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RESPONSE OF FLAX (*LINUM USITATISSIMUM*) TO NANO-NPK AND EMG-1 IN GROWTH, OIL CONTENT, AND ACTIVE COMPOUNDS

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SUMMARY

The presented study determined the effects of nano-NPK and biofertilizer EMG-1 on the growth and oil traits and active compounds of *Linum usitatissimum* L. The experiment ensued in a randomized complete block design with a split-plot arrangement, two factors, and three replications. It transpired in 2022 at the Alsada City, Babel, Iraq. The first factor was foliar application of nano-NPK fertilizer with four concentrations (0.0, 0.7, 0.10, and 0.15 ml L^{-1}). The second factor was biofertilization of EMG-1 (0.0, 5, 10, and 15 ml L^{-1}), mixed with soil with a 1-cm incision made near the rhizosphere area. The results indicated a positive role of nano-NPK and biofertilization EMG-1 in improving growth and oil yield traits in flax (*L. usitatissimum*). The application of nano-NPK (0.15 ml L^{-1}) and biofertilization EMG-1 (15 ml L^{-1}) showed a significant increase in the flax's plant height, fruit branches, oil yield, oil percentage, and linolenic and oleic acids.

Keywords: Flax (*Linum usitatissimum* L.), Nano-NPK, EMG-1, growth and oil traits, linolenic acid, oleic acid

Key findings: The results indicated the positive role of nano-NPK (0.15 ml L^{-1}) and biofertilization EMG-1 (15 ml L^{-1}) in enhancing the flax's plant height, fruit branches, oil yield and percentage, and linolenic and oleic acids.

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INTRODUCTION

Medical plants provide a basis for the development of the drug industry due to positive effects of active biological compounds used in treating various diseases with favorable marketing prices (Pan *et al*., 2009; Guan-Yu *et al*., 2016). It further boosts the cultivation of a wide range of specialized plants, especially if accompanied by establishing production lines of pharmaceutical industries dependent on the resulting effective materials from these plants. This requires the management of agricultural policy related with the cultivation of medical plants and their production development (Mohammadi-Sartang *et al*., 2017).

Flax (*Linum usitatissimum* L.) belongs to the family Linaceae, with cultivations in various regions worldwide to obtain its seeds with high oil content (30%–45%) and naturally occurring essential fatty acids like linolenic and oleic acids. The flax oil benefits various industrial fields, such as, varnishes and paints (Jarak *et al*., 2006). Flax oil is also suitable for human consumption because of unsaturated fatty acids—Omega-3, Omega-6, and Omega-9 (Oomah, 2001). The flax seed production is about 2.5 million tons per year globally. Canada leads the world for flax seed production, followed by China, India, America, Ethiopia, and Egypt (34%, 25.5%, 9%, 8%, 3.5%, and 2.2%, respectively). All other countries produce less than the above leading countries (Darzi *et al*., 2012).

Nanotechnology is one of the important and proposed solutions to reduce the environmental pollution. The economic costs arise from the use of chemical fertilizers to increase agricultural production; however, large amount of food at a lower cost and lower rates of energy consumption can result from nanotechnology (Al-Ibrahemi *et al*., 2020). The effectiveness of nanofertilizers may exceed other traditional fertilizers, as nanofertilizers can serve either as a substitute for traditional fertilizers or as carriers for their components. It is due to the distinctive characteristics of increasing control over the work of the steering mechanism. Nanofertilizers' surface area and particles are tiny compared with conventional fertilizers. Therefore, nanofertilizers are vital in plant nutrition, whether sprayed on the vegetative system or added to the soil through different types of ground treatments, as these nanofertilizers proved more soluble and active than the traditional fertilizer molecules (Drostkar *et al*., 2016).

Determining the optimal level of nitrogen, phosphorus and potassium, corresponding with crop requirements, is one of the critical strategies for managing crops, as these macroelements positively contribute to crop improvement. The basis of the biological functions relies on plant growth and development, which needs nutrients in the soil in sufficient quantity. Often, adding fertilizers help meet the plant's need, as the plant use 50% of traditional fertilizers, with the rest wasted during evaporation, leaching, and sedimentation, reducing the net benefit and increasing environmental pollution (Pérez, 2017).

