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Vermicompost liquid fertilisation affects the vegetative growth of African rose plum seedlings grafted on various rootstocks

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Abstract

This study was conducted at the Horticultural Research Institute, Agricultural Research Center in Giza, Egypt, over two successive seasons (2019 & 2020) using seedling African-rose plum grafted onto two different rootstocks: Nemaguard and Marianna 26-24. The primary aim of this project was to investigate the impact of vermicompost liquid fertilization on the vegetative growth patterns of African rose plums grafted onto distinct rootstocks. The seedling African rose plums were subjected to soil application with vermicompost diluted five times, foliar application with vermicompost diluted ten times, a combination of soil and foliar treatments with vermicompost, and a control group. The study's findings revealed that all vermicompost treatments significantly improved African rose plum's vegetative growth parameters and leaf mineral content compared to the control group. Noteworthy were the results from the combination treatment of soil and foliar applications, which exhibited the highest growth parameters and leaf mineral content levels. Furthermore, the study observed a substantial impact on endogenous hormone levels across all the treatments investigated. These results underscore the positive effects of employing vermicompost liquid fertilization on the morphological and physiological characteristics of the treated African rose plum seedlings. The outcomes of this study hold promise for the application of vermicompost-based practices in the development of organic farms, gradually reducing dependency on chemical fertilization while concurrently fostering environmental sustainability.

Key words: Vermicompost, foliar and soil treatments, African-rose, rootstocks, plum

Introduction

Plum (*Prunus domestica*) belongs to the group of deciduous fruits commonly known as stone or drupe fruit (Gregory, 1993). The cultivated area in Egypt reached 26666 feddan with productivity of 14775 tons (Food and Agriculture Organization of the United Nations, 2018). Rootstocks play a major role in modern orchards. Combining the desirable features of two different plants by grafting can produce contrasting growth effects. This difference can be established by comparing rootstocks' relative significance for precocity, growth, canopy management techniques, and tree size control (Khalil and Kassem, 2020).

Nemaguard (peach rootstock) is resistant to root-knot nematodes but has high chilling requirements (825 hr at < 7°C) (Mahmoud 2009), Mariana 26-24 plum rootstock (*Prunus cerasifera* Ehrh. x *Prunus munsoniana* White &Hedr.) is slightly dwarfing, moderately resistant to phytophthora crown gall, root rot, and oak root fungus, tolerates wet soils, root-knot nematode resistant. It is widely adapted to different soil types and moisture conditions, good for most cultivars (Southwick *et al.*, 1999).

Vermiwash is a liquid collected after the passage of water through a column of worm action. It is a mixture of excretory products, mucus secretion of earthworms, and micronutrients from the soil organic molecules. It contains nitrogen as a nitrogenous excretory product and some minerals such as P, K, Mg, Fe and growth-promoting hormones and essential enzymes infusing resistance in plants. It is applied as a foliar spray and transported to leaves, shoots, and other plant parts in the natural ecosystem. It contains various enzymes protease, amylase, unease and phosphatase. These are beneficial for the growth and development of plants. The microbial study of vermiwash has shown that it contains nitrogen-fixing bacteria, like *Azotobacter, Agrobacterium* (Kaur *et al.*, 2015).

In addition, vermicompost (VC) is attributed to plant growth-regulating substances (PGRS) acting directly on the plants' physiology, providing more significant growth and development. Earthworm intestine contains a wide range of microorganisms, enzymes, and hormones that are transferred to VC during the organic waste degradation. These microorganisms produce significant amounts of PGRS, such as auxins, gibberellins, cytokinins, abscisic acid, and ethylene. These five classic hormones modulate various stages of vegetative growth development (Kist *et al.*, 2019).

Recently, the agriculture sector aimed to produce organic foods according to agricultural sustainability strategy to avoid the harmful mineral fertilization side effect. Using the natural products of earthworm "VC" that feed on organic waste is considered more useful for ecology maintenance and can be used in organic fertilization. This study compares two of the most important peach rootstocks that are used extensively under the conditions of the Egyptian environment and the extent of their impact on the morphological and physiological characteristics of the grafted variety and how to promote those young trees by using one of the forms of organic fertilization without resorting to the use of chemical fertilizers. Thus, the main purpose of this

investigation was to see the effect of VC liquid fertilization on the vegetative growth behavior of African rose plum budded on two different rootstocks and to explore establishing farms by replacing chemical fertilization with organic means to preserve the environment and maintain consumer health.

