RESPONSE OF SUNFLOWER TO PHOSPHORINE AND CEREALIN INOCULATION UNDER LOW NP-FERTILIZER LEVELS

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ABSTRACT

Two field experiments were conducted at the Experimental Farm, Faculty of Agriculture, Tanta University, at Kafr El-Sheikh, Egypt during 1999 and 2000 seasons. The objective of this investigation was aimed to study the response of sunflower to inoculation with biological nitrogen fixation bacteria (Cerealin) and phosphate dissolving bacteria (Phosphorine) under low nitrogen fertilizer levels i.e., (15, 30 and 45 kg N/fed.) and two phosphorous level i.e., (0 and 15 kg P_2O_5/fed.). A split plot design with four replication was used. The main findings could be summarized as follows:

Increases in nitrogen rate up to 45 kg N/fed. with or without phosphorus fertilizer significantly increased plant height, head diameter, 100-seed weight, seed husk percent, seed yield/plant as well as seed and oil yields/fed. in both seasons. Whereas, seed oil content was significantly decreased by increasing nitrogen rate. On the other hand, application of phosphorous fertilizer at the rate of 15 kg P_2O_5/fed. at the same level of applied nitrogen had no significant effect on all studied characters, except seed oil content in the two seasons.

The obtained data reveal that the inoculation of sunflower seed with N_2-fixing bacteria (Cerealin) or with phosphate dissolving bacteria (Phosphorine) or with combined of the two biofertilizers significantly enhanced all the studied traits over the control, except plant height, seed husk percent and seed oil content in both seasons. Seed inoculation with combined of Phosphorine and Cerealin gave the highest values of most studied traits.

It could be concluded that under the conditions of this investigation, inoculation of sunflower seed with Phosphorine +

Cerealin along with application of NP-fertilizer at the rate of 45 kg N + 15 kg P_2O_5/fed. is recommended for increasing yield of sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus*, L.) is becoming an increasingly important source of edible vegetable oil throughout the world due to its no cholestrol and high unsaturated fatty acids content (Leland, 1996). It receives considerable attention in Egypt due to its short growing season and it can be grown well under the low fertility soils as in the newly reclaimed areas. Sunflower could be one of the main oil crops suggested to solve edible vegetable oil shortage in the country. One important aspect of sunflower production is plant nutration as high productivty can only be achieved if plants are properly fed. The practical side of plant nutration is fertilizer application. Recently, attention was paid also to biological nitrogen fixation and phosphate dissolving bacteria because of increasing costs of non- organic nitrogen and phosphorus fertilizer and their environmental hazards.

Many reports indicated that application of nitrogen fertilizer increased plant height, head diameter, seed husk percentage, 100-seed weight, seed yield/plant as well as seed and oil yields/fed. (Anton *et al.*, 1995; Mohamed, 1996; Mohamed, 1997; El-Essawy and Mohamed, 1998; Keshta and El-Kholy, 1999; Abou Khadrah *et al.*, 2000; Basha, 2000; El-Tabbakh, 2000; Abou-Ghazala *et al.*, 2001; Abdo *et al.*, 2002 and Abou Khadrah *et al.*, 2002). On the other hand, several investigators of them reported that increasing nitrogen level decreased seed oil content.

Phosphorus is well known to be one of the most important major nutrint- elements for plant growth and development. In this respect, Mohamed (1996) and El-Tabbakh (2000) observed that increases in phosphorus rate up to 30 kg P_2O_5/fed. increased seed and oil yields/fed., seed weight/head and 100-seed weight. Also Khalil (1997) stated that increasing phosphorus application to sunflower resulted in a significant increase in seed yield and its components in addition to plant height.

