

SABRAO Journal of Breeding and Genetics 57 (3) 1254-1263, 2025 http://doi.org/10.54910/sabrao2025.57.3.36 http://sabraojournal.org/ pISSN 1029-7073; eISSN 2224-8978



BIOSYNTHESIS OF SILVER NANOPARTICLES USING MORINGA LEAF EXTRACT EFFECT IN GROWTH, YIELD, AND CAROTENE CONTENT OF MAIZE (*ZEA MAYS* L.)

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SUMMARY

Determining the effect of foliar spraying of moringa silver nanoparticles synthesized from aqueous leaf extract of *Moringa oleifera* on the growth, yield, and carotene content of four genotypes of maize (*Zea mays* L.) was this study's aim. The research, carried out during the spring of 2022–2023, occurred at the Governorate of Kerbala, Kerbala, Iraq. The experiment, conducted in a randomized complete block design (RCBD), had a split-plot arrangement and three replicates. The main plots included the foliar application of moringa silver nanoparticles with four concentrations (0, 200, 400, and 600 mg L⁻¹), while the subplots comprised four maize genotypes, i.e., NadH-9055, NadH-362, NadH-386, and NadH-315. The results showed the nano-moringa extracts (400 and 600 mg L⁻¹) caused a significant increase in leaf area and 500-grain weight, while an increased concentration (600 mg L⁻¹) exceeded for grain yield and total carotene content. The outcomes also revealed the maize genotype Nadh-315 was superior in leaf area compared with the rest of the genotypes. The genotype Nadh-362 was excellent in the 500-grain weight and grain yield. The results enunciated that moringa silver nanoparticles positively affected the growth traits of maize genotypes that differed in their color intensity, which positively reflects on yield traits and carotene content.

Keywords: Maize (*Z. mays* L.), genotypes, moringa silver nanoparticles, growth and yield traits, leaf area, carotene content

Key findings: The concentration of moringa silver nanoparticles (600 mg L⁻¹) emerged superior in maize grain yield and carotene content. The maize cultivar NadH-9055 also excelled over other genotypes for most traits.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: April 21, 2024; Accepted: September 28, 2024. © Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2025

Citation: Al Yasari AT, Farhood AN (2025). Biosynthesis of silver nanoparticles using moringa leaf extract effect in growth, yield, and carotene content of maize (*Zea mays* L.). *SABRAO J. Breed. Genet.* 57(3): 1254-1263. http://doi.org/10.54910/sabrao2025.57.3.36.

INTRODUCTION

Maize (*Zea mays* L.) occupies a great importance after wheat and rice, as a food source worldwide. In addition to its biochemical compounds giving it economic prominence, these have led to a trend to improve the existing cultivars from a nutritional viewpoint. Maize grains contain vitamins C, E, and B, in addition to folic, oleic, and linolenic acids and potassium, which have a key role in the human's daily diet (Kumar and Jhariya, 2013).

Numerous cultivars maize are widespread globally and differ in seed color, including yellow, red, black, and purple, resulting from varied carotenoid contents in its grain (Dragičević et al., 2022). Total carotenoids vary in the seeds depending on their colors. Carotenoids provide nutritional and functional values as a source of provitamin A, lutein, and zeaxanthin. These compounds are beneficial for protecting against eye and cardiovascular diseases, as well as illnesses associated with aging, working as antioxidants in the immune system (Shah et al., 2016).

Nanofertilizers are crucial in plant nutrition, whether used through foliar application on leaves, or added through ground treatments. Nanofertilizers boost the activity of photosynthesis by increasing the chlorophyll content in leaves, aside from increasing the ability of crops to withstand various abiotic stresses, boosting crop resistance to different Additionally, diseases. it also enhances biologically active substances in crop plants (Guru et al., 2015).

The moringa leaf extract contains some nutrients, hormones, and vitamins, aside from secondary receptors (Khan *et al.*, 2020). Maize crop spraying every two weeks with moringa leaf extract caused a significant increase in leaf area and plant height (Ali *et al.*, 2011). Batool *et al.* (2019) reported that maize spraying with moringa leaf extract appeared very effective in increasing the chlorophyll a and b contents in leaves, as well as, the carotene content in grains. Maswada *et al.'s* (2018) findings revealed spraying maize crops with moringa leaf extract caused a notable rise in the number of cob seed rows, the grains per row, 100-grain weight, and grain yield. Maize crops with foliar application of moringa leaf extract displayed a significant increase in the number of grains per cob, and eventually, grain yield (Shehu and Okafor, 2017). Therefore, based on the above discussion, the presented study aimed to determine the effect of moringa leaf extract or silver nanoparticles on maize growth, productivity, and carotene content in maize seeds, which contributes to increasing the harvest quality.

