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CONTENT

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INFLUENCE OF THE COVID-19 PANDEMIC ON THE IMPORT AND EXPORT OF ROSES IN SERBIA

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ABSTRACT

Roses are an important part of the flower production in Serbia. The rose cut flowers, grafted plants and rootstocks are produced for both domestic and international market. During the past three years, Covid-19 pandemic influenced production and consumption of roses worldwide, so the aim of this study was to determine how the pandemic influenced export and import of roses in Serbia. In order to obtain more accurate data, the roses trade in Serbia during pandemic years (2020 and 2021) was compared to the average data for five year period before the pandemic (2015-2019). The rose plants (both grafted and rootstocks) have considerably larger share in rose export compared to cut roses. Before the pandemic the average export of rose plants was 1104.72 metric tons with average value of 2.28M (USD). However, in 2021 the quantity of exported plants remained almost the same (1102.7 tons) but the value increased by 41.9% (3.23M). The export markets also changed. For example, Russia was the main importer of Serbian rose plants before the pandemic. However export to Russia decreased by 52.16% while export to EU rose by 244% in 2021. The export of cut roses also increased considerably during the pandemic by 648%, from an average 0.03M before pandemic to 0.2M in 2021. In Serbia, the pandemic did not have a negative impact on roses production and trade, and export increased considerably. However, international market may change again after the pandemic and it is important to work on improving roses production in Serbia and increasing the competitiveness on international markets.

Keywords: *cut roses production, roses trade, grafted roses market, roses rootstock plants.*

INTRODUCTION

Roses, chrysanthemums, and carnations are often considered as traditional cut flowers, and they are the most represented and economically the most important on cut flowers world market. Besides, rose is a very popular garden plant or potted plant, worldwide (Darras, 2021; Roberts *et al.* 2003; Zlesak, 2006; Debener and Byrne, 2014). In Serbia, roses are also an important part of the flower production, and rose cut flowers, grafted plants and rootstocks are produced for both domestic

and international markets. According to The Observatory of Economic Complexity (OEC), Serbian exports of roses reperesented 1.36% of the world's roses market, and 2% of the European roses market in 2020 with the value of 2.7M (USD) (https://oec.world/en/profile/hs92/roses).
During the past three years, after the Covid-19 pandemic started, prophylactic measures, such as the social isolation and lockdowns were conducted in the majority of countries, having impact not only to human health, but also on the economy with the drop in consumption, reduction of the production system, income-generating activities were reduced, commercial establishments were temporary closed and the unemployment occured in some sectors. The floriculture was also influenced by the pandemic, including production and consumption of roses worldwide (Clair *et al.*, 2021; Anacleto *et al.*, 2021; Lamm *et al*. 2021; Lioutas and Charatsari, 2021). For this reason, the aim of this study was to determine how the pandemic influenced export and import of roses in Serbia.

MATERIAL AND METHODS

The official statistical databases of Serbia were used in this study. The data on the export and import of roses (both plants and cut flowers) given in tons and USD were obtained from the databases of the Republic Bureau of Statistics (https://data.stat.gov.rs/?caller=SDDB), for the period 2015-2021. The trend analysis was used to calculate trends for the years 2020 and 2021 in order to determine expected change in export and imports based on five years data (2015-2019) collected before the pandemic and to compare obtained data with real data collected for the pandemic years 2020 and 2021. Microsoft Excel 2007 was used for trend analysis. Also, in order to obtain more accurate data, the roses trade in Serbia during the pandemic years (2020 and 2021) was compared to the average data for the five year period before the pandemic (2015-2019).

RESULTS AND DISCUSSION

The data for the most important markets, those with the largest share in the export or import were presented in Tables 1, 2, 3 and 4. The rose plants (both grafted and rootstocks) have a considerably larger share in rose export compared to cut roses. For the observed period (2015-2021), total export of rose plants ranged from 1.9M (USD) to 3.2M (Table 1) while the value of rose cut flowers export ranged from 0.003M to 0.2M (Table 2). On the contrary, the import of the rose plants was significantly lower, not exceeding 0.3 M while the value of the import of rose cut flowers ranged from 0.97M to 3.14M during the same period.
The total export of rose plants (in tons) did not change significantly during 2015-2019 with an average export of 1104.7 tons. The amount of exported plants dropped by 4% in 2020 compared to the average amount for the period of 2015-2019, but it decreased by 22.7% compared with the estimated expected value for 2020 according to trend analysis (Table 1). However, the average price per ton

increased from average 2064 USD/ton (2015-2019) to 2536 USD/ton (2020) and 2935 USD/ton (2021), which is higher than estimated by trend analysis. In that way, the value of export increased by 41.9% (3.23M) in 2021 compared to the average value of 2.28M for the years before the pandemic.

Table 1. Export of rose plants from Serbia during 2015-2021

Year	European union		CEFTA		Russia		World		World	
	t	USD*	t	USD*	t	USD*	t	USD*	t	USD*
2015	202.6	527.9	102	186.4	606.7	1199.8	994.4	2104.0	Estimated values based on 2015-2019 data	
2016	300.4	662.1	85.9	167.5	415.1	873.1	897.9	1883.0		
2017	449.3	879.1	99.3	175.0	463.8	1001.7	1136.4	2267.8		
2018	583.9	986.7	121.4	277.0	419.2	1036.5	1212.8	2529.8		
2019	652.2	1099.4	123	276.4	446.4	1088.1	1282.1	2620.4		
2015-19*	**437.7**	**831.0**	**106.3**	**216.5**	**470.2**	**1039.8**	**1104.7**	**2281.0**		
2020	630.4	1404.3	135.7	305.6	214.2	649.5	1060.3	2689.2	1371.8	2784.9
2021	709.3	2025.1	144.4	360.5	157.8	497.2	1102.7	3236.9	1460.8	2952.8

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period. CEFTA: Central European Free Trade Agreement

The export markets also changed. For example, Russia was the main importer of Serbian rose plants before the pandemic, but the amount of exported plants decreased considerably from an average of 470.2 tons to 214.2 tons in 2020 and 157.8 tons in 2021. In that way, the average value of export to Russia for the period of 2015-2019 decreased by 52.16% in 2021. However, although the value of exports to the EU rose by 244% in 2021 compared to the average value for the period of 2015-2019, we cannot conclude that this growth was influenced by the pandemic, because the export to EU had a tendency of growth during the observed period, and trend analysis showed that an increase of export to the EU was expected.

The rose plants are mostly imported to Serbia from EU, the amount of imported plants has been increasing over years, but the pandemic did not influence the import because the amount of plants (in tons) is not significantly different from the estimated amount according to trend analysis (Table 2).

Table 2. Import of rose plants to Serbia during 2015-2021

Year	European union		World		World	
	t	**USD***	**t**	**USD***	**t**	**USD***
2015	28.5	59.7	28.9	60.6	Estimated values based on 2015-2019 data	
2016	12.5	35.0	12.5	35.0		
2017	39.0	129.3	39.0	129.7		
2018	69.1	280.9	69.1	280.9		
2019	80.4	212.0	80.4	212.0		
2015-19*	**45.9**	**143.4**	**46.0**	**143.6**		
2020	86.5	294.9	86.5	294.9	93.86	308.53
2021	91.4	309.3	91.4	309.3	109.82	363.12

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period

Table 3. Export of rose cut flowers from Serbia during 2015-2021

Year	European union		CEFTA		World		World	
	t	**USD***	**t**	**USD***	**t**	**USD***	**t**	**USD***
2015	21.3	57.6	8.7	19.3	30	76.9	Estimated values based on 2015-2019 data	
2016	0.0	0.0	0.0	0.0	0	0		
2017	0.0	0.0	1.3	2.7	1.3	2.7		
2018	37.6	79.2	0.0	0.0	37.6	79.2		
2019	6.1	10.7	0.0	0.0	6.1	10.7		
2015-19*	**13.0**	**29.5**	**2.0**	**4.4**	**15.0**	**33.9**		
2020	20.5	158.7	0.0	0.0	20.5	158.7	11.94	35.88
2021	27.4	219.8	0.0	0.0	27.4	219.8	10.92	25.24

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period

The amount of exported rose cut flowers from Serbia is low and irregular over years before the pandemic (Table 3). However, export of rose cut flowers increased considerably during pandemic years compared to the period before the pandemic, by 648%, from average 0.03M before pandemic to 0.2M in 2021, more than it was estimated by trend analysis. Besides, the average price of 2260 USD per metric ton increased considerably to 7741 USD in 2020 and 8022 in 2021 (table 3), which is significantly higher than the prices of imported rose cut flowers which were: 1752 USD (average before pandemic), 3049 USD (2020) and 3542 USD (2021) (Table 4).

Table 4. Import of rose cut flowers to Serbia during 2015-2021

Year	**European union**		**CEFTA**		**Ethiopia**		**Kenya**		**World**		**World**	
	t	**USD***	**t**	**USD***	**t**	**USD***	**t**	**USD***	**t**	**USD***	**t**	**USD***
2015	346.9	665.5	26.6	50.7	92	56.8	260.8	173	742.4	975.4	Estimated values based on 2015-2019 data	
2016	370.2	558.5	24.2	47.5	54	56.8	442.4	550.9	844.4	1089.7		
2017	228.2	503.0	24.5	48.7	54	56.8	442.4	550.9	766.7	1237.2		
2018	312.7	716.1	20.1	43.0	68.5	157.1	400.3	572.3	817.5	1570.7		
2019	269.9	949.3	14.8	33.4	95.2	490.2	533.7	732.6	931.6	2314.9		
2015-19*	**305.6**	**678.5**	**22.0**	**44.7**	**72.74**	**163.54**	**415.92**	**515.94**	**820.5**	**1437.6**		
2020	133.2	524.7	0.4	1.2	84.9	476.9	426.6	911.2	657.2	2003.8	925.9	2385.6
2021	148.8	589.6	0.0	0.0	98.1	640.1	612.0	1738.8	886.7	3141.1	961.1	2701.6

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period

In Serbia, import of cut flowers decreased in 2020 probably because of the lower demand due to lockdowns, but in 2021 import increased (Table 4). A relatively small amount of cut roses is imported from the EU, the roses are mostly imported from Ethiopia and Kenya. During 2021, imports from Ethiopia and Kenya increased, while imports from the EU decreased (Table 4).

Anacleto *et al.* (2021) stated that the pandemic considerably influenced the floriculture sector, both production systems and stores and flower shops, creating a disconnection between all sectors of the production chain. Similarly, Bulgari *et al.* (2021) concluded that there was a reduction in the demand of cut flowers during the COVID-19 pandemic due to lockdowns and lack of social events, and Lioutas and Charatsari (2021) reported that a great amount of cut flowers was unsold or donated, at the beginning of pandemic in the Netherlands, while demand for indoor plants increased (Pérez-Urrestarazu *et al.* 2021), which correspond with the decrease in cut roses import to Serbia during 2020.

CONCLUSIONS

The pandemic considerably influenced the floriculture sector worldwide, including production and distribution of roses. The production of rose cut flowers, grafted plants and rootstocks is an important part of the floriculture sector in Serbia. The pandemic influenced demand for cut roses in Serbia, and import of rose cut flowers decreased in the first year of the pandemic (2020). However, export of both cut roses and rose plants increased considerably during pandemic years. Also, the export market for rose plants changed, and export of rose plants to Russia decreased by 52.16% while export to the EU rose by 244% in 2021. However, it is important to work on improving roses production in Serbia and increasing competitiveness on international markets, because international markets may change again after the pandemic.

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URBAN AND PERI-URBAN AGRICULTURE IN BURKINA FASO AND NIGER: A BIBLIOMETRIC ANALYSIS

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ABSTRACT

Urban and peri-urban agriculture (UPA) has been recently put forward as a means to address many challenges such as food insecurity, climate change and poverty. This is particularly relevant in developing countries, facing the dramatic consequences of these challenges, such as Burkina Faso and Niger in Sahelian West Africa. Research is needed for the development of UPA. However, comprehensive analyses about the landscape of research dealing with UPA are oftentimes lacking especially in developing countries. In this context, the present review provides a bibliometric analysis of the scholarly literature addressing UPA in Burkina Faso and Niger. It draws upon a search performed in June 2022 on the Web of Science. The bibliometric analysis focuses on sources/journals, research areas, authors and organisations/affiliations. It suggests that while interest in research on UPA is increasing, the annual output of articles remains low. Furthermore, the research field is quite multidisciplinary but mainly falls under the area of agriculture thus focusing on biological and environmental sciences, while social sciences and economics are generally overlooked. The analysis also shows that a large share of studies on UPA is carried out by scholars affiliated with universities and research centres based outside Burkina Faso/Niger and even West Africa, especially in Germany. This might denote the weakness of the research system and the lack of structured research projects/programs on UPA in both countries. Investments in research, development and innovation are needed to bridge the existing knowledge gap and unlock the potential of UPA in addressing the challenges that both countries face. Since challenges and opportunities are quite similar, multi-country and regional research programmes on UPA would be highly beneficial in the Sahel and West Africa.

Keywords: *urban agriculture, urban food systems, Sahel, West Africa, bibliometrics.*

INTRODUCTION

Urban and peri-urban agriculture (UPA) can be defined as "an industry that produces, processes and markets food and fuel, largely in response to the daily demand of consumers within a town, city or metropolis, on land and water dispersed throughout the urban and peri-urban area, applying intensive production methods, using and reusing natural resources and urban wastes, to yield a diversity of crops and livestock" (UNDP, 1996). Several studies suggested that UPA can contribute to addressing many challenges. Indeed, UPA has several environmental, social and economic benefits. From the socio-economic point of view, it contributes to food and nutrition security (El Bilali et al., 2013; Levasseur et al., 2007; Orsini et al., 2013), livelihoods and income generation (Levasseur et al., 2007; Orsini et al., 2013) as well as social inclusion and reduction of gender inequalities (El Bilali et al., 2013; Orsini et al., 2013). From the environmental standpoint, UPA has positive effects in terms of waste reduction and recycling (Orsini et al., 2013), biodiversity conservation (Orsini et al., 2013), air quality improvement (El Bilali et al., 2013; Orsini et al., 2013) as well as the reduction of the environmental impacts related to food transport and storage (Orsini et al., 2013). However, several constraints hamper the development of urban agriculture in developing countries; these relate, inter alia, to insufficient government support, difficult market access, insecure land tenure, limited access to production factors/inputs, and inequality issues (Houessou et al., 2020).

The multifaceted benefits of UPA are particularly relevant for developing countries facing different sustainable development challenges such as Burkina and Niger, two landlocked countries in Sahelian West Africa. Both countries have low human development (UNDP, 2019) and are affected by multiple forms of malnutrition (FAO et al., 2021). Agriculture, with a significant contribution to the gross domestic product (GDP) and employment (World Bank, 2021), is extensive, poorly mechanized and vulnerable to climate change (El Bilali, 2021b). Climate change represents a challenge for agriculture (Mainardi, 2011; USAID, 2017) and is also an important driver of poverty, livelihoods vulnerability and food insecurity (El Bilali, 2021a).

Research is needed for the development of UPA. Nevertheless, despite its numerous benefits and recognised potential to help addressing several challenges, comprehensive analyses about the landscape of research dealing with UPA are oftentimes lacking especially in developing countries. In this context, the present review provides a bibliometric analysis of the literature on UPA in Burkina Faso and Niger.

METHODS

The paper draws upon a systematic review of all documents indexed in the Web of Science (WoS) and follows the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009). A search was performed in June 2022, using the following search string: *(urban OR city OR town OR ville OR urbain) AND (agriculture OR farming OR garden OR horticulture OR* *growing OR animal OR élevage OR pastoralism) AND (Burkina OR Niger OR Sahel OR "West* Africa" OR "Afrique occidentale" OR "Afrique de l'Ouest")*. Three inclusion/eligibility criteria were considered: geographical coverage (viz. the document deals with Burkina Faso and/or Niger); thematic focus (viz. the main topic is UPA); and document type (viz. only journal articles, book chapters or conference papers were selected; letters to editors, commentaries, notes and/or reviews were excluded). Only documents that met all three criteria were considered eligible and included in the review.

The search on WoS returned 524 documents. However at first, 190 documents were screened out based on the titles as they do not refer to Burkina Faso and/or Niger; documents covering wider geographical areas (e.g. Sahel, West Africa, Sub-Saharan Africa,) or those where the geographical scope is not reported in the title were kept for further scrutiny. Secondly, additional 256 documents were excluded based on the abstracts not meeting at least one of the inclusion/eligibility criteria. Finally, 8 documents were discarded after the analysis of full texts, including reviews. Therefore, 70 documents were included in the systematic review.

The selected articles underwent a bibliometric analysis focusing on sources/journals, research areas, authors, affiliation organisations and affiliation countries. Also an analysis of the geography of research on UPA (cf. where research was performed) was carried out. Both analyses were informed by the methodology used by El Bilali (2021).

RESULTS AND DISCUSSION

The analysis of the selected documents suggests that the annual output of articles fluctuates a lot from one year to another; in the considered period (2001-2022), it ranges from nil in some years (2003, 2004) to a maximum of 12 in 2019 followed by 10 in 2012. Meanwhile, the average annual output in the period 2001-2022 is very low viz. about 3 documents per year. The peak in the number of publications in 2019 might suggest that interest in research on UPA is increasing.

As for *sources*, the analysis of the results (Table 1) shows that the maximum number of articles was published in *Nutrient Cycling in Agroecosystems* (7 articles) followed by the *Journal of Plant Nutrition and Soil Science* (4 articles) and *Landscape and Urban Planning* (4 articles). Nevertheless, the findings of the research on UPA in Burkina Faso and Niger were published in 45 further sources and journals, which suggests that there are no specific publication outlets. The majority of the selected articles (40 articles, 57.1%) can be linked to the *research area* of agriculture. Further important research areas include environmental sciences - ecology (22 articles, 31.4%), science technology (8 articles), urban studies (8 articles) and geography (6 articles). However, the selected documents can be categorized in 27 research areas – including public administration, veterinary sciences, food science technology, physical geography, plant sciences, anthropology, sociology, biotechnology, business economics, development studies, forestry, nutrition dietetics, parasitology and zoology – which shows that research on UPA is multidisciplinary. It can be argued that while biological and environmental sciences are sufficiently addressed, social sciences and economics are generally overlooked. The bibliometric analysis shows that the most prominent, productive *authors* in the research field are Andreas Buerkert (29 articles, 41.4%), Eva Schlecht (18 articles, 25.7%), Désiré Jean-Pascal Lompo (10 articles, 14.3%) and Luc Hippolyte Dossa (7 articles, 10.0%). However, the fact that many authors have only one article dealing with UPA in Burkina Faso and/or Niger might indicate a lack of consistency in the research field i.e. even authors dealing with the topic do that in a sporadic rather than a systematic way. This, in turn, might be due to the absence of structured research projects/programs on UPA in both countries.

The analysis of *affiliation countries* suggests that the most active country in the research field is Germany (42 articles, 60.0%). Interestingly, slightly more than half of the selected documents (36 articles, 51.4%) are authored by Burkinabe researchers. Affiliation West African countries also include Ghana (10 articles), Benin (6 articles), Nigeria (3 articles), Mali, Niger and Senegal (2 articles each). However, many publications are authored by researchers based outside West Africa; either in Africa (e.g. Cameroon, Kenya, Madagascar, Tanzania, Zimbabwe), Europe (e.g. Austria, Belgium, England, France, Italy, Netherlands, Sweden, Switzerland), North America (e.g. USA, Canada) or Oceania (e.g. Australia). This shows, on the one side, that there is a network of researchers working on UPA from different countries but might be, on the other side, considered an indicator of the weakness of the agricultural knowledge and innovation system (AKIS) in Burkina Faso and, especially, Niger.

Many of the prominent *organisations* in the research field are based outside Burkina Faso/Niger and even West Africa, especially in Germany. These organisations include the University of Kassel (Germany), University of Gottingen (Germany), Ruhr University Bochum (Germany), Université Catholique Louvain (Belgium), Wageningen University and Research (Netherlands), INRAE (National Research Institute for Agriculture, Food and Environment - France), University of Freiburg (Germany), University of Natural Resources and Life Sciences (Austria) and CIRAD (Agricultural Research Centre for International Development - France). The most relevant funding agencies are also based in Germany (e.g. Federal Ministry of Education Research – BMBF, German Research Foundation - DFG, Volkswagen, Federal Ministry for Economic Cooperation and Development - BMZ, German Academic Exchange Service - DAAD). There are also some international organisations and regional research centres dealing with research on UPA such as CGIAR (Consultative Group on International Agricultural Research), *Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide* (CIRDES) and International Water Management Institute (IWMI). However, many organisations in Burkina Faso/Niger or West Africa are active in research on UPA in Burkina Faso (e.g. *Institut de l'Environment et de Recherches Agricoles* – INERA, University Ouaga I Pr Joseph Ki-Zerbo, Univ. Polytech Bobo Dioulasso, Univ. Dedougou), Benin (e.g. University of Abomey Calavi), Ghana (e.g. University for Development Studies, University of Ghana) and Nigeria (e.g. Ahmadu Bello University).

Table 1. Bibliometrics of the analysed literature: top ten journals, research areas, authors, countries and organizations.

Journals/sources (a*)	Research areas (b*)	Authors (c*)	Countries and territories (d*)	Organisations (e*)
Nutrient Cycling in Agroecosystems (7)	Agriculture (40)	Buerkert A. (29)	Germany (42)	University Kassel (36)
Journal of Plant Nutrition and Soil Science (4)	Environmental sciences - Ecology (22)	Schlecht E. (18)	Burkina Faso (36)	University Gottingen (19)
Landscape and Urban Planning (4)	Science technology (8)	Lompo D. J. P. (10)	Ghana (10)	INERA (12)
Cahiers Agricultures (3)	Urban studies (8)	Dossa L. H. (7)	Belgium (7)	Univ. Joseph Ki-Zerbo (12)
Sustainability (3)	Geography (6)	Diogo R. V. C. (6)	Benin (6)	Ruhr University Bochum (8)
Agricultural Systems (2)	Public administration (5)	Marschner B. (6)	France (6)	CGIAR (7)
Environment and Urbanization (2)	Veterinary sciences (5)	Stenchly K. (6)	Netherlands (6)	University Abomey Calavi (6)
Human Ecology (2)	Food science technology (4)	Abdulkadir A. (5)	Austria (3)	Univ. Polytech Bobo Dioulasso (6)
International Journal of Agricultural Sustainability (2)	Physical geography (4)	Akoto-danso E. K. (5)	England (3)	Univ. Dev. Studies (5)
Land Use Policy, Outlook on Agriculture, Tropical Animal Health and Production (2)	Plant sciences (4)	Compaore E., Nyarko G. (5)	Kenya, Nigeria, Switzerland, USA (3)	Université Catholique Louvain, Wageningen University (5)

* Figures in brackets refer to the number of documents by a journal (a), research area (b), author (c), affiliation country (d), or affiliation organization (e).

The analysis of the geography of the research on UPA shows that the lion's share of the documents deals with Burkina Faso; indeed, 59 out of the 70 selected documents (84.2%) deal with Burkina Faso, either separately or with other countries. Meanwhile, only 13 documents (18.5%) address urban agriculture in Niger. Only a few documents deal with UPA in both countries (Brinkmann et al., 2012; Cissé et al., 2005). There are also some regional studies that encompass different West African countries: Benin, Burkina Faso and Ghana (Probst et al., 2012); Benin, Burkina Faso, Côte d'Ivoire, Mali, Mauritania, Niger and Senegal (Cissé et al., 2005); Burkina Faso and Ghana (Akoto-Danso et al., 2019; Manka'abusi et al., 2020); Burkina Faso, Ghana and Mali (Bellwood-Howard et al., 2021); Burkina Faso, Mali and Nigeria (Dossa et al., 2015); and Burkina Faso, Mali, Niger and Nigeria (Brinkmann et al., 2012). In the case of Burkina Faso, the focus is mainly on the two largest cities in the country viz. Ouagadougou and Bobo-Dioulasso.

