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Effect of organic pest control products on Arbuscular Mycorrhizal colonization in Bulgarian rose plantations: A two-year field study

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Abstract

This two-year field study aims to investigate the impact of organic pesticides used in organic Damask rose (*Rosa damascena* Mill.) fields on Arbuscular Mycorrhizal Fungal (AMF) colonization. Conducted in the renowned Rose Valley of Bulgaria, specifically in the village of Kliment, the experiment employed a randomized complete block design with two rows of 21 plants each in organic certified plots. The results revealed low AMF colonization in the first year, ranging between 14.78% and 20.89%, with no significant differences between treatments. In the second year, while no significant differences were observed between treatments (ranging from 48.00% to 76.49%), there was a notable increase in AMF colonization compared to the initial sampling. The study concluded that specific organic pesticides, including Neemazal, Limocide, Phytosev, and Nano sulfur, had minimal negative effects on AMF colonization. These findings contribute to understanding the implications of organic farming practices on AMF and soil health in the context of Damask rose cultivation.

Keywords: Arbuscular mycorrhiza, oil-bearing rose, organic production.

Introduction

Mycorrhizal association is a natural symbiotic relationship between endophytic fungi and several higher plant species. It poses a number of beneficial effects that ranges from improvement of basic growth parameters (Nadeem et al., 2014) and biotic and abiotic stress mitigation (Abdel-Salam et al., 2017; Begum et al., 2019). Under the paradigm of organic farming, such natural interactions are capitalized to help attain an economical sustainable and ecologically sound system which has the potential to outperform conventional production systems (Gamage et al., 2023).

Bulgaria is among the biggest exporter and producer of rose oil with the highest quality. Roses in particular, an economic and culturally important crop, is grown in about 5.269 ha of land and contributes most of the total contribution of the cut-flower industry (Ministry of Agriculture, 2022). Various products such as essences and cut-flowers are also important commodities produced by Bulgarian farmers (Tineva and Nencheva, 2021). Both the quantity and quality of these products are affected by basic growth parameters such as primal and lateral root and shoot growth, number of floral primordia and inflorescence induction time. Bulgarian rose production continues to fluctuate as a result of unstable climatic patterns, shorting cultivation cycles and degrading soil conditions. For instance, in 2022 there has been a recorded decrease in the total rose petal and oil production due to higher amount of precipitations. Arbuscular Mycorrhizal Fungi (AMF) can improve soil health and functioning leading to a more resilient system and affect the plant's growth

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parameters by enhancing root nutrient absorption capacity and serve as a natural protection against other pathogenic microorganisms (Begum et al., 2019).

Under the EU provisions and regulations, certain organically based biocontrol agents are allowed to be used. Their compounds may range from natural extracts (e.g. Neem Extract) to biosynthetic compounds and volatiles. The extent of influence of these compounds on the below-ground ecosystem is not yet fully explored and its influence on its functioning which has a significant influence of the quantity and quality of produce (Hage-Ahmed, 2019). This study evaluated the degree of mycorrhizal colonization in organically grown roses in village Kliment, Bulgaria supplemented by various pesticides permissible for organic production under EU regulations.

Material and Methods

Description of the location

The experiment was carried out with organic Damasc Rose (*Rosa damascena* Mill.) in the region of the village Kliment, Bulgaria (W: 42.59699739096776, L: 24.682717358466093) for two consecutive years, 2022 and 2023. The village of Kliment is part of the famous Rose Valley of Bulgaria, where is concentrated the oil-bearing rose production. The region is surrounded by mountains and well protected from north cold winds. The rivers Stryama and Tundzha provide good water supply for the rose plantations.

Site characterization

The soil cover in the Rose Valley is represented by deluvial noncalcareous sediments (Todorova et al., 2020). The colluvium is presented by rock fragments consisting of sand, silt, and clay, which are collected at the base of steep slopes. According to the World Reference Base for Soil Resources (WRB) the soils in the region are classified as *Fluvisols*. In these deluvial soils, the content of organic matter naturally decreases over time. The pH reaction of the soil is acid, which is characteristic for this soil type.

Soil analysis

Soil samples have been taken annually from the layer 0-30 cm, to determine the pH and content of mineral nitrogen, available phosphorus (P_2O_5) and exchanged potassium (K_2O) and analyzed in the accredited laboratory complex at the Agricultural University, Plovdiv. In the present investigation the pH values were determined by potentiometric method and the values ranged between 4.37 in 2022 to 5. 74 in 2023. Because of liming made by farmer owner of the plantation soil pH was increased in the second year. The humus content for the upper horizons by Turin varied between 3.32 to 3.87%. The mineral nitrogen content was determined by the Kjeldahl method as the values ranged between 14.47 to 15.68 mg kg^{-1} for the first and the second year respectively. Available potassium and available phosphorus content was evaluated using the Egner-Riem method. In terms of mobile phosphorus content, the values were in the range of 12.67 mg 100 g^{-1} in 2022 to 12.85 mg 100 g^{-1} in 2023. The content of available potassium ranged between 16.60 mg 100 g^{-1} and 17.27 mg 100 g^{-1}. The content of SOC was measured according to the Nikitin-modified Tyurin method using the spectrophotometric procedure at the wavelength of 590 nm (Slepetiene et al., 2023). For the study period the values of SOC varied between 14.9 mg g^{-1} and 15.7 mg g^{-1}.