Biofertilizers serve to improve the soil fertility and reduce production costs, while lessening the risk developed by mineral fertilizers (Tamas *et al*., 2010). Plants need essential nutrients, such as nitrogen, phosphorus, and potassium. Nitrogen and phosphorus are the basic components for building nucleic acids, as they are readily available for absorption by plants (Banchio *et al*., 2009). Teruo Higa developed effective microorganisms (EMG-1) at the University of Ryukyu, Japan, which includes more than three microorganisms (*Rhodopseudomonas, Lactobacillus planetarium, L. casei, L. fermentum, L. delbrueckii*, and *Saccharomyces cerevisiae*).

These microorganisms, in turn, increase the diversity of microbial influence in soil and plants (Sharma, 2002). Biofertilization improves the ecosystem of the soil and crop plants and leads to supplying the nutrients to plants to improve their production and quality (Kader *et al*., 2002). Based on the above discussion, the presented study sought to determine the effects of nano-NPK and EMG-1 on the growth-oil traits and active compounds of flax (*L. usitatissimum* L.).

MATERIALS AND METHODS

The relevant experiment on flax (*L. usitatissimum* L.) commenced in 2022 at the Alsada City, Babel, Iraq. Considering the impact of nanofertilizer combined with biofertilization on growth, oil, and active compounds of flax, the trial comprised a randomized complete block design (RCBD) with a split-plot arrangement, two factors, and three replications. The first factor was the nanofertilizer (NPK) concentrations (0.0, 0.7, 0.10, and 0.15 ml L^{-1}) sprayed on the leaves one month after germination. The second factor was biofertilization, which involved mixing the EMG-1 with soil and making a 1-cm incision close to the rhizosphere area. An area pollination only occurred once throughout the growing season with four concentrations of EMG-1 (0.0, 5, 10, and 15 ml L^{-1}). The harvest of flax plants ensued at the end of April 2023 at the physiological maturity following the measurement of growth features. The assessment of several chemical and physical properties of soil used soil samples collected from the experimental field by following the procedure, as outlined by Al-Yasssiry *et al*. (2024) (Table 1).

Data recorded and statistical analysis

The data recording included the plant height (cm), fruit branches per plant, seed oil percentage, and yield. Extraction of volatile oil from flax used a Clevenger apparatus, with 50 g of flax powder and 150 ml of 70% chloroform placed in a round volume flask of 500 ml for 20 hours. Measuring the active chemical compounds (linolenic and oleic acids) also continued using a high-performance liquid chromatography (Al-Ghazali *et al*., 2023; Salman *et al*., 2023). Following data collection and tabulation, a statistical analysis on all examined features proceeded in accordance with the experiment design using the Genestat program. The arithmetic averages comparison employed the Least Significant Difference ($LSD_{0.05}$) test.

RESULTS AND DISCUSSION

Plant height

Results revealed significant differences among the concentrations of nano-NPK and EMG-1 and their interactions for plant height in flax (Table 2). The nano-NPK at the concentration of 0.15 ml L^{-1} showed the maximum plant height (77.00 cm), followed by EMG-1 concentration of 15 ml L^{-1} (76.25 cm). However, the interaction between nano-NPK (0.15 ml.L^{-1}) and EMG-1 (15 ml L^{-1}) gave the leading and highest mean for plant height (84.00 cm). The recorded lowest mean for plant height (61.00 cm) appeared in the control treatment.

Nanoparticles have a remarkable role in providing nutrients to flax plants, which eventually increased the chlorophyll formation and light construction, resulting in better vegetative growth (Grover *et al*., 2012). It may also be due to the considerable role of EMG-1 fertilizer in providing nitrogen and phosphorus elements that further divide and expand cells and improve plant growth and dry matter accumulation, contributing to an enhanced the plant stature (Al-Rawi, 2010).

Table 1. Chemical and physical properties of the experimental soil.

Cl	5.54 mm L^{-1}
K	1.27 mm L^{-1}
Mg	3.58 mm L^{-1}
Ca	4.19 mm L^{-1}
Na	1.28 mm L^{-1}
S	2.59 mm L^{-1}
NH_4N	26.24 mg kg^{-1}
PH	7.38

Table 2. Effect of Nano-NPK and EMG-1 and their interaction on plant height in flax.

EMG-1 (ml L^{-1})	Nano-NPK (ml L^{-1})				Means (cm)
	Control	0.7	0.10	0.15	
Control	61	64	68	70	65.75
5	64	69	73	75	70.25
10	67	70	76	79	73.00
15	69	72	80	84	76.25
Means (cm)	65.25	68.75	74.25	77.00	-

$LSD_{0.05}$ Nano-NPK: 1.45, EMG-1: 1.73, Interaction: 1.71

Table 3. Effect of Nano-NPK and EMG-1 and their interaction on fruit branches per plant in flax.

