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Responses of Two Sunflower (*Helianthus annuus* L.) Varieties to Inoculation with Azotobacter and Bacillus under Different NPK and Organic Manure Fertilizers Treatments

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KEY WORDS:
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ABSTRACT

Due to the environmental problems occurring from using chemical fertilizers, several considerations have been taken to the application of biofertilizers in the agriculture system. An experiment was implemented during seasons of 2020 under the environmental conditions of Basrah Governorate, Iraq, to compensate for sunflower inorganic NPK needs by biological and organic matter fertilizers. The experimental treatments were organized in a strip-split plot design, with each treatment being replicated three times. The vertical plots were allocated to the two sunflower Varieties (Shumoos and Aqmar). The horizontal plots were subjected to the organic matter fertilizer rates (control "without organic matter" and 16 tons ha^{-1} of chicken manure). While the sub-plots were allowed to five treatments of inorganic NPK and biological fertilizers (F0: control "without any fertilizers"; F1: NPK 120:80:100; F2: Biofertilizer "Azotobacter chroococcum" as a source of N plus PK; F3: Biofertilizer "Bacillus polymexa" as a source of P plus NK; F4: Biofertilizer "Azotobacter + Bacillus" plus K). the obtained results showed that Shumoos Varieties was found to outperform Aqmar genotype in the following characters: plant height, leaf area index, percentage of empty grain, number of grains per plant, 1000-achenes weight, the grain yield of ha, and oil yield per ha. The differences were 6.24, 22.26, 8.99, 10.86, 15.13, 27.72, and 4.22%, respectively. In addition, the findings unequivocally demonstrated that the application of 16 tons of chicken manure per hectare recorded the highest values of all the features under study. It could be recommended that for maximizing the productivity and oil content of sunflower achenes yield, sowing Shumoos genotype besides applied 16 tons ha^{-1} of chicken manure plus F3: biofertilizer "Bacillus polymexa" as a source of P plus NK under ecological conditions of study area.

استجابة صنفين من محصول زهرة الشمس (*Helianthus annuus* L.) للتلقيح ببكتيريا Azotobacter وBacillus تحت معاملات مختلفة من NPK والسماد العضوي

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الخلاصة

نظراً للمشاكل البيئية الناجمة عن استخدام الأسمدة الكيماوية فقد تم الأخذ بعين الاعتبار عدة اعتبارات عند تطبيق الأسمدة الحيوية في النظام الزراعي، حيث تم تنفيذ تجربة خلال موسم الصيف لعام 2020 في ظل الظروف البيئية لمحافظة البصرة في العراق لتعويض احتياجات محصول زهرة الشمس من العناصر المعدنية والعضوية والبيولوجية، وقد تم ترتيب المعاملات بتصميم القطع المنشقة على شكل قطاعات بثلاث مكررات، حيث تم تخصيص القطع الرأسية لصنفي زهرة الشمس (شموس وأقمار)، أما القطع الأفقية فقد تم تطبيق مستويات التسميد العضوي عليها (السيطرة "بدون مادة عضوية" و16 طن هكتار$^{-1}$ من روث الدجاج). في حين تضمنت القطع الفرعية خمس معاملات من الأسمدة غير العضوية NPK والأسمدة البيولوجية (F0: السيطرة "بدون أي أسمدة"؛ F1: NPK 120:80:100؛ F2: السماد الحيوي "Azotobacter chroococcum" كمصدر للنيتروجين بالإضافة إلى PK؛ F3: السماد الحيوي "*Bacillus polymexa*" كمصدر للفوسفور بالإضافة إلى NK؛ F4: السماد الحيوي "Azotobacter + Bacillus" بالإضافة إلى K). أظهرت النتائج المتحصل عليها تفوق صنف شموس على صنف أقمار في الصفات التالية: ارتفاع النبات، مؤشر مساحة الورقة، نسبة الحبوب الفارغة، عدد الحبوب لكل نبات، وزن 1000 حبه، حاصل الحبوب للهكتار، وحاصل الزيت للهكتار. كانت الفروق 6.24، 22.26، 8.99، 10.86، 15.13، 27.72، و4.22%. بالإضافة إلى ذلك، أظهرت النتائج بشكل واضح أن تطبيق 16 طنًا من روث الدجاج لكل هكتار سجل أعلى القيم لجميع السمات قيد الدراسة. يمكن التوصية بأنه لتعظيم إنتاجية ومحتوى الزيت في محصول عباد الشمس، فإن زراعة الصنف Shumoos بالإضافة إلى تطبيق 16 طنًا من روث الدجاج بالإضافة إلى F3: السماد الحيوي "Bacillus polymexa" كمصدر للفوسفور بالإضافة إلى النيتروجين الطبيعي في ظل الظروف البيئية لمنطقة الدراسة.

