Effect of Different Nano-NPK fertilizers on Vegetative Growth Parameter and Soil Microbial Activity of Fig Crop Under Different Irrigation Regims

Abstract:

World face several challenges the most decisive climatic changes , freshwater poverty and poor usage efficiency for natural resources (soil & water) particularly in developing countries.

Applying Nanotechnology may represent a smart mechanism toward sustainable agricultural. Much efforts have been exerted to utilizing nano-technology and producing agro-chemicals in nano-form i.e, nano-fertilizers and nano-pesticides.

Current work that carried out in greenhouse belong pomology department , National Research Centre during 2020/2021, aims to assessing impact of nano-fertilizers on growth performance and soil microbial activity under different drought stress levels. NPK-nano-fetrilizer was applied on uniform one-year old fig seedlings, as foliar application at two levels (200 and 400ppm) compared with traditional NPK fertilizer, under three levels of water regimes (once, twice and three times irrigation weekly. Obtained results indicated that under drought stress nano-fertilizers enhanced fig seedlings growth performance and nutrient content. Moreover, nano-fertilizer raised antioxidant enzyme activity that work on scavenging active oxygen species and thereby reinforce drought stress tolerance in plants. Besides, nano-fertilizer had a positive impact on soil microbial under low soil moisture. This study came in chain of studies which proved the efficiency of nano-fertilizer under drought stress with no negative impact on environments under this study conditions. This study concluded that nano-fertilizer has a bright future particularly under challenges that face the world (climatic changes, poverty of water resources, soil degradation and global food famine risk with fast growing of population) toward sustainable agriculture with low risk on environment.

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Key words: Fig seedlings, Nano-NPK, Drought stress tolerance, antioxidant enzyme activity, soil health

Introduction

The world suffers from the limited and deteriorating natural resources needed for agricultural activities, leading to an increase in the food gap. The situation becomes more catastrophic in developing countries with rapid population growth and limited water resources. Hence, farmers strive to use more doses and different types of fertilizers (and pesticides) to increase the productivity of their crops in order to reduce the gap between production and consumption. Although traditional mineral fertilization gives quick positive results in terms of increased production, unfortunately, it is associated with serious environmental and health problems when being overused. Based on these health and environmental concerns, scientists are constantly looking for alternatives to the traditional fertilizers to achieve food security for the rising population with the minimum adverse effects **(Erisman et al. 2008; Ali et al., 2021; Astaneh et al. 2021)**.

One of the alternatives to the conventional chemical fertilizers is the application of nanotechnology and nanoparticles. Nanomaterials can be synthesized through various physical, chemical, and biological approaches. The small size of nanoparticles (<100 nm) gives them a high surface area to volume ratio and thus acquires unique properties useful in many agricultural, industrial, and medical applications. Numerous novel nanomaterials (nanoparticles, nanoformulations, nanocomposite, nanoemulsion and nanoencapsulation) have been developed for improving food quality and safety, crop growth, pest control and monitoring environmental conditions **(Chippa 2019; Darwesh et al., 2019; He et al., 2019; Matter et al., 2021)**.

The application of "Nano-Fertilizers" as a "smart nutrient delivery system" to plants can increase the overall plant growth dynamics as well as enhance soil fertility. These advantages could be attributed to the unique physicochemical properties of the applied nanoparticles, which enable high absorbance and reactivity **(Sidorowicz et al., 2019)**. Based on the nutrient needs of plants, nano fertilizers are classified into three categories: macro nano-fertilizers, micro nano-fertilizers, and nano-particulate fertilizers **(Chhipa and Joshi 2016)**. They provide nutrients to plants in an available form, thus increasing nutrient uptake by plants, and boosting plant production. The relevant features of nano-fertilizers are shown in the study by **(Guru et al., 2015)**: (1) delivering the appropriate nutrients for enhancing plant growth through foliar and soil applications, (2) being low-cost and sustainable sources of plant nutrients, (3) having a high fertilization efficiency; and (4) playing a key role in preventing pollution.