Material and methods

The investigation was carried out during two successive seasons (2019 & 2020) at the Horticulture Research Institute farm, Agricultural Research Center, Giza, Egypt. This study aimed to enhance the growth of African rose on Nemaguard and Mariana rootstocks seedlings as affected by different treatments of VC fertilization.

African rose grafted on Nemaguard and Mariana 26-24 rootstocks received four doses of VC treatments, which was diluted by tap water as follows; soil treatment by VC diluted five times (200 cm^2/L water), foliar treatment by VC diluted ten times (100cm^2/L water), combination of above two treatmenta and control. The analysis of VC is presented in Table 1.

Table 1. Chemical characteristics of liquid VC

Parameter	Content
pH	7.57
EC (ds/m)	2.65
Total N (%)	1.65
CoD (mg/L)	6.73
BoD (mg/L)	12.65
Total P (%)	1.42
Total K (%)	1.01

VC treatments: VC soil application was used as direct soil applications through a flood irrigation system for each seedling. Grafted plants grown in plastic bags were irrigated one day before adding soil application of VC, starting from the first of March to the October end. The application was applied by adding 100 cm^2 from diluted fertilizer solution every 15 days until the end of the growing season (October end).

The VC foliar application was carried out 24 hours after conducting the soil application of VC by adding 100 cm^2 of dilute VC liquid to each seedling every 15 days using a spray pump (capacity of 5 L) starting from the emergence of leaves during March until the end of the growing season at the end of October.

Vegetative growth parameters: Stem diameter (cm/year) was measured at the end of each season as the increment in stem diameter (above 3 cm from the grafting area) (Soliman, 2020). Then, it was calculated as a difference between the stem diameter at the end of the season and the initial stem diameter at the beginning.

Leaf area (cm^2): Six mature leaves were taken at the third node from the base of the main stem of the seedlings for estimating leaf area (model 1203, CID, Inc., USA). The leaves number per shoot was counted at the end of each study season.

Chlorophyll analysis: The leaf chlorophyll content was estimated using SPAD 502 meter (Minolta Co., Osaka, Japan). Thirty leaves per seedling were randomly taken starting from the seventh leaf from the base of bearing shoots around the crown. Measurements were carried out in the beginning of May during both study seasons. The C/N ratio was estimated according to the following equation.

C/N ratio = Total carbohydrates/Total Nitrogen

Endogenous hormones levels

Endogenous hormones: IAA, GA_3 and ABA and total free amino acids % were determined in the second season using 5.0g fresh weight of leaves at Mid- July. Samples were frozen in a cold aqueous methanol 80% (v/v). Then it was adjusted to 20 mL/g and stored at 2°C for 48 hours. Hormones were extracted according to Wasfy and Orrin (1975). Determining plant hormones and total free amino acids % as a Lysine was carried out using High-Performance Liquid Chromatography (HPLC). Finally, hormones were determined according to AOAC (2005).

Statistical analysis: The experimental design was arranged in a Randomized Complete Block Design. The grafted African rose on different rootstocks (Nemaguard or Mariana) was factor (A) and the treatment of VC was factor (B). Each treatment in this study contained three replicates and each replicate had five plants. Statistical analysis was carried out according to Snedecor and Cochran (1989) using analysis of variance. The significance is determined using LSD values at 0.05 levels (Gomez and Gomez, 1984).

Results and discussion

Vegetative growth parameters

Stem diameter (cm/year): Data in Table 2 show that the growth of African rose (stem diameter) was significantly affected by rootstock, VC, and their interactions**.** Stem diameter increased

Table 2. Effect of vermicompost treatments on stem diameter (cm/year) and number of leaves /shoot of African- Rose grafted on Nemaguard and Mariana rootstocks 2018-2020

