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The effect of bio-fertilization with *Pseudomonas fluorescens* bacteria and nano-zinc on the active compounds, physiological traits, and productivity of flaxseed (*Linum usitatissimum* L.)

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P A P E R I N F O

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A b s s t r a c t

This research aims to examine the effects of bio fertilization with *Pseudomonas fluorescens* bacteria and nano-zinc fertilization on the active compounds and productivity of flaxseed (L. *usitatissimum* L.), at 2025. The current flax experiment was carried out in the Iraqi city of Alhindia in Kerbala. A randomized complete block design (RCBD) with split-plot arrangement, two factors, and three replications was used to set up the amounts of nano-zinc (control, 0.10, 0.20, 0.25 mL^{-1}) that were applied to the leaves one month following germination that were the initial factor. The second factor was bio-fertilization, which entailed combining soil and *P.fluorescens* and cutting a 1-cm hole near the rhizosphere. During the growth season, four doses of *P. fluorescens* (control, 25, 50, and 100 mL^{-1}) , indicate the results the concentrations of *P. fluorescens* 100 mL^{-1} and nano-Zinc 0.25 mL^{-1} varied significantly at plant height, fruit branches, seed oil percentage and yield oleic and linolenic acids.

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1. INTRODUCTION

Flax seed (*Linum usitatissimum* L.) is considered one of the most important commercial crops because it includes active substances that are necessary for the food and pharmaceutical sectors, such as proteins, lignans, and unsaturated fatty acids (especially alpha-linolenic acid). This oil seed crop, like others, has certain environmental and nutritional problems that affect its chemical quality and production. As a result, modern agricultural methods that increase its physiological efficacy and active compound content are required (Green *et al.*, 2020).Because *Pseudomonas* bacterial strains can fix nitrogen, release growth promoters like auxins and cytokinins, and improve soil nutrient absorption, one of the most promising techniques for boosting plant growth and productivity efficiency is biofertilization with these strains (Kloepper *et al.*, 2004). It has been demonstrated that *P. fluorescens*, in particular, increases photosynthetic efficiency and stimulates the production of secondary metabolites, both of which enhance the qualitative characteristics of crops (Bhattacharyya & Jha, 2012).Because of their high ability to penetrate plant tissues and increase the bio availability of micro nutrients, nano-fertilizers like nano-zinc have gained more attention in light of agricultural nanotechnology advancements. This is because they promote several vital physiological processes, such as the

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formation of chlorophyll, the activation of enzymes, and the improvement of plant resistance to environmental stresses (Rastogi *et al.*, 2017). According to research, nano-zinc may also facilitate more efficient plant metabolism and result in the presence of more potent chemicals, especially in aromatic and medicinal crops (Dimkpa *et al.*, 2012).This study aims to examine the potential interactions between nano-zinc fertilization and bio-fertilization with Pseudomonas bacteria, as well as their combined effects on the physiological and productive traits of flax seed plants, with a focus on how much they react to increasing the content of bio-active compounds. This study is a preliminary step toward examining sustainable farming methods that enhance the productivity and quality of this vital crop in light of climate change and environmental stressors.

2. MATERIALS AND METHODS

2.1.examine the effects of bio-fertilization with *P. fluorescens* bacteria and nano-zinc fertilization on the active compounds and productivity of flaxseed , at 2025, the current flax (L. *usitatissimum* L.) experiment was carried out in the Iraqi city of Al-hindia in Kerbala. A randomized complete block design (RCBD) with split-plot arrangement, two factors, and three replications was used to set up the study. The first factor was the amounts of nano-zinc (control, 0.10, 0.20, and 0.25 mL^{-1}) sprayed on the leaves one month after germination. The second component was bio-fertilization, which entailed combining soil and *P.fluorescens* and cutting a 1-cm hole near the rhizosphere. During the growth season, four doses of *P. fluorescens* (0.0, 25, 50, and 100 mL^{-1}) were used to pollinate the region just once. After the growth characteristics were measured, the flax plants were harvested on 24/4/2025 when flax reached physiological maturity (Al-Yasssiry *et al.* 2024, Al-Masaoodi *et al.* 2025)

2.2. The studied traits

Plant height: The height of the plant was measured using a ruler by taking five random plants.fruit branches : The fruit branches of the flax plant were calculated from each experimental unit.

2.3. Seed oil percentage and yield.

After the harvest season, the flax seeds were collected and ground using an electric grinder for each treatment, then placed in special tubes and stored in the refrigerator for the purpose of extracting the volatile oil (Rabee *et .al.*, 2023).Using a soxlet apparatus, extract the volatile oil from flax seeds. For 24 hours 100 g of flax plant and 300 mL of 80% hexan were placed in a round flask (1000 mL) . Additionally, high-performance liquid chromatography was used to measure the active chemical compounds (oleic and linolenic acids) (Al-Ibrahemi *et al*., 2025).

