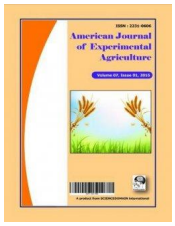

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Response of Sunflower (*Helianthus annuus* L.) to Spraying of Nano Silver, Organic Fertilizer (Algastar) and Salicylic Acid and Their Impact on Seeds Content of Fatty Acids and Vicine

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This experiment is carried out on Sunflower (*Helianthus annuus* L.), Spanish class (Viki) in one of the fields for the period from 05/03/2013 until 18/06/2013, where the shoots are sprayed after period of 65 days of its cultivation with 25 and 50 ml/l of nanosilver, 0.75 and 1.5 g/l of organic fertilizer (Algastar) and with the use of salicylic acid in two concentrations 60 and 120 mg/l in addition to the comparison treatment (spraying with distilled water only). The field experiment is laid as a factorial experiment which included three factors in RBCD design with three replications (3×3×3×3) that included three factors, the averages of transaction are compared by using LSD (least significant difference at 0.05%).

Results of the experiment have shown that spray shoots with 50 ml/l of nanosilver, 1.5 g/l of organic fertilizer (Algastar) and 120 mg/l of salicylic acid gave the highest positive effects of unsaturated fatty acids while the same high concentration of factors reduced the content of the most of the saturated fatty acids and vicine.The three way interaction has affection on field experiment in some studied traits positively while it has no significant effect on seeds content of vicine and fatty acids (Palmitic, Stearic, Archaic, Myristic, Linoleic and Oleic).

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1. INTRODUCTION

Sunflower plant (*Helianthus annuus* L.) is considered as an annual oil crops that are grown in the spring and autumn. This plant belongs to the family Compositae (Asteraceae) [1,2].

The original inhabitant of the plant is in the southwest of the United States America. It is known in Asia, and it is believed that the Indian tribes are the first ones in the United States who have worked in the cultivation of the sunflower and then they have transferred to the southwestern United States and Europe by the Spaniards and moved it to Russia, where it began easily spread, since the year 1860 [3].

Sunflower crop is characterized by its third sequence since it is important for the production of oils after peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.) and the global production of sunflower seeds has increased from 26-31 million tons between 2004-2006 [4].

The sunflower leaves are used in the Caucasus for the treatment of malaria fever, and its seeds are considered as a diuretic and expectorant which are used in the bronchial infections, cold and cough and the importance of this plant in the world [5]. Particularly, we must use the best methods and new technologies. On the level of Iraq because of their low productivity in Iraq and among these technologies is the one that is provided by technical science "technologies minor" or Nanotechnology, in which nanomaterials are used in sizes ranging from 1-100 nm [6].

The usage of nanosilver particles is considered to be one of the most popular and widely used methods at the commercial level because of the positive effects on plants, as well as it is the only nano available in this time in Iraq [7,8].

On one hand, Organic fertilizer (Algastar) also is regarded as an organic, natural fertilizer that extracted from marine extracts which is a rich in amino acids and natural hormones and it consists of nitrogen (1%) potassium (18%) alginic acid more than 10%, sulfur 1.3% , plant growth regulators and organic matter 45%. This can contribute to an increase in the proportion of germination and work to produce strong seedlings, and increase productivity and fruit quality, and it is valid for use with all types of crops.

On the other hand, the use of salicylic acid has an important role in enhancing the plant positive growth, in addition to its being one of the growth regulators with a phenolic nature. It participates in the regulation of many physiological processes in the plant, such as the closure of stomata, photosynthesis, uptake of ions, bio-inhibition of ethylene, and stress tolerance and throw off the free radicals and its negative effects [9,10]. Therefore, many reasons have pushed the researcher for this study like the importance of nutritionally and its use in the production of unsaturated vegetable oils with a special importance in human health, and to increase the yield of winning the unity of space and the lack of information on the effects of silver nanoparticles on the plant. Thus, the aim of this study is to find the effect of combinations of the above factors mentioned and its interventions in the fatty acids.