CONCLUSIONS

To the best of our knowledge, this is the first paper that provides a comprehensive overview of the landscape of research on UPA in Burkina Faso and Niger by analysing the bibliometrics of the research field. The analysis of the selected literature suggests that interest in research on UPA in Burkina Faso and Niger is increasing but the annual output of articles remains low. While research on UPA is mainly within the research area of agriculture, it is linked to 27 research areas, which shows that it is rather multidisciplinary. However, the focus is mainly on biological and environmental sciences, while social sciences and economics are generally overlooked. The scrutiny of authors, affiliation countries and affiliation organisations suggests that a large share of studies on UPA is carried out by researchers affiliated with universities and research centres based outside Burkina Faso/Niger and even West Africa, especially in Germany (e.g. University of Kassel, University of Gottingen, Ruhr University Bochum). This might denote the weakness of the research system in Burkina Faso and, especially, Niger. Moreover, the funding of the research on UPA is dominated by foreign agencies, especially from Germany. This, in turn, might indicate the absence of structured research projects/programs on UPA in both countries. Meanwhile, the analysis of the geography of the research on UPA shows that the lion's share of the selected documents deals with Burkina Faso. Only a few documents address UPA in both countries or West Africa. In the case of Burkina Faso, the focus is mainly on Ouagadougou and Bobo-Dioulasso.

So that UPA contributes to addressing the many challenges that both countries face – such as food insecurity and malnutrition, climate change and poverty – investments in research, development and innovation are needed. In this regard, the present paper sets the table and prepares the ground for such an endeavour as it provides a baseline for future research in the field. It shows the research gaps but also the institutions and researchers that have been active in the field and that can be approached and involved in future research programmes. In general, since challenges and opportunities are quite similar, regional programmes on UPA would be highly beneficial in the Sahel and West Africa regions.

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NEGLECTED AND UNDERUTILISED SPECIES (NUS): AN ANALYSIS OF STRENGTHS, WEAKNESSES, OPPORTUNITIES AND THREATS (SWOT)

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ABSTRACT

Despite the growing attention to the neglected and underutilised species (NUS) as a valid instrument to promote not only sustainable agriculture and food systems but also sustainable development in rural areas, attempts to move from good intentions to effective actions have been limited. This is due, among others, to a gap in knowledge about the myriads of existing NUS and their potential. In this context, the present paper provides a comprehensive analysis of the strengths, weaknesses, opportunities and threats (SWOT) of crop NUS. Data for the SWOT analysis were retrieved through a review of the literature carried out in June 2022 on the Web of Science. Strengths relate, inter alia, to adaptability to harsh, marginal conditions, tolerance to biotic and abiotic stresses (e.g. pests and diseases, drought) and low external input requirements of NUS as well as their high medicinal and nutritional values combined with widespread culinary traditions. Weaknesses regard low productivity as well as difficult access to quality seeds, inputs, technologies and knowledge. Higher demand from consumers as well as increasing attention to sustainability and resilience in the whole agri-food system and agroecology represent opportunities for the promotion of NUS to address challenges such as food and nutrition insecurity and poverty. Climate change, biodiversity loss and genetic erosion, land and agroecosystem degradation, loss of traditional knowledge and heritage, and competition from commercial crops are among the main threats to NUS. The SWOT of NUS outlined in this work should inform evidence-based policies and strategies for the promotion of NUS, especially in developing countries. They should also guide the undertakings and actions of all stakeholders interested in the development of NUS value chains.

Keywords: *orphan crops, NUS, SWOT analysis, sustainable agriculture, rural development.*

INTRODUCTION

Crops can be divided into staple, commercial crops and underutilized, neglected and orphan ones (cf. neglected and underutilized species - NUS) (Li & Siddique, 2018). Padulosi et al. (2013) posit that "*Neglected and underutilized species (NUS) are those to which little attention is paid or which are entirely ignored by agricultural researchers, plant breeders and policymakers*" (p. 5). More than 7,000 crop species have been either cultivated and domesticated for food, or collected from the wild during the course of human history (FAO, 1998; Garn & Leonard, 2009). Nevertheless, only about 150 species are cultivated commercially (FAO, 1995; Prescott-Allen & Prescott-Allen, 1990), so tens of thousands of edible plant species remain relatively 'underutilized' (Chivenge et al., 2015).

The enhancement and promotion of NUS have been reported to contribute to climate change adaptation and mitigation (Mabhaudhi et al., 2019), agro-biodiversity conservation (Padulosi et al., 2013), environmental integrity and health (Mabhaudhi et al., 2019), food and nutrition security (Padulosi et al., 2013; Ulian et al., 2020), human health (Tadele, 2018), and rural livelihoods sustainability and resilience (Kour et al., 2018; Padulosi et al., 2013). Mabhaudhi et al. (2019) argue that with adequate research, NUS could play an important development role in the Global South and that research is also needed to advocate for policies and strategies as well as investments in orphan crops. Indeed, many challenges hinder the mainstreaming of NUS (Mabhaudhi et al., 2019). To promote NUS, barriers against their mainstreaming have to be identified and thoroughly analysed through research (Baldermann et al., 2016). Padulosi et al. (2013) postulate that "*Neglect by agronomic researchers and policy makers, genetic erosion, loss of local knowledge, marketing and climate change are major challenges to the sustainable use of NUS*" (p. 6). Likewise, Williams and Haq (2002) enumerate among the constraints to NUS development: lack of interest by farmers, researchers and extension agents, limited germplasm availability, and lack of technical information and tailored national policies. Therefore, research, innovation and development are highly needed to unlock the potential of NUS (Mabhaudhi et al., 2017), especially in developing countries (Chivenge et al., 2015).

In a recent systematic review on orphan crops in Burkina Faso and Niger, El Bilali (2020) puts that "*despite the noted benefits of orphan crops in addressing multiple challenges—such as climate change, livelihoods vulnerability and poverty, food and nutrition insecurity, and biodiversity loss and ecosystem degradation—current gaps in knowledge and research hinder the capacity to promote and exploit these crops in both countries*" (p. 7). Such a negative assessment is not limited to Burkina Faso and Niger and can be extended to many other countries, especially developing ones. Indeed, the real potential of NUS is largely unknown due to the lack of scientifically-sound and comprehensive assessments. Some studies on the use and potential NUS exist but systematic, comprehensive evaluations are generally lacking. This regards the strengths and opportunities as well as the weaknesses and threats, which hinders initiatives and undertakings to valorise and promote them. In this context, the present review paper is meant to fill this gap by providing a comprehensive SWOT analysis of NUS.

MATERIAL AND METHODS

SWOT analysis is a technique for strategic planning and strategic management used to help identifying strengths, weaknesses, opportunities, and threats related to a business, project, activity, etc. (Benzaghta et al., 2021; Gürel & Tat, 2017). It is sometimes called situational assessment or situational analysis (Weihrich, 1982). This technique is designed for use in the preliminary stages processes of decision-making (Silva, 2005). SWOT analysis is intended to identify the internal and external factors that are either favourable or unfavourable to achieving the objectives. The name SWOT is an acronym for the four components encompassed in the technique: *Strengths*: characteristics giving an advantage over others; *Weaknesses*: characteristics considered as disadvantages relative to others; *Opportunities*: elements in the environment that could be exploited to one's advantage; and *Threats*: elements in the environment that could cause trouble and problems. The result of SWOT analysis is usually presented in the form of a matrix (Ansoff, 1980). Internal factors and characteristics are usually classified as strengths and weaknesses, while external ones are placed under opportunities and threats (Gürel & Tat, 2017; Minsky & Aron, 2021). The classification of internal factors as strengths or weaknesses is not universal and depends on the objectives. While SWOT analysis is a widely used technique (Benzaghta et al., 2021), it has some limitations (Hill & Westbrook, 1997) that relate, inter alia, to the usefulness/usability of its outputs as well as biases that might stem from how it is performed and who is involved in the process.

Data for the SWOT analysis of NUS were collected through a systematic review (Moher et al., 2009; Page et al., 2021). A search was carried out on the Web of Science (WoS) on June 4th, 2022, using the following search string: *("SWOT" OR "strength" OR "weakness" OR "opportunity"* OR "threat"*) AND *("neglected and underutilised species" OR NUS OR "neglected species" OR "neglected and underutilized crop" OR "neglected crop" OR "abandoned crop" OR "abandoned species" OR "alternative crop" OR "alternative species" OR "local crop" OR "local species" OR "lost crop" OR "lost species" OR "minor crop" OR "minor species" OR "niche crop" OR "niche species" OR "orphan crop" OR "orphan species" OR "traditional crop" OR "traditional species" OR "underdeveloped crop" OR "underdeveloped species")*. The search returned 497 records/documents whose eligibility was checked against two criteria: addressing NUS and providing SWOT elements.

The screening of titles and abstracts as well as, in case of doubt, the scrutiny of full-texts led to the exclusion of 449 documents. Out of these, 322 documents were excluded because they do not deal with neglected and underutilized species

(NUS)[1]. Further documents deal with major commercial crops such as soybean, fava bean, rice, maize/corn, pear, chestnut, pepper, banana, tobacco, and lettuce. Other articles deal with forest trees such as eucalyptus. Also, documents referring to local and minor arthropod and insect species as well as fish, bird and animal species were discarded. Further 122 documents were excluded because they do not address SWOT analysis. Consequently, 48 documents resulted eligible and provided data for the SWOT analysis of NUS.

RESULTS AND DISCUSSION

The analysis of the selected documents shows that the geographical coverage of the studies ranges from global to local through regional and national levels. Interestingly, many studies deal with developing regions and countries in Africa (e.g. Benin, D. R. Congo, Guinea, Ethiopia, Ghana, Madagascar, Mozambique, Nigeria), Asia (e.g. India, Sri Lanka) and Latin America (e.g. Bolivia, Paraguay, Peru). While many studies address NUS in general, others focus on specific botanical families/groups (e.g. legumes, medicinal and aromatic plants, grains) or, even species (e.g. African nightshade, baobab, amaranth, bambara groundnut, breadfruit, cassava, cowpea, emmer, enset, fonio, miracle plant, moringa, oat, oca, pigeon pea, pitaya, quinoa, sesame, tef).

The literature reports many *strengths* of NUS (Table 1). One of the main strengths is their adaptability to grow in harsh conditions. These include marginal, poor and nutrient-depleted soils/lands as well as areas characterised by difficult climatic conditions such as mountains. This high adaptability confers on the NUS the capability of fitting into multiple niches in crop production systems. In addition, many NUS seem adapted to cultivation in low-input systems as they have low requirements in terms of fertilisers and agrochemicals. Moreover, their tolerance to pests and diseases reduces the need to resort to pesticides. Indeed, the tolerance of NUS to biotic (pests and diseases) and abiotic/environmental stresses (e.g. drought) is widely recognized. NUS are also claimed to be tolerant to drought and resilient to extreme climate events, which makes them an important ally in the context of climate change and variability. Certainly, NUS perform well even in the case of harsh climatic conditions or low external inputs; some studies report that local NUS landraces outperform modern, improved varieties (Mondo et al., 2021). Other strengths of NUS are the high diversity of genetic resources available at the local level as well as the easiness of their propagation and cultivation by local

[1] For instance, some documents refer to some forest tree species, such as *Pinus (e.g. Pinus attenuata*, *Pinus radiata*, *Pinus oocarpa*, *Pinus ponderosa*), or National University of Singapore (NUS), National University of Samoa (NUS), networked universities (NUS), non-uniform in-situ stress (NUS), neuromuscular ultrasound (NUS), nonlinear unified strength (NUS), non-uniform sampling (NUS), normalized updraft strength (NUS), never users (NUS), normal nutritional status (NUS), nerve ultrasonography (NUS), new urbanist subdivision (NUS), next-generation sequencing (NUS), new urbanization strategy (NUS), non-autistic university students (NUS), National Health Service (NUS), rather than neglected and underutilised species (NUS).

communities that already possess considerable traditional knowledge. Many scholars point out the outstanding nutritional benefits of NUS and highlight their nutritional properties (Assogbadjo et al., 2021; Kodahl & Sørensen, 2021). Indeed, many NUS have high nutrient density and high contents of proteins (cf. legumes) as well as bioactive, health-enhancing and health-protecting compounds such as calcium, iron, potassium and zinc, fatty acids (omega-3 and -6 fatty acids), anti-oxidants, etc. These characteristics confer them also good sensory properties and acceptance by consumers.

The *weaknesses* of NUS regard all the phases of the food supply chain from production to consumption through processing and distribution. At the production level, the main problem relates to low yield and productivity, especially when compared to modern, commercial varieties (Giuliani et al., 2009). This can be due to the low potential of the local varieties used as it might be a direct consequence of the difficult access to and low use of inputs (e.g. fertilisers) when cultivating NUS. Low yield and productivity lead to low production levels and this makes it difficult for NUS to benefit from economies of scale, which has negative implications in terms of the organisation and development of their value chains; indeed, value chains are often either disorganized or non-existent. Another constraint at the production level regards difficult access to quality seeds and propagation materials. This is due to the low volume of seeds as well as the lack of seed improvement programs. Difficult access to information is another problem faced by producers, which affects negatively their agronomic skills and knowledge. At the processing level, there is a lack of adequate technologies, which determines difficulties in processing, especially for smallholders, as well as drudgery problems. All the above-mentioned constraints affect product distribution and consumption. Indeed, there are only a few products available on the market and their quality does not always meet the consumers' expectations. Furthermore, low volumes marketed and lack of economies of scale push prices up and affect product affordability.

The *opportunities* reported in the analysed scholarly literature relate to the increasing recognition of NUS as an important asset for the sustainability and resilience of agri-food systems as well as to address numerous challenges such as food and nutrition insecurity, malnutrition, livelihoods vulnerability and poverty in the context of climate change. This positive trend is due, among others, to the growing attention to sustainable and green systems such as agroecology. Local NUS are also increasingly promoted by the government as a strategy for food self-sufficiency and import substitution. The success of NUS is also explained by the growing attention to health, which determines the adoption of more sustainable, diversified diets that are mainly plant-based. This, in turn, determines an increase in demand for a variety of NUS and products based on them in domestic as well as export markets. Also the versatility and diversity of uses of NUS as food and feed as well as medicinal or pharmaceutical, cosmetic and magico-therapeutic ones open up many avenues of opportunities for the development of NUS value chains. Some NUS are even considered 'functional foods' or 'superfoods'. Further opportunities for NUS arise from the development of technologies, especially in breeding, which leads to the development of improved varieties. While research and development on NUS remain below commercial crops, there is a growing number of national and international, collaborative and multi-stakeholder projects, programmes and initiatives that deal with them. This corresponds to an increase in investments and funding from national governments, international organisations as well as philanthropic organisations.

Threats to NUS are environmental, economic, socio-cultural and political. One of the most important threats to NUS relates to the loss of diversity. The erosion of genetic resources is caused by many phenomena including climate change, which makes local conditions unsuitable for the cultivation of some NUS by affecting rainfall patterns as well as temperature regimes. Some scholars point out the increasing adoption of modern, improved varieties as one of the causes of the erosion of traditional genetic resources (Balemie & Singh, 2012; Moscoe & Emshwiller, 2016). What is even more alarming is that the erosion of genetic resources is combined with the erosion of the collective and traditional cultural heritage and knowledge relating to NUS. In fact, there are serious problems regarding the transmission of knowledge about NUS in rural areas to new generations, which are often less interested in agriculture and prefer to migrate to cities. In general, an important threat relates to the negative perception of NUS as ‘foods of the poor’ as well as to the changing culture and socio-cultural structure in rural areas. Indeed, NUS products are often labelled by youth as ‘backward’ and ‘unmodern’. This is particularly alarming given the ongoing erosion of local food cultures and the loss of traditional dietary habits caused by the standardization of local food cultures arising from globalization as well as the global use of fast-food or ‘junk food’ that favours standard and low-quality ingredients. Furthermore, low attention to NUS in agricultural policies and research programmes implies that they are ill-equipped to compete with commercial crops. In general, the lack of knowledge and data about NUS remains one of the main constraints hampering attempts to unlock their potential in effective development endeavours. Some scholars enumerate alien, invasive pests and diseases among the threats to NUS (Chater et al., 2018; Shah et al., 2019), especially in the context of a lack of knowledge about their interaction with orphan crops and damages they might cause in local conditions. A further threat stems from the green revolution approaches pushing productivity in agriculture thus favouring high-yielding, improved cultivars to the detriment of local, traditional farmer varieties and landraces.

Table 1. SWOT analysis of NUS based on the literature review.

Strengths (S)	**Weaknesses (W)**
Climate resilience, drought tolerance Adaptability to cultivation on marginal and poor soils/lands, tolerance to salinity and sodicity Vast genetic resources and diversity in	Difficult access to quality seeds and propagation material Low agronomic skills and knowledge of producers Low yield and production levels

landraces Resistance/tolerance to pests and diseases Adaptability to local agroecosystems and low-input conditions Easiness of propagation and potential cultivation High contents of nutrients and health-enhancing compounds Good sensory properties and consumer acceptability Long-term storability	Variation in product quality Lack of appropriate storage, processing and packing technologies Limited processing knowledge Disorganized or non-existent value chains and markets Low market value discouraging investment High consumer prices due to a lack of economies of scale Low consumption in production areas
Opportunities (O)	**Threats (T)**
Recognized contribution to addressing major challenges such as food and nutrition insecurity and poverty High genetic variability within the cultivated and wild relatives Improved knowledge of genetics and biotechnologies Modern technologies in breeding, development of improved varieties Increasing use and demand of NUS in diversified, sustainable diets Significant industrial opportunities (food, feed, etc.) Growing attention and new market potential associated with nutritional and health properties Availability of a local market and appreciation by local consumers Increased recognition of agro-biodiversity in policies as a strategic asset Development of pro-poor and fair trade value chains Policies promoting the inclusion of NUS in school feeding programs Increasing investments and funding for research and development projects and programmes Increasing international collaboration on NUS	Climate change and variability Degradation of arable lands Invasive pests and diseases Erosion of the cultural heritage and knowledge relating to NUS New Green revolution approaches pushing to increase productivity in agriculture Loss of biodiversity due to the adoption of modern, improved varieties, and competition from commercial, commodity crops Changes in the socio-cultural structure: abandonment of agriculture by young people and migration from rural areas Changes in consumer taste and preferences, eroding local food cultures and loss of traditional dietary habits Consumer unawareness about NUS Negative cultural perceptions about NUS as the ‘foods of the poor’ Decline of government support for and intervention in agriculture and rural areas Inadequate funding for and investment in research, development and extension Discriminating agricultural and trade policies

The classification of the different items is not unanimous or universal and can change from one source to another depending, inter alia, on the concerned NUS and context as well as the ideological settings and worldviews of scholars. For instance, while some scholars consider very high genetic variation within the species as a strength since it allows the species to adapt to different conditions and environments and can be used to develop varieties with different features and traits, others consider it as a weakness that hampers standardisation so the development of commercial cultivars as well as opportunities for processing. Similarly, genetics is considered by some scholars as an opportunity to allow the conservation and genetic improvement of NUS while it is seen by others as a threat that can lead to the further erosion of the genetic resources relating to the NUS by favouring modern, commercial cultivars to the detriment of traditional, local varieties.

CONCLUSIONS

The present review provides a comprehensive analysis of the strengths, weaknesses, opportunities and threats (SWOT) of NUS with a particular focus on crops. The analysis of the scholarly literature shows that research on NUS is growing and expanding both in terms of geographical coverage (with more attention devoted to developing countries) and the range of NUS species addressed; nevertheless, it also clearly shows that comprehensive SWOT analyses in the NUS field are still hard to find. Therefore, the present review is timely and fills an existing knowledge gap. While the classification of the different items is far from being unanimous and depends, among others, on the concerned NUS, which makes generalisation difficult, some general trends emerged from the literature review. In general, strengths relate to the good agronomic performance of NUS even in unfavourable growing conditions. Indeed, most NUS are adapted to harsh, marginal conditions, tolerant to biotic and abiotic/environmental stresses, and require low external inputs. This makes NUS suitable for cultivation by smallholders in remote rural areas. The main weaknesses of NUS regard low yield and productivity, which determines a low level of production and, consequently, underdeveloped value chains. Moreover, producers have difficulty in access to quality seeds and propagation material, inputs, technologies for production and processing as well as information and knowledge on good agronomic practices. The analysis of the scholarly literature shows that there is increasing recognition in policies and strategies of the role of the NUS in the sustainability and resilience of agri-food systems as well as their contribution to addressing challenges such as food and nutrition insecurity, livelihood vulnerability and poverty. Further opportunities for the promotion of NUS stem from the increasing demand from consumers as they are becoming more aware of their benefits, especially in nutritional and health terms. Meanwhile, NUS are threatened by many trends and phenomena in the environmental, economic and socio-cultural realms. One of the most important threats regards the loss of biodiversity and the erosion of genetic resources that are caused by the degradation of lands and agroecosystems as well as climate change and variability. However, NUS are also threatened by societal and socio-cultural changes that determine the loss of their value among young generations as well as the erosion of the traditional, local knowledge relating to their cultivation, storage, processing, preparation and consumption in local communities.

The present SWOT provides general information that are valid for most of NUS but not necessarily for all. Therefore, contextualisation is needed to make it fit for specific cases. Nevertheless, the work provides valuable insights and scientifically sound evidence that should inform policies and strategies aiming at the valorisation and promotion of NUS. Moreover, the proposed SWOT analysis provides a baseline that can guide the actions and endeavours of all stakeholders and actors involved in the development of the value chains of NUS.

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POLLEN FORMATION IN SUNFLOWER HYBRIDS ON THE BASIS OF CYTOPLASMIC MALE STERILITY

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ABSTRACT

In externally fertile plants, the quality of the pollen may be different, and it is possible to distinguish the so-called "semi-fertile" forms. This is typical for sunflower F_2 hybrid populations from crossing CMS PET1 line and restorer lines. We have characterized the fertility/sterility manifestation in a sample set of 17 F_2 and 7 F_3 genotypes derived from a cross between the CMS line VIR 116A and a restorer line VIR195. The segregation pattern for fertility/sterility in the original F_2 population of 262 genotypes fitted three (fertile): one (sterile) model that supports the hypothesis on controlling the fertility restoration by a single locus (putatively *Rf1*). Each plant was phenotyped under field conditions and genotyped using SSR marker ORS511 linked the to the *Rf1* locus. After flowering, the fertile or sterile plants were registered. The pollen of fertile and chimeric plants was stained with acetocarmine. F_1 plants produced appr. 90% of fertile pollen. Among F_2 plants examined, five plants were classified as purely fertile, seven as sterile, three as semi sterile, and two as chimeric ones. Among the F_3 plants three fertile, three sterile, and one chimeric plant have been noted. The chimeras possessed fertile flowers in the 1st to 3rd cycles of flowering, and further to the center the flowers were sterile. In most cases, fertile and chimeric plants possessed the ORS511 marker whereas sterile plants lacked the marker. The occurrence of the marker in sterile genotypes, and the absence of it in fertile ones can be a consequence of recombination events.

Keywords: *pollen fertility, CMS, sunflower, SSR marker ORS511*, *Rf1* gene.