Statistical analysis

Following data collection and tabulation, every feature that was looked at was statistically analyzed using the Gnestat program in compliance with the experiment design. The LSD0.05 test was used to compare the arithmetic averages (Al-Rawi ,2010).

Plant height

Based on the results at table (1), the concentrations of *P.fluorescens* and nano-Zinc varied significantly. The highest plant height (68.25 cm) was observed with *P. fluorescens* at a concentration of 100 mL^{-1}, followed by nano zinc at a concentration of 0.25 mL^{-1} (65.75 cm), the interaction between nano-Zinc (0.25 mL^{-1}) and *P. fluorescens* 100 mL^{-1} highest mean for plant height (72cm). The control treatment had the lowest mean plant height (51 cm).

Fruiting branches branch.plant^{-1}

The concentrations of *P. fluorescens* and nano-Zinc varied significantly, according to the results at table (2). The highest Fruiting branches (98.39 branch.plant^{-1}) was observed with *P. fluorescens* at a concentration of 100 mL^{-1}, followed by nano zinc at a concentration of 0.25 mL^{-1} (97.53 branch.plant^{-1}). the interaction between nano-Zinc (0.25 m L^{-1}) and *P. fluorescens* 100 mL^{-1} highest mean for Fruiting branches (101.65 branch.plant^{-1}) . The control treatment had the lowest mean Fruiting branches (88.76 branch.plant^{-1}).

Oil yield tons ha^{-1}

Indicate the results at table (3) concentrations of *P. fluorescens* and nano-Zinc varied significantly. The highest Oil percentage (0.293 tons ha^{-1}) was observed with *P. fluorescens* at a concentration of 100 mL^{-1}, followed by nano zinc at a concentration of 0.25 mL^{-1} (0.291 tons ha^{-1}) the interaction between nano-Zinc (0.25 mL^{-1}) and *P. fluorescens* 100 ml L^{-1} highest mean for Oil percentage (0.312 tons ha^{-1}). The control treatment had the lowest mean Oil percentage (0.233 tons ha^{-1}).

Oil percentage%

according to the results at table (4) The concentrations of *P. fluorescens* and nano-Zinc varied significantly,. The highest Oil percentage (33.63 %) was observed with *P. fluorescens* at a concentration of 100 mL^{-1}, followed by nano zinc at a concentration of 0.25 mL^{-1} (32.36 %) the interaction between nano-Zinc (0.25 mL^{-1}) and *P. fluorescens* 100 mL^{-1} highest mean for Oil percentage (36.45 %). The control treatment had the lowest mean Oil percentage (24.65 %).

Linolenic acid%

Based on the results at table (5), the concentrations of *P. fluorescens* and nano-Zinc varied significantly. The highest Linolenic acid (29.49 %) was observed with *P. fluorescens* at a concentration of 100 mL^{-1}, followed by nano zinc at a concentration of 0.25 mL^{-1} (28.71 %) ,the interaction between nano-Zinc (0.25 mL^{-1}) and *P. fluorescens* 100 mL^{-1} highest mean for Linolenic acid (32.65 %) . The control treatment had the lowest mean Linolenic acid (20.65 %).

Oleic acid

According to the results at table (6), the concentrations of *P. fluorescens* and nano-Zinc varied significantly. The highest Oleic acid (19.68 %) was observed with *P. fluorescens* at a concentration of 100 mL^{-1}, followed by nano zinc at a concentration of 0.25 mL^{-1} (18.69 %) the interaction between nano-Zinc (0.25 mL^{-1}) and *P. fluorescens* 100 mL^{-1} highest mean for Oleic acid (21.67 %) . The control treatment had the lowest mean Oleic acid (12.45 %).

DISCUSSION

The results of the study showed that the physiological and yielding properties of flax seed plants were significantly improved by bio fertilization with *P. fluorescens* bacteria. This is explained by the bacteria's ability to enhance the absorption of micro nutrients and encourage the production of natural growth regulators such as cytokinins and auxins, which in turn initiate vital plant processes (Bhattacharyya & Jha, 2012). These bacteria also improve the metabolic efficiency of the plant by increasing the amount of chlorophyll in the leaves. This increases photosynthetic efficiency, which encourages

Because zinc is believed to be an essential component for the synthesis of numerous enzymes and proteins involved in vital functions like cellular respiration and the formation of nucleic acids, research on the effects of nano-zinc fertilization shows that it significantly increases the metabolic efficiency of plants (Dimkpa *et al*., 2012). According to Rastogi *et al*. (2017), the nano form of zinc has a higher bio-availability and is simpler to absorb through the surfaces of roots and leaves than traditional forms, which leads to a quicker and more effective physiological response. The zinc nano form has a higher bio-availability and is simpler to absorb through the surfaces of roots and leaves than conventional forms, leading to a quicker and more effective physiological response.

