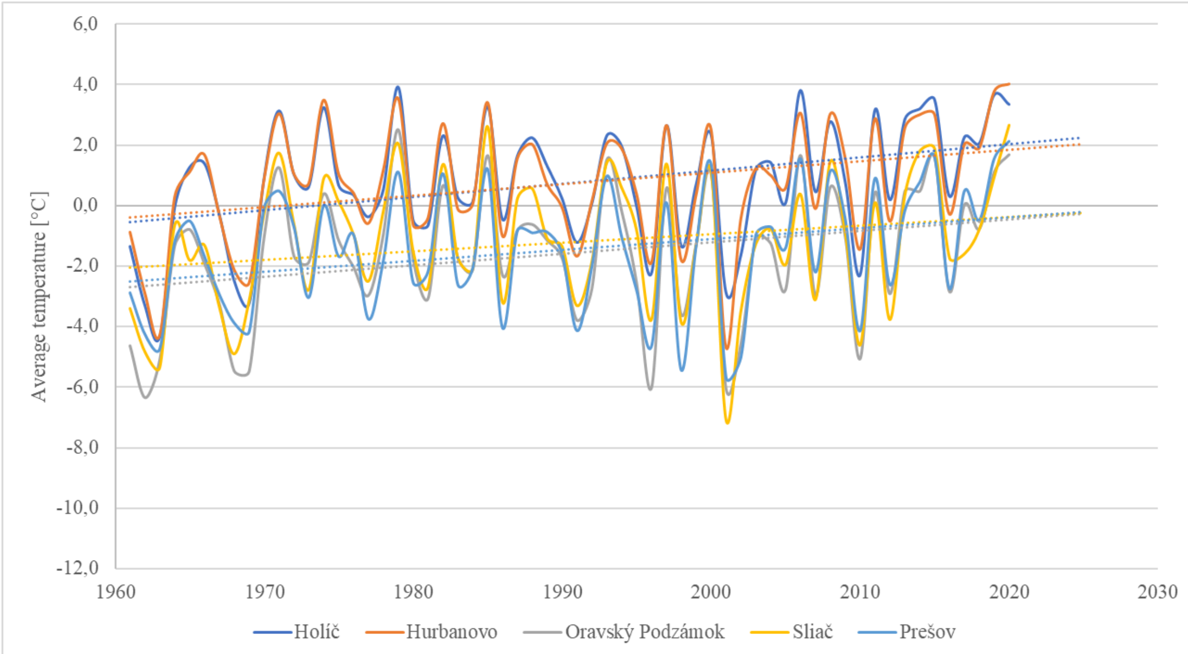

Figure 2 Development of average monthly temperatures in December (*dotted lines represent linear trends for individual stations*) (Minárik M., 2024)

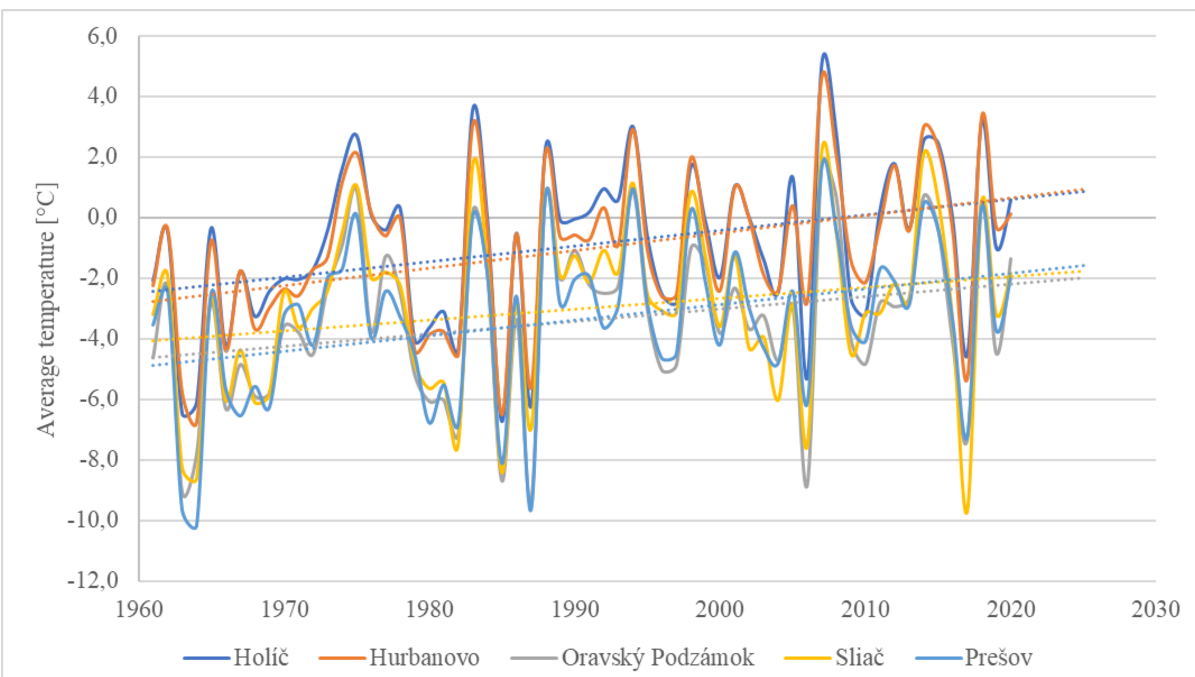

Figure 3 Development of average monthly temperatures in January (*dotted lines represent linear trends for individual stations*) (Minárik M., 2024)

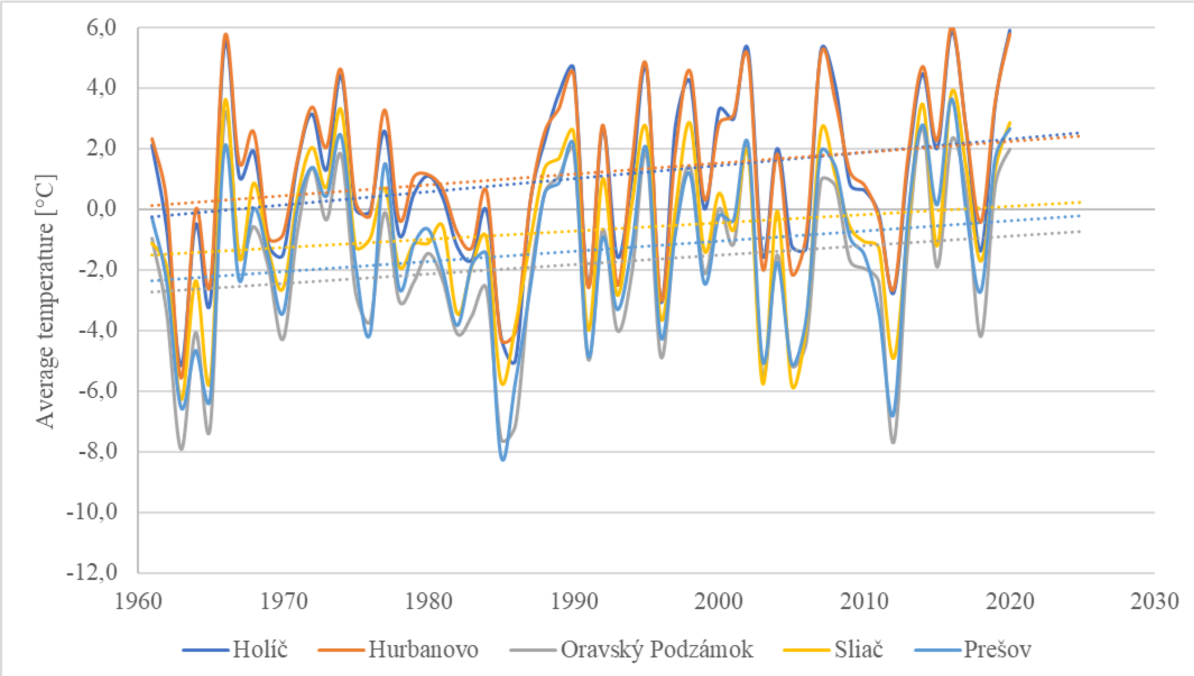

Figure 4 Development of average monthly temperatures in February (*dotted lines represent linear trends for individual stations*) (Minárik M., 2024)

As we can see from Table 1, the phenological phases in field crops begin to take place when the air temperature reaches a minimum of 5,0 °C and an average of 7,5 °C. For overwintering cereals, the effect of temperature on the phenological phase of fruiting has not been demonstrated. Temperature only affects the stalking phase. The average temperature at the end of winter, i.e. in February, gradually increases. At the Hurbanovo and Holíč stations already reaches a value of approximately 6,0 °C. The other stations, which are further north are currently averaging around 3,0 °C. In the first two mentioned above, it is expected that winter stalking can be expected as early as February and March. However, this puts these crops at risk as there is still a high probability of later frosts, which could adversely affect the development of the winter crops. At other stations, it can be expected that within a couple of years average temperature at the end of the winter period of 5,0 °C, which would mean higher threat to plants. As it is much higher in the northern parts of the probability of cold weather arriving in early spring.

As for the selected fruit species, Table 1 shows that they need a higher temperature to start the phenological phase of the first flowering. Since apricots need a temperature of approximately 6,5 °C to start flowering, there is a great risk, since, as already mentioned, the average temperature at the end of the winter period is already reaching 6,0 °C at some stations. With further warming, there is a high risk that the plantings of fruit species will deteriorate, as they will start flowering at the end of February and the beginning of March. Flowering fruit species are at risk from later cold spells and frosts. If the flowers freeze, the harvest is minimal or non-existent. Cherry and currant need warmer temperatures for first flowering, so they are not as much at risk as apricots now.

4. Discussion

Václav Lednický in his paper on the effect of temperature on the phenology of fruit trees states that in 1979 the earliest flowering of apricot in Slovakia was recorded on 2.4. For cherry it was 9.4. and for red currant 3.4. However, the average dates of onset were later by about 2 - 3 weeks, i.e. in the last decade of April. Similar was the case for winter rye, which continued to grow from about the beginning of April (Lednicky V., 1979, p. 9-16).

On the contrary, Snopková et al. (2024) report that in the western part of the territory the beginning of apricot and the first leaves of red currant were observed as early as the end of February, in the rest of the territory in the beginning of March. In the second decade of March the apricots were already in full bloom. Locally, in some locations, frost damaged the flowering apricots in the second half of March. There was also sporadic damage to cherry trees. Furthermore, the first decade of March saw the beginning of stalking of winter wheat and rye, as well as the elongation of winter rape. Compared to the long-term phenological normal (1991-2020), the onset of individual phenological phases was earlier by two, sometimes three weeks (Snopková Z. et al., 2024). Compared to 1979, this is an earlier onset by 3-4 weeks.

Mrekaj, Jakubíková and Kajaba in their work dealt with the onset of flowering of bird cherry for the period 1990 - 2020. They found that for the period under study, the date of onset of flowering has a decreasing trend, i.e. it occurs at an earlier date than in the past. On average, the shift to earlier periods ranged from 13 to 15 days. It also notes that the shift in phenological phases also occurred in other crops, especially field crops (Mrekaj, Jakubíková, Kajaba, 2020, p. 130-134).

5. Conclusion

The aim of our paper was to find out how the temperatures develop during the winter season in Slovakia, whether they have an increasing or decreasing trend. Finally, our aim was to find out the effect of these temperatures on the earlier onset of flowering in selected fruit species and winter crops. The results show that the average monthly temperatures in winter months have an increasing trend, so further increases can be expected in the future. In the context of winter crops, there is a shift in the onset of the different phenological phases due to increasing average temperatures. Flowering in fruit species is also shifting to an earlier period. In both cases, the delay in the onset of the selected phenological phases creates a risk of compromising development and, consequently, yields due to later cold weather and frosts. Based on the above, increased financial costs can be expected in the future for the provision of frost protection for fruit species. Unfortunately, due to the huge areas under cultivation, field crops cannot be frost-protected, as large amounts of money would have to be spent, which would ultimately not be effective.

Our findings are also consistent with those of other authors who have shown in their studies that the onset of the different phenological phases of the species under study can be coordinated.

Acknowledgment
This publication was funded by the Grant Agency of the Slovak University of Agriculture (GA SPU), project No. 10-GA-SPU-2024 and by the Slovakia's recovery and resilience plan, project No. 09I03-03-V05-00018 – Early Stage Grants at SUA in Nitra.

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ARTICLE HISTORY:
Received 20.05.2024
Received in revised form 28.05.2024
Accepted 30.05.2024

SUSTAINABILITY OF PLANTATIONS OF FAST-GROWING ENERGY PLANTS FROM THE POINT OF VIEW OF THEIR ECOSYSTEM SERVICES AND THE USE OF UNUSED AGRICULTURAL LANDS

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Abstract

Human existence is dependent on ecosystems, their goods and services. Goods provided by ecosystems include food, feed, textile materials, pharmaceuticals. Ecosystem services necessary for human society represent e.g. maintenance of the hydrological cycle, air water filtration, climate regulation, storage and cycle of nutrients, provision of biotopes, cultural or spiritual values. Without these and many other goods and services, there is no real human life and certainly no economic development. The analysis of selected ecosystem services was carried out based on the classification according to Bastian and Grunewald (2015), while we started from the group of supply ecosystem services and included a socio-cultural ecosystem service, specifically – information services. We also connected the analysis with the identification of ecosystem services according to Prokopová (2010), where we evaluated the ecosystem service in relation to a specific agroecosystem – a plantation of fast-growing energy plants. The analysis of ecosystem services is complemented by an overview study of the potential based on the research results of various authors. Data on the soil fund were processed according to the database of the Soil Science and Conservation Research Institute as of 31.12.2019. Plantations of fast-growing energy plants represent local agroecosystems in regions where they mainly perform production functions. They provide supply services in the form of biomass production, which subsequently has energy and non-energy uses. For the cultivation of fast-growing energy plants in the conditions of Slovakia, suitable soils are included in soil quality groups 5-9. The area of soil quality group 5-9 is represented at the level of all regions of the Slovak Republic. The highest potential in the cultivation of fast-growing energy plants with regard to specific land areas at the regional level is in the Prešov region (99.95 %), the Žilina region (99.89 %), the Košice region (99.51 %), the Banská Bystrica region (98, 94 %) and the Trenčín region (83.02 %). Regions with an area of soil quality group 5-9 below 50 % of the total area of agricultural land include Bratislava region (46.35 %), Nitra region (39.56%) and Trnava region (32.7%).

Keywords: ecosystem services, plantation, fast-growing plants, agricultural land

1. Introduction

Human existence is dependent on ecosystems, their goods and services. Goods provided by ecosystems include food, feed, textile materials, pharmaceuticals. Ecosystem services necessary for human society represent e.g. maintenance of the hydrological cycle, air water filtration, climate regulation, storage and cycle of nutrients, provision of biotopes, cultural or spiritual values. Without these and many other goods and services, there is no real human life and no economic development at all (Kanianska et al., 2016).

