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EFFECT OF SOME BACTERIAL TYPES, FERTILIZERS, AND NANOFERTILIZERS ON IMPROVING BIOCHEMICAL TRAITS AND BIOACTIVE COMPOUNDS IN SAFFLOWER PLANT

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ABSTRACT

Background: Safflower (*Carthamus tinctorius L.*) belongs to the family Asteraceae (Li and Mundel, 1996). It is an erect annual herbaceous plant with a growth period ranging from 100 to 200 days depending on the cultivar. It is cultivated in two seasons: autumn and spring. Safflower oil is considered suitable for human consumption, and the oil content in seeds ranges between 25–40%. It contains high levels of unsaturated fatty acids, particularly linoleic and oleic acids Linoleic acid may constitute up to 75% of the oil content. **Methodology** A field experiment was conducted in one of the farms of Al-Musayyib District affiliated with the Directorate of Agriculture during the 2022–2023 growing season to evaluate the efficiency of bacterial biofertilizer and NPK nanofertilizer in improving certain biochemical traits and bioactive compounds of safflower (*Carthamus tinctorius* L.). The experiment was carried out using a Randomized Complete Block Design (R.C.B.D.) as a factorial experiment (2 × 3 × 4): including two safflower cultivars, three concentrations of biofertilizer (0, 10^7 , and 10^9 CFU ml^{-1}):and four concentrations of nanofertilizer (0, 0.5, 1.0, and 1.5 ml L^{-1}).The **results** indicated a significant increase in the average values of the bioactive compounds (Apigenin, Acacetin, Luteolin, and Quercetin). The studied safflower cultivars differed significantly in their morphological and chemical characteristics. The local cultivar recorded the highest flavonoid contents of Luteolin and Quercetin, with carbohydrate percentages reaching 6.792% and 6.853%, respectively. The Indian cultivar showed a significant increase in carbohydrate concentration in the vegetative parts by 2.134%, protein content in seeds by 1.247%, Acacetin concentration by 8.631%, and Apigenin concentration by 25.361%.The concentration of 1.5 ml L^{-1} nanofertilizer produced a significant increase in seed protein percentage and in the average concentrations of the bioactive compounds Apigenin, Acacetin, Luteolin, and Quercetin, with increases of 31.930%, 23.820%, 26.577%, and 18.951%, respectively. The binary interaction between experimental factors achieved the highest significant increase at the interaction between 1.0 ml L^{-1} nanofertilizer and 10^8 CFU ml^{-1} biofertilizer.The triple interaction among experimental treatments showed that applying 1.0 ml L^{-1} nanofertilizer combined with 10^8 CFU ml^{-1} bacterial biofertilizer for both cultivars produced the highest significant increase in most of the studied traits.

KEYWORDS: Bio fertilizer, CFU; Nanofertilizer; Safflower.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) belongs to the family Asteraceae.[1] It is an erect annual herbaceous plant with a growth period ranging from 100 to 200 days depending on the cultivar. It is cultivated in two seasons: autumn and spring. Safflower oil is considered suitable for human consumption, and the oil content in seeds ranges between 25–40%. It contains high levels of unsaturated fatty acids, particularly linoleic and oleic acids.[2,3] Linoleic acid may constitute up to 75% of the oil content.

Its flowers are also used for dyeing silk and cotton fabrics, while safflower seed cake is considered a good animal feed due to its high protein content ranging from 20–40%.[4]

The use of bacterial biofertilizers contributes to improving soil fertility and reducing production costs, in addition to minimizing the risks associated with mineral fertilizers. The objective of using these fertilizers is to provide essential nutrients such as nitrogen, phosphorus, and potassium, which are important for plant biosynthetic processes.[5,6]

Bacteria such as *Rhizobium* and *Azotobacter* convert atmospheric or clay-adsorbed nitrogen into organic nitrogen using the enzyme Nitrogenase.[7] *Bacillus megaterium* dissolves insoluble phosphate compounds and releases phosphorus in a form available for plant absorption.[8-10] Furthermore, *Bacillus mucilaginosus* releases potassium from complex compounds found in soil minerals, making it readily available for plant uptake.[11-13]

This study aims to determine the response of safflower plants to bacterial biofertilizers and their effects on the studied growth traits, and also to identify the optimal concentration of bacterial biofertilizers through their interactive effects on the studied traits, in addition investigate the interaction effect between nanofertilizer and bacterial biofertilizer on flavonoid compounds in safflower plants.