On the other hand, drought stress is a critical challenge for growing crops in arid and semi-arid regions. However, the provision of essential nutrients needed by plants plays an important role in drought tolerance. Although the mechanism of drought resistance is not yet sufficiently clear, it is expected that improving the activities of antioxidants and enzymes (e.g.

peroxidases and polyphenol oxidases) will improve tolerance to abiotic stress (i.e. drought) in plants **(Mustafa et al., 20.18; Ahanger et al., 2021)**.

In this study, the role of the commercial nano-fertilizers (NPK in nano-form) at different doses in improving the drought tolerance of an economical fruit crop, figs, was studied. In addition, the influence of the nano-fertilizers on fig growth and some of its key enzymatic activities, as well as the overall soil microbial activities have been studied under different water regimes.

Material and Methods:

Plant Material:

This work was carried out in the experimental research green house at National Research Centre, Dokki, Giza, Egypt during seasons 2020 and 2021. For this purpose, healthy one- year-old Fig and almost uniform seedlings of Black Mission cv. About 126 seedlings of fig were divided into 6 groups. These groups were subjected to nano-fertilizer treatments as shown in Table (1):

Each treatment is applied under three different irrigation regims (once, twice, and three times irrigation weekly). Current work was continued from March to September of each season's growth.

Nano-fertilizer preparation:

Nano-fertilizers had been produced by Dr. Hassan Sharway (chemical engineering and pilot plant Dept., Engineering Division, NRC).

The production steps of the nano-fertilizer are as follows: Addition of 20/20/20 NPK fertilizer in water and stirring till complete dissolution. Addition of citric acid and stirring till complete dissolution. Addition of sodium carbonate with vigorous stirring till an ash like solution formation,adjusting pH to 5. Nano-fertilizer morphology shape and size of the obtained nano fertilizer were characterized by means of a JEOL-JEM-1200 Transmission Electron Microscope (TEM). The TEM sample was prepared by adding a drop of the obtained nano fertilizer after sonication for 15minutes, on a 400 mesh copper grid coated by an amorphous carbon film and lifting the sample for drying in air at room temperature. The average diameter of the fertilizer particles was determined from the diameter of 100 nano-particles found in several chosen areas in enlarged microphotographs.

Measurements:

I. **Vegetative growth parameters** Average leaf areas (cm^2) by Intelligent Leaf area meter (Android)serial No. 19504/49u700591, fresh and dry weight of leaves (g) were measured. A chlorophyll reading (SPAD) was recorded by using a Minolta chlorophyll meter (Spad – 501). Leaf water content was calculated based on differences between fresh and dry weight of leaves.

II. **Leaf nutrient content analysis**: Leaf samples were dried in a ventilated oven at 70 ^{o}C to a constant weight. Samples were grinded in stainless steel mill with 0.5 mm sieve and kept in plastic containers for chemical analysis. The samples (1 g of each sample) were dry-ashed in a muffle furnace at 450 ^{o}C for 6 hours. Macronutrients were extracted using the dry ashing digestion method according to Chapman and Pratt, (1979). The ash was dissolved in HCl (2N). Nitrogen was determined by using the Kjeldahl method, and phosphorus was photometrically determined in the digested solution using vanado-molybdate color reaction according to the method described by Jackson, (1973). Potassium was measured in the digested suspension using the Flamephotometer, (Eppendorof, DR Lang).

Polyphenol oxidase (PPO) and peroxidase (POX) isoforms

For the assay of antioxidant enzymes, peroxidase (POX) and polyphenol oxidase (PPO) were extracted based on the method described in (**Stagemann *et al.*, 1985**). 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**).

Measurement of total microbial activity of soil

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 was stopped by adding 15 mL of chloroform/methanol (2:1) and vortexing for 1 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).

Data Statistical analysis:

Means were represented as the average of replicates of two seasons (as a combined analysis of two seasons). The least significant difference (LSD5%) test was used to compare among the means of treatments according to (Snedecor and Cochran, 1992).

Results and Discussion:

Table 2 showed impact of nano-fertilizer application at two levels (200 & 400 ppm) on leaf fresh weight of fig seedlings. The fresh weight of leaves was decreased with reduction in both of irrigation rate and fertilization rate. The highest fresh weight of leaves was observed when fig seedlings received irrigation at a rate of 3 times/week and fertilizer at 500 ppm fertilizer.