INTROUCTION

After peanut and soybean plants, *Helianthus annuus* L. (sunflower), it regarded as the 3rd most significant sources of vegetable oil crops. Its oilseed has excellent nutritional qualities that are beneficial for humans (45–55% oil content, 30–35% protein content, 18–19% carbohydrates, 72.5% linoleic acid, 16.2% oleic acid, 60% polyunsaturated fatty acids, and several vitamins, including A, D, E, and K) (King, 2000; Ramulu *et al*., 2011; Amirian and Nassiri, 2013). The FAO estimates that Iraq's entire sunflower cultivation covered around 6000 hectares and produced a total of 20,000 tons in 2022. In contrast, the world's total area under cultivation was around 26.533 million hectares, yielding a combined weight of 47.863 tons. Generally, there exists a huge difference between the production and consumption of oil across the world, and this gap is continually widening as a result of the ongoing growth in the global population. Use of additional fertilizers to generate Varieties with a high sunflowers yield is one of the technical techniques utilized to boost sunflower production. Application of too much nitrogen fertilizers also resulted in a reduction in achene output, quality, yield, and nitrogen usage efficiency (Alsubaihi, and Al-

Ani,(2020). Recently, employing biofertilizers has become popular in part because it reduces the use of agrochemicals, which are used in large quantities and have a detrimental effect on human health and ecological damage (El-Kholy *et al*., 2005).

Biofertilizers Fertilizers contain helpful bacteria that can inhabit the rhizosphere enhances and facilitates plant growth by improving the provision or accessibility essential nutrients to plants (Vessey, 2003). Additionally, the cost of using NPK mineral fertilizers has been increasing. So, it is important to discover different NPK sources to prevent these problems. Increasing the utilisation of biofertilizers, it can reduce reliance on chemical fertilizers and mitigate their adverse of environmental effects. The process of capturing atmospheric nitrogen and the synthesis of compounds that promote plant development can be significantly influenced by them. Thus, biofertilization Plays a vital function in the formation and use of sustainable agricultural methods for reducing environmental pollution and the degradation of the natural world (Akbari *et al.*, 2011; Jalilian *et al*., 2012). The existence of microbial communities as Azotobacter and Bacillus in the soil or rhizosphere of promotes plant growth by facilitating the cycling and accessibility of nutrients; creates a variety of antibiotic structures; it may grow and reproduce on plant roots, colonizing them quickly by passively dispersing on the lengthening cells; it stays near to the root tip which stimulates systemic resistance by releasing volatile organic compounds and encourages plant and root development by releasing phytohormones and extracellular enzymes (Swain and Ray, 2009); Enhancing root health during the growth phase by outcompeting root infections, and increasing nutrient absorption (Al-Dulaimi *et al*., 2020; Ahmed, 2022); it lowers plant ethylene levels by deaminating 1-aminocyclopropane,-1-carboxylic acid -(ACC), which is ethylene's direct precursor because of this reaction (ACC plus ACC deaminase), less ethylene is produced in the form of α-ketobutyrate and ammonia the decreased in ethylene production impacted on pathogen defense, germination, cell elongation, leaf senescence, flower abscission, and fruit ripening (Ryu *et al*., 2004; Penrose and Glick, 2003).