Using the biological fertilizer such as phosphate dissolving bacteria and bio- nitrogen fixation bacteria aimes mainly to reduce using of meniral fertilizers in order to reduce the environmental pollution (Nawar, 1994). Radwan (1996) observed that inoculation of sunflower seed with phosphate dissolving bacteria significantly increased number and weight of seeds/head, head diameter and seed index in addition to sunflower growth attributes. The results obtained by Keshta and El-Kholy (1999) indicated that the application of inorganic nitrogen and bionitrogen fertilizers for sunflower increased plant height, head diameter, 100- seed weight, seed yield/fed. and seed oil content. Sharief *et al.* (2000) reported that applying either the fertilization regimes (Cerealin + Phosphorine) along with 50 kg N/fed. or 70 kg N/fed. (as control) for wheat produced the heaviest grain weight, number of grains/spike, number of spikes/m^2 and grain yield/fed. Abou Khadrah *et al.* (2002) found that inoculation of sunflower seed with phosphorine as biofertilizer significantly increased head diameter, number of seeds/head, seed oil content, seed yield/plant as well as seed and oil yields/fed., but it did not show any significant effect on plant height, 100- seed weight and seed husk percent.

The objective of this study is to evaluate the response of sunflower plants to Phosphorine (phosphate dissolving bacteria) and Cerealin (bio- nitrogen fixation bacteria) under low NP-fertilizer levels.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm, Faculty of Agriculture, Tanta University at Kafr El-Sheikh Governorate, Egypt during the two successive sunflower growing seasons, 1999 and 2000. The soil of the experimental fields was clay in texture with pH of 8.1, organic matter, 1.72% and containing 18.44, 12.37 and 282 ppm available N, P and K, respectively. The preceding crop was wheat in the two seasons. A split plot design with four replicates was used with six treatments from combinations between three nitrogen levels (15, 30 and 45 kg N/fed.) and two phosphorus levels (0 and 15 kg P_2O_5/fed.) as main plots and four seed inoculation treatments, viz., without inoculation (control), inoculation with Phosphorine, inoculation with Cerealin and inoculation with mixture of Phosphorine and Cerealin as sub plots. The following combination levels of NP in kg N and P_2O_5/fed. were used:

1) $N_{15}P_0$, 2) $N_{15}P_{15}$, 3) $N_{30}P_0$, 4) $N_{30}P_{15}$, 5) $N_{45}P_0$, 6) $N_{45}P_{15}$.

Each sub plot consisted of 5 ridges each 3.5 in length and 60 cm in width with 20 cm between hills. Seed of sunflower hybrid "Vidoc" were sown during the latest week of May in both seasons. Calcium super phosphate (15.5% P_2O_5) at the above mentioned levels was applied before sowing. Nitrogen fertilizer was fully given as Urea (46% N) after thinning and just before the first irrigation. Phosphorine is a commercial biofertilizer containing active phosphate dissolving bacteria and Cerealin is also a commercial biofertilizer containing active bio-nitrogen fixation bacteria, produced by the General Organization for Agricultural Equalization Fund, Ministry of Agriculture and Land Reclamation. The wetted sunflower seed was thoroughly inoculated with different inoculation treatments just before planting. Arabic gum was used as an adhesive agent. Soil was directly irrigated after sowing to provide suitable moisture for the inocula. Other cultural practices for growing sunflower were conducted as recommended.

At harvest, ten guarded plants were taken at random from each sub plot and the following characters were determined: plant height (cm), head diameter (cm), 100-seed weight (g), seed yield (g/plant), seed husk percentage and seed oil content. Seed oil content was determined according to A.O.A.C. (1980) using soxhlet apparatus and diethyl ether as a solvent. Heads of the two inner ridges of each sub plot were harvested and left two weeks until fully air dried by sunshine and seed yield was calculated as t/fed. Oil yield (kg/fed.) was determined by multiplying seed yield (kg/fed.) by seed oil content.

Data of both seasons were subjected to the proper analysis of variance according to Snedecor and Cochran (1980). For comparison between means, Duncan's multiple range test was done (Duncan, 1955). All statistical analysis were performed using analysis of variance technique by means of "IRRISTAT" computer software package.