MATERIALS AND METHODS

The experiment transpired during the spring of 2022-2023 at the Governorate of Kerbala, Kerbala, Iraq, to know the effect of foliar spray application of moringa silver nanoparticles on the growth, vield, and carotene content of four genotypes of maize (Zea mays L.). The experiment had a randomized complete block design (RCBD) with a split-plot arrangement and three replicates. The main plots included foliar application of moringa silver nanoparticles with four concentrations (0, 200, 400, and 600 mg L⁻¹), sprayed every two weeks until physiological maturity of the crop. The subplots comprised four maize genotypes, i.e., NadH-9055, NadH-362, NadH-386, and NadH-315, with different color intensity (Figure 1). The growing of maize genotype seeds continued in mixed sandy soil. The preparation of the moringa leaf nano-extract and its use followed the procedure of Mashamaite et al. (2022).

Moringa plant extract test

In determining the size of the nanoparticles in moringa leaf extract, the study used a field emission scanning electron microscope (FE-SEM). The number of active groups of the nano and non-nano extracts' estimation utilized Fourier transform infrared spectroscopy (FTIR). The estimation of active compounds of the non-nano extract also proceeded by gas chromatography (GC) (Saeed, 2020).

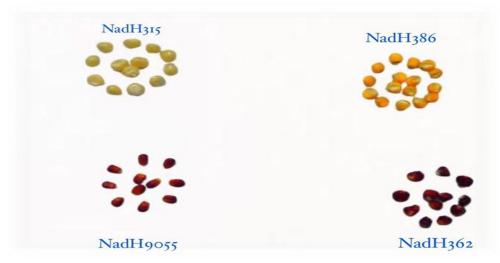


Figure 1. Seed colors of the maize genotypes.

Data recorded

The leaf area and carotene content measurement ensued in the maize genotype leaves. The 500-grain weight and the grain yield also reached calculation. In addition, estimating the total carotene concentration in grains happened for the maize genotypes. The leaf area (cm² plant⁻¹), measured from an average of five plants and then averaged, applied the method by Maddonni and Otegui (1996), as per the following equation.

Leaf area = square of the length of the leaf under the petiole x 0.75 (one leaf)

The concentration of carotene (mg g⁻¹ fresh weight of leaves), estimated according to the method of Lichtenthaler (1987), comprised taking 1 g of fresh leaves and cutting them into small pieces. Then crushing them used a ceramic mortar by adding 20 ml of acetone (80%) and retaining in the refrigerator until the next day. After completing the volume to 50 ml of distilled water, placing the solution in the centrifuge for five minutes had the speed of 1000 rpm. The obtained extract placed in a spectrophotometer bore a reading at a wavelength of 452 µm and was calculated according to the following equation.

Carotene (mg g^{-1}) = (1000×Abs₄₅₂)-(1.9×chla)-(63.14×chlb)/214

For the 500-grain weight (g), counting 500 grains from each sample of five plants harvested in each experimental unit and weighing them with a sensitive electric balance. Grain yield (Mg ha⁻¹) calculation also ensued by weighing the yield of one plant taken as an average of five plants harvested in each experimental unit multiplied by the plant density. Total carotenoids (ma kq⁻1) computation proceeded from the addition of an extracted sample of 3 g of seed powder and 20 ml of acetone (80%), then, left for 24 h (Haborone, 1973; Ijarotimi et al., 2013). Then, filtering the samples, determining total carotenoids and beta-carotene used highquality liquid chromatography technology.