Vermicompost (VC) treatment	Stem diameter (cm/year)						Number of leaves /shoot					
	Rootstock first season			Rootstock second season			Rootstock first season			Rootstock second season		
	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean
Soil addition 1VC/5 Water	0.90	0.46	0.68	1.00	0.55	0.78	25.00	25.00	25.00	27.00	27.00	27.00
Foliar spraying 1VC/10Water	0.93	0.53	0.73	1.10	0.57	0.84	27.00	27.90	27.45	35.00	33.00	34.00
Soil addition + Foliar spraying	1.00	0.53	0.76	1.60	0.64	1.12	38.33	38.33	38.33	45.00	41.00	43.00
Control	0.80	0.30	0.55	0.81	0.30	0.56	21.66	20.00	20.83	22.00	23.00	22.50
Mean	0.90	0.45	--	1.12	0.51	--	28.00	27.81	--	32.25	31.00	--
LSD (5%)	First season: Rootstocks (A) = 0.03, VC (B) = 0.05, A x B =0.07						First season: Rootstocks (A) = ns, VC (B) = 1.29, A x B =1.82					
	Second season: Rootstocks (A) =0.04, VC (B) = 0.06, A x B =0.08						Second season: Rootstocks (A) =0.98, VC (B) = 1.39, A x B =1.97					

dramatically in the African rose on Nemaguard compared to that grafted on Mariana during both seasons. The double treatment of VC (soil addition and foliar spraying) showed the best stem diameter under study in both seasons.

The interaction between rootstocks and VC treatments cleared that African rose on Nemaguard with the double treatment of VC (soil addition and foliar spraying) showed the best results than other interactions under this study.

Ram *et al.* (2007) reported that stem diameter significantly increased when guava plants were fertilized by VC at 5 kg/tree. Also, Ansari and Sukhraj (2010) stated that combining organic fertilizers VC and vermiwash improved plant growth parameters. Sharma *et al.* (2017) found that when using VC at 25 kg/tree, biofertilizer at 40 g/tree, FYM at 30 kg/tree, and compost at 15 kg/tree, VW at a 1:10 dilution and cow urine at 1:10 dilution significantly improved plant growth of nectarine plants.

Leaf number /shoot: The data presented in Table 2 indicates that the number of leaves per shoot was strongly influenced by the rootstock, VC, and their interactions, with the exception of the rootstock effect observed during the initial season. In the subsequent season, there was a notable difference in leaf production between plants grafted on Nemaguard and those grafted on Mariana, with the former exhibiting a greater abundance of leaves. The application of VC through both soil addition and foliar spraying exhibited the highest leaf count compared to other treatments in both seasons. The present study revealed that the combination of African rose on Nemaguard rootstock with VC, applied through both soil addition and foliar spraying, resulted in the largest number of leaves compared to other interactions examined.

The present results are in line with Solanki *et al.* (2019), who reported that a maximum increase in tree canopy volume of 52.72% was supplemented by the combination of VC at 15 kg/tree treatment with 75% of the recommended dose of fertilizers. Ansari *et al.* (2019) reported that the highest number of leaves was found in plants grown in 50 g of VC, which was added to each bag treated with 25 earthworms introduced into the containers' soil with 200 g cattle dung + 150 g lime leaves. Also, Tamang and Manivannan (2020) study the organic fertilizer VC on growth in Sikkim Himalaya. They found that the highest number of leaves in Bhaise using VC+ VAM+ Cap1+Trichoderma treatment.

Leaf area (cm^2): Results in Table 3 show that leaf area was significantly affected by rootstocks, VC,and their interactions. In both seasons, the leaf area increased dramatically in African rose grafted on Nemaguard compared to Mariana.

The combination treatment of VC (soil addition of VC, 1 L/5 L water and foliar spraying of VC 1L/10L water) showed the best leaf area than other treatments during both seasons. The interaction between rootstock and VC showed that African rose on Nemaguard with VC (soil addition and foliar spraying) showed the best leaf area than other interactions under this study.

Solanki *et al.* (2019) found that the largest leaf area, 32.17 cm^2 was recorded in trees supplemented by the combination of VC at 15 kg/tree treatment with 75% of the recommended dose of fertilizers. Also, Nidhika and Thakur (2017) reported that the leaf area was 13.12 cm^2 when inorganic fertilizers in combination with VC, was used in plum cv. Santa Rosa.