RESULTS AND DISCUSSION

A. Effect of NP- fertilizer level:

Results presented in Tables (1 and 2) show a significant response in yield and its attributes of sunflower plants to nitrogen levels in both seasons. Increasing nitrogen level from 15 to 45 kg N/fed. with or without phosphorus fertilizer resulted in a highly significant increase in plant height in the two seasons. This increase might be explained in terms of more cell division or/ and cell elongation of stems. Head diameter showed a progressive increase with increasing nitrogen level. It is widely recognized that adequate nitrogen increase growth more than the ratio of development leading to larger head diameter. These results are similar to those reported by Mohamed (1997), El-Essawy and Mohamed (1998), Abou-Ghazala *et al.* (2001) and Abou Khadrah *et al.* (2002).

Each increment of applied nitrogen up to 45 kg N/fed. in both seasons was accompanied by significant increases in seed yield/plant and 100-seed weight (Tables 1 and 2). Such findings could be attributed to the promoting effect of nitrogen on seed setting and the seed size. These results are in full agreement with those recorded by Anton *et al.* (1995), Mohamed (1996), Basha (2000) and Abdo *et al.* (2002).

As shown in Tables (1 and 2), seed husk percent was significantly increased with increasing N-level in both seasons. The increase in seed husk percent by application of N-fertilizer is in agreement with the results obtained by Mohamed (1997), El-Essawy and Mohamed (1998), Abou Khadrah *et al.* (2000) and Abou Khadrah *et al.* (2002).

According to the results presented in Tables (1 and 2), each nitrogen increment resulted in a significant decrease in seed oil content. At the low nitrogen nutrition the oil content was higher but the seed yield and the seed size were depressed. This beneficial effect of the lower level of nitrogen nutrition on the oil content probably resulted because the earlier senescence of leaves, obseved in this treatment, reduced the rate of seed filling during seed maturation. Similar results were obtained by Mohamed (1996), Mohamed (1997), Keshta and El-Kholy (1999) and Abou-Ghazala *et al.* (2001).

Table (1): Effect of NP-fertilizer level and seed inoculation with two biofertilizer on some characteristics of sunflower plants during 1999 and 2000 seasons.

Factor	Plant height (cm)		Head diameter (cm)		100-seed weight (g)		Seed husk %	
	1999	2000	1999	2000	1999	2000	1999	2000
NP-fertilizer level (A):								
N_{15} P_0	149.37c	153.71c	17.51c	18.05c	4.83d	5.03c	20.34c	19.94d
N_{15} P_{15}	150.15c	154.20c	17.57c	18.11c	4.94cd	5.11c	20.75bc	20.26cd
N_{30} P_0	156.80b	159.70b	18.27b	18.75b	5.23bc	5.69b	21.12b	20.48bc
N_{30} P_{15}	157.10b	160.11b	18.33b	18.82b	5.32b	5.77b	21.53a	20.89b
N_{45} P_0	162.60a	164.66a	19.92a	20.24a	5.58ab	6.39a	21.75a	21.50a
N_{45} P_{15}	162.55a	165.03a	20.00a	20.36a	5.68a	6.50a	21.84a	21.63a
F- test	**	**	**	**	**	**	*	*
Inoculation (B):								
Uninoculated	154.23	156.63	18.25c	18.76c	5.01c	5.49c	21.10	20.59
Phosphorine	156.10	158.93	18.54bc	19.03b	5.22bc	5.71b	21.16	20.74
Cerealin	157.05	161.21	18.66b	19.07b	5.30ab	5.80ab	21.35	20.96
Ph. + Cer.	158.33	161.51	18.95a	19.37a	5.52a	5.99a	21.27	20.84
F-test	NS	NS	*	*	*	*	NS	NS
Interaction	NS	NS	*	*	NS	NS	NS	NS

*, ** and NS indicate $P<0.05$, $P<0.01$ and not significant, respectively.
Means followed by the same letter within columns are not significantly different at the 5% level, using Duncan's multiple range test.