Azotobacter is a free-living fixer that can grow well in the rhizosphere of sunflowers and many other field crops, fixing an average of 10 to 20 kilograms of nitrogen per ha per year (Salhia, 2010; Ahmed, 2021). Azotobacter strains might potentially be employed to enhance plant nutrition because they aid in the uptake of micronutrients like Fe and Zn as well as macronutrients like N and P by seed. Azotobacter seed inoculation has a significant impact on yield growth by feeding crops with nitrogen. The inoculated sunflower seeds with Bacillus subtilis strain had a positive effect on growth characters (height plant and leaf area index), yield and its components percentage of empty seeds, number of seeds per plant, weight of 100-seed) Also, the percentage of oil. Additionally, the utilisation of the Bacillus M-13 strain has a beneficial effect on the growth characteristics, productivity, and quality of sunflowers. The synergistic impact of applying 100 kg P_2O_5 per hectare along with Bacillus resulted in the most significant enhancement in oil production. However, maximum sunflower seed yield achievable with P fertilizer was obtained by using approximately 50 kg P_2O_5 per hectare in combination with Bacillus, as reported by Ekin (2010), Maziyar *et al*. (2011), and Pramanik and Bera (2013). The study showed that applying Azotobacter bacterium to sunflower seeds resulted in a substantial enhancement in growth, yield, yield components, and oil content. Patra, (2013); Hindy *et al*., (2022) showed that combined inoculation of Bacillus and Azotobacter besides the application of 40 kg P_2O_5 per ha for hybrid sunflower significantly increased the height of plant and index of leaf area, as well as yield attributes, yield, and oil content. N, P, K Essential nutrients Plants rely heavily on photosynthesis to facilitate their growth, development, and output and are found in inorganic fertilizer components. A key limiting issue for crop productivity all around the world is commonly a nitrogen (N) deficit (Dwayyeh *et al*., 2023; Abdullah and Mohammed, 2022; Aljaberi *et al*., 2023). Because protein and nucleic acids serve as the primary building blocks and sources of information for each cell, nitrogen plays a crucial function in the structure of these molecules in plants. Additionally, chlorophyll contains nitrogen, which helps plants use photosynthesis to convert solar energy into usable electricity for their cells. Hence, the plant's production of protein, amino acids, protoplasm, and chlorophyll is contingent upon the quantity of nitrogen it receives. Additionally, it affects photosynthetic activity, leaf area, and cell size (Dordas and Sioulas, 2008). To ensure that sunflowers grew as much as possible, balanced fertilization of each was crucial (Patil *et al*., 2009; Handayati and Sihomobing, 2019). The application of 120-90-60 kg per ha of NPK significantly increased sunflower growth characteristics, grain and biological yields (Sitawati, 2018; Saleh, 2021). Another field experiment revealed that the recommended application rate of NPK fertilizer is 150-75-50 kg per hectare, producing the highest grain production (Patil *et al*., 2009). The height of plant, the biological yield, seed yield, and seed oil content, were all significantly impacted by the amounts of nitrogen, phosphorous and potassium rates (Patil *et al*., 2009; Mollashahi and Fanaei, 2013). To enhance the soil's capacity to retain water and nutrients, organic elements like chicken manure have been added (Bigelow *et al*., 2004). Organic matter directly impacts crop growth and yield by providing nutrients. Additionally, it indirectly affects soil by altering its physical characteristics, which can improve the conditions for root formation and stimulate plant development (Bandyopadhya *et al*., 2010; Khodaei-Joghan *et al*., 2018). Moreover, increases amount of nutrients and organic matter in the soil, raises its pH and CEC, and encourages the activity of soil organisms (Maziyar *et al*., 2011). Namvar and Shojaei, (2012) study demonstrated that the combination of chemical nitrogen application and biofertilizer inoculation using Nitroxin, a biological fertilizer containing Azotobacter sp., at a rate of one litre per 30 kg of seed, had a noteworthy influence on various aspects of sunflower growth, including characteristics, grain yield, yield components, and oil yield. The application of the maximum nitrogen fertilizer dosage (200 kg N ha^{-1}) in combination with biofertilizer inoculation resulted in the attainment of the highest measurements for plant height, stem and head diameter, number of grains per head, grain yield, oil output, and biological yield. Toosi and Azizi (2014), revealed that application of ammonium sulphate as a source of nitrogen fertilizer beside Nitroxin biofertilizer recorded a highest of heigh plant, leaf area per plant, yield and yield components as well oil percentage and oil yield. Aljanabi (2021) Stated that adding various amounts of nitrogen fertilizer and inoculating with Azotobacter significantly increased plant height, leaf area index and dry of weight, concentration of nitrogen, and the quantity of nitrogen absorption in vegetative portion of sunflower plants compared with control treatment. Akbari *et al*., (2011) demonstrated that applying nitrogen fertilizer (260 kg N per ha) and farmyard manure (32 ton per ha) together beside biofertilizer using Azotobacter gave superior outcomes than using either approach alone for growth characters, grain and biological yields of sunflower plants. Regarding the effect of the interactions between sunflower Varieties and biofertilizers and organic manure, all studied treatment had positive effect on plant height, leaf area, grain and biological yields as well as oil percentage (Datta *et al*., 2017; Khandekar *et al*., 2018; Hafez *et al*., 2021).

Productivity and oil percentage of sunflowers may therefore be increased by employing appropriate agricultural practices, such as sowing the right genotype, implementing a good fertilization plan using some organic matter, such as chicken manure, and inoculating the plants with specific strains of Azotobacter and Bacillus. This study aims to examine the impact of two different Varieties of sunflower (Helianthus annuus L.) when inoculated with Azotobacter and Bacillus and subjected to various treatments of NPK and organic manure fertilizers. The study will be conducted under identical environmental circumstances in the experimental region.

MATERIAL AND METHODS

Two experiments field were conducted in South Iraq, specifically in Basrah (30° 22′ 0″ N and 47° 22′ 0″ E), during the 2020 season. The purpose of these experiments was to investigate the effects of inorganic NPK, biological, and organic manure fertilizers on the growth characteristics, yield, attributes, and seed oil content of two sunflower Varieties.

