

ROLE OF BIO AND NANO FERTILIZATION IN INCREASING GROWTH AND PRODUCTIVITY OF SPINACH IN SANDY SOIL

Ghada A.Z. Ibrahim

Department of Soil Fertility and Microbiology, Desert Research Center, Cairo, Egypt

E-mail: ghada_micro@yahoo.com

Lysinibacillus macroides is a plant growth promoting bacteria (PGPB) and phosphate solubilizing bacteria (PSB). Nano-phosphozinc is a nano-compound used to increase yield of grown plants (Spinach) and decrease the added doses of mineral fertilizers. When compared to controls, the results showed that using treatment with 50 ppm of nano-phosphozinc + *L. macroides* increased fresh weight by approximately 169.08 and 77.47% for fresh matters, respectively, and increased dry weight by approximately 273.3 and 84.27% for the first and second cuts, respectively. *L. macroides* produced organic acids, phytohormones, acidic and alkaline phosphatase enzymes and it had a phosphate solubilizing activity. *L. macroides* could survive nano-phosphozinc concentrations of up to 15 ppm. It is important to do more studies on the effects of nanomaterials on a long time to know its accumulation on human body, soil, plant, micro-organism activities and environment.

Keywords: spinach, *Lysinibacillus macroides*, phosphate solubilizing bacteria, Plant growth promoting bacteria , nano-fertilizers

INTRODUCTION

In order to bridge the gap between agricultural productivity and food consumption, land reclamation must be increased. It is necessary to expand the area under cultivation in sandy soils, primarily in arid and semi-arid regions. In such soils, crop output is constrained and affect soil fertility and nutrient availability. Egyptian soils have alkaline pH values as a result of the high quantities of calcium and magnesium, which limit the availability of phosphorus and molybdenum. To boost the productivity of these soils, it was required to investigate the factors that increase the soils' capacity to hold water and nutrients, such as fertilization and the application of micronutrients. *Lysinibacillus* spp. shows the capacity to convert fixed inorganic phosphorus compounds into soluble P form that can be easily absorbed by plants (Gupta and Prakash, 2020).

Phosphorus is a necessary component for plant life. Through solubilization and mineralization processes, phosphate-releasing bacteria can convert phosphate to a bioavailable form (Behera et al., 2017). Because Egyptian soil is alkaline, many nutrients are inaccessible to plants. Nano-fertilizers offer benefits as environmentally friendly and low-cost sources of nutrients for plants, have a high supplemental fertilization method, complement mineral fertilizers, and safeguard the environment from pollution risks, according to Yaseen et al. (2020). Previous research has shown that P-solubilizing bacteria generate a variety of metabolites and enzymes that dissolve P (Rafi et al., 2019), although P solubilization occurs through acidification (Bakhshandeh et al., 2017 and Rafi et al., 2019).

Phosphorus solubilizing activities of *Pseudomonas* which successfully demonstrated positively reconditioned with the creation of organic acids (Zeng et al., 2017). *Lysinibacillus fusiformis* produce organic acids (fumaric, lactic, malic, citric, succinic and propionic acid) and phytohormones as [indol acetic acid (IAA) and abscisic acid (ABA)] that are important for plant elongation and growth. Through phosphate solubilization, different organic acids as malic acid, lactic acid and acetic acid were also detected in the broth culture through HPLC analysis (Behera et al., 2017). Many *Lysinibacillus* strains can solubilize more important insoluble minerals, such as potassium, iron and zinc (Vendan et al., 2010; Verma et al., 2016 and Naureen et al., 2017). Organic acids, hydrolytic enzymes, and metal chelator compounds from these *Lysinibacillus* spp. were reported to facilitate the conversion of insoluble minerals into bio-available forms (Trivedi et al., 2011 and Naureen et al., 2017).

The aim of this work is to evaluate the importance of nano-fertilizer with replace and reduce the quantities of mineral fertilizers using the suitable biofertilizer strain and estimate its effect on the growth and yield of spinach (*Spinacia oleracea*) groan plants.

MATERIALS AND METHODS

1. Distribution of Particle Sizes of Nano-Phosphozinc

Nano-phosphozinc was kindly provided by National Center for Radiation Research and Technology, Cairo, Egypt. Dynamic light scattering (DLS, Malvern Zetasizer Nano-ZS Nano Series) is used to measure the size of nanoparticles. The substance is made up of zinc sulphate and sulfuric acid that have been dissolved in glycerol. A sample of about 0.1 mg was dissolved in 10 ml of water and sonicated for 30 minutes with 10-second on-and-10-second off cycles. In the Central Lab of the Desert Research Center, the samples discreted in water and the size measurements were done at 25°C and a scattering angle of 90°/173°. Nano-phosphozinc (contains 18% P from phosphoric acid and 10% Zn from zinc sulphate where solvent in glycerin) was obtained according to Ibrahim and Hegab (2022).

2. Bacteria Used

It was identified as *Lysinibacillus macroides* strain LMG 18474, accession no. (NR 114920.1), and was isolated from olive mill wastewater (OMWW) (Omar and Ibrahim, unpublished).