EMG-1 (ml L^{-1})	Nano-NPK (ml L^{-1})				Means (#)
	Control	0.7	0.10	0.15	
Control	91.45	98.85	99.32	99.56	97.30
5	93.87	99.54	100.23	100.45	98.52
10	96.54	100.45	100.56	100.83	99.60
15	100.45	100.67	100.96	102.54	101.16
Means (#)	95.58	99.88	100.27	100.85	

$LSD_{0.05}$ Nano-NPK: 1.56 , EMG-1: 1.47, Interaction: 1.94

Fruiting branches per plant

The nano-NPK and EMG-1 concentrations and their interactions revealed significant differences for flax fruiting branches (Table 3). The nano-NPK concentration (0.15 ml L^{-1}) exhibited the highest mean for the number of fruiting branches (100.85 branches $plant^{-1}$), while the EMG-1 concentration (15 ml L^{-1}) also gave the comparable and utmost number of fruit branches (101.16 branches $plant^{-1}$). The interactions between nano-NPK (0.15 ml L^{-1}) spraying and EMG-1 (15 ml L^{-1}) gave the maximum mean of fruit branches (102.54 branches $plant^{-1}$) and excelled their individual applications. The lowest mean of fruit branches (91.45 branches $plant^{-1}$) resulted in the control treatment.

The nutrients provided by nano-NPK and EMG-1 have many functions in the plant's biological activities, as these elements interfere in the process of cell division and protein formation (Bahar *et al*., 2021). The biofertilizer role by containing nutrients and by straining it to release organic acids and auxin, which have the potential to form roots, shoots, and cytokinins, are main contributors in the basic processes. In turn, these biological processes are crucial in increasing plant growth and developing a strong root mass (Sing and Pathak, 2003).

Oil yield

Results revealed notable variations among the individual use of nano-NPK and EMG-1 and their interactions for flax seed oil yield (Table 4). The nano-NPK concentration (0.15 ml L^{-1}) enunciated the premiere seed oil yield (0.292 t ha^{-1}), followed by the EMG-1 concentration (15 ml L^{-1}), with a mean seed oil yield of 0.297 t ha^{-1}. However, the interaction between nano-NPK (0.15 ml L^{-1}) and EMG-1 (15 ml L^{-1}) emerged with the leading and highest mean value for seed oil yield (0.319 t ha^{-1}). In comparison, the lowest mean of oil yield resulted in the control treatment (0.243 t ha^{-1}). This may refer to the effects of more nutrient absorption, which is key in increasing chlorophyll and protein (Aziz, 2021). It has led to an increase in atmospheric nitrogen stabilization, an increase in organic analysis, and the production of materials to help accelerate plant growth, which enhanced the plant's capacity to retain additional organic compounds (Jilani, 1997).

Table 4. Effect of Nano-NPK and EMG-1 and their interaction on the oil yield in flax.

EMG-1 (ml L^{-1})	Nano-NPK (ml L^{-1})				Means (t ha^{-1})
	Control	0.7	0.10	0.15	
Control	0.243	0.254	0.269	0.273	0.260
5	0.253	0.275	0.279	0.285	0.273
10	0.274	0.284	0.288	0.291	0.284
15	0.283	0.287	0.298	0.319	0.297
Means (t ha^{-1})	0.263	0.275	0.283	0.292	

$LSD_{0.05}$ Nano-NPK: 1.73, EMG-1: 1.83, Interaction: 1.38

Table 5. Effect of Nano-NPK and EMG-1 and their interaction on the oil percentage in flax.

EMG-1 (ml L^{-1})	Nano-NPK (ml L^{-1})				Means (%)
	Control	0.7	0.10	0.15	
Control	25.87	26.84	29.83	30.76	28.33
5	27.73	29.65	30.83	32.65	30.22
10	29.75	31.84	33.85	35.73	32.80
15	30.74	32.65	34.76	37.84	33.100
Means (%)	28.52	30.25	32.32	34.25	

$LSD_{0.05}$ Nano-NPK: 1.65, EMG-1: 1.49, Interaction: 2.56

Table 6. Effect of Nano-NPK and EMG-1 and their interaction on the linolenic acid in flax.