RESULTS AND DISCUSSION

Diagnosis of active compounds

The GC-MS chromatographic analysis of moringa leaf extract showed the presence of 30 peaks, which indicates 30 chemical compounds are present. The specific plant mass spectra of moringa leaves extracted with hexane occurred. Among the identified chemical compounds observed with a positive effect on crop plants were Pentadecan, Tetradecane, Hexadecane, and n-Hexadecanoic

Active compounds	R.T	Pk
Pentadecan	2.928	1
Tetradecane	6.750	2
Heptadecane	8.654	4
Heneicosan	14.020	8
Docosane	14.817	10
Phytol	19.638	15
n-Hexadecanoic acid	21.377	20
9,12,15Octadecatrienoic acid (Z,Z,Z)	23.541	22

Table 1. Active compounds of the moringa leaf extract and their harms.

acid. These compounds are considerably potential growth promoters, in addition to their enhancing the plant's ability to resist biotic and abiotic stresses. The secondary metabolic compounds and volatile compounds (Heptadecane, Heneicosan, and Docosane) also identified tended to be responsible for the plant's ability to withstand biotic and abiotic stress conditions. The detection of the compound Phytol has also revealed its importance in the formation of the chlorophyll pigment, as well as a compound believed to be an antioxidant, 9,12,15-Octadecatrienoic Acid (Z,Z,Z) (Table 1) (Gutbrod et al., 2019; Devi et al., 2021).

FE-SEM

The FE-SEM (field emission scanning electron microscopy) examination could provide highresolution imaging down to the nanometer level, and this allows for analyzing the details of nanoparticles. With the stable magnification, it grants for seeing the finer details of nanoparticles and estimating their sizes. In addition to their structure, more information is available about their properties. Likewise, the distribution of nanoparticles reveals their ability to perform three-dimensional imaging. Therefore, in the synthesis of nanoparticles, it is critical to control the size of the particles, as well as, their shape and size distribution. The nanoparticles' synthesis resulted from developing an extract using silver nitrate. Examining the nanoparticles used the FE-SEM to obtain the shape of the moringa silver nanoparticle samples and the size of the particles (Figure 2). The crystalline structure of the nanoparticles was visible by FE-SEM. It

was evident that the average size of the nanoparticles ranged from 32.46 to 39.18 nm. The shape of the nanoparticles was irregular to circular and polygonal. From the average size of the particles, it was clear that the prepared extract was in the nanoparticle dimension.

FTIR spectrum

Using the FTIR spectrum helped determine the effective groups of active components found in the non-nano and nano-extract, as per the curves' values in the infrared region (Nandiyanto et al., 2019). Eleven effective groups appeared, while in the nano-extract, 13 active groups emerged. By passing the extract to the IR area, the functional groups of the components got separated based on their degree of availability. The results confirmed the presence of the groups, viz., alcohol, aromatic, thiol, alkenes, alkyl, and carbonyl in the non-nano extract (Table 2). However, the examination results of the nano-extract confirmed the presence of all thiol groups, alkenes, alkynes, alkyl groups, silicon oxy, alcohols, aromatic groups, carbonyls, and secondary aminos (Table 3). Enhanced active groups have a positive role in crop plants in several aspects. It enhances plant growth and its antioxidant activity. This, in turn, helps in fighting free radicals and reducing harmful effects in plant cells and tissues. It also leads to increasing the nutritional value, as it contains nutrients and minerals. Amino acids increase the nutritional value of plant products, and the active groups also lead in improving the general health of the plant by preventing diseases (Shousha et al., 2019).

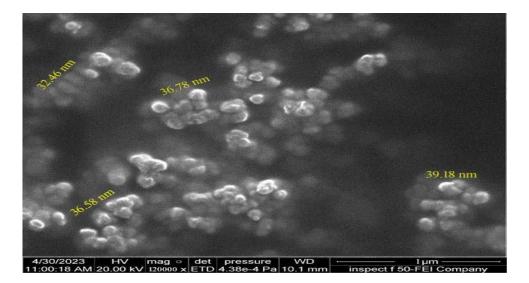


Figure 2. Size and shape of nanoparticles in the moringa leaf extract using FESEM.

Wave number (cm ⁻¹)	Functional group	Phytocompounds identified	
482	S-S	Thiol	
725	C-C	Aromatic compounds	
844	C-H	Aromatic	
1109	C-0	Alcohols	
1377	C-H	Alkyl	
1462	C-H	Alkyne	
1649	C=C	Alkyne	
1741	C=0	Carbonyl	
2852	C-H	Alkyne	
2922	C-H	Alkyne	
3473	О-Н	Alcohol	

Table 2. Effective groups of non-nano moringa leaf extract.

