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Synergistic Effects Of Sodium Azide Mutagen And Nano – Potassium Fertilizer On Growth And Yield Traits Of Canola

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Abstract
The aims of this study were to evaluate the effect of three concentrations of sodium azide (SA) 0%, 0.02%, 0.04% under three levels of Nano-potassium (Nano-K) fertilizer including (0, 50, 100) ppm were added as foliar application after (25, 50, and 75) days after seedling, and their interactions on some of canola growth and yield traits such as plant height, leaf area, chlorophyll content, 1000-seeds weight, oil yield, and seed yield. All treatments were distributed as a factorial experiment using randomized complete block design (RCBD) with three replicates at $P \leq 0.05$. The statistical analysis revealed that significant effects have been observed on all measured traits except chlorophyll content. Results showed that 0.04% of (SA) gave the highest plant height of (155.13) cm, leaf area of (3190.66) cm^2, 1000-seed weight of (2.414) g, oil yield of (556.26) Kg/ha, and seed yield of (1167.9) Kg/ha compared to control treatments which recorded (145.08)cm, (2536)cm^2, (2.353)g, (427.34)Kg/ha, and (938.4)Kg/ha respectively. Furthermore, 100 ppm of Nano-K fertilizer gave the highest plant height of (153.47) cm, leaf area of (3137.9)cm2, 1000-seed weight of (2.407)g, oil yield of (531.87) Kg/ha, and seed yield of (1185.7) Kg/ha in compared with the control treatments. The interaction between (0.04% SA + 100 ppm Nano-K fertilizer) was superior in most of the traits studied and recorded highest plant height of (157.58) cm, leaf area of (3515.9)cm^2, 1000-seed weight of (2.450)g, oil yield of (589.77) Kg/ha, and seed yield of (1375.4) Kg/ha over than all treatments in addition to the control.
Keywords: *Canola, Brassica napus, Sodium azide, Nano-potassium fertilizer.*

INTRODUCTION

Canola (Brassica napus), ranks second in the production of global oilseed, third vegetable oil in world consumption(1). Thus it is the fifth economically important crops following rice, wheat, cotton, and maize(2). Brassica belongs to the cruciferous family (Brassicaceae / Cruciferae), it is utilized in the production of biodiesel, fodder, and edible oils (3). Canola seeds compose of various components, like oil, protein, carbohydrates, vitamins, minerals, ash, crude fiber, moisture content (4). Also has a bioactive compounds like anti-inflammatory, antioxidant, and anticancer effects (5,6).Oil is the most important component which represent 40–50% of the seed weight (7,8). Due to its healthy fatty acid profile, it is considered as one of the healthiest edible oils. It contains the least amount of saturated fats (~7%), and a high content of monounsaturated fatty acids like oleic acid (~60%), and the essential polyunsaturated fatty acids such as omega-6 and omega-3 that the body cannot produce and play a vital role in lowering cholesterol and reducing the risk of heart disease, (9,10). In addition, canola ranking second in global production of meal, a by-product of oil extraction, is used for animal feed because of its high protein content (11). In producing countries, Canola is an important economic resource, the globally largest producer of canola is Canada, whereas grown on more than 20 million hectares annually and constitutes an important part of the national agricultural economy (12). Europe and Australia , have adopted sustainable farming strategies depend on precise soil management and fertilization, in addition to the use of mutagens and genetic improvements to improve productivity (13). Canola can be modify the rate of water evaporation and stimulating the formation of some anti-stress enzymes which enable canola to adopt with water shortage (14).

Chemical mutagens is represented as an active way in enhancing the crop productivity. Sodium azide is a chemical mutagen acts as alkylating agent as it is the most strongful mutagen in plant. Its application on plants is easy, cheap, and generate mutations to enhance their properties. The mutagen efficiency based on many parameters like pH, concentrations, soaking into water, treatment duration, and temperature. It create point mutation, damage the chromosomes and then produce plant tolerance for many conditions (15).