INTRODUCTION

Cultivated sunflower (*Helianthus annuus* L.) is one of the main oilseed crops. The industrial production of high seed yields is based on heterotic interlinear hybrids obtained using the phenomenon of cytoplasmic male sterility. The CMS trait manifests itself in certain combinations of mutant (usually chimeric) mitochondrial genes resulting from rearrangements of the mitochondrial genome and nuclear genes called *Rf* (Restoration of fertility) pollen restoration genes (Anisimova, Gavrilenko, 2017; Anisimova, 2020). Fertility restoration genes are present in

some varieties and lines that can participate in crossing for heterosis to obtain high-yielding hybrids. In sunflower, lines with PET1-type of cytoplasmic male sterility (CMS) are currently used worldwide for producing F_1 hybrid seeds. The effects of CMS are suppressed when functional alleles of *Rf* genes are included in the genotype. Based on this hypothesis, fertility should be restored in the first generation of hybrids, and the second generation should segregate for this trait. It is known that the F_2 plants can differ in the pollen characteristics including pollen fertility indices and morphometric parameters. According to recent studies, some externally fertile plants from F_2 segregating populations may not be completely fertile. As a result, an F_2 population can be highly heterogeneous for pollen characteristics (Karabitsina *et al.,* 2019; Voronova and Gavrilova, 2019). Therefore, in the present study we checked the fertility distribution of pollen grains by cytological methods.
Externally fertile plants may not be fully fertile, resulting in heterogeneous pollen. Based on this, the purpose of our study was to carry out phenotyping, genotyping and analyze the fertility of hybrid plants by cytological methods.

MATERIAL AND METHODS

Field experiments were conducted in 2021 at Pushkin and Pavlovsk Laboratories of the VIR (St. Petersburg, Pushkin). F_2 and F_3 hybrid seeds from crossing a CMS PET1 inbred line VIR 116A with fertility restorer line-VIR 195 were sown at the end of May.
During the growing season, phenological observations were carried out, visual assessment of-fertility / sterility trait was performed, and the damage of plants by pests and diseases was monitored. Moreover, leaf material for DNA isolation was collected at the 2-3 pairs of true leaves. For cytological examination, part of head was collected and fixed in FAA solution. To assess pollen fertility, a modified acetocarmine staining method was used (Voronova and Gavrilova, 2019).
DNA was isolated from frozen leaves. Genomic DNA was isolated by the modified CTAB method (Li *et al.,* 2007).
The obtained DNA fractions were stored at a temperature of +4 to -20 ° C. The concentration and quality of DNA preparations were determined by electrophoresis in 1% agarose gel. In order to identify the genotypes of hybrid plants for alleles of Rf1 locus, the microsatellite diagnostic marker ORS511 was used.
For PCR, a standard reaction mixture was used (volume – 25 µl, but could vary slightly), which included 1.5 µl of DNA template, 15.95 µl of H2O, 2.5 µl of 10X reaction buffer, 2.4 µl of 2.5 mM dNTP, 1.25 µl of 50 mM MgCl2, for 0.5 µl of forward and reverse primers at a concentration of 10 pM, 0.4 µl of Taq polymerase (5U/µl).
The amplification products were separated by electrophoresis on 1.5% agarose gel and stained with 0.05% ethidium bromide solution. The gels were visualized using the Gel Doc XR+ Bio Rad gel documentation system in ultraviolet light.

Pollen fertility was evaluated in the field (phenotypically) by the presence of normally developed anthers containing pollen and on cytological preparations stained with acetocarmine.

Cytological analysis was performed using a Zeiss Axio Imager.Z1 microscope with an AxioVision digital camera and Zen 2.1 software. The percentage of fertile pollen grains was calculated based on the analysis of at least 12 visual fields at a 20-fold magnification.

RESULTS AND DISCUSSION

We have characterized the fertility/sterility manifestation in a sample set of 17 F_2 and 7 F_3 genotypes derived from a cross between the CMS line VIR 116A and a restorer line VIR195.

As previously defined, F_1 hybrid plants produced appr. 90% of fertile pollen. The segregation pattern for fertility/sterility in the original F_2 population of 262 genotypes fitted three (fertile): one (sterile) model that supports the hypothesis on controlling the fertility restoration by a single locus (putatively *Rf1*) (Anisimova *et al.,* 2021).

Each plant was phenotyped under field conditions. After flowering, the fertile or sterile plants were registered (Fig. 1, *1-3*).

Among F_2 plants examined, five plants were classified as highly fertile, seven as sterile, three plants had intermediate phenotype and marked as sterile/fertile. They had visible anthers, but the anther tube was not as well developed as in truly fertile plant. Moreover, the two plants were classified as chimeric ones. The chimeras possessed fertile flowers in the 1st to 3rd cycles of flowering, and further to the center the flowers were sterile (Fig. 1, 2). Among the F_3 plants three fertile, three sterile, and one chimeric plant have been noted.

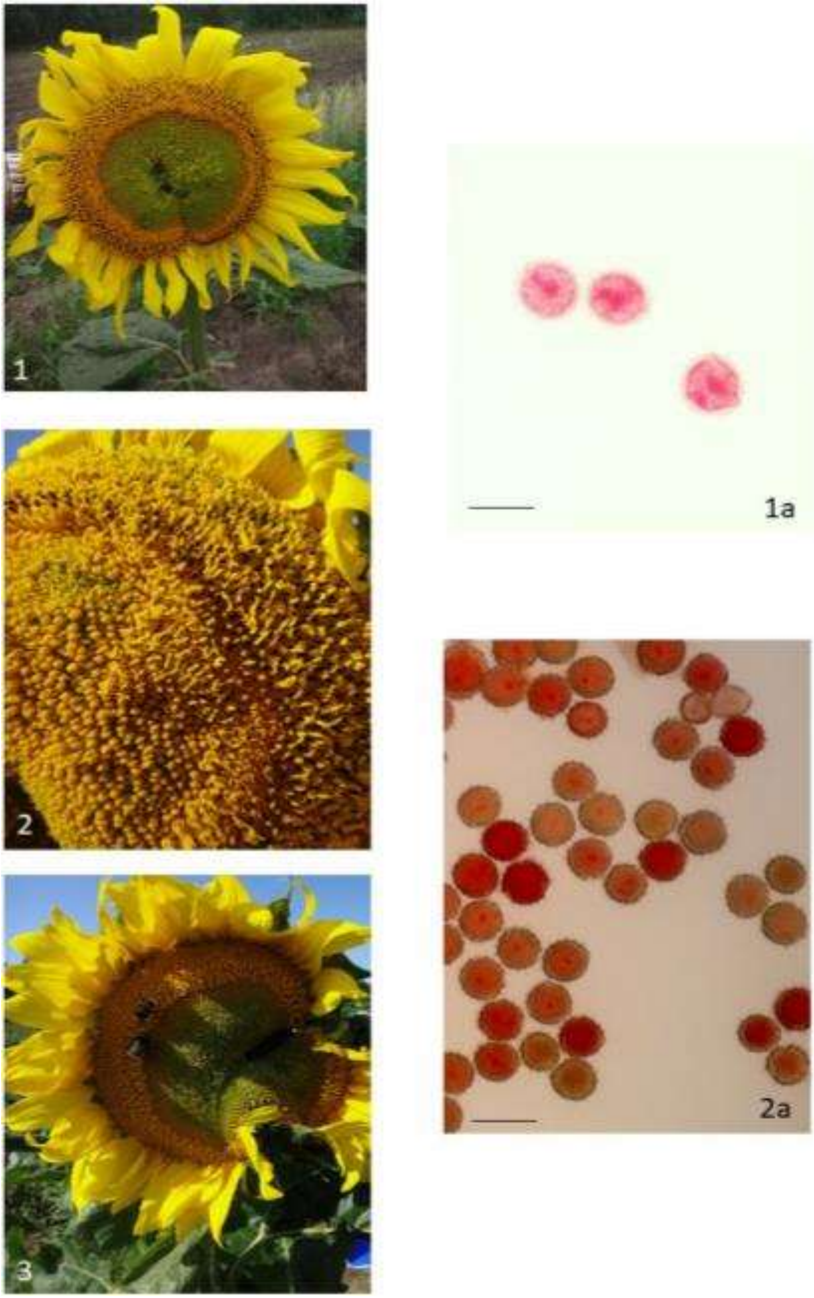

Figure 1. F_2 plants from cross combination VIR 116A × VIR 195: 1) head of a fertile plant, 1a) fertile pollen, 2) head of a chimeric plant, 2a) pollen of a chimeric plant, 3) head of a sterile plant (St. Petersburg, Pushkin, 2021)

The presence of chimeric plants seems especially interesting to us, because in the early stages (flowering 1-3 circles) they look like truly fertile ones. If we did not carry out monitoring until the end of flowering, then these plants would be counted as ordinary fertile ones.
The pollen of fertile and chimeric plants was stained with acetocarmine (Fig. 1, *1a*, *2a*). When pollen was evaluated by staining with acetocarmine, it is customary to say not about stained or not stained, but about fertile and sterile pollen grains. We considered the pollen grains of normal and enlarged size with a uniformly stained cytoplasm as a fertile one; unstained and heterogeneously stained pollen grains of all sizes, as well as stained grains of a much smaller size (micropollen), were classified as sterile ones.
In general, the analysis of pollen by the acetocarmine method revealed a significant diversity among hybrid plants by coloration and the diameter of pollen grains. The range of variations of these parameters was from highly fertile plants with a fertility rate of 85-97%, through a group of plants with medium fertility of 72-74%, to low-fertile plants with a pollen fertility rate of 10-56% (Fig. 2).
Highly fertile plants have the pollen that is aligned with diameter. In the pollen of plants with reduced fertility, there is a significantly greater heterogeneity in diameter compared to highly fertile forms. Also, in general, a decrease in the total amount of pollen was noted in plants with reduced fertility.

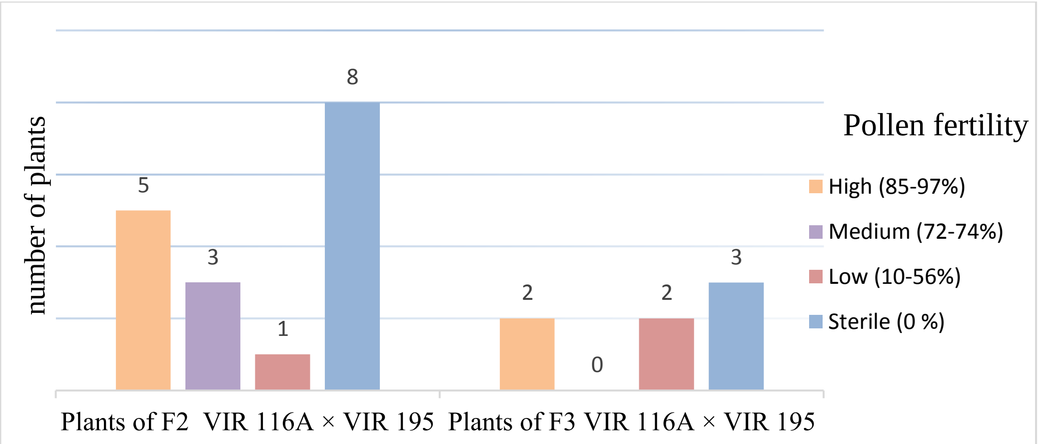

Figure 2. Distribution of plants by pollen fertility

In total there were only 7 highly fertile (HF) plants (44%), medium fertile (MF) - 3 (23%), low fertile (LF) - 3 (23%) and 11 sterile plants. The distribution according to the level of fertility among the F_2 plants was as follows: 5 HF, 3 MF and 1 LF. Plants from F_3 generation had 2 HF, 0 MF, and 2 LF. In general, there is a tendency that highly fertile plants (HF) belong to plants with reduced fertility (MF + LF) in a ratio of 1 : 1 (Fig. 2).
Also, the ratio of sterile and fertile plants is close to 1:1. Of course, we do not yet have enough material for convincing statistics, but we plan to continue research to

clarify this issue. Similar cases with partially fertile pollen have already been found earlier in lines with CMS. For example, in some plant species, along with classes of fertile/sterile plants, semi-fertile or semi-sterile ones were also observed. Such plants were characterized by reduced pollen fertility. The so-called “pollen deficiency” effect has been repeatedly noted by authors on different plant species (Elkonin, 2005; Sinha *et al.*, 2013; Karabitsina *et al.*, 2019), but there is still no consensus on the reason for this phenomenon. For example, variants of microsporogenesis with partial degeneration of tapetum cells and young microspores were described in cultivated sunflower. It leads to the appearance, in addition to aborted pollen grains, and a certain amount of normal pollen (Kovacik and Sykorova, 1979).

Most plant was genotyped using SSR marker ORS511 linked the to the *Rf1* locus (Table 1). Unfortunately, clear data could not be obtained for several plants. In most cases, fertile and chimeric plants possessed the ORS511 marker whereas sterile plants lacked the marker. The occurrence of the marker in sterile genotypes, and the absence of it in fertile ones can be a consequence of recombination events.

Table 1. Distribution of SSR marker ORS 511 and pollen fertility index among F_2 and F_3 hybrid plants from cross combination VIR 116A x VIR 195

№	Plant number	Amount of fertile pollen grains, in %	Plant phenotype	Presence of the ORS 511 marker
	F_2 VIR 116A × VIR 195			
1	1	0	Sterile	no data
2	5	0	Sterile	–
3	9	0	Sterile	–
4	10	0	Sterile	–
5	16	0	Sterile	–
6	3	0	Sterile	+ (?)
7	8	0	Sterile	+ (?)
8	7	0	Sterile/fertile	–
9	11	55	Fertile	no data
10	2	72	Sterile/fertile	+
11	14	73	Fertile	+
12	15	74	Chimeric	+
13	12	85	Fertile	no data
14	17	86	Fertile	+
15	4	87	Sterile/fertile	– (?)
16	6	92	Fertile	no data
17	13	93	Chimeric	no data
	F_3 VIR 116A × VIR 195			
1	20	0	Sterile	–
2	21	0	Sterile	–
3	24	0	Sterile	–

4	22	10	Fertile	no data
5	18	56	Fertile	no data
6	19	82	Fertile	+
7	23	97	Chimeric	+

CONCLUSIONS

We found that long-term monitoring (until the flowering of the head is completed) and cytological analysis of pollen (determination of fertility by the acetocarmine method) makes it possible to reveal interesting features of the F_2 and F_3 progeny from crossings of the CMS line VIR 116 with the pollen fertility restorer line VIR 195. Hybrid plants not only differentiated for fertile and sterile in appearance, but chimeric plants were found among the fertile ones. Also, among the fertile plants, heterogeneity was found in the quality of pollen, which made it possible to distinguish highly fertile, medium fertile, and low fertile forms.

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BIOCHAR BASED BACTERIAL BIOFERTILIZER PROMOTES GROWTH AND MITIGATES COPPER TOXICITY IN *BRASSICA OLERACEA*

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ABSTRACT

The interest to bacterial biofertilizers (BFs) is currently increasing since their application is environment friendly and cheaper in comparison with chemical fertilizers. BFs application reduces the metals availability in the soil through chelation, thereby reducing the toxicity of pollutants. The aim of the research was to study the effect of BF based on biochar and metal tolerant plant growth promoting rhizobacteria (PGPR) on growth parameters, copper accumulation and lipid peroxidation level in *Brassica oleracea* L. *Bacillus altitudinis* strain TF16a isolated from the rhizospheric soil of *Tussilago farfara* L. growing close to copper smelter was used to prepare the inoculum. To obtain BF, liquid inoculum of PGPR (10^8 CFU mL^{-1}) was mixed with sterile substrate. Plants were grown in pot for 30 days. In the control substrate and with the addition of BF, the copper content was two times lower than the maximum permissible concentration for Russia. It was found that combined action of BF with Cu had a positive effect on the growth parameters in *B. oleracea*. Copper application led to the significant increase in its accumulation in shoot and root which was accompanied by the increase in malondialdehyde content in cabbage leaves. The combined action of BF and Cu reduced Cu accumulation and mitigated peroxidation processes. Thus, it can be concluded that biochar based biofertilizer promoted growth and alleviated copper toxicity in *B. oleracea*.

Keywords: *Bacillus altitudinis, cabbage, biochar, plant growth, agrobiotechnology* .

INTRODUCTION

One of the global tasks of our time is to increase the productivity of cultivated plants, as well as to provide the population with high-quality and safe food. The uncontrolled use of chemical fertilizers and pesticides in the agricultural sector leads to the pollution of ecosystems with various pollutants, including heavy metals, and poses a threat to human health (Dubey and Nidhi Shukla, 2014; Yadav et al., 2017). In this regard, the search for safe biological products to stimulate plant growth without harming the environment is becoming increasingly important. One of the promising directions for improving agricultural technologies is the use of biofertilizers based on plant growth promoting bacteria (PGPB), which include both endophytic and rhizobacteria (Lugtenberg and Kamilova, 2009; Rana et al., 2011; Glick, 2012; Maleva et al., 2017; Kumar et al., 2021). In addition, the use of biochar (BC), a carbon-rich material produced by pyrolysis, gasification or hydrothermal carbonization from various organic wastes, is becoming increasingly popular in the agricultural sector (Gascó et al. 2019). The numerous studies have shown that the addition of BC leads to an improvement in the structure and physicochemical characteristics of the soil, promotes the development of soil microflora, and reduces the toxic effect of organic and inorganic pollutants (Lehmann, 2011; Wang et al., 2019). The use of BC allows not only the efficient use of industrial waste, but also a significant increase in crop yields, on an average by 10–30% (Sean 2020; Borisova et al., 2021). It has been shown that BC addition to the soil reduces the availability of metals including copper and their toxic effects on plants, thereby allowing their use for phytostabilization purposes (Kamran 2020). However, to date, data on the use of biochar as a carrier for PGPB are fragmentary.

Copper occurs in nature in different forms and it is one of the most used non-ferrous metals in industries. Wastes from metallurgical enterprises are the main sources of air, water and soil pollution with copper (Rehman et al., 2019; Kumar et al., 2020). Copper is an essential trace element for the growth and development of plants, since it is part of the active complex of enzymes that are involved in respiration, photosynthesis and many other key metabolic processes (Printz et al., 2016). It involved also in lignin synthesis and metabolism of carbohydrates and proteins (Printz et al., 2016; Tugbaeva et al., 2022). At the same time copper deficiency or excess can have a negative effect on agricultural plants (Mwamba et al., 2016; Tugbaeva et al., 2021; 2022).

The aim of the work was to study the effect of biofertilizer based on biochar and metal tolerant PGP rhizobacteria *Bacillus altitudinis* strain TF16a on growth parameters, copper accumulation and lipid peroxidation level in *Brassica oleracea* L.

MATERIALS AND METHODS

As a carrier material for bacteria in bioformulation, a biochar was chosen, made by "DianAgro" (Novosibirsk, Russia) by the method of oxygen-free pyrolysis using high-density birch wood and representing 100% charcoal grade A (according to GOST 7657-84). Such wood produces good biochar due to higher percentage of carbon and minimum ash content. A liquid culture of *Bacillus altitudinis* strain TF16a (10^8 CFU mL^{-1}) was used as an inoculum for the preparation of bacterial biofertilizer (BF). This strain was previously isolated from the rhizosphere of *Tussilago farfara* L., growing closed to the copper smelter, and selected on the basis of the best Cu-tolerance and PGP attributes.

The pot-scale experiments were done on *Brassica oleracea* L. (variety Express F1) which were grown from seeds in 100 mL plastic pots in the growing chamber for 30 days with photoperiod 14:10 (day:night), light intensity – 130±20 μM m^{-2} s^{-1}, humidity – 55±5%, and temperature –23±2 °C. The experiment included four treatments: 1 – control (peat based soil substrate, S); 2 – substrate with the addition of 5% BF; 3 – substrate with addition of 100 mg kg^{-1} Cu (sulfate form); 4 – substrate with the addition of 5% BF and 100 mg/kg Cu (BF+Cu). Three seeds were planted in each pot, a total of 8 pots were used for each treatment, the experiment was repeated two times. The following growth parameters of *B. oleracea* were measured: number of seedlings, seed germination rate, shoot and root length, leaf area, fresh and dry biomass per plant.

To calculate the leaf area, a specialized program JMicroVision software (version 1.2.7) was used (Roduit, 2019). The level of lipid peroxidation was assessed by the amount of malonic dialdehyde (MDA) according to the generally accepted method (Heath, Packer, 1968). The copper content in shoot and root of *B. oleracea* was determined using the atomic absorption spectrometer AA240FS (Varian Australia Pty Ltd., Mulgrave, Victoria, Australia) after wet digestion in HNO_3 and calculated per dry weight (DW).

All data were analyzed statistically by analysis of variance using Statsoft Statistica 8.0. Data are presented as mean values ± standard errors (SE). ANOVA analysis of variance was used to determine the individual and joint effects of BF and Cu. The significance of differences between the treatments was assessed using Tukey's HSD test. Different alphabetical letters indicate significant difference between the treatments.

RESULTS AND DISCUSSION

A significant decrease in the rate of seed germination was observed in the treatment with copper. At the same time the maximum number of seeds germinated with the combined action of BF with copper (BF+Cu) (Fig 1a). Single application of BF and Cu led to a decrease in leaf area compared to the control by 15 and 28%, respectively. Combined action of BF and Cu (BF+Cu) increased leaf area of *B. oleracea* by 14% as compared to the control and by 50% compared to the single action of BF (Fig 1b). Moreover, the highest value of shoot length was also noted for this combined treatment, while the single application of BF or Cu had the inhibitory effect (Fig. 1c). The similar trend was also previously noted for *Brassica napus* by Dąbrowska et al. (2017), they reported that the combined action of biofertilizer with zinc and cadmium led to the increase in the length of its roots and stems.

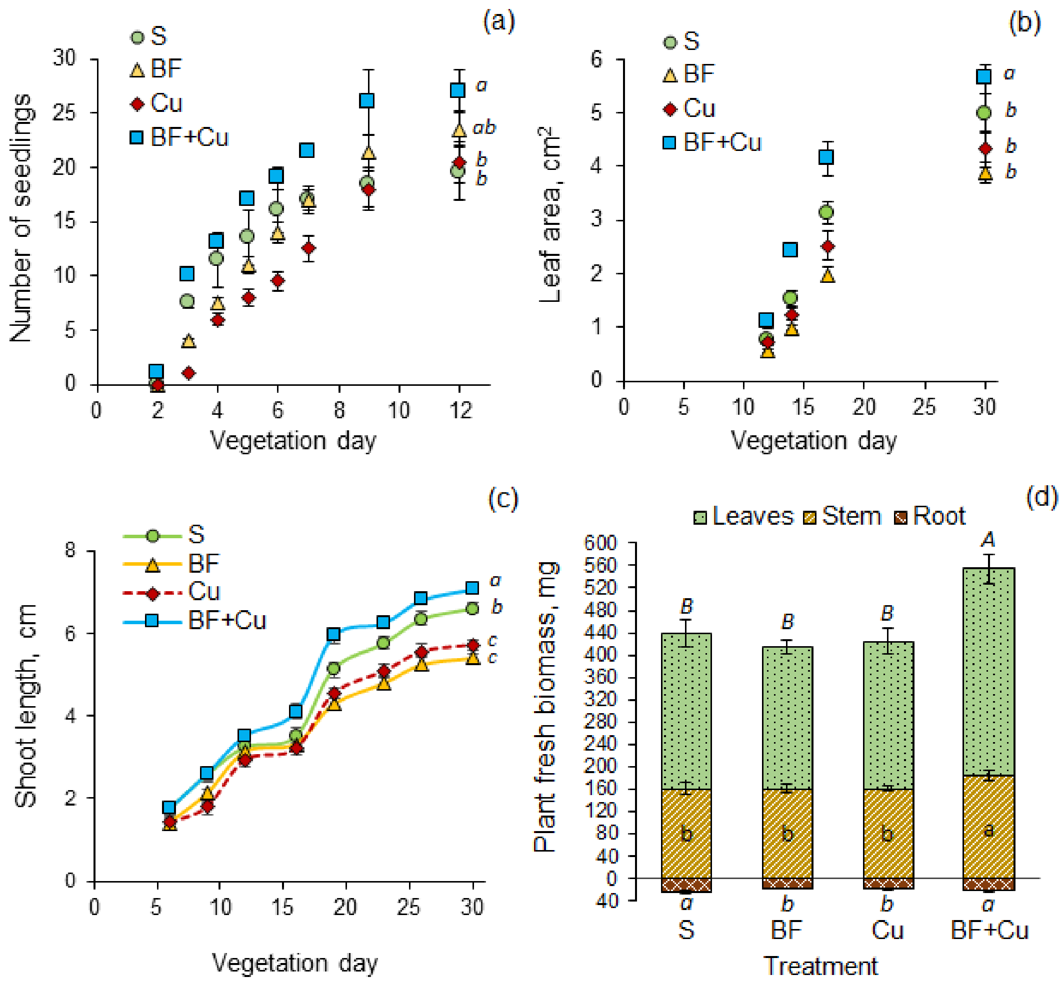

Figure 1. Number of seedlings (a), leaf area (b), shoot length (c) and fresh biomass (d) of *B. oleracea*.