Wheater conditions

The oil-bearing rose is a plant of the cool climate. The soft winter, the long and humid spring and the cool summer which are characteristic of the area, create the ideal conditions for growing the crop. The air in the closed valley fields contributes to the formation of abundant dew during rose-picking and protects rose oil from evaporation. Another important characteristic of the valley is the big temperature amplitude, which is necessary for the staking and the growth of the buttons, as well as for the storage of the formed essential oil. The climate is transitional continental and annual precipitation amount is 930 mm. The climatic conditions during the years of the experiment are presented in detail in Table 1. The evenly distributed rainfall and moderate average monthly temperatures create good conditions for the development of rose plants.

Table.1. Climate conditions

Year	Temperature (^{0}C)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2022	3.2	7.1	9.2	12.0	16.0	22.7	26.0	26.5	20.0	12.4	10.2	4.5
2023	1.5	6.4	9.6	14.0	17.3	24.2	28.0	28.3	21.7	15.0	13.4	4.6
long-term average	0.5	2.2	5.1	10.0	16.5	20.8	24.5	24.5	17.4	14.3	8.5	2.0
	Precipitation (mm)											
2022	55.1	35.6	58.0	62.3	68.8	62.0	41.7	45.3	85.4	92.0	78.7	105.5
2023	61.0	73.3	90.2	80.0	120.2	115.4	60.3	62.2	95.8	103.9	62.3	92.0
long-term average	70.0	80.4	72.8	72.0	110.4	50.5	40.0	50.5	100.2	80.0	115.0	90.0

Experimental setup

Since the experiment is established with a five-year-old rose plantation, the purpose of the study is to determine the presence of natural mycorrhiza. No mycorrhizal fungus inoculation was applied. Oil-bearing rose is a perennial plant and the right agrotechnical approach is of great importance for the optimal development and prolonged exploitation of the plantation. In the spring, with the beginning of the growing season, the inter-rows were plowed at a depth of 18-20 cm. The soil has been cultivated twice before the period of blooming at a depth of 5-6 cm. The nutritional regime of the roses was improved through the application of 2% solution of the leaf fertilizer Acramet Ultra® (N – 12.5%, P_2O_5 - 5.7 % K_2O - 11%; S-2.9%; B-0.35%; Cu- 0.025%; Mg-0.48%; Mn-0.028%; Zn-0.125%; Fe - 0.026%; Mo-0.024%; ultratrace elements – cobalt, chromium, vanadium). The product was applied twice in 15 days before flowering. Weed control was achieved mechanically. In autumn the area was fertilized annually with 20 t ha^{-1} organic manure, which is ploughed with the last tillage. Before the last cultivation in October 2022, the soil was limed (ground limestone in a dose of 3t ha^{-1}) to adjust the pH value. Due to lack of built irrigation systems, the area was not irrigated, as the producer relied on the drought resistance of the plant and the uniform distribution of rainfall in the Rose Valley.

Four organically certified insect pest control products, Phytosev®, Limocide® , Nano sulfur (Calcium polysulfide-CaS5 (20nm)), Neemazal® and one biological agent- *Chrysoperla carnea* (Stephens) were employed as treatments. Each plot in the field had two rows of plants (21 plant in total). The plot was 6 m in length and 3 m in width. The field was set up in a randomized complete block design in four replications. Rows were spaced 3 m apart, with 0.40 m inter row space. In order to determine the percentage of Arbuscular mycorrhiza fungal colonization, root samples were taken during the picking period on 26th of May during the first year (2022) and on 5th of June during the second year (2023). The products have been applied three times in the interval of 10 days in April and May depending on the weather but not later than 10 days before the harvest of the rose flowers at the respective doses shown in Table 2. The bioagent *Chrisoperla carnea* has been released 3 times during the springtime depending on the aphid's population.