Nanotechnology presents a promising solution for crops productivity increase and environmental hazards decrease. By utilizing Nano-powders and Nano particles, can delay fertilizers release. The high activity of Nano- particles due to the big active area density and big specific surface area, which simplify fertilizers absorption that produced in Nano standard (16). Potassium considered the major nutrient for growth and physiological actions of plant, such as activation of enzymes, photosynthesis, osmotic control, transfer of energy, transport of phloem, movement of stomata, synthesis of protein, metabolic of nucleic acid and vitamins, cation-anion balance and stress resistance resulting in improve yield and quality (17).

Despite the growing importance of canola as a promising crop, its cultivation in Iraq remains neglected. With rapid progress in chemical mutagenesis and technologies of Nano-fertilizers, local researches lack studies integrating these approaches to improve canola growth and productivity. So this study aims to investigate the effect of chemical mutagen sodium azide on growth and yield traits, evaluate the impact of Nano- potassium fertilizer which is added as foliar application , and assess their interaction.

2. MATERIALS AND METHODS

A field experiment was conducted at research station of Agriculture college, University of Anbar in Al-Buaitha area during the fall season of 2023 in a sandy clay mixture soil, some of whose chemical and physical characters are shown in Table (1). .

Table 1. Chemical and physical characters of experiment soil for the agricultural season (2023)

sequence	Type of analysis	Unit of measurement	Appreciation
1	PH degree	----	7.14
2	Electrical conductivity (EC)	Micro centimeter/m	1423
3	Ready potassium (K)	mg/kg	110.40
4	Ready phosphorus (P)	mg/kg	65.3
5	Ready nitrogen (N)	mg/kg	0.25
6	TDS	ppm	711
7	NaCl	%	2.6
8	Clay	%	29
9	Silt	%	12
10	Sand	%	59
11	Histology	----	Sandy clay mixture

Plant materials and treatments

Canola seeds were soaked in sodium azide (NaN_3) solutions (0, 0.02%, 0.04%), for twenty four hours at room temperature while untreated seeds were presoaked in distilled water for the same period of soaking and used as control. The treated seeds were rinsed with running tap water to remove mutagen. Solutions of Nano- potassium fertilizer were prepared (0, 50, and 100 ppm).

Seeds sowing

Treated seeds along with control were sown in the field on November 2023. The seeds were planted in plots, each plot containing five lines . After seeds germination seedlings were thinned. Crop service operations were carried out, including irrigation and weeding, whenever necessary. The treatments Nano-K fertilizers were applied after (25, 50 and 75) days from sowing . At the maturity stage (150 days after sowing) in April 2024, the plants were harvested manually using sickles when the mustards turn brown. The following characters were measured : plant height (cm), leaf area (cm^2), chlorophyll content, average of 1000-seed weight (g), oil yield (Kg per ha) and seed yield (Kg per ha).

Statistical analysis

The field experiment was performed in a factorial experiment using a randomized complete-block design (RCBD) replicated three times per treatment. Data were statistically analyzed by two tests i.e. analysis of variation (ANOVA) and least significant difference (LSD) at probability level (0.05) using the electronic Statistical program Genstat (18).

RESULTS AND DISCUSSION

1.Plant height

Table 2. Effect of sodium azide (SA) and Nano-Potassium fertilizer on plant height (cm) of canola

K fertilizer	NaN_3 Concentrations			Mean
	0	0.02	0.04	
0	145.08	144.25	149.50	146.28
50	143.91	151.08	158.33	151.10
100	146.25	156.58	157.58	153.47
Mean	145.08	150.63	155.13	
L.S.D 5%	K	NaN_3	Interaction	
	2.20	2.56	4.44	

Results presented in Table 2. Showed that plant height significantly effected with increasing levels of sodium azide. The highest mean value was obtained at addition the highest dose of SA (0.04%) which gave (155.13 cm) compared to (145.08 cm) was recorded in control. This can be due to that sodium azide may generate stimulated mutations which result in substitution of base pairs particularly (GC – AT) causing changes in amino acid, which change protein function but does not abolish its function as frame shift or deletion mutation mostly do, leading to improve a broad variation of yield composition and morphological traits compared to normal plants (19).This finding is in agreement with (20), who obtained phenotypic mutations that lead to an increase of plant height in cowpea plant using sodium azide.