The term ecosystem was introduced by the English ecologist Tansley in 1935. He understood it as a system in which all communities of organisms (both plant and animal) are interrelated, together with the complex of all physical and chemical factors that create the environment of these organisms. Over the years, the meaning of the term ecosystem has been modified, and even today it is possible to come across many definitions. For example, in Act no. 17/1992 Coll. about the environment, the ecosystem represents a functional system of living and non-living components of the environment, which are connected to each other by the exchange of substances, the flow of energy and the transfer of information, and which interact and develop in a certain space and time. In Act 543/2002 Coll. on nature and landscape protection, rocks, minerals, relief, soil, water, air, flora, fauna and anthropogenic objects and substances are considered to be components of the ecosystem.

The concept of ecosystem services arose as a response to global developments in the 20th century, associated with pressure on ecosystems, natural resource stocks and environmental quality. According to UN forecasts, the world population will exceed 9 billion (in 2050) inhabitants of the Earth and this will multiply the already existing pressure that is exerted on ecosystems, the environment, or natural resources provided to the human population. This will not be the case in Slovakia either (Považan, Filčák (eds.), 2020).

The concept of ecosystem services began to be accepted at the international level relatively recently, in the 1990s. The proposal of the Millennium Ecosystem Assessment (MEA) project, which applied the concept of ecosystem services (Kušíková, 2013), was approved. In the framework of MEA, ecosystem services were defined as benefits provided to human society by natural ecosystems, more broadly understood as ecosystem processes that sustain human life (MEA, 2005).

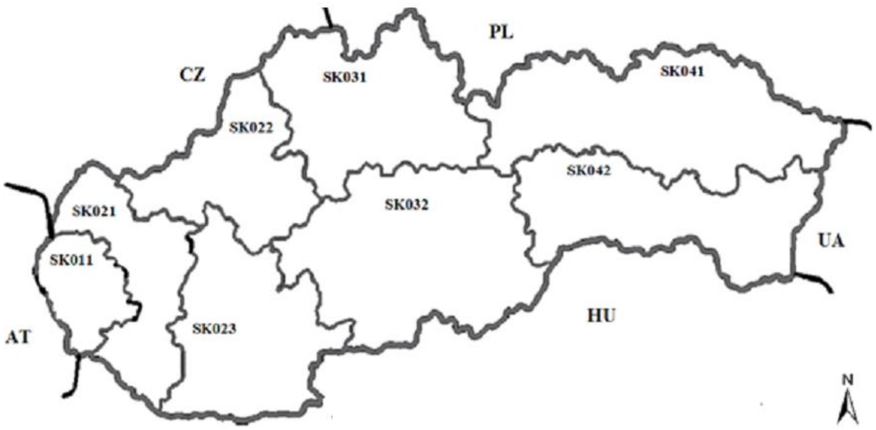
The aim of the presented contribution is the definition of plantations of fast-growing energy plants from the point of view of their ecosystem services, the identification of soils suitable for the establishment of plantations in the regions of Slovakia with an emphasis on the need to perceive ecosystem services as a factor that significantly affects human well-being.

2. Material and methods

The analysis of selected ecosystem services was carried out based on the classification according to Bastian and Grunewald (2015), while we started from the group of supply ecosystem services and also included a sociocultural ecosystem service, specifically information services. We also connected the analysis with the identification of ecosystem services according to Prokopová (2010), where we evaluated the ecosystem service in relation to a specific agroecosystem – a plantation of fast-growing energy plants. The analysis of ecosystem services is complemented by an overview study of the potential based on the research results of various authors.

Of the total area of Slovakia (4,903,500 ha), agricultural land occupies 2,401,693 ha, which represents 49.16%. For the analysis of the suitability of growing fast-growing plants at the level of the regions of Slovakia, the distribution of the area of Slovakia based on NUTS 3 (*Nomenclature of Territorial Units for Statistics*) was used. Based on the use of this methodology, the territory of Slovakia was divided into 8 regions (respecting the regionalization based on VÚC - 8 regions) (Figure 1).

The soil factor was expressed as the area of agricultural land suitable for growing fast-growing plants. The data were processed according to the database of the Soil Science and Conservation Research Institute as of 31.12.2019.



Legend: SK011 – Bratislava, SK021 – Trnava, SK022 – Trenčín, SK023 – Nitra, SK032 – Banská Bystrica, SK031 – Žilina, SK042 – Košice, SK041 – Prešov, AT – Austria, HU – Hungary, UA – Ukraine, PL – Poland, CZ – Czechia

Figure 1 Regions of Slovakia based on NUTS 3

3. Results and discussion

Plantations of fast-growing energy plants represent local agroecosystems in regions where they mainly perform production functions. Plantations of fast-growing energy plants primarily provide supply services in the form of biomass production, which subsequently has both energy and non-energy uses. This type of cultural ecosystem represents an alternative to growing mainly on agriculturally unused land (Gaduš et al., 2023).

In the analysis of agroecosystems of fast-growing energy crops, the concept of ecosystem services is used as a general term that includes the interconnection of ecological processes and the provision of benefits to humans (Mace et al., 2013).

It is about providing those ecosystem services that will benefit and benefit human society. As stated by Holland et al. (2015), much attention was paid to the analysis of ecosystem services of plantations of energy grasses Arundo donax, Miscanthus, as well as combinations of other energy grasses and short-rotation woody plants (Salix, Populus). The analysis confirmed that agro-ecosystems of fast-growing energy plants, which produce raw material for second-generation biofuels, have both positive and negative impacts on ecosystem services.

Bastian and Grunewald (2015) classify plantations of fast-growing energy plants in the group of renewable natural materials, namely renewable energy sources, from the point of view of the classification of supply ecosystem services. It is biomass produced from purposefully grown fast-growing energy plants and waste. The selected indicator is evaluated from the point of view of the produced crop (t.ha^{-1}) and the amount of energy

(MJ.ha^{-1}). From the perspective of socio-cultural ecosystem services, it is possible to include plantations of fast-growing energy plants also within the group of information services, specifically in the field of education and scientific knowledge, where it is an opportunity to acquire knowledge about natural relationships, processes and development, apply scientific research and technological innovations. As an example, it is possible to mention the functioning connection of natural, semi-natural ecosystems and agro-ecosystems in the country and the application of traditional technological procedures (traditions) in local (local) soil and climate conditions. The selected indicator evaluating this area defines the forms of land use and its naturalness.

Maes et al. (2013) state in the case of cultural ecosystem services according to international classifications (they can also be defined for plantations of fast-growing energy plants):

- educational and educational values (MEA provides a classification that is recognized worldwide and used in sub-global assessment),
- information for cognitive development (The economics of Ecosystem and Biodiversity "TEEB" provides an updated classification based on the MEA classification, which is used in national TEEB studies throughout Europe),
- other cultural outputs - existence and heritage - (Common International Classification of Ecosystem Services "CICES", which provides a hierarchical system based on the MEA and TEEB classification and which is adapted for the needs of accounting).

According to Prokopová (2010), we can assign plantations of fast-growing energy plants to the type of supply ecosystem service. It is a specific agroecosystem service: photosynthesis, primary and secondary production. The plant community plays an important role in the process of photosynthesis, the capture of solar energy by the surface of the leaves and the conversion of inorganic substances into organic ones. Indicators pointing to this process are Leaf Area Index (LAI), biomass and production. Another important service of the agroecosystem is the supply of biochemical and genetic material. It is about the differentiation of species and the preservation of diversity in the country. The role of plant communities lies in the creation and preservation of species. The indicator that defines this role is biodiversity at the level of communities, populations and genes. According to the author, the regulatory type of ecosystem service is also an important type, specifically the service: binding CO_2 (mitigating the greenhouse effect). The plant community plays an important role in the process of photosynthesis, the capture of solar energy by the surface of the leaves and the conversion of inorganic substances into organic substances and complements the importance in terms of carbon fixation in plants and in the soil. The indicator defining this role is biomass, production and carbon content in biomass and in soil.

Haines-Young and Potschin (2011) also define the class energy within the supply ecosystem services, within it the group biological renewable energy sources, the service type renewable energy sources of plant origin, and indicative benefits include firewood, energy plants (e.g. Miscanthus), oil, etc.

Cultivated biomass of fast-growing energy plants should replace non-renewable carbon materials and support carbon sequestration and other ecosystem services, such as improving soil and water quality, reducing erosion, and increasing biodiversity habitats (Evangelou et al., 2012).

Milner et al. (2016) confirmed that these ecosystems also provide regulatory services – protection against diseases and pests, pollination, improvement of soil and water quality. Compared to annual crops, plantations of fast-growing energy plants increase biodiversity due to the lack of seasonal tillage and reduced pesticide concentrations (Jørgensen, 2011).

The role of soil in fulfilling ecosystem services is unquestionable. Soil contributes significantly to ecosystem services, mainly through the functions it performs naturally. They bring direct and indirect benefit to people, fulfilling their needs. Soil is involved in ecosystem services mainly in the production of biomass, climate regulation (carbon storage), bioremediation of waste and toxic substances, protection against erosion and preservation of genetic resources (Kanianska et al., 2016).

Slovakia's share of biomass use is less than 1 %. The Slovak Republic developed and adopted the National Action Plan for renewable energy sources according to Directive no. 2009/28/EC on the promotion of the use of energy from renewable energy sources. In addition to waste biomass from agricultural and forestry production, in the regions of Slovakia it is planned to use targeted, for this purpose cultivated so-called energy trees and grasses. This targeted cultivation of the so-called of energy biomass will ensure the diversification of sustainable use of renewable energy sources at the regional level. Energy plantations are also directly related to the interaction of soil and biomass in relation to the sustainable use of the rural part of the country with the aim of strengthening the overall productivity of agriculture. The benefit of growing fast-growing energy plants is also the development of economic activities focused on the use of energy biomass, especially in rural areas and with regard to biodiversity and the environment (Kotrla et al., 2017; Kotrla, Prčík, 2018).

According to Act no. 220/2004 Coll. on the protection and use of agricultural land and on the amendment of Act no. 245/2003 Coll. on integrated prevention and control of environmental pollution and on amendments and additions to some laws of the Slovak Republic, all agricultural lands are classified into 9 soil quality groups according to their affiliation to the BPEJ. The best quality belongs to the 1st group and the least quality to the 9th group. For the cultivation of fast-growing energy plants in the conditions of Slovakia, those soils that are included in the 5-9 soil quality group are suitable. The area of soil quality group 5-9 is represented at the level of all regions of the Slovak Republic. The highest potential in the cultivation of fast-growing energy plants with regard to specific land areas at the regional level is in Prešov region (99.95 %), Žilina region (99.89 %), Košice region (99.51 %), Banská Bystrica region (98.9 4%) and the Trenčín region (83.02 %). Regions with an area of soil quality group 5 to 9 below 50 % of the total area of agricultural land include the Bratislava region (46.35 %), Nitra region (39.56 %) and Trnava region (32.7%) (Mandalová et al., 2017).

To ensure food self-sufficiency, Slovakia must have a minimum area of agricultural land of 1,367,853 ha, which represents about 56% of currently registered agricultural land in Slovakia. In addition to primary land, the soil fund in Slovakia consists of secondary land, which occupies 696,038 ha, which represents about 29 % of the currently registered agricultural land in Slovakia, and the rest of the land, which should preferably be used for alternative agricultural use, for growing fast-growing energy plants and for various non-biological purposes (sports, tourism and recreation). The other agricultural land fund occupies 368,587 ha, which represents about 15 % of currently registered agricultural land in Slovakia (Figure 2).

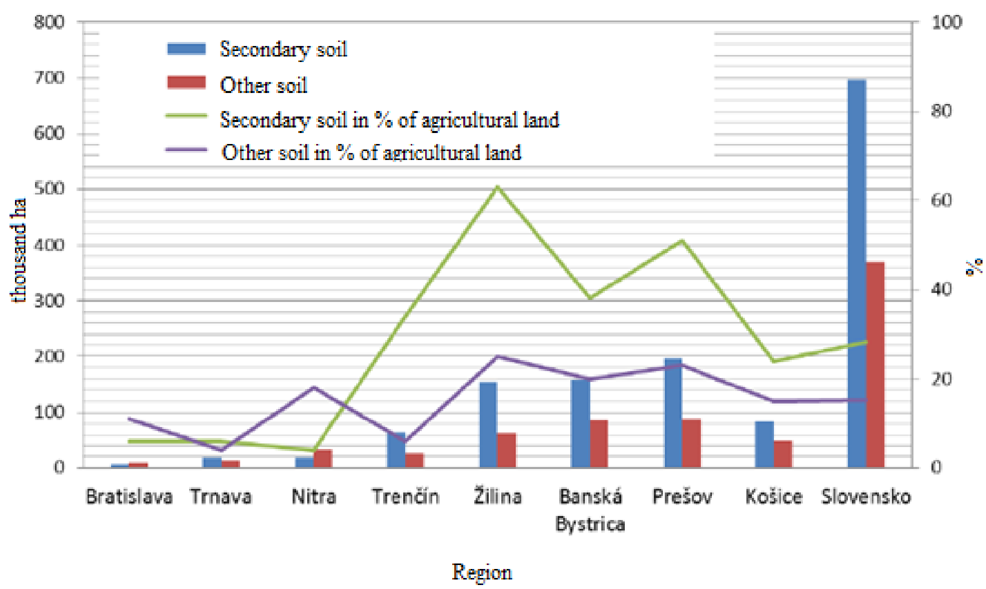


Figure 2 The area of agricultural land usable for the cultivation of fast-growing energy plants in the regions of Slovakia (Prčík, 2021)

Intensive cultivation of fast-growing plants for energy use must be carried out on areas that can be used for this purpose. These are areas that are not directly linked to food production. On the other hand, it is the fact that by growing fast-growing energy plants, they could obtain a higher degree of economic and ecological use (Gaduš et al., 2023).

From the point of view of diversifying the use of alternative energy sources, purposefully grown fast-growing energy plants are important. Cultivation of such plants on agricultural land also depends on other factors, such as geographical, ecological and climatic, in individual regions, cultivation technologies and intensification factors (Grundmann et al., 2013).

4. Conclusion

Plantations of fast-growing energy plants represent local agroecosystems in regions where they mainly perform production functions. From the point of view of the classification of supply ecosystem services, they are included in the group of renewable natural materials, namely renewable energy sources. From the perspective of socio-cultural ecosystem services, it is possible to include plantations of fast-growing energy plants also within the group of information services, specifically in the field of education and scientific knowledge, where it is an opportunity to acquire knowledge about natural relationships, processes and development, apply scientific research and technological innovations. Another important service of the agro-ecosystem of plantations of fast-growing energy plants is the supply of biochemical and genetic material. It is about the differentiation of species and the preservation of diversity in the country. The role of plant communities consists in the creation and preservation of species, plantations also provide regulatory services - protection against diseases and pests, pollination, improvement of soil and water quality. Compared to annual crops, plantations of fast-growing energy plants increase biodiversity due to the lack of seasonal tillage and reduced pesticide concentrations. The area of soil quality group 5-9 is represented at the level of all regions of the Slovak Republic. The highest potential in the cultivation of fast-growing energy plants with respect to specific land areas at the regional level is in the Prešov region (99.95 %), the Žilina region (99.89 %), the Košice region (99.51 %), the Banská Bystrica region (98, 94 %) and the Trenčín region (83.02%). Regions with an area of soil quality group 5 to 9 under 50 % of the total area of agricultural land include Bratislava region (46.35 %), Nitra region (39.56 %) and Trnava region (32.70 %).

Acknowledgment

This study was supported by the project Visegrad Grants ID #22310328 and by the Science Grant Agency under the contract No. VEGA 1/0559/23.