Table 3. Effective	e groups of moringa	leaf nano-extract.
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Wave numb	Functional group	Phytocompounds identified	
480	S-S	Thiol	
623	C-H	Alkanes	
1037	S=0	Silicon oxy	
1112	C-0	Alcohol	
1392	C-H	Alkyl	
1456	C=C	Aromatic	
1637	C=C	Alkyne	
1745	C=0	Carbonyl	
2382	C≡C	Alcayenne	
2854	C-H	Alkyne	
2924	C-H	Alkyne	
3437	N-H	Secondary amino	
3751	O-H	Alcohol	

Leaf area

The results showed spraying moringa silver nanoparticles with two concentrations (400 and 600 mg L⁻¹) caused a significant increase in leaf area, with averages of 4643.81 and 4662.81 cm² plant⁻¹, respectively, versus the spraying with water only (4116.91 cm² plant⁻¹) (Table 4). As for the concentration of 200 mg L⁻¹, the average leaf area reached 4329.41 cm² plant⁻¹. The increased leaf area due to enhanced concentration of moringa silver nanoparticles can be due to the contents of compounds. Among these active are tetradecane and pentadecane that promote growth (Table 1), thus, enhancing the leaf area, as a result of cell elongation and division (Table 1) (Ali et al., 2011; Blom et al., 2011; Abbas et al., 2013; Chattha et al., 2015).

The results further revealed the maize genotypes significantly differed in leaf area (Table 4). The cultivar Nadh-315 showed the highest average leaf area per plant, reaching 5409.08 cm² plant⁻¹. Meanwhile, the three other maizes Nadh-9055, Nadh-362, and Nadh-386 gave averages of 3574.84, 4035.90, and 4732.72 cm² plant⁻¹, respectively. The difference in genotypes for leaf area may be

referring to the varied genetic make-up of the genotypes, as well as, their varying growth period.

Moreover, the findings indicated a notable interaction between spraying treatments with moringa silver nanoparticles and maize genotypes for the leaf area. The interaction of spraying treatment with moringa silver nanoparticles (600 mg L^{-1}) and the maize genotype Nadh-315 gave a higher average for leaf area ($5851.20 \text{ cm}^2 \text{ plants}^{-1}$) than the control treatment with the genotype Nadh-9055, giving a low average for leaf area ($3472.60 \text{ cm}^2 \text{ plants}^{-1}$).

Leaf carotene content

The results revealed moringa nano-extract (600 mg L⁻¹) foliar application substantially increased the carotene content (1.56 mg g⁻¹), while the three other concentrations (0, 200, and 400 mg L⁻¹) showed the lowest averages (1.12, 1.21, and 1.37 mg g⁻¹, respectively) (Table 5). The rise in the concentration of carotene at moringa silver nanoparticle concentration of 600 mg L⁻¹ can be because of its ability with the presence of the compound Phytol. It causes an enhancement in the

Table 4. Effect of moringa silver nanoparticles on the leaf area of maize genotypes.

Concentrations	Genotypes			Genotypes Means				Means
(mg L ⁻¹)	Nadh-9055	Nadh-362	Nadh-386	Nadh-315	(cm ² plant ⁻¹)			
0	3472.60	3647.70	4067.75	5279.60	4116.91			
200	3603.83	4049.55	4691.20	4973.04	4329.41			
400	3601.79	4401.65	5037.75	5532.50	4643.42			
600	3612.15	4044.70	5134.20	5851.20	4662.81			
Means (cm² plant ⁻¹)	3574.84	4035.90	4732.72	5409.08				

Table 5. Effect of moringa silver nanoparticles on the average concentration of carotene (mg g^{-1} fresh weight) for four maize genotypes.

Concentrations		Genotypes Means (Means (mg g ⁻¹
(mg L ⁻¹)	Nadh-9055	Nadh-362	Nadh-386	Nadh-315	fresh weight)
0	1.30	1.24	1.22	1.13	1.12
200	1.36	1.30	1.32	1.19	1.21
400	1.26	1.44	1.44	1.26	1.37
600	1.75	1.57	1.48	1.45	1.56
Means (mg g ⁻¹ fresh weight)	1.43	1.38	1.36	1.26	
	ilver nanoparticles	s = 0.134, Genotyp	es = 0.078, Interac	tions = 0.176	

content of various pigments, including chlorophyll and carotene pigments, in crop plants (Gutbrod *et al.*, 2019; Zhang and Ervin, 2004).