3. Greenhouse Experiment

Seeds of spinach (variety Thessaloniki) were provided by Agricultural Research Center, Ministry of Agriculture and Land Reclamation (MALR), Cairo, Egypt. Throughout this work, germination test was carried out to make sure of the viability of seeds. Pots experiment was conducted in pots filled with sandy soil from Balaza research station area in Greenhouse of Desert Research Center, Egypt. Spinach plant (*Spinacia oleracea*) was the task crop and the treatment which used was soil drench.

A greenhouse pot experiment was set up during 2021/2022 to study the response of spinach plants to fertilization with different rates of nanophosphozinc with and/or without bio fertilization. *L. macroides* was added as a soil drench to pots containing 10 kg of sandy soil from Balaza (29° 25' N 31° 15' E). Surface sandy soils (0-30 cm) from Balaza were air dried, sieved through a 2 mm sieve and was packed in 10 kg pots.

Each pot was amended with full recommended doses of 2.5 g N (as ammonium nitrate, 33.3%), 0.75 g K₂O (as potassium sulphate, 48.5%) and with half recommended dose of 1 g P₂O₅ (as super phosphate, 15.5%). The nitrogen fertilizers were added in two doses: the first one after 3 weeks from planting and the second one after 2 weeks from the first one.

Seeds of spinach were surface sterilized and cultivated in pots, 15 seeds per pot were planted. Plants were harvested after 30 days of cultivation (1st cut) and after 60 days of cultivation (2nd cut). The fresh and dry weights for both cuts were recorded. The plant materials (shoots) were dried at 70°C for 3 days, and the dry matter yield was recorded. The pots were arranged in complete block randomized design with 3 replicates (Cochran and Cox, 1987).

The physical and chemical prosperities of the studied soil (Table 1) were carried out according to Chapman and Pratti (1961).

Table (1). The chemical and physical properties of the studied soil

Particle size distribution %			Soil texture	EC (ds/m)	pH	Soluble cations (meq/l)				Soluble anions (meq/l)			
Sand	Silt	Clay				Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻ (mg/l)	SO ₄ ⁻	Cl ⁻
90.5	7.1	4.5	Sand	4.58	7.92	19.33	2.67	12.99	10.76	-	2.864	25.26	17.62

The studied treatments were

1. Control (uninoculated and non-nano phosphozinc fertilizers)
2. 50% of the recommended dose of super phosphate (for all treatments)
3. 50% of the recommended dose of super phosphate + *L. macroides*
4. Application of 12.5, 25, 50 and 100 ppm of nano-phosphozinc fertilizer (50 ml) individually
5. Application of 12.5, 25, 50 and 100 ppm of nano-phosphozinc with the biofertilizer *L. macroides*.

The addition of nano-phosphozinc was 12.5 ml/L, 25 ml/L, 50 ml/L and 100 ml/L from the main nano-solution. Then, 20 ml were added per treatment and 50 ml (10^8 CFU/ treatment) of biofertilizer *L. macroides* were applied on plant. The first and second cuttings of dried plant elements were wet digested (Thomas et al., 1967). Micro Kjeldahl was used to determine nitrogen using the ammonium molybdate-ascorbic acid technique (Watanabe and Olsen, 1965), K was determined using a flame photometer, and Zn was evaluated using an atomic absorption spectrophotometer (Jenway Model 6105 UV/ Vis spectrophotometer). The plant growth parameters determined were fresh and dry weights of biological yield (g/pot). The total biomass/g fresh and dry weights were calculated.

4. Effect of Nano-Phosphozinc Inoculation with *L. macroides* on Chemical Analysis

NPK content in shoots were determined by modified Kjeldahl method (Chapman and Pratt, 1961), while phosphorus was measured according to Watanabe and Olsen (1965) and potassium content determined by using flame photometer, and Zn was determined in acid digested solution, which was prepared according to Cottenie et al. (1982).

5. Microbiological Analysis**5.1. Phosphate solubilizing activities of bacterial strains included the following****5.1.1. Change in the pH**

Using Bunt and Rovira broth medium (Bunt and Rovira, 1955), pH was adjusted to 6.8 and inoculated with bacteria, after incubated for 3 days at 30°C, then pH value was estimated.

5.1.2. Total Phosphorus determination

Pikovskaya broth media was used to determine total phosphorus solubilizing by *L. macroides* after inoculation and incubation for 3 days at 30°C using molybdenum blue method of Pikovskaya (1948), then total P was estimated according to Watanabe and Olsen (1965).

5.1.3. Quantitative phosphate determination

Bunt and Rovira medium was used to estimate the clear zone of phosphate solubilizing by bacterium used.

5.1.4. Phosphatase enzyme activity

One enzyme unit of phosphatase was defined as amount of enzyme

that hydrolyzed 1 mM of p-nitrophenol/hour. The sample determined at Soil, Water and Environmental Research Institute, Agricultural Research Center. After six days of incubation at 35°C, 0.5 ml inoculum from Pikovskaya broth media were used. Phosphorus was measured in free-cell supernatant for acidic and alkaline phosphatase activity according to Tabataba and Bremner (1969).