The experimental treatments were organised using a strip-split plot design, with each treatment being replicated three times. Vertical plots were allocated to sunflower Varieties (Shumoos and Aqmar). The horizontal plots were subjected to the organic matter fertilizer rate (control “without organic matter” and 16 tons ha^{-1} of chicken manure). While the sub-plots were allowed to five treatments of inorganic NPK and biological fertilizers (F0: control “without any fertilizers”; F1: NPK 120:80:100; F2: Biofertilizer “Azotobacter chroococcum” as a source of N plus PK; F3: Biofertilizer “Bacillus polymexa” as a source of P plus NK; F4: Biofertilizer “Azotobacter + Bacillus” plus K). Azotobacter bacteria with the population (1.78 x 109 cell/ml of broth) was isolated from soil planted with wheat nearby, the same area where the sunflower was tested later, for this purpose, a total of 30 grams of fresh soil was fully saturated by adding sterile water, followed by the addition of 3 grams of mannitol to the resulting soil paste. Next, the soil paste was transferred and compacted into grains with a diameter of 3 mm in a Petri plate using a sterile spatula. Each Ashby-Sucrose agar plate had a total of 16 soil grains, with three replicates for each soil sample. The plates were placed in an incubator set at a temperature of 28°C for 7 days. then Azotobacter was multiplied using the sucrose mineral salt culture which consists of sucrose (10 g L^{-1}), $FeSO_4.7H_2O$ (0.02 g L^{-1}), CaCO3 (3.0 g L^{-1}), $MnSO_4.H_2O$ (0.02 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), MoO_3 (0.01 g L^{-1}), $MgSO_4.7H_2O$ (0.2 g L^{-1}), KI (0.01 g L^{-1}), $CaSO_4$ (0.1 g L^{-1}) and H_2O (1000 ml) as stated by (Aquilanti *et al*., 2004; Jimenez *et al*., 2011).

Bacillus bacteria with the population (6.65 × 109 cell/ml of broth) was also isolated from the same area, grown, a 10-gramme soil sample was mixed with 90 milliliters of sterile normal saline and subjected to a temperature of 80°C for 10 minutes to destroy vegetative organisms, as described by Travers *et al*. in 1987. Cellulase-producing bacteria were obtained by isolating them using the dilution spread plate technique on CMC agar media, where CMC served as the only carbon source. The colonies present on the CMC agar plates were gathered and a quantitative assay technique was employed to ascertain the cellulase activity of the chosen bacterial isolates in Bikovsky's medium, which consists of sucrose (5 g L^{-1}), glucose (0.5 g L^{-1}), yeast extract (2 g L^{-1}), peptone (0.75 g L^{-1}), $(NH4)_2SO_4$ (0.5 g L^{-1}), $FeSO_4$ (0.01 g L^{-1}), KH_2PO_4 (0.5 g L^{-1}), MgSO4

(0.5 g L^{-1}), CaCl2 (10% 5 ml), H2O (1000 ml) and agar (15 g L^{-1}) Additionally, the pH of the medium was modified to 7.5 in test tubes, as specified by Sethi *et al*. (2013). The cellulase activity of each culture was assessed by quantifying the quantity of reducing sugars released using a DNS approach (Miller, 1959).

Calcium superphosphate, which contains 20% P_2O_5, was applied at a rate of 400 kg ha^{-1} (equivalent to 80 units of P_2O_5 ha^{-1}) during seedbed preparation. Nitrogen fertilizer, in the form of urea containing 46% N, was applied at a rate of 260.8 kg ha^{-1} (equivalent to 120 units of N ha^{-1}) in two equal portions before the first and second irrigations. In addition, potassium fertilizer was sprayed at a rate of 227.27 kg ha^{-1} (equivalent to 100 units of K_2O ha^{-1}) in the form of potassium sulphate, which contains 44% K_2O. This application was done in one portion, together with the second component of nitrogen.

The experimental units were prepared by adding chicken manure, an organic fertilizer, to the soil surface. The manure was then mixed into the soil using a hack. Chemical analysis was conducted on the organic poultry manure, according to AOAC (2016) was available nitrogen 0.625%, available phosphorous 0.077%, available potassium 0.270%, organic matter 41.22%, pH 7.52 and EC 0.78 dsm^{-1}.

Experimental unit area was 10.5 m^2 comprised five ridges, each 3.0 m length, and 0.7 m width. Samples of soil were randomly collected pre-sowing at 0-30 cm depth to clarify physical and chemical soil analysis. The soil texture was clayey loam, pH (7.53), EC (6.12 dSm^{-1}), organic matter (11.31 gm kg-1), CaCO3 (207 gm kg-1) available N, P, K contents of were (0.075, 19.7, 245 ppm), respectively as average of both seasons. In both seasons previous crop was wheat. Achenes of sunflower plant were regular hand sowing at hill spacing 25cm on one side 70 cm ridge, after a period of three weeks from sowing, the number of plants was reduced to one per hill to produce the optimum plant density (57142 plant ha^{-1}).