Table 2. Description of the applied biological products

Product name	Manufacturer	Active substances	Applied amount
NeemAzal® T/C	Trifolio-M GmbH	azadirachtin A -1%; azadirachtin B, C, D, D-0.5 %; Nime-substance – 2.5 %	4 g ha^{-1}
Limocid ®	Vivagro	60 g/L orange oil	2000 ml ha^{-1}
Nano sulfur ®	Bio fertilizer Ltd Bulgaria	Calcium polysulfide – CaS5 (size 20 nm) – 230 g/l.	300 ml ha^{-1}
FytoSave®	Fytofend S.A	oligosaccharides COS-OGA 12.5 g l^{-1}	200 ml ha^{-1}

Root sample collection

For the assessment of the percentage of arbuscular mycorrhizal root colonization (Betancur-Agudelo et al., 2021), rose roots have been sampled from the field experiment after harvest in 2022 and 2023. Three 500 g soil samples (0–20 cm depth) pro variant were collected from randomly selected rose plants during the intensive vegetation period. Fine roots were rinsed free from soil and 2 cm down the hypocotyl, 2 cm root segments were used for the further staining procedure for determination of the mycorrhiza.

Root clearing and staining

A modified staining procedure (Phillips and Hayman, 1970) was performed on the fine rose root samples taken from Plovdiv, Bulgaria. 5-10 mL of 10% KOH was added to falcon tubes containing the root samples. The samples were then placed into a water bath at 70°C for 20 min. This was done to remove the natural pigments and facilitate easier visualization of AMF structures; excess KOH solution was then discarded afterwards, and the samples were rinsed with distilled water. 10 mL of 3.5% H_2O_2 was added to remove any remaining pigments. The samples were then placed in a water bath at 70 °C for 5 minutes. Afterwards, the samples were rinsed with 1:1 water : vinegar solution to acidify the roots for better staining results. 5-10 mL of 5% vinegar: ink solution was used to stain the roots. Samples were placed in a water bath at 70 °C for 20 mins.

Data gathering

Gridline intersection method (Newman, 1966; Giovanetti, 1980) was used to estimate the degree of AMF colonization, an improvised counting plate was made by drawing 10 mm x 10 mm grid on its surface using a scalpel. Root samples were then placed on the counting plate and the number of positive and total intersections was assessed. Degree of root colonization was calculated using the formula;

$$\text{Root Colonization Percentage} = \frac{\text{Number of AMF Positive Intersections}}{\text{Total Number of Intersections}} \text{x } 100\%$$

Data analysis

Statistical analysis was performed using Statistical Tool for Agricultural Research (STAR). Colonization percentage 2022 and 2023 were analyzed using one-way ANOVA, least significant differences were assessed using Tukey's HSD Test.

Results and Discussion

Site Characterization

Organic agriculture is the answer to the need for sustainable agriculture and biodiversity conservation. The main challenges which are facing Bulgarian rose growers are resistance to diseases, combined with appropriate genotype conservation and plant propagation in order to maintain the necessary traditional aroma and chemical composition of rose oil (Chalova et al., 2017). Due to the lack of resistance of R. damascena to the major diseases and pests and the prohibition on the use of chemical treatments the cultivation of healthy plants in organic farming is difficult. When developing the plant protection strategy the rose growers rely mainly on self experience. These study aims to observe if the tested products, which are effective against the main pests and deseas of roses have negative action on the mycorrhizal colonization. There is a lack of information about the presence of AMF in the rose plantations in the Rose Valley of Bulgaria. AMF are of great importance especially in organic farming systems because they are not only improving the soil nutritional properties, but also increasing the crop yields (Gosling et al., 2006).

AVM Colonization

During the initial phases of the project (2022), results reveal low AMF colonization ranging in between 14.78 ± 4.44 % to 20.89 ± 14.89 % showing no significant differences between treatments. No-significant differences between treatments was also observed (48.00 ± 11.00 % to 76.49 ± 8.21 %) during the second sampling (2023), but AMF colonization increased sigficantly as compared to initial sampling (Figure 1).