2. MATERIALS AND METHODS

The experiment is carried out from period 05/03/2013 until 18/06/2013 in the College of Agriculture /University of Wasit which is like a piece characterized as agricultural field, amounting to 364 m^2 (40.5 m length × 9 m width). It is divided into three sectors and the area of each sector amounted to 121.5 m^2 (40.5 m length × 3 m width) and every sector contains 27 panels with a total of 81 panels, the area of each plate (1.5 m × 3 m) in which one panel contains 20 seeds. The sowing of the seeds have happened after Sixty five day and the resulted plant shoots are sprayed within 25 and 50 ml/l of nanosilver and 0.75 and 1.5 g/l of Algastar and salicylic acid 60 and 120 mg/l in addition to the control treatment.

A manual sprinklers are used (1 liter) capacity, for each concentration of previously prepared solution already mentioned, the comparison treatment has been sprayed by distilled water only.

The harvested plants are measured by the following characteristics: Extraction, isolate and diagnose of vicine in seeds according to [11], estimation of some fatty acids (saturated and unsaturated) in the seeds according to [12].

2.1 Statistical Analysis

Statistical analysis is carried out by three-way ANOVA using SPSS. The data sets are analyzed by using randomized completely block design (RCBD) with three replicates of each treatment (3×3×3×3). Least significant difference (p<0.05)

values are calculated for comparisons of treatment means [13].

3. RESULTS AND DISCUSSION

3.1 Estimation of Vicine (mg/g)

The obtained results are shown in Table (1) and it is used nanosilver which leads to reduce the content of vicine in seeds. With increasing the concentrations of particles nanosilver from 25 ml/l to 50 ml/l, the vicine content decreased from 0.4885 to 0.5681 mg/g. It also organic fertilizer (Algastar) has shown a clear reduction content of vicine in seeds with increasing concentrations used as given level of 1.5 g/l (0.5348 mg/g) in comparing with the least concentration and treatment comparison that gave 0.5785 and 0.6393 mg/g respectively. Besides, the use of salicylic acid at different levels resulted in clear reduction content of vicine in seeds and the used concentration of caused reduction in vicine reached to 0.5485 mg/g for 120 mg/l compared with concentration of 60 mg/l and the treatment of comparison which gave 0.5856 and 0.6185 mg/g, respectively.

The two way interaction between nanosilver particles and organic fertilizer (Algastar) in its high concentrations is established vicine content reached to 0.4289 mg/g, which differed significantly from the combination that consists of 50 ml/l nanosilver and 0.75 g/l organic fertilizer which gave 0.4778 mg/g and the comparison treatment that gave 0.7400 mg/g. On one side, the two way interaction between nanosilver particles and salicylic acid, and between the organic fertilizer and salicylic acid on the other side and the three way interaction among the parameters previously mentioned didn't achieve any effect in the vicine seeds content.

3.2 Saturated Fatty Acid Palmitic (µg/ml)

The results of Table (2) have shown that the treated plants with nanosilver particles lead to significant reduction in saturated fatty acid

Table 1. The effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction with each other on content of vicine (mg/g) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	0.7733	0.7500	0.6967	0.7400
	0.75	0.7267	0.7000	0.6700	0.6989
	1.5	0.6933	0.6467	0.6067	0.6933
25	0	0.6467	0.6100	0.6000	0.6467
	0.75	0.5900	0.5567	0.5300	0.5589
	1.5	0.5667	0.5167	0.4967	0.5267
50	0	0.5933	0.5667	0.5167	0.5589
	0.75	0.5100	0.4900	0.4333	0.4778
	1.5	0.4667	0.4333	0.3867	0.4289
Average salicylic acid (mg/l)		0.6185	0.5856	0.5485	0.0209
LSD %0.05		0.0120			
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		0.7311	0.6989	0.6578	0.6959
25		0.6011	0.5611	0.5422	0.5681
50		0.5233	0.4967	0.4456	0.4885
Two way interaction		N.S			0.0120
Organic fertilizer (g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer (g/l)**
		0	**60**	**120**	
0		0.6711	0.6422	0.6044	0.6393
0.75		0.6089	0.5822	0.5444	0.5785
1.5		0.5756	0.5322	0.4967	0.5348
Two way interaction		N.S			0.0120