The area of field experimental was full prepared after the inoculated and non-inoculated seeds of sunflower at the rate of 12 kg ha^{-1} were sown on the 24th of July in 2020 seasons. All other prescribed agricultural methodologies for cultivating sunflowers were adhered to in accordance with the guidelines set forth by the Iraq Ministry of Agriculture, with the exception of the variables being investigated.

At 60 days after sowing (DFS), we selected five plants at random from the outer edges of each plot to measure the leaf area index (LAI). The leaf area (cm^2) was estimated by calculating it using the equation published by Schneiter (1978).

The formula to calculate the value of LA is $LA = (L \times B \times 0.6683) - 2.45\ cm^2$.

Let L represent the length of a leaf and B represent the breadth of a leaf.

The LAI was determined by dividing the leaf area (measured in cm^2) by the land area occupied by a single plant (measured in cm^2 for each density examined).

During the harvesting stage at 105 DFS, five plants were randomly selected from ridges of each plot and left to air dry for approximately one week. The following characteristics were subsequently evaluated: plant height in centimeters, number of achenes per head, weight of 1000 achenes in grams, and achenes weight in tons per hectare. The achenes weight was estimated by harvesting all plants from the three inner ridges, calculating the weight based on a moisture content of 12-13%, and then converting it to tons per hectare. The oil content was determined using a

Soxhlet apparatus using petroleum hexane as an organic solvent. Seed samples were collected from each subplot, cleaned, and finely crushed into a powder using a grinder following the AOAC (2016) guidelines.

The data were subjected to statistical analysis using the MSTAT statistical program, employing a strip-split plot design with three replicates, as outlined by Gomez & Gomez (1984). In addition, the treatment means were compared using the Least Significant Difference (LSD) test at a significance threshold of 5%, as indicated by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The field experiment was performed with summer sunflower plants during the 2020 growing season, two Varieties of sunflower plants were involved, two organic fertilizer rates of chicken manure, and five fertilizer treatments were tested. According to the ANOVA Analysis Test, we have found that sunflower Varieties were the most significant factor affecting all studied characters, whereas the organic fertilizer rates of poultry manure were the significant factor affecting most of the studied characters, with the exception (empty seeds %, no of seeds plant^{-1} and oil %), whereas treatments of inorganic NPK and biological fertilizers was the significant factor affecting most of studied characters, with exception (empty seeds and oil %) ($p < 0.01$), (Table 1). Varieties × organic fertilizer rates of chicken manure interaction were also highly significant most studied traits except (plant height, LAI and 1000 seeds weight and oil %) ($p < 0.01$).

Table 1. Summarized results of variance analysis for the examined characteristics.

S.O.V.	D.F	plant height (cm)	LAI	Empty seeds %	No of seeds plant^{-1}	1000 seeds weight (gm)	Seeds yield (kg ha^{-1})	Oil %	Oil yield (kg ha^{-1})
Block	2	221.7	0.357	6.63	609.4	0.43	10976.2	0.119	1012.1
Varieties	1	1455.3**	5.667**	10.58*	108655.6**	1458.393**	6665066.7**	1089.9**	50055.9**
Organic	1	3397.5**	1.852**	0.96ns	3473.2ns	6.902*	131770.3*	0.163ns	19386.0*
Fertilizers	4	2189.2**	0.431*	4.52ns	23844.5**	4.832*	307671.9**	7.363ns	42506.6**
GO	1	48.8ns	0.079ns	11.97*	17953.9**	0.172ns	254801.6**	0.263ns	33007.2**
GF	4	233.0ns	0.07ns	4.87ns	196.1ns	7.614**	6927.8ns	0.490ns	1011.3ns
OF	4	420.69*	0.228ns	0.571ns	2322.1ns	1.554	33853.3ns	1.101ns	11984.1*
GOF	4	204.9ns	0.079ns	2.63ns	4019.8ns	4.345*	37008.8ns	0.659ns	9867.9ns
Error	38	154.3	0.141	2.553	4011.6	1.451	31985.3	4.706	4032.5
C.V.%		**7.64**	**12.33**	**16.37**	**8.70**	**1.72**	**6.97**	**5.23**	**7.91**