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POSTER SECTION

https://doi.org/10.15414/2024.9788055227559

VISUALIZING COLOR PROFILES OF *AGARICUS BISPORUS* BY E-EYE

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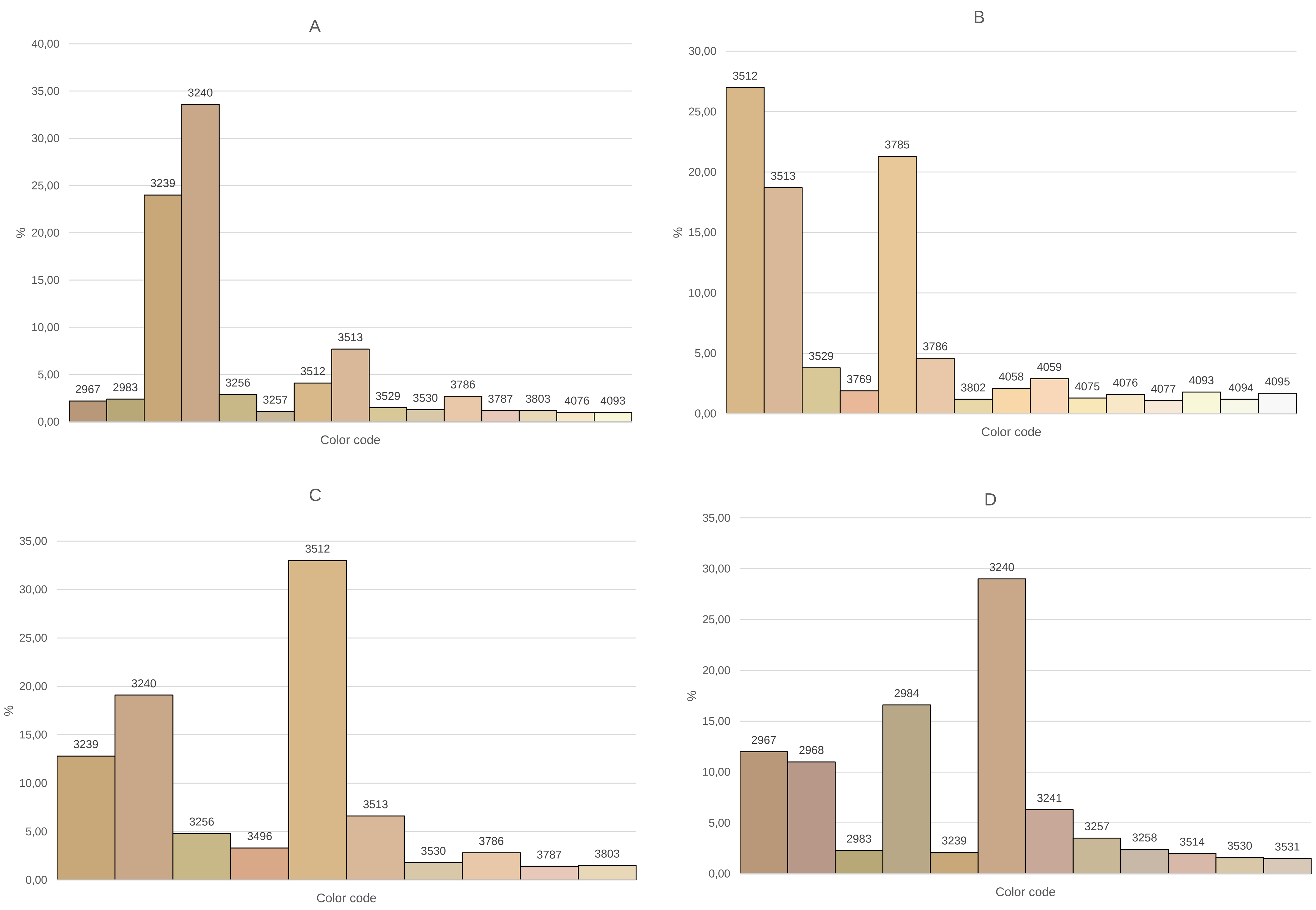
INTRODUCTION

Agaricus bisporus, commonly known as champignon or button mushroom, is one of the world's most popular and widely cultivated edible fungi (Muzzarelli et al., 2012). The button mushroom is highly valued for its versatility, mild flavor and culinary adaptability (Cardoso et al., 2021). The color of the button mushroom plays a crucial role in its visual appeal, consumer preference, and marketability (Castellanos-Reyes et al., 2021). Understanding and quantifying its color profile can provide valuable insights into its quality, freshness, and even nutritional content. Moreover, advancements in technology, such as electronic eye systems, have enabled precise measurement and visualization of the color profiles of *Agaricus bisporus*. The aim of this study was the evaluation of color profiles of *Agaricus bisporus* using electronic eye IRIS VA400.



MATERIAL AND METHODOLOGY

- Four samples of white canned *Agaricus bisporus* (A-D) in salty brine were purchased from the Slovak market.
- Only the caps of samples were evaluated for the color profiles using an electronic eye (e-eye) IRIS VA400 (Alpha M.O.S., France.
- Only colors presented in amount >1% were identified.
- Three fruiting bodies were randomly selected from each sample (A-D) and color profiles were obtained in triplicates (n=9) and visualized using AlphaSoft programme (Alpha M.O.S., France) (Štefániková et al., 2019).

RESULTS & DISCUSSIONS

The color profiles of evaluated samples are presented in Figure 1. There were 15 colors identified in samples A and B. The least colors were identified in sample C (10 colors). Color coded as 3240 was the major color in sample A (33.6%) and D (29.0%) and the 2nd major color in sample C (19.1%). On the other hand, color coded as 3512 was identified as major color in samples B (27.0%) and C (33.0%). To authors best knowledge there are no studies evaluating the color profiles of canned *Agaricus bisporus* using an electronic eye. Only a limited number of studies have evaluated the color of white *Agaricus bisporus* using Chroma Meter (Mohapatra et al., 2010) and image processing techniques (Zhao et al., 2023), primarily focusing on fresh samples.

CONCLUSION

- In this pilot study we evaluated color profiles of four (A-D) white canned *Agaricus bisporus* (champignon) mushroom samples.
- Electronic eye IRIS is able to analyze and visualize the color profiles of mushroom samples.
- The color profiles of evaluated samples had different color representation in the range 10-15 colors with percentage representation >1%.
- In the future we plan to use the e-eye to evaluate mushroom color profiles.

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ACKNOWLEDGEMENTS

This research was funded by the project of Ministry of Education, Research, Development and Youth of the Slovak Republic project VEGA No. 1/0602/22 „Possibilities of elimination of xenobiotics in edible forest fruits from environmentally polluted areas„, we thank the Operational Program Research and Innovation: "Support of research activities in the ABT RC" 313011T465, co-financed by the European Regional Development Fund, and the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

https://doi.org/10.15414/2024.9788055227559

SCIENCE OF YOUTH 2024

18th International Scientific Conference

May 30th-31st, 2024, Nitra, SLOVAKIA

FLAVORED HONEY AS A MARKET DRIVER: EXPLORING PERCEPTIONS AND PREFERENCES FROM YOUNG WOMEN'S PERSPECTIVE

Kristína Predanócyová, Peter Šedík
Slovak University of Agriculture in Nitra

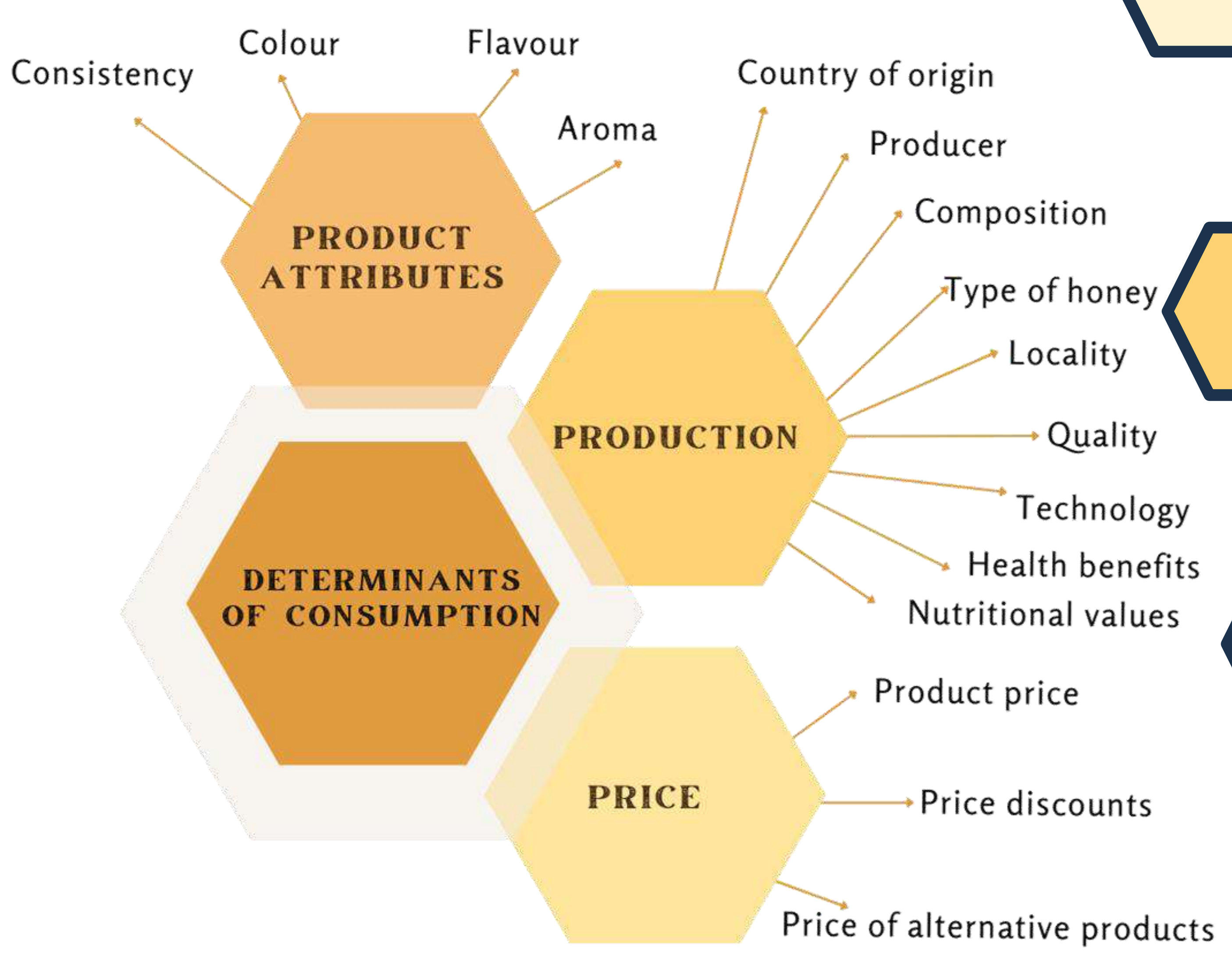
INTRODUCTION

Recently, the honey market has been determined by several consumer trends and faces many challenges, mainly in terms of sustainability, locality, guarantees of origin and quality, and health. It is known that the consumption of honey has a decreasing tendency, especially among young consumers. To support the consumption of honey, it is advisable to implement measures that will be acceptable to consumers, especially the younger generation. One of the possibilities is to enrich honey with various flavors that have a beneficial effect on the health of consumers and are attractive to consumers, and the flavored honey can be a substitute for spreads, jams, or it can be used to flavor yogurts. For the future development of the honey market and the launch of new flavored honeys, it will be crucial to understand young consumers, their taste preferences, their perception of trends in gastronomy and nutrition, or their perception of innovation in the food industry. Based on the mentioned, the aim of the research is to identify consumer acceptance and factors determining the potential consumption of flavored honeys from the point of view of young women.

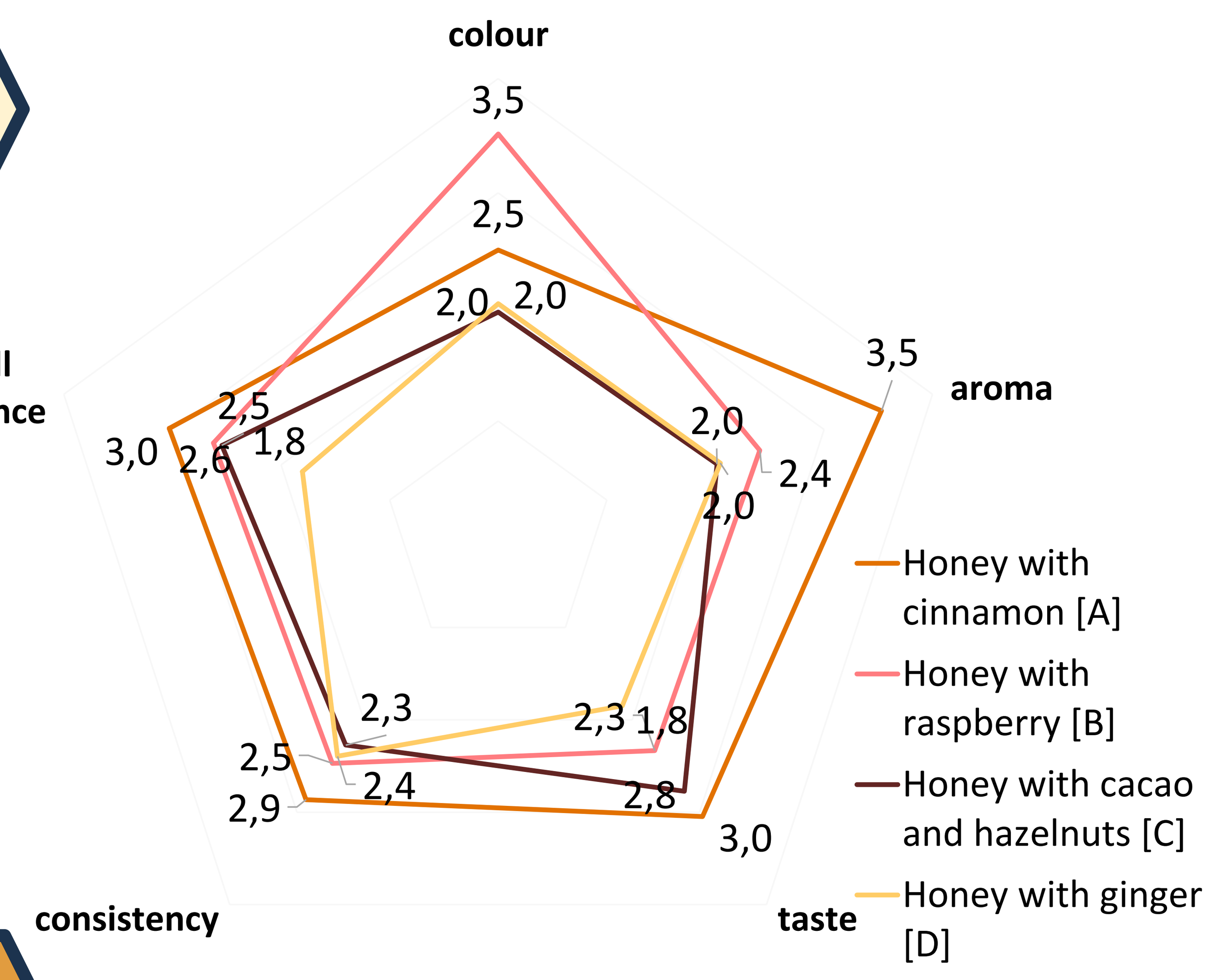
RESEARCH

The research is based on a consumer survey extended by the sensory evaluation of samples of flavored honeys. The research was carried out in person in 2023 on a sample of 66 young women under the age of 35. The aim of the research is to identify consumer behavior and determinants of the purchase and consumption of flavored honeys, as well as to reveal the preferences of young women towards flavored honeys on a sample of 4 flavored honeys (cinnamon, ginger, raspberry, cocoa) and the attributes of the honeys were evaluated, specifically taste, aroma, color, consistency and overall acceptance.