With respect to the effects of Nano-K fertilizer treatments, results indicated that a significant increase in plant height with increasing application dose of Nano-K fertilizer. The highest mean value was obtained at addition (100 ppm) of Nano-K which gave (153.47 cm) compared to control which recorded (145.08 cm). This may be because of Nano-K fertilizers have been shown to enhance growth through improved nutrient uptake efficiency, osmotic regulation, and photosynthetic activity, stress tolerance, reduce nutrient leaching and improve soil structure. Furthermore, since Nano-fertilizers are more active than mineral fertilizers, foliar application with NPK Nano fertilizer significantly contributed to the increase in most growth traits (17,21,22). The results agreed with (23), who showed that plant height of "Nubaria 2" cultivar increased significantly by fertilizing with foliar Nano-fertilizers (NPK + micro nutrient). As well as it agreed with [24] who reported a significant increase in plant height by applying different rates of NPK Nano fertilizers.

Regarding to the double interactions effect between (sodium azide + Nano-K), results indicated a significant effect on plant height and recorded highest mean value (157.58 cm) under addition (0.04% SA + 100 ppm Nano-K) over than all the treatments with the control which recorded (145.08 cm). This can be attributed to the interaction between the action of sodium azide as genetic stimulating and Nano-potassium fertilizer as physiological enhancing, resulting in a synergistic effect, ultimately leading to significant increase in the height of plant compared to the single treatments or the control.

2. Leaf area

Table 3. Effect of sodium azide (SA) and Nano-Potassium fertilizer on leaf area (cm^2) of canola

K fertilizer	NaN_3 Concentrations			Mean
	0	0.02	0.04	
0	2536	2909.7	2996.5	2814.1
50	2617.1	2700.9	3059.6	2792.5
100	2762.4	3135.5	3515.9	3137.9
Mean	2638.5	2915.36	3190.66	
L.S.D 5%	K	NaN_3	Interaction	
	54.2	54.2	93.9	

As shown in Table 3.The results Showed that leaf area significantly effected with increasing levels of sodium azide. The highest mean value was obtained at addition the highest concentration of treatment (0.04%) which gave (3190.66 cm^2) compared to (2536 cm^2) was recorded in control. The stimulative impact of sodium azide may be due to division of cell rates also action of growth hormones such as auxin (25). This is agreed with (26), who found that leaf area of Eruca sativa treated with sodium azide was increased as compared to control after 60 days of sowing.

With respect to the effects of Nano-K fertilizer treatments, results indicated that leaf area significantly increased with increasing application dose of Nano-k fertilizer. The highest mean value was obtained at addition (100 ppm) of Nano-K which gave (3137.9 cm^2) compared to control which recorded (2536 cm^2). This is may be due to that potassium is a key element in regulating stomatal conductance, osmotic balance, and carbohydrate partitioning—all critical for leaf expansion. When applied in Nano-form, potassium shows higher bioavailability and uptake efficiency, which directly promotes larger leaf surface area for enhanced light interception. Moreover, Nano-fertilizers have been shown to improve nutrient use efficiency and reduce leaching losses, resulting in better vegetative performance and greater leaf area (17,21,22).This agreed with [27], who reported that Nano fertilizer improve performance of plant in terms of extremely high absorption, improved higher photosynthesis, and an increase in the leaf area.

Regarding to the double interactions effect between (sodium azide + Nano-K), findings indicated a significant effect on leaf area and recorded highest mean value (3515.9 cm^2) under addition (0.04% SA + 100 ppm Nano-K) over than all treatments in addition with control which gave (2536 cm^2). This can be explained by an interaction of both genetic and physiological mechanisms. Thus, the synergistic effect of sodium azide and Nano-K fertilizer leads to genetic stimulating of leaf development and improved physiological nutrient uptake, which resulting in a significant increase in leaf area compared to individual treatments and the control.