5.2. Organic acid determination

The organic acids were determined by quantitative and qualitative analyses as following:

5.2.1. Quantitative analysis of organic acids

This method was applied according to Kumar et al. (2014).

5.2.2. Qualitative analysis of organic acids by HPLC

This analysis was carried out by Central Lab of Desert Research Center. Organic acids were also analyzed by High-Performance Liquid Chromatography using HPLC Ultimate 3000 Thermo dionex, Germany, (Hanifi and Elhadramy, 2007).

5.3. Phytohormones

The auxin indole-3-acetic acid (IAA) was determined by HPLC Ultimate 3000 Thermo dionex, Germany, at Central Lab of Desert Research Center.

6. Effect of Nano-Phosphozinc Inoculation with *L. macroides* on Bacterial Counts and Dehydrogenase Activity in Spinach Rhizosphere

Microbial densities in spinach soil rhizosphere samples were conducted on different media as following:

6.1. Ashby medium

Ashby medium (Abd El-Malek and Ishac, 1968) was used for counting of nitrogen fixers by M.P.N technique and was calculated using Cochren's tables (Cochran, 1950). Total microbial counts were determined by nutrient agar medium (Jacobs and Gerstein, 1960). For phosphate dissolving bacteria, counts were done using medium agar of Bunt and Rovira.

6.2. Soil dehydrogenase activity

Soil dehydrogenase activity (gTPF /g dry soil /24 hours) was analyzed using 2, 3, 5-triphenyl tetrazolium chloride (TTC) which turns into triphenyl formazan (TPF). Briefly, 5 g of fresh soil were incubated at 37°C for 24 hours in 5 ml of a TTC solution (5 g TTC in 0.2 mol/L Tris-HCl buffer, pH 7.4). Two drops of concentrated H₂SO₄ were immediately added after the incubation to end the reaction. The sample was then blended with 20 ml of methanol and shaken for 1 hour at 200 rpm, followed by filtering to extract TPF. Optical density of the filtrate was measured at 485 nm using spectrophotometer (Casida, 1977).

6. Statistical Analysis

Data of the present work were statistically analyzed and the differences between the means of the treatments were considered significant when they were more than the least significant differences (L.S.D) at the 5%

level. The obtained data were subjected to statistical analysis by ANOVA using the method described by Snedecor (1966).

RESULTS AND DISCUSSION

1. Distribution of Particle Sizes of Nano-Phosphozinc

According to the average data on particle size distribution, nano-phosphorus particles have a size of 19.2 nm (Ibrahim and Hegab, 2022).

2. Bacteria Used

Lysinibacillus macroides strain LMG 18474, accession no. (NR 114920.1), this bacterium was utilized as plant growth promoting (PGP) and P- solubilizing agents in this work, this agree with Ahsan and Shimizu (2021), who estimated that the interest of beneficial bacteria is increasing due to its non-toxic and eco-friendly properties. *Lysinibacillus* bacteria species are gram-positive, spore-forming, and motile. This genus was formerly known as *Bacillus* spp. and belonged to the family Bacillaceae in the Firmicutes languages. In recent years, *Lysinibacillus* species have attracted the attention of researchers as potential alternatives to agrochemicals in combating and promoting plant development and disease.

3. Greenhouse Experiment

Response of spinach to inoculation with *L. macroides* and fertilization with nano-phosphozinc fertilizers was evaluated by measuring the different parts of spinach plant shoots as shown in Table (2). Generally, spinach shoots responded positively and significantly to inoculation of the studied bacterial type relative to those non-inoculated (control). This increase may be attributed to the ability of the studied microbe to convert insoluble P to soluble P by producing many organic acids which reduced soil pH especially around rhizosphere areas (root zone), thereby increasing the availability of most essential macro- and micronutrient. These results clarify the beneficial and importance role of biofertilization to increase the growth and yield of the growing plant. These results agree with Posso et al. (2017), who estimated that one of the methods by rhizosphere bacteria to solubilize the phosphorus linked to insoluble mineral compounds in soil is the production of organic acids. This procedure is a significant biotechnological option, particularly in soils where there is considerable nutrient fixation, which is a circumstance that happens frequently in tropical regions. This also agrees with Aguirre-Monroy et al. (2019), who studied the given capacity of *Lysinibacillus sphaericus*, (a gram-positive, mesophilic, and spore-forming bacteria) to fix nitrogen, nitrify, and solubilize phosphorus. This increases the soil nutrients utilized for plant growth.

The findings showed that when compared to control soils, soils with additional *L. sphaericus* had substantial variations in ammonium, nitrites, nitrates, phosphate, and IAA concentrations. It is suggested that *L. sphaericus*

could be effective in boosting nutrients and promoting plant development. The increase induced by 50 ppm nano-phosphozinc + *L. macroides* for fresh matters were 169.08 and 77.47% in the 1st and 2nd cuts, respectively. The corresponding increases of dry matter yield were 273.3 and 84.27% for the 1st and 2nd cuts, respectively.

Table (2). Plant growth parameters of *Spinacia oleracea*.