MATERIALS AND METHODS

Bacterial Isolation

The current study included the collection of 90 bacterial samples from different clinical sources: 30 burn samples, 30 wound samples, and 30 urine samples obtained from different hospitals in Baghdad city.

Diagnosis of Isolates

Cultural Diagnosis

Cultural identification was performed based on the phenotypic characteristics of bacterial isolates cultured on blood agar medium. Colonies growing on this medium showed complete β-hemolysis. Colonies growing on mannitol salt agar appeared as spherical clusters, concave in shape, with smooth non-irregular edges and diameters ranging from 0.1–0.8 mm, and demonstrated mannitol fermentation ability.

Microscopic Diagnosis

Microscopic examination was carried out using Gram staining. The isolates appeared as Gram-positive, non-spore-forming *Staphylococcus* species.

Biochemical Tests

The following biochemical tests were conducted:

1. Oxidase test
2. Catalase test
3. Coagulase test

The diagnosis was subsequently confirmed using the VITEK system.

Statistical Analysis

The data were tabulated using a Randomized Complete Block Design (R.C.B.D.) for statistical analysis. Significant differences among treatments were determined using analysis of variance (ANOVA):followed by the Least Significant Difference (L.S.D.) test at a probability level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect on Seed Protein Percentage

Table (1) demonstrates a significant increase in seed protein concentration in safflower plants. The Indian cultivar outperformed the local cultivar in protein percentage, recording 26.80% with an increase of 1.25%. These results are consistent with those reported by Ahmed et al. (2012) regarding the effect of organic and biofertilizers on the growth and yield of fenugreek cultivars.

The bacterial biofertilizer concentration of 10^8 CFU ml^{-1} recorded the highest mean value (27.88%):representing an increase of 11.52% compared with untreated plants. Likewise, the nanofertilizer concentration of 1 ml L^{-1} achieved the highest mean value (27.49%):with an increase of 3.52% relative to untreated plants.

The interaction between cultivar and bacterial biofertilizer significantly increased seed protein content. The Indian cultivar treated with 10^8 CFU ml^{-1} recorded the highest mean value (27.99%):with an increase of 12.36% compared with untreated plants. Similarly, the interaction between cultivar and nanofertilizer concentration showed that the Indian cultivar at 1 ml L^{-1} achieved the highest value (27.68%):representing a 7.37% increase over untreated plants.

The triple interaction among the experimental factors revealed that the Indian cultivar treated with 1 ml L^{-1} nanofertilizer and 10^8 CFU ml^{-1} biofertilizer recorded the highest mean value (29.42%):with an increase of 19.35% compared with untreated plants.

Table (1): Bio fertilizer x Nano-fertilizer effect on seed protein (%) in safflower.

Mean interaction of type X Nano-fertilizer concentration	Dilution level of bio fertilizer C.F.U.ml^{-1}			Concentration level of Nano-fertilizer ml. L^{-1}	Types
	10^8	10^4	0		
25.78	26.34	26.34	24.65	0	**Indian**
26.57	27.83	27.03	25.11	0.50	
27.68	29..42	28.41	25.20	1	
27.18	28.34	28.22	24.96	1.5	
25.40	26.15	25.28	24.77	0	**local**
26.32	27.60	26.65	24.71	0.50	
27.30	28.78	27.45	25.66	1	
26.87	28.52	26.86	25.22	1.5	
0.07	0.12 L.S.D=0.05				
Type mean	**Type x Bio fertilizer dilution interaction C.F.U ml^{-1}**				**Types**
26.80	27.99	27.50	24.91		**Indian**
27.47	27.76	26.56	25.09		**Local**
0.03	0.06 L.S.D=0.05				
Mean Nano-fertilizer Concentration ml. L^{-1}	**Concentration level of Nano-fertilizer ml. L^{-1} x Dilution level of bio fertilizer C.F.U.ml^{-1} interaction**				**Concentration level of Nano-fertilizer ml. L-1**
25.59	26.25	25.81	24.71	0	
26.44	27.72	26.84	24.78	0.5	
27.49	29.10	27.93	25.43	1	
27.02	28.43	27.54	25.09	1.5	
0.05	0.08 L.S.D=0.05				
	27.88	27.03	25.00		**Effect of mean bio fertilizer dilution C.F.U ml^{-1}**
	0.04 L.S.D=0.05				

Effect on Carbohydrate Concentration in Vegetative Parts

Results presented in Table (2) indicate a significant increase in carbohydrate concentration in the vegetative parts of safflower plants. The Indian cultivar surpassed the local cultivar by recording 14.36%, representing a 7.62% increase compared with untreated plants.