RESULTS



- low consumption of honey
- consumer awareness of the existence of flavored honey
- ingredient into cereal and oat porridge
- honey enriched with fruit
- up to 6 Euros per 250 g packaging
- recommendations



CONCLUSION

The research is a reflection on current trends in the honey market and the related challenges it faces. The results of the research indicate that young women are aware of the existence of flavored honey and most of them have already had the opportunity to consume flavored honey, but especially with propolis, royal jelly, pollen, ginger, or cinnamon. From the point of view of sensory evaluation, fruit-flavored honeys with raspberries were positively evaluated. Among honeys with spices, honey with cinnamon was the best rated. The key determinant of the purchase and consumption of flavored honey is quality, important reasons for consumption are taste and health benefits, and in terms of attractiveness of use, it is suitable for spreads or as an addition to cereal or oat porridge. The research results are key for further research in the field of flavored honeys, but also for producers in the development of new products and their launch on the market, including the creation of a suitable marketing strategy. It is important that consumers should have an increased awareness of the existence of flavored honeys and their health benefits, therefore the results are also applicable for policy makers and public health support. In the future, it is possible to extend the research to other age generations and focus on identifying differences between different age cohorts.

ACKNOWLEDGEMENTS

This publication was supported by grant number 14-GASPU-2021 "Analysis of consumer behaviour towards honeys enriched with health-promoting substances", by support within the operational program Research and Innovation for project: Support of research activities in RC ABT, 313011T465, co-financed from of the European Regional Development Fund and by the Operational Program Integrated Infrastructure within the project: Demand-driven Research for the Sustainable and Innovative Food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

https://doi.org/10.15414/2024.9788055227559

SCIENCE OF YOUTH 2024

18th International Scientific Conference

30 – 31 May 2024, Nitra, SLOVAKIA

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Crown reduction as potential tool in alleviating drought stress in initial phase of seedlings development

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INTRODUCTION

The increasing frequency and severity of droughts worldwide, driven by climate change, significantly impact forest ecosystems. These changes pose a particular threat to forest establishment, particularly affecting seedlings—both planted and naturally regenerated. The successful development and growth of high-productivity forest stands are significantly dependent on the quality of these seedlings. The most critical period during reforestation is when the seedlings adjust their morphology and physiology to adapt to new environmental conditions. Transplanted seedlings are exposed to conditions that differ significantly from those in the nursery environment, which presents unique challenges for their initial adaptation and survival. Low-cost and easily applicable techniques are essential in reforestation efforts. In this study, we examine the practice of reducing crown length. Our assumption is based on the observation that trees typically possess more leaves than are necessary for their normal development. The most readily observed effects of dry conditions on seedlings are changes in their growth and survival. Therefore, to explore how CR (crown reduction) might alleviate drought effects in a controlled environment, we employed automatic dendrometers in an experiment on two contrasting tree species: broadleaf sessile oak (*Quercus petraea* (Matt.) Liebl.) and conifer Norway spruce (*Picea abies* (L.) Karst. Dendrometers provide a valuable tool for monitoring the water status of seedlings, including tree water deficit (TWD), which indicates reliance on stem water reserves.

Our study evaluated the following hypothesis:

(i) CR treatment is expected to enhance soil water availability by reducing the water use of seedlings;

(ii) Seedlings subjected to CR will exhibit improved stem water status, consequently leading to a decrease in TWD;

(iii) Under CR treatment, seedlings growing in conditions with reduced water availability, will achieve growth rates comparable to those of seedlings not receiving this treatment.

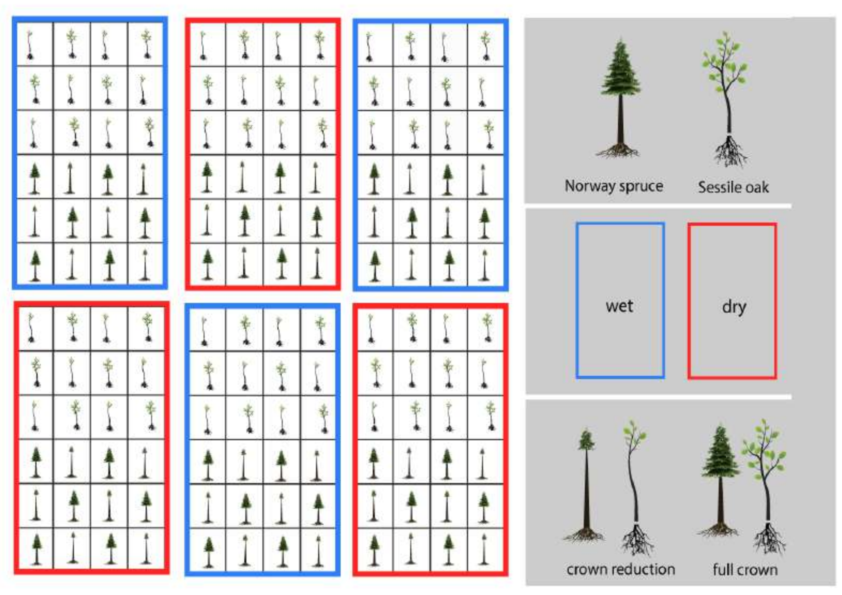

Figure 1. Design of experiment. Blocks circled in blue and red indicate containers with pots of well-watered (wet) and water-stressed (dry) seedlings, respectively.

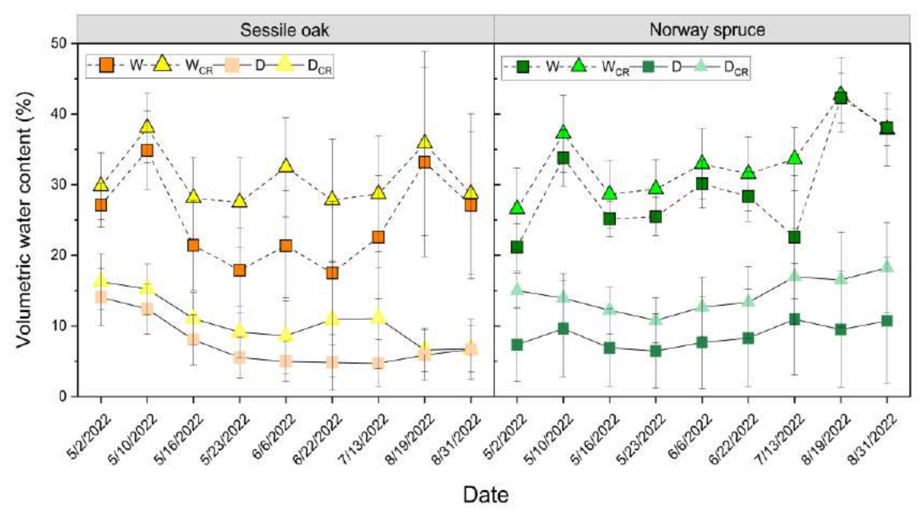

Figure 2. Volumetric water content – (VWC; %).

MATERIALS & METHODS

- The experiment was designed for 3-year old seedlings of Sessile oak and Norway spruce
- The study was conducted on the campus of the Czech Globe (Brno, Czech Republic 49°11'N, 16°35'E).
- Seasonal totals of approximately 800 mm for the wet treatment and approximately 200 mm for the dry treatment, covering the period from March until October (2022).
- Immediately after planting, the seedlings were equipped with automatic dendrometers (PDS40P, EMS Brno, Czech Republic) to monitor continuous stem diameter variations. These variations were observed at a resolution of 0.62 µm, with measurements taken and stored at 1-hour intervals

RESULTS & DISCUSSION

- The seedlings watering scheme significantly increased VWC in wet conditions compared to dry, with levels nearly four times higher ($p < 0.0001$; **Fig. 2**). CR treatment consistently increased VWC in both wet and dry conditions for both species; in wet conditions, the increase ranged from 12-24%, while in dry conditions, the differences were even greater, ranging from 42-67%.
- In sessile oak seedlings, the highest GRO values were observed under wet treatment conditions, while significantly lower values were recorded under dry conditions ($p < 0.001$; **Fig. 3a**). Despite different moisture levels, CR treatment did not significantly affect sessile oak GRO in either wet ($p = 0.379$) or dry conditions ($p = 0.983$). TWD in sessile oak was significantly higher under dry conditions ($p < 0.001$; **Fig. 3b**). CR treatment led to a significant increase in TWD, regardless of whether conditions were wet or dry ($p < 0.001$). Finally, the lowest TWD values were observed under wet conditions without CR, while the highest was in dry conditions with CR (**Fig. 3b**).
- Norway spruce seedlings exhibited comparable GRO across both wet and dry conditions, regardless of CR application (**Fig. 4a**). Notably, seedlings in dry conditions without CR significantly underperformed compared to those in wet conditions, with or without CR ($p < 0.05$). Concerning TWD, only dry conditions resulted in a significant increase for seedlings without CR ($p < 0.001$), while CR-treated seedlings showed TWD levels comparable to those in wet conditions ($p > 0.05$).
- Seedlings growth observations revealed that Norway spruce seedlings initiated growth earlier and sustained it for longer than sessile oak (**Fig. 5**). Sessile oak seedlings experienced 81 growing days under wet conditions and 58 under dry conditions. Regardless of moisture, CR led to a consistent duration of 55 growing days in sessile oak. In contrast, Norway spruce seedlings maintained longer durations across all treatments, with CR extending the number of growing days to 110 in wet conditions and 122 in dry conditions. Notably, under CR, spruce seedlings growing days were up to twice as long as those of sessile oak (**Fig. 5**).

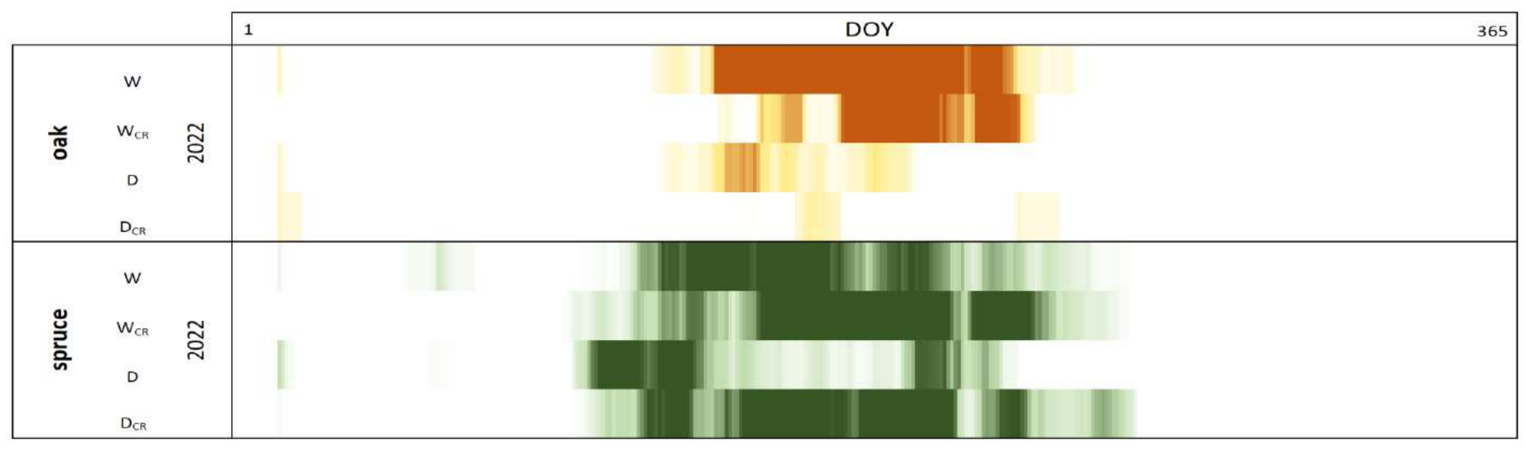

Figure 5. Displays the annual growth phenology (number of growing days) for sessile oak and Norway spruce seedlings within all different applied treatments during the growing season 2022.

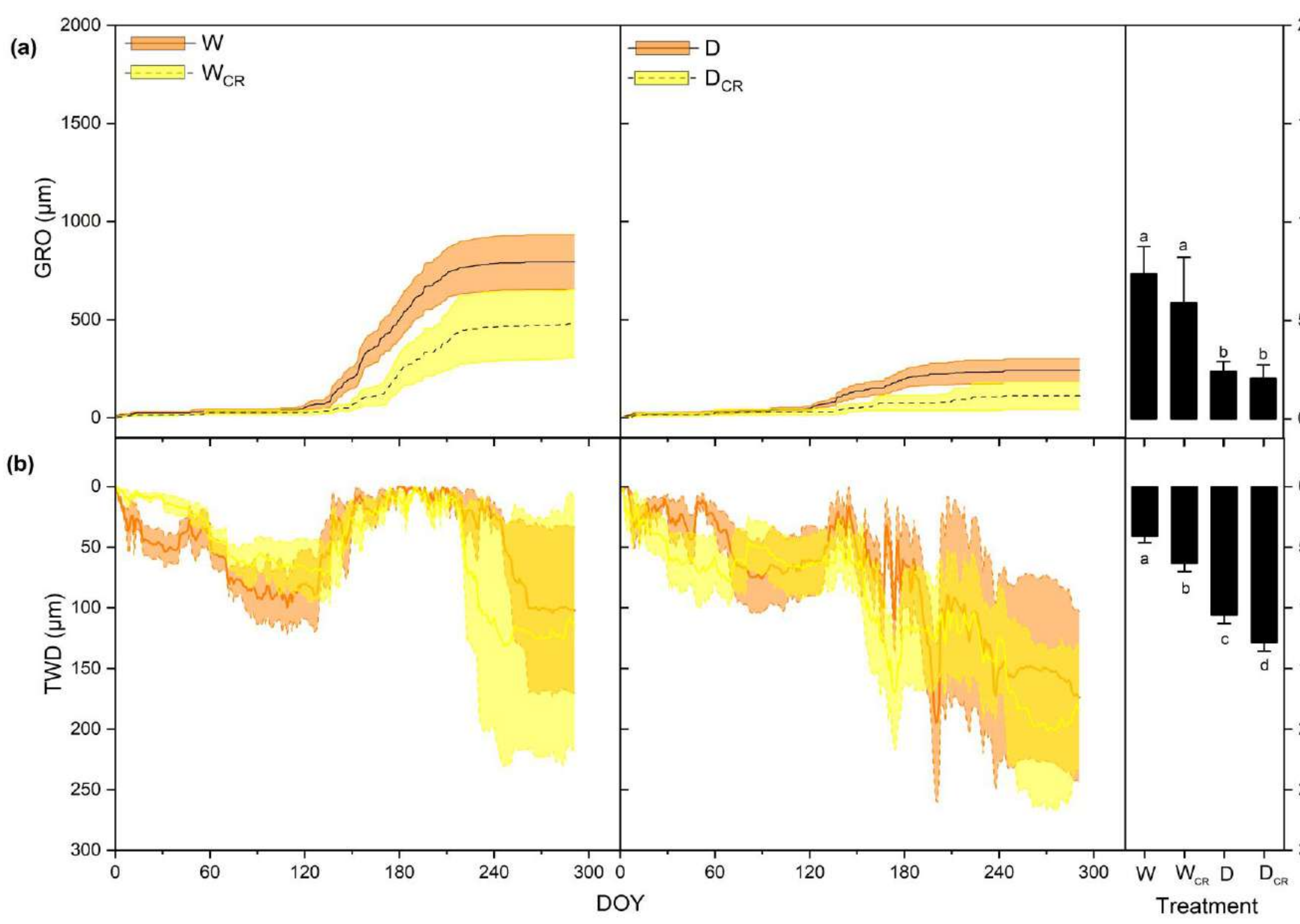

Figure 3. Seasonal growth dynamic of sessile oak seedlings in the growing season 2022.