The application of VC to improve growth characteristics very well discussed earlier (Arancon *et al.*, 2004; Najar and Khan, 2013; Sharma *et al.* (2017). Release of nutrients is considered with the physiological stage, which resulted in enhanced nutrient uptake which was reflected in all growth parameters (Musmade *et al.*, 2010). Canopy volume increased with the combined application of VC and VW (Rekha *et al.*, 2013).

Chlorophyll content (SPAD): The main effect of experimental treatments showed that the leaf content total chlorophyll was significantly affected by rootstock, VC, and their interactions (Table 3). Leaf total chlorophyll content was highest in African rose grafted on Nemaguard compared to graft on Mariana in both seasons. Moreover, the double treatment of VC (soil addition and foliar spraying) showed better results than other treatments during both seasons. Also, the interaction between rootstock and VC cleared that African rose on Nemaguard with VC (soil addition and foliar spraying) showed better results than other interactions under this study.

These results are in harmony with Karmegam and Daniel (2008) that total chlorophyll content of leaves of *L. purpureus* was significantly higher in VC applied plots and VC + chemical fertilizer applied plots ($P<0.05$) than in the control.

C/N ratio: The African rose C/N ratio as affected by different rootstocks, VC, and the interactions between them are presented in Table 4. Data indicated that the African rose on Nemaguard

Table 3. Effect of vermicompost treatments on leaf area (cm^2) and total chlorophyll content of African-Rose grafted on Nemaguard and Mariana rootstocks 2019-2020

Vermicompost (VC) treatment	Leaf area (cm^2)						Total chlorophyll content					
	Rootstock first season			Rootstock second season			Rootstock first season			Rootstock second season		
	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean
Soil addition 1VC/5 Water	18.16	17.83	17.99	22.23	19.83	21.03	35.30	33.40	34.35	37.11	36.71	36.91
Foliar spraying 1VC/10Water	23.50	18.33	20.91	28.70	20.30	24.50	40.00	37.15	38.57	40.48	38.10	39.29
Soil addition + Foliar spraying	24.00	20.50	22.25	30.30	25.17	27.74	40.80	38.97	39.88	44.30	39.20	41.75
Control	14.66	11.30	12.98	12.10	11.30	11.70	26.30	23.40	24.85	28.10	25.30	26.70
Mean	20.08	16.99	-	23.22	19.15		35.60	33.23	--	37.50	34.83	--
LSD (5%)	First season: Rootstocks (A) =1.05, VC (B) = 1.48, A x B =2.10 Second season: Rootstocks (A) =1.31, VC (B) = 1.85, A x B =2.62						First season: Rootstocks (A) =0.71, VC (B) = 1.00, A x B =1.42 Second season: Rootstocks (A) =0.81, VC (B) = 1.14, A x B =1.61					

produced a significant effect on C/N ratio compared to that on Mariana in both seasons. Moreover, the double treatment of VC (soil addition and foliar spraying) showed the best C/N ratio than other treatments during both seasons.

Also, the interactions between rootstock and VC cleared that African rose on Nemaguard with VC (soil addition and foliar spraying) showed better results than other interactions under this study. These results are in parallel with Fayed (2005) found that chicken manure treatments or compost with bio fertilization showed the highest results on C/N ratio. Moreover, Abdou (2010) revealed that the C/N ratio significantly increased by adding bio-fertilizer, humic acid, and compost tea to organic manure in pear.

Table 4. Effect of vermicompost treatments on C/N ratio of African-Rose grafted on Nemaguard and Mariana rootstocks 2019-2020

Vermicompost (VC) treatment	Rootstock first season			Rootstock second season		
	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean
Soil addition 1VC/5 Water	18.86	17.47	18.16	19.36	16.19	17.77
Foliar spraying 1VC/10Water	20.15	17.60	18.87	21.21	18.35	19.78
Soil addition + Foliar spraying	20.24	18.22	19.23	21.23	19.80	20.51
Control	17.25	17.41	17.33	17.52	17.50	17.51
Mean	19.12	17.31	--	19.83	17.96	--
LSD 5%	First season: Rootstocks (A) = 0.34, VC (B) = 0.49, A x B =0.69 Second season: Rootstocks (A) =0.47, VC (B) = 0.67, A x B =0.94					

Endogenous hormones: African rose endogenous hormones were significantly affected by rootstock, VC, and the interactions between them (Table 5). GA_3 and total amino acid (%) increased dramatically in African rose on Nemaguard compared to Mariana's. Also, the double treatment of VC (soil addition and foliar spraying) promoted the accumulation of GA_3 and total amino acid (%) more than other treatments under study in the second season. The interaction between rootstock and VC cleared that African rose on Nemaguard with VC (soil addition and foliar spraying) showed the best leaf content of GA3 and amino acid than other interactions under this study.