The nanofertilizer treatment at 1 ml L^{-1} showed the most pronounced effect, producing the highest mean value of 14.66%, with a 6.54% increase relative to distilled-water-treated plants.

The interaction between cultivar and bacterial biofertilizer demonstrated that the Indian cultivar at 10^8 CFU ml^{-1} achieved the highest value (14.72%):corresponding to a 7.13% increase compared with untreated plants.

The interaction between bacterial and nanofertilizers was also significant, as the concentration of 1 ml L^{-1} nanofertilizer combined with 10^8 CFU ml^{-1} bacterial biofertilizer recorded the highest carbohydrate concentration (15.13%):representing a 4.06% increase compared with untreated plants.

The triple interaction among experimental factors significantly affected carbohydrate concentration in vegetative parts. The Indian cultivar treated with 10^8 CFU ml^{-1} bacterial biofertilizer and 1 ml L^{-1} nanofertilizer recorded the highest value (15.16%):with an increase of 8.29% compared with untreated plants.

Table (2): Bio fertilizer x Nano-fertilizer effect mean carbohydrate concentration in safflower vegetative growth.

Mean interaction of type X Nano-fertilizer concentration	Dilution level of bio fertilizer C.F.U.ml^{-1}			Concentration level of Nano-fertilizer ml. L^{-1}	Types
	10^8	10^4	0		
13.94	13.98	13.83	14.00	0	**Indian**
14.27	14.88	14.40	13.52	0.50	
14.68	15.16	15.12	13.76	1	
14.52	14.94	14.94	13.66	1.5	
13.52	14.02	13.54	13.08	0	**local**

13.88	14.82	13.62	13.18	0.50	
14.60	15.08	14.88	13.93	1	
14.25	14.52	14.22	14.06	1.5	
0.12	0.20	L.S.D=0.05			
Type mean	**Type x Bio fertilizer dilution interaction C.F.U ml^{-1}**				**Types**
14.36	14.72	14.56	13.74		**Indian**
14.06	14.63	14.03	13.57		**Local**
0.06	0.10	L.S.D=0.05			
Mean Nano-fertilizer Concentration ml. L^{-1}	**Concentration level of Nano-fertilizer ml. L^{-1} x Dilution level of bio fertilizer C.F.U.ml^{-1} interaction**				**Concentration level of Nano-fertilizer ml. L-1**
13.76	14.00	13.69	14.54	0	
14.07	14.86	14.04	14.32	0.5	
14.66	15.13	14.96	13.84	1	
14.38	14.74	14.58	13.85	1.5	
0.08	0.14			L.S.D=0.05	
	14.68	14.32	13.64		**Effect of mean bio fertilizer dilution C.F.U ml^{-1}**
	0.07	L.S.D=0.05			

Effect on Flavonoid Compounds

Tables (3, 4, 5, and 6) demonstrate significant increases in the flavonoid compounds Quercetin, Luteolin, Acacetin, and Apigenin in safflower seeds.

The local cultivar showed superior contents of Quercetin and Luteolin compared with the Indian cultivar, recording 29.87 and 19.49 µg g^{-1}, respectively, with increases of 6.79% and 6.85%. In contrast, the Indian cultivar recorded significantly higher levels of Acacetin and Apigenin, reaching 34.36 and 17.35 µg g^{-1}, with increases of 8.63% and 25.34%, respectively.

The bacterial biofertilizer concentration of 10^8 CFU ml^{-1} resulted in the highest levels of Quercetin, Luteolin, Acacetin, and Apigenin, recording 32.41, 22.18, 38.58, and 18.62 µg g^{-1}, respectively. These represented increases of 33.77%, 58.66%, 41.11%, and 63.91% over untreated plants.

The nanofertilizer concentration of 1 ml L^{-1} significantly increased the concentrations of these flavonoids by 24.35%, 32.50%, 26.55%, and 38.05%, respectively, compared with untreated plants.