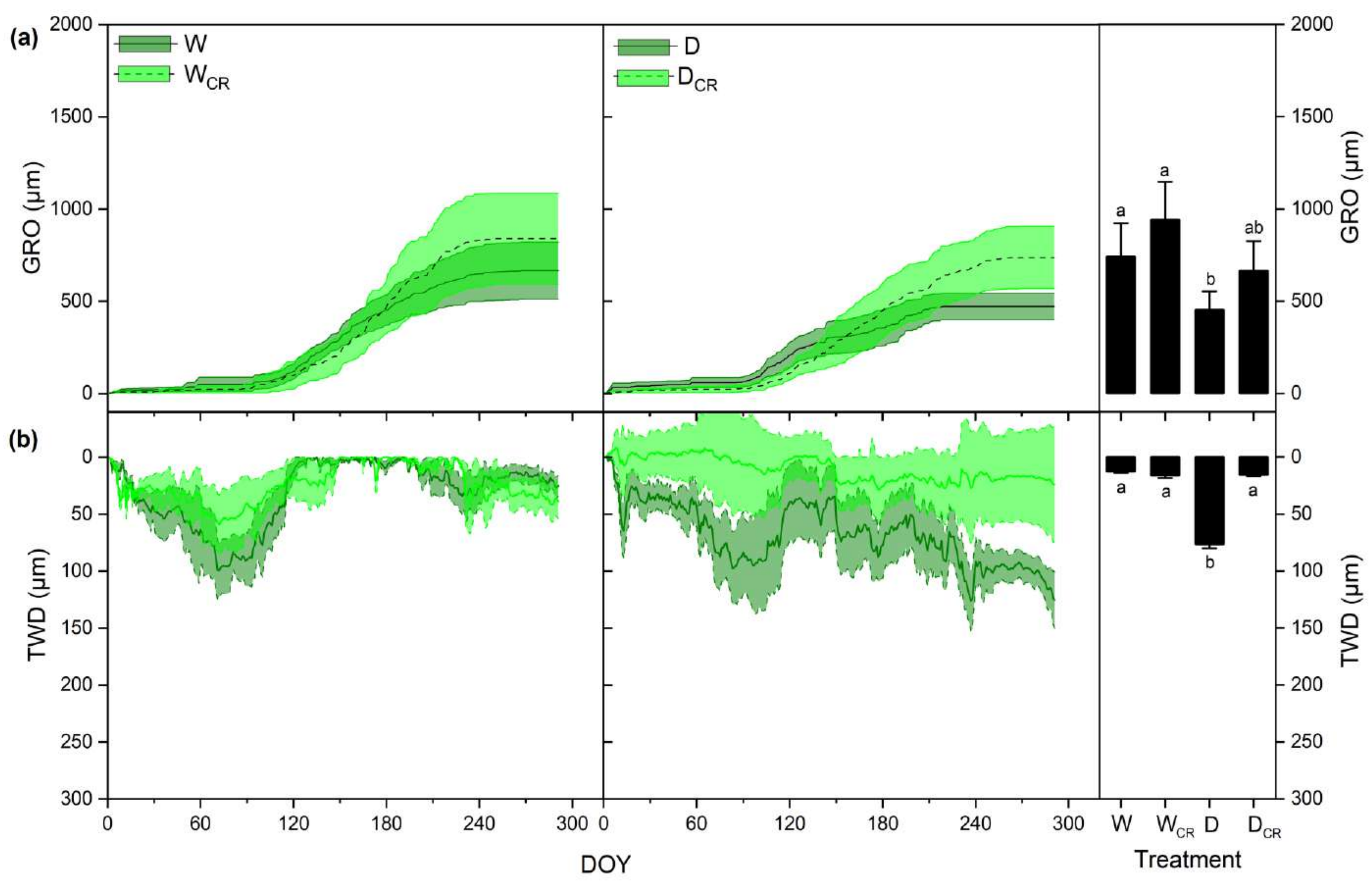

Figure 4. Seasonal growth dynamics of Norway spruce seedlings in the growing season 2022.

CONCLUSION

Our study investigated how CR and drought conditions affect the growth and drought resilience of Norway spruce and sessile oak seedlings, conducted in pot experiments. We found that CR significantly enhances drought stress tolerance in Norway spruce, increasing the number of growth days under both wet and dry conditions. Conversely, this treatment did not benefit sessile oak seedlings and adversely affected their growth when applied outside of drought conditions. The results highlight the importance of selectively applying CR, suggesting its use should be limited to periods of anticipated drought to avoid potential negative impacts on growth. While the treatment shows promise, especially for Norway spruce, field experiments are necessary to confirm these results and to test the efficacy of CR on different species. This research highlights the need for simple and cost-effective strategies to enhance seedling survival and establishment as climate change and large-scale disturbances continue to challenge forest ecosystems. A deeper understanding of how seedlings respond to environmental stressors is crucial for developing effective management strategies and selecting suitable species for reforestation projects across Europe. These insights are essential for forest managers tasked with mitigating abiotic stresses and adapting to changing climatic conditions.

ACKNOWLEDGEMENTS

This research was funded by IGRAČEK MENDELU project CZ.02.2.69/0.0/0.0/19_073/0016670, with registration number SGC-2021-013. J.A. benefited from the Internal Grant Agency of Mendel University in Brno under grant number 040/2021.

https://doi.org/10.15414/2024.9788055227559

SUA Slovak University of Agriculture in Nitra

SCIENCE OF YOUTH 2024

18th International Scientific Conference
30 - 31 May 2024, Nitra, SLOVAKIA

WHAT PROPERTIES CAN ESSENTIAL OILS SHOW AGAINST SPANISH SLUGS

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INTRODUCTION

About 150 species and varieties of cultivated plants are damaged by slugs (Kozłowski (2005). For 10 years, Slovakia has been fighting the most dangerous Spanish slug (Zając et al. (2017), Cejka et al. (2006)).

The Spanish slug (*Arion vulgaris*) can grow up to 6-15 centimeters in length, has mainly a bright brown, yellowish, sometimes gray color with stripes on the body. They have become a common problem in many European countries, including Slovakia. These slugs cause significant damage to gardens, crops and ecosystems, making them a real threat to gardeners, farmers and environmentalists (Adomaitis et al. 2022).

Land mollusks are one of the most destructive agricultural pests worldwide, the management of which depends on synthetic molluscicides. However, many of these molluscicides are harmful to nontarget organisms. Hence, there is a need to develop alternative ecofriendly molluscicides that are less impactful toward nontarget organisms. Thus the necessity to evaluate essential oils as natural repellent and (or) attractant substances were appeared (Abobakr et al. (2022).

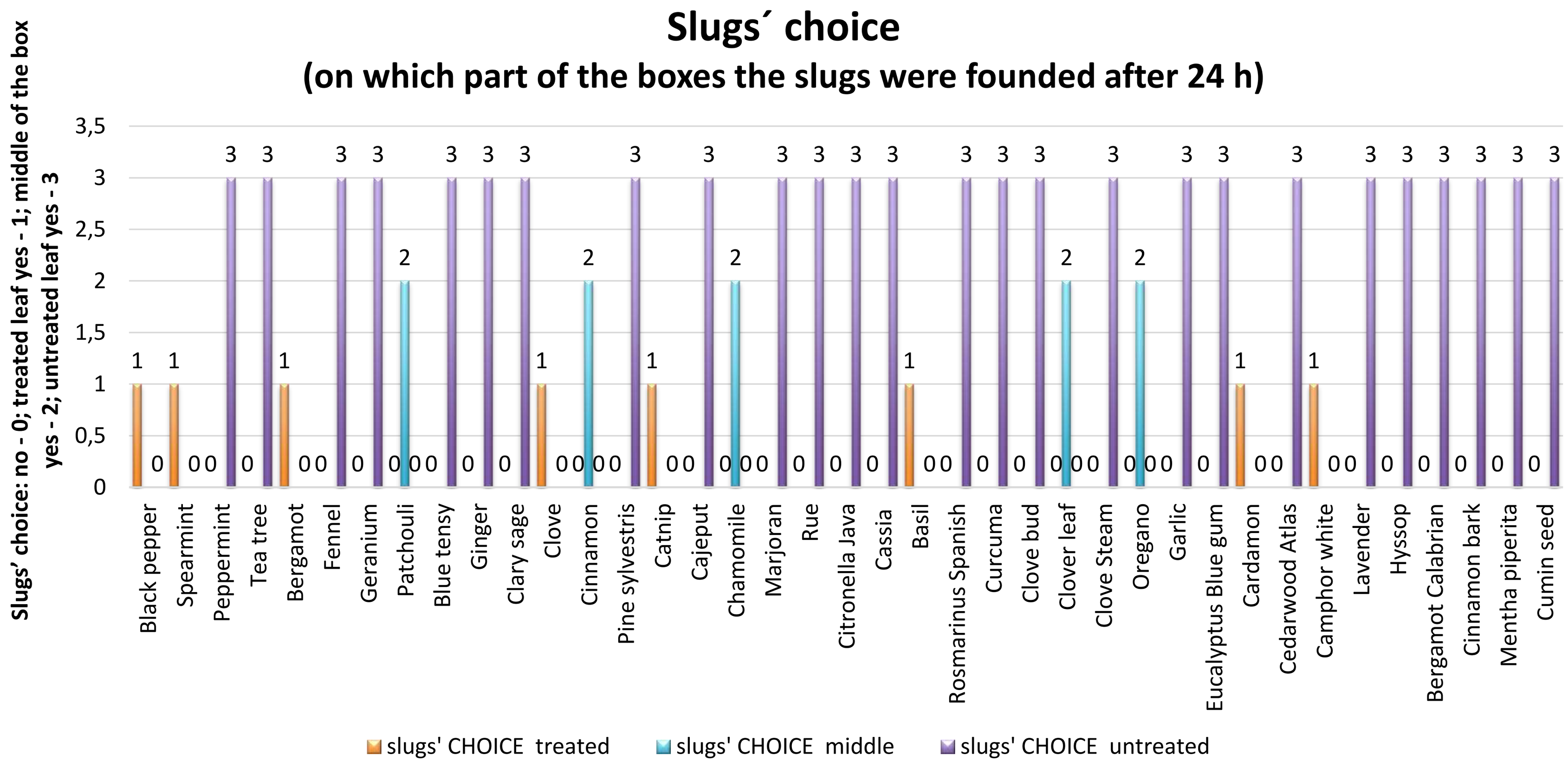

Figure 1. Slugs´ choice (on which part of the boxes the slugs were founded after 24 h)

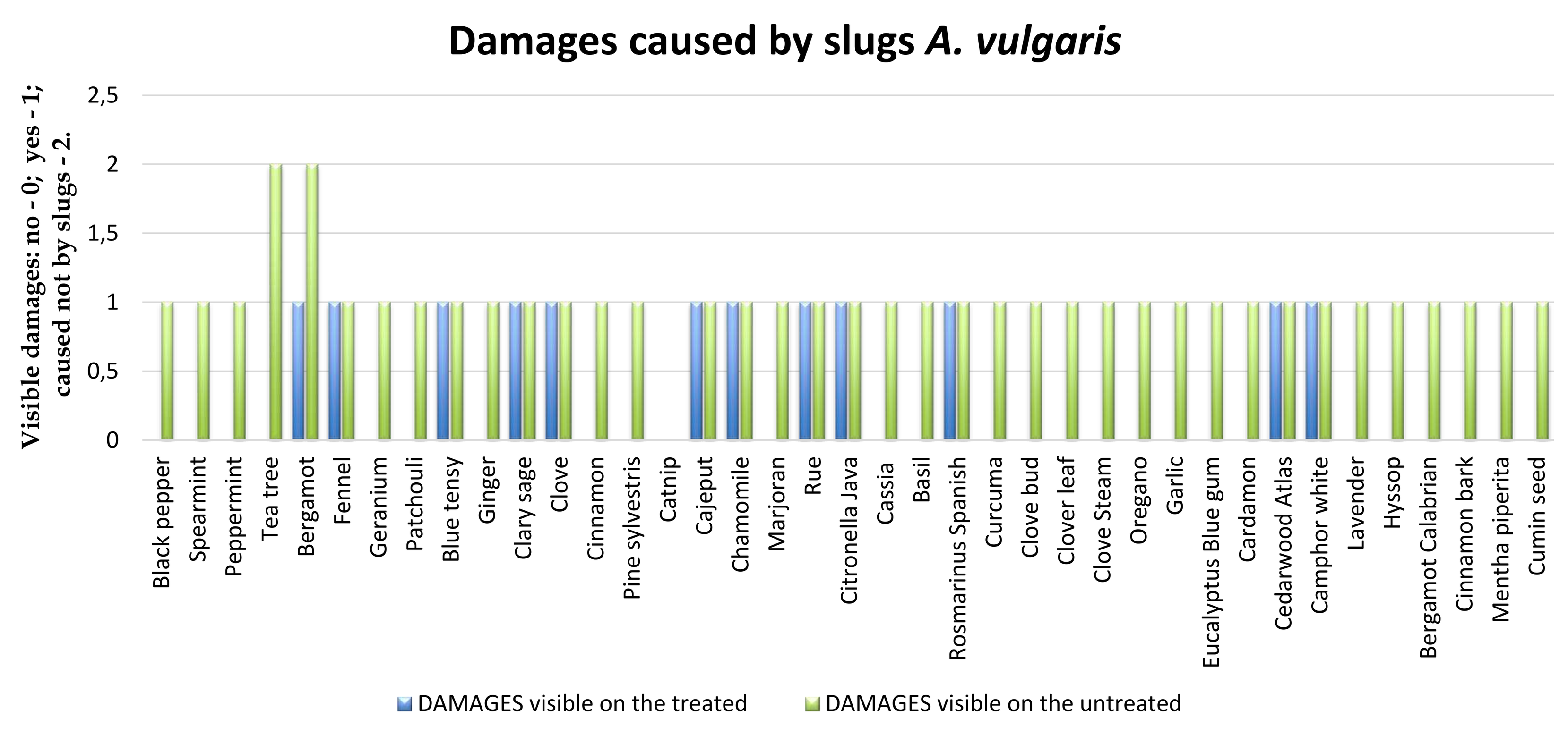

Figure 2. Damages caused by slugs *A. vulgaris*

RESEARCH

This study was carried out at the Laboratory of Entomology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra.