(Palmitic) as the rate reached 18.93 µg/ml when using the concentration of 50 ml/l, which differed significantly from the use of 25 ml/l concentration, and it gave 21.08 µg/ml from Palmitic acid compared with control which amount of 33.76 µg/ml. Organic fertilizer also achieved a significant decrease in saturated fatty acid (Palmitic) when using a different concentrations: The concentration of 1.5 g/l gave 19.86 µg/ml, which is significantly different from the use of 0.75 g/l and the concentration which gave 23.45 µg/ml comparing to control treatment 30.46 µg/ml. Spraying with salicylic acid showed reduction in fatty acid (Palmitic), when using the concentration 120 mg/l that gave 21.12 µg/ml comparing to the treatment control 28.05 µg/ml also differed significantly when spraying with 60 mg/l, which gave 24.59 µg/ml saturated fatty acid (Palmitic).

Furthermore, it has been shown that the two way interaction between nanosilver particles and organic fertilizer concentrations in which the combination of 25 and 50 ml/l of nanosilver particles with 1.5 g/l of organic fertilizer (Algastar) gave the lowest level of saturated fatty acid Palmitic in seeds reached to 17.29, 14.76 µg/ml which is the lowest level if it is compared with all other treatment combinations of overlap, including control treatment amounting to 40.07 µg/ml.

The two way interaction between the nanosilver particles and salicylic acid has shown that the superiority of the combination consisting of 50 ml/l nanosilver particles and 120 mg/l of salicylic acid, which gave the lowest level reached to 16.79 µg/ml, which is not significantly different from the combination of 25 ml/l of nanosilver with 120 mg/l of salicylic acid 18.02 µg/ml or the combination consisting of 50 ml/l of particles nanosilver with 60 mg/l of salicylic acid amounting 18.93 µg/ml, but they are less than all other interaction factors including control treatment amounting to 39.36 µg/ml.

The three way interaction has no significant effect on the studied factors in the saturated fatty acid Palmitic of the sunflower seeds, as well as the two way interaction between organic fertilizer and salicylic acid.

Table 2. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction with each other on content of saturated fatty acid Palmitic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer(Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	44.53	40.73	34.96	40.07
	0.75	38.47	32.84	29.75	33.68
	1.5	35.09	26.45	20.99	35.09
25	0	31.75	27.45	22.38	31.75
	0.75	20.37	19.31	16.59	18.76
	1.5	19.05	17.73	15.09	17.29
50	0	26.48	24.30	21.57	24.12
	0.75	19.37	17.46	16.89	17.91
	1.5	17.35	15.05	11.90	14.76
Average salicylic acid (mg/l)		28.05	24.59	21.12	2.165
LSD %0.05		1.250			
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		39.36	33.34	28.56	33.76
25		23.72	21.50	18.02	21.08
50		21.07	18.93	16.79	18.93
Two way interaction		2.165			1.250
Organic fertilizer(g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer (g/l)**
		0	**60**	**120**	
0		34.25	30.83	26.30	30.46
0.75		26.07	23.20	21.08	23.45
1.5		23.83	19.75	15.99	19.86
Two way interaction		N.S			1.250

3.3 Saturated Fatty Acid Stearic (µg/ml)

The obtained results in Table (3) have shown the effects of factors and its interventions in Stearic. Besides, the using of the concentrations of 25 and 50 ml/l of nanosilver particles causes a significant decrease in fatty acid about 1.259 µg/ml, 1.095 µg/ml respectively, as compared to the treatment control amounting to 2.875 µg/ml with no significant difference between each other. The organic fertilizer has led to decrease in the saturated fatty acid Stearic with it concentrations 0.75 and 1.5 g/l which are 1.599 µg/ml and 1.407 µg/ml respectively, with no significant difference from each other, but they have shown a significant difference from the control treatment which is 2.223 µg/ml.

The use of salicylic acid has led to significant effect in saturated fatty acid by its concentrations 60 and 120 mg/l as it reached 1.669 µg/ml and 1.500 µg/ml respectively, both of them do not differ significantly from each other, but they disagree with control treatment which is 2.060 µg/ml.

The two way interaction between the nanosilver particles and organic fertilizer (Algastar) have shown that all combinations contain nanosilver concentrations 25 and 50 ml/l with organic fertilizer (Algastar) with all its concentrations which are significantly lower than the treatment organic fertilizer (Algastar) without the use of nanosilver.