**Significant at a 0.01 level, *Significant at a 0.05 level, and ns denotes non-significant.

Results clearly detected that, studied sunflower Varieties i.e. Shumoos and Aqmar significantly differed and resulted the highest averages of plant height (167.49 cm), leaf area index (3.35), the percentage of empty achenes (10.18%), number of achenes per plant (770.45), 1000-achenes weight (75.02 g), achenes yield per ha (2898.55 kg ha^{-1}), oil yield of ha (1077.20 kg ha^{-1}) absorbed for Shumoos compared with Aqmar genotype as presented in Table 2. However, Shumoos genotype recorded a lowest averages oil percentages (37.18) as compared with Aqmar genotype (45.71%). It could be noticed that Shumoos exceeded Aqmar Varieties by 6.24, 22.26, 8.99, 12.41, 15.13, 29.86 and 5.66% in height of plant, index leaf area, the percentage of empty grain, grains number per plant, 1000-achenes weight, grain yield of ha and oil yield per ha. The high grain production per hectare of the Shumoos genotype can be linked to the genetic characteristics that arise from the genotype's genetic makeup relationships. Furthermore, variations in their genetic makeup and their interaction with the environmental circumstances that exist during the growing season resulted in an increase in the leaf area index and yield attributes. This rise was evident in the higher production of achenes and oil per hectare. The results produced from this study partially align with those seen and discussed by (Datta *et al*., 2017; Khandekar *et al*., 2018).

Organic matter fertilizer “chicken manure “had a significant effect on height of plant, leaf area index, number of seeds per plant, seeds and oil yields per ha as presented in Tables 2. On the other hand, empty seed percentage, weight of 1000 seed and oil percentage not significantly affected by organic fertilization. The data clearly showed that application of 16 tons per ha of chicken manure recorded the highest values of all character’s understudies. The positive effects due to using organic matter resulted from its directly affect crop growth, yield by giving nutrients, and indirectly by altering the physical characteristics of the soil, so improving the conditions for root formation and facilitating plant development (Bandyopadhyay *et al*.,2010; Khodaei-Joghan *et al*., 2018). In addition, improves the amount of nutrients and organic matter in the soil, raises its pH and CEC, and encourages the activity of soil organisms (Maziyar *et al*.,2011). It is noticed that using chicken manure as a source of organic fertilizer at 16 tons per ha increased plant height by (9.70%), LAI by (12.19%), percentage of empty seeds by (2.69%), seeds number of plant by (2.11%), weight of 1000 seeds by (0.97%), seeds yield per ha by (3.72%), oil % by (0.24%) and yield per ha by (3.48%) during growing summer season. These results are confirmation with thus resulted by (Khandekar *et al*., 2018; Hafez *et al*., 2021).

Except for the percentage of empty seeds, weight of 1000 seeds (g) and oil percentage, the findings showed that fertilizer treatments were significant on all studied traits as shown in Table 2. The statistical analysis showed that plants which received F1: NPK 120:80:100 resulted in the highest plant height (178.85 cm), LAI (3.19), number of seeds per plant (778.89 cm), weight of 1000 seeds (70.20 g) and seeds yield per ha (778.89 kg). The increase in above mentioned traits resulted from using N, P, K may be due to these elements, which are critical nutrients for sunflower plant development (Dong *et al*., 2012; Altai *et al*., 2024). Because protein and nucleic acids serve as the primary building blocks and sources of information for each cell, chlorophyll contains nitrogen, which helps plants use photosynthesis to transfer energy from sunlight to their tissues, affects photosynthetic activity, leaf area index, and cell size (Dordas and Sioulas, 2008). These results in line with obtained by (Patil *et al*., 2009; Stiawati, 2018; Mollashahi *et al*., 2013). Plants received F3: Biofertilizer “Bacillus polymexa” as a source of P plus NK came The F1 and F3 fertilizer treatments showed no significant changes and were both ranked in the second position.

Table 2. Plant height (cm), LAI, % empty seeds %, no of seeds plant^{-1}, weight of 1000 seeds (g), seeds yield ha^{-1}, Oil % and yield ha^{-1} as affected by sunflower Varieties, organic matter, inorganic NPK and biological Fertilizers during 2020 growing season.