The single action of BF as well as Cu did not affect fresh biomass of both *B. oleracea* aboveground and underground organs (Fig. 1d). However, the combined action of BF with Cu increased shoot biomass of *B. oleracea* by 26% compared to the control, mainly due to the increase in leaf weight. At the same time, the root weight practically did not change (Fig. 1d).

Similar trend was observed for dry biomass. In the control substrate and with the addition of BF, the copper content was 25 mg kg-1 which was 2.2 times lower than the maximum permissible concentration for Russia (Bakradze et al., 2008). After 30 days of *B. oleracea* vegetation at single copper treatment plants accumulated up to 24 and 27 mg Cu kg^{-1} DW in shoots and roots, respectively (Fig. 2a). The addition of BF did not affect the Cu accumulation by plants in comparison with the control. Combined effect of BF with copper (BF+Cu) significantly reduced its content in the roots and almost completely prevented the metal accumulation in the shoots which is of particular importance when growing this leaf vegetable on Cu-contaminated soils. The decrease in copper accumulation can be explained by metal sorption by rhizobacteria that was previously shown by other authors (Aust et al., 1985).

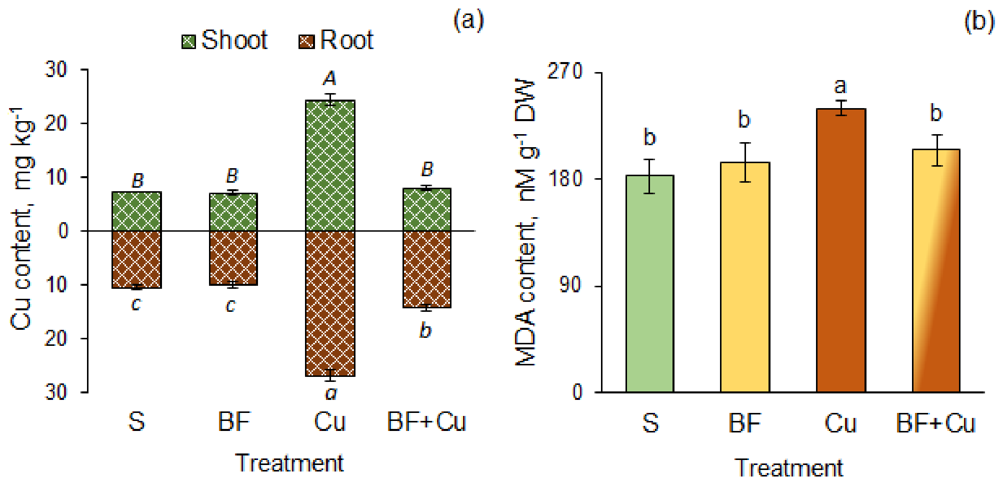

Figure 2. The copper accumulation in shoot and root of *B. oleracea* (a) and the malondialdehyde content in plant leaves (b).

The calculation of the copper bioconcentration factor (BCF) in shoots and roots of *B. oleracea* showed that cabbage does not belong to copper accumulators, since in all treatments BCF was significantly less than 1. When adding biofertilizer, the BCF value did not change considerably.

In all treatments, Cu was predominantly accumulated in the roots, as evidenced by the low translocation coefficient (<1). The addition of BF prevented the translocation of the metal into *B. oleracea* shoots to an even greater extent.

It is known that an excess of heavy metals can cause oxidative stress in plants due to the generation of reactive oxygen species (ROS) and disruption of the native conformation of enzymes. Since copper is a redox active metal, it is involved in the direct generation of ROS, such as superoxide radical, hydrogen peroxide, and hydroxyl radicals (Aust et al., 1985). The intensity of lipid peroxidation processes is usually judged by the content of malonic dialdehyde. It was noted that the addition of copper led to a significant increase in the content of MDA in the leaves of *B. oleracea* by 32 % (Fig. 2b). At the same time, when copper was added together with BF, the MDA content did not significantly differ from the control. This is in good agreement with a decrease in the accumulation of copper, which led to a decrease in the process of peroxidation.

Thus, the greatest positive influence on the *B. oleracea* growth parameters was observed in the BF+Cu treatment. This fact is confirmed by the results of a two-way ANOVA, which showed that the most significant effect on studied growth parameters was exerted by the combined action of copper with biofertilizer ($p < 0.01$). The absence of the noticeable effect at single BF addition on the cabbage growth allows us to suggest that the PGP activity of used bacterial strain depended on copper concentration in the soil.

CONCLUSIONS

The study of the effect of biofertilizer based on metal tolerant PGP rhizobacteria *Bacillus altitudinis* strain TF16a and biochar on growth parameters and copper accumulation in *Brassica oleracea* L. are presented. The combined application of BF and copper had a positive effect on the germination rate, leaf area, shoots length, biomass value of cabbage. The addition of copper led to a significant increase in its accumulation in shoot and root and in the content of MDA in the leaves of *B. oleracea*. However, when copper was added together with BF, the Cu content and lipid peroxidation level did not significantly differ from the control. Thus, it can be concluded that biochar based biofertilizer promoted growth and alleviated copper toxicity in *Brassica oleracea*. Biofertilizer usage suggest an environmentally sustainable approach to increase crop production contributing substantially in development of agrobiotechnology.

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CONTENT OF RISK ELEMENTS IN TECHNOSOLS AND THEIR INFLUENCE ON SELECTED SOIL PARAMETERS

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ABSTRACT

Mining bodies as remains of mining activities are a source of risk elements that contaminate individual components of the environment and may seriously danger human health. The mining area of Dubnik opal mines (Eastern Slovakia) is known for mining gold, silver, and antimony, but above all, it is a world-famous opal deposit. The study aims to determine the content of risk elements (As, Cd, Cu, Fe, Hg) in soils taken from mining bodies (6 heaps of mining material, 6 open mining pits) and to evaluate their impact on the activity of soil enzymes (urease, acid, and alkaline phosphatase, fluorescein diacetate, and β-glucosidase), nutrient content (Na, K, Mg, Ca) and soil reaction (pH). The aim of the research was also to compare the state of pollution between two types of mining bodies and the influence of pollution on selected soil characteristics. The content of hazardous substances in the soils reached extremely high and above the limit values, especially on the heaps of mining material. Urease was evaluated as the most sensitive soil enzyme while ß-glucosidase showed the highest resistance to contamination. The content of Na, K, and Mg was significantly higher on the heaps of mining material compared to open mining pits.

Keywords: *Opal mines, Soil enzymes, Former mining area, Slovakia.*

INTRODUCTION

Current and former mining activities in Slovakia focused on ore processing, have significantly affected the quality of the environment. In Slovakia, a total of 68 probable and 310 confirmed environmental loads are registered, of which 21 are sludge ponds and 78 localities are affected due to mining or processing activities. Polluted localities affect all components of the environment and the health of living organisms, including humans. Risk elements from mining activities have been shown several times to cause carcinogenic, mutagenic, and respiratory diseases (Agyemang and Duah, 2016). The spread of dust particles from heaps of the waste material, the leaching of acid mine waters and emissions from ore processing plants contribute to the reduced quality of the soil, which is nevertheless economically exploited by the population. Such contaminated soil loses its quality, which is reflected in its reduced fertility (Kelepertzis, 2014), and is also a source of hazardous substances that enter agricultural products and are stored in the bodies of consumers (Kowalska *et al.*, 2018). Soil enzyme activity is an important indicator of soil quality because it can respond very quickly to environmental stress. Several studies have shown that increasing content of risk elements in the soil, has a negative effect on the activity of soil enzymes (Wyszkowska, 2010). For this reason, enzymes are often used as bioindicators of soil quality (Demková *et al.*, 2015). Soil enzymes are also heavily involved in the nutrient cycle in the soil and their subsequent transformation to be acceptable to plants (Li *et al.*, 2016). The nutrient content in the soil is changing very rapidly depending on external factors (Alghobar and Suresha, 2017). The object of the study was the former mining area Dubnicke opal mines. The area is characterised by a number of open mining pits and heaps of waste material.

The aim of the study was to determine the content of risk elements in soils in the Dubnicke opal mines area and their impact on the activity of soil enzymes, the content of nutrients in the soil, and the soil reaction.

MATERIAL AND METHODS

The research was realized during the summer of 2020 in the area of Dubnik opal mines (East Slovakia). Soil samples (5-15 cm) were taken from 6 heaps of waste material and 6 open mining pits. Approximately 500g of soil was taken at each sampling point, which was transported to the laboratory in plastic bags. A part of soil was frozen and then used fresh to determine soil enzyme activity. The second part of the soil was dried at room temperature and sieved through a sieve with a mesh size of 2 mm. The pH of the soil in 0.01 M $CaCl_2$ solution was determined using a pH meter inoLab pH 720 WTW. Soil urease activity (URE) was determined according to Khaziev (1976). Acid (ACF) and alkaline phosphatase (ALF) activity were determined according to Grejtovský *et al*., (1991). Fluorescein diacetate (FDA) activity was determined according to Green *et al*. (2006). *B*-glucosidase (BG) activity was determined according to Eivazi and Tabatabai (1988). The content of risk elements (As, Cd, Cu, Hg, Fe) as well as the content of nutrients (Ca, Na, Mg, K) was determined using an ICP-OES Agilent 720 instrument (Agilent Technologies, Germany). All statistical operations were performed using the R studio program (R studio Team, 2016). The data were logarithmically transformed before analysis. The Spearman's correlation coefficient was used to determine the relationship between risk elements, soil enzyme activity, nutrients, and soil reaction. Mann-Whitney U test was used to determine the differences in soil characteristics between two types of sampling sites.

RESULTS AND DISCUSSION

The content of risk elements determined at sampling localities together with the limit values (Act no. 220/2004 Coll. of Laws) is given in Table 1. The limit value of Cd, Fe, and Hg was exceeded at all sampling sites, both open mining pits and heaps of waste material. High contents of As, but also Ni were found in 2007 in the sediments of the water reservoir located in the west direction of the evaluated area (Brehuv, 2007). Pyrite (iron ore, Fe_2S) is one of the main iron ores in the area and is usually separated from precious metals as unnecessary (waste) material and is deposited on heaps of waste mining material (Willis, 2006). Salomon and Foster (1984) stated in their study that the release of Fe into the soil environment contributes to the low pH that was found on all heaps of mining material (Table 2). Arsenic is very often released from sludge, but also from dust particles that enter the air during the smelting of ores containing Cu, Zn, Pb, Au, or Ag (Molnár *et al.*, 2010).

Table 1. Descriptive statistics expressing the content of the risk elements determined at two types of mining bodies.

	Open mining pits (min-max(average±st.dev)	**Heaps of waste material** (min-max(average±st.dev)	**Limit value***
As	6.35-27.3(13.4±9.42)	5.10-464(213±189)	25
Cd	2.08-12.9(5.75±4.34)	5.31-17.2(10.9±4.82)	0.7
Cu	5.91-21.7(12.8±6.11)	6.59-38.3(22.7±12.9)	60
Fe	6708-32039(15505±10384)	15558-38737(27103±9453)	550
Hg	13.4-27.4(21.6±5.05)	32-91.2(55.1±25.7)	0.7

*Act no. 220/2004 Coll of Laws.

The soil reaction in open mining pits ranged between 3.60 to 7.42 and on the heaps between 3.20-3.40 (Table 2). According to the classification by Čurlík and Šefčík (1999), the soil in the mining pits can be characterized as extremely acidic to alkaline, while the soil on the heaps of waste material was in all cases extremely acidic. Soil enzymes showed lower activity on the heaps compared to mining pits, which is closely related to the higher content of risk elements on the heaps (Table 2.). Hinojosa *et al.* (2004) confirmed that the impact of soil pollution has a significant effect on decreasing enzyme activity. Gao *et al.* (2010) found that compared to other enzymes, soil URE and phosphatase activity responds more significantly to the presence of risk elements in the soil system.

Table 2. Descriptive statistics expressing the values of soil properties determined at mining bodies.

	Open mining pits (min-max(average±st.dev)	**Heaps of waste material** (min-max(average±st.dev)
Ca	344-3605(1543±1464)	308-2365(1272±844)
Na	211-409(303±85.1)	260-736(529±199)
K	1012-2327(1766±540)	2065-9534(5015±3244)
Mg	631-1081(879±199)	1464-4191(2614±1153)
URE	0.37-0.73(0.59±0.15)	0.07-0.22(0.13±0.06)
ACP	42.8-241(107.2±69.8)	29.1-105(74.7±32.8)
ALP	28.0-169(62.9±47.9)	32.8-96.6(63.5±26.1)
FDA	8.90-57.4(17.3±17.9)	0.00-2.44(0.81±1.15)
BG	11.7-158(101±63.8)	38.7-86.6(61.5±19.6)
pH	3.6-7.42(5.25±1.55)	3.20-3.40(3.34±0.04)

According to the results of the nonparametric Mann-Whitney U test (Table 3.), the content of Hg was significantly higher on the heaps of waste material. Other risk elements reached higher values at heaps compared to mining pits, but the differences were not significant. URE and FDA reached significantly higher values at open mining pits comparing heaps. Soil URE is closely related to the presence of plants, and both types of mining bodies are characterized by a very rare occurrence of vegetation (Polacco, 1977). The contents of other risk elements reached higher values at heaps of mining material compared to mining pits, but the differences were not significant. Heaps of mining material are highly likely to contain risk elements that pollute and threaten the surrounding environment (Rasemann, 2015), while pollution in open mining pits is often associated with water pollution - in terms of acid mine drainage waters (Singovszka *et al.*, 2016). Han *et al.* (2018), who studied the content of the soil in areas polluted by rail transport found increased content of Ca and Mg in the most polluted localities. Alghobar and Suresca (2017), who monitored soil properties under the influence of different amounts of sludge, also confirmed that while the content of K and Na did not change significantly, the content of Ca and Mg increased with increasing sludge content. In our case, the more polluted heaps of waste material showed significantly higher values of N, K and Mg comparing open mining pits.

Table 3. The results of Mann-Whitney U test expressing the differences in the risk element contents, activity of soil enzymes, soil nutrients and soil pH between two types of mining bodies.

Soil characteristics		**U**	**Z**	**p**	**Soil characteristics**		**U**	**Z**	**p**
As	Between mining bodies	12.0	-0.88	0.37	Mg	Between mining bodies	0.00	-2.72	0.001**
Cd		8.00	-1.56	0.12	URE		0.00	2.80	0.003**
Cu		8.00	-1.53	0.14	ACP		12.0	0.88	0.37
Fe		8.00	-1.52	0.12	ALP		14.0	-0.56	0.54
Hg		0.00	-2.80	0.004*	FDA		0.00	2.80	0.001**
Ca		16.0	0.24	0.80	BG		12.0	0.88	0.37
Na		6.00	-1.84	0.05*	pH		0.00	2.80	0.002**
K		4.00	-2.16	0.03*	BG		12.0	0.88	0.37

*p<0.05; **p<0.01

Correlation relationships between soil characteristics are listed in Figure 1. Risk elements correlated significantly positively between themselves (Hg correlated significantly only with As). As in the study by Árvay *et al.* (2017), also in our case, it was confirmed that the high content of risk elements has an inhibitory effect on the soil reaction. Soil pH gave a negative correlation with all evaluated heavy metals. FDA and BG correlated positively with soil pH, even URE gave a significant positive correlation with pH. ACP, ALP gave with pH negative correlation. Nutrients correlated with pH differently, while Mg, K, and Na gave with pH negative correlation, Ca correlated with pH positively. A significant positive correlation was found between nutrients and risk elements. In the case of Na, K the correlation with risk elements was significant. Between soil enzymes and risk elements, almost in all cases negative correlation was found. Additionally, URE was found the most sensitive to environmental stress. In line with our findings, Wyszkowska *et al.* (2010) have found that soil urease responded most sensitively to the presence of risk elements in the soil system. Martinez-Toledo *et al.* (2017) noted a strong correlation between the FDA, and URE, as well as between KF, AF, and BG. In our case, significant positive correlation between FDA – URE, and ACP-ALP was determined. The correlations between the other enzymes (themselves) were not significant.

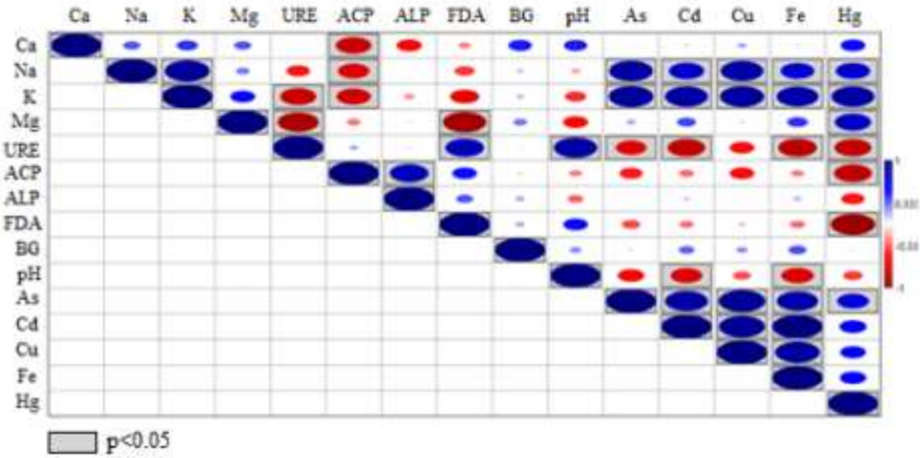

Figure 1. Correlation relationship between evaluated risk elements, nutrients, soil pH and soil enzymes (regardless the type of mining body).

CONCLUSION

The high content of risk elements in the soil environment is a serious environmental problem not only in former mining areas but also in the vicinity of industrial plants or major transport hubs. Poor soil quality has a negative effect on the quality of agricultural production, toxic substances enter the food chain and endanger human health. Among the evaluated elements, Cd, Hg, and Fe exceeded the limit values on all sampling sites. Soil enzyme activity decreased with the increasing content of risk elements in the soil, which was confirmed by the negative correlation between individual enzymes and risk elements. Soil reaction values ranged from extremely acidic to alkaline, with the lowest (most acidic) values were recorded at the most polluted heaps of waste material. The individual nutrients reacted differently to the content of hazardous substances in the soil, while the content of K and Na did not change significantly due to the influence of hazardous substances, the content of Ca and Mg reached the highest values at the most polluted sampling sites.

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ISOPRENOID CONTENT OF PEACH *PRUNUS PERSICA* AND APRICOT *PRUNUS ARMENIACA* FRUIT CUTICULAR WAXES

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ABSTRACT

Peach (*Prunus persica* L. Batsch) and apricot (*Prunus armeniaca* L.) are very popular drupe-type fleshy fruits from Rosaceae family, important to the agricultural economies of many countries. The majority of Rosaceae fruits are characterized with a relatively high content of isoprenoids (triterpenoids and steroids) occurring in their surface cuticular waxes. However, the data concerning the occurrence of these compounds in peaches and apricots are scarce. The aim of the present study was the determination of isoprenoid content in the cuticular waxes of peach var. 'Redhaven' and apricot var. 'Somo' cultivated in Poland. Chloroform-soluble wax extracts obtained from fruit samples were fractionated with the use of preparative adsorption chromatography and analyzed by gas chromatography-mass spectrometry (GC-MS). The profile of the identified compounds was similar in both fruits. However, some quantitative and qualitative differences were noticed. Triterpenoid acids (betulinic, oleanolic and ursolic acids) were the predominating fraction of isoprenoids identified in cuticular waxes of both fruits, however, they were 4-fold more abundant in peach. Moreover, dihydroxy acid of oleanane-type, maslinic acid, was identified only in peach, whereas ursane-type corosolic acid only in apricot wax. The fraction of the neutral triterpenoids was similar, composed primarily of oleanane- and ursane-type alcohols and aldehydes. Typical profile of steroids was identified in both fruits, however, stigmasterol was dominating in peach, whereas sitosterol in apricot. The total contents of isoprenoids in peach (approx. 90 μg/mg of wax extract) and apricot (approx. 27 μg/mg) cuticular waxes were found to be significantly less than in other Rosaceae fruits.

Keywords: *apricot, cuticular wax, peach, steroids, triterpenoids.*

INTRODUCTION

Occurrence of isoprenoids, including triterpenoids and steroids, in fruit surface cuticular wax is a well-known feature; however, our understanding of the relevance of these compounds for fruit quality traits is still rather preliminary. Regarding various biological activities of triterpenoids, their presence in fruit cuticle might be of interest due to their potential role in protection against abiotic and biotic stresses, mechanical toughness of the fruit peel, surface texture, general fruit appearance, post-harvest quality and shelf-life duration (Szakiel *et al*. 2012; Trivedi *et al*. 2019; Lara *et al*. 2015, 2019). Moreover, anti-inflammatory, antimicrobial, antiviral, cardio- and hepatoprotective, and potentially anti-cancer properties of triterpenoids make their occurrence in fruits valuable for the consumers (Szakiel *et al*. 2012; Dashbaldan *et al*. 2020). As constituents of cuticular waxes, triterpenoids and steroids can be very abundant in some fruits, while occurring only in trace amounts in others (Dashbaldan *et al*. 2019). The diversity of fruit cuticular wax composition deserves to be further explored, particularly regarding edible fleshy fruits. The Rosaceae family is one of the major angiosperm families, with many economically important fruit crop plants bearing various types of fruits, e.g., follicles, capsules, nuts, achenes, drupes and accessory fruits (Butkevičiūtė *et al*. 2018; Dashbaldan *et al*. 2020, 2021). Many Rosaceae fruits have been characterized in terms of isoprenoid content of their cuticular waxes, however, the data concerning the triterpenoid and steroid content in peaches (*Prunus persica* L. Batsch) and apricots (*Prunus armeniaca* L.) are scarce. Meanwhile, peach and apricot are very popular drupe-type fleshy fruits, important to the agricultural economies of many countries. Therefore, the aim of the present study was the qualitative and quantitative determination of isoprenoid content in the cuticular waxes of peach var. 'Redhaven' and apricot var. 'Somo' cultivated in Poland, through targeted GC-MS metabolomic profiling of chloroform-soluble wax extracts.

MATERIAL AND METHODS

Peach (*Prunus persica* L. Batsch) var. 'Redhaven' and apricot (*Prunus armeniaca* L.) var. 'Somo' were cultivated in a private orchard in Rudno (51°471 N, 15°410 E), western Poland (Lubusz Voivodeship). The samples of fully ripened fruit were collected in 2020 in three independent replicates (i.e., from different trees) for each species. Cuticular waxes were extracted as described previously (Dashbaldan *et al.* 2019, 2020) by incubation of entire intact fruit in appropriate volume of chloroform (permitting the full dipping of fruit sample) with gentle stirring for 30 s at room temperature.

Evaporated extracts of chloroform-soluble cuticular waxes were fractionated by adsorption preparative thin-layer chromatography (TLC) on 20 cm × 20 cm glass plates coated manually with silica gel 60H (Merck). The solvent system chloroform:methanol 97:3 (v/v) was applied for developing. Two fractions were obtained as described earlier (Woźniak *et al*. 2018): free (non-esterified) steroids and triterpenoids, and triterpenoid acids. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols (R_F 0.3-0.9) were directly analyzed by GC-MS, whereas fractions containing triterpene acids (R_F 0.2-0.3) were methylated with diazomethane. Nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpenoid acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether,and held at 2 °C for 24 h.

