Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS **Asian Journal of Plant Sciences**

ISSN 1682-3974
DOI: 10.3923/ajps.2022.339.346

Research Article
Agrophysiological Variation in Flax Affected by Folic Acid and Ag Nanoparticles Foliar Applications

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Abstract
Background and Objective: Nanotechnology can be used to improve food security by increasing crop production. Nanoparticles are tiny particles that have the potential to boost the growth and yield of various plants including the flax plant. So, the present work was conducted to evaluate the efficiency of foliar application of folic acid, Ag nanoparticles on improving the growth, yield quantity and quality of flax plants under sandy soil conditions. **Materials and Methods:** Thus, two field experiments were carried out at the experimental station of the National Research Centre, Al-Nubaria district El-Behira Governorate, Egypt, in the 2019-2020 and 2020-2021 winter seasons. Foliar application of different concentrations of folic acid at rates of (50, 100 and 150 ppm) and Ag NPs (0.0, 20, 40 and 60 ppm) was carried out twice. **Results:** The results indicating that all the applied treatments showed highly significant variations in all the investigated growth parameters (shoot length, shoot fresh and dry weight, root length and fresh weight). There were significant increases in all biochemical parameters due to all concentrations used in all treatments. **Conclusion:** The promoting effect reached maximum at 100 mg L^{-1} of folic acid and 40 mg L^{-1} of Ag NPs foliar application and for all biochemical characters and yield quantity and quality.

Key words: Flax, folic acid, Ag-nanoparticles, productivity, quality, sandy soil, vegetable oils

Citation: Sadak, M.S., B.A. Bakry and M.F. El-Karamany, 2022. Agrophysiological variation in flax affected by folic acid and Ag nanoparticles foliar applications. Asian J. Plant Sci., 21: 339-346.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is one of the important plants, its seeds show a very high antioxidant activity and are increasingly proposed as an important source of oil and antioxidants. The flax plant has been grown as a dual-purpose plant (as a fibre and oil source) in many countries as Egypt[1,2]. Regarding the national economy, flax plays a prominent role through its large possibilities of exportation and fabrication[3,4]. The need for traditional edible oils increased due to population growth over the world and an increase in the demand for the plant. Thus it is required to find new renewable resources of vegetable oils. It can be mentioned that flax seeds produce a vegetable oil known as flaxseed or linseed oil. Oil quality is usually valued according to the content of Essential Fatty Acids (EFAs) as α-linolenic (omega-3) to linoleic (omega-6) unsaturated fatty acids, and it is among the biggest suppliers of omega-3[5].

Folic acid (vitamin B_9) is one of the most prominent B-complex vitamins, it is a water-soluble vitamin. Folic acid is active in plants in its reduced form as tetra-hydro-folic acid (tetra-hydro-folate) and tetra-hydro-folic coenzymes[6-8]. These folic acid derivatives have different functions, in photosynthesis[9], biochemical conversions of nitrogen, carbon, and sulfur as well as synthesis and catabolism of protein amino acids[10] and nucleic acids[11,12]. Emam *et al.*[13] stated that vitamin treatments did not only stimulate oil production but also activated the antioxidative properties of flax seeds in terms of increasing the endogenous contents of glutathione, ascorbic acid and total phenols. However, the observed stimulation of oil production was found to be at the expense of carbohydrate and protein accumulation in vitamin-treated flax plants.

Nowadays nanotechnology uses are increasing in various fields like information technology, energy, the medical sector, and agriculture. Nanotechnology has proved its ability to solve problems in agriculture and related industries. The use of nanotechnology in agriculture is expected to have many environmental benefits[14]. Many studies on NPs release have risen on how the release would affect ecosystem health and human safety[15,16]. Increasing nanoparticles (ENPs) are exploited in most applications and products due to their physico chemistry. Pathway of ENPs entry into plants mainly via stomata or cuticle[17], ENPs have a positive or negative effect. It has been reported that ENPs can play a role in enhancing energy harvesting for photosynthesis, electron transport, photoreduction activity of PSII, and oxygen evolution[18,19]. Under certain conditions, plants can generate natural mineralized nanoparticles required for plant growth[20]. Silver nanoparticle Ag NPs are the most commonly used manufactured nanoparticles in a wide range of commercial products[21]. Ag NPs have been linked to crop yield improvement in agriculture. Several studies have shown that suitable concentrations of Ag NPs have an important role in improving seed germination[22,23] plant growth[24-26], enhancing photosynthetic quantum efficiency and chlorophyll content[24,27], and increasing water and fertilizer use efficiency[28].