Treatments		Fresh weight (g)	Dry weight (g)	
First cut	Control	20.70f	1.50d	
	With bacterium	<i>Lysinibacillus macroides</i>	25.70e	2.03d
		12.5 ppm nano-phosphozinc + <i>L. macroides</i>	29.06de	4.76ab
		25 ppm nano-phosphozinc + <i>L. macroides</i>	45.70b	5.00a
		50 ppm nano-phosphozinc + <i>L. macroides</i>	55.70a	5.60a
		100 ppm nano-phosphozinc + <i>L. macroides</i>	8.53g	1.76d
	Without bacterium	12.5 ppm nano-phosphozinc	26.40e	1.60d
		25 ppm nano-phosphozinc	37.70c	3.50c
		50 ppm nano-phosphozinc	33.00d	4.00bc
		100 ppm nano-phosphozinc	12.20g	1.90d
LSD (0.05)		4.3369	0.9847	
Second cut	Control	58.60f	7.76e	
	With bacterium	<i>Lysinibacillus macroides</i>	70.60cd	7.93e
		12.5 ppm nano-phosphozinc + <i>L. macroides</i>	63.70e	8.66d
		25 ppm nano-phosphozinc + <i>L. macroides</i>	74.30c	8.70d
		50 ppm nano-phosphozinc + <i>L. macroides</i>	104.00a	14.30a
		100 ppm nano-phosphozinc + <i>L. macroides</i>	44.14g	7.80e
	Without bacterium	12.5 ppm nano-phosphozinc	60.50ef	9.83c
		25 ppm nano-phosphozinc	68.16d	9.60c
		50 ppm nano-phosphozinc	85.30b	11.60b
		100 ppm nano-phosphozinc	63.30e	8.83d
LSD (0.05)		4.0617	0.3505	

Control = irrigated by tap water (without nano or bio fertilizers)

50% P = 50% recommended dose of mineral phosphorus

Increasing application rate of nano-phosphozinc significantly increased the yield of spinach at the 1st and 2nd cuts by the addition 50 ppm nano-phosphozinc + *L. macroides*. At the 1st cut, dry weight increased to 233.3% by using treatment of 25 ppm nano-phosphozinc + *L. macroides*. This behavior may be explained on the basis of bacterial effects on nutrients availability, hormones stimulating to plant growth, vital enzymes and synergistic effect of the microorganisms. In this respect, Rafique et al. (2017) included that *Lysinibacillus fusiformis* significantly increased spinach growth by enhancing yield. The decrease after the addition of the high rates of nano-phosphozinc (100 ppm nano-phosphozinc and 100 ppm + *L. macroides*) may be attributed to unbalance of nutrients in root zone (rhizosphere) of spinach plant, which led to the antagonistic effects among the nutrients in the

rhizosphere and by then decreasing the absorption of the essential nutrients which decrease the growth nearly. The same balance was found for the dry matter yield, where increases reached about 6.67, 133.33 and 166.67% by the addition of 12.5, 25 and 50 ppm of nano-phosphozinc, while the decrease of yield reached about 66.67% by addition of 100 ppm. The obtained data at the 2nd cut took another trend, when adding 25 ppm of nano-phosphozinc. This agrees with Jyolsna et al. (2021), who studied that *L. macrides* as plant growth-promoting bacteria promotes plant growth by dissolving insoluble minerals, producing phytohormones and secreting enzymes that resist pathogen attack. The potential of *L. macrides* in promoting the growth of *S. lycopersicum* by increasing stem length and terminal leaf length and width was evaluated. Passera et al. (2021) studied that *L. fusiformis* strain S4C11 *in vitro* exhibits the capacity to release fixed inorganic phosphorus compounds into and in plant estimate a plant-advantageous microbe, the genus *Lysinibacillus* was studied and employs *in vitro*, *in vivo*, and in plant oncoming to detect *L. fusiformis* strain S4C11, isolated from an apple tree roots in north Italy.

4. Effect of Nano-phosphozinc Inoculation with *L. macroides* on Chemical Analysis in Shoot and Soil

Table (3) shows that the increase of elements (P and Zn in shoot) for the first cut was induced by *L. macroides* using the treatment of 50 ppm nano-phosphozinc + *L. macroides* and reached 224.84% and 117.74%, comparing with controls. Treatment of 25 ppm nano-phosphozinc + *L. macroides* gave high value of P of shoot (209.84%). For the second cut, the highest results of elements (P and Zn in shoot) were achieved using the treatment of 50 ppm nano-phosphozinc + *L. macroides* (162.43% and 108.6%) comparing with control. There was a decrease by adding the high rate of nano-phosphozinc, which may be attributed to unbalance of nutrients in root zone (rhizosphere) of spinach plant that led to the antagonistic effects among the nutrients in the rhizosphere and by then decrease the absorption of the essential nutrients which decrease the growth.