In regard to leaf dry weight, Table 2 showed that leaf dry weight has the same trend as leaf fresh weight, whereas it decreased with decreasing levels of irrigation (from 3 times/week to 2 or 1 time /week) and fertilization rate from 500 ppm (NPK) to 400 and 200 ppm of nano-fertilizers. Besides, the highest leaf dry weight was observed when fig seedlings received fertilizer at 500 ppm (NPK), and the irrigation rate was 3 time /week.

For leaf water content, data in Table 2 showed that this parameter follows the same trend as leaf weight, whether fresh or dry weight). Whereas leaf water content increased with increasing both of irrigation rate and fertilizer levels and the highest leaf water content was recorded with applying irrigation rate at 3 times /week in parallel wih fertilizer at 500 ppm as NPK.

In addition, data in Table (2) showed that leaf area increased with an increasing level of irrigation rate 3 times/week, compared with 1 and 2 irrigation times /week. Furthermore, leaf area influenced with applying nanofertilizer, whereas applying nanofertilizer at 400 ppm recorded the highest value of leaf area followed by 200 ppm of nano-fertilizer in comparison to 500 ppm (NPK). In respect to leaf area under a combination of irrigation levels and different fertilizer treatments, obtained results showed an increase in leaf area with increasing levels of nano-fertilizers and irrigartion times. The highest leaf area (24125.8 mm^2) was achieved when fig seedlings received 3 times irrigation and 400 ppm of nano-fertilizers compared with 500 ppm NPK and 3 times irrigation/week **Furthermore, fig seedlings that received 200 ppm nano-fertilizers in combination with 3 times weekly irrigation had greater leaf area than those that received 500 ppm NPK at the same irrigation level.** Under different levels of irrigation that were less than 3 times/week, the same trend was noticed. However, fig seedlings that received 400 ppm nano-fertilizer recorded the highest leaf area value, followed by 200 ppm nanofertilizer and the control treatment (that received 500 ppm NPK) came in the last rank at the same level of irrigation. These results incdicated that applying nanofertilizer may result in enhancing leaf area under regim water compared with control treatment.

Besides, data in Table (2) showed that applying nano-fertilizers led to an increase in chlorophyll content compared with control treatment (500 ppm NPK). Moreover, increasing the level of applied nano-fertilizer from 200 to 400 ppm resulted in increasing chlorophyll content in leaves. In respect to impact of irrigation times/week on chlorophyll content , data revealed that chlorophyll content in leaves increased with increasing times of irrigation/seedling/ week. Applying nano-fertilizer at 400 ppm recorded the highest value of chlorophyll compared with other treatments at the same level of irrigation. Also, applying nano-fertilizer at 200 ppm came in second rank after 400 ppm of nano-fertilizer treatment at the same level of irrigation, and applying NPK at 500 ppm (control) came in the last ranks.

Data in Table (3) showed that irrigation three times weekly recorded the highest N content (2.8%) compared with irrigation once or twice weekly. Besides, data in this table revealed that 400 ppm of nano fertilizer resulted in the highest leaf N content (2.87%) compared with 200 ppm nano-fertilizer or 500 ppm of NPK. Also, applying nano-fertilizer at 400 ppm recorded the highest value of N content in leaves, followed by nano-fertilizer at 200 ppm at the same level of irrigartion (once, twice ,and three times weekly).

For P content, data in Table (3) revealed that P content increased with increasing irrigation times form one to three times weekly. Also, applying nano-fertilizer at (400 ppm) produced the highest content of P followed by nano-fertilizer at 200 ppm. In regard to, the impact of the combination of irrigation treatments and nano-fertilizer treatments, data showed that applying nano-fertilizer at 400 ppm came in the first rank followed by nano-fertilizer at 200 ppm at the same level of irrigation. Meanwhile, control treatment (500 ppm NPK) recorded the lowest value of P content compared with nano-fertilizers treatments at the same level of irrigation.