3.Chlorophyll Content

Table 4. Effect of sodium azide and Nano-Potassium fertilizer on Chlorophyll content (SPAD unit) of canola

K fertilizer	NaN_3 Concentrations			Mean
	0	0.02	0.04	
0	50.80	47.80	49.62	49.41
50	50.51	49.85	50.97	50.44
100	51.76	50.60	50.43	50.93
Mean	51.02	49.42	50.34	
L.S.D 5%	K	NaN_3	Interaction	
	ns	ns	ns	

As shown as in Table 4. The results showed that chlorophyll content did not show significant differences regarding the application of sodium azide, Nano-K fertilizer, or their interactions. This can be attributed to the relatively low concentrations used, which may not basically influence chlorophyll synthesis under the experimental conditions. As chlorophyll content is often genetically stable (28). In addition, chlorophyll synthesis is impacted by various physiological and environmental parameters, such as stage of plant development, balance of nutrient, and light intensity, which may be reduced treatments impact in this experiment..

4. 1000-Seed Weight

Table 5. Effect of sodium azide (SA) and Nano-potassium fertilizer on 1000-seed weight (g) of canola

K fertilizer	NaN_3 Concentrations			Mean
	0	0.02	0.04	
0	2.353	2.383	2.397	2.378
50	2.373	2.387	2.397	2.386
100	2.380	2.390	2.450	2.407
Mean	2.369	2.387	2.414	

L.S.D 5%	K	NaN_3	Interaction
	0.00754	0.00754	0.01306

Results presented in Table 5. Showed that 1000 seed weight significantly effected with increasing levels of sodium azide. The highest mean value was obtained at addition the highest concentration of treatment (0.04%) which gave (2.414 g) compared to (2.353 g) was recorded in control. This can be attributed to that sodium azide generates point mutation in the plant genome through metabolite and then produced protein possess different function. The formed mutant plants are able to withstand undesirable conditions, and have induced stress tolerance, reduced agronomic input, longer shelf life, and improved yield compared to normal plant (29).This agreed with (30), who showed that 1000 seed weight was increased under different doses of sodium azide (31) also recorded an increase in 1000-seed weight at higher treatment.

With respect to the effects of Nano-K fertilizer treatments, results indicated that 1000-seed weight significantly increased with increasing application dose of Nano-K fertilizer. The highest mean value was obtained at addition (100 ppm) of Nano-K which gave (2.407 g) compared to control which recorded (2.353 g). This may be due to that potassium plays a crucial role in enzyme activation, carbohydrate translocation, and osmotic regulation during seed filling. Nano-formulated potassium ensures higher bioavailability and uptake efficiency, thereby promoting more efficient transport of photo assimilates to developing seeds, resulting in increased seed mass (17, 21, 22). This agreed with (23) who reported that 100 seed weight of "Nubaria 2" cultivar increased significantly by fertilizing with foliar Nano-fertilizers (NPK + micro nutrient).

Regarding to the double interactions effect between (SA + Nano-K), findings showed a significant effect on 1000 seed weight and recorded highest mean value (2.450 g) under addition (0.04% SA + 100 ppm nano-k) over than all treatments in addition with control which gave (2.353 g). This can be attributed to the interaction of both genetic and physiological mechanisms, which lead to a synergistic impact of sodium azide and Nano-K fertilizer results in a significant increase in 1000 seed weight compared with single treatments or control.

5.Seed yield

Table 6. Effect of sodium azide and Nano-Potassium fertilizer on seed yield (Kg/ha) of canola

K fertilizer	NaN_3 Concentrations			Mean
	0	0.02	0.04	
0	938.4	993.9	1034.5	988.9
50	982.5	1020.4	1094	1032.3
100	1004.6	1177	1375.4	1185.7
Mean	975.2	1063.8	1167.9	
L.S.D 5%	K	NaN_3	Interaction	
	8.49	8.49	14.70	

Results presented in Table 6. Showed that seed yield significantly effected with increasing levels of sodium azide. The highest mean value was obtained at addition the highest concentration of treatment (0.04%) which gave (1167.9 kg/ha) compared to (938.4 kg/ha) was recorded in control. This effect could be explained by sodium azide mutagenesis can create point mutation in the DNA of plant through metabolite and thus formed protein has different function which can make the mutant plant produced have improved yield able to withstand a range of undesirable conditions, and reduced agronomic inputs (29). This agreed with (32), who reported that moderate concentration of sodium azide significantly increased the number of seeds/ pod and pod length in Brassica napus.