Concerning IAA and ABA % (Table 6), African rose on Mariana rootstock had the highest IAA and ABA percentage compared to that grafted on Nemaguard. Regarding VC treatments, the control treatment appeared to have the highest values of IAA and ABA compared to the VC treatments. The interaction of African rose growing on Mariana under control treatment showed the highest IAA and ABA % compared to African rose growing on Nemaguard comparing other treatments of VC.

The obtained results of endogenous hormones of GA_3 and total amino acid are in line with Broz *et al.* (2016) who reported that VCs can enhance plant growth by the production of plant growth-regulators hormone. Kaur *et al.* (2015) found that Vermiwash contains nitrogen as nitrogenous excretory product and growth-promoting hormones. VC could produce plant growth regulators such as gibberellins (Atiyeh *et al.*, 2002; Manivannan, 2004; and Ramamoorthy, 2004).

Furthermore, the application of VC significantly affected the

Table 5. Effect of vermicompost treatments on endogenous hormones GA_3 percentage and total amino acid percentage of African rose grafted on Nemaguard and Mariana rootstocks

Vermicompost (VC) treatment	GA_3 (%)			Total amino acid (%)		
	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean
Soil addition 1VC/5 Water	0.090	0.070	0.080	0.18	0.12	0.15
Foliar spraying 1VC/10Water	0.430	0.320	0.370	0.22	0.16	0.19
Soil addition + Foliar spraying	0.870	0.520	0.700	0.30	0.19	0.25
Control	0.010	0.005	0.007	0.10	0.06	0.80
Mean	0.350	0.220	--	0.20	0.13	--
LSD 5% level for	GA3: Rootstocks (A) = 0.039, VC (B) = 0.055, A x B =0.078 Total amino acid (%): Rootstocks (A) =0.01, VC (B) = 0.02, A x B =0.03					

Table 6. Effect of vermicompost treatments on endogenous hormones IAA percentage and ABA percentage of African rose grafted on Nemaguard and Mariana rootstocks

Vermicompost (VC) treatment	IAA (%)			ABA (%)		
	African rose on Nemaguard	African rose on Mariana	Mean	African rose on Nemaguard	African rose on Mariana	Mean
Soil addition 1VC/5 Water	0.530	0.710	0.620	1.69	1.87	1.78
Foliar spraying 1VC/10Water	0.090	0.110	0.100	1.07	1.09	1.08
Soil addition + Foliar spraying	0.002	0.002	0.002	1.00	1.00	1.00
Control	0.850	0.980	0.915	2.53	3.80	3.16
Mean	0.368	0.450	--	1.57	1.94	--
LSD (5%)	IAA: Rootstocks (A) = 0.043, VC (B) = 0.061, A x B =0.087 ABA (%): Rootstocks (A) =0.11, VC (B) = 0.16, A x B =0.23					

growth promotion due to the high level of nutrients besides some growth-stimulating substances excreted by earthworms in their casts. It has been demonstrated that soil microflora like fungi, bacteria, and actinomycetes could produce plant growth-promoting substances such as gibberellins during the VC process (Arancon *et al.*, 2004). Better growth of plants was attributed to the slow release of nutrients for absorption with additional nutrients like giberellin by applying organic inputs like VC in combination with vermiwash (Ansari, 2008).

From the results mentioned above and discussions, it could be concluded that the growth behavior of African rose grafted on Nemaguard rootstock was better than grafted ones on Mariana rootstock. Also, African rose plants appeared to have good promotion of all growth characters, C/N ratio, Chlorophyll and GA_3, and amino acid leaf content as affected by VC fertilization. We recommend using the combination treatment of VC by soil addition (1L VC/5L water) plus foliar sparing by vermicompost (1L VC/10L water) to obtain the best growth for the African rose in the orchard. The obtained results are useful to promote and enhance the growth parameters in fruit trees and avoiding mineral fertilization.

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