However, the present work was conducted to evaluate the efficiency of foliar application of folic acid, Ag nanoparticles on improving the growth, yield quantity and quality of flax plants.

MATERIALS AND METHODS

Study area: Two field experiments were carried out at the experimental station of the National Research Centre, Al-Nubaria district El-Behira Governorate-Egypt, in the 2019/2020 and 2020/2021 winter seasons. The soil of the experimental site was sandy. Mechanical, chemical and nutritional analysis of the experimental soil is reported in Table 1 according to Carter and Gregorich[29].

Methodology: The experimental design was a complete randomized block design with 3 replications, to investigate the effect of foliar application with 3 levels of folic acid at rates of (50, 100 and 150 mg L^{-1}) and Ag NPs (0.0, 20, 40 and 60 mg L^{-1}) on growth, seed yield and yield components of flax (Letwania-9) cultivar.

Flax seeds of Letwania-9 cultivar were sown in the mid of November, 2019/2020 and 2020/2021 winter seasons in rows 3.5 m long, and the distance between rows was 20 cm apart, plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The seeding rate was 2000 seeds m^{-2}. Pre-sowing, 150 kg per fed of calcium super-phosphate (15.5% P_2O_5) were used. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% N at a rate of 75 kg per fed in 5 equal doses. Potassium sulfate (48% K_2O) was added at 2 equal doses of 50 kg per fed. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Foliar application of different concentrations of Ag NPs (0.0, 20, 40 and 60 ppm) and folic acid at rates of (50, 100 and 150 ppm) was carried out twice, where plants were sprayed after 45 and 60 days from sowing. Water deficit (by skipping one irrigation after the 2 foliar applications) aiming to avert spray treatments washing. Plant samples were taken after 75 days from sowing for measurements of growth characters and some biochemical parameters.

Table 1: Some physical and chemical characteristics of the experimental soil

Sand											
Course 2000-200 (μ %)		Fine 200-20 (μ %)		Silt 20-0 (μ %)			Clay<2 (μ %)				Soil texture
47.46		36.19		12.86			4.28				Sandy
Chemical analysis											
				Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
pH 1:2.5	EC (dSm^{-1})	$CaCo_3$	OM (%)	Na^+	K^+	Mg^+	Ca^{++}	CO_3^-	HCO_3^-	Cl^-	SO_4^-
7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89
Nutritional analysis/Available nutrients											
Macro element (ppm)				Micro element (ppm)							
N	P		K	Zn		Fe			Mn		Cu
52	12.0		75	0.14		1.4			0.3		0.00

Growth traits of plant height (cm), shoot fresh and dry weight (g)/plant, root length (cm), fresh and dry (g)/plant, were measured. At harvest, flax plants were harvested when signs of full maturity appeared, after removing capsules carefully, the following flax traits were measured, plant height (cm), fruiting zone length (cm), technical stem length (cm), number of fruiting branches/plant, number of capsules/plant, biological yield/plant (g), seed yield/plant (g), and 1000-seeds wt. (g), were recorded on random samples of ten guarded p l ants in each plot. Also, straw yield (ton per fed), seed yield (kg per fed) and biological yield (ton per fed) and oil yield (kg per fed) were studied. Some biochemical analysis of the seed yield was done such as oil (%), carbohydrates (%).

Chemical analysis: Photosynthetic pigments contents (chlorophyll a, b and carotenoids) in fresh leaves were estimated using the method of Lichtenthaler and Buschmann[30]. Indole acetic acid content was extracted and analyzed using the method of Hansen and Halkier[31]. Total phenol content was measured as described by William and Metzger[32]. Seed oil content was determined using Soxhlet apparatus and petroleum ether (40-60°C) according to Das *et al.*[33]. Carbohydrates were determined calorimetrically according to the method of Pak and Simon[34].

Statistical analysis: Combined analysis of variance of complete randomized block design for the substances and their concentrations across the two seasons were performed using the method described by Lawal[35]. since the trend was similar in both seasons the homogeneity test Bartlett's equation was applied and t he combined analysis of the two seasons was done according to the method[36]. Means were compared by using the Least Significant Difference (LSD) at 5 and 1%.

$$\text{Feddan} = 4200 \ \text{m}^2 \text{ (Local area meter)}$$

RESULTS

Effect on growth parameters: Folic acid (50, 100 and 150 mg L^{-1}) and silver nanoparticle Ag Nps (20, 40 and 60 mg L^{-1}) with all concentrations showed highly significant increases in growth parameters of flax plant such as shoot length, shoot fresh and dry weight, root length and fresh weight except root length treatment with 50 mg L^{-1} folic acid and 20 mg L^{-1} Ag NPs the increases were not significant as compared with control plants as shown in Table 2. Regarding treatment with folic acid 100 mg L^{-1} was the most effective concentration on all the studied parameters compared with the other used concentrations. While 40 mg L^{-1} was the most effective concentration of Ag NPs compared with the other Ag NPs concentrations. Folic acid with 100 mg L^{-1} showed the highest significant increases in shoot length (68.67 cm), shoot fresh weight (4.37 g), shoot dry weight (0.59 g). While Ag NPs showed the highest increases in root length (13.67 cm) and root fresh weight (0.49 g) compared with other treatments.