Table (4) shows that the increase of the element P in soil was 128.3 ppm and Zn in soil was 156.87 ppm induced by *L. macroides*, comparing with controls, at the first cut. At the second cut, P and Zn in soil were 124.2 ppm and 394.16 ppm, respectively, comparing with controls. These results agree with Amadi et al. (2021), who reported that the biofertilizers are eco-friendly fertilizers that are produced via degradation of wastes by microorganisms. Nitrogen, phosphorus and potassium were determined using standard analytical methods. The treatment supplemented with *L. macroides* showed significantly higher ($P \leq 0.05$) amount of phosphorus, potassium and total organic carbon than the control. Thus, *L. macroides* is a better biofertilizer producer. This agrees with Rafique et al. (2017), who studied that *Lysinibacillus fusiformis* significantly increased plant growth by improving nutrients uptake (N, P, and K) in maize plant. The increments in plant growth

are mainly attributed to the P-solubilization by bacterial strain in the soil. Bacterial interaction may form soluble-P which resulted in 0.87% absorption to the plant leaves in *L. fusiformis* strain 31 MZR + sawdust biochar-amended maize on D65 and estimated plants inoculated with sawdust biochar + *L. fusiformis* strain 31MZR increased N (32.8%), P (72.5%), and K (42.1%) than control on 65 days. Only *L. fusiformis* strain 31MZR increased N (23.1%) and P (61.5%) than control and shows the significant interaction of PSB and biochar in nutrient uptake. Behera et al. (2017) determined nitrogen, phosphorus, potassium and total protein to be as indicator of any up normal changes in the plant by using the new component of nano.

5. Microbiological examination

5.1. The phosphate solubilizing activities of bacterial strain

5.1.1. Change in the pH

After three incubation days at 35°C in lab, the pH of Bount and Rovera broth medium decreased from 6.8 to 4.98.

5.1.2. Total Phosphorus determination

Total phosphorus of *L. macroides* was 5.83 ppm.

5.1.3. Amount of phosphate determination

Fig. (1) shows that the bacteria used gave a clear zone around the growth on Bunt and Rovera agar medium. This agrees with Zeng et al. (2017), who studied that the P solubilizing activities of *Pseudomonas* that successfully demonstrated positively reconditioned with the creation of organic acids.

5.1.4. Phosphatase enzyme activity

Positive phosphatase enzyme was produced by *L. macroides*, the acidic phosphatase was equal (1.63 ug P nitrophenol/ml/h) and alkaline phosphatase was equal (1.3 ug P nitrophenol / ml / h). Margalef et al. (2017) detected that the data of place scale of phosphatase-activity ranged by different strains were between 0.01 and 79 µmol/g/h, with a mean of (11.6 ± 0.8 µmol/g/h) and were divided over the seven continents. Abdelgalil et al. (2021) studied that the highest alkaline phosphatase was produced in optimum growth conditions by using *Lysinibacillus* sp.

5.2. Organic acids

The organic acids were determined by two procedures, quantitatively and qualitatively by HPLC (Fig. 2).

5.2.1. Quantitative analysis of organic acids

For the analysis of total organic acids, the deep red color of sample means a positive result of organic acid produced by bacterium.

Table (3). Effect of treatments on the concentration of nutrient in spinach shoot.

Treatments		Nitrogen in shoot (%)	Phosphorus in shoot (%)	Potassium in shoot (%)	Zinc in shoot (ppm)	
First cut	Control	1.540i	1.139d	0.265g	0.578h	
	<i>Lysinibacillus macroides</i>	2.382e	1.479cd	0.239h	0.776e	
	12.5 ppm nano-phosphozinc + <i>L. macroides</i>	2.408d	1.88bcd	0.368b	0.713f	
	25 ppm nano-phosphozinc + <i>L. macroides</i>	2.498b	3.525a	0.303d	0.856d	
	50 ppm nano-phosphozinc + <i>L. macroides</i>	2.556a	3.7a	0.434a	1.257a	
	100 ppm nano-phosphozinc + <i>L. macroides</i>	1.423j	1.52cd	0.210j	0.583h	
	Without bacteriu	12.5 ppm nano-phosphozinc	2.467c	1.60bcd	0.280f	0.710f
	25 ppm nano-phosphozinc	2.295f	2.326b	0.291e	0.902c	
	50 ppm nano-phosphozinc	1.967g	2.146bc	0.233i	1.083b	
	100 ppm nano-phosphozinc	1.910h	1.9bcd	0.306c	0.687g	
LSD (0.05)		0.0226	0.7954	2.348	0.0107	
Second cut	Control	1.685b	1.11d	0.240c	0.626g	
	<i>Lysinibacillus macroides</i>	2.556b	1.58cd	0.338c	0.966d	
	12.5 ppm nano-phosphozinc + <i>L. macroides</i>	2.004b	1.9bc	0.468c	0.357h	
	25 ppm nano-phosphozinc + <i>L. macroides</i>	2.179b	2.633ab	0.680c	0.743f	
	50 ppm nano-phosphozinc + <i>L. macroides</i>	4.764a	2.913a	2.900a	1.306a	
	100 ppm nano-phosphozinc + <i>L. macroides</i>	5.868a	1.682cd	0.724c	0.854e	
	Without bacteriu	12.5 ppm nano-phosphozinc	2.469b	1.73cd	0.670c	1.093c
	25 ppm nano-phosphozinc	2.992b	1.88bc	0.649c	0.655g	
	50 ppm nano-phosphozinc	2.585b	1.779cd	0.604c	1.219b	
	100 ppm nano-phosphozinc	2.266b	1.965bc	1.850b	1.208b	
LSD (0.05)		1.7030	0.7694	0.5561	0.0555	

Control = irrigated by tap water (without nano or bio fertilizers)
(50% P) = 50% recommended dose of mineral phosphorus

Table (4). Effect of treatments on nutrients in spinach soil rhizosphere.