Moreover, data in Table (3) showed that K content increased with increasing irrigation times from one to three times weekly (0.67 to 0.8% respectively). Also, applying nano-fertilizer at (400 ppm) produced the highest content of K (0.88%), followed by nano-fertilizer at 200 ppm (0.745). In regard to, the impact of combination of irrigation treatments and nano-fertilizers treatments, data showed that applying nano-fertilizer at 400 ppm came in the first rank, followed by nano-fertilizer at 200 ppm at the same level of irrigation. Meanwhile, control treatment (500 ppm

NPK) recorded the lowest value of K content compared with nano-fertilizers treatments at the same level of irrigation.

In addition, data in Table (3) indicated that Ca content raised with increasing times of irrigation from twice to three times weekly (0.92 to 0.96). Also, applying nano-fertilizer at (400 ppm) produced the highest content of Ca (1.1%) followed by nano-fertilizer at 200 ppm (0.92%). In regard to, the impact of combination of irrigation treatments and nano-fertilizers treatments, data showed that applying nano-fertilizer at 400 ppm came in the first rank with value of Ca content (1.1%), followed by nano-fertilizer at 200 ppm (0.925) at the same level of irrigation.. Meanwhile, control treatment (500 ppm NPK) recorded the lowest value of Ca content (0.75 to 0.87%), compared with nano-fertilizers treatments at the same level of irrigation.

Finally, Table (3) showed that Mg content raised with increasing times of irrigation from one to three times weekly (0.217 to 0.253 % respectively). Also, applying nano-fertilizer at (400 ppm) produced the highest content of Mg (0.25%), followed by nano-fertilizer at 200 ppm (0.24%). In regard to, the impact of combination of irrigation treatments and nano-fertilizers treatments, data showed that applying nanofertilizer at 400 ppm came in the first rank, followed by nano-fertilizer at 200 ppm at the same level of irrigation. Meanwhile, control treatment (500 ppm NPK) recorded the lowest value of Mg content compared with nano-fertilizers treatments at the same level of irrigation.

These results were in the same line of findings of Mustafa et al., 2018; Agrawal and Rathore 2014).

Besides, Vafa *et al*.,2015] observed that nano-Zn treatment induced an increae in content of chlorophyll, essential oil, P, and the antioxidant capacity of rice (Weisany *et al*., 2012). Antioxidants are secondary metabolites produced by plants under adverse situations, i.e., drought, salt, and nutritional deficiency. The nano-fertilizer supplies enough nutrients to improve antioxidant activity in plant cells (Benzon *et al*., 2015).

These positive impacts of nano-fertilizers may be attributed to what was mentioned by Wiesner *et al*., (2006) and Chugh *et a*l., (2021), who reported that the nanoscale particles (nano-fetrilizers) are smaller in size and may be absorbed with different dynamics from those in bulk particles or ionic salts, which has significant benefits. The reduced size of nano-fertilizers through physical/chemical means enhances their surface–mass ratio in order to allow an increase in the absorption of nutrients by roots, thereby leading to an increase in metabolic processes in plants that enhance of plant growth performance.

Influence of Nano-fertilizer on peroxidase (POX) and polyphenol oxidase (PPO) isozyme activities under different irrigation regims:

The data represented in Figure (3) and Table (4) showed that response some isozyme activities to nano-fertilizers application under water regim, whereas peroxidase scored the highest number of bands under one time irrigation per week with nano-fertilizers application (400 and 200 ppm), compared with treatment 500 NPK, whether under 3 or 1 times irrigation /week. Also, the same trend was noticed with polyphenol oxidase activity whereas the number of bands increased under 1 time irrigation/week and nanofertilizer (200 and 400 ppm) compared with 500 NP treatment a 1 or 3 times irigation / week.