Photosynthetic pigments, IAA and phenolics: Regarding photosynthetic pigments of flax plants grown under drought stress in sandy soil is represented in Table 3. Data show that folic acid foliar application at rates of (0, 50, 100, 150 mg L^{-1}) or Ag NPs foliar application at rates of (0, 20, 40, 60 mg L^{-1}) caused significant increases in chlorophyll a, chlorophyll b, carotenoids and total pigments, IAA and phenol contents of flax plants. Regarding foliar application with folic acid

Table 2: Effect of folic acid and Ag NPs foliar applications levels on growth parameters of flax plants at 75 days after sowing combined of two seasons

	Shoots			Roots	
Treatments (mg L^{-1})	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)
Control	56.33	2.14	0.29	11.00	0.19
Folic acid					
50	65.33	3.07	0.42	12.00	0.27
100	68.67	4.37	0.59	13.33	0.48
150	66.67	4.09	0.56	13.33	0.37
Ag NPs					
20	64.00	2.95	0.40	11.67	0.36
40	67.67	4.00	0.54	13.67	0.49
60	63.33	3.29	0.45	11.67	0.49
$LSD_{0.05}$	2.02	0.28	0.04	0.79	0.05
$LSD_{0.01}$	3.90	0.53	0.07	1.53	0.09

Table 3: Effect of Ag NPs and folic acid foliar applications levels on biochemical characters of flax plants at 75 days after sowing combined of two seasons

	Photosynthetic pigments (μg g^{-1} fresh weight)					
Treatments (mg L^{-1})	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments	IAA (μg/100 g FW)	Phenolic (mg/100 g FW)
Control	1009.7	529.3	249.9	1788.9	22.92	64.29
Folic acid						
50	1377.5	626.0	286.5	2290.0	25.68	84.79
100	1760.4	654.9	338.8	2754.1	47.73	106.68
150	1480.1	606.7	321.3	2408.2	41.58	86.90
AgNPs						
20	1177.2	620.2	277.0	2074.4	34.14	75.31
40	1454.3	659.4	319.9	2433.6	43.93	87.51
60	1409.8	681.9	282.5	2374.1	37.36	100.21
$LSD_{0.05}$	18.3	14.5	3.5	39.5	0.50	1.12
$LSD_{0.01}$	36.0	28.10	6.70	77.66	0.98	2.17

100 mg L^{-1} gave the highest values of chlorophyll a, chlorophyll b, carotenoids, total pigment, IAA and phenolics contents compared with the other concentrations (50 and 150 mg L^{-1}). While, the highest promotive effect of Ag NPs was obtained from foliar treatment with 40 mg L^{-1}, in chlorophyll a, carotenoids, total pigments, IAA and phenolics. While 60 mg L^{-1} gave the highest increases in chlorophyll b content of flax plants. Moreover, Folic acid with 100 mg L^{-1} was the most effective treatment followed by 40 mg L^{-1} Ag NPs compared with control plants and the other treated plants.

Change in yield and yield components: Table 4 and 5 show the effect of foliar application at different concentrations of folic acid or Ag NPS on yield parameters of flax plants grown under the newly reclaimed sandy soil. Data clearly show that foliar application with different levels increased significantly yield and its components such as plant height (cm), fruiting zone length (cm), technical stem length (cm), number of fruiting branches/plant, number of capsules/plant, biological yield/plant (g), seed yield/plant (g), straw yield (ton per fed), seed yield (kg per fed), biological yield (ton per fed), 1000 seeds weight (g), oil (%), carbohydrates (%) and oil yield (per fed). Folic acid with 100 and Ag NPs 40 mg L^{-1} were the most effective treatments compared with the other used concentrations. The increase in yield components of flax in response to different treatments relative to untreated plants might result from the increased number of fruiting branches/plant, the number of capsules/plant and 1000 seed weight (g).