The two way interaction between the nanosilver particles and salicylic acid has a significant effect on seeds content of saturated fatty acid Streaic, such combinations containing nanosilver with 60 and 120 mg/l salicylic acid caused a significant decrease in the Stearic comparing with other treatments that didn't include nanosilver paticles. While the two way interaction between organic fertilizer and salicylic acid have shown no significant impact. Also the three way interaction between the factors didn't achieve any significant effect on the saturated fatty acid Stearic in plant seeds.

3.4 Saturated Fatty Acid Myristic (µg/ml) in the Seeds

The obtained results in Table (4) have shown that the addition of nanosilver with concentrations of 25 and 50 ml/l caused a

Table 3. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction with each other on content of saturated fatty acid Stearic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	4.307	3.907	3.173	3.796
	0.75	3.357	2.493	2.187	2.679
	1.5	2.660	1.947	1.843	2.660
25	0	2.223	1.313	1.183	2.223
	0.75	1.150	1.107	1.047	1.101
	1.5	1.100	1.110	1.096	1.102
50	0	1.657	1.167	1.077	1.300
	0.75	1.057	1.020	0.973	1.017
	1.5	1.033	0.953	0.917	0.968
Average salicylic acid (mg/l)		2.060	1.669	1.500	0.3413
LSD %0.05		0.1971			
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		3.441	2.782	2.401	2.875
25		1.491	1.177	1.109	1.259
50		1.249	1.047	0.989	1.095
Two way interaction		0.3413			0.1971
Organic fertilizer(g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer(g/l)**
		0	**60**	**120**	
0		2.729	2.129	1.811	2.223
0.75		1.854	1.540	1.402	1.599
1.5		1.598	1.337	1.285	1.407
Two way interaction		N.S			0.1971

significant decrease in saturated fatty acid of Myristic (5.11 µg/ml and 4.95 µg/ml, respectively) with no significant difference from each other, but they disagreed with control treatment which is 7.52 µg/ml. The use of organic fertilizer with concentrations of 0.75 and 1.5 g/l led to a significant decrease in saturated fatty acid Myristic reached to 5.53 µg/ml and 4.79 µg/ml respectively (with no significant difference from each other), but they disagreed with control treatment which is 7.25 µg/ml. Also salicylic acid didn't achieve any significant effect.

On one hand, the two way interaction between the nanosilver particles and organic fertilizer (Algastar) has shown that all combinations of concentrations nanosilver particles (25 and 50 ml/l) with (0.75 and 1.5 g/l) of orgnic fertilizer didn't differ from each other significantly, but it is less in comparing with the treatment that didn't include nanosilver particles or organic fertilizer which is 10.51 µg/ml. on the other hand, the two way interactions between nanosilver particles and salicylic acid concentration and organic fertilizer with salicylic acid didn't achieve any significant effect in the saturated fatty acid Myristic. Also the three way interaction of studied factors have not any significant influence in the saturated fatty acid Myristic.

3.5 Saturated Fatty Acid Archaic (µg/ml)

Results in Table (5) have shown the saturated fatty acid Archaic in seeds which decreased significantly with increasing the used concentrations of used nanosilver particles as it is reached to 0.125 µg/ml when used the concentration of 50 ml/l, which is significantly different from the concentration of 25 ml/l, which reached 0.282 µg/ml and the treatment of control amounting to 0.535 µg/ml. The use of organic fertilizer (Algastar) showed effect on saturated fatty acid Archaic reached to 0.209 µg/ml when using 1.5 g/l, which didn't differ from the least concentration 0.75 g/l and it gave 0.261 µg/ml but it differed significantly from the control treatment which is 0.473 µg/ml. Salicylic acid with the concentration of 120 mg/l achieved a reduction in seed content of saturated fatty acid Archaic reached to 0.252 µg/ml, which is significantly different from the use of 60 mg/l as it