Treatments		Phosphorus in soil (ppm)	Zinc in soil (ppm)
First cut	Control	3.85j	0.953g
	<i>Lysinibacillus macroides</i>	4.46h	1.882c
	12.5 ppm nano-phosphozinc + <i>L. macroides</i>	6.26c	1.553d
	25 ppm nano-phosphozinc + <i>L. macroides</i>	5.35g	2.042b
	50 ppm nano-phosphozinc + <i>L. macroides</i>	8.79a	2.448 a
	100 ppm nano-phosphozinc + <i>L. macroides</i>	6.13d	1.499d
	12.5 ppm nano-phosphozinc	4.19i	1.085f
	25 ppm nano-phosphozinc	5.89e	1.145f
	50 ppm nano-phosphozinc	5.48f	1.849c
	100 ppm nano-phosphozinc	7.43b	1.308e
LSD (0.05)		0.0878	0.0774
Second cut	Control	7.23d	1.524i
	<i>Lysinibacillus macroides</i>	7.79d	1.945h
	12.5 ppm nano-phosphozinc + <i>L. macroides</i>	15.14a	2.699f
	25 ppm nano-phosphozinc + <i>L. macroides</i>	14.67a	7.043b
	50 ppm nano-phosphozinc + <i>L. macroides</i>	16.21a	7.531a
	100 ppm nano-phosphozinc + <i>L. macroides</i>	12.83b	3.28e
	12.5 ppm nano-phosphozinc	8.9cd	2.272g
	25 ppm nano-phosphozinc	9.65c	5.619d
	50 ppm nano-phosphozinc	14.62a	5.772c
	100 ppm nano-phosphozinc	16.02a	2.012h
LSD (0.05)		1.6935	0.1212

Control = irrigated by tap water (without nano or bio fertilizers)
 (50% P) = 50% recommended dose of mineral phosphorus


Fig. (1). Clear zone of phosphate dissolving *L. macroides* on Bunt and Rovira medium.

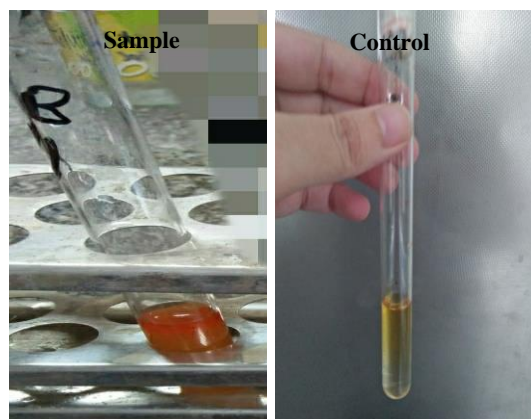


Fig. (2). The positive amount production of organic acid by *L. macrolides*.

5.2.2. Qualitative analysis of organic acids by HPLC

Data in Table (5) and Fig. (3) show that *L. macroides* produced many organic acids: formic, lactic, malic, citric, succinic and propionic acids. They are important for decreasing pH value of rhizosphere zone and releasing the phosphorus and other elements around plant rhizosphere. Becky et al. (2020) found that biofertilizers are microbial formations made of domestic plant growth-promoting rhizobacteria (PGPR) what can normally progress plant growth directly or indirectly, through the production of socialization of soil nutrients and phytohormones.

5.3. Phytohormones

It is noticed that *L. macroides* produced IAA phytohormone and the amount equals 0.0473 ppm, as shown in Fig. (4). This hormone is necessary of elongation of plant. This agrees with the study of Rafique et al. (2017), which estimated that the phytohormones produced from PGP abilities such as IAA, gerbilline and cytokinin production by *Lysinibacillus fusiformis*, and agrees with Becky et al. (2020), who found that biofertilizers are microbial formations made of domestic PGPR what can normally progress plant growth directly or indirectly, through the production of socialization of soil nutrients, phytohormones and siderophores.

5.4. Effect of Nano-Phosphozinc inoculation with *L. macroides* on Bacterial Counts and Dehydrogenase Activity in Spinach Rhizosphere

Table (6) shows that *L. macroides* induced the highest results of soil microbial activity, dehydrogenase activity "TTC" ($76 \mu\text{g TPF} / \text{g dry soil} / 24 \text{ h}$), total microbial counts ($120 \times 10^4 \text{ CFU} / \text{g dry soil}$), nitrogen fixers counts ($44 \times 10^4 \text{ MPN} / \text{g dry soil}$) and phosphate dissolving bacteria counts ($60 \times 10^3 \text{ CFU} / \text{g dry soil}$) for the first cut by using the treatment of 25 ppm nano-phosphozinc + *L. macroides*, comparing with control.