Analyses were performed with the use of an Agilent Technologies 7890A gas chromatograph (GC–MS) (Perlan Technologies). The system was equipped with a 5975C mass selective detector, a G4513A autosampler, and a 30 m × 0.25 mm i.d., 0.25-μm, HP-5MS UI column (Agilent Technologies, Santa Clara, CA, USA). The following temperature program was applied: the start at 160 °C (2 min), an increase to 280 °C at 5 °C/min, and the final temperature of 280 °C held for 44 min. The other employed parameters were as follows: the carrier gas (helium, 1 mL/min), inlet and FID (flame ionization detector) temperature 290 °C; quadrupole temperature 150 °C; ion source temperature 230 °C; EI ionization energy 70 eV; scan range, m/z 33–500; MS transfer line temperature 275 °C; FID gas: hydrogen 30 $mL \cdot min^{-1}$; air 400 mL/min. Wiley 9th ED. and NIST 2008 Lib. SW Version 2010 were used in GC-MS data analysis. Individual compounds were identified by comparing their mass spectra with library data, and/or their chromatographic mobility and corresponding mass spectra with those of authentic standards. Quantitation was conducted with a FID detector and performed using an external standard method based on calibration curves determined for authentic standards of ursolic acid methyl ester, α-amyrin and stigmasterol. Data were presented as the means ± standard deviation of three independent samples and subjected to one-way analysis of variance (ANOVA), the differences between means were evaluated using Duncan's multiple-range test. Statistical significance was considered to be obtained at $p<0.05$ (Dashbaldan *et al*. 2021).

RESULTS AND DISCUSSION

The composition of the identified triterpenoids and steroids was similar in chloroform-soluble cuticular wax extracts obtained from both peach and apricot fruit samples. The list of the compounds and the results of the quantification of the individual compounds are presented in Table 1.

Table 1. The content of triterpenoids and steroids in peach (*Prunus persica* L. Batsch) var. 'Redhaven' and apricot (*Prunus armeniaca* L.) var. 'Somo' fruit cuticular waxes. Results are referenced to wax extract mass and expressed as the mean ± SD of three independent samples.

Compound	Peach 'Redhaven'	Apricot 'Somo'
	Content (µg/mg wax extract)	
Triterpenoid acids:		
betulinic acid	1.84 ± 0.20[a*]	4.64 ± 0.48[b]
oleanolic acid	38.42 ± 4.06[a]	2.64 ± 0.30[b]
ursolic acid	40.83 ± 5.01[a]	9.29 ± 1.14[b]
olean-2,12-dien-28-oic acid	1.22 ± 0.08[a]	0.36 ± 0.05[b]
ursa-2,12-dien-28-oic acid	2.94 ± 0.22[a]	0.81 ± 0.10[b]
corosolic acid	n.d.**	1.08 ± 0.08
maslinic acid	0.42 ± 0.04	n.d.
sum of triterpenoid acids	*85.67*	*20.88*
Neutral triterpenoids		
α-amyrin	1.05 ± 0.12[a]	0.61 ± 0.07[b]
β-amyrin	0.23 ± 0.02[a]	0.22 ± 0.03[a]
α-amyrenone	0.07 ± 0.01[a]	0.11 ± 0.01[b]
erythrodiol	0.08 ± 0.02[a]	0.06 ± 0.01[a]
uvaol	0.36 ± 0.04[a]	0.19 ± 0.02[b]
oleanolic aldehyde	0.13 ± 0.04[a]	0.29 ± 0.03[b]
ursolic aldehyde	0.35 ± 0.05[a]	0.46 ± 0.06[a]
sum of neutral triterpenoids	*2.27*	*1.94*
Steroids:		
campesterol	0.39 ± 0.05[a]	0.23 ± 0.04[b]
cholesterol	0.06 ± 0.01[a]	0.31 ± 0.03[b]
sitosterol	0.05 ± 0.01[a]	2.66 ± 0.32[b]
stigmasterol	0.97 ± 0.12[a]	0.10 ± 0.01[b]
sitostenone	0.05 ± 0.01[a]	0.25 ± 0.03[b]
tremulone	0.07 ± 0.01[a]	1.05 ± 0.12[b]
sum of steroids	*1.63*	*4.60*
Total	89.57	27.42

*Means in rows not sharing a common letter are significantly different ($p<0.05$).
**n.d., not detected.

As in the majority of other Rosaceae (Dashbaldan *et al*. 2020, 2021), triterpenoid acids belonging to the three types of carbon skeletons, i.e., lupane-, oleanane- and ursane-type groups, were found; with their typical representatives, namely betulinic, oleanolic and ursolic acids. Moreover, the analogs of oleanolic and ursolic acids with an additional double bond in position 2, i.e., olean-2,12-dien-28-oic acid and ursa-2,12-dien-28-oic acids, were also identified. The difference in triterpenoid composition between peach and apricot was the presence of 2,3-dihydroxy analogs of oleanolic and ursolic acids; i.e., oleanane-type maslinic acid was identified in peach, whereas ursane-type corosolic acid in apricot. In turn, no compositional differences were noticed in the fractions of the neutral triterpenoids and steroids. The basic ursane- and oleanane-type alcohols with one hydroxyl group (α- and β-amyrins) and two hydroxyl groups (uvaol and erythrodiol), as well as respective aldehydes (ursolic and oleanolic) were identified in both peach and apricot cuticular waxes, accompanied by one ketone, α-amyrenone. The steroid fraction was composed of typical sterols, i.e., campesterol, cholesterol, sitosterol and stigmasterol, as well as two steroid ketones, sitostenone and tremulone.The total contents of both groups of isoprenoids, i.e., triterpenoids and steroids, were found to be relatively low in peach and apricot cuticular waxes as compared to other Rosaceae fruit crops analyzed previously, e.g., apple (Andre *et al*. 2012; Butkevičiūtė *et al*. 2018; Sut *et al*. 2019), black chockeberry (Dashbaldan *et al.* 2020), cherry (Peschel *et al.* 2007), pear (Kolniak-Ostek 2016), plum (Huang *et al*. 2022) or rose (Dashbaldan *et al*. 2020, 2021). The total isoprenoid content in peach cuticular waxes did not exceed 100 μg/mg, i.e., 10% of chloroform-soluble wax extract, whereas in such fruits like apple it could reach 50% of the extract. The total isoprenoid content in apricot cuticular waxes was 3.3-fold lower than in peach.

Triterpenoid acids constituted the major fraction of cuticular wax isoprenoids, reaching 96% and 76% of the total content of all quantified compounds in peach and apricot, respectively. In turn, steroids were the minor fraction (1.8%) in peach fruit cuticular waxes, whereas they constituted 17% in apricot waxes.

Ursolic acid was the predominating compound in both peach and apricot cuticular waxes (48% and 44% of the fraction of triterpenoid acids, respectively). The second abundant acid was oleanolic acid (45% of the fraction) in peach, whereas betulinic acid (22%) in apricot. Among the neutral triterpenoids, α-amyrin was the most abundant in both fruits (46% and 31% of the fraction in peach and apricot, respectively). In turn, the predominating sterol was stigmasterol in peach (60% of steroid fraction), whereas sitosterol in apricot (58%).

The results obtained in this study and reported previously (Dashbaldan *et al*. 2020) point to some similarities in the isoprenoid content of fruit cuticular waxes in Rosaceae crops. One of such common features is the presence of lupane-, oleanane- and ursane-type triterpenoids, whereas in other taxonomic families, e.g., Ericaceae, the composition of triterpenoids was found to be more complex, containing compounds of several other carbon skeletons (Dashbaldan *et al*. 2019). Another common feature is the predominance of the triterpenoid acid fraction, observed in numerous Rosaceae fruit crops bearing various fruits as drupes (Peschel *et al*. 2007, Lino *et al*. 2020, Huang *et al*. 2022) or accessory fruits (Andre *et al*. 2012, Butkevičiūtė *et al.* 2018, Kolniak-Ostek 2016, Sut *et al*. 2019, Dashbaldan *et al*. 2020).

However, comparing the isoprenoid content in such Rosaceae drupes as cherry (Peschel *et al*. 2007), plum (Huang et al. 2022) or even nectarine (Lino et al. 2020), revealed that the amounts of these compounds (particularly triterpenoids) in peach and apricot cuticular waxes are significantly lower. It might be due to the fruit surface properties and the morphological features of epidermis (e.g., the presence

of trichomes forming a dense indumentum, in which such volatile compounds as monoterpenoids can be accumulated). Perhaps it is also related to the general plant defense strategy, based either on the accumulation of triterpenoids (in a free form, or as glycosides called saponins) or other bioactive constituents belonging to various classes of plant metabolites.
The observed difference between the isoprenoid content in cuticular waxes of peach and apricot revealed that it is difficult to correlate this feature directly with plant taxonomy. The significant differences in triterpenoid and steroid profiles exist even within one genus of Rosaceae family, i.e., Prunus. Moreover, such differences can be observed also among different varieties and cultivars of crop plants (Andre *et al*. 2012; Butkevičiūtė *et al.* 2018; Kolniak-Ostek 2016; Sut *et al*. 2019; Huang *et al.* 2022), therefore, the taxonomic classification of any fruit crop might not be relevant to predict the isoprenoid content of its cuticular waxes, and it requires a detailed case study.

CONCLUSIONS

This report presents the isoprenoid content of peach and apricot fruit cuticular waxes, and it complements the data concerning the occurrence of these compounds in surface layer of Rosaceae fruit crops. Some described features, e.g., the presence of lupane-, oleanane- and ursane-type compounds, the predominance of the triterpenoid acid fraction, seem to be typical for the majority of Rosaceae fruits characterized so far. However, the total isoprenoid contents in peach and apricot cuticular waxes are significantly lower than in other Rosaceae fruits, including other drupes. Therefore, it can be concluded that the isoprenoid content of fruit cuticular waxes is related mainly to the fruit surface properties and the morphological features of epidermis (e.g., the presence of surface trichomes), whereas it is difficult to clearly connect the isoprenoid composition and quantity with the fruit crop taxonomy or the type of fruit (e.g., drupes, accessory fruit, berries).

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SOIL ELECTRICAL CONDUCTIVITY IN RELATION TO SOIL MICROCLIMATE AND SOIL RESPIRATION UNDER WHEAT AND BARLEY LAND COVERS

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ABSTRACT

Soil electrical conductivity (EC) is an important indicator of soil health. It affects crop yields and suitability, many soil properties like plant nutrient availability, and activity of soil microorganisms which influence key soil processes including the emission of greenhouse gases such as carbon dioxide i.e. soil respiration. While it is well known that major drivers of soil EC are soil temperature and moisture content, less is known on the relation between soil EC and respiration. Therefore, the objectives of this research were to determine relation of soil EC and soil microclimate (soil temperature and moisture), as well relation of soil EC and soil respiration under three different land covers. A study on soil EC, microclimate and respiration under bare soil, winter wheat and winter barley was carried out from November 2020 until July 2021 on experimental field near Osijek city, continental Croatia. The results showed that EC is more related to soil microclimate elements i.e. soil temperature and soil moisture content than on soil respiration. Between 17% and 47% of EC can be explained by soil microclimate elements and none i.e. only 4% to 12% by soil respiration.

Key words: *soil EC, soil* CO_2 *efflux, soil temperature, soil moisture content, Croatia.*

INTRODUCTION

Soil electrical conductivity (EC) is a measure of the amount of salts in soil. EC levels are indicator of soil health and can serve as an indirect indicator of important soil physical and chemical properties like the amount of water and water-soluble nutrients available for plant uptake. EC is affected by land use, cropping, irrigation, application of fertilizer, manure, and compost. Furthermore, factors affecting EC include soil minerals, climate, and soil texture, *i.e. studies have shown that soil water content and concentration, soil temperature, clay content and mineralogy, cation exchange capacity (CEC), and organic matter content are among the dominating soil properties affecting EC (Rhoades et al., 1976; Sheets & Hendrickx, 1995;* Wei et al., 2013*). Soil EC affects crop suitability and yields, plant nutrient availability, and* activity of soil microorganisms which influence key soil processes including the emission of greenhouse gases such as nitrogen oxides, methane, and carbon dioxide i.e. *soil respiration.* Soil respiration is considered to be the largest terrestrial–atmospheric carbon exchange, it is part of global carbon cycle which dynamic presents key issue of global warming. Therefore, any alterations in soil respiration could alter the climate warming (Cox et al., 2000). Although soil microclimate i.e. soil temperature and soil moisture content are considered to be main controlling factors of soil respiration (Cox et al., 2000; Rastogi et al., 2002; Luo and Zhou, 2006; Wan et al., 2007; Bilandžija et al., 2016; Bilandžija et al., 2021, Zhen et al., 2022), understanding of EC role and its impact on soil respiration is important for discerning how carbon balances may shift (Rietz and Haynes, 2003; Xie et al., 2009). EC can affect soil respiration through strong effects on the microorganisms including effects on microbial biomass, population, community structure and activity (Sardinha et al., 2003; Vincent et al, 2006). Soil microorganism activity declines as EC increases and this impacts important soil processes such as residue decomposition, nitrification, denitrification and soil respiration. However, contradictory results have been obtained for the effects of EC on soil respiration rate (Rietz and Haynes, 2003). Therefore, the objective of the study is to determine the relation of EC and soil microclimate (temperature and moisture) i.e. relation of EC and soil respiration.

MATERIALS AND METHODS

Experimental site, soil properties, climate conditions and agrotechnical measures

A study on soil EC and soil microclimate i.e. soil respiration under different land covers was conducted from November 2020 until July 2021 on experimental field near Osijek city in Croatia (φ = 45° 31' 56.47" N, λ = 18° 44' 16.07" E; 90m a.s.l.). The experiment includes three different land cover:

- ✓ BS - bare soil;
- ✓ WB - winter barley (*Hordeum vulgare* L.): Rex, Lord, Barun and Panonac cultivars
- ✓ WW - winter wheat (*Tritucum aestivum* L.): Srpanjka, Renata, El Nino and Kraljica cultivar.

More on wheat and barley cultivars can be found in Bilandžija et al. (2021) and AIO (2022).

Soil at the experimental site has silty clay texture, water holding capacity amounts 37.7%, air holding capacity 10.2%, soil porosity 47.8%, and bulk density 1.39 g cm^{-3}. Soil pH_{KCl} amounts 7.24, soil contains 2.3% of humus, 0.11% of total nitrogen, 1.25% of total carbon, 0.06% of total sulphur, 17.9 mg of P_2O_5, and 15.5 mg of K_2O per 100 g of soil.

Osijek area has continental climate, with an average annual air temperature of 11.7 °C and average annual precipitation amount of 707 mm in the period 1991–2018. During the studied vegetation season average air temperature was 10.8 °C and precipitation amounted 650 mm. More on climate conditions and agroclimatic factors during period 1991–2018 can be found in Bilandžija and Martinčić (2020/2021) and during vegetation growing season in Bilandžija et al. (2021.).

All agrotechnical measures for winter wheat and barley i.e. reduced tillage, fertilization, planting/harvesting dates, weed and pest control were done according to the good agricultural practices. More on the applied agrotechnical measures can be found in Bilandžija et al. (2021).

Measurement of CO2 concentrations and electrical conductivity

Data on soil respiration rates are obtained from Bilandžija et al. (2021). Soil CO_2 concentrations and microclimate elements were measured once per month during vegetation growing season (November – July). Due to unfavourable weather conditions, measurements were not conducted during two winter months (December – January). Measurements of soil CO_2 concentrations were conducted by portable infrared carbon dioxide detector (GasAlertMicro5 IR, 2011) and *in situ* closed static chamber method was used. More on the measurement procedure can be found in Bilandžija et al. (2021). The aim of the research also requires reliable data on *electrical conductivity, soil moisture and soil temperature* in the soil surface layer (10 cm depth). E*lectrical conductivity (dS/m), soil moisture content and soil temperature* were determined by IMKO HD2 instrument (probe Trime, Pico64, 2011) in the vicinity of each chamber once per month along with determination of soil respiration. Total number of soil CO_2 concentrations, microclimate and EC measurements during vegetation season was 7 per experiment treatment (once per month). Each treatment of wheat and barley includes 4 different cultivars of wheat/barley and all measurements were conducted in three repetitions. Therefore, total number of measurements for WB and WW treatment amounts 84 (7 months' x 4 cultivars x 3 repetitions). Total number of measurements for BS treatment amounts 42 as all measurements were conducted in 6 repetitions (7 months' x 6 repetitions).

Statistical analyses

Correlation analyses between EC and soil temperature, moisture, and respiration is contucted by Microsoft Excel programme. The strength of the correlation coeficients is interpreted according to the Roemer-Orphal scale (Table 1).

Table 1. Roemer-Orphal scale

correlation strength	**Correlation coefficient (r)**
non	0.0 – 0.1
very weak	0.1 – 0.25
weak	0.25 – 0.4
moderate	0.4 – 0.5
strong	0.5 – 0.75
very strong	0.75 – 0.9
full	0.9 – 1.0

Source: Vasilj (2000)

RESULTS AND DISCUSSION

EC and soil temperature

During the studied period, strong negative linear correlation for bare soil was determined between EC and soil temperature (r = -0.64) (Figure 1). Furthermore, strong negative linear correlation between EC and soil temperature was also determined for wheat (r = -0.60) and barley (r = -0.69) (figure 1). According to the coefficient of determination, 40%, 36% and 47% of EC depend on soil temperature under bare soil, wheat and barley, respectively (Figure 1). Other studies have also found soil temperature to have an effect on EC (McKenzie et al., 1989, Slavich and Petterson, 1990, Sudduth et al., 2001), while Brevik et al. (2004 determined that linear regression analysis is indicating no correlation between soil temperature in the upper soil layer (10cm) and EC values

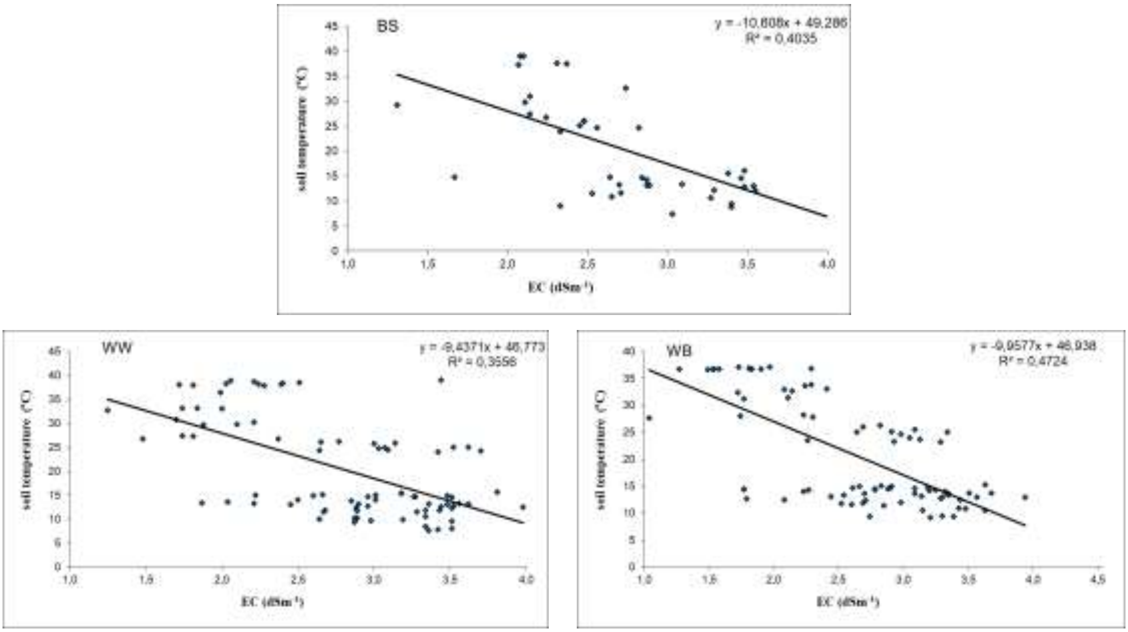

Figure 1. Correlation of EC and soil temperature for bare soil, wheat and barley covers

EC and soil moisture content

Between EC and soil moisture content, moderate positive linear correlation was determined for bare soil (r = +0.41) and strong positive linear correlation for wheat (r = +0.64) and barley (r = +0.56) (Figure 2). According to the coefficient of determination, 17%, 41% and 31% of EC depend on soil moisture under bare soil, wheat and barley, respectively (Figure 2). Positive correlation of EC and soil moisture content was determined by Mueller et al. (2003), and also other previous studies determined a simple linear relationship between EC and soil moisture content (Kachanoski, et al., 1988; Sheets, and Hendrickx, 1995).

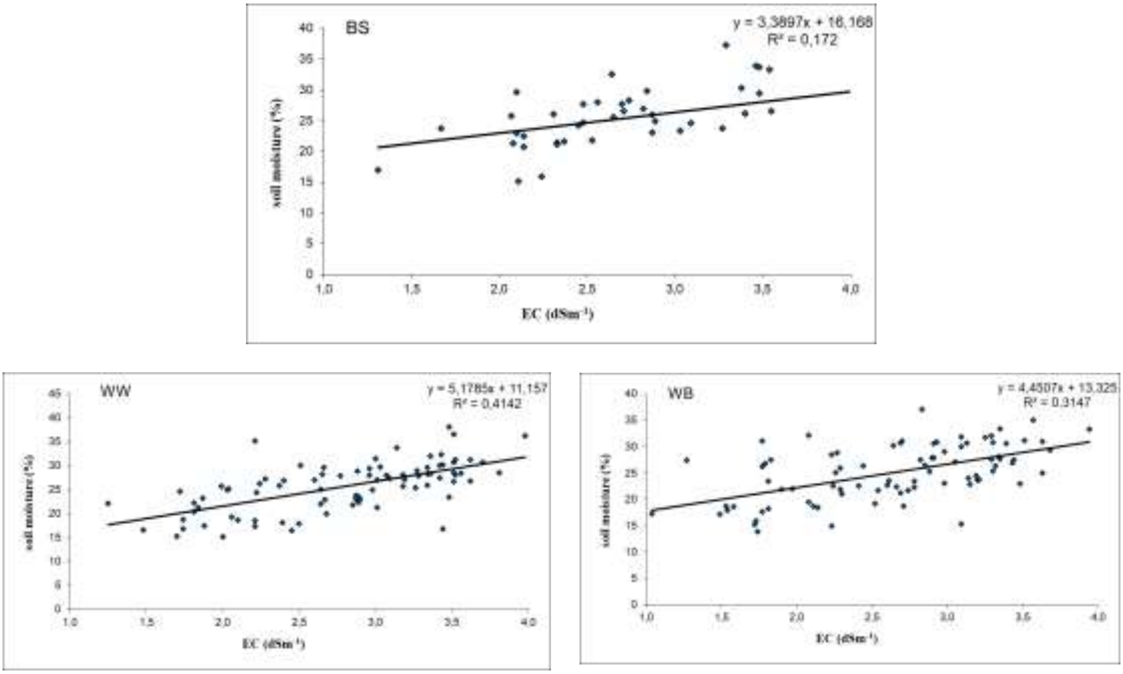

Figure 2. Correlation of EC and soil moisture content for bare soil, wheat and barley covers

EC and soil respiration

Considering EC and soil respiration, non to weak negative linear correlations was determined. For bare soil, non correlation was determined between soil respiration and EC (r = -0.08). Furthermore, weak negative linear correlation was determined between EC and soil respiration for wheat (r = -0.35), and very weak negative linear correlation for barley (r = -0.20) (Figure 3). According to the coefficient of determination, soil respiration does not depend on EC under bare soil i.e. only 4% and 12% of soil respiration depends on EC under barley and wheat covers, respectively (Figure 3). This may be attributed to the depressive effects of EC on the microorganisms. Similar results for the inhibition effects of EC on soil respiration have been obtained in many other previous studies (Xie et al., 2009; Rietz and Haynes,2003; Sardinha et al., 2003; Lai et al., 2012).