Table 4. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction with each other on content of saturated fatty acid Myristic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	11.50	9.75	10.28	10.51
	0.75	6.59	6.15	5.91	6.22
	1.5	6.06	7.56	3.91	6.06
25	0	6.74	5.14	4.91	6.74
	0.75	5.56	4.48	6.94	5.66
	1.5	5.19	3.63	3.36	4.06
50	0	6.74	5.23	4.94	5.64
	0.75	4.89	5.05	4.22	4.72
	1.5	5.98	4.38	3.08	4.48
Average salicylic acid(mg/l)		6.58	5.71	5.28	2.25
LSD %0.05		N.S			
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		8.05	7.82	6.70	7.52
25		5.83	4.42	5.07	5.11
50		5.87	4.88	4.08	4.95
Two way interaction		N.S			1.29
Organic fertilizer(g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer(g/l)**
		0	**60**	**120**	
0		8.33	6.71	6.71	7.25
0.75		5.68	5.23	5.69	5.53
1.5		5.74	5.19	3.45	4.79
Two way interaction		N.S			1.29

gave 0.300 µg/ml and differed from the control treatment 0.390 µg/ml. The two way interaction between concentrations nanosilver particles and organic fertilizer (Algastar) has shown that the combinations of concentrations nanosilver particles (25 to 50 ml/l) with (0.75 and 1.5 g/l) of organic fertilizer gave a lower level of saturated fatty acid Archaic in seeds, in comparing to the treatment control amounting to 0.801 µg/ml, or in comparing to the use of 0.75 and 1.5 g/l of organic fertilizer without the use of nanosilver which is 0.444% and 0.360% respectively.

The two way interaction between nanosilver and salicylic acid explained that combinations of nanosilver with concentrations of 25 or 50 ml/l with 60 and 120 mg/l of salicylic acid caused reduction in saturated fatty acid significantly when compared with the control treatment which included salicylic acid (from without nanosilver).

Two way interaction between the concentrations of organic fertilizers and salicylic acid also showed all combinations of organic fertilizer (0.75 and 1.5 g/l) with concentrations of salicylic acid (60 and 120 mg/l) caused result in saturated fatty acid Archaic less than the control treatment amounting to 0.620 µg/ml or with the use of 60 or 120 mg/l of salicylic acid without organic fertilizer 0.470 µg/ml and 0.322 µg/ml respectively. The three way interaction between the affecting factors didn't show any significant effect in the saturated fatty acid Archaic.

3.6 Unsaturated Fatty Acid Linoleic (µg/ml)

Table (6) shows the significance of effect study of factors and its interventions in the unsaturated fatty acid Linoleic. Results in the table have shown that the use of nanosilver with 50 ml/l led to a significant increase in the proportion of unsaturated fatty acid Linoleic amounted to 26.59 µg/ml in comparing to the control of treatment, while the concentration of 25 ml/l of nanosilver has led to a significant increase in the acid above by 16.54 µg/ml when it is compared to the control treatment. Its worth to mention, that the previously mentioned treatments differ from each other significantly.

Table 5. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction with each other on content of saturated fatty acid Archaic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	1.047	0.817	0.540	0.801
	0.75	0.487	0.393	0.453	0.444
	1.5	0.407	0.310	0.363	0.407
25	0	0.663	0.457	0.310	0.663
	0.75	0.327	0.210	0.143	0.227
	1.5	0.167	0.133	0.127	0.142
50	0	0.167	0.137	0.117	0.140
	0.75	0.110	0.110	0.113	0.111
	1.5	0.137	0.133	0.100	0.123
Average salicylic acid (mg/l)		0.390	0.300	0.252	
LSD %0.05		0.055			0.096
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		0.647	0.507	0.452	0.535
25		0.386	0.267	0.193	0.282
50		0.138	0.127	0.110	0.125
Two way interaction		0.096			0.055
Organic fertilizer(g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer (g/l)**
		0	**60**	**120**	
0		0.620	0.470	0.322	0.473
0.75		0.308	0.238	0.237	0.261
1.5		0.237	0.192	0.197	0.209
Two way interaction		0.096			0.055

The use of salicylic acid also led to a significant increase in the unsaturated Linoleic acid where it reached 22.04 µg/ml and 23.96 µg/ml for treatment of salicylic acid 60 and 120 mg/l, respectively both of which differed from each other and were higher than the treatment of control, which differed from them, amounting to 20.17 µg/ml.

