Impact of Nano-Fertilizers on Growth Performance of Fig crop and Soil Health

Abstract

The current study aimed to study the effect of foliar application of nano-NPK fertilizer to one-year-old Fig trees (cv. Black Mission) in terms of tree growth performance as well as on soil microbial activity. The investigated concentrations of nano-NPK were 100, 200, 300, 400 and 500 ppm in addition to the control treatment applied for two seasons. The obtained results for both seasons indicated that all concentrations of nano-fertilizers significantly improved parameters of vegetative growth and soil health compared to the control group. However, the highest values for leaf wet and dry weight, leaf area, and leaf water content were recorded as a result of foliar fertilization at a concentration of 300 ppm. While 400 and 500 ppm nano-NPK gave the highest values for the contents of N, P, K, Ca, Mg and chlorophyll in the leaves. On the other hand, the highest bacterial activity was found in Fig's rhizosphere subjected to foliar application with nano-NPK at concentrations of 300, 400 and 500 ppm. In addition, the highest POX and PPO isozyme activities were scored in 200 and 300 ppm. In general, foliar fertilization with nano-NPK can be recommended as it enhances vegetative growth, leaf chlorophyll and mineral contents without negative impact on soil microbial activity.

Keywords: Black Mission Fig, Nanofertilizing, Vegetative growth, Enzyme activities, Soil Micribial Activity.

Introduction

In Egypt, it has become necessary to expand the cultivated areas of fruit crops to meet the increasing food needs in line with the population increment. Due to global climatic changes water resources of the world have been limited and found scarcity of fresh water that needed to enlarge cultivated area especially in arid and semi-arid regions such as in Egypt. Thus, increasing the cultivation area of low water demanding and fertilizer consuming fruit crops like figs, olives and date palms should be adopted as new agricultural policy in Egypt. The fig fruits are highly nutritive because of their high content of sugars, vitamins, and minerals. It is a deciduous tree that grows under a wide range of soil and climate and tolerates different environmental conditions. However, most cultivated area with figs in Egypt do not follow any irrigation or fertilizing program that fulfill water and nutritive requirements for best tree growth and high yield with enhanced fruit quality. According to El-Shazly *et al.* (2014), low growth and production of fig trees is apparently due to unbalanced or insufficient fertilization and insufficient irrigation.

Mineral fertilization is the common fertilzer type used in traditional agriculture to improve the yield and quality of fruit crops. However, mineral fertilzers often lead to adverse environmental effects, upset the ecological balance and make plants more susceptible to diseases and pests. Nowadays, the clean agriculture is getting more attention for reducing environmental problems as well as improving structure and increasing soil fertility by using different organic and biofertilizers. The use of nano-fertilizers is the most important application of nanotechnology in agriculture to improve plant growth, yield and fruit quality parameters. In the recent years, the use of nano-fertilizers is becoming increasingly important in modern agricultural applications as alternative to traditional fertilizers. It is considered as a new approach to increase agricultural production with high quality, environmental safety, biological support, and financial stability (El-Saadony *et al.*, 2021). Nano-fertilizers are aimed to make nutrients more available for plant by increasing nutrient use efficiency and to reduce the quantity of added fertilizer which consequently would reduce cultivation costs and eliminate environmental pollution especially soil and ground-water resources. Nevertheless, the use of nano-fertilizers on fruit trees is reported to contributes very effectively in increasing tree productivity and improving fruit quality by enhancing nutrients management and maintaining soil properties. Nano-fertilizers are applied in very low doses with high absorption rate compared to other fertilizers without negative effects on plant growth and nutritional status, as well as on the environmental (Mustafa *et al*., 2018; Qureshi *et al.*, 2018; Al-Hchami and Alrawi, 2020).

Recently, several researchers investigated the impact of using different nano-fertilizers on fruit trees growth and productivity (Sabir *et al*., 2014; Davarpanah *et al.*, 2016; Roshdy and Refaai, 2016; Abd El-Razek *et al*., 2017; Mohamed *et al*., 2017 and Wassel *et al*., 2017). They studied the response of different fruit crops to either the soil or foliar application of nano-fertilizers indicating the positive influence of nano-fertilzers in increasing tree yield, improving fruit quality, and ensuring crop sustainability. Also, they found a good positive correlation between the applied rates of the nano-fertilizers and the tree vegetative growth and its productivity.

Black Mission fig is one of the promising common fig varieties in Egypt especially in the reclaimed area and in accordance with the above discussed, the present work aims to study the effect of applying the nano-fertilizer treatments on growth performance of one year old Black Mission plants as well as the fertilizer influence on the soil microbial activity.