The outcomes further disclosed remarkable differences among the maize genotypes for leaf carotene content (Table 5). The genotype Nadh-9055 showed the highest average (1.43 mg g^{-1} fresh weight), while the cultivar Nadh-362 recorded an average of 1.38 mg g⁻¹ fresh weight. The two other genotypes, Nadh-386 and Nadh-315, appeared with the averages of 1.36 and 1.26 mg g⁻¹ fresh weight, respectively. The superiority of the Nadh-9055 genotype in carotene content may be due to its superiority in the expression of the number of genes, such as *psbA*. This gene encodes the D1 protein, considered an essential component of the Photosystem (PSII). The Photosystem has a remarkable role in photosynthesis and the generation of carotenoids (Elbasan et al., 2024).

The results also exhibited a meaningful interaction effects between the spraying treatments with moringa silver nanoparticles and the maize genotypes in carotene content. The spraying treatment with a concentration of 600 mg L⁻¹, when interacting with the maize genotype Nadh-9055, gave the supreme average of carotene content (1.75 mg g⁻¹ fresh weight) versus the control treatment with genotype Nadh-315, recording the least average carotene content (1.13 mg g⁻¹ fresh weight).

500-grain weight

The results signified spraying moringa silver nanoparticles (600 mg L^{-1}) caused a relevant increase in the 500-grain weight (103.08 g)

(Table 6). However, it did not differ significantly from the moringa silver nanoparticles (400 mg L⁻¹), which recorded an average of 101.42 g compared with the spraying with water only (87.16 g). The increase in the 500-grain weight can be because the moringa silver nanoparticle concentration of 600 mg L⁻¹ led to an increase in the leaf area (Table 4), which, in turn, heightened the process of photosynthesis. This effect also led to an increase in the nutrients' accumulation in grains, energy production, ATP formation, and the production of sugars, proteins, and nucleic acids contained in the grains, enhancing the grain size and weight (Darren *et al.*, 2000).

The maize genotypes showed significant differences for 500-grain weight (Table 6). The maize genotype Nadh-362 gave the highest average of 500-grain weight (118.50 g), while the genotype Nadh-9055 showed the lowest average (82.58 g). The two other genotypes, Nadh-38 and Nadh-315, recorded average values of 89.33 and 95.67 g, respectively. The increased 500-grain weight in the maize genotype Nadh-362 could refer to its increased chlorophyll content, which boosted growth and grain weight.

Significant interaction effects were evident between the spraying treatments with moringa silver nanoparticles and the maize genotypes for the 500-grain weight (Table 6). The spraying treatment with moringa silver nanoparticles (400 mg L⁻¹), when interacted with the genotype Nadh-362, gave the utmost average of 500-grain weight (124.67 g) compared with the control treatment with the maize genotype Nadh-386, which showed the lowest average for the said trait (70.64 g L⁻¹).

Table 6. Effect of moringa silver nanoparticles on the weight of 500 grains (g) of four maize genotypes.

Concentrations	Genotypes			Maama (a)		
(mg L ⁻¹)	Nadh-9055	Nadh-362	Nadh-386	Nadh-315	— Means (g)	
0	78.67	109.67	70.64	89.67	87.16	
200	76.33	120.33	87.33	93.67	94.42	
400	83.33	124.67	98.67	99.00	101.42	
600	92.00	119.33	100.67	100.33	103.08	
Means (g)	82.58	118.50	89.33	95.67		
LSD _{0.05} Moringa s	ilver nanoparticles	s = 5.225, Genotyp	es = 4.949, Interac	tions = 9.537		

Grain yield

The moringa nano-extract (600 mg L⁻¹) foliar application caused a significant increase in grain yield (6.26 Mg ha⁻¹), while the concentration of 400 gave an average of 5.56 Mg ha⁻¹ versus the spraying with water only (4.21 Mg ha⁻¹). It also significantly differed from the concentration of 200 mg L^{-1} (Table 7). The increased grain yield due to enhanced concentration of the moringa silver nanoparticles (600 mg L⁻¹) can refer to its superiority in the 500-grain weight (Table 6). Consequently, an increase in the number of grains per ear can also reflect a positive increase in grain yield (Al-Sahuki and Al-Alusi, 2006). Mvumi et al. (2013) and Kamran et al. (2016) also found an elevation in grain yield by spraying maize moringa with silver nanoparticles.

The results further showed maize genotypes markedly differed in grain yield (Table 7). The genotype Nadh-362 showed the maximum average of grain yield (7.86 Mg ha⁻¹), while the two other genotypes, Nadh-386 and Nadh-315, provided the lowest averages (3.01 and 3.81 Mg ha⁻¹, respectively). The superiority of the genotype NadH362 in grain yield is due to factors related to the genetic

makeup. There could be an apparent difference among the genotypes in the yield-related traits and the influence of environmental factors, which also affect various traits linked to an increased grain yield (Singh and Kumar, 2017).