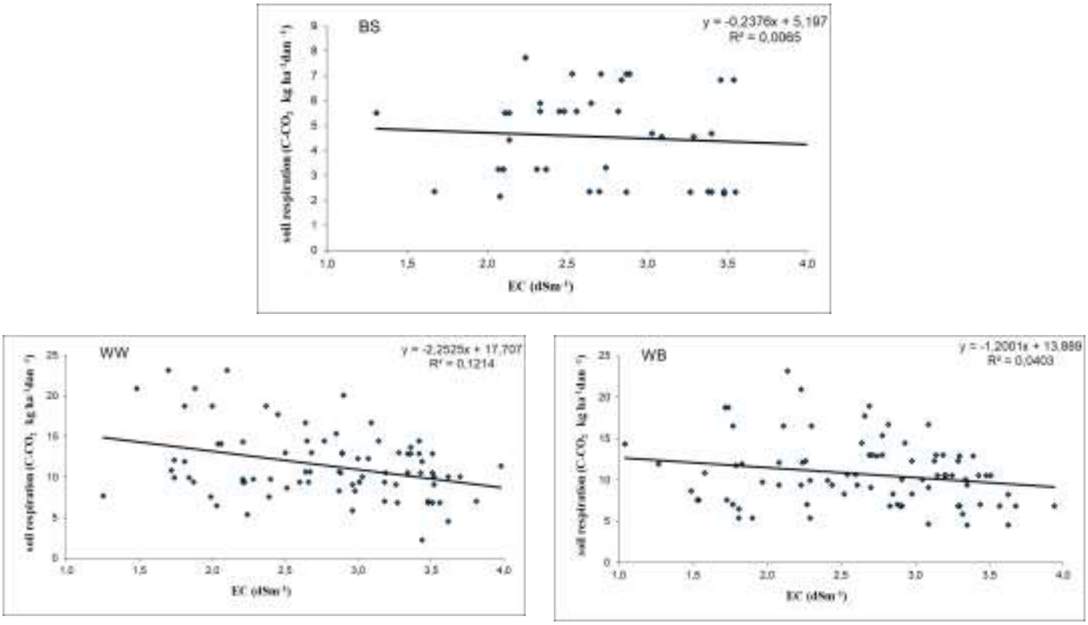

Figure 3. Correlation of EC and soil respiration for bare soil, wheat and barley covers

CONCLUSIONS

In a study on EC and its relation to soil temperature (10 cm depth), soil moisture content (10 cm depth) and soil respiration under three different land covers, conducted during 2020/2021 vegetation season on experimental field in continental Croatia, the following was determined:

- ✓ EC and soil temperature: strong negative linear correlation for all three studied land covers (bare soil, wheat and barley)
- ✓ EC and soil moisture: moderate positive linear correlation for bare soil, strong positive linear correlation for wheat and barley
- ✓ EC and soil respiration: none correlation for bare soil, weak negative linear correlation for wheat, and very weak negative linear correlation for barley

The obtained results indicate that EC is more related to soil microclimate elements i.e. soil temperature and soil moisture content than on soil respiration. Between 17% and 47% of EC can be explained by soil microclimate elements and none i.e. only 4% to 12% by soil respiration.

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INFLUENCE OF BIOGAS DIGESTATE, WOOD ASH AND THEIR MIXTURES ON THE YIELD AND QUALITY OF CUCUMBERS

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ABSTRACT

The biogas digestate can be as alternative of synthetic fertilizers in agricultural practice. Without additives, the drying of digestate can be unprofitable. The adding of wood ash to digestate dehydration process gives the opportunity for soil liming as well as for soil enrichment with nutrients. Within the framework in the Latvian National Research project, it was necessary to compare digestates from various raw materials and to test the possibility of mixing them with ash for using in cultivation of fast-growing crops. The research aimed to evaluation the influence of biogas digestate and wood ash on the yield and quality of cucumbers in polycarbonate greenhouse. The experiment was provided in 2020, using 11 fertilization treatments as well as peat (pH_{KCl} 5.5) as control. Starting of experiment, no significant differences between acidy of substrates were observed, but at the end of investigations, pH_{KCl} varied from 6.8 till 7.5, that was non-optimal for cucumbers growing. The development of plants under treatments was not significantly different ($p>0.05$). During experiment, cucumbers were harvested 23 times. The count of fruits per plant, depending on variant, per each harvesting varied from 1 till 9 (maximal result was observed for digestate from pig manure and horse manure). The significant influence of fertilization variant to cucumbers yield was observed ($p <0.05$). The organoleptic parameters were not differed significantly throughout the growing season ($p>0.05$).

Keywords: *biofertilizers, fertilization treatment, digestate, wood ash, fast-growing vegetables.*

INTRODUCTION

The content of organic substances in soil is an important indicator of soil quality. It impacts the attraction of chemicals, the formation of soil structure as well as the regime of humidity and air in the soil (Kvasoviene – Petraityte et al., 2019). An uneven soil use, different cultivation and fertilisation practices affect the soil structure, its agrochemical properties and fertility (Koszel & Lorencowicz, 2015; Caruso et al., 2018).

The percentage of soils with an insufficient content of organic matter and pHKCl lower than 5.5 is increasing every year in Latvia. It negatively affects the fertilisation effect as well as the crop yield.

By statistical data, there are currently 49 biogas plants operating in Latvia with a total installed electricity capacity of approximately 56 MW. Currently, 41 agricultural biogas stations use a total of 1.85 million tons of raw materials per year. Not only manure, but also corn silage, water sewage treatment plant slime, grain refuse is used as resources in biogas production in Latvia (Priekulis et al., 2016). In agriculture, there is a high quantity of organic wastes suitable for biogas production (Dubrovskis & Adamovics, 2012; Tampere & Viiralt, 2014; Kall et al., 2016). That is why the composition of locally produced digestate is variable.

The biogas is used for production of electric energy and heat (in form of hot water), but the digestate is mainly used as a liquid fertilizer and is incorporated into the soil (Kalnina et al., 2018).

Digestate can be defined as a liquid from anaerobic digestion of a biodegradable feedstock, it contains nitrogen, phosphorus, potassium (Dubrovskis & Kotelenec, 2014; Koszel & Lorencowicz, 2015; Kall et al., 2016). Usually, it is a semi-solid mass that consists of a semi-degraded plant material, the biomass of microorganisms and a slurry (if it was used in biogas production process). The dry matter content of digestate makes approximately 5-10% (Slepetiene et al., 2016).

Because of the digestate`s large water content, the transportation costs are relatively high – not only in Latvia, but also in other European countries (Dubrovskis and Kotelenec, 2014; Auburger et al., 2015; Kuusik et al., 2017). For that reason, the separation and drying of digestate is used. Without additives (for example, calcium carbonate - CaCO3), the drying of digestate can be unprofitable. The adding of wood ash to digestate`s dehydration process gives the opportunity for soil liming as well as for soil enrichment with bioavailable P, K, Ca, Mg and other macro- and micronutrients (Augusto et al., 2008; Schiemenz and Eichler-Lőbermann, 2010; Libiete et al., 2016). The chemical properties of ash are depending on many factors, including subsidiary fuel type, combustion system and season.

Research of Bulgarian scientists indicated that for lettuce (*Lactuca sativa* L.) under varying doses of biofertilisers, compared with untreated soil, the best development and quality options can be obtained applying 15% of digestate that contain 70% of pig manure (Kathijotes et al., 2015).

In Italy, digestate was evaluated as an alternative nutrient solution in the hydroponic cultivation of baby leaf lettuce (*Lactuca sativa* L.). In total, three combinations (agriperlite + liquid digestate, solid digestate + standard solution and pelleted digestate + standard solution) enhanced plant growth by affecting the root, the shoot and the total dry weight in all investigated experiments (+32%, +40% and +29% respectively). Based on the obtained results, digestate represents a sustainable and alternative growing media or a nutrient solution for the production of baby leaf lettuce cultivated in a hydroponic system (Ronga et al., 2019).

In the reason of increase of the mineral fertilizers' price as well as in the system of organic crop production, the biogas digestate, wood ash and their mixtures can be as alternative of synthetic fertilizers in agricultural practice. Within the framework of the Latvian National Research project, it was necessary to compare digestates from various raw materials used by the project partners and to test the possibility of mixing them with ash for using in cultivation of fast-growing crops, including cucumbers.

The aim of this research was to evaluate the influence of biogas digestate and wood ash mixtures on the yield and quality of cucumbers in polycarbonate greenhouse.

MATERIALS AND METHODS

The experiment was started in spring 2020 in the laboratory of Horticulture and Apilogy of the Latvia University of Life Sciences and Technologies. Experiment was provided in polycarbonate greenhouse. Seeds of cucumber hybrid cultivar 'Mirabelle' F1 (Seminis/Monsanto) were sown in biodegradable peat pots at 9^{th} May. At the stage of first leaves (27^{th} May) seedlings were replanted in vegetation pots (15 L) filled with peat (producer Laflora LTd., pH_{KCl} 5.5) mixed with 11 different fertilizers.

Cucumber plantation was created using different fertilization treatments with cattle (from JSC "Ziedi JP" = GD) and pig (from LLC "Latvi Dan Agro" (CD) and LLC "Mežacīruļi" (organic agicultural system, MCD) manure digestate and woodchip ash (from LLC "Fortum" = P) in different ratios (digestate - 2500 g per vegetation pot, ash – 200 g per vegetation pot, digestate to wood ash – in proportions 3:1 and 4:1). Horse manure (as traditionally practiced treatment) from university`s horse training farm "Mušķi" (ZM) – 650 g per vegetation pot. The same peat substrate ("Laflora" LTd., pH_{KCl} 5.5) was used as control (K).

Figure 1. Cucumbers under fertilization treatments in experimental greenhouse

Each treatment was added to peat substrate 2 weeks before planting of seedlings.

During experiment, automatic ventilation, irrigation as well as phytosanitary measures were provided. Forming of plants was made by traditional scheme. As the aim of the trial was to investigate the efficacy of the fertilizer`s variant, microelements were not added to substrates.

In the period of experiment, growth and yield dynamic was explored. Fruits were harvested regularly at the size of 12 cm. Data about the number of fruits per plant was collected. Organoleptic test (appearance, color, aroma, taste and aftertaste) for each harvest was provided, using scoring system from 1 (minimal value) to 5 (maximal value).

RESULTS AND DISCUSSION

During growing period the development of plants was not significantly different ($p>0.05$): at 11.06 the average length was about 45.35 cm, at the phase of fruit ripening (26.06) – about 1.25 m, but at the end of the vegetation – about 2.58 m.

Fig. 2. Cucumbers at the start of flowering.

First harvesting was provided at 25.06.2020, the last – at 31.07.2020., that in comparison with other experiments, provided in Latvia, was relatively short period. In total, fruits were picked 23 times, each 2-3 days. At previous investigations, provided at the same greenhouse, in average, cucumbers were harvested 33 times, that is by 10 times more than in our experiment (Sivicka *et al*., 2018). For each harvesting, the count of fruits per plant, depending on variant, was from 1 till 9 fruits (maximal results were showed by CD and ZM), but average count was 1.3 fruits per plant. The average weight of one fruit was 50.79 g, ranging from 37.5 (control) to 153.3 g (MCD) during the experiment.

Table 1. Quantitative parameters of cucumbers` yield

Fertilization treatment	Digestate and wood ash ratio in the mixture	Average count of fruits per plant	Total count of fruits per m^2	Average yield, kg per plant	Total yield, kg per m^2
Pig manure digestate (CD)	1:0	39	118	3.67	11.02
	3:1	29	87	2.78	8.30
	4:1	27	81	2.52	7.56
Pig manure digestate (MCD, organic agricultural system)	1:0	29	86	2.53	7.58
	3:1	33	99	2.89	8.64
	4:1	34	101	3.13	9.41
Cattle manure digestate (GD)	1:0	29	88	2.79	8.37
	3:1	23	69	2.31	6.94
	4:1	26	79	2.48	7.44
Ash (P)	1:0	25	75	2.29	6.86
Horse manure (ZM)	1:0	26	82	2.41	7.84
Control (K)	1:0	28	85	2.56	7.96

For all period of experiment, 1213 fruits and 97.91 kg of cucumbers` yield were harvested. The maximal total count of cucumbers per m^2 was harvested from CD variant (118), also MCD + P 4:1 and 3:1 was showed relatively high results (101 and 99). Similar results by variants were observed also for total yield, kg per m^2. It means, that higher total yield was characterized by bigger count of fruits per variant.

During the experimental period, the yield of cucumbers was more than 6 kg per m^2 for all variants. The variants such as CD, MCD + P 4: 1, MCD + P 3: 1 showed the highest results. The significant influence of fertilization`s variant to cucumbers` yield was observed ($p < 0.05$). For comparison, in previous experiments (using both synthetical and organic fertilizers), the total yield per unit area was reached 12.81 kg per m^2 – only variant with CD (11.02 kg per m^2) was close to this result (Sivicka *et al.*, 2018).

Non-standard cucumbers were detected in all variants except the P (ash), JPGD, MCD and MCD + P in a 4: 1 ratio (only standard cucumbers were detected in these variants). Per each harvesting time, 1-2 non-standard cucumbers from different experimental variants were detected, but no correlation was observed with fertilizer`s variant.

A

B

Fig. 3. Fruits prepared for organoleptic test:
A – 27.06.2020, B – 20.07.2020.

The total count of non-standard yield during the trial was 1.81%, which is low result. Non-standard products are usually produced under the influence of the microclimate, with insufficient water and nutrient supply in hot weather, rapid fruit swelling and fruiting on warm nights as well as deformed cucumbers during pollination of parthenocarpic hybrids (as ‘Mirabelle’ F1 is) by bees from the laboratory`s apiary.
The organoleptic parameters were not differed significantly throughout the growing season, except for the color (it became much duller at the end of the experiment). The average organoleptic score was 4.3 points. It was observed, that for by age younger assessors, the lower count of points was given to the appearance, because they were "worried" about the coarse warts of ‘Mirabelle’ F1 cucumbers.

CONCLUSION

By complex of quantitative indices, highest results were observed for fertilization treatments such as pig digestate (CD) and pig manure digestate (MCD from organic agricultural system) with ash 4:1. It is necessary to continue this research for exploring the influence of fertilization treatment on growing and yield period of cucumbers. The influence of digestate`s type on the acidy changes of substrate should be observed much more carefully.

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EFFECT OF FERTILIZATION ON UPTAKE OF MACROELEMENTS WITH SUNFLOWER BIOMASS IN A POT EXPERIMENT WITH HAPLIC VERTISOL

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ABSTRACT

The study was conducted in the conditions of pot experiment with Haplic Vertisol soil. The aim was to evaluate the effect of different norms and combinations of nitrogen, phosphorus, potassium and silicon fertilizers in the soil and their impact on the content and uptake of some main macro elements with sunflower biomass. The test culture was an early to medium-early hybrid Sunflower (*Helianthus annuus L.*) - Sumiko HTS. The experiment includes 16 variants of fertilization with 3 repetitions. Data are obtained on the yield of fresh and absolutely dry biomass from the above-ground part and the content of N, P, K, Si, Ca and Mg in the resulting dry biomass from plants. According to the experimental data obtained, the content and uptake of the examined macro elements with the sunflower biomass are significantly influenced by the imported norms and combinations of fertilizers. The highest is the uptake of nitrogen in the variants with the following norms: N_{200}, N_{300} and N_{400}.N uptake is the highest also in comparison with all other values of the examined elements.It is established that the changes in the macroelements uptake significantly follow changes in the quantities of the relevant elements in dry biomass in the variants of the experiment. With an increase in fertilization norms, not only the content of N, P, and Si is increased, but also the uptakes with sunflower biomass.This trend is expressed to a lesser extent with potassium.

Key words: *fertilizers, rates, export by sunflower biomass. "Helianthus annuus, L." and "macro elements uptake.*

INTRODUCTION

Sunflower is a major oilseed crop in Bulgaria. The nutrient requirements for yield formation depend both on the specific soil and climatic conditions of the area and on several other factors. Sunflower responds well to nitrogen fertilization, excess nitrogen lowers oil content and lowers plant resistance to disease. Appropriately balanced phosphorus and potassium fertilization increase yield and oil content. The potassium requirements of sunflower are high - 7-11 kg per 100 kg of seed, and increases the absolute weight and fat content of the seeds. Experiments conducted in our country show an increase in fat yield under the influence of potassium fertilization(Nikolova, 2010).Determining the optimal nutrient regime for crops requires establishing the export and consumption of nutrients to form a unit of production, and their balance in the soil for different soil conditions (Nenova, Mitova,2018; Mitova, Dinev 2012). Establishing nutrient balance is an effective method for assessing nutrient use by crops.In this way, the negative consequences of improper fertilization could be avoided. Furthermore, balanced nutrition plays a key role in obtaining stable yields of high quality. This crop is also distinguished by its exceptional micronutrient requirements. Very important is also the growth of the stem, which must be resistant to lodging and provide a continuous transfer of metabolites to the growing seeds, and here is mainly the role of Si. In our country, the use of silicon fertilizers is poorly developed. Silicon (Si) is not classified as an essential element for plants, but numerous studies have described its beneficial effects under various soil and climatic conditions, including low levels of plant-available forms of silicon. The application of Si shows the potential to increase the availability of nutrients in the rhizosphere and their uptake by plants (Pavlovic et al., 2021). Plant species vary greatly in their ability to accumulate Si, with values ranging from 0.1% to 10% Si (Epstein, Bloom, 2008). Consequently, some plant species are minimally affected by the introduction of Si compared to others (Coskun et al., 2019).

The main objective of the study was to determine the content and export of macronutrients (N, P, K, Si, Ca, and Mg) with biomass of sunflower (*Helianthus annuus* L.), under the influence of increasing levels of fertilization with nitrogen, phosphorus, potassium, and silicon under the conditions of a growing experiment on haplicvertisol.

MATERIALS AND METHODS

A vegetation fertilizer trial with a test crop of an early hybrid of sunflower (Helianthus annuus L.) - Sumiko HTS of Sinjenta was set up and established. The initial soil is leached smolnitza supplied by the experimental field in Bozhurishte, Sofia region.According to the classification of soils in Bulgaria (Koinov, 1987), it is defined as haplicvertisol(FAO, 2015). It is characterized by a close to neutral soil reaction in the plowing horizon (pH_{H2O}- 6,4,pH_{KCl} - 5.6), with a high content of total (0,217%) and mineral nitrogen (40,32 mg N/kgsoil). The soil has a low supply of mobile phosphorus (1,92 мg P_2O_5/100 gsoil) and better available potassium content (30,86mg K_2O /100 g soil).Before sowing the seeds, fertilizers with different amounts of active substances in mg/plant were added to the experimental pots of 3 kg capacity, as presented in Table 1. Five sunflower seeds were sown, leaving 3 plants in each pot at a later stage. On the 67th day of vegetation in the budding phase, the plants were harvested, weighed, and prepared for chemical analysis. The content (% a. b.w.) of macronutrients N, P, K, Si, Ca, and Mg in sunflower plant biomass was determined by acid digestion and ICP readings (5800 ICP - OES system - Agilent). The export of the tested elements with the plant production was determined.The experiment included 16 fertilization treatments in 3 replications. It is a multifactorial scheme with four factors varied at 5 levels (Sadovski, 2020). Table 1 shows the experimental design and the imported amounts of the active substance of the macroelements used in mg/pot.

Table 1. Scheme of a pot experiment and quantities of active substance in mg/pot:

1.$N_0P_0K_0Si_0$ -Control	9. $N_{200}P_{160}K_{140}Si_{2000}$
2.$N_0P_{160}K_{140}Si_{800}$	10. $N_{200}P_{160}K_{140}Si_{800}$
3.$N_{400}P_{160}K_{140}Si_{800}$	11. $N_{300}P_{240}K_{70}Si_{400}$
4. $N_{200}P_0K_{140}Si_{800}$	12. $N_{300}P_{80}K_{210}Si_{400}$
5.$N_{200}P_{320}K_{140}Si_{800}$	13. $N_{300}P_{80}K_{70}Si_{1200}$
6. $N_{200}P_{160}K_0Si_{800}$	14. $N_{100}P_{240}K_{210}Si_{400}$
7.$N_{200}P_{160}K_{280}Si_{800}$	15. $N_{100}P_{240}K_{70}Si_{1200}$
8.$N_{200}P_{160}K_{140}Si_0$	16. $N_{100}P_{80}K_{210}Si_{1200}$

The following soil analyses were performed before and after the vegetation experiments: pH - potentiometric in H_2O and KCl (Arinushkina, 1962); total and mineral nitrogen content - Bremner and Kinney method (Bremner,1965a, Bremner,1965b); mobile forms of phosphorus and potassium (P_2O_5 и K_2O) - by the acetate-lactate method (Ivanov, P.,1986); organic carbon (humus) content - according to Turin's method (Kononova, 1963).
Statistical analysis of the obtained results was done with Statgraphics statistical product (ANOVA). Fisher's method for comparison of means at least significant difference (LSD) was used to detect differences between the variants studied.

RESULTS AND DISCUSSION

From the obtained data, it was reported that the lowest fresh weight is in the control variant and the highest in the variantsV11 ($N_{300}P_{240}K_{70}Si_{400}$)-345,708g, B12 ($N_{300}P_{80}K_{210}Si_{400}$)-330,576gand B3 ($N_{400}P_{160}K_{140}Si_{800}$) -328,248g.It is evident that these are the variants with the high norms of nitrogen, the differences between them were insignificant. Similar results are also obtained for other plants (Vasileva, V. & Ilieva, A., 2017).Noticeably lower are the weights in variants B2($N_0P_{160}K_{140}Si_{800}$)abd B14($N_{100}P_{240}K_{210}Si_{400}$) without N and low N norm. The combinations of different rates and types of mineral fertilizers used failed to emit the most favorable combination that influenced the plant's fresh and dry weight index at the budding stage of sunflower in the pot experiment.It can be said that N_{300} and N_{200} in combination with lower rates of the other macronutrients are most conducive to sunflower development. It is noteworthy that the highest result was not achieved in the variant with the highest nitrogen rate (N_{400}).It may noticed that the dry weight of the total biomass obtained from 1 plant per pot varies in proportion to the corresponding fresh weight in the different variants.

As a result of the conducted pot fertilizer experiment and the one-way ANOVA analysis of the data for the amount of plant biomass on the 67th days, the leading role of nitrogen fertilization in the norm of 200 mg/per pot was established (the difference between the variants are at the high level of significance $P \leq 0.001$) (Table 2).Nitrogen is an essential nutrient that determines the growth of oilseeds and increases the amount of protein and yield.The accumulation of biomass in sunflower is associated with the absorption of nutrients during the whole growing season (Hassan, F., & Kaleem, S., 2014).