Seed and oil yields/fed. were responsed positively and significantly to increasing nitrogen level up to 45 Kg N/fed. This is fairly true for both seasons. It is evident from Tables (1 and 2) that each increment of applied nitrogen resulted in a significant increase in seed and oil yields/fed. The increase in seed yield/fed. due to nitrogen application could be attributed to the contribution of larger head diameter, higher seed yield/plant and heavier 100-seed weight. Since the oil content decreased with increasing N- level, the increase in oil yield, despite the decrease in oil content could be ascribed to the increase in seed yield/fed. This indicates that any decrease in seed oil content is offset by a higher seed yield. The role of N in enhancing seed and oil yields/fed. of sunflower was reported by Basha (2000), El-Tabbakh (2000), Abou-Ghazala *et al.* (2001), Abdo *et al.* (2002) and Abou Khadrah *et al.* (2002).

Table (2): Effect of NP-fertilizer level and seed inoculation with two biofertilizer on some characteristics of sunflower plants during 1999 and 2000 seasons.

Factor	Seed yield (g/plant)		Seed yield (t/fed.)		Seed oil content		Oil yield (kg/fed.)	
	1999	2000	1999	2000	1999	2000	1999	2000
NP-fertilizer level (A):								
$N_{15} P_0$	46.90c	51.69c	1.055d	1.141c	41.60b	41.72b	439.33d	468.91e
$N_{15} P_{15}$	48.00c	51.40c	1.164c	1.193c	41.76a	41.87a	486.24c	499.98d
$N_{30} P_0$	52.67bc	54.40bc	1.301b	1.333b	40.75d	40.88d	530.45b	544.99c
$N_{30} P_{15}$	54.78b	56.72b	1.332b	1.379b	40.92c	41.06c	545.40b	566.59b
$N_{45} P_0$	58.26ab	59.50ab	1.506a	1.549a	40.50f	40.70e	610.02a	631.02a
$N_{45} P_{15}$	61.14a	62.48a	1.531a	1.582a	40.70e	40.58d	623.66a	646.26a
F- test	**	**	**	**	*	*	**	**
Inoculation (B):								
Uninoculated	48.17c	52.54c	1.197c	1.199c	41.03	41.15	490.71c	492.45c
Phosphorine	52.67b	55.25b	1.299b	1.341b	41.19	41.31	534.65b	549.52b
Cerealin	54.26b	56.08b	1.329b	1.402b	40.79	40.98	541.71ab	573.39ab
Ph. + Cer.	59.41a	60.28a	1.434a	1.510a	41.15	41.28	589.67a	623.12a
F-test	**	*	*	*	NS	NS	*	*
Interaction	*	NS	**	**	NS	NS	**	*

*, ** and NS indicate P<0.05, P<0.01 and not significant, respectively.
Means followed by the same letter within columns are not significantly different at the 5% level, using Duncan's multiple range test.

The data presented in Tables (1 and 2) show that application of phosphorus fertilizer at the rate of 15 kg P_2O_5/fed. at the same level of applied nitrogen had no significant effect on all studied characters, except seed oil content in both seasons. It is evident from the presented data that application of 15 kg P_2O_5/fed. at the same N-level tended to increase plant height, head diameter, 100-seed weight, seed yield/plant as well as seed and oil yields/fed. but the differences between the two treatments of phosphorus fertilizer were not great enough to reach the 5% level of significance. Available P content in the soil of the experimental site was (12.37 ppm) and this may be fair enough to face sunflower plant requirements. In this connection, Mohamed (1996) and El-Tabbakh (2000) found that increasing phosphorus rate up to 30 kg P_2O_5/fed. significantly increased 100-seed weight, seed weight/head as well as seed and oil yields/fed. Also Khalil (1997) stated that increasing phosphorus application to sunflower resulted in a significant increase in seed yield and its components in addition to plant height.