EMG-1 (ml L^{-1})	Nano-NPK (ml L^{-1})				Means (%)
	Control	0.7	0.10	0.15	
Control	21.76	22.85	24.76	25.76	23.78
5	23.76	24.84	26.74	28.95	26.07
10	25.76	26.76	29.76	30.64	28.23
15	27.94	28.65	31.65	34.76	30.75
Means (%)	24.81	25.78	28.23	30.03	

$LSD_{0.05}$ Nano-NPK: 0.65, EMG-1: 1.84, Interaction: 2.63

Oil percentage

For oil percentage, the nano-NPK and EMG-1 individual applications and their interactions provided remarkable differences (Table 5). The treatment with nano-NPK concentration at 0.15 ml L^{-1} showed the topmost mean of oil percentage (34.25%), followed by the treatment with EMG-1 concentration at 15 ml L^{-1}, also giving a supreme mean of oil percentage (33.100%). However, like interactions in other traits, for oil percentage the interaction between nano-NPK (0.15 ml L^{-1}) and EMG-1 (15 ml L^{-1}) gave the highest mean of oil percentage (37.84%), excelling the individual use of these treatments. In comparison, the lowest mean of oil percentage (25.87%) appeared in the control treatment. The EMG-1 positively reduced the soil acidity and density and enhanced the resistance of plant roots (Sing and Pathak, 2003). Similar with other microorganisms, the release of EMG-1 fertilizer is through enzymes around the root area via metabolic processes carried out by microorganisms that stimulate plant vegetation and increase nutrient stocks (Phillips, 2009).

Linolenic acid

Outcomes revealed significant differences among the nano-NPK and EMG-1 concentrations and their interactions for linolenic acid (Table 6). The individual use of nano-NPK concentration (0.15 ml L^{-1}) and EMG-1 (15 ml L^{-1}) indicated the highest means of linolenic acid (30.03% and 30.75%, respectively). However, the interaction

Table 7. Effect of Nano-NPK and EMG-1 and their interaction on the oleic acid in flax.

EMG-1 (ml L^{-1})	Nano-NPK (ml L^{-1})				Means (%)
	Control	0.7	0.10	0.15	
Control	14.75	15.74	16.43	17.73	16.16
5	15.74	17.64	18.64	19.63	16.91
10	16.84	18.54	19.65	20.74	18.94
15	17.45	22.64	24.73	26.84	22.92
Means (%)	16.19	18.64	19.86	21.24	

$LSD_{0.05}$ Nano-NPK: 1.49, EMG-1: 1.36, Interaction: 1.94

between nano-NPK (0.15 ml L^{-1}) and EMG-1 (15 ml L^{-1}) showed the maximum mean of linolenic acid (34.76%) and excelled the individual doses of these two treatments. The lowest mean of linolenic acid (21.76%) appeared in the control treatment. It is a belief that oxygen is vital in reducing plant stress, leading to an increased concentration of active biological compounds. These compounds enhance their concentration within plant tissues, increase their physiology and green growth, and provide the plants with the largest amount of manufactured material (Bahar *et al*., 2021; Amangaliev *et al*., 2023; Bakry *et al*., 2024; El-Bassiouny *et al*., 2024). The biofertilizer role by containing nutrients and by straining it to release organic acids and growth organization can form roots, shoots, and cytokinin, participating in the basic processes carried out inside the plants (Rai, 2006).

Oleic acid

Findings indicated marked variances among the nano-NPK and EMG-1 concentrations and their interactions for oleic acid (Table 7). The treatment of nano-NPK concentration (0.15 ml L^{-1}) exhibited the highest mean for oleic acid (21.24%), and the EMG-1 (15 ml L^{-1}) emerged with the highest value for oleic acid (22.92%). However, the interaction between nano-NPK (0.15 ml L^{-1}) and EMG-1 (15 ml L^{-1}) gave the leading value for oleic acid (26.84%), and even surpassed the individual use of nano-NPK and EMG-1. In comparison, the lowest mean of oleic acid (14.75%) occurred with the control treatment. Nano-NPK acts to enhance the active compounds due to the increased velocity of biological reactions and the production of growth materials. The process of nutrients absorbency was significantly superior with the application of nano-NPK and EMG-1, improving the quality of the manufactured organic products in the plants (Aziz, 2021).

CONCLUSIONS

The nano-NPK and EMG-1 with higher concentrations have significant effect on growth parameters, seed oil, and active compounds (linolenic and oleic acids) in flax (*L. usitatissimum* L.). Moreover, the interaction between nano-NPK (0.15 ml L^{-1}) and EMG-1 (15 ml L^{-1}) revealed the leading and highest mean for plant height, fruiting branches per plant, oil yield, oil percentage, and linolenic and oleic acids.

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