The populations of Spanish slugs *A. vulgaris* (Stylommatophora: Arionidae) in amount of 117 adults were collected from the ground at a local field of the crop garden in Nitra (Slovakia) 48°18'16.7"N 18°05'41.3"E in May 2023. The adults of both sexes were used in the experiments.

The study was carried out under the laboratory conditions at the temperature of 20 ± 1°C, humidity 70 ± 1%, and /light/dark 16:08/ h. Essential oils were obtained from a Mystic Moments Inc. (UK) as commercial essential oils.

The experiment was conducted using plastic boxes 20 x 10 cm. The bottom of each box was covered by wet cotton to avoid the desiccation of slugs. Fresh lettuce leaves were divided on two pieces: one was without essential oil solution, second was marked by paper clip and sprayed by 1% essential oils solution in quantity 10 mL. It was used 39 essential oils. One slug was placed in the middle of the box; the box was covered by nylon mesh to avoid the slugs' escape. The experiment consisted of 3 replications (pic. 1 and 2).

RESULTS & DISCUSSIONS

Essential oils showed diverse effects on slugs' choice, same as on the nutritional preferences of slugs. According the obtained results three oils showed **attractant properties**, slugs were obtained on the treated leaf and damages were visible on these treated leaves: *Citrus bergamia* (bergamot), *Eugenia caryophyllata* (clove) and *Cinnamomum Camphora* (Camphor white). *Matricaria chamomilla* essential oil also showed attractant properties with only difference that slugs were choose the middle of the box, but damages were available on the treated leaf.

Absolutely **repellency properties** e.g. when slugs choose untreated leaves and damages were visible only on untreated leaves showed 17 essential oils: Mentha piperita Peppermint, Pelargonium graveolens, Zingiber officinale, Pinus sylvestris, Origanum majorana, Cinnamomum cassia, Curcuma longa, Eugenia caryophyllata (bud and steam), Allium sativum, Eucalyptus globulus, Lavandula angustifolia, Hyssopus officinalis, Citrus bergamia Calabrian, Cinnamomum zeylanicum, Mentha piperita var. officinalis Sole, Cuminum cyminum.

Picture 1. Experimental boxes with slugs and EOs solutions

Picture 2. Lettuce leaves sprayed by *Melaleuca cajuputi* EO.

CONCLUSION

In conclusion, slugs choose treated by black pepper, spearmint, bergamot, clove, catnip, basil, cardamom and camphor white essential oils leaves. *A. vulgaris* were ostend in the middle of the research box after using patchouli, cinnamon, chamomile, clover leaf and oregano essential oils. All the rest oils were not chosen by slugs, means showed repellent activity.

ACKNOWLEDGEMENTS

This research was financially supported by the Operational Program Integrated Infrastructure, cofinanced by the European Regional Development Fund (ERDF) (project Smart Farm 313011W112).

https://doi.org/10.15414/2024.9788055227559

SCIENCE OF YOUTH 2024

18th International Scientific Conference
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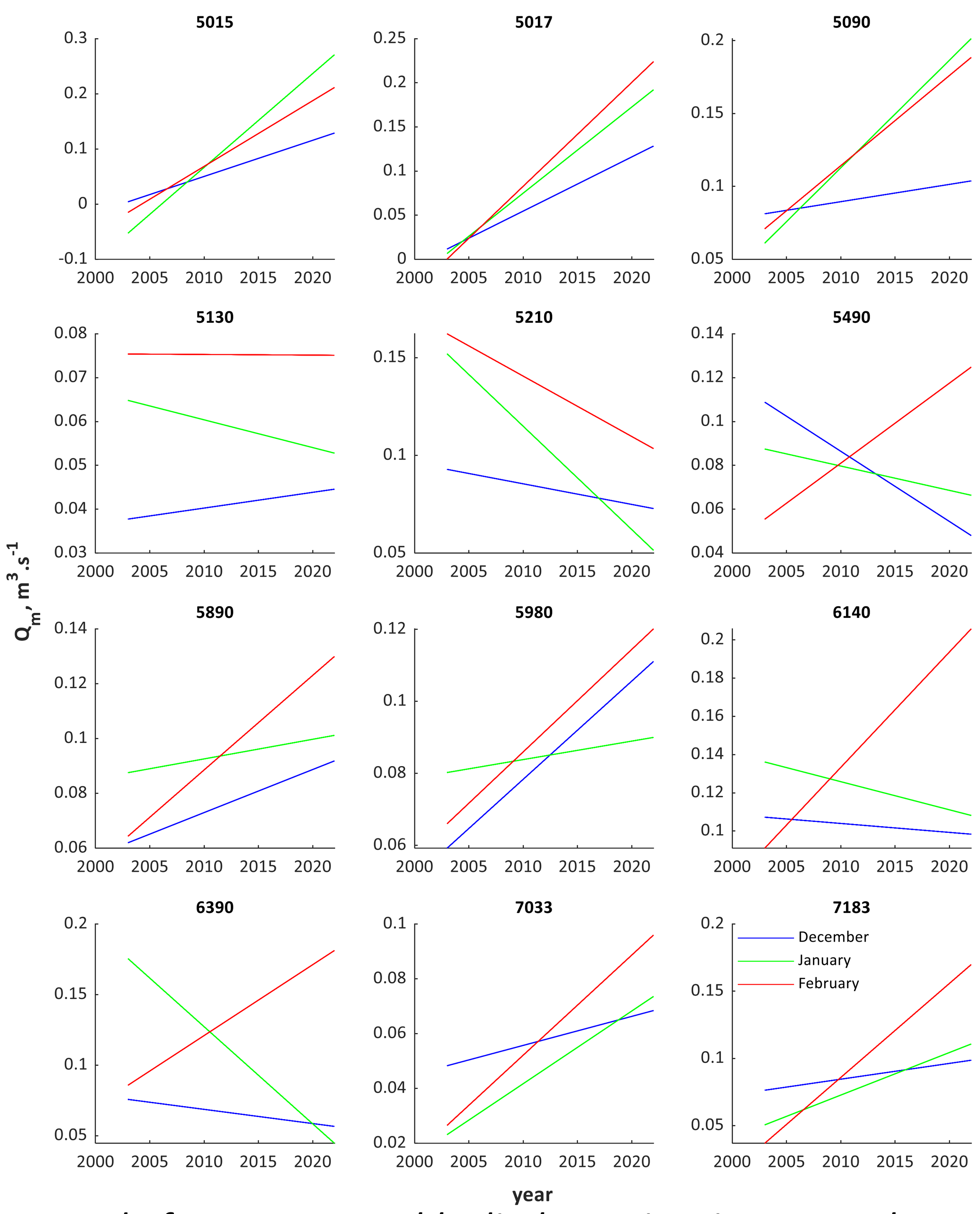
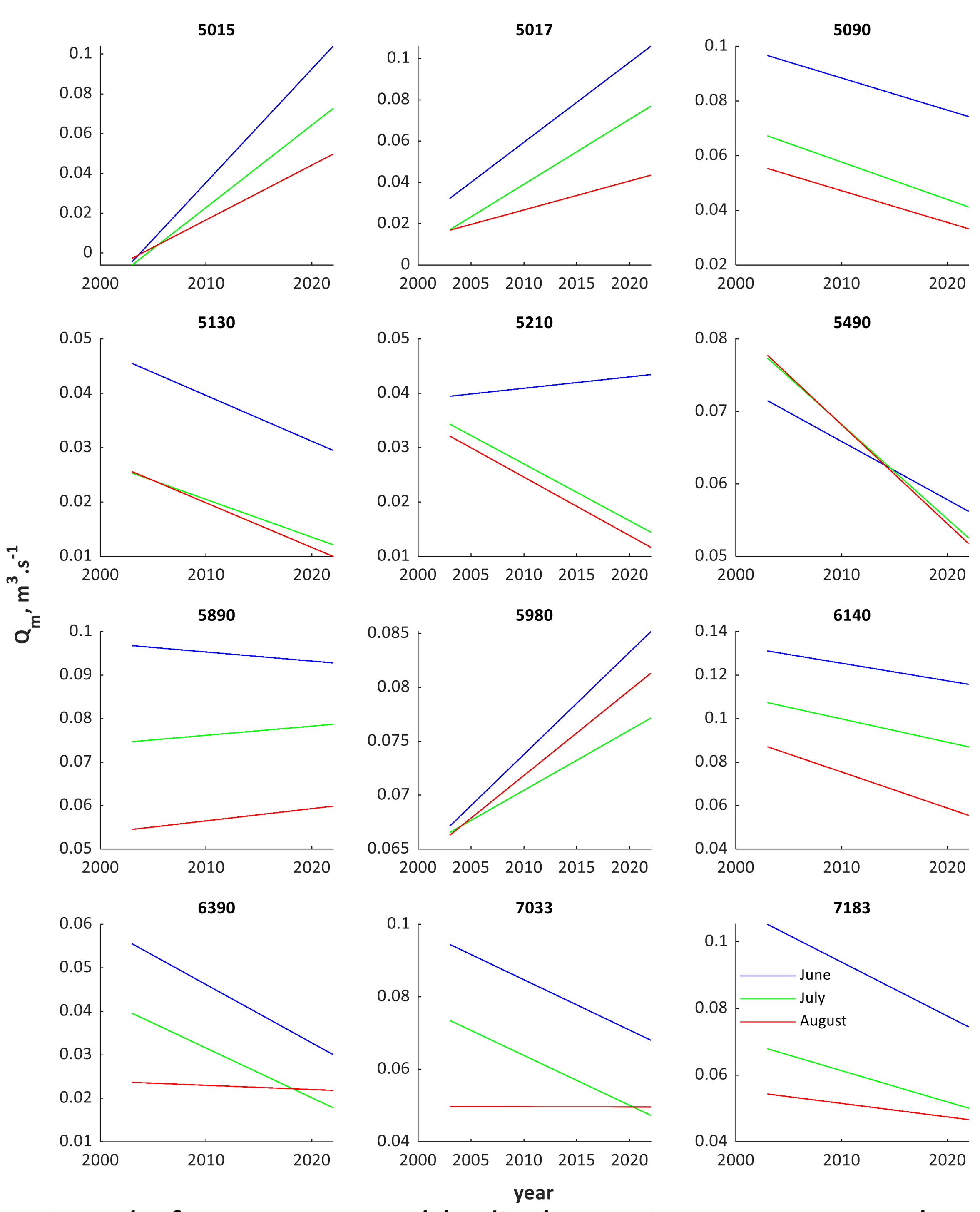
SPU Slovenská poľnohospodárska univerzita v Nitre
SPU·FZKI Fakulta záhradníctva a krajinného inžinierstva

TREND OF MONTHLY DISCHARGE IN THE CHATCHMENTS UNDER 10 km^2

Tatiana Kaletova
Slovak University of Agriculture in Nitra



Trend of average monthly discharge in winter months



Trend of average monthly discharge in summer months

INTRODUCTION

Anthropogenic climate change has influenced mean and extreme river flow, while it has increased flood risk or dry river bed, thus having severe implications worldwide. There are reported declining trends for the annual discharge volume of rivers and flooding, increasing the disparity between wet and dry alterations. It is noteworthy that several studies have shown that the water resources will probably continue the decreasing trend in the future, causing amplification of droughts and aridity.

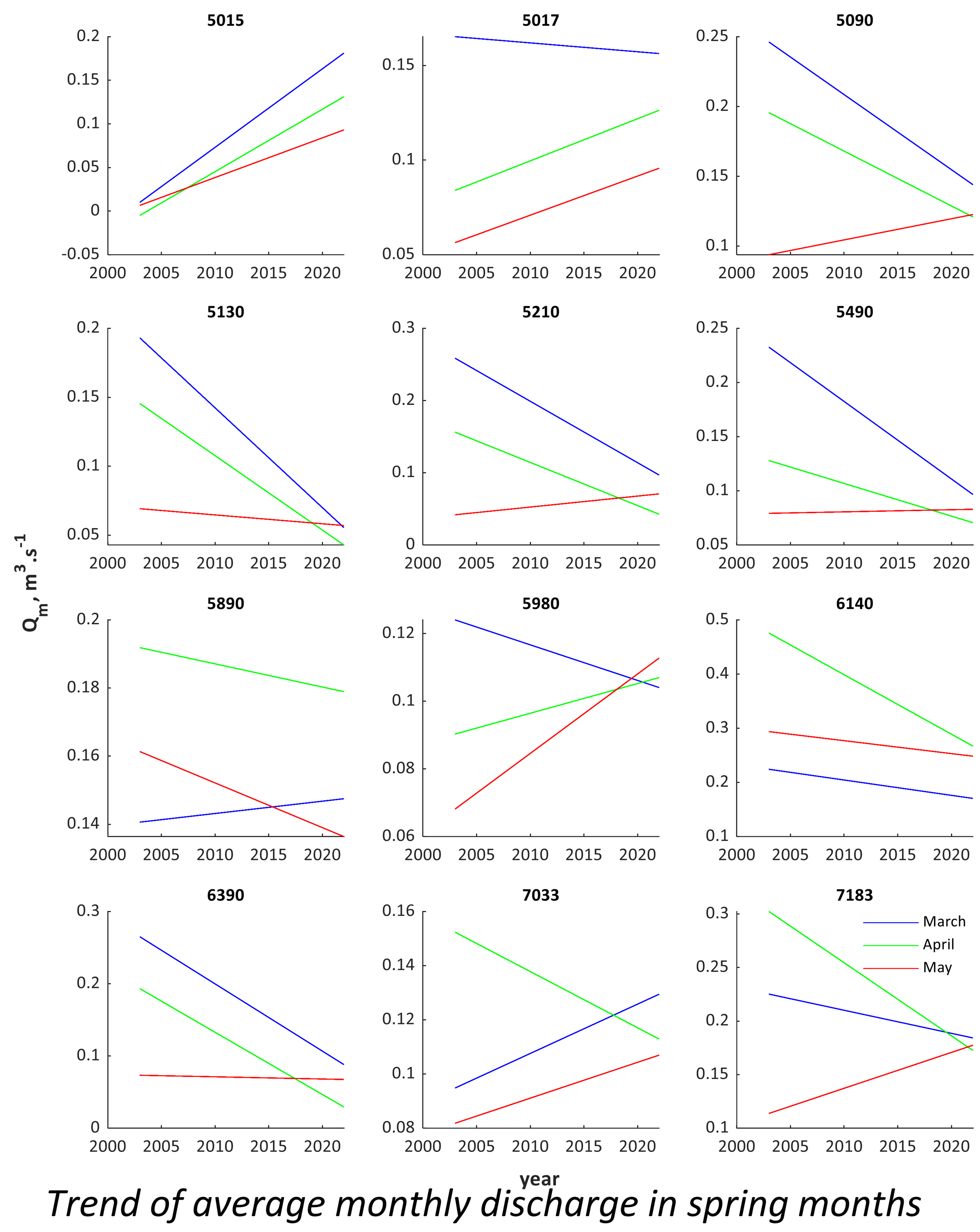
The Water Framework Directive (2000/60/EC) consider only basins with area over 10 km^2. However, the number of smaller catchments is not negligible. Therefore, the aim of this study is to analyse the trend of monthly discharges in the gauging station with a catchment area up to 10 km^2 in Slovakia.