B. Effect of seed inoculation:

Data in Tables (1 and 2) clearly reveal that inoculation of sunflower seed with phosphate dissolving bacteria (Phosphorine) or with N_2-fixing bacteria (Cerealin) or with both biofertilizers significantly enhanced all the studied traits over the control (uninoculated), except plant height, seed husk percent and seed oil content in 1999 and 2000 seasons. It is also clear from the present data that the seed inoculation with Phosphorine + Cerealin gave the highest values of head diameter, seed yield per plant and per feddan as well as the highest values of 100-seed weight and oil yield/fed. Generally, results pointed out to a beneficial effect of seed inoculation with N_2-fixing or/and phosphate dissolving bacteria on yield and most yield components might be attributed to the fixation of nitrogen, the soluble mono- calcium phosphate and/or to the production of certain plant growth promoting substances by these bacteria as well as supplying sunflower plants with its requirements during different growth stages. The increase in sunflower seed yield, resulted by the inoculation with

Phosphorine and Cerealin might be due to the favourable effect of biofertilizers on plant height, head diameter and seed yield/plant. Also, the increase in oil yield might be attributed to greate seed yield/fed. The previous results are in good accordance with those reported by Radwan (1996); Keshta and El- Kholy (1999); Sharief *et al.* (2000) and Abou Khadrah *et al.* (2002).

C. Effect of interaction:

The interaction between NP-fertilizer level and inoculation treatment had a significant effect on head diameter, seed and oil yields/fed. in both seasons as well as on seed yield/plant in the first season (Tables 1 and 2). Data presented in Table (3) as well as Fig. (1 and 2) for seed yield/fed. reveal that the maximum head diameter (20.36 and 20.62 cm), seed yield/plant (66.41 g/plant), seed yield/fed. (1.676 and 1.705 t/fed.) and oil yield/fed. (684.43 and 698.42 kg/fed.) were recorded when sunflower seed was inoculated with Phosphorine + Cerealin in addition to applied NP-fertilizer at the rate of 45 kg N + 15 kg P_2O_5/fed. in both seasons, respectively. Whereas the lowest of these traits were given by the control treatment (without inoculation) + 15 kg N/fed. in the two seasons. On the light of the obtained results, it could be concluded that under the conditions of this investigation, enrichment of sunflower plants with NP-fertilizer at the rate of 45 kg N + 15 kg P_2O_5/fed. combined with Phosphorine + Cerealin inoculation gave a considerable increase in sunflower yield and its attributes.

Table (3): Effect of interaction between NP-fertilizer level and seed inoculation treatment on some characteristics of sunflower plants during 1999 and 2000 seasons.