This data indicated that with applying traditional fertilizers (NPK), enzyme activities (particularyl antioxidant enzymes : peroxidase and polyphenol oxidase) has been affected and decreased with a decrease number of irrigation times /week. This trend changed when nano-fertilizers were applied and these enzyme activities increased with the decreaseing number of irrigations /week from 3 to 1 time weekly which may give good evidence that these nano-fertilizers may be an effective tool to reinforce drought stress tolerance in plants via raising anti-oxidance enzyme activities (i.e .peroxidase and polyphenol oxidase) that play a role in scavenging active oxygen species, which emerge when plants exposed to drought stress and cause damage to plant cell organs. These results were agreed with those reported by Mustafa *et al*., (2018). They indicated that higher activities of peroxidase and polyphenol oxidase enzymes resulted from applying nano-fertilizer at all levels (100 to 400 ppm nanofertilizers) compared with conventional fertilizers (NPK 500 ppm) which recorded the lowest activities of these enzymes with (two isoforms). This may be attributed to the increased ratio of surface to volume of the nano-fertilizes which reinforces the efficiency and their role in metabolic processes and as co-enzymes (Chhipa, 2017).

Moreover, the role of nano-copper in improving miaze growth was studied by Adhikari *et al*., 2016 and their results indicate that the nano-particles of copper could enter into the plant cell, easily be assimilated by plants and also enhance its growth by regulating the different enzyme activities.

Impact of nano-fertilizer on soil microbial activity different irrigation regims

Rhizosphere microorganisms play an important role in agricultural soils that includes nutrient facilitation, production of plant growth stimulants, bioremediation of hazardous materials, and disease control. Total bacterial enzyme activity is an important parameter of soil quality. It reflects the activity of the microbial population, which give an indirect indication of soil nutrition and fertility. In this method, the enzymes produced by microbial populations in soil (such as proteases, lipases, and esterases) are capable of cleavage the colorless fluorescein diacetate into fluorescein (with a measurable fluorescent color) **(Abou-Shanab et al., 2012; Rashid et al., 2016; Backer et al., 2018; Patle et al., 2018)**. In the current study, the effect of using nano-fertilizer (NPK) for fig plants on the activity of microorganisms in the soil was investigated under three levels of irrigation (once, twice, and three times a week) fig 4. The effect of fertilizers can be attributed to the direct use of nutrients that reach the soil by microbes. In addition, plant nutrition affects the microbial activity of the soil indirectly by stimulating root exudates that contain microbial growth stimulants.

According to results illustrated in Figure (4), both levels of nano fertilizer (200 and 400 ppm) resulted in higher microbial activity in the fig rhizosphere compared to the control treatment (500 ppm mineral NPK). However, reducing the irrigation rate from three times a week to two and once a week reduces the microbial activity in the soil. These results demonstrate the positive effect of NPK nanofertilizers in reducing the effect of drought on fig plants. These findings and recommendations are important in arid and semi-arid regions to overcome the negative effects of drought on economic plants.

Generally, Agrawal and Rathor (2014) mentioned that the positive morphological impacts of nanomaterials include improved germination percentage and rate; length of root and shoot, and their ratio; and total vegetative biomass of seedlings, along with enhancement of physiological parameters like enhanced photosynthetic activity and nitrogen metabolism in many crop plants. Furthermore, they attributed these positive effects of nanomaterials to changes in their properties compared to the original bulk, whereas Chen and Yada (2011) reported that nanoparticles have enhanced reactivity due to enhanced solubility, a higher proportion of surface atoms relative to the interior of a structure, unique magnetic/optical properties, electronic states, and catalytic reactivity that differ from equivalent bulk materials.Also, they reported that numerous studies indicated that nano-technology holds the promise of controlled release of agrochemicals and site targeted delivery of various macromolecules needed for improved plant disease resistance, efficient nutrient utilization, and enhanced plant growth.

Conclusion:

Applying nano-technology as a promising futuristic agri-technologies holds many aspects toward ensuring food security and sustained agricultural development. The agro-nanotech innovations offer a new concept of "low input but maximum output" based agro-farming are well aligned with the desired crop production. Therefore, nanotechnology has a significant role to play in the improvement of the efficiency of agro-chemicals as well as with the safety and healthy of agro-ecosystem under environmental adversities mainly climatic changes.

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Table 1: Nano-fertilizers treatments used in this study.