Material and Methods

Plant material and experimental design:

This work was carried out in the experimental research greenhouse of National Research Centre (NRC), Dokki, Giza, Egypt during 2020 and 2021 on healthy one years old fig plants cv. Black Mission grown in pots size No.30 in mixed soil (2 sand : 1 peat moss). The nano-fertilizer (conventional N.P.K-fertilizer in Nano form) produced by NRC was used. About 90 plants uniform as possible were subjected to six nano-fertilizer treatments as follows:

T1: plants not receiving any nano-fertilizer (Control = 500 ppm conventional fertilizer).

T2: plants received 100 ppm nano-fertilizer.

T3: plants received 200 ppm nano-fertilizer.

T4: plants received 300 ppm nano-fertilizer.

T5: plants received 400 ppm nano-fertilizer.

T6: plants received 500 ppm nano-fertilizer.

All plants received its fertilizer dose twice weekly starting from March until September each season. The experiment was carried out as randomized complete block design RCBD with six treatments and three replicates per treatment and five plant per replicate, i.e. (6 treatments x 3 replicates x 5 plants= 90).

Nano-fertilizer production:

The using nano-fertilizer prepared by addition of conventional compact fertilizer (20:20:20 N.P.K) in water and stirring till complete dissolution, then put the citric acid and stir until it is completely dissolved, after that add sodium carbonate with vigorous stirring until it becomes an ash-like formation solution, and in the end the pH is adjusted to 5. Morphology (size and shape) of the obtained nano-fertilizer particles was characterized according to the method described by Mustafa *et al*. (2018). The average particles diameter was (15 to 54 nm) represents about (70 %) of nano-fertilizer structure which is in agreement with the nano-scale safety standards.

Measured parameters:

For measuring the effect of the different treatments on the vegetative growth, average leaf area (cm^2) was estimated, leaf fresh and dry weight (g) were measured. In the meantime, leaf water content was calculated as the difference between fresh and dry weight. Also, in a fresh sample of leaves, leaf chlorophyll content was recorded using Minolta chlorophyll meter (SPAD – 501). In addition, leaf mineral content was determined at the end of the experiment in both seasons. Leaf samples were dried in a ventilated oven at 70°C to constant weight and then were grinded in stainless steel mill with 0.5 mm sieve and kept in paper bags for chemical analysis. One gram of each sample was dried to ash in a muffle furnace at 450 °C for 6 hours and nutrients were extracted using digestion method according to Chapman and Pratt (1978). Nitrogen was determined by using the Kjeldahl method, phosphorus was photometrical determined according to the method described by Jackson, (1973). Potassium was measured using the Flame photometer, (Eppendorof, DR Lang). Calcium and magnesium were determined by Perkin Elmer Atomic Absorption Spectrophotometer. All nutrients concentration were expressed as percent. Moreover, the activity of two antioxidant enzymes; peroxidase (POX) and polyphenol oxidase (PPO) in the leaves was evaluated in 2021 season. Both POX and PPO were extracted according to the method described by Stagemann *et al*. (1985) and PPO and POX isozymes were separated by Native-polyacrylamide gel electrophoresis (Native-PAGE). The activities of POX and PPO were determined according to Baaziz *et al*. (1994). In the meantime, soil microbial activity was investigated to evaluate the health status of the growth media. Soil samples were analyzed using the standard procedures in the laboratory at Microbial Genetics, National Research Centre (NRC).

The total microbial enzyme activities of soils were estimated based on the rate of fluorescein diacetate (FDA) hydrolytic activity according to the method described by **Patle et al., (2018)** with some modifications. In brief: Two grams of rhizosphere soil samples were placed (in triplicates) into 50-mL capped centrifuge tubes. A volume of 15 mL potassium phosphate buffer (60 mM, pH 7.6) and 0.2 mL of 0.1% FDA (in acetone) were added to initiate the reaction. Tubes were incubated horizontally at 30°C for 20 min in a rotary shaker. After incubation and color development, the reaction stopped by adding 15 mL of chloroform/methanol (2:1) and vortexing for one min. Tubes were subjected to centrifuge (5000 rpm for 10 min) to spindown soil and turbidity and separate chloroform layer. The developed colored fluorescein in the chloroform layer was spectrophotometrically measured at 490 nm against fluorescein standers. Total soil microbial activity was expressed as FDA hydrolysis values (µg of released fluorescein g^{-1} soil).