Table 2. Influence of fertilization rate and fertilizer combinations on the yield of fresh biomass from the aboveground part of sunflower plants (One-way ANOVA analysis)

Fresh weight of Sunflower plants (g/ per pot)

Variants	Bozhurishte-67 day	
1. $N_0P_0K_0Si_0$	29,82	a
2. $N_0P_{160}K_{140}Si_{800}$	50,46	b
3. $N_{400}P_{160}K_{140}Si_{800}$	76,43	c
4. $N_{200}P_0K_{140}Si_{800}$	80,70	d
5. $N_{200}P_{320}K_{140}Si_{800}$	89,23	e
6. $N_{200}P_{160}K_0Si_{800}$	90,80	e
7. $N_{200}P_{160}K_{280}Si_{800}$	94,67	f
8. $N_{200}P_{160}K_{140}Si_0$	97,40	fg
9. $N_{200}P_{160}K_{140}Si_{2000}$	97,43	fg
10. $N_{200}P_{160}K_{140}Si_{800}$	98,97	gh
11. $N_{300}P_{240}K_{70}Si_{400}$	100,10	gh
12. $N_{300}P_{80}K_{210}Si_{400}$	101,67	h
13. $N_{300}P_{80}K_{70}Si_{1200}$	108,63	i
14. $N_{100}P_{240}K_{210}Si_{400}$	109,43	i
15. $N_{100}P_{240}K_{70}Si_{1200}$	110,20	i
16. $N_{100}P_{80}K_{210}Si_{1200}$	115,23	j
Average	**90,69**	
Stnd. deviation	**22,167**	
Stnd. error	**2,78**	
LSD≥95%		

Under the influence of mineral nutrition, significant changes occur in the amount of nutrients absorbed by the biomass of sunflower plants (Table 3).The content of total N in the dry biomass of plants varies from 1,67in the control to 2.87% in variant B11 ($N_{300}P_{240}K_{70}Si_{400}$). Approximately in the same order is the content of total N in variants B4 ($N_{200}P_0K_{140}Si_{800}$), B12($N_{300}P_{80}K_{210}Si_{400}$) and B13($N_{300}P_{80}K_{70}Si_{1200}$). The average content of total N in the seventh variants with norm N200 and in the three variants with norm N300 is 2.1 and 2.48%,respectively.The application of mineral nitrogen in the soil in most cases is accompanied by an increase in the content of N in plants(Hara, Sonoda, 1979; Atanassova, 2005; Kolota, Chohura, 2015; Nenova, Mitova, 2018, Vasileva, Ilieva., 2017). This trend was also confirmed in the current experiment.

The content of phosphorus in sunflower plants is significantly lower than the nitrogen content and varies less depending on the combinations of rates and types of mineral fertilizers applied- from 0.26 inthecontrolto 0.30% inthefertilized variants.The phosphorus content is the highest in the B11($N_{300}P_{240}K_{70}Si_{400}$) variant, which contains the highest norm of triple superphosphate.There is only a very slight tendency to increase the phosphorus content in the plants with increasing fertilizer rate in Haplic Vertisol.

The potassium content is slightly higher than the phosphorus content and ranges from 1.07 in the control to 2.14% in the variant with the potassium norm (K_{140}). Other authors have reported similar results. Summarized results from a large number of experiments show that "economically" nitrogen, high phosphorus, and abundant potassium fertilization are suitable for sunflower cultivation. On magnesium-poor soils, both yields and oil content can be increased by magnesium fertilization (Nikolova, 2010).

The calcium content is high and ranges from1.21 to 1.96% but it is significantly higher in the fertilized variants. It cannot be argued that the higher fertilization rates lead to a higher accumulation of Ca in sunflower plants.The change in the content of Mg varies similarly, but the amount in the control variant is lowher - 0.36%, and in the fertilized variants it is between 0.37 and 0.77%.It can be concluded that fertilization has a significantly lower effect on the accumulation of Mg in sunflower plants.

Of interest is the Si content, which increases from 0.0112% in the control variant to 0.0285% in the variant with the highest Si rate.The combinations of norms and fertilizers used in the experiment do not establish a direct relationship between increasing the accumulation of Si in plants with increasing the imported Si level.In the studies by Peixoto, et al., 2022, a higher total leaf area of Si-treated plants leads to increased overall CO_2 uptake by the plant.Plants treated with Si have an increase of 24-39% in biomass yield.

Table 3. Content of total N, P, K, Si, Ca, and Mg in sunflower biomass (in% of absolutely dry weight) by variants (pot experiment on haplicvertisol, 2021).

Variants	N	P	K	Si	Ca	Mg
			%			
1.$N_0P_0K_0Si_0$	1,67	0,26	1,96	0,0112	1,78	0,36
2.$N_0P_{160}K_{140}Si_{800}$	1,00	0,23	1,87	0,0292	1,71	0,34
3.$N_{400}P_{160}K_{140}Si_{800}$	1,93	0,29	1,68	0,0700	1,96	0,69
4.$N_{200}P_0K_{140}Si_{800}$	2,84	0,24	2,14	0,0348	1,93	0,60
5.$N_{200}P_{320}K_{140}Si_{800}$	2,05	0,27	1,07	0,0830	1,25	0,43
6.$N_{200}P_{160}K_0Si_{800}$	2,65	0,29	1,61	0,0514	1,85	0,60

7.$N_{200}P_{160}K_{280}Si_{800}$	2,02	0,26	1,80	0,0263	0,16	0,50
8.$N_{200}P_{160}K_{140}Si_{0}$	1,83	0,26	1,59	0,0263	1,61	0,66
9.$N_{200}P_{160}K_{140}Si_{2000}$	1,69	0,24	1,53	0,0285	1,70	0,60
10.$N_{200}P_{160}K_{140}Si_{800}$	1,60	0,26	1,50	0,0290	1,76	0,61
11.$N_{300}P_{240}K_{70}Si_{400}$	2,87	0,30	1,52	0,0304	1,76	0,77
12.$N_{300}P_{80}K_{210}Si_{400}$	2,33	0,23	1,91	0,0320	1,92	0,63
13.$N_{300}P_{80}K_{70}Si_{1200}$	2,25	0,19	1,50	0,0267	1,62	0,67
14.$N_{100}P_{240}K_{210}Si_{400}$	1,77	0,27	2,01	0,0362	1,79	0,52
15.$N_{100}P_{240}K_{70}Si_{1200}$	1,47	0,28	1,74	0,0278	1,67	0,51
16. $N_{100}P_{80}K_{210}Si_{1200}$	1,83	0,21	1,50	0,0207	1,21	0,37

Based on the obtained dry biomassand the content of N, P, K, Si, Ca, and Mg in it(Table 3), the export from the soil of the studied elements was calculated.From the obtained results it is evident that the changes in the exports of the studied macroelements significantly follow the changes in the quantities of the respective elements in the dry biomass according to the variants of the experiment. As the fertilization rates increase, not only the content but also the exports of N, P, K, and Si increase.

Figures 1 to 4 show the regression curves of the exports of N, P, K, and Si depending on the imported quantities of the respective elements, presented as polynomials of the second degree.

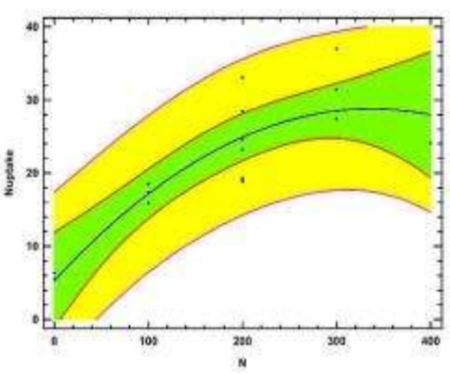

Fig. 1. Nitrogen export

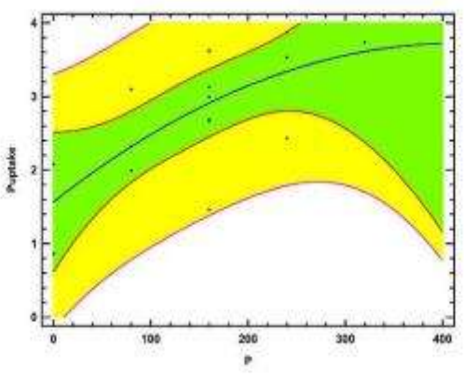

Fig.2. Phosphorus export

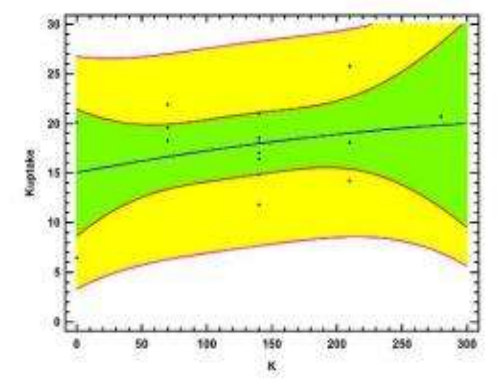

Fig. 3. Potassium export

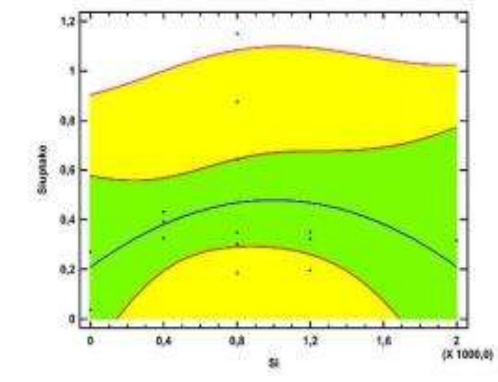

Fig. 4. Silicon export

Table 4 is compiled by conversion following the imported fertilization rates and the average values of exports with the relevant macronutrients, the patterns established for N, P, K, and Si are very clear. From this table, we can summarize that the difference between the imported quantities of active substances with fertilizers and the export with the obtained biomass is significant. This means that in the variants with high fertilization rates, large amounts of nutrients are available, which will be able to ensure the nutrition of sunflower even after the "R4" phase until the end of the growing period.

Table 4. Average export of N, P, K, and Si with biomass of sunflower (kg.da^{-1}) by variants and rates of fertilization (kg.da^{-1})

norms of N	average export of N	norms of P	average export of P	norms of K	average export of K	norms of Si	average export of Si
0	5,50	0	0,86	0	6,45	0	0,04
30	28,74	24	2,47	21	19,94	120	0,38
60	33,14	48	2,88	42	16,82	240	0,54
90	34,48	72	3,28	63	21,93	360	0,29
120	33,38	96	3,74	84	20,7	600	0,32

CONCLUSION

The leading role of nitrogen fertilization with high norms of 200, 300, and 400 mg/pot and silicon 800 mg/pot was established as a result of the experiment carried out on a haplicvertisol in a vegetation house and from the single-factor dispersion analysis of the values for the amount of biomass of sunflower plants on the 67th day, (the proven difference between variants is at a high confidencelevel $P \leq 0.001$).

According to the experimental data obtained on the fresh and absolutely dry biomass from the above-ground part, the content and export of the studied macroelements (N, P, K, Si, Ca and Mg) with biomass are significantly influenced by imported soil fertilization norms and combinations. The highest is the nitrogen exports in the N200, N300 and N400 variants. Exports of nitrogen with sunflower biomass are the highest compared to the exports of all other elements examined. There is only a very slight tendency to increase the phosphorus content in the plants with increasing fertilizer rate in haplic vertisol.

Changes in exports of the macroelements examined have been found to follow changes in the quantities of the relevant elements in dry biomass in the variants of the experiment. With an increase in fertilization rates, not only the content is increased, but also the exports of N, P, and Si. In potassium, this trend is expressed to a lesser extent.

The calcium content is high and ranges from1.21 to 1.96% but it is significantly higher in the fertilized variants. The change in the content of Mg varies similarly, but the amount in the control is lower - 0.36%, and in the fertilized variants it is between 0.37 and 0.77%. It can be concluded that fertilization has a significantly lower effect on the accumulation of Mg in sunflower plants.

Si content increases from 0.0112% in the control variant to 0.0285% in the variant with the highest Si rate.The combinations of norms and fertilizers used in the experiment do not establish a direct relationship between increasing the accumulation of Si in plants with increasing the imported Si level.

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RESPONSE OF SOIL CHEMICAL PROPERTIES TO BIOCHAR AND NITROGEN APPLICATION IN A FIELD EXPERIMENT

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ABSTRACT

A two-year field experiment was carried out with maize (*Zea mays* L.) at two biochar (BC) doses of (5 and 10 t. ha^{-1}) and two nitrogen fertilizer rates (130 and 260 kg. ha^{-1}) in Tsalapitsa village (Plovdiv) on Alluvial-meadow soil (Fluvisol). The aim of the study was to find out the influence of biochar as an aftereffect, as well as nitrogen fertilization on soil physicochemical and agrochemical properties. In order to observe the effect of biochar in the second year, the variants from the first year ($B_{(1)}$5N130, $B_{(1)}$10N130, $B_{(1)}$5N260 and $B_{(1)}$10N260, without the controls K1N130 and K2N260 were left without the addition of biochar, and for the second year, new variants were set up according to the same scheme. During the vegetation of maize, the influence of biochar as the aftereffect was observed with increase in pH values (0.2 - 0.3 units) in comparison with the control variants (without biochar, nitrogen fertilization only), which was confirmed by its application in the second year. There were positive changes in the mineral N content of the studied treatments as the aftereffect, compared to the controls and the second-year treatments, which may be related to the ability of the biochar to fix nitrogen and protect it from leaching, especially on vulnerable soils such as the Fluvisol studied. A slight increase in organic carbon (0.09-0.32 %) was also found at the lower N rate (130 kg. ha^{-1}) in the aftereffect variants. In these variants, a slight increase in cation exchange capacity, exchangeable Ca and the degree of base saturation was also observed. Biochar utilization had little effect on the investigated properties of the Fluvisol. Applying the lower doses of biochar in longer term would have a more significant impact.

Keywords: *pH, cation exchange capacity, organic carbon, soil mineral nitrogen, second year.*

INTRODUCTION

Globally, agriculture is identified as one of the most significant sources of anthropogenic loading on soils and water, causing negative effects on environmental components such as biodiversity loss, soil acidification, erosion, salinization, surface and groundwater pollution, eutrophication, increased gas emissions, etc. The need to implement effective measures to improve soil quality and mitigate climate change requires the use of soil improvers (Lal, 2004). Nowadays, the recovery of nutrients through the reintegration of organic waste into agriculture is a sustainable alternative that can contribute to the restoration of the natural balance of environment (Griffin *et al.*, 2003).

The Alluvial - meadow soil in the study area is characterized by light soil texture, poor water holding capacity and relatively high-water permeability. There is a relatively high-water exchange between the layers, which creates conditions for active migration of chemical elements in the profile. For this reason, the agricultural practices applied are of significant importance in regard to the nitrogen uptake. Studies have shown that the use of biochar improves physico-chemical, water-physical, biological properties and increases yields, as well as potential means of sequestering carbon in soil (Bista *et al.*, 2019, Liang *et al.*, 2006; Joseph *et al.,* 2010). Biochar can increase soil C storage and influence soil quality and function. Compared to other organic amendments, biochar has greater stability whereby it increases soil fertility, water holding capacity, crop growth and development (Yadav *et al.*, 2018). The aim of the study was to find out the influence of biochar as an aftereffect in relation with nitrogen fertilization on soil physicochemical and agrochemical properties.

MATERIALS AND METHODS

The two – year experiment (2019 and 2020) was conducted at the experimental field in the village of Tsalapitsa (Plovdiv region), in Bulgaria. The soil texture is sandy clay loam and the soils in the area are classified as Fluvisol in the WRB (2015) classification. Biochar was produced from oak peels at a pyrolysis temperature of 400 °C. The basic properties of the topsoil (0–0.15 m) are: pH (H_2O) 6.1, CEC 16.7 cmol.kg^{-1}, soil organic carbon (SOC) 0.68%, total N 0.052%, available N 20.17 mg.kg^{-1}, available P 17.13 mg.100^{-1} g, available K 22.40 mg.100^{-1} g. Biochar properties were: pH (H_2O) 9.7, organic C content 49 %, total N 0.59%, available N 72 mg.kg^{-1}, K 499.6 mg.100^{-1} g, available P 43.4 mg.100^{-1} g.

Treatments with two rates of BC (5 and 10 t.ha^{-1}) and two rates of nitrogen fertilizer (130 and 260 kg.ha^{-1}) in the first year (2019) were set. Each level of BC application was tested against each level of nitrogen fertilizer application. To observe the effect of BC in the second year (2020), the variants from the first year (without controls) are left for observation without adding BC, and 6 new variants are set for the second year according to the same scheme (Table 1).

Table 1. Scheme of variants

Variants	BC t. ha^{-1}	N kg. ha^{-1}	Variants	BC t. ha^{-1}	N kg. ha^{-1}
	from 1 st year - after effect			from 2 nd year	
$B_{(1)}$5N130	5	130	K1N130	0	130
$B_{(1)}$10N130	10	130	B $_{(2)}$5N130	5	130
			$B_{(2)}$10N130	10	130
$B_{(1)}$5N260	5	260	K2N260	0	260
$B_{(1)}$10N260	10	260	$B_{(2)}$5N260	5	260
			$B_{(2)}$10N260	10	260

Note: $_{(1)}$ - treatments from the previous year (without BC application, only with mineral fertilizers) for observation on the BC aftereffect$_{;\,(2)}$ - treatments with BC application during the second year.

Soil samples were taken during development of maize (at the 10-12 leaf phenophase - about 45 days after biochar and nitrogen fertilizer were applied in the second year) from the treatments. Mineral nitrogen was analysed by the method of Kjeldahl (Methods of Soil Analysis, 1982) and available P and K following the methods of (Ivanov, 1984). The physico-chemical soil properties were determined by the method of Ganev and Arsova (1980). Soil organic matter content and composition were obtained by a modified method of Turin (oxidation with dichromate and H_2SO_4 in a thermostat at 120 °C, 45 min., Ag_2SO_4 catalyst and back titration with $(H_4)_2SO_4$. $FeSO_4.6H_2O$ and Kononova - Belchikova method (Filcheva and Tsadilas, 2002).

RESULTS AND DISCUSSION

The results of the soil chemical analysis showed that pH values in the control treatments ranged from 5.6 (K2N260) to 6.1 (K1N130). After addition of BC, the pH values increased by 0.2 - 0.4 units. It was interesting to note the more pronounced aftereffect of BC, in the first year, compared to the control with the higher nitrogen rate. Many authors indicated that the application of BC increases pH of soil for acid soils (Pandian *et al.*, 2016, Zhao *et al.*, 2018, He *et al.*, 2021). Furthermore, previous studies of the relationship between soil pH and biochar dose have identified complex interactions (Hailegnaw *et al.*, 2019), requiring the long-term study of soil response.

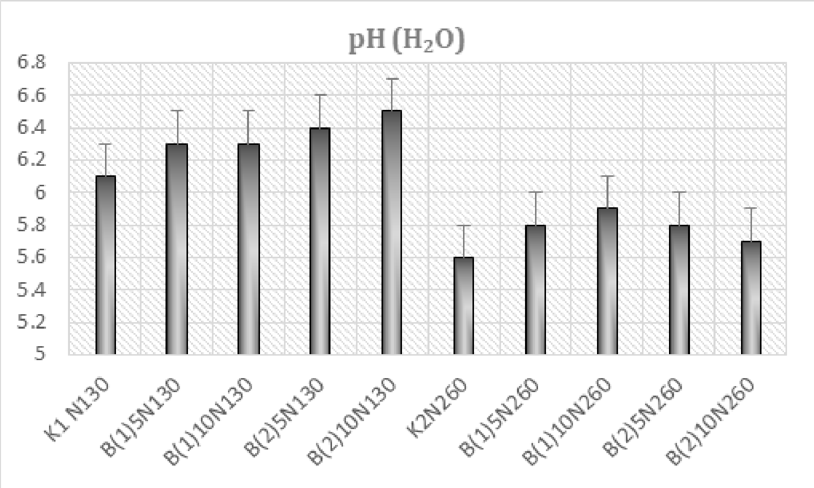

Fig. 1. Values of pH in Fluvisol by treatments during maize development

It is known that the cation exchange capacity (CEC) depends on soil texture, the organic matter content, and also the type of clay minerals that make up the fine-grained part soils. The Alluvial-meadow soil studied contains a relatively small amount of clay, and as can be seen from the data the sorption capacity values are low. Interestingly, the highest values of the cation exchange capacity were recorded in the variants with $B_{(1)}5N130$ and $B_{(1)}10N130$ aftertreatment - 16.5 and 16.6 $cmol.kg^{-1}$ compared to the other variants studied (Table 2). According to (Karim *et al.*, 2020) BC is a porous material, with high surface area and significant negative charges. These are properties that can significantly increase the cation exchange capacity of soil and retain nutrients and water. CEC were found to have a positive correlation with total C content (r=0.85, P < 0.001) as a linear relationship with the biochar applied.

Table 2. Physico-chemical characteristics of Fluvisol by variants during the development of maize.

Variants	pH_{H2O}	EC	CEC	CEC_{CA}	CEC_A	$exch.H_{8.2}$	exch. Al	exch. Ca	exch. Mg	BS
		Ms/cm				$cmol.kg^{-1}$				%
K1N130	6.1	0.07	16.2	12.0	4.2	3.0	0	11.0	2.0	81.48
$B_{(1)}5N130$	6.3	0.08	16.5	12.6	3.9	2.8	0	11.8	2.0	83.03
$B_{(1)}10N130$	6.3	0.08	16.6	12.7	3.9	2.8	0	11.9	2.0	83.13
$B_{(2)}5N130$	6.4	0.09	16.2	13.3	2.9	1.6	0	12.4	2.1	90.12
$B_{(2)}10N130$	6.5	0.09	16.2	13.3	2.8	1.5	0	12.4	2.0	90.69
K2N260	5.6	0.06	16.0	11.1	4.9	4.0	0.5	9.5	1.8	71.87
$B_{(1)}5N260$	5.8	0.063	16.0	11.8	4.2	3.7	0.2	10.2	1.8	75.87

$B_{(1)}$10N260	5.9	0.063	16.2	12.4	3.8	3.5	0.1	10.5	1.9	78.40
$B_{(2)}$5N260	5.8	0.06	16.2	11.7	4.5	3.7	0.4	10.5	1.9	77.16
$B_{(2)}$10N260	5.7	0.06	16.2	11.7	4.5	3.7	0.5	10.0	1.8	74.07

The degree of base saturation (BS) increased compared to the controls, and this trend was more noticeable in the variants with a lower N rate and incorporation BC in the second year. The total acidity (exch.$H_{8.2}$), which covered all adsorbed cations with acidic functions, decreased relative to the controls with increasing pH values. In the variants with high nitrogen rate, acidification and the occurrence of exchange acidity were observed because even high biochar levels cannot provide sufficient basic cations (Ca, Mg) for precipitation of Al^{3+} to neutralise strongly acid exchangeable sites (CECca).

The application of a significant amount of nitrogen and other nutrients causes the accumulation of residual mineral nitrogen in the soil profile, which is a potential source of changes in the soil solid and liquid phase. The studied Alluvial-meadow soil is characterized by a light texture and accelerated water flow, which makes it more vulnerable to nitrate leaching from the soil. The addition of biochar is thought to have different, and sometimes conflicting, effect on nitrogen content and movement across the soil profile. Many researchers (Laird *et al.*, 2010; Libutti *et al*, 2016; Borchard *et al.*, 2019) found that the addition of biochar to arable soils can reduce nitrate and phosphate leaching. This may be explained by an increase in the anion exchange capacity of biochar, although the mechanisms are not yet well understood. In other studies (Yao *et al.*, 2012; Hollister *et al.*, 2013) found a limited ability of biochar to retain nitrate from the soil solution; furthermore, the authors believe that the material from which biochar is derived and the type of soil have a significant impact on nitrogen uptake.