The interaction between the local cultivar and bacterial biofertilizer concentration of 10^8 CFU ml^{-1} achieved the highest mean values for Quercetin, Luteolin, and Acacetin, with increases of 30.84%, 56.02%, and 53.33%, respectively, compared with control plants. Conversely, the Indian cultivar recorded the highest Apigenin content at 16.55 µg g^{-1}, representing a 64.78% increase compared with untreated plants.

The interaction between cultivar and nanofertilizer concentration revealed that the local cultivar treated with 1 ml L^{-1} nanofertilizer produced the highest Quercetin and Luteolin concentrations, recording 32.52 and 21.31 µg g^{-1}, respectively, with increases of 20.04% and 28.45% compared with untreated plants. The Indian cultivar combined with 1.5 ml L^{-1} nanofertilizer produced the highest Acacetin concentration (37.46 µg g^{-1}):representing a 24.83% increase over untreated plants. The same cultivar treated with 1 ml L^{-1} nanofertilizer recorded the highest Apigenin concentration (19.23 µg g^{-1}):with an increase of 41.50% compared with untreated plants.

The triple interaction significantly affected flavonoid content. The local cultivar treated with 10^8 CFU ml^{-1} bacterial biofertilizer and 1 ml L^{-1} nanofertilizer recorded the highest concentrations of Luteolin and Acacetin, reaching 25.28 and 47.03 µg g^{-1}, respectively, with increases of 111.73% and 73.48% compared with untreated plants. Similarly, the Indian cultivar treated with 1 ml L^{-1} nanofertilizer and 10^8 CFU ml^{-1} bacterial biofertilizer recorded the highest Apigenin concentration (22.77 µg g^{-1}):representing a 102.22% increase compared with untreated plants.

Table (3): Effect Bio fertilizer x Nano-fertilizer and Two safflower types and their interations on mean Quercetin (Flavonoids) in seeds (µg g^{-1})

Mean interaction of type X Nano-fertilizer concentration	**Dilution level of bio fertilizer C.F.U.ml^{-1}**			**Concentration level of Nano-fertilizer ml. L^{-1}**	**Types**
	10^8	10^4	0		
23.65	26.17	22.89	21.93	0	**Indian**
28.32	32.24	29.40	23.32	0.50	

30.59	34.02	33.65	24.14	1	
29.32	32.85	32.29	22.81	1.5	
27.09	30.05	28.39	22.83	0	local
28.85	33.26	27.14	26.17	0.50	
32.52	36.50	34.05	27.01	1	
31.02	34.28	32.39	26.40	1.5	
0.07	0.13	L.S.D=0.05			
Type mean	**Type x Bio fertilizer dilution interaction C.F.U ml^{-1}**				**Types**
27.97	31.31	29.53	23.07		**Indian**
29.87	33.52	30.48	25.62		**Local**
0.04	0.06	L.S.D=0.05			
Mean Nano-fertilizer Concentration ml. L^{-1}	**Concentration level of Nano-fertilizer ml. L^{-1} x Dilution level of bio fertilizer C.F.U.ml^{-1} interaction**				**Concentration level of Nano-fertilizer ml. L-1**
25.38	28.10	22.62	22.41	0	
28.59	32.74	28.26	24.77	0.5	
31.56	35.25	33.38	25.59	1	
30.19	33.56	32.33	24.68	1.5	
0.05	0.09				L.S.D=0.05
	32.41	30.01	24.34		**Effect of mean bio fertilizer dilution C.F.U ml^{-1}**
	0.05	L.S.D=0.05			

Table (4): Effect Bio fertilizer x Nano-fertilizer and Two safflower types and their interations on mean Luteolin (Flavonoids) in seeds (μg g^{-1})