Statistical analysis:

Data were analyzed as one way analysis of variance (ANOVA) and means were represented as combined analysis of both seasons. Data were statistically analyzed using the SAS (Statistical Analysis System) version 9.1 according to Gomez and Gomez (1984). The least significant difference (L.S.D) at 0.05 was used to compare among the means of the different treatments according to (Snedecor and Cochran, 1989).

Results and Discussion:

Vegetative growth

Combined analysis data in Table (1) showed that all nano-fertilizer treatments significantly increased leaf fresh and dry weight compared with the control. The nano-fertilizer at 300 ppm (T_4) gave the highest value of leaf fresh weight (22.88 g) and dry weight (8.28 g) compared with all other treatments followed by T_6 (500 ppm). However, the lowest values of leaf fresh & dry weights noticed with T_5 treatment (400 ppm) as compared with both nano-treatments (100 and 200 ppm). The highest leaf area was recorded with $T_{3,}$ T_4 and T_6 without significantly differences among each other, while, the control treatment recorded significantly the lowest value. These results were in harmony with those obtained by Haggag *et al*. (2018 a) working on Aggizi olive seedlings, Mustafa *et al*. (2018) on Sultani fig cultivar and Abdelaziz *et al*. (2019) on Keitte mango trees. They found that fertilization with nano-fertilizer improved vegetative growth of fruit crops comparing with control (conventional fertilizer).

Leaf water and chlorophyll content

Data presented in Table (2) cleared that all nano-fertilizer treatments increased leaf water and chlorophyll contents in comparison with the control. Nano-fertilizer at (300 ppm) resulted in significantly higher leaf water content than those of all other treatments except T_2. Moreover, the T_2 treatment indicated significantly higher leaf water content than T_5 and the lowest water content of leaves was recorded in the control. Also, the same trend was noticed for leaf chlorophyll content as control treatment recorded the lowest chlorophyll value comparing with all nano-fertilizer treatments. Both levels (400 and 500 ppm) of nano-fertilizer produced significantly the highest leaf chlorophyll content in comparison with 100, 200 and 300 ppm treatments. However, applying 300 ppm (T_4) resulted in significantly lower chlorophyll content than 200 ppm (T3). These results agreed with those obtained by Sabir *et al*. (2014), Zagzog *et al*. (2017) and El-Sayed -Esraa (2018). They showed that applying nano-fertilizers increased leaf content of chlorophyll.

Leaf mineral content

Regarding leaf mineral content, results in Table (2) reveled that all nano-treatments enhanced all recorded N, P, K, Ca, Mg values compared to the control. The application of 500 ppm (T_6) gave the highest value of leaf N, P, K, Ca and Mg content as compared with all other treatments, with the control treatment indicating significantly the lowest values. Moreover, no significant difference obtained between adding 100 ppm (T2) and the untreated control treatment in leaf K content. Moreover, nano-fertilizer at 300 (T4) and 400 ppm (T5) significantly increased the percentage of leaf nitrogen than 100 ppm (T2) and 200 ppm (T3). The same trend was observed with leaf K, Ca and Mg contents. However, the values of N, Ca and Mg percent were significantly differing among T_4 and T_5 treatments. The T_5 treatment had significantly higher leaf P content than those of $T_{2,}$ T_3 and T_4. Meanwhile, applying 100 ppm (T_2) gave significantly lower leaf N, P and K than applying 200 ppm (T_3). Similar results were reported by different investigators (Roshdy and Refaai, 2016; Haggag *et al*., 2018 b; Abdelaziz *et al*., 2019 and Abdel-Hak *et al*., 2019) working on different fruit species. They reported that nano-fertilizer treatments had positive influence in increasing leaf mineral content.

In general, the enhanced growth and increased mineral content in obtained data could be due to the effect of the nano-fertilizer in encouraging various metabolic process mainly photosynthesis that leads to higher photosynthates accumulation and more dry matter production. The results also revealed that application of nano-fertilizer significantly increases leaf chlorophyll and mineral contents over control. This might be due to improving availability and uptake of elements and enhancing nutrient use efficiency. Nano-fertilizers have very small particles which are smaller than root and leaf pores. Small particles mean larger surface area that facilitate fertilizer absorption and thus it would be more easily absorbed into plant roots with high rate of absorption (Sekhon, 2014; Qureshi *et al*., 2018; Zahedi *et al*., 2019, Al-Hchami and Alrawi, 2020; Verma *et al*., 2022).