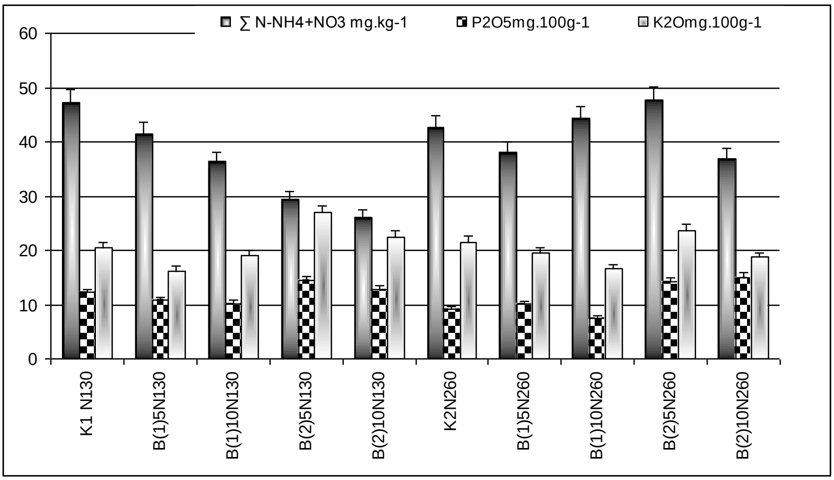

Fig. 2. Content of mineral nitrogen, available phosphorus and potassium (mg.kg^{-1}) in Fluvisol by variants in maize cultivation

The agrochemical soil data showed that the mineral nitrogen content of the control treatments ranged in the interval (47.23- 42.62 mg.kg^{-1}). It was observed that in the $B_{(1)}$5N130 and B(1)10N130 after-treatments, the mineral nitrogen content was higher (41. 47 mg.kg^{-1}) at the lower rate of BC compared to the treatment with higher amount of BC ($B_{(1)}$10N130 36. 29 mg.kg^{-1}). In the other two treatments ($B_{(2)}$5N130 and $B_{(2)}$10N130) in the second year, the mineral nitrogen content was about 1.5 times lower (Fig. 2). In the variants with nitrogen rate of 260 kg ha^{-1}, no significant difference was observed in the mineral nitrogen content of the soil, perhaps reflecting the more significant effect of fertilisation. The studies of Li *et al.*, (2019a), Paetsch *et al.*, (2018) found that monitoring N in soil can effectively reveal the impact of biochar on agroecosystems during its application, which is associated with aging processes and changes in its functional groups and specific surface area.

From the results obtained for the available phosphorus content, it was found that they did not vary significantly. It was necessary to note its higher content in the variants in the second year studied, with the highest values reaching $B_{(2)}$10N260 (15.05 mg.100g^{-1}). It is known that many factors significantly affect the availability of phosphorus in soil, such as soil solution pH, adsorption reactions, organic matter, phosphatase activity, etc., and that the organic inputs and their interaction with soil phosphorus content are not well understood. Regarding the potassium content (Fig. 2), the highest values were observed in the variants with the low doses of BC ($B_{(2)}$5N130 and $B_{(2)}$5N260, 26. 9 mg. 100g^{-1} and 23.7 mg.100g^{-1}, respectively). In their research Bista *et al.*, (2019) found the better effectiveness of lower doses of BC on the properties of certain soil types.

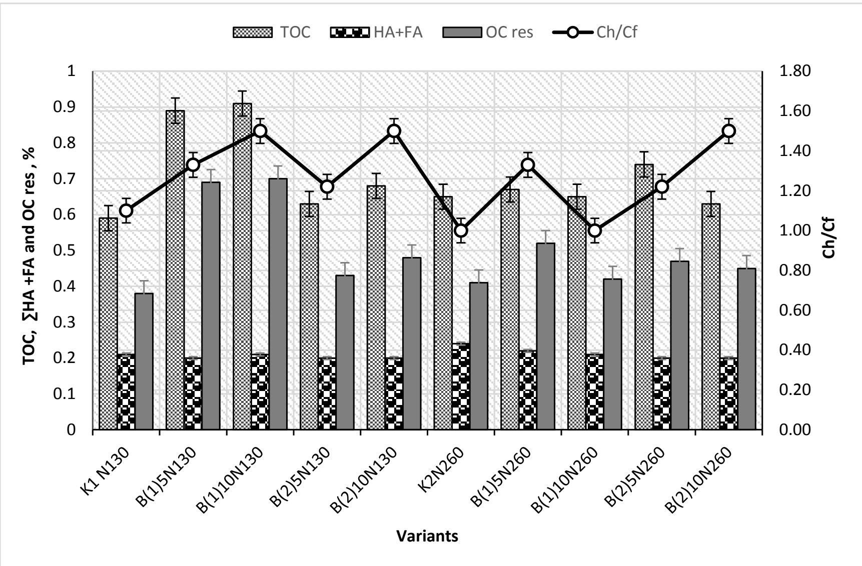

Fig. 3. Content and composition of TOC in Fluvisol by variants

The studied soil was characterized by a low content of humus (1.17 %) of humate-fulvic type (Ch/Cf = 1), low content of humic acids, 100 % bound with Ca and weak degree of humification (13.5 % of total carbon) (Benkova *et al.*, 2022). The results for total carbon content and organic matter composition (Fig. 3), indicate that there was a slight increase in carbon, and this change was the highest (0. 89 and 0. 91 %) in the variants with low dose of N in the first year, $B_{(1)}$5N130 and $B_{(1)}$10N130. The humus system in soil changed from humate-fulvate (C_{HA}/C_{FA} 0.5 - 1) to fulvate - humate type (C_{HA}/C_{FA} 1.5) (Orlov, 1985). Biochar application to soil resulted in an increase in C_{HA}/C_{FA} ratio and hence soil organic matter quality compared to controls. The application of biochar increased the amount of humin from 0. 38 % in the control to 0. 69-0. 70 % in the variants $B_{(1)}$5N130 and $B_{(1)}$10N130 in the first year. In other studies, Uzoma *et al*. (2011), Van Zwieten *et al*. (2010) and Oladele *et al.* (2018) also observed an increase in soil TOC after the application of biochar. Van Zwieten *et al*. (2010) found that biochar significantly increased total soil C in the range of 0.5 – 1.0 %. Oladele *et al.* (2018) reported that biochar amendments to soil significantly increased total C with increasing application rates.

CONCLUSIONS

The obtained results indicate that an increase in pH values was found in the BC and lower N rate treatments. There was divergent behaviour between mineral nitrogen content, available forms of phosphorus and potassium and applied BC rates. The mineral-N was lower at the higher rate of BC (10 $t.ha^{-1}$) than at the lower dose (5 t.ha-1) after the first year, and it is possible that biochar ageing processes alter its influence on soil physico-chemical properties compared to recently applied biochar. A higher available phosphorus content was observed in the variants of the second year studied compared to the after-effect variants. The results showed that the C content was higher in the biochar treatments compared to the controls (K1N130, and K2N260), with the highest values obtained in the first year of the experiment, when biochar was applied at the low rate of 130 $kg.ha^{-1}$ N as an after-treatment. A slight increase in cation exchange capacity, exchangeable calcium and degree of base saturation was also found in the same variants. These results indicate that long-term additions of BC have the beneficial effects on the soil physico-chemical and agrochemical properties. Further research is needed to clarify the complex influence between fertilization rates and BC doses on light-textured soils such as Alluvial-meadow soils, due to their vulnerability to anthropogenic loading.

ACKNOWLEGDEMENT

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ANTIFUNGAL ACTIVITY OF POMEGRANATE PEEL EXTRACTS AGAINST *FUSARIUM OXYSPORUM F.SP.RADICIS –LYCOPERSICI*

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ABSTRACT

The objective of our study was the antifungal power test of the pomegranate peel aqueous extract PPE, on a phytopathogenic fungal strain of tomato "*Fusarium oxysporum f.sp.radicis-lycopersici*". For this purpose, a test was carried out, by the direct contact method in PDA potato dextrose agar medium, on the phytopathogen, in different concentrations of PPE (1%, 2%, 3%, 5%). Antifungal activity was evaluated by the estimation of the mycelial growth, the mycelial inhibition rate and the spore count. Our results showed a remarkable and highly significant antifungal activity against the studied phytopathogen, an inhibition index of 100% with the 3% and 5% concentrations was noted. The effectiveness of PPE could be the subject of investigation and exploitation in the integrated control of the tested fungus that causes much damage to the tomato crop.

Key words: *PPE, Fusarium oxysporum f.sp.radicis-lycopersici, mycelial growth, rate inhibition, sporulation.*

INTRODUCTION

Plant pathogens reduce crop yield and quality, whose control in traditional agricultural production systems relies mainly on the application of fungicides (Romanazzi et al.,2012; De Corato et al., 2016), which reduces the stability and sustainability of agricultural production (Larsen et al., 2017; Fones et al., 2020). However, the fungicides application presents a serious risks to human and environmental health, with negative impacts on non-target microorganisms (Sanzani et al.,2010; Gill and Garg , 2014).

Several alternative methods have been proposed to inhibit the growth of several pathogens fungal and achieve crop disease control, especially plant extracts (biosourced products) (Romanazzi et al.,2012; De Corato et al., 2016; Mari et al., 2016; Palou et al., 2016; Van Lenteren et al., 2018). These natural extracts possess a biological antifungal compounds that influence the mycelial growth, the sporulation rate and the germination, varying from a fungistatic effect to complete inhibition. In laboratory tests, the bioactive compounds of plant extracts applied in their raw state or as a fraction, inhibit partially or totally the fungal growth, affecting the colony development when applied at low concentrations (Mahlo et al., 2010; Castillo et al., 2012; Cerqueira et al., 2016).

In this regard, pomegranate peel extracts PPE (*Punica granatum L., Punicaceae*) have emerged as a very promising source of antifungal substances, for the plant control and food pathogens. The phytochemical screening of different parts of the pomegranate fruit; including peel, arils and seeds revealed a high predominance of polyphenols in the peel part (Orak et al., 2012). This explains their particular use in traditional medicine (Shaygannia et al., 2016). Numerous scientific researchers have demonstrated the therapeutic and antioxidant activity of pomegranate peel extracts (PPE), against many serious diseases; including cancer, inflammation, diabetes, cardiovascular diseases, etc. (Li et al., 2016; Stojanovi et al., 2017; Du et al., 2019).

Fusarium crown and root rot is a soil-borne disease, with the potential to limit productivity in glasshouse and field tomato crops. The causal agent is *Fusarium* oxysporum *f. sp. radicis-lycopersici*. Fungicides have limited potential benefit for most *Fusarium* diseases. (Sierotzki and Ulrich, 2003).

The aim of this study was the *in-vitro* evaluation of the antifungal activity of the pomegranate peel extracts PPE, against plant pathogenic fungi: *Fusarium oxysporum f.sp.radicis-lycopersici* by the evaluation of the mycelial growth, the inhibition rate of mycelial growth and the spore count.

MATERIALS AND METHODS

Plant material. The plant material choice was based on the pomegranate peel (*P.granatum Linn*), the pomegranate fruit was collected in the Sidi-Ali-Benyoub locality (Table 1) located at 25 km to the south of the Sidi-Bel-Abbés city, the peel has been authenticated, the reference specimens were sent to the plant taxonomy laboratory ,at the environment department of the natural and life sciences faculty, in Djillali Liabés university of Sidi-Bel-Abbés, for identification and confirmation, where the peel samples were deposited and coded (Kanoun et al.,2014 a; Kanoun et al., 2014 b; Kanoun et al., 2016).

Table 1. Bioclimatic aspects of the study area

Characters	Longitude	Latitude	Altitude	Climate
Sidi Ali Benyoub	00:76408°	35:26978°	1061 m	Semi-arid dry and cold

Plant powder preparation. The fruit was washed with tap water; the peel has been dried in the shade, away from moisture and light, at room temperature for one month on a wooden plate. After drying, they have been crushed in a traditional mortar ,then pulverised with a manual grinder, until a very fine powder was obtained, this latter was stored in the refrigerator at +4°C in a hermetically sealed container, it will be used later for the studied extract preparation (Kanoun et al., 2014 a; Kanoun et al., 2014 b; Kanoun et al., 2016).

Aqueous extracts preparation (hot). The peel powder weighing 400 g was added to 1000 ml of distilled water and boiled under reflux for 6 hours at a temperature of 200°C at 500 rpm. After 6 hours of agitation, the cooled decoctate was filtered on Whatman paper (N°3) under reduced pressure, the separated marc reated with a second extraction, by mixing 500 ml distilled water with 900 ml of moistened marc, under the same operating conditions as previously; the resulting supernatant was concentrated by a freeze dryer (lyophiliser) of Tel Star type at a temperature of -45°C and a pressure of 4.10^{-2} millibars (Kanoun et al.,2014 a). The different experimental groups in comparison with the control are distributed as follows : Group 1 : control ; Group 2 : PPE1% ; Group 3 : PPE 2% ; Group 4 : PPE 3% ; Group 5 : PPE 5%.

Mycelial growth evaluation. The plant extract was diluted in DMSO (dimethyl sulfoxide) to obtain the concentrations of (0%,1%,2%,3% and 5%). 1ml of each concentration was added to each Petri dish containing PDA medium (potato dextrose agar), a rate of 15 ml per dish was introduced (Jamil et al., 2002; Pande et al., 2011).

After the medium solidification, the inoculation was performed with explants of 5 mm diameter obtained from a 7-day culture (from a fungal suspension adjusted to 9×10^5 spores/ml, with a sterile cutter. These explants are positioned in the Petri dishes centre containing increasing doses of the extract (Kolai et al., 2012; Kanoun et al., 2014 a).

The evaluation of mycelial growth was determined by the Rapilly method (Rapilly, 1968), which consists of measuring the mycelium diameter during seven days, using the following formula: L= D-d/2 (L: mycelial growth, D: colony diameter, d: explant diameter). The mycelial growth means were calculated by the following formula: V (mm/day) = (Ln – Ln-1) / n (V: Mean mycelial growth, Ln, Ln-1,.. were the mycelial growths and n,n-1,n-2..etc: Number of days) (Benzohra et al., 2011; Kanoun et al., 2014 a).

Evaluation of the mycelial growth inhibition level. The results obtained from the mycelial growth estimation were expressed as a percentage (%) of the mycelial growth compared to the control, according to the formula described by Leroux and Credet (Leroux and Credet, 1978). T (%) = (L-I /L) x 100 (T: inhibition rate, L: mycelial growth of the control expressed in cm and I: mycelial growth of the treated fungi expressed in cm) (Kanoun et al.,2014 a).

Sporulation test. The evaluation of sporulation was carried out according to the principle of the method used by Maslouhy (Maslouhy, 1989). This test was performed by washing the entire Petri dish containing the fungus with 10 ml of sterile distilled water, to remove all the spores, then the obtained suspension was transferred in a flask filled with 50 ml sterile distilled water, the number of spores for each sample was counted by the Mallassez cell ,under the optical microscope.

Statistical analysis. The results are expressed as means and their standard error (X±ES). The comparison of means was performed using the t "Student" -test. The differences are considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Mycelial *growth*. According to our results illustrated in (Figure.1), the mycelial growth reaches a maximum of 37 mm in the control group and a minimum of 10 mm in the PPE 5% group at D7. Similarly, in the PPE 3% and PPE 5% groups, mycelial growth did not start until the second day, on the other side, the extracts become more active from a dose of 3% and the mycelial growth in the PPE 5% group was stopped at 10 mm at D4, and remained stable until the end of the experiment (D 7).The mycelial growth kinetics of the control group was clearly superior to that of the experimental groups with the different concentrations of PPE (1%, 2%, 3%, 5%).

According to (Figure.2) , it can be observed that, the mean diameter of mycelium in the control group was 20.21± 12.71 mm, which was higher than the experimental groups PPE 1%, PPE 2%, PPE 3%, PPE 5% with values of (12.42±7.72 mm; 11.07±7.65 mm; 8.85 ± 5.59 mm; 6.78± 4.26 mm) respectively. Our results show that extracts at different concentrations have an inhibitory effect on mycelial evolution.

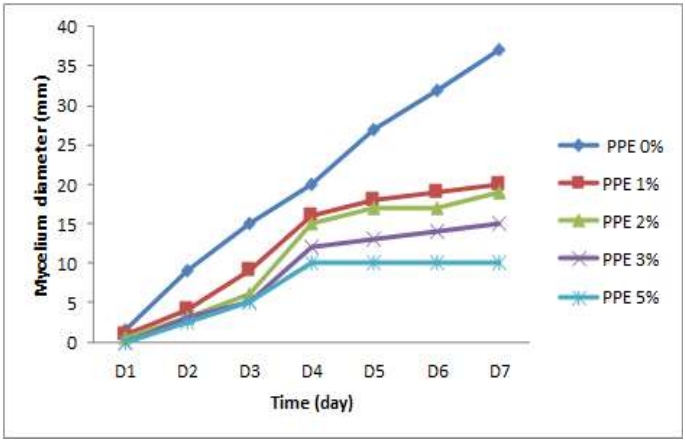

Figure 1. Kinetics of the mycelial diameter evolution according to the different concentration of PPE during 7 days

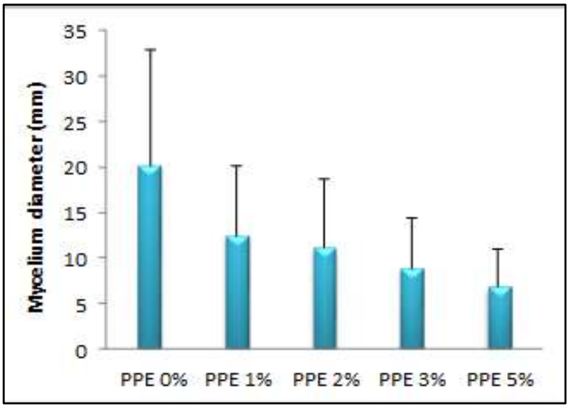

Figure 2. Means myceliums diameters in the experimental groups compared to the control

Inhibition rate of mycelial growth. Our results confirm that both groups (PPE 3%, PPE5%) presented a maximum inhibition rate at D1 which was 100%, while the PPE1% group showed a rate of 20% which was the lowest. It can also be seen that, the inhibition rate kinetics, for all experimental groups was decreasing from D2 to D4, then an increase from D5 to D7 (Figure .3).
Our results confirms the proportional relationship between the PPE dose and the inhibition rate (Figure.4), with a maximum of (70.52± 15.12%) in the PPE 5% group and a minimum of (39±10.98 %) in the PPE1% group.

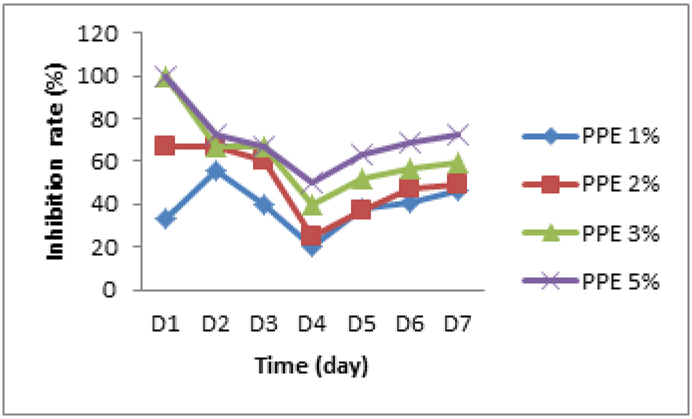

Figure 3. Kinetics of mycelial growth inhibition rate according to the different concentration of PPE during 7 days

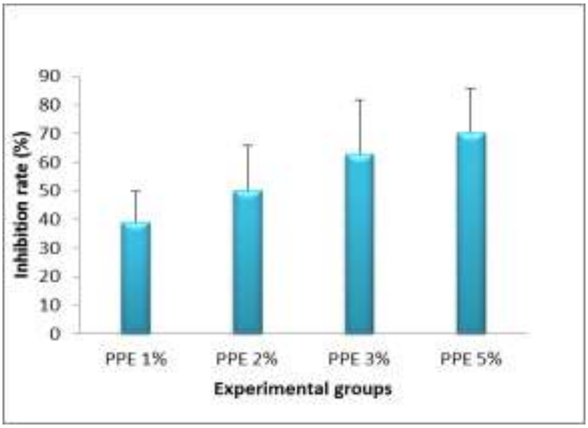

Figure 4.Means of mycelium inhibition rate in experimental groups exposed to PPE

Spore count evaluation. We also proceeded to the sporulation test of this fungus, according to our results we found a decrease in spore count compared to the control, for all extract concentrations tested (Table 2). This reduction corresponded to the mycelial growth decrease, due to their extract exposure, an inverse relationship was also found between the spores count and the extract concentration.

Table 2 . Spores count according to the different concentration of PPE

Groups	**Spores count (.10^5)**
Control	9
PPE1%	8.3
PPP2%	7.56
PPP3%	4.06
PPP5%	3.08

Many researchers highlight the PPE inhibitory activity against the mycelial growth germination of most plant fungal pathogens ,including *Botrytis cinerea*, *Penicillium digitatum*, *Penicillium expansum*, *Penicillium italicum*, *Alternaria alternata*, *Stemphylium botryosum*, *Colletotrichum acutatum sensu stricto*, *Fusarium oxysporum*, *Aspergillus parasiticus*, *Monilinia laxa* and *Monilinia fructigena* (Tayel et al., 2009; Oraki et al., 2011; Glazer et al., 2012; Rongai et al., 2015; Li Destri Nicosia et al., 2016; Rongai et al., 2017; Pangallo et al., 2017; Rongai et al., 2018).
Our results were coherent with those found by Al-Askar (2012), in which pomegranate peels significantly reduced the *Fusarium oxysporum* growth at different graduated concentrations. However, Glazer et al. (2012) confirmed the inhibitory effect of aqueous PPE on mycelial growth of *Alternaria alternata*, *Stemphylium botryosum* and *Fusarium spp*. However, the extract is ineffective

against *Penicillium expansum*, *Penicillium digitatum* and *Botrytis cinerea*. Similarly, Sudharsan et al. (2019) confirmed a similar results regarding the conidial germination and hyphal growth of the mycotoxigenic fungi *Aspergillus flavus* and *Fusarium proliferatum* .

The mechanisms by which the PPE bioactive components exert their activity have not been completely elucidated **(**Al-Zoreky, 2009; Oraki et al., 2011; Nuamsetti et al., 2012). The level of antifungal activity can considerably vary depending on extract type and pathogen species. For example, an ethanolic PPE can completely inhibit the germination of *Botrytis cinerea* conidia and *Colletotrichum acutatum*, while it was less effective against *Penicillium digitatum* and *Penicillium expansum*, with a reduction rate of 91.0% and 82.7%, respectively (Li Destri Nicosia et al., 2016; Pangallo et al., 2017).

Several studies correlated the PPE antifungal activity to their high concentration of polyphenols, particularly punicalagins and ellagic acids. Rongai et al. (2012) found that punicalagins are responsible for the inhibition of the *Fusarium oxysporum f. sp. lycopersici* mycelial growth, and highlighted that PPE are among the most effective plant extracts in preventing the *Fusarium oxysporum* germination.

According to some studies (Foss et al., 2014; Akhtar et al., 2015). The PPE polyphenolic compounds have the ability to combine with fungal cell membrane proteins, increasing cell permeability, which causes cell death . Furthermore, the PPE can decrease the pH gradient around the cell membrane and cause the cell death by increasing permeability (Rongai et al., 2018; Singh et al., 2019). Morphological changes in hyphae, including curling, twisting, and collapsing (microscopic observation) were detected in *Fusarium sambucinum* mycelium treated with methanol PPE (Elsherbiny et al., 2016). Cell empty cavities and disintegration of cytoplasmic organelles were also observed. In addition, an abnormal mycelial structure was detected in *Monilinia laxa and Monilinia fructigena* species following treatment with PPE (Elkhetabi et al., 2020).

CONCLUSION

This study showed the clear antagonistic effect of PPE at different concentrations towards *F. oxysporum f. sp. radicis- lycopersici*, indeed, *in-vitro* confrontation tests (on culture medium) between *F.oxysporum f.sp.radicis- lycopersici* and PPE, revealed an inhibition of the tested pathogen mycelial growth. This inhibition is dose-dependent.

Based on these results, it is of primary interest to use PPE as a biological control agent against *Fusarium* root and crown blight of tomato caused by *F.oxysporum f. sp.radicis- lycopersici*, especially since there are relatively few chemicals active against this pathogen. On the other hand, it is necessary to test PPE on different phytopathogenic agents, in order to identify the action spectrum to develop an alternative to the use of synthetic fungicides, with the objective of substituting them with a biological treatments based on plant extracts.

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