Characters / Treatments	Plant height (cm)	LAI	Empty seeds %	No. of seeds plant^{-1}	1000-seed weight (g)	Seeds yield (kg ha^{-1})	Oil %	Oil yield (kg ha^{-1})
A. Sunflower Varieties performance:								
G_1	167.49a	3.35a	10.18a	770.45a	75.02a	2898.55a	37.18b	1077.20a
G_2	157.64b	2.74b	9.34b	685.34b	65.16b	2231.97b	45.70a	1019.43b
B. Organic matter fertilizer rates:								
O_1	155.04b	2.87b	9.63	720.28	69.75b	2518.40b	41.39	1030.34b
O_2	170.09a	3.22a	9.89	735.50	70.43a	2612.13a	41.49	1066.29a
C. Inorganic NPK and biological Fertilizers:								
F_0	145.69c	2.74b	10.52	663.43c	69.14b	2319.00d	41.60	964.23c
F_1	178.85a	3.19a	9.67	778.89a	69.90ab	2733.61a	41.91	1130.47a
F_2	159.28b	3.07a	10.26	759.38ab	70.20a	2674.18ab	40.15	1061.07b
F_3	155.51bc	3.02a	9.20	714.52bc	70.34a	2524.82c	42.17	1034.91b
F_4	173.48a	3.22a	9.16	723.23b	70.86a	2574.68bc	41.38	1050.92b
LSD$_{(P<0.05)}$	**10.26**	**0.31**	**NS**	**52.23**	**0.99**	**147.49**	**NS**	**52.37**

Results indicated that plants received F3: Biofertilizer "Azotobacter chroococcum" as a source of N plus PK recorded the maximum values of oil percent (42.17 %). the highest value of oil yield per ha (1130.47 kg) resulted from applied F1: NPK 120:80:100. The growth, yield and its quality increased due to using Azotobacter microorganisms maybe due to its encourages plant growth through manufacturing of hormones that control growth, including as auxins, cytokinin, and giberellic acid (GA3); promotes nutrient uptake, protects plants from phytopathogens; colonizing them quickly by passively dispersing on the lengthening cells; Enhancing root health during the growth phase by outcompeting pathogens, that affect the roots and increasing nutrient absorption (Swain and Ray, 2009; Al-Dulaimi *et al*., 2020; Abido *et al*., 2023). These results are in agreement with those obtained by (Akbari *et al*., 2011; Toosi and Azizi, 2014; Aljanabi, 2021). On the other hand, the lowest value of empty seeds percentage was resulted when sunflower plant was received F3 fertilizer treatment (9.16 %) compared with F0 fertilizer treatment. It's noticed that using F1, F2, F3 and F4 increased plant height by (22.76, 9.32, 6.74 and 19.07%), LAI by (16.42, 12.04, 10.21 and 17.51%), number of seeds per plant by (17.40, 14.46, 7.70 and 9.01%), weight of 1000 seeds by (1.09, 1.53, 1.73 and 2.48%), seed yield per ha by (17.87, 15.31, 8.87 and 11.02%) and oil yield per ha by (17.24, 10.04, 7.33 and 8.99%) respectively, When compared to the F0 control treatment. However, the percentage of empty seed were decreased by (8.07, 2.47, 12.54 and 12.92%) due to using F1, F2, F3 and F4 fertilizer treatments as compared with using F0 fertilizer treatment. These results are in harmony with those obtained by (Datta *et al*., 2017; Hafez *et al*., 2021).

As presented in Table 3 the three-way interaction between sunflower Varieties, organic matter fertilizer and fertilizer treatments had a significant effect only on 1000 seeds weight. Results showed that using sunflower Varieties "Shumoos" under the dose of 16 tons ha^{-1} of chicken manure as well as application of F1: NPK 120:80:100 produced the height values of plant height, no of seeds plant-1, seeds yield (kg ha^{-1}) and oil yield (kg ha^{-1}). Whereas, LAI and 1000 seeds weight were produced with Shumoos Varieties under the dose of 16 tons ha^{-1} of chicken manure beside using F4: Biofertilizer "Azotobacter + Bacillus" plus K. on the other hand, Aqmar genotype beside using 16 tons ha^{-1} of chicken manure as well as application of F0: control "without any fertilizers" resulted the height percent of empty seeds. In addition, the highest average of oil % resulted from using Aqmar genotype, application of 16 tons ha^{-1} of chicken manure plus application of F4: Biofertilizer "Azotobacter + Bacillus" plus K. These results are consistent with those obtained by (Datta *et al*., 2017; Hafez *et al*., 2021).

Table 3. Plant height (cm), LAI, % empty seeds %, no of seeds $plant^{-1}$, weight of 1000 seeds (g), seeds yield ha^{-1}, Oil % and yield ha^{-1} as affected by the three way interaction between sunflower Varieties, organic matter, inorganic NPK and biological Fertilizers during 2020 growing season.