NP level	Seed inoculation treatment	Head diameter (cm)		Seed yield (g/plant)	Seed yield (t/fed.)		Oil yield (kg/fed.)	
		1999	2000	1999	1999	2000	1999	2000
N_{15} P_0	1	17.18p	17.77m	41.61q	0.990u	0.918p	412.45n	383.09m
	2	17.43o	18.01L	45.37p	1.034t	1.117o	432.35m	443.10L
	3	17.61n	18.12L	47.58n	1.068s	1.210mn	442.13m	498.08k
	4	17.83m	18.31k	53.06k	1.130q	1.322jkl	470.42k	551.39hij
N_{15} P_{15}	1	17.11p	17.77m	41.79q	1.089r	0.954p	455.51L	399.92m
	2	17.59n	18.13L	46.65o	1.139p	1.167n	478.66jk	490.67k
	3	17.64n	18.15L	48.86m	1.175n	1.274L	487.36j	530.08j
	4	17.93L	18.40jk	54.69hi	1.251m	1.379hi	523.42i	579.26fg
N_{30} P_0	1	17.98L	18.47j	47.55n	1.146o	1.184mn	466.64kl	383.92k
	2	18.18k	18.65i	51.91L	1.301L	1.313kl	531.43i	538.35ij
	3	18.31ij	18.77hi	53.18k	1.321k	1.361ijk	535.72hi	553.81hi
	4	18.60h	19.13g	58.04ef	1.438g	1.473def	588.02e	603.90de
N_{30} P_{15}	1	18.01L	18.49j	48.50m	1.183n	1.221m	484.05j	501.13k
	2	18.27j	18.76hi	54.39ij	1.325j	1.373hig	544.04gh	565.55gh
	3	18.36i	18.82h	55.47gh	1.365i	1.417gh	556.01g	579.54fg
	4	18.70g	19.22g	60.76d	1.456f	1.505cde	597.52de	620.12cd
N_{45} P_0	1	19.59f	20.02f	53.53jk	1.373i	1.446fg	555.91g	587.49efg
	2	19.83e	20.26de	57.35f	1.483e	1.522cd	603.17d	620.93cd
	3	19.97d	20.16ef	58.69e	1.512d	1.554bc	606.79cd	629.99bc
	4	20.27b	20.51ab	63.49b	1.655b	1.677a	674.22a	685.66a
N_{45} P_{15}	1	19.66f	20.05f	56.04g	1.401h	1.468efg	569.68f	599.18def
	2	19.94d	20.36cd	60.35d	1.513d	1.557bc	618.27bc	638.56bc
	3	20.06c	20.41bc	61.76c	1.535c	1.596b	622.25b	648.87b
	4	20.36a	20.62a	66.41a	1.676a	1.705a	684.43a	698.42a

Means followed by the same letter within columns are not significantly different at the 5% level. using Duncan's multiple range test.

Where : (1) Uninoculated (2) Phosphorine (Ph)

(3) Cerealin (Cer) (4) Ph. + Cer.

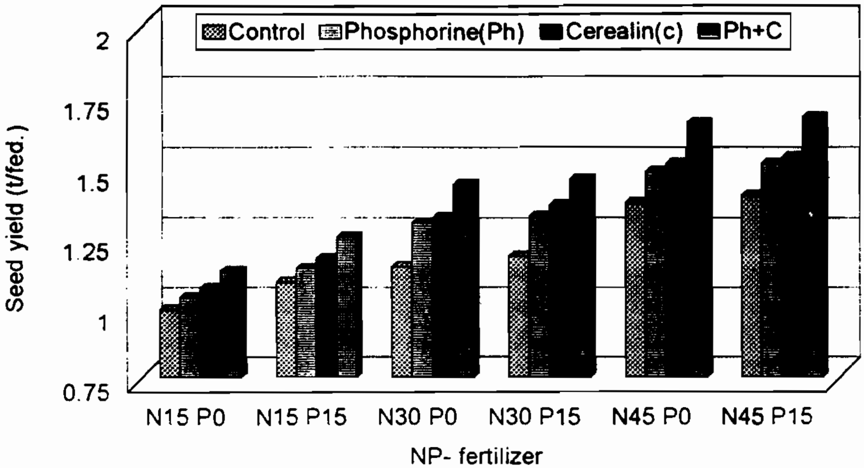

Fig. (1): Effect of interaction between NP-fertilizer level and seed inoculation on seed yield in 1999 season.