Table (5). Organic acids secreted by *L. macrolides*.

Standard organic acids	RT (min)	Organic acids (mg/100 ml)
Formic acid	5.346	7.52
Lactic acid	7.471	8.65
Malic acid	8.717	8.22
Citric acid	11.153	7.87
Succinic acid	12.610	4.41
Propionic acid	13.370	6.48

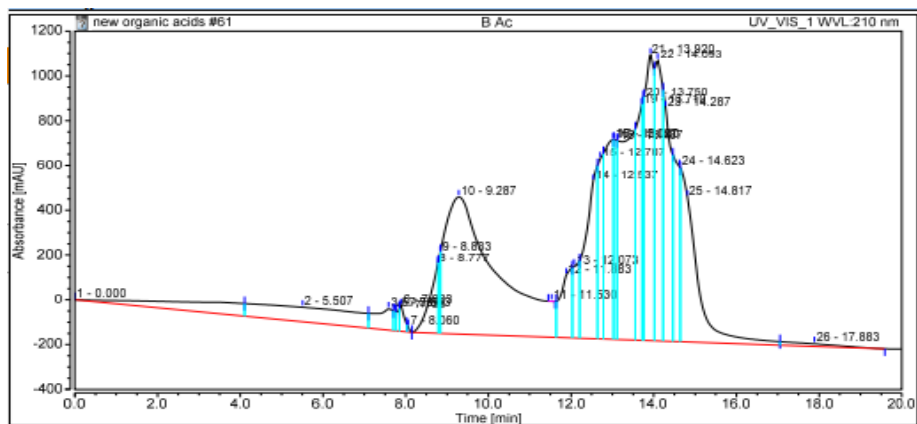


Fig. (3). Organic acids produced by *L. macrolides*.

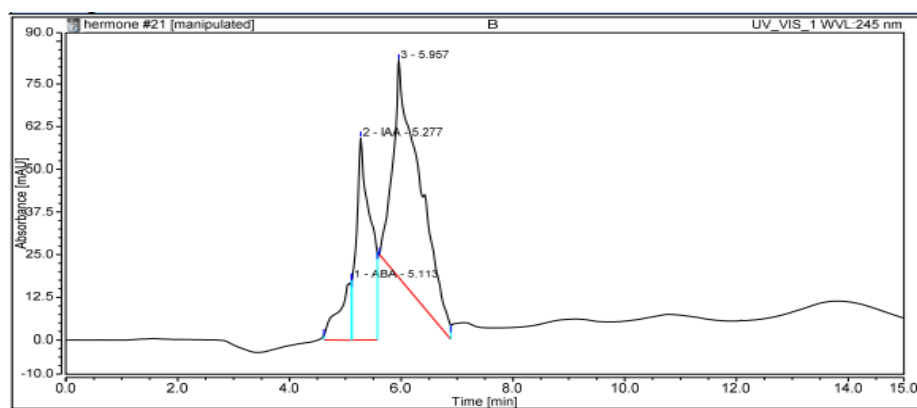


Fig. (4). Phytohormones produced by *L. macrolides*.

Table (6). Rhizosphere microbial counts and dehydrogenase enzymes activity.

Treatments		PDB counts (CFU/g dry soil)	Nitrogen fixers (MPN/g dry soil)	Total count (CFU/g dry soil)	Dehydrogenase enzyme ($\mu\text{g TPF/g}$ dry soil/24 h)
First cut	Control	30*10 ²	62*10 ²	96*10 ²	26.2fg
	<i>Lysinibacillus macroides</i>	38*10 ³	50*10 ³	78*10 ⁴	58.6c
	12.5 ppm nano-phosphozinc	52*10 ²	89*10 ³	125*10 ³	52.3d
	+ <i>L. macroides</i>				
	25 ppm nano-phosphozinc	60*10 ³	44*10 ⁴	120*10 ⁴	76a
	+ <i>L. macroides</i>				
	50 ppm nano-phosphozinc	49*10 ³	38*10 ³	112*10 ⁴	67.7b
	+ <i>L. macroides</i>				
	100 ppm nano-phosphozinc	35*10 ²	35*10 ²	36*10 ²	25g
	+ <i>L. macroides</i>				
Without bacterium	12.5 ppm nano-phosphozinc	30*10 ³	45*10 ³	80*10 ³	28.8f
	25 ppm nano-phosphozinc	52*10 ²	50*10 ³	88*10 ³	45e
	50 ppm nano-phosphozinc	30*10 ³	153*10 ³	60*10 ⁴	60.9c
	100 ppm nano-phosphozinc	30*10 ²	68*10 ³	200*10 ³	28f
L.S.D. 0.05%		-	-	-	2.7799
Second cut	Control	34*10 ³	45*10 ²	80*10 ³	24h
	<i>Lysinibacillus macroides</i>	35*10 ³	36*10 ³	98*10 ³	26.8g
	12.5 ppm nano-phosphozinc	54*10 ³	31*10 ³	200*10 ³	35.7d
	+ <i>L. macroides</i>				
	25 ppm nano-phosphozinc	96*10 ³	51*10 ³	35*10 ⁴	68a
	+ <i>L. macroides</i>				
	50 ppm nano-phosphozinc	44*10 ³	32*10 ³	175*10 ³	55b
	+ <i>L. macroides</i>				
	100ppm nano-phosphozinc	35*10 ³	55*10 ³	63*10 ³	31f
	+ <i>L. macroides</i>				
Without bacterium	12.5 ppm nano-phosphozinc	46*10 ³	60*10 ³	100*10 ³	30.5f
	25 ppm nano-phosphozinc	30*10 ³	73*10 ³	70*10 ³	33.6df
	50 ppm nano-phosphozinc	58*10 ³	62*10 ²	112*10 ³	48.7c
	100 ppm nano-phosphozinc	35*10 ³	60*10 ³	115*10 ³	32
L.S.D. 0.05%		-	-	-	2.5262