Leaf peroxidase (POX) and polyphenol oxidase (PPO) activities

It was noted from data in Table (3) and Figure (1) that the POX activities analysis scored the relative mobility (*Rf*) values ranging from 0.401 to 0.828, while the *Rf* values of PPO enzymes were from 0.373 to 0.768. The highest enzymes activities of POX and PPO were observed in fig plants treated with both levels of nano-fertilizer at 200 and 300 ppm. However, the lowest enzyme activities were recorded with the other nano-treatments and the control. These results are in agreement with those obtained by several researchers who worked on nano-fertilizers. Mustafa *et al.* (2018) indicated that the highest enzyme activities of POX and PPO resulted from applying nano-fertilizer at doses ranging from 100 to 400 ppm, compared to applying 500 ppm traditional fertilizer. This may be attributed to the increased ratio of surface to volume of the nano-fertilizes that reinforces the absorption efficiency, as well as their role in enhancing metabolic processes working as co-enzymes (Chhipa, 2017). Moreover, the positive effect of nano-fertilizers on reactive oxygen species (ROS) generation mechanism is mentioned by El-Saadony *et al.* (2021) and Verma *et al*. (2022). Also, nano-fertilizers are reported to play an important role in increasing anti-oxidative enzymes such as peroxidase, superoxide dismutase (SOD) and catalase, which constantly scavenge ROS (Upadhyaya *et al.,* 2015).

Soil microbial activity

Regarding health characters of the growing media, obtained data presented in Figure (2) showed that applying nano-fertilizer at all levels produced higher values microbial activity in soil comparing with control. The highest total microbial activity was found in soil treated with nano-fertilizer at 300, 400 and 500 ppm. This result might be due to reduce the quantity of added fertilizer which eliminate soil environment pollution. Also, nano-fertilizer make elements more available to plant without negative impact on root growth and root zone. So, it doesn't harm to soil microorganisms, and enhances the activity of soil microflora. All of these suggestions reflect the positive role of nano-fertilizers on soil microbial activity, consequently soil health. This suggestion agreed with those found by (Verma *et al*., 2022) they cleared that the nano-fertilizers significantly improve soil quality. The use of nano-fertilizer in the agricultural field preserves the soil. It reduces their pollution by reducing the amount of fertilizer used (Al-Hchami and Alrawi, 2020).

Conclusion

It could be concladed from the peresent study that application of nano-fertilizers would be promissing in improving plant growth as well as soil microbial activity and a dose of 300 and 400 ppm could be the best recommended in fertilzing fig plants. Besides, this study might be considered one in a series of reseaches conferming the safety of using nano-fertilizers as an alternative to conventional fertilizers without harmful influence on the soil environment.

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Table (1): The effect of nano-fertilizer on leaf fresh and dry weight, and leaf area of Black Mission fig plants as combined analysis for the 2020 and 2021 seasons.

Treatments	**Leaf fresh weight**	**leaf dry weight**	**Leaf area**
	(g)		**(mm^2)**
T_1 Control	13.00 f	6.41	50.91
T_2 (100 ppm)	19.55 c	7.80	65.49
T_3 (200 ppm)	18.14 d	7.56	100.49
T_4 (300 ppm)	22.88 a	8.28	97.95
T_5 (400 ppm)	16.25 e	7.26	75.23
T_6 (500 ppm)	20.60 b	8.15	119.22
L.S.D at 0.05	0.91	0.28	22.77

Means were represented as average of replicates.

Table (2): The effect of nano-fertilizer on leaf water, chlorophyll and mineral contents of Black Mission fig plants as combined analysis for the 2020 and 2021 seasons.

Treatments	**leaf water content**	**Chlorophyll Content**	**N**	**P**	**K**	**Ca**	**Mg**
	(%)	**SPAD**	**(%)**				
T_1 Control	50.38	34.33	2.00	0.22	0.57	0.87	0.21

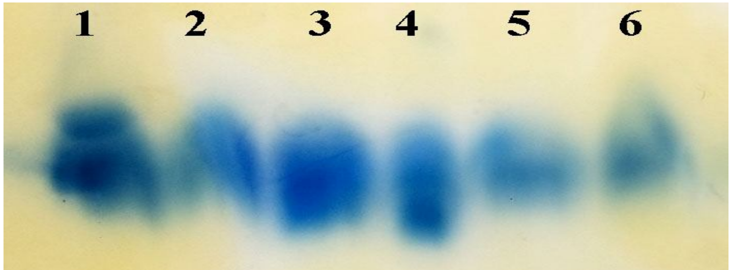
T_2 (100 ppm)	62.15	46.72	2.40	0.27	0.57	0.91	0.23
T_3 (200 ppm)	58.32	47.20	2.50	0.28	0.72	0.92	0.23
T_4 (300 ppm)	63.81	41.20	2.70	0.28	0.75	0.97	0.24
T_5 (400 ppm)	55.29	54.15	2.90	0.29	0.77	1.01	0.25
T_6 (500 ppm)	58.31	54.83	3.00	0.31	0.80	1.25	0.26
L.S.D at 0.05	4.5	5.7	0.1	0.01	0.03	0.02	0.01

Means were represented as average of replicates.