Similarly, significant interaction effects occurred between the spraying treatments with moringa silver nanoparticles and the maize genotypes for grain yield (Table 7). The spraying treatment with moringa silver nanoparticles ($600 \text{ mg } \text{L}^{-1}$) with genotype Nadh-362 showed the highest average grain yield (9.62. Mg ha⁻¹) compared with the control treatment with the genotype Nadh-386, exhibiting the lowest average (2.50 Mg ha⁻¹).

Total carotene content in grains

The results revealed spraying with moringa silver nanoparticles (600 mg L⁻¹) remarkably raised the total carotene concentration of grains and gave the maximum average (287.951 mg kg⁻¹) compared with the control treatment, which showed the lowest average (240.245 mg kg⁻¹) (Table 8). The two other concentrations of moringa silver nanoparticles (200 and 400 mg L⁻¹) provided averages of 252.780 and 262.052 mg kg⁻¹, respectively.

Concentrations		G	Genotypes Moan (Mg		
(mg L ⁻¹)	Nadh-9055	Nadh-362	Nadh-386	Nadh-315	── Mean (Mg ha ⁻¹)
0	4.87	6.44	2.50	3.03	4.21
200	5.71	7.21	2.70	3.22	4.71
400	6.88	8.23	2.91	4.23	5.56
600	6.65	9.62	3.93	4.76	6.26
Means (Mg ha ⁻¹)	6.03	7.86	3.01	3.81	
LSD _{0.05} Moringa s	ilver nanoparticles	s = 0.580, Genotyp	es = 0.604, Interac	tions = 1.142	

Table7. Effect of moringa silver nanoparticles on grain yield (Mg ha⁻¹) of four maize genotypes.

Table 8. Effect of moringa silver nanoparticles on the average concentration of total carotene (mg kg⁻¹) for four maize genotypes.

Concentrations		G	enotypes		— Means (mg kg ⁻¹)		
(mg L ⁻¹)	Nadh-9055	Nadh-362	Nadh-386	Nadh-315			
0	666.319	285.932	11.319	0.410	240.245		
200	673.160	324.804	12.709	0.446	252.780		
400	711.128	324.089	12.531	0.458	262.052		
600	832.759	305.465	13.139	0.440	287.951		
Means (mg kg ⁻¹)	720.091	310.072	12.425	0.438			
LSD _{0.05} Moringa sil	LSD _{0.05} Moringa silver nanoparticles = 16.9807, Genotypes = 17.4124, Interactions = 33.0398						

The increase in total carotene concentration may be due to the enhanced concentration of moringa silver nanoparticles, as it stimulates and improves the quality of plant products and the concentration of important nutrients, including carotenoids (Yuniati *et al.*, 2022).

The maize genotypes enunciated significant differences for total carotenoids (Table 8). Genotype Nadh-9055 appeared with the highest average of carotene (720.091 mg kg⁻¹), while three other maize genotypes, Nadh-362, Nadh-386, and Nadh-315, showed averages of 310.072, 12.425, and 0.438 mg kg⁻¹, respectively. The superiority of the genotype Nadh-9055 could be because of its genetic potential and the presence of some genes believed to be responsible for increasing the concentration of carotenoids in maize, such as beta-carotene hydroxylase and zeta carotene (Harjes et al., 2008; Yan et al., 2010; Wang et al., 2020).

A noticeable interaction between the spraying treatments with moringa silver nanoparticles and the maize genotypes for total carotene concentration emerged (Table 8). Notably, the yellow corn genotypes differed in the extent, as affected by the moringa silver nanoparticles. The spraying treatment (600 mg L^{-1}) with the genotype Nadh-9055 showed the ultimate average in total carotene content (832.759 mg kg⁻¹) compared with the control treatment with genotype Nadh-315, which gave the lowest average for the said trait (0.410 mg kg⁻¹).

CONCLUSIONS

The silver nanoparticles moringa with increased active compounds positively affected the grain yield and carotene concentration in four maizes (Z. mays L.) genotypes. The moringa leaf nano- extract (600 mg L⁻¹) showed a positive effect on the studied traits, as well as, the maize cultivars responding differently to extract concentrations. Thus, the study recommended growing the maize genotype Nadh-362 with moringa silver nanoparticles (600 mg L⁻¹) for better results.

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