For the second cut, the use of the treatment of 25 ppm + *L. macroides*, compared to the control, produced the highest results for soil microbial activity dehydrogenase activity "TTC" (68 g TPF / g dry soil / 24 h), Total microbial counts (35*10⁴ CFU / g dry soil), nitrogen fixers counts (51*10³ MPN / g dry soil), and phosphate dissolving bacteria counts (96*10³ CFU/g dry soil). This agrees with Jyolsna et al. (2021), who reported that the rich microbial root diversity of the pea plant and the use of *L. macroides* from a non-conventional source improve the diversity of PGPR available for

agricultural practices. More research is needed to discover the growth-promoting mechanism and to explore the plant-microbe interaction pathway. Also, this agrees with Gupta and Prakash (2020), who reported that it is necessary to evaluate the effect of slow reduced nano-fertilizer on the enzyme activity in soil and soil microbial communities. The effect of nano-fertilizers on activity of microorganisms could be estimated by measurement of soil breathing and enzymatic activities. This review discusses the impacts of nano-fertilizers on soil microbial activity, covering both positive and negative outcomes. Despite the fact that microbial communities are a sensitive and significant target for assessing the risks of the environment of nanoparticle, there is currently little evidence available on the impact of nano-fertilizers on the soil microbiome.

CONCLUSION

Lysinibacillus macroides is a plant promoting bacteria (PGPB) that solubilizes phosphate produces phytohormones such as IAA, as well as many organic acids like formic acid, lactic acid, malic acid, citric acid, succinic acid, propionic acid and others. This bacterium reduces pH of plant rhizosphere zone in alkaline Egyptian soils making elements available for plant absorption and increasing productivity of spinach. Nano-phosphozinc is a chemical compound that provides an easily absorbed source of phosphorus and in smaller amounts compared to mineral fertilizers.

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دور التسميد الحيوي والنانوي في زيادة نمو وإنتاجية السبانخ في التربة الرملية

غادة أمين زكي إبراهيم

قسم خصوبة وميكروبيولوجيا الأراضي، مركز بحوث الصحراء، القاهرة، مصر

أثبتت بكتيريا الريزوسفير المحفزة لنمو النبات والمذيبة للفوسفات *Lysinibacillus macroides* كفاءة عالية في زيادة إنتاجية السبانخ خلال حشتين وبإضافة مركب النانو فوسفوزنك معها. وقد أجريت تجربة أصص في تربة رملية غير مالحة وكانت الإضافة أرضية بالنسبة للبكتيريا ولمركب النانو فوسفوزنك والذي تمت اضافته بتركيزات مختلفة. نظراً لأن البكتيريا كانت تنتج عدد من المركبات الحيوية كالأحماض العضوية والتي أدت لإنخفاض pH التربة في منطقة ريزوسفير السبانخ مما أدى إلى زيادة في إمتصاص العناصر خاصة الفوسفور. كما تنتج البكتيريا أيضاً بعض الهرمونات النباتية مثل الأندول أسيتك أسيد الهام لاستطالة النبات، بالإضافة لإنزيم الفوسفاتيز القاعدي والهامضي. مما أدى لزيادة في امتصاص النبات للعناصر وانعكس على نمو النبات بالزيادة مقارنة بالكونترول. أشارت النتائج إلى زيادة المحصول البيولوجي للسبانخ باستخدام المعاملة ٥٠ جزء في المليون من مركب النانو فوسفوزنك + البكتيريا المستخدمة. كما أعطت نفس المعاملة أعلى نسب من العناصر بالمجموع الخضري والتربة بعد الحصاد. لكن أفضل نتائج للعد الميكروبي بالريزوسفير والنشاط الميكروبي بالتربة والمقدر بإنزيم الديهيدروجيناز كانت عند استخدام ٢٥ جزء في المليون من سماد النانو فوسفوزنك + البكتيريا المستخدمة. من الأهمية أنه من الضروري مواصلة الدراسات والأبحاث على تلك المواد النانومترية عموماً لمعرفة الآثار الناتجة عن استخدامها ومدى ترسبها وتراكمها بالتربة، النبات أو جسم الإنسان على المدى الطويل وأيضاً تأثير ذلك على البيئة.