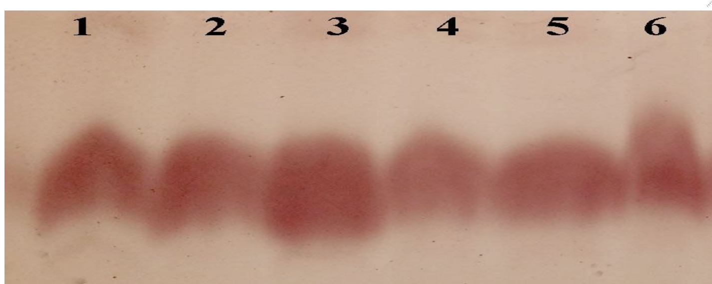
Table (3): The effect of nano-fertilizer application on POX and PPO enzyme activities in Black Mission fig leaves in 2021 seasons.

Relative mobility (*Rf*)	**Nano-fertilizer**					
	POX iso-enzymes					
	Control	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**500 ppm**
0.401	1	0	0	0	0	0
0.524	1	1	1	1	1	1
0.667	0	1	1	1	0	0
0.828	0	0	1	1	0	0
The total number of bands= 4	**2**	**2**	**3**	**3**	**1**	**1**
	PPO iso-enzymes					
0.373	0	0	0	0	0	1
0.448	1	1	1	1	1	1
0.548	1	1	1	1	1	1
0.647	1	1	1	1	1	1
0.768	0	0	1	0	1	0
The total number of bands = 5	**3**	**3**	**4**	**3**	**4**	**4**
The total number of POX and PPO bands = 9	**5**	**5**	**7**	**6**	**5**	**5**

Rf= The relative mobility, 0= absence of band, 1= presence of band



POX



PPO

Figure (1): POX and PPO profiles of fig leaves cv. Black Mission treated with nano-fertilizers. Lane 1= control; lane 2= 100 ppm; lane 3= 200 ppm; lane 4: 300 ppm; lane 5= 400 ppm; lane 6= 500 ppm.

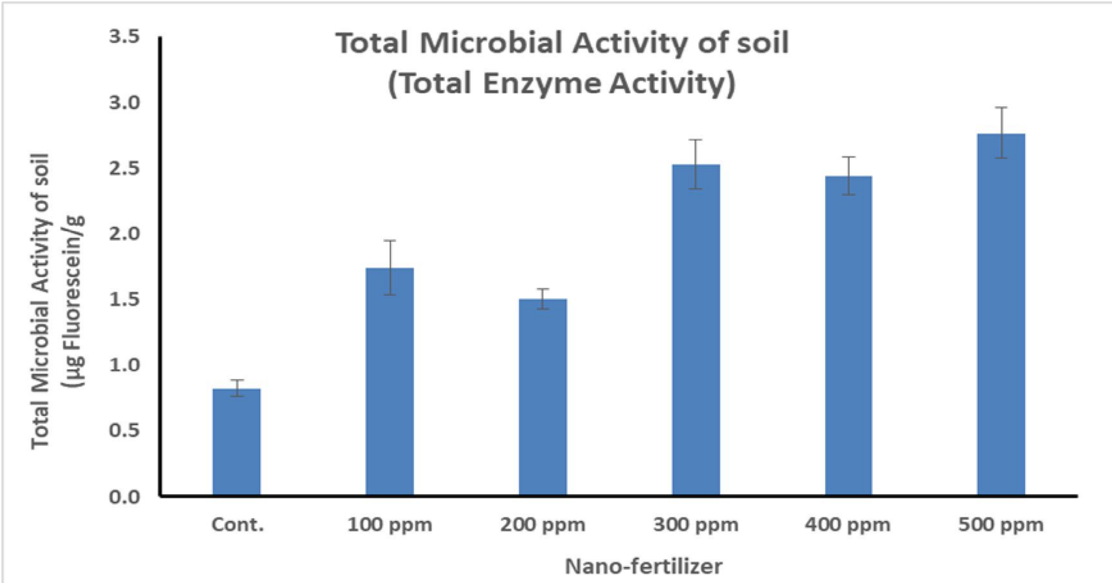


Figure (2): Effect of nano-fertilizer on microbial activity in soil as a result of combined analysis for the 2020 and 2021 seasons.