With respect to the effects of Nano-K fertilizer treatments, results indicated that seed yield significantly increased with increasing application dose of Nano-K fertilizer. The highest mean value was obtained at addition (100 ppm) of Nano-K which gave (1185.7 kg/ha) compared to control which recorded (938.4 kg/ha).This may be attributed to that potassium plays a critical role in activation of enzyme, photosynthesis, osmosis regulation, energy transfer, phloem transport, movement of stomata, synthesis of protein, nucleic acids and vitamins metabolism, cation-anion balance and stress resistance resulting in improve yield and quality (17, 21, 22). The results agreed with (33,34)who reported that application by K and G power calcium nanoparticles in Broad bean showed significantly increased seed yield.

Regarding to the double interactions effect between (SA + Nano-K), results indicated a significant effect on seed yield and recorded highest mean value (1375.4 kg/ha) under addition (0.04% SA + 100 ppm Nano-K) over than all the treatments in addition to control which gave (938.4 kg/ha). This can be explained through combination of genetic and physiological mechanisms, causing in a synergistic effect of sodium azide and Nano-K fertilizer which may be responsible for the significant increase in seed yield compared with individual treatments or control.

6. Oil yield

Table 7. Effect of sodium azide and Nano-Potassium fertilizer on oil yield (Kg/ha) of canola

K fertilizer	NaN_3 Concentrations			Mean
	0	0.02	0.04	
0	427.34	482.38	503.26	470.99
50	455.57	515.44	575.58	515.53
100	477.83	527.84	589.77	531.87
Mean	453.58	508.55	556.26	
L.S.D 5%	K	NaN_3	Interaction	
	3.330	3.330	5.769	

Results presented in Table 7. Showed that oil yield significantly effected with increasing levels of sodium azide. The highest mean value was obtained at addition the highest concentration of treatment (0.04%) which gave (556.26 kg/ha) compared to (427.34 kg/ha) was recorded in control. It is possible that sodium azide creates point mutations which lead to substitution of base pairs particularly (GC – AT) resulting changes in amino acid, which change protein function but does not abolish its function as frame shift or deletion mutation mostly do, leading to stimulate a broad variation of yield composition and morphological traits compared to normal plant (19, 29). This

agreed with (32), who reported that moderate concentration of SA significantly increased the number of seeds/ pod and pod length which increased the crop productivity and oil yield in Brassica napus.
Regard with effects of Nano-K fertilizer treatments, results indicated that oil yield significantly increased with increasing application dose of Nano-K fertilizer. The highest mean value was obtained at addition (100 ppm) of Nano-k which gave (531.87 kg/ha) compared to control which recorded (427.34 kg/ha). It is possible that potassium plays a vital role in enzyme activation, translocation of photo assimilates, and energy metabolism required for fatty acid synthesis in developing seeds. Nano-formulated potassium improves nutrient uptake efficiency and promotes better allocation of carbohydrates and minerals to oil-producing tissue (17, 21, 22). This result is in agreement with (33), who indicated that application by K and G power calcium nanoparticles in Broad bean showed significantly increased in pod number per plant, seed number per pod, 100 seeds weight, which increase the seed productivity.
Regarding to the double interactions effect between (NS + Nano-K), results found a significant effect on oil yield and recorded highest mean value (589.77 kg/ha) under addition (0.04% NA + 100 ppm Nano-K) over than all the treatments with the control which gave (427.34 kg/ha) This can be attributed to a synergistic effect of sodium azide and Nano-K fertilizer which may be result significant increase in oil yield compared to individual treatments in addition to control.

CONCLUSION AND FUTURE PERSPECTIVES

This study showed that the combined application of sodium azide and Nano-potassium fertilizer substantially improved canola growth, seed yield, and oil yield compared with single treatments, while chlorophyll content relatively remained stable. These results highlight a clear synergistic effect and show that integrating chemical mutagenesis with nanofertilization considers a feasible and scalable approach to improve canola productivity and oil quality. Future studies should focus on optimizing treatment doses, validating results in various environments, and examining underlying physiological and molecular mechanisms in crop production.

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