Two way interaction between nanosilver particles and organic fertilizer has shown a significant positive impact in increasing the unsaturated fatty acid Linoleic, when used the combination consisting of 50 ml/l of nanosilver with 1.5 g/l of organic fertilizer, which reached to 29.56 µg/ml and differed significantly from the rest of the other combinations including the comparison treatment amounting to 12.96 µg/ml.

The two way interaction between fertilizer and salicylic acid combination consisting of high concentrations achieved a 1.5 g/l of organic fertilizer and 120 mg/l of salicylic acid distinctive in the unsaturated fatty acid Linoleic 26.58 µg/ml, and the combination constituent of 0.75 and 1.5 mg/l organic fertilizer and 120 and 60 mg/l of salicylic acid have achieved a high amount reached to 23.48 µg/ml and 16.68 µg/ml and 24.23 µg/ml respectively, and both of them disagreed with each other and differed from the rest of the other combinations including control treatment.

On one side, the two way interaction between the nanosilver and salicylic acid didn't have any significant effect in unsaturated fatty acid Linoleic. The two way interaction between the nanosilver and salicylic acid on the other side and three way interaction between the studied factors didn't achieve any significant effect on unsaturated fatty acid Linoleic.

3.7 Unsaturated Fatty Acid α- Linolenic (µg/ml)

From the data presented in Table (7) that the use of nanosilver concentrations 25 and 50 ml/l led to a significant increase in the amount of unsaturated fatty acid α- Linolenic. The use of

Table 6. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction which each other on content of unsaturated fatty acid Linoleic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	9.14	12.77	16.99	12.96
	0.75	15.88	17.50	18.16	17.18
	1.5	17.67	19.76	20.97	17.67
25	0	18.96	21.06	22.90	18.96
	0.75	22.30	23.91	25.05	23.75
	1.5	23.68	23.50	26.06	24.42
50	0	21.95	24.11	25.58	23.88
	0.75	25.42	26.35	27.24	26.34
	1.5	26.55	29.43	32.69	29.56
Average salicylic acid (mg/l)		20.17	22.04	23.96	1.313
LSD %0.05		0.758			
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		14.23	16.68	18.71	16.54
25		21.65	22.82	24.67	23.05
50		24.64	26.63	28.50	26.59
Two way interaction		N.S			0.758
Organic fertilizer(g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer(g/l)**
		0	**60**	**120**	
0		16.68	19.31	21.82	19.27
0.75		21.20	22.59	23.48	22.42
1.5		22.63	24.23	26.58	24.48
Two way interaction		1.313			0.758

organic fertilizer (Algastar) showed effect on saturated fatty acid α- Linolenic reached at 0.419 µg/ml when using 1.5 g/l, which didn't differ from the least concentration 0.75 g/l, and it gave 0.390 µg/ml but it differed significantly from the control treatment which is 0.113 µg/ml. Further, the use of salicylic acid also led to a significant increase in the unsaturated Linoleic acid where it reached to 0.452 µg/ml and 0.326 µg/ml for treatment of salicylic acid 120 and 60 mg/l, respectively both of which differed from each other and they are higher than the treatment of control, which differed from them, amounting to 0.247 µg/ml.

There is no significant effect for the two way interaction between nanosilver and organic fertilizer (Algastar) on the unsaturated fatty acid α-Linolenic. The two way interaction between nanosilver and salicylic acid have shown that the combination constituent of 50 ml/l of nanosilver with the use of 60 and 120 mg/l of salicylic acid gave the highest amount of unsaturated fatty acid (α-Linolenic) amounted to 0.646 µg/ml and 0.629 µg/ml respectively.

Also the combination of 25 ml/l of nanosilver with 120 mg/l of salicylic acid achieved a high amount of 0.603 µg/ml and despite the lack of difference in the last three combinations from each other, but they differed significantly from the most of other combinations and treatment control which was 0.121 µg/ml. The two way interaction between organic fertilizer and salicylic acid effects in saturated fatty acid (α-Linolenic) has shown that the combination consisting of 0.75 g/l of organic fertilizer with 120 mg/l of salicylic acid gave the highest unsaturated fatty acid (α-Linolenic) 0.562 µg/ml, which is significantly different from all other combinations. The three way interaction between nanosilver and organic fertilizer and salicylic acid increased α-Linolenic when it is used the combination consisting of 50 ml/l of nanosilver with 0.75 g/l of organic fertilizer and salicylic acid with 120 mg/l which reached to 903µg/ml and differed significantly from the rest of the other combinations including the comparison treatment amounting to 0.135 µg/ml.