RESEARCH

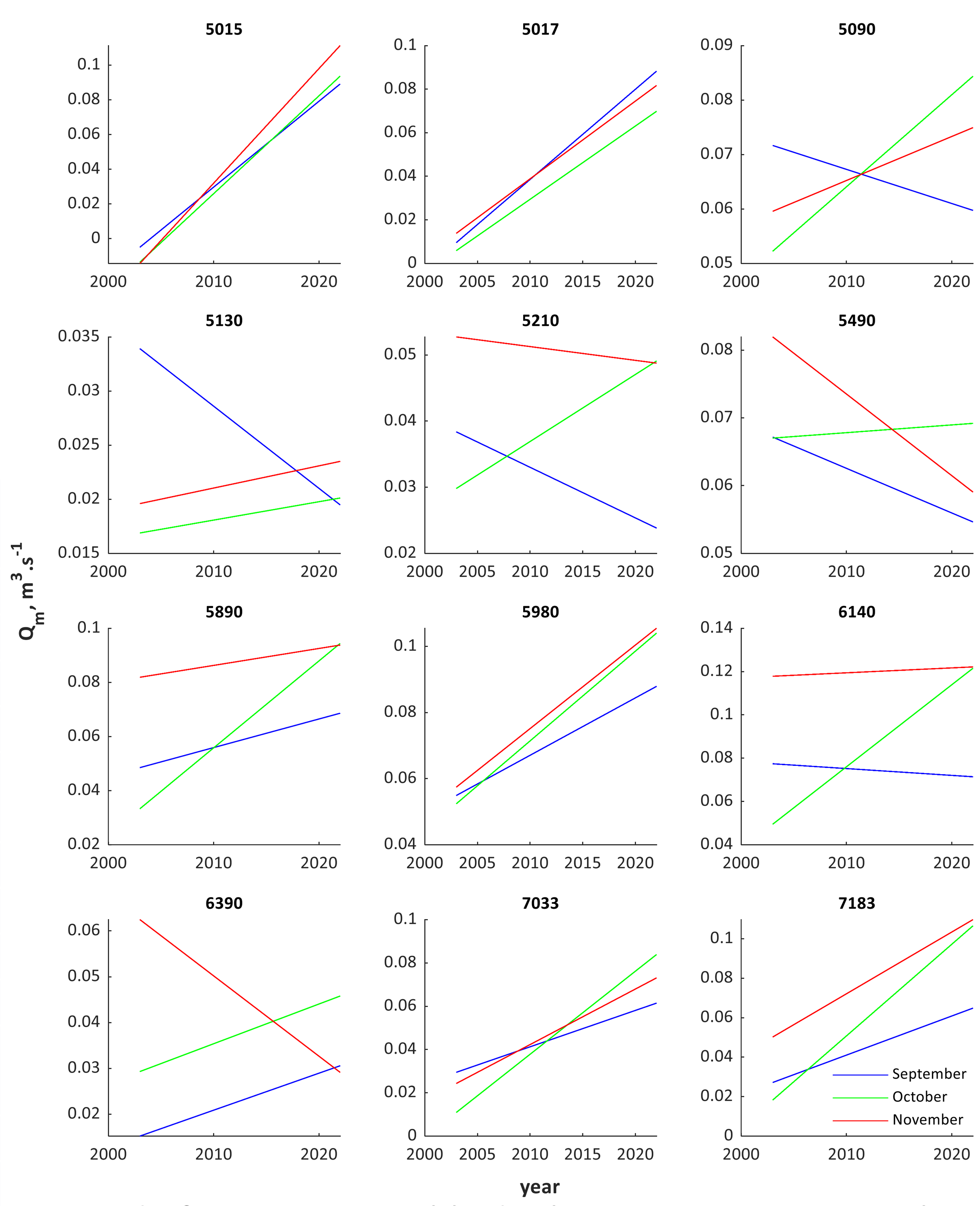
Gauging stations with catchment area up to 10 km^2 were selected for the analysis. Catchments are in western and middle part of Slovakia. Average monthly discharge data free available for the use were download from the webpages of Slovak Hydrometeorological Institute. Continuously, the linear trend for each month of 2003-2022 were calculated.

Catchment parameters of selected gauging stations

Gauging station	Watercourse	Position, rkm	Area, km^2	Main basin
5130 Spariská	Vydrica	11.5	7.25	Dunaj
7033 Čierny Balog	Brôtovo	3.3	9.28	Hron
7183 Hriňová	Hukava	0.3	9.96	Hron
5015 Turá Lúka	Svacenický jarok	0.1	6.85	Morava
5017 Brestovec	Brestovský potok	0.2	9.19	Morava
5090 Kuchyňa	Malina	42.05	7.94	Morava
5490 Podtureň	Jamníček	0.1	7	Váh
5890 Turany	Čiernik	0.24	2.9	Váh
5980 Háj	Somolický potok	2	8.54	Váh
6140 Martin	Pivovarský potok	1.9	8.83	Váh
6390 Vydrná	Petrínovec	2.1	8.63	Váh
5210 Modra	Vištucký potok	22.15	9.88	Váh



Trend of average monthly discharge in spring months



Trend of average monthly discharge in autumn months

RESULTS & CONCLUSION

There is a visible decrease trend in most of the catchment in the spring and summer months, except May. The downward trend itself may not be the problem; however, the problem is the shape and a possible minimal value. Such a rapid decrease is visible at station 5490 Podtureň, where the average value in the second decade was approximately half lower than in the previous decade. Trend line in the station 5015 Turá Lúka in July and August is highly affected by the average monthly discharge in 2018. Other years the average values are about 0.001 m^3.s^{-1}, and the riverbed is dry for several days. We can expect more frequent dry riverbeds in the stations 5130 Spariská and 5210 Modra in July and August next years. On the other hand, in February and October there is an opposite trend which can bring a better condition for the water fauna and flora. More-less stabilised average monthly values are in stations 5130 Spariská in February, 6390 Vydrná and 7033 Čierny Balog in August.

The problem which is not very well visible based on the average monthly data are both extremes, floods and drought. Even the average values look fine and the increasing trendlines are more often, the reality is a lot of time created by one flood and several drought events. Therefore, it is possible that most of these small catchments will be totally dry mainly in July, August and September.

ACKNOWLEDGEMENTS

The author acknowledge Slovak University of Agriculture in Nitra and Scientific Grant Agency project VEGA 1/0681/24 their material and financial support.

https://doi.org/10.15414/2024.9788055227559

SCIENCE OF YOUTH 2024

18th International Scientific Conference
30 - 31 May 2024, Nitra, Slovakia

SPU·FZKI
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The Disintegration Test of PLA/PHB-Based Materials Under Industrial Composting Conditions

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INTRODUCTION

Bioplastics have risen to prominence as a sustainable alternative to traditional plastics, primarily due to their renewable origin and potential to reduce environmental pollution. An important advantage of bioplastics is their compostability, which allows them to decompose under specific conditions, reducing landfill waste and pollution.

RESEARCH

The disintegration test was carried out at the municipal composting plant in Nitra and followed the certified methodology "Methodology for the assessment of the degradation of degradable polymeric materials in real composting conditions" developed at Mendel University in Brno (see Vaverková et al., 2014). On the basis of this methodology, the degree of decomposition was determined.

We weighed the samples individually on a digital balance and made a photographic record of each sample. For our research we selected 6 types of products. We had 2 samples of each studied species and 1 sample that was after 6 days of composting in an electric composter. In total, we tested 18 samples (see Table 1.). As reference samples, we used two pieces of filter paper of circular shape with a diameter of 150 mm, which were uncoated and un-smoothed, in accordance with the certified methodology. The prepared samples were placed in the compost pile and then covered with a layer of compost, see Figure 1.

Table 1. Characteristics of samples subjected to the disintegration test

Sample	Material Specification	Sample Specification
A1	bicomposite cup	original sample
A2	bicomposite cup	original sample
A(6)	bicomposite cup	sample after 6 days in the composter
B1	festival cup	original sample
B2	festival cup	original sample
B(6)	festival cup	sample after 6 days in the composter
C1	VI.cup	original sample
C2	VI.cup	original sample
C(6)	VI.cup	sample after 6 days in the composter
D1	thermoforming film	original sample
D2	thermoforming film	original sample
D(6)	thermoforming film	sample after 6 days in the composter
E1	film	original sample
E2	film	original sample
E(6)	film	sample after 6 days in the composter
F1	film	original sample
F2	film	original sample
F(3)	film	sample after 6 days in the composter
G1	filter paper	reference sample
G2	filter paper	reference sample

Fig. 1. Placement of samples in the compost pile

RESULTS & DISCUSSIONS

Based on the mass differences of the samples before and after the test, the degrees of decomposition of the foil samples (D, E and F) that have completely decomposed during the test can be considered as positive results in the disintegration test. The reference samples in the form of cellulose filter paper (G1, G2) did not reach the required degradation level (90 %) as can be seen in Fig.2.

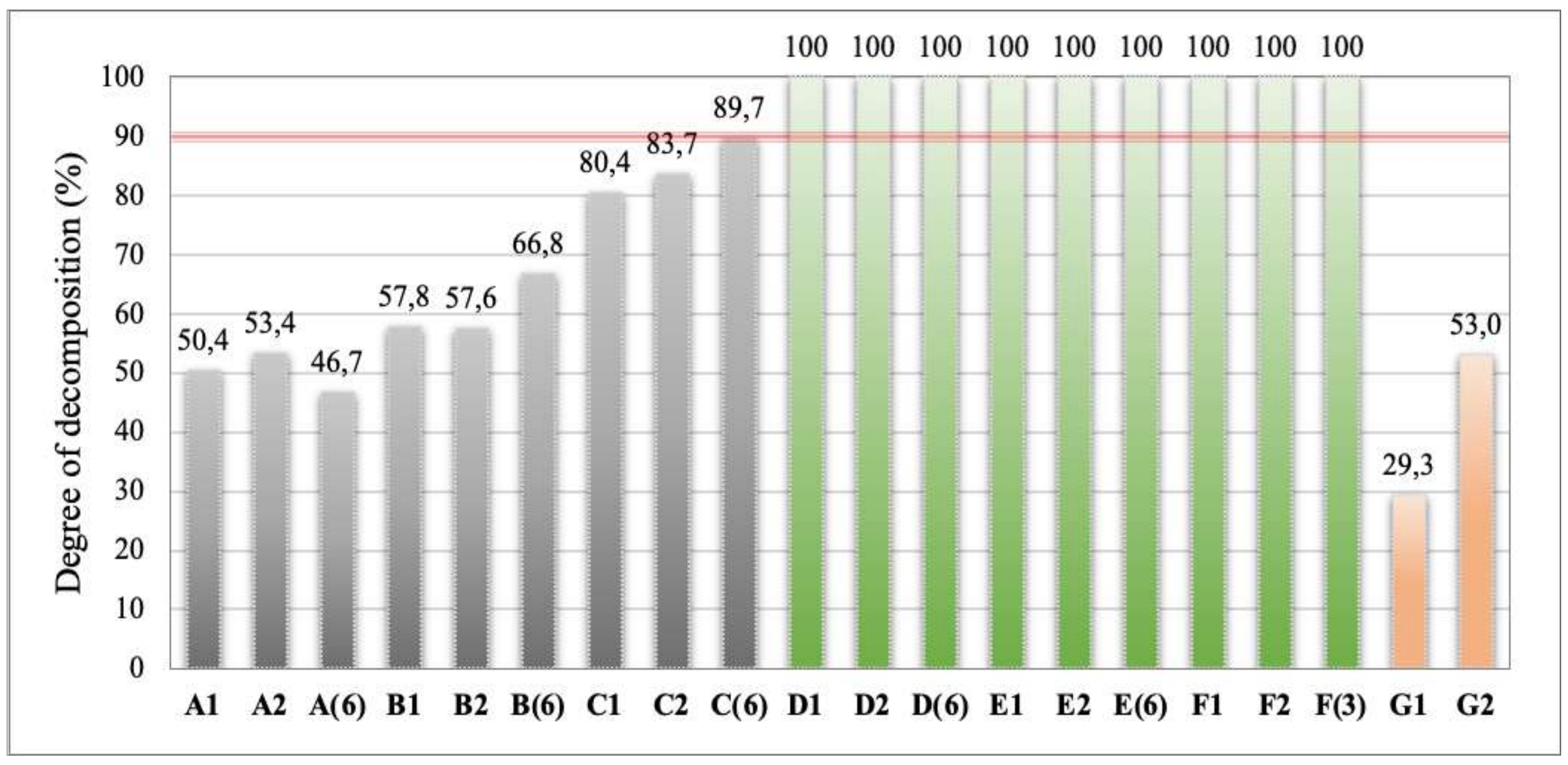

Fig. 2. Degree of decomposition of the individual samples tested

The reason for the slow decomposition of the reference sample is the relatively low moisture content of the composting layer in the first half of the test (below 30 % by volume), therefore 100 % decomposition of the films under such unfavourable conditions is considered to be an interesting and positive result. This fact is reinforced by comparison with the same disintegration test of other bioplastic products from 2019 (Slavikova, 2020), where complete disintegration of the same reference sample occurred. The moisture content in the first stages of composting in 2020 was very low in contrast to 2019. This may be the reason why in 2020 the compost was not active enough for biodegradation of organic material. The cellulose paper from the 1st test (from 2019) was completely decomposed after 12 weeks of composting. In the 2nd test in 2020, after 12 weeks of low moisture composting, more than 50% of the cellulose paper weight was identified and the paper was in its original shape.

A study by Dutch researchers (Zee, Molenveld, 2020) describes a similar observation that compostable products (e.g. tea bags, thicker pots, coffee capsules) decomposed even faster in the compost than e.g. paper or orange peels and could not be found after eleven days from the start of the composting process.

CONCLUSION

The rate of the disintegration of individual compostable product types is different under real composting facility conditions and does not only depend on material composition of a specific product, but also on a composition of the composting pile and the used composting technology. The Nitra city composting facility can process all tested products safely as all of the undisintegrated pieces are being separated during the sowing of the final compost and returned into the composting process.

ACKNOWLEDGEMENTS

This poster presents the results obtained by the authors within the research project Demand-driven Research for Sustainable and Innovative Food, Drive4SIFood 313011V336 thanks to the support of the Operational Programme Integrated Infrastructure, which is co-financed by the European Regional Development Fund.