Rank correlation coefficients: Biological yield (ton per fed), straw yield (ton per fed) and/ or seed yield (kg per fed) of flax showed a very strong and positive correlation with other traits and yield components, namely, shoot length (cm), shoot fresh wt. (g), shoot dry wt. (g), root length (cm), root fresh wt. (g), chlorophyll a, chlorophyll b, total pigments, IAA, phenolic, technical stem length (cm), fruiting zone length (cm), plant height (cm), no. of fruiting branches/plant, no. of capsules/ plant, 1000 seeds wt. straw yield (ton per fed) and seed yield (kg per fed) showed significant and positive ($p \leq 0.05$) correlation with fruiting zone length (cm),

Table 4: Effect of Ag NPs and folic acid foliar applications on seed yield and yield components of flax plants grown under sandy soil conditions combined of two seasons

Treatments (mg L^{-1})	Plant height (cm)	Fruiting zone length (cm)	Technical stem length (cm)	No. of fruiting branches/plant	No. of capsules/plant	Biological yield/plant (g)	Seed yield/ plant (g)
Control	66.00	13.33	52.67	18.00	33.00	0.87	2.83
Folic acid							
50	73.33	15.00	58.33	23.67	35.00	1.08	3.45
100	75.00	15.67	59.33	27.33	38.33	1.38	4.07
150	70.33	17.00	53.33	26.33	34.33	1.41	3.12
Ag NPs							
20	71.67	17.33	54.33	26.33	32.67	1.01	3.22
40	77.00	20.33	56.67	21.00	42.67	2.64	4.16
60	75.00	20.33	54.67	28.00	39.67	1.65	3.04
$LSD_{0.05}$	2.57	1.07	2.05	1.64	1.37	0.09	0.22
$LSD_{0.01}$	4.97	2.08	3.96	3.18	2.65	0.18	0.43

Table 5: Effect of Ag NPs and folic acid foliar applications on seed yield and yield components of flax plants grown under sandy soil conditions combined of two seasons

Treatments (mg L^{-1})	Straw yield (ton per fed)	Seed yield (kg per fed)	Biological yield (ton per fed)	1000-seeds weight (g)	Oil (%)	Carbohydrates (%)	Oil yield (kg per fed)
Control	1.68	347.92	2.03	4.79	32.79	29.43	114.08
Folic acid							
50	2.49	527.39	3.01	5.06	34.01	31.01	179.38
100	2.92	660.73	3.58	5.80	35.73	32.83	236.10
150	2.32	533.56	2.86	5.04	34.65	31.65	184.86
Ag NPs							
20	2.57	472.29	3.04	5.19	33.79	31.12	159.59
40	2.96	584.51	3.54	6.04	35.79	32.46	209.18
60	2.15	495.10	2.65	5.12	34.72	32.82	171.91
$LSD_{0.05}$	0.01	0.01	0.01	0.13	0.31	0.87	1.70
$LSD_{0.01}$	0.02	0.02	0.02	0.25	0.43	1.19	2.34

Table 6: Rank correlation coefficients between biological yield, straw yield and/ or seed yields per fed with other studied traits for flax

Traits	Straw yield (ton per fed)	Seed yield (kg per fed)	Biological yield (ton per fed)
Shoot length (cm)	0.79**	0.77**	0.80**
Shoot fresh wt. (g)	0.67**	0.73**	0.69**
Shoot dry wt. (g)	0.67**	0.73**	0.69**
Root length (cm)	0.55**	0.61**	0.57**
Root fresh wt. (g)	0.67**	0.84**	0.71**
Chlorophyll a	0.74**	0.69**	0.75**
Chlorophyll b	0.75**	0.81**	0.77**
Carotenoids	0.73**	0.74**	0.74**
Total pigments	0.78**	0.75**	0.79**
IAA	0.67**	0.83**	0.71**
Phenolic	0.66**	0.65*	0.67**
Technical stem length (cm)	0.59**	0.63**	0.61**
Fruiting zone length (cm)	0.38*	0.33	0.38*
Plant height (cm)	0.68**	0.68**	0.69**
No. of fruiting branches/plant	0.33	0.49**	0.36
No. of capsules/plant	0.52**	0.57**	0.54**
Biological yield/plant (g)	0.56**	0.51**	0.56**
Seed yield/plant (g)	0.85**	0.79**	0.86**
Straw yield (ton per fed)	-	0.89**	1.00**
Seed yield (kg per fed)	0.89**	-	0.92**
Biological yield (ton per fed)	1.00**	0.92**	-
1000-seeds wt. (g)	0.85**	0.77**	0.85**

*Significant at 0.05 and **Significant at 0.00, combined data of 2 seasons

seed yield (kg per fed) showed very strong and positive ($p \leq 0.01$) correlation with no. of fruiting branches/plant.