Code	Nano-fertilizers Treatments (Treatment Details of Fertilizers and Nano-Fertilizers)
(T_0)	Seedlings received 500 ppm NPK doses twice weeks
(T_1)	Seedlings received 400 ppm nano-fertilizers doses twice weeks
(T_2)	Seedlings received 200 ppm nano-fertilizers doses twice weeks

Table (2): Effect of interaction between nano-fertilizer and water regim on some growth parameters of fig crop

Treatments Parameters	Treatments					
	No of Irrigation/ week	**Control 500 ppm NPK (T0)**	**400ppm (T1)**	**200ppm (T2)**	**Mean**	**(A) X (B)**
Leaf F.W(g)	**1**	15.37	11.94	14.03	**13.78 b**	**0.88**
	2	16.87	12.85	14.35	**14.69 b**	
	3	18.75	17.07	14.85	**16.89 a**	
	Mean	**17.00 a**	**13.95 b**	**14.41 b**		
Leaf D.W (g)	**1**	6.77	5.65	5.82	**6.08 b**	**0.28**
	2	6.64	5.74	6.06	**6.15 b**	
	3	6.80	6.84	6.27	**6.64 a**	
	Mean	**6.73 a**	**6.08 b**	**6.05 b**		
Leaf water content	**1**	55.93	52.69	58.51	**55.71 c**	**1.44**
	2	60.64	55.32	57.73	**57.90 b**	
	3	63.76	59.88	57.76	**60.47 a**	
	Mean	**60.11 a**	**55.97 c**	**58.00 b**		
Leaf Area (mm^2)	**1**	9016.2	16407.3	11850.8	**12424.8 c**	**780.9**
	2	12514.0	22800.2	15888.7	**17067.6 b**	
	3	19340.9	24125.8	21278.4	**21581.7 a**	
	Mean	**13623.7 c**	**21111.1 a**	**16339.3 b**		
Leaf chlorophyll content (SPAD)	**1**	35.13	42.05	40.3	**39.16 c**	**1.47**
	2	37.73	43.10	40.23	**40.36 b**	
	3	39.70	47.50	42.05	**43.08 a**	
	Mean	**37.52 c**	**44.22 a**	**40.86 b**		

Table (3): Effect of interaction between nano-fertilizerand water regim on nutreints content in fig crop

	No of Irrigation/ week (A)	Treatments (B)				LSD $_{0.05}$ (A) X (B)
		Control 500 ppm NPK (T_0)	400ppm (T_1)	200ppm (T_2)	Mean	
N (%)	1	2.4	2.7	2.5	2.53 c	0.06
	2	2.4	2.9	2.8	2.70 b	
	3	2.5	3.0	2.9	2.80 a	
	Mean	2.43 c	2.87 a	2.73 b		
P (%)	1	0.1	0.18	0.12	0.13 c	0.01
	2	0.12	0.23	0.14	0.16 b	
	3	0.18	0.35	0.19	0.24 a	
	Mean	0.13 c	0.25 a	0.15 b		
K (%)	1	0.57	0.82	0.62	0.67 c	0.02
	2	0.57	0.87	0.82	0.75 b	
	3	0.67	0.95	0.77	0.80 a	
	Mean	0.60 c	0.88 a	0.74 b		
Ca (%)	1	0.87	1.1	0.92	0.96 a	0.02
	2	0.75	1.1	0.92	0.92 b	
	3	0.87	1.1	0.92	0.96 a	
	Mean	0.83 c	1.10 a	0.92 b		
Mg (%)	1	0.2	0.23	0.22	0.217 c	0.01
	2	0.23	0.26	0.25	0.247 b	
	3	0.25	0.26	0.25	0.253 a	
	Mean	0.23 c	0.25 a	0.24 b		

Table 4: Impact of nano-fertilizer on plant enzyme activity (peroxidase and poly phenol oxdase) under drought stress :

Rf	Impact				Nano-fertilizer				
	200-1	**400-1**	**500-1**	**200-2**	**400-2**	**500-2**	**200-3**	**400-3**	**500-3**
0.401	1	1	0	0	0	1	1	0	1
0.524	1	1	1	1	1	1	1	1	1
0.667	1	1	0	1	1	1	1	0	1
0.828	1	1	0	0	0	1	0	0	0
Total number of bands = 4	**4**	**4**	**1**	**2**	**2**	**4**	**3**	**1**	**3**
					PPO isozymes				
Rf	**200-1**	**400-1**	**500-1**	**200-2**	**400-2**	**500-2**	**200-3**	**400-3**	**500-3**
0.373	1	1	0	1	0	0	0	0	0
0.448	1	1	1	1	1	1	1	1	1
0.548	1	1	1	1	1	1	1	1	1
0.647	1	1	1	1	1	1	1	1	1
0.768	1	1	0	1	1	0	1	1	1
Total number of bands = 5	**5**	**5**	**3**	**5**	**4**	**3**	**4**	**4**	**4**
Total number of POX and PPO bands= 9	**9**	**9**	**4**	**7**	**6**	**7**	**7**	**5**	**7**