Characters / Treatments	plant height (cm)	LAI	Empty seeds %	No of seeds $plant^{-1}$	1000 seeds weight (g)	Seeds yield (kg ha^{-1})	Oil %	Oil yield (kg ha^{-1})
$F_0G_1O_1$	151.8	3.06	10.03	676.8	73.70	2492.6	38.10	949.4
$F_0G_1O_2$	154.8	3.25	9.97	746.9	72.03	2766.3	37.70	1041.6
$F_0G_2O_1$	138.9	2.27	10.17	665.5	64.53	2146.1	46.58	999.4
$F_0G_2O_2$	137.3	2.38	11.90	564.6	66.30	1871.2	46.29	866.4
$F_1G_1O_1$	183.0	3.60	10.70	801.5	74.50	2984.1	37.70	1125.6
$F_1G_1O_2$	193.7	3.19	10.77	839.4	76.60	3212.8	37.80	1214.9
$F_1G_2O_1$	170.8	2.84	8.70	735.2	64.40	2366.5	46.04	1089.0
$F_1G_2O_2$	167.9	3.13	8.50	739.5	64.11	2371.1	46.11	1092.4
$F_2G_1O_1$	150.3	3.60	10.33	774.2	74.88	2899.3	36.39	1055.9
$F_2G_1O_2$	166.2	3.59	10.30	822.2	74.60	3065.9	35.91	1101.2
$F_2G_2O_1$	147.4	2.46	9.60	753.1	65.33	2458.7	44.38	1090.3
$F_2G_2O_2$	173.2	3.02	10.77	688.1	66.00	2272.9	43.91	996.9
$F_3G_1O_1$	144.4	3.05	10.30	735.4	74.90	2755.0	37.00	1018.8
$F_3G_1O_2$	180.3	3.55	9.70	783.2	76.30	2986.5	37.50	1119.6
$F_3G_2O_1$	143.1	2.53	8.00	668.1	65.37	2183.6	46.00	1004.0
$F_3G_2O_2$	154.1	2.94	8.80	671.4	64.80	2174.2	45.88	997.3
$F_4G_1O_1$	165.8	3.17	11.13	739.8	75.70	2801.6	36.77	1029.2
$F_4G_1O_2$	184.5	3.89	8.53	785.1	77.00	3021.5	36.91	1115.8
$F_4G_2O_1$	154.9	2.53	7.37	653.3	64.20	2096.7	44.92	942.0
$F_4G_2O_2$	188.8	3.27	9.60	714.7	66.56	2379.0	46.92	1116.7
F. test ($P<0.05$)	NS	NS	NS	NS	1.99	NS	NS	NS

A Pearson's correlation analysis was conducted to examine the associations between the observed variables under study. The relationship between important agronomic variables was analyzed by Pearson correlation. Medium and positive correlation was detected between the plant height (PH) and leaf area index (LAI) (R2= 0.666**), seed number of plant (NS) (R2= 0.644**), seed yield (SY) (R2= 0.573**) and oil yield (OY) (R2= 0.770**). The relationships were also positive between the LAI and NS (R2= 0.744**), 1000-seed-weight (TSW) (R2= 0.758**), SY (R2= 0.821**) and OY (R2= 0.600**). We found very strong positive correlations between NS and SY (R2= 0.926**) and OY (R2= 0.903**), and positive correlation was also revealed between NS and TSW (R2= 0.664**). A robust positive association was discovered between TSW and SY (R2= 0.895**) and SY and OY (R2= 0.747**). The correlations between oil percentage (O%) and all studied traits were negative, they were significant in the case of LAI, NS, TSW and SY (R2 values were -0.738; -0.678; -0.960 and -0.885, respectively). We did not find any relationship between the percentage of empty seed and other characteristics (Table 4).

Table 4. Correlations between variables, and their relationships. Abbreviations: PH: plant height, LAI: Leaf area index, ES%: empty seed %, NS: No of seeds plant^{-1}, TSW: 1000 seeds weight, SY: Seeds yield (kg ha^{-1}), O%: oil % and OY: Oil yield (kg ha^{-1})

	PH	LAI	ES%	NS	TSW	SY	O%	OY
PH	1.00							
LAI	0.666**	1.00						
ES%	-0.005-	0.108	1.00					
NS	0.644**	0.744**	0.066	1.00				
TSW	0.383	0.758**	0.414	0.664**	1.00			
SY	0.573**	0.821**	0.249	0.926**	0.895**	1.00		
O%	-0.270-	-0.738-**	-0.369-	-0.678-**	-0.960-**	-0.885-**	1.00	
OY	0.770**	0.600**	-0.046-	0.903**	0.422	0.747**	-0.356	1.00

* The Pearson Correlation is statistically significant at the 0.05 level. ** The Pearson Correlation is statistically significant at the 0.01 level.

CONCLUSION

The results of this study showed that the Shumoos Varieties were found to outperform the Aqmar genotype in the following characters: plant height, leaf area index, percentage of empty grain, grains number of plant, 1000-achenes weight, grain yield of ha, and oil yield per ha. The differences were 6.24, 22.26, 8.99, 10.86, 15.13, 27.72, and 4.22%. In addition, the findings.

CONFLICT OF INTEREST

The authors assert that there are no conflicts of interest related to this manuscript.

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