Because the combined treatment (bio-fertilization + nano-zinc) showed a clear advantage over the other treatments in terms of increasing the amount of active compounds like lignans and unsaturated fatty acids in the seeds and improving growth indicators like branch count, dry weight, and leaf area, the study emphasizes the positive relationship between bio-fertilization and anon-fertilization. This interaction results from the complementary functions of the two components; zinc stimulates the enzymatic activities that enable the plant to convert basic materials into active molecules with substantial economic value, while pseudomonas enhances the absorption of micro-nutrients like zinc (Green *et al.*, 2020).

Since dual applications of nano materials and bio-fertilizers have shown positive interactive effects in enhancing the chemical and productive properties of crops including basil, fenugreek, and black cumin, these findings are consistent with previous studies on other crops (Rastogi *et al*., 2017; Dimkpa *et al*., 2012). Incorporating these options into complete fertilization management programs is crucial since the goal is to maximize farmers' economic and productive returns while decreasing their reliance on conventional chemical fertilizers that have negative environmental effects.

According to the study's findings, flax seeds' physiological traits and bio active compound content can be successfully improved, as well as their quality and productivity, by combining nano-zinc fertilization and bio-fertilization with Pseudomonas bacteria. This opens up new possibilities for the application of this technique in sustainable agricultural systems.

CONCLUSION

Bio-fertilization with *P. fluorescens* bacteria and Nano-zinc fertilization led to a significant improvement in vegetative and active compounds.

TABLE 1. study for *P.fluorescens* and nano-zinc fertilization on the plant height .

P. fluorescens ml L^{-1}	Nano Zinc (ml L^{-1})				Means
	0	0.10	0.20	0.25	
0	51	55	57	60	55.75
25	54	58	61	63	59
50	59	63	65	68	63.75
100	64	67	70	72	68.25
Means	57	60.75	63.25	65.75	

$LSD_{0.05}$ for Nano Zinc = 1.25 *P. fluorescens*=1.85 Interaction:2.64

TABLE 2. study for *P.fluorescens* and nano-zinc fertilization on the Fruiting branches .

P.fluorescens ml L^{-1}	Nano Zinc (ml L^{-1})				Means
	Control	0.10	0.20	0.25	
Control	88.76	90.43	92.45	93.45	91.27
25	91.65	93.45	95.67	96.46	94.31
50	93.76	96.45	97.45	98.56	96.56
100	94.67	97.56	99.67	101.65	98.39
Means	92.21	94.47	96.31	97.53	
LSD$_{0.05}$ for Nano Zinc = 1.48 *P. fluorescens*=1.84 Interaction: 2.75					

TABLE 3. study for *P.fluorescens* and nano-zinc fertilization on the oil yield.

P. fluorescens ml L^{-1}	Nano Zinc (ml L^{-1})				Means
	Control	0.10	0.20	0.25	
Control	0.233	0.246	0.257	0.268	0.251
25	0.244	0.267	0.275	0.286	0.268
50	0.256	0.274	0.295	0.296	0.280
100	0.276	0.286	0.298	0.312	0.293
Means	0.252	0.268	0.281	0.291	
LSD$_{0.05}$ for Nano Zinc =1.36 *P. fluorescens*=1.86 Interaction: 3.75					

TABLE 4. study for *P.fluorescens* and nano-zinc fertilization on the oil percentage**.**

P.fluorescens ml L^{-1}	Nano Zinc (ml L^{-1})				Means
	Control	0.10	0.20	0.25	
Control	24.65	25.74	26.87	28.45	26.43
25	26.76	27.47	28.84	30.76	28.46
50	28.45	29.54	31.65	33.76	30.85
100	30.65	32.65	34.76	36.45	33.63
Means	27.63	28.85	30.53	32.36	
LSD$_{0.05}$ for Nano Zinc = 1.34 *P. fluorescens*=1.74 Interaction:2.59					

TABLE 5. study for *P.fluorescens* and nano-zinc fertilization on the linolenic acid**.**

P.fluorescens ml L^{-1}	Nano Zinc (ml L^{-1})				Means
	Control	0.10	0.20	0.25	
Control	20.65	21.65	22.45	23.65	22.10
25	22.65	24.65	25.76	27.87	25.01
50	24.65	26.65	28.45	30.65	27.60
100	26.49	28.45	30.35	32.65	29.49
Means	23.61	25.35	26.75	28.71	
LSD$_{0.05}$ for Nano Zinc = 1.34 *P. fluorescens*=1.84 Interaction: 2.76					

TABLE 6. study for *P.fluorescens* and nano-zinc fertilization on the oleic acid.

P. fluorescens ml L^{-1}	Nano Zinc (ml L^{-1})				Means
	Control	0.10	0.20	0.25	
Control	12.45	13.76	14.35	15.85	14.10
25	13.56	14.76	16.76	17.46	15.64
50	15.74	16.46	18.45	19.76	17.60
100	17.65	18.76	20.63	21.67	19.68
Means (cm)	14.85	15.89	17.62.	18.69	
LSD$_{0.05}$ for Nano Zinc =1.56 *P. fluorescens*=1.94 Interaction: 2.56					

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