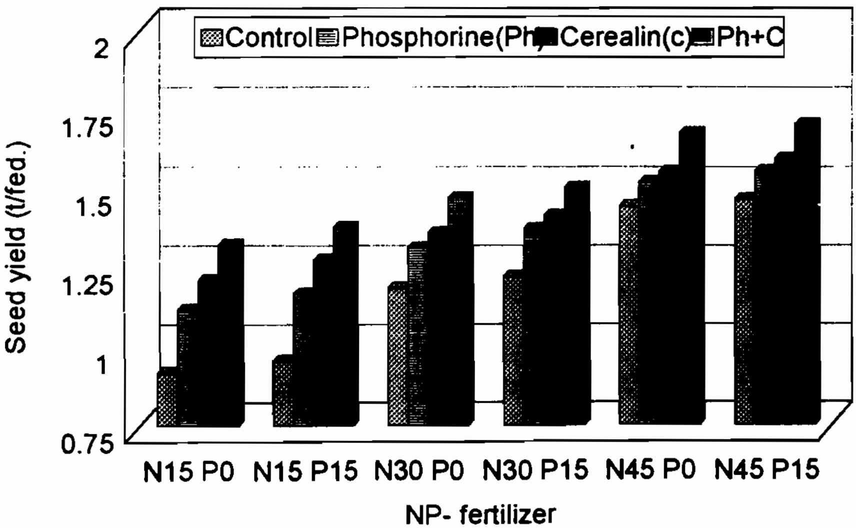

Fig. (2): Effect of interaction between NP-fertilizer level and seed inoculation on seed yield in 2000 season.

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الملخص العربى

إستجابة عباد الشمس للتلقيح بالفسفورين والسيريالين تحت مستويات السماد الأزوتى والفوسفاتى المنخفضة

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قسم المحاصيل – كلية الزراعة بكفرالشيخ – جامعة طنطا – مصر

أجريت تجربتان حقليتان بالمزرعة البحثية بكلية الزراعة بكفرالشيخ – جامعة طنطا- مصر خلال موسمى ١٩٩٩، ٢٠٠٠ بهدف دراسة استجابة هجين عباد الشمس فيدوك للتلقيح بالبكتريا الحيوية المثبته للنيتروجين (السيريالين) والبكتريا الميسرة للفوسفات (الفسفورين) تحت مستويات منخفضة من الأزوت (١٥، ٣٠، ٤٥ كجم أزوت/فدان) ومستويين من الفسفور (صفر، ١٥ كجم فو٢أ٥/فدان). وقد استخدم تصميم القطع المنشقة فى أربع مكررات فى تنفيذ التجارب. ويمكن تلخيص أهم النتائج المتحصل عليها فيما يلى :

أدت الزيادة فى مستوى التسميد الزوتى حتى ٤٥ كجم أزوت/فدان مع أو بدون التسميد الفوسفاتى الى زيادة معنوية فى كل من ارتفاع النبات، قطر القرص، وزن الـ ١٠٠ بذرة، النسبة المئوية للقشرة، محصول البذور للنبات وكذلك محصول البذور والزيت للفدان بكلا موسمى الدراسة. بينما نقص محتوى الزيت بالبذور معنويا بزيادة مستويات التسميد الأزوتى. ومن ناحية آخرى فلم يكن لإضافة التسميد الفوسفاتى بمعدل ١٥ كجم فو٢أ٥/فدان عند نفس المستوى من التسميد الأزوتى أى تأثير معنوى على أى صفة من الصفات المدروسة فيما عدا محتوى الزيت بالبذور فى كلا الموسمين.

أوضحت النتائج المتحصل عليها أن تلقيح بذور عباد الشمس بالبكتريا المثبته للنيتروجين حيويا (السيريالين) أو بالبكتريا الميسرة للفسفور (الفسفورين) أو بهذين السمادين الحيويين أدى الى زيادة معنوية فى كل الصفات المدروسة مقارنة بالكنترول فيما عدا إرتفاع النبات، النسبة المئوية للقشرة، محتوى الزيت بالبذور بكلا الموسمين. وقد أعطت معاملة التلقيح بخليط من الفسفورين والسيريالين أعلى القيم لمعظم الصفات المدروسة.

وتحت ظروف هذا البحث يمكن أن نستخلص أن تلقيح بذور عباد الشمس بكل من الفسفورين والسيريالين وإضافة السماد الأزوتى والفوسفاتى بمعدل ٤٥ كجم أزوت + ١٥كجم فو٢أ٥ للفدان يمكن أن ينصح به لزيادة محصول عباد الشمس.