Rf= The relative mobility: 0= Absence of band   1= Presence of band

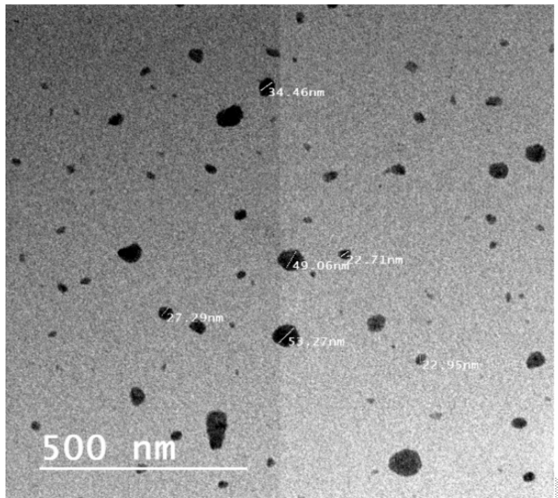


Figure. (1) Below shows the nano-fertilizer particles' size by Electronic microscope in NRC.

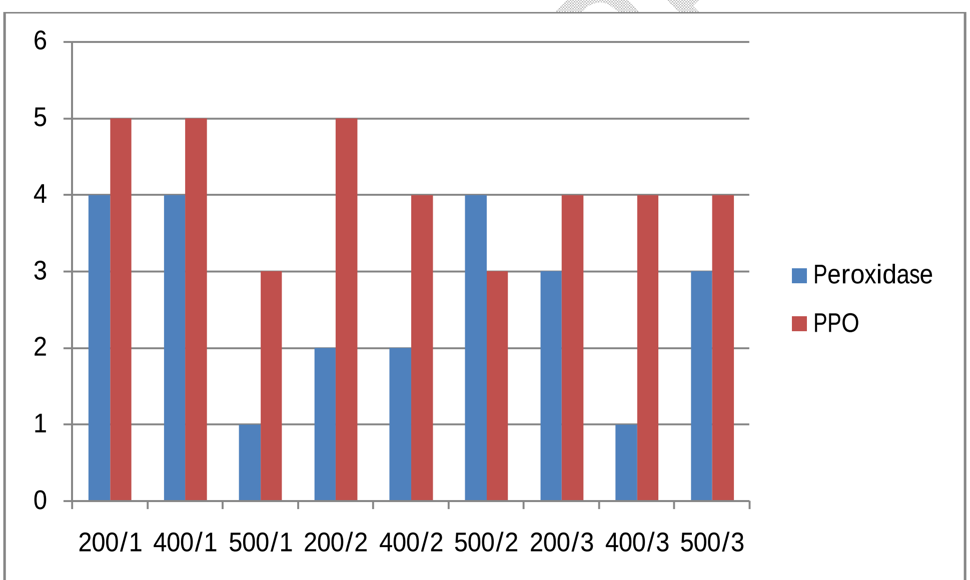
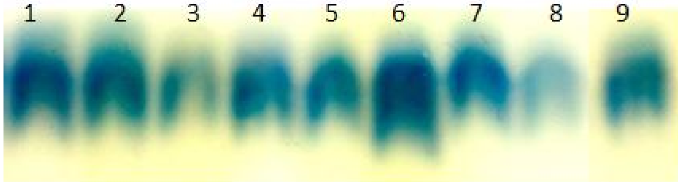