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https://doi.org/10.15414/2024.9788055227559

SCIENCE OF YOUTH 2024

18th International Scientific Conference

30 – 31 May 2024, Nitra, SLOVAKIA

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ACCESSIBILITY ANALYSIS OF GREEN AND OPEN SPACES TO RESIDENTIAL AREAS IN THE URBAN DISTRICT OF CHRENOVÁ, NITRA

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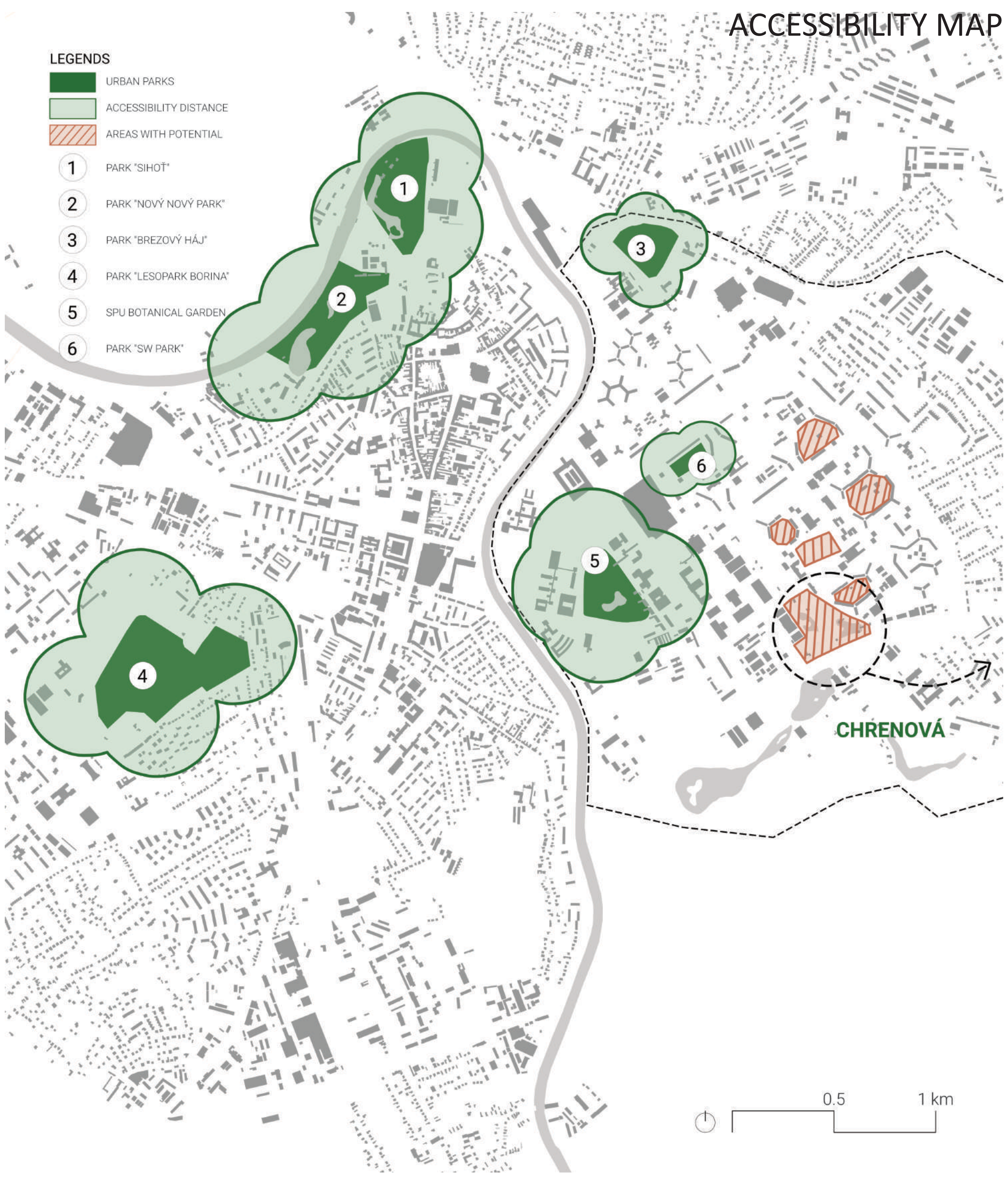
INTRODUCTION

Urban green and open spaces represents an important component of the city sctructure. They have huge recreational value for residents because it forms a group of freely availaible and vital elements in the urban landscape. Therefore, the accessibility of green spaces to residential complexes is crucial for the inhabitants and the green infrastruture itself. Green infrastructure is a planned network of natural and semi-natural areas (Tóth, 2024), and in urban green space planning, it is necessary to strategically plan green networks in such a way that they interconnect with each other. The accessibility of green space is also included among the indicators of urban sustainability, especially park areas for inhabitants (Dobrucká, 2013). The research focuses on the city of Nitra and its accessibility of parks to residential complexes.

ACCESSIBILITY MAP



RESEARCH

The city of Nitra has five larger parks, which are mostly more than 5 hectares in size. The complete analysis is based on the analysis of accessibility to these selected areas to the residential areas of the Chrenová urban district. Based on the recommended standards of the Vienna manual "STEP2025", accessibility should be within 250 m (parks up to 5 ha) and within 500 m (parks larger than 5 ha). These standards were applied as part of the accessibility analysis.

RESULTS & DISCUSSIONS

The result of the research is an accessibility map, which shows that the city of Nitra does not meet the German standards and indicators of sustainable urban development. The residential area of Chrenová does not currently have a larger park despite having a high percentage of green space coverage. In the analysis we also evaluated areas with high potential for creating a smaller or larger park and at the same time we tried to create a scenario of a sustainable park for the inhabitants of Chrenova using the "Research by Design" method. There are many more of these spaces on which this research will continue to focus.

CHRENOVÁ PARK PROPOSAL

CONCLUSION

Urban green and open spaces are undoubtedly important because they form the urban structure and create a network of green infrastructure elements. City of Nitra does not completely meet the greening standards which was proven in this research but it does have several potential areas to be redeveloped into parks which is a future finding for further development of this research. The research also presents a suitable design for a scenario of a smaller park that would serve the inhabitants of Chrenová and at the same time partially meet the Viennese green space standards.

ACKNOWLEDGEMENTS

This poster was funded by the EÚ NextGenerationEU project n. 09I03-03-V02-00043, KEGA 004SPU-4/2023 KR:EK:IN, BIN SGS02_2021_013 RelmaGIne and KEGA 038SPU-4/2024 U:INT - White Spaces and Ephemeral Urban Interventions.

https://doi.org/10.15414/2024.9788055227559

SCIENCE OF YOUTH 2024

18th International Scientific Conference
30 - 31 May 2024, Nitra, Slovakia

SUA Slovak University of Agriculture in Nitra

Case study of rainfall-runoff parameters of Critical Profile Návojovce in Partizánske

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[1] University Agricultural Company SPU, s.r.o., [2] Slovak University of Agriculture in Nitra

INTRODUCTION

On May 4th, 2024, an erosion event occurred in Navojovce's cadastral area following a 15-minute heavy rainfall that resulted in 30 mm of precipitation (Facebook: Chceme zmenu v Partizanskom, 2024). Climatologist Prof. Milan Lapin described the storm as a severe weather event with a 50 to 100-year return period, typical for the middle and lower parts of Slovakia (TA3, 2024). This event primarily resulted in severe erosion rather than flooding, causing drainage channels to clog and fail, which led to streets and cellars being flooded up to 30 cm of sediments. The incident underscored the importance of proactively identifying and mapping vulnerable locations, as exemplified by the pre-existing national web map service in Slovakia, titled "Critical Profiles and Their Catchments" (https://www.fzki.uniag.sk/sk/kriticke-profily/, https://www.geoportal.sk/sk/sluzby/mapovy-klient-zbgis/kriticke-profily.html).

RESEARCH

The erosion event was analyzed at the Návojovce-732-KP_II Critical Profile (Muchová et al., 2023), located in the municipal district of Návojovce-Partizanske within the Trenčín Self-Administrative Region, Partizánske District. To obtain accurate runoff estimations, the contributing area and spatial parameters of the critical profile were updated using a Digital Elevation Model (DEM 5.0) with a 1x1 m cell resolution. **Erosion Risk Assessment**: The Universal Soil Loss Equation (USLE) (Wischmeier and Smith, 1978) was employed to estimate water erosion. Soil erosion risk level is defined by the proportion of the USLE calculation and the allowable soil loss according to STN 75 4501. Technic norm was used to classify the degree of erosion risk into five categories: 1) Slight to light; 2) Moderate; 3) Severe; 4) Very severe; and 5) Extremely severe. **Flow rate estimation:** Peak discharge and total runoff was calculated using DesQ-MaxQ (http://www.desq-maxq.cz/), a hydrological model designed for small catchments. The model considered flood wave characteristics for 50 and 100-year design rainfall, with precipitation values of 61.7 mm and 65.9 mm respectively for Topoľčany station, provided by the Slovak Hydrometeorological Institute. Input data for the model included: area and average slope of catchment, length and average slope of thalweg, followed by CNII values and surface roughness

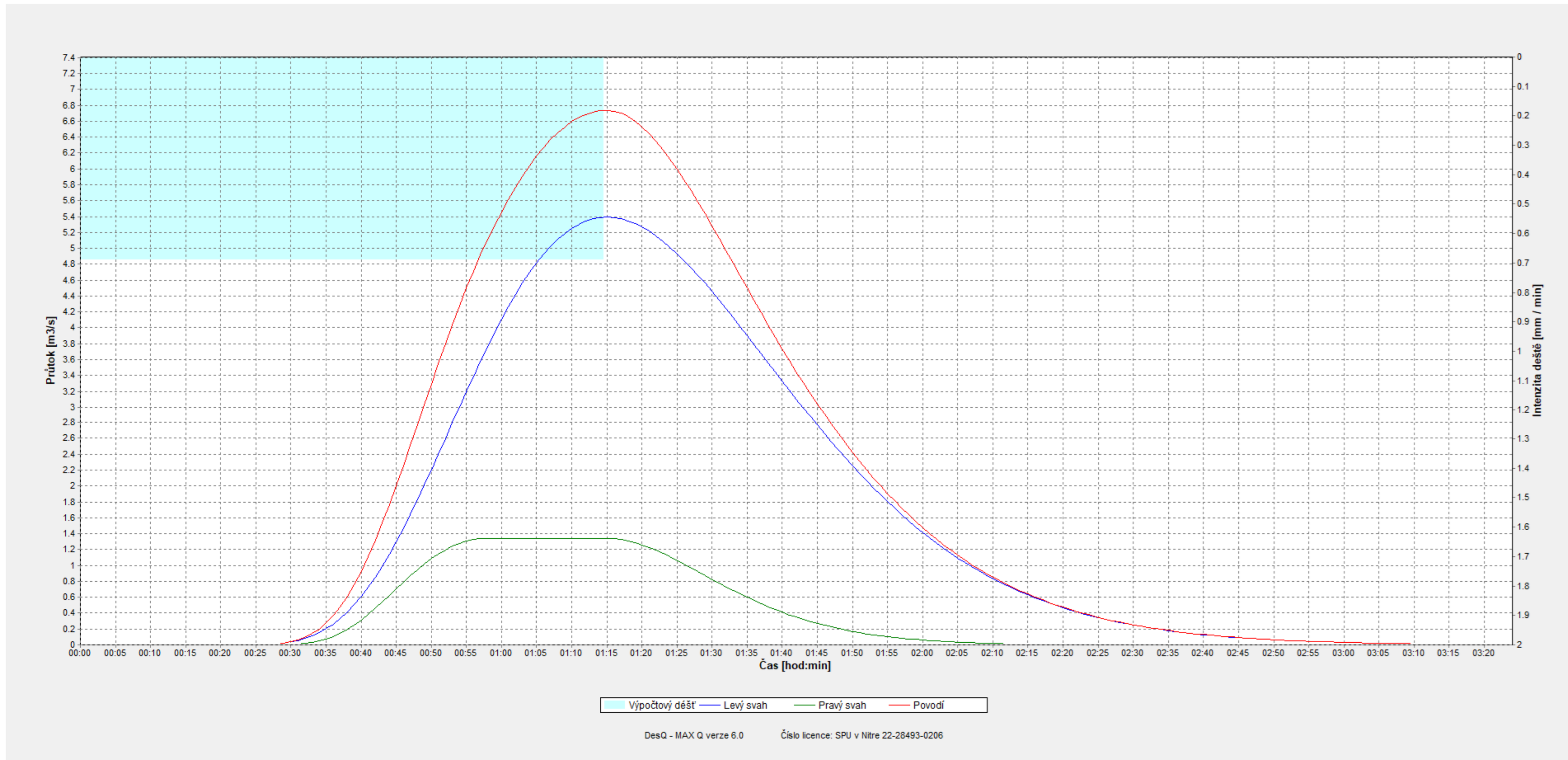

Figure 1. Design hydrograph for N100 years return period

Table 1. Results of DesQ-MaxQ model

	N-year maximum discharge and volume of flood wave					Units
N	5	10	20	50	100	(years)
H_{zN}	42.9	49	54.4	61.7	65.9	(mm)
Q_N	2.12	3.04	4.08	5.64	6.84	($m^3.s^{-1}$)
W_N	12.9	15.3	17.6	20.5	22.6	($10^3.m^3$)

RESULTS & DISCUSSIONS

The SERL revealed that the majority of the study area is highly susceptible to erosion. Specifically, 66% of the area falls under "Severe" and "Very Severe" erosion risk categories. This susceptibility is largely due to the intense rainfall events and the bare soil conditions (excluding vegetation cover factor C) at the time of assessment. The high percentages in these categories indicate a critical need for erosion control measures. The DesQ-MaxQ model indicates substantial peak discharge and flood wave volumes, with a 50-year event resulting in 5.64 $m^3 \cdot s^{-1}$ peak discharge and 20.5 x 10^3 m^3 flood volume, and a 100-year event showing 6.84 $m^3 \cdot s^{-1}$ and 22.6 x 10^3 m^3 respectively. These values highlight the inadequacy of current measurements such as drainage systems and most importantly unmaintained dry water dam to handle extreme events, leading to potential flooding and damage.

Figure 2. Soil erosion risk level (SERL)

Table 2. Resulting balance of SERL share in area and percentage

SERL	Area (ha)	Percentage (%)
1) Slight to light	24.89	16.08
2) Moderate	21.34	13.78
3) Severe	48.06	31.04
4) Very severe	54.16	34.98
5) Extremely severe	6.37	4.11

CONCLUSION

The erosion event in Návojovce on May 4, 2024, highlighted the vulnerability of the region to severe weather events and the inadequacy of existing drainage infrastructure. Despite the brief duration of the rainfall, its intensity was enough to cause significant erosion, filling drainage channels with sediments and leading to localized flooding. Analysis using the Universal Soil Loss Equation (USLE) and hydrological modeling via DesQ-MaxQ confirmed that the majority of the area is at high risk of severe erosion, particularly during extreme rainfall events.´The study underscored the necessity for robust erosion control measures and the maintenance of existing infrastructure, such as dry water dams, to mitigate the impacts of future events. The findings point to a critical need for proactive measures, including the identification and mapping of vulnerable locations, and the implementation of erosion control practices to protect the community and its infrastructure from similar occurrences in the future.

ACKNOWLEDGEMENTS

This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic and the Slovak Academy of Sciences (No. 1/0244/21).

Veda mladých 2024 - Science of Youth 2024

PROCEEDINGS OF PAPERS FROM A SCIENTIFIC CONFERENCE

Nitra, Slovakia
30. – 31.05.2024

Held under the auspices
prof. Ing. Dušan Igaz, PhD., dean of FHLE SUA in Nitra.

Editors: Ing. Vladimír Kišš, PhD., Ing. Mária Tárníková, PhD.

Publisher: Slovak University of Agriculture in Nitra

Edition: first

Year of publication: 2024

Form of publication: online

Not reviewed at the Publishing House of SUA in Nitra.

ISBN 978-80-552-2755-9

DOI: https://doi.org/10.15414/2024.9788055227559