3.8 Unsaturated Fatty Acid Oleic (µg/ml)

Results in Table (8) have shown that the concentration of nano silver has significantly impact on unsaturated fatty acid Oleic, as the

Table 7. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction which each other on content unsaturated fatty acid α-Linolenic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar) (g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	0.135	0.126	0.119	0.126
	0.75	0.139	0.128	0.116	0.128
	1.5	0.160	0.150	0.140	0.160
25	0	0.124	0.120	0.405	0.124
	0.75	0.130	0.114	0.667	0.304
	1.5	0.110	0.361	0.737	0.402
50	0	0.663	0.407	0.383	0.484
	0.75	0.118	0.661	0.903	0.561
	1.5	0.647	0.870	0.600	0.706
Average salicylic acid (mg/l)		0.247	0.326	0.452	N.S
LSD %0.05		0.113			
Three way interaction		0.339			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		0.121	0.135	0.125	0.127
25		0.145	0.199	0.603	0.315
50		0.476	0.646	0.629	0.584
Two way interaction		0.196			0.113
Organic fertilizer(g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer (g/l)**
		0	**60**	**120**	
0		0.129	0.218	0.302	0.216
0.75		0.307	0.301	0.562	0.390
1.5		0.306	0.460	0.492	0.419
Two way interaction		0.196			0.113

concentration of 50 ml/l recording the highest amount 49.90 µg/ml, which differed significantly in comparing to the concentration of 25 ml/l, which gave 46.35 µg/ml while the treatment control is 26.20 µg/ml. The use of organic fertilizer also has significant effect on organic fertilizer respectively. The impact of spraying salicylic acid treatment with 120 mg/l achieved the highest rate of unsaturated fatty acid Oleic which is 45.57 µg/ml, that differed significantly from the use of concentration of 60 mg/l from which achieved 40.43 µg/ml and 36.45 µg/ml treatment control.

The two way interaction between nanosilver and organic fertilizer (Algastar) has shown that all combinations of nanosilver concentrations 25 and 50 ml/l with 0.75 and 1.5 g/l of organic fertilizer caused a significant increase in the amount of unsaturated fatty acid Oleic comparing to the treatment control. The higher amount of the Oleic acid is achieved when the combination consists of 50 ml/l nanosilver with 1.5 g/l of organic fertilizer and it is reached at 59.54 µg/ml which is the highest among all interaction factors or in comparison to the control 17.32 µg/ml.

On one hand, the two way interaction between nanosilver and salicylic acid and the two way interaction between of organic fertilizer and salicylic acid on the other hand and three way interaction between the three factors for the experience have not any significant effect in the amount of unsaturated fatty acid Oleic.

The impact of the factors in this study which result in reducing the seed content as in shown in Table (1) may possibly be due to the use of materials and components that are available compounds in the plant to improve the vegetative and the content of the plant which is not enough for vicine manufacturing and increase the quantity of the seeds. This can be explained by the impact of nanosilver or organic fertilizer or salicylic acid as single or combined to disrupt the main ingredient in vicine Orotic acid compound which is responsible for the formation of pyrimidine ring and giving the toxicity of a compound vicine formation [14,15].