Fig 2: showed the total number of bands or both of peroxidase (POX) and polyphenol oxidse (PPO) as response for nano-fertilizer treatments under different irrigation levels : whereas 1; received 200 ppm nano-fertilizers and 1 time irrigation weekly, 2 received 400 ppm nano-fertilizers and 1 time irrigation weekly, 3 received 500 ppm NPK fertilizers and 1 time irrigation weekly, 4; received 200 ppm nano-fertilizers and twice irrigation weekly, 5 received 400 ppm nano-fertilizers and twice rrigation weekly, 6 received 500 ppm NPK fertilizers and twice rrigation weekly, 7; received 200 ppm nano-fertilizers and 3 times irrigation weekly, 8 received 400 ppm nanfertilizers and 3 times irrigation weekly and 9 received 500 ppm nano-fertilizers and 3 times irrigation weekly.

A



A

B

B

Fig 3: a: Effect of nano-fertilier on peroxidase enzyme activities under water regim. B: effect of nano-fetilizer on polyphenol oxidase enzyme activityies nder water regim. : whereas 1; received 200 ppm nano-fertilizers and 1 time irrigation weekly, 2 received 400 ppm nano-fertilizers and 1 time irrigation weekly, 3 received 500 ppm NPK fertilizers and 1 time irrigation weekly, 4; received 200 ppm nano-fertilizers and twice irrigation weekly, 5 received 400 ppm nano-fertilizers and twice rrigation weekly, 6 received 500 ppm NPK fertilizers and twice rrigation weekly, 7; received 200 ppm nano-fertilizers and 3 times irrigation weekly, 8 received 400 ppm nanfertilizers and 3 times irrigation weekly and 9 received 500 ppm nano-fertilizers and 3 times irrigation weekly.

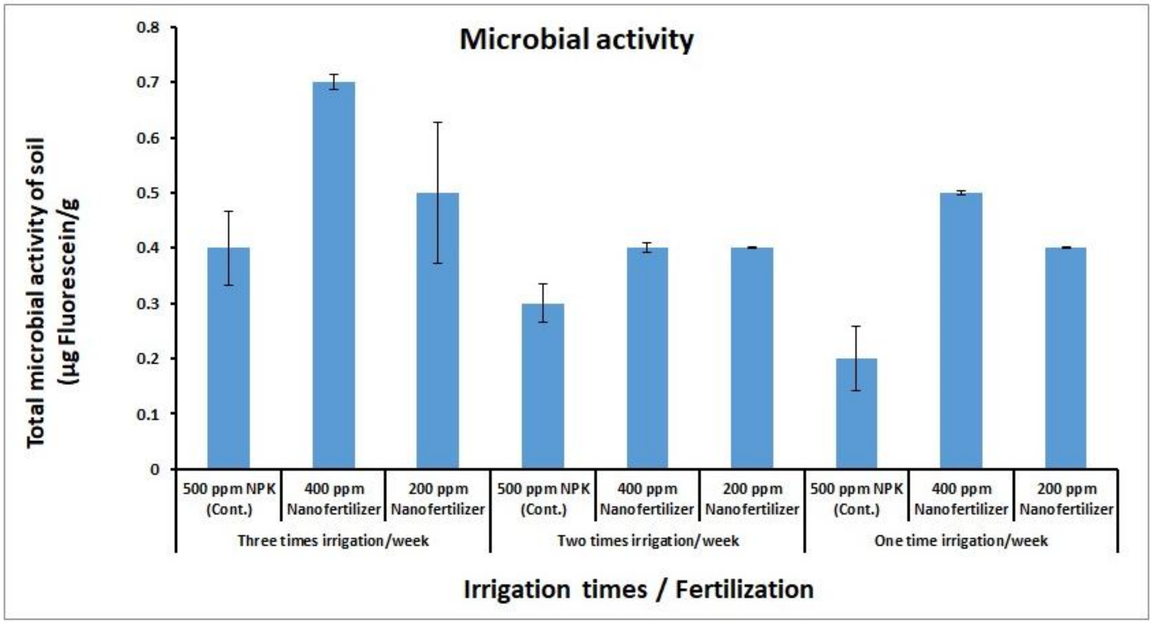


Fig. 4: Influences of diferent nano-fertilizer doses under different irrigation regims on the total microbial activity of fig rhizosphere soils.