Mean interaction of type X Nano-fertilizer concentration	**Dilution level of bio fertilizer C.F.U.ml^{-1}**			**Concentration level of Nano-fertilizer ml. L^{-1}**	**Types**
	10^8	10^4	0		
14.81	17.17	15.32	11.94	0	**Indian**
18.27	21.31	20.15	13.33	0.50	
20.27	24.13	22.57	14.14	1	
19.61	23.02	22.17	13.62	1.5	
16.59	19.69	17.37	12.17	0	**local**
19.98	23.24	21.42	15.27	0.50	
21.31	25.28	23.09	15.55	1	
20.12	23.66	21.32	15.38	1.5	
0.07	0.13	L.S.D=0.05			
Type mean	**Type x Bio fertilizer dilution interaction C.F.U ml^{-1}**				**Types**
18.24	21.41	20.08	13.24		**Indian**
19.49	22.95	20.82	14.71		**Local**
0.04	0.06	L.S.D=0.05			
Mean Nano-fertilizer Concentration ml. L^{-1}	**Concentration level of Nano-fertilizer ml. L^{-1} x Dilution level of bio fertilizer C.F.U.ml^{-1} interaction**				**Concentration level of Nano-fertilizer ml. L-1**
15.69	18.40	16.37	12.31	0	
19.12	22.28	20.81	14.28	0.5	
20.79	24.70	22.85	14.82	1	
19.86	23.34	21.77	14.48	1.5	
0.06	0.09				L.S.D=0.05
	22.18	20.44	13.98		**Effect of mean bio fertilizer dilution C.F.U ml^{-1}**
	0.05	L.S.D=0.05			

Table (5): Effect Bio fertilizer x Nano-fertilizer and Two safflower types and their interations on mean Acacetin in seeds (µg g^{-1})

Mean interaction of type X Nano-fertilizer concentration	Dilution level of bio fertilizer C.F.U.ml^{-1}			Concentration level of Nano-fertilizer ml. L^{-1}	Types
	10^8	10^4	0		
30.01	32.79	30.14	27.11	0	**Indian**
34.24	39.33	35.33	28.06	0.50	
36.72	38.03	38.62	30.52	1	
37.46	40.60	38.47	30.32	1.5	
27.16	29.56	27.15	24.78	0	**local**
29.47	32.37	31.08	24.96	0.50	
35.63	47.08	34.05	25.77	1	
34.33	42.91	31.58	25.51	1.5	
1.73	3.00 L.S.D=0.05				
Type mean	**Type x Bio fertilizer dilution interaction C.F.U ml^{-1}**				**Types**
34.36	38.44	33.64	29.00		**Indian**
31.63	38.73	30.97	25.26		**Local**
0.87	1.50 L.S.D=0.05				
Mean Nano-fertilizer Concentration ml. L^{-1}	**Concentration level of Nano-fertilizer ml. L^{-1} x Dilution level of bio fertilizer C.F.U.ml^{-1} interaction**				**Concentration level of Nano-fertilizer ml. L-1**
28.59	31.18	28.65	25.95	0	
31.86	35.85	33.21	26.51	0.5	
36.18	44.06	36.34	28.15	1	
35.40	43.26	35.03	27.92	1.5	
1.23	2.12			L.S.D=0.05	
	38.58	33.29	27.34		**Effect of mean bio fertilizer dilution C.F.U ml^{-1}**
	1.06 L.S.D=0.05				

Table (6): Effect Bio fertilizer x Nano-fertilizer and Two safflower types and their interations on mean Apigenin in seeds (µg g^{-1})

Mean interaction of type X Nano-fertilizer concentration	Dilution level of bio fertilizer C.F.U.ml^{-1}			Concentration level of Nano-fertilizer ml. L^{-1}	Types
	10^8	10^4	0		
13.59	16.32	13.18	11.26	0	**Indian**
17.58	21.19	19.07	12.46	0.50	
19.23	22.77	18.67	13.15	1	
19.01	22.54	18.57	12.90	1.5	
11.61	13.02	11.99	9.80	0	**local**
14.03	16.64	15.54	9.90	0.50	
15.53	19.06	17.16	10.37	1	
14.21	17.42	15.15	10.14	1.5	
0.07	0.13 L.S.D=0.05				
Type mean	**Type x Bio fertilizer dilution interaction C.F.U ml^{-1}**				**Types**
17.35	20.72	18.87	12.45		**Indian**
13.84	16.55	14.94	10.05		**Local**
0.037	0.064 L.S.D=0.05				
Mean Nano-fertilizer Concentration ml. L^{-1}	**Concentration level of Nano-fertilizer ml. L^{-1} x Dilution level of bio fertilizer C.F.U.ml^{-1} interaction**				**Concentration level of Nano-fertilizer ml. L-1**
12.59	14.68	12.59	10.57	0	
15.81	18.93	17.32	11.42	0.5	
17.38	20.93	19.43	11.84	1	
16.61	19.99	18.32	11.56	1.5	
0.05	0.09			L.S.D=0.05	
	18.62	16.90	19.36		**Effect of mean bio**

			fertilizer dilution C.F.U ml^{-1}
0.05	L.S.D=0.05		

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