The influence on the qualitative oil or oil quality by using the factors in this study confirms the importance of such factors as reducing fatty

Table 8. Effect of nanosilver, organic fertilizer (Algastar) and salicylic acid and interaction which each other on content unsaturated fatty acid Oleic (µg/ml) in the seeds

Particles nanosilver (ml/l)	Organic fertilizer (Algastar)(g/l)	Salicylic acid (mg/l)			Two way interaction between nanosilver and organic fertilizer
		0	60	120	
0	0	13.12	16.77	22.06	17.32
	0.75	21.47	29.33	34.86	28.55
	1.5	26.93	32.75	38.50	26.93
25	0	32.21	37.67	44.58	32.21
	0.75	45.17	49.75	50.00	48.31
	1.5	49.65	50.92	57.24	52.60
50	0	40.68	43.66	47.78	44.04
	0.75	42.84	45.50	49.99	46.11
	1.5	55.98	57.53	65.11	59.54
Average salicylic acid (mg/l)		36.45	40.43	45.57	2.256
LSD %0.05		1.303			
Three way interaction		N.S			
Nanosilver (ml/l)		**Salicylic acid (mg/l)**			**Average nanosilver (ml/l)**
		0	**60**	**120**	
0		20.50	26.29	31.81	26.20
25		42.34	46.11	50.61	46.35
50		42.34	46.11	50.61	49.90
Two way interaction		N.S			1.303
Organic fertilizer (g/l)		**Salicylic acid (mg/l)**			**Average organic fertilizer(g/l)**
		0	**60**	**120**	
0		42.34	46.11	50.61	33.17
0.75		36.49	41.53	44.95	40.99
1.5		44.19	47.07	53.62	48.29
Two way interaction		N.S			1.303

acids and saturated Palmitic, Stearic, Myristic and Archaic as in shown in Tables (2, 3, 4, 5) and increase the unsaturated fatty acids Linoleic, α-Linolenic and Oleic as in shown in Tables (6, 7, 8).

Moradkhani et al. [16] also have reached that treatment salicylic acid on leaf lipid metabolism is probably have a relation with chlorophyll synthesis, photosynthetic activity and carbon supply of sunflower plants. Salicylic acid can also work in increasing the percentage of unsaturated fatty acids accompanied by the decrease in saturated fatty acids that proved the quality of oil. Polyunsaturated fatty acids from soybean oil are essential for human diet because of lowering the risk of heart diseases related to cholesterol oxidation. In addition, consumption of Oleic, Linoleic and α-Linolenic acids decreases the level of low density lipoprotein (LDL) in human blood [17]. Abd El-Razek et al. [18] have concluded that the foliar spray with salicylic acid on olive trees working on control of biosynthetic pathways leading to the production of specific metabolites as essential oils which are controlled by enzymes, which in turn are mainly effected by growth regulators.

Moreover, the impact of the two way interaction on nanosilver and organic fertilizer, especially the high combinations of each one is 50 ml/l of nanosilver with 1.5 g/l of organic fertilizer to the positive effects of the increase in unsaturated fatty acids Linoleic, Oleic reached to 128.08% and 243.76%, respectively, in comparing to the treatment comparison for each one of them. while the combination of interference showed 50 ml/l of nanosilver with 0.75 g/l of organic fertilizer and 120 mg/l of salicylic acid increasing in the proportion of fatty acid α-Linolenic reached to 568.88%, or more than five times from the comparison treatment, which showed that there is no difference from the combination that consists of 50 ml/l of nanosilver with 1.5 g/l of organic fertilizer and 60 mg/l of salicylic acid, which are the other side that recorded an increase in saturated fatty acid α-Linolenic reached to 544.44% .Thus, it is possible to be an alternative combination.

4. CONCLUSIONS

All the factors have worked with their single effect and the two way interaction as consisting of nanosilver and organic fertilizer has worked with its high concentrations in reducing the amount of vicine in the sunflower seeds.

The use of nanosilver led to a reduction in the amount of saturated fatty acids Palmitic, Stearic, Myristic, Archaic concentration when using 50 ml/l that reached to 60.76%, 61.90%, 38.16% and 76.60% respectively, in comparing to the treatment comparison for each one of them, while nanosilver worked alone to increase the amount of unsaturated fatty acid Linoleic, α-linolenic and Oleic, when using the concentration of 50 ml/l which reached to 60.76%, 35.80% and 90.45%, respectively, in comparing to the treatment comparison for each of them.

The impact of two way interaction between nanosilver and organic fertilizer, especially the high combinations of 50 ml/l of nanosilver with 1.5 g/l of organic fertilizer led to a positive effects in increasing the unsaturated fatty acids Linoleic Oleic, amounted to 128.08% and 243.76% respectively, in comparing to the treatment comparison for each on of them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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