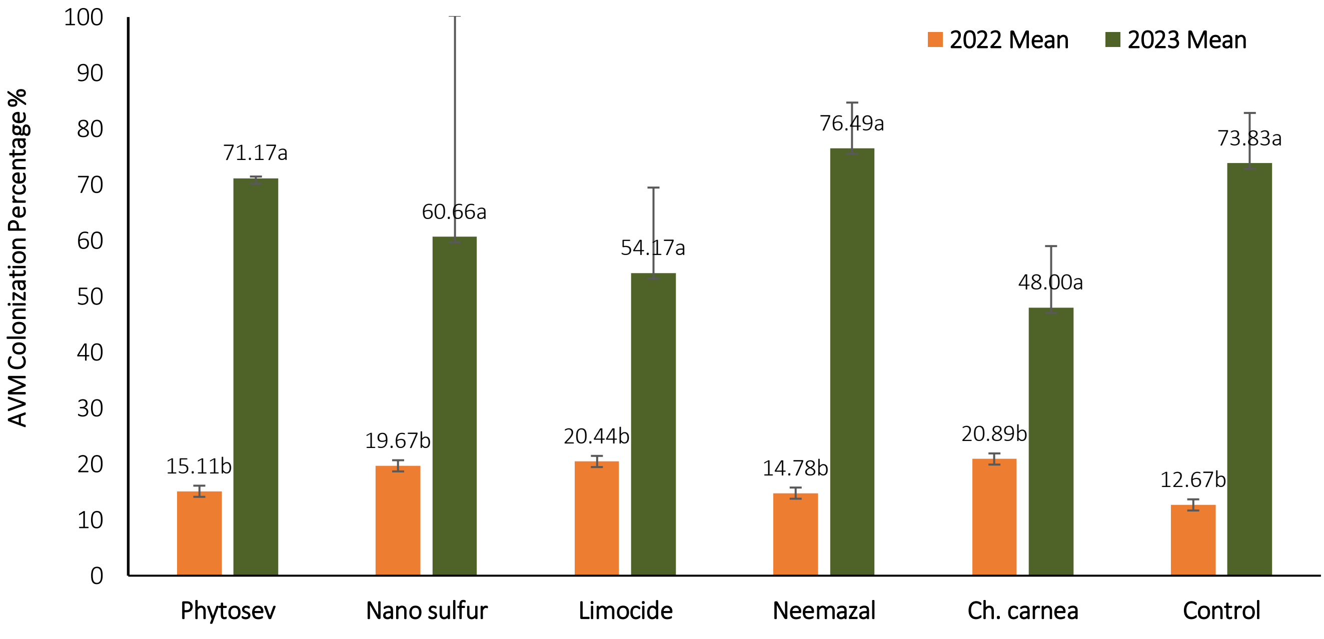

Figure 1. Rose Roots AMF Colonization Percentage

Pesticides can alter soil microbiological and chemical properties depending on several factors but the major of them are the amount of active ingredient which enters the soil system, the degradability of active substance and the mode of action of the active substance in relation to key physiological processes (Hage-Ahmed 2019; Tian et. al., 2019). Soil conditions especially hydraulic conductivity, organic matter content and texture significantly affect the amount of active ingredient in the soil (Yu et al., 2010; Mosquera-Vivas et al., 2023). There are relatively few studies related to the effect of Neemazal, Limocide, Phytosev and Nano sulfur on mycorrhizal species, but significant reduction in mycelial growth has been observed in some commonly fungal genera as these compounds affect key physiological processes such as chitin synthesis and ergosterol production (Tian et. al., 2019; Kilani-Morakchi et al., 2021; Jian et. al., 2023). Gopal et al (2006) stated, that azadirachtin applied in higher doses has negative effect on fungi and nitrifying bacteria. The field application of azadirachtin had no significant influence on mycorrhizal colonization but modified the structure of the AMF community (Ipsilantis et al., 2012). The same author observed the stimulating action of terpenes on the mycorrhizal colonization. When applied in combination with AMF inoculation azadirachtin could increase the root colonization (Bharadwaj and Sharma, 2006). Organic plant protection products are increasingly used as they respect the principles of sustainable agriculture. Few studies have investigated the action of those products on non-target organisms, such as the soil microbiome and the AMF. Some authors reported stimulatory effect of insecticides on arbuscular mycorrhizal fungal colonization, as well as on plant growth (Schweiger and Jacobsen, 1997; Schweiger et al., 2001).

The absence of significant differences in the mycorrhizal colonization of the treated plants with respect to the control plants and the significant increase in AMF colonization from 2022-2023 presents that with the application of compounds for pest control allowed for use in organic crop production, there is minimal effect on mycorrhizal colonization (Figure 1). This result can also be explained by the relationship between pesticide interference on mycorrhizal on both pre- and post-symbiotic phases. Since treatments were applied on an already established rose plantation, it can be said that a symbiotic community has already been established prior to the study. The more acidic soil reaction and the higher amount of precipitation in 2022 could also have negative effect on the mycorrhizal fungi colonization rate, but the influence must be further investigated.

Conclusion

The present study is the first which investigated the effect of biological products for plant protection on the AMF density in organic oil-bearing rose plantation. The results prove the presence of natural AMF on the roots of *Rosa damascena* Mill. The applied products didn't negatively affect the AMF density, but their action on non-target organisms should be further investigated. Probably the more acid soil reaction and the higher amount of precipitations during the first year of the experiment had left to lower mycorrhizal fungi colonization rate, but there is a need of further investigation and observation to make a concrete conclusions, because various factors could affect the soil rhizobium. Further experiments will investigate the impact of organic products on the colonization ability and the community structure of AMF on oil-bearing roses roots.

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