On the contrary, biological yield (ton per fed) and or straw yield (ton per fed) showed negative correlations with number of fruiting branches/plant, seed yield (kg per fed) also showed negative correlation with fruiting zone length Table 6. This indicates the importance of these traits in the yield and yield component of flax plants under sandy soil conditions.

DISCUSSION

The promotive effect of different treatments of either folic acid or Ag NPs on the growth and development of flax plants illustrate their important role in biochemical and physiological processes in plant cells. Folic acid treatments enhance agronomic performance and the other growth traits of the flax plant (Table 2). Dawood *et al.*[37] and Al-Maliky *et al.*[38] confirmed the positive role of folic acid on the growth traits of the faba bean plant. These increases in growth traits caused by folic acid could be related to its content of the most prominent of B complex vitamins besides its essential biochemical function in amino acid metabolism and nucleic acid synthesis[12], increasing cell division and expansion, biosynthesis of bioregulators as IAA and chlorophyll[39] as well as, nutrient absorption[40]. The role of folic in such activities was demonstrated by an increase in the total vegetative growth as well as an increase in-plant weight and dry matter accumulation.

These results of Ag NPs effect on flax plant are in good harmony with those obtained by Latif *et al.*[41], El-Batal *et al.*[42] and Jurkow *et al.*[43] they found that treatment different plants with Ag NPs have a significant impact on growth parameters. Shelar and Chavan[23] stated that appropriate concentrations of Ag NPs play an important role in enhancing seed germination and plant growth. This enhancing effect of silver nanoparticles with different levels could be due to its effect in blocking ethylene signalling in flax plants[44]. Moreover, the stimulating effect of Ag NPs on the growth and physiological attributes of the plant depends on the size and shape of the nanoparticle.

Exogenous treatment of folic acids caused significant increases in various constituents of photosynthetic pigment (Table 3). These increases may be ascribed with the function of folic acid as a central cofactor for one-carbon transfer reactions which are involved in many cellular reactions such as synthesis of chlorophyll and the photorespiration cycle[45]. Moreover, folic coenzymes play a role in the biosynthesis of glycine, and an increased level of free glycine starts the development of plant porphyrins and their derivatives, chlorophylls[10]. In this regard, Dawood *et al.*[37] and Al-Maliky *et al.*[38] stated that foliar treatment of folic acid increased chlorophyll content in the faba bean plant.

Table 3 showed the significant increases in all photosynthetic pigment constituents of flax plants treated with Ag NPs with different concentrations. Those results incongruent with those of Farghaly and Nafady[46] and Latif *et al.*[41] they concluded that Ag NPs improved photosynthesis and attributed these effects to the change of nitrogen metabolism. Moreover, the increased contents of photosynthetic pigments constituents could increase the rate of photosynthesis, due to which there was more production of the photosynthesis process, which in turn increased the weight and growth and productivity of plant as it was observed in our study.

Foliar treatment of folic acid caused significant increases in yield and its components of flax plants grown under the sandy soil. These increases might be due ascribed to the increase in nutrient uptake and/or assimilation due to vitamin application[47]. Stakhova *et al.*[48] stated that folic treatment promoted th e formation of the dependent amino acids and increases the yield and quality of the seeds of pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.). Moreover, strawberries, Li *et al.*[49] stated that folic acid is one of the most important micronutrients and has many forms, but the only folic acid form has cofactor activity. In this respect, a few pieces of literature reported that exogenous folic acid has a positive effect on the growth, yield and quality of some plants such as flax[13], winter wheat[50] white button mushroom[51].

Foliar treatment of flax plants with Ag NPs showed significant increases in yield and its components (Table 4 and 5). Silver is an excellent growth simulator[52]. Similar results of enhancing the role of Ag NPs treatments were obtained on mung bean by Najafi and Jamei[53] and Razzaq *et al.*[54] on the wheat plant. These increases in yield and yield components might be attributed to the increases in growth parameters, photosynthetic pigments, and IAA of treated flax plants.

CONCLUSION

This study showed the effect of foliar treatment of folic acid and Ag NPs on flax plants under sandy soil. Different concentrations increased plant growth, photosynthetic pigments, IAA, phenolics contents and yield quantity and quality. Among different concentrations,100 and 40 mg L^{-1} were the most effective treatments of folic acid and Ag NPs, respectively.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of folic acid and Ag NPs foliar treatments that can be beneficial for increased plant growth, photosynthetic pigments, IAA, phenolics contents and yield quantity and quality. This study will help the researchers and farmers to maximize the flax productivity under sandy soil conditions by using 100 mg L^{-1} of folic acid and 40 mg L^{-1} of Ag NPs foliar application and for all biochemical characters and yield quantity and quality.

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