Effect of Seeds Bio-inoculation and Nitrogen Fertilization Levels on Yield and Its Components of Three Soybean Cultivars

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ABSTRACT

Two field experiments were conducted at the Agriculture Research Station, Alexandria University, Egypt, in 2022 and 2023, to clarify the effect of seed bio-inoculation (without and with) and three levels of nitrogen fertilization 48, 96 and 144 kg N/ha on yield and its components of three soybean cultivars, i.e. Giza 35, Giza 111 and Giza 83. Obtained results showed a significant three-way interaction among the studied factors for most traits measured in both seasons, harvest index in the first season only and number of branches/plant in the second season only. Plants fertilized with the 144 kg N/ha or 96 kg N/ha with bio-inoculation of seeds gave the highest significant values for the studied traits. Also, Giza 35 cultivar was the most responsive. However, the least significantly values of the studied traits were obtained from Giza 83 cultivar fertilized with 48 kg N/ha without seed bio-inoculation. Conversely harvest index trait showed an opposite trend to the other traits, as increasing the nitrogen fertilization level led to a decrease in the harvest index, while decreasing the nitrogen level without or with seed bio-inoculation gave the highest harvest index in the first season. Therefore, it was concluded that under the conditions of this study, bio-inoculation of seeds with non-symbiotic atmospheric nitrogen-fixing bacteria maximizes the benefit from nitrogen fertilizer and saving of about 33% (48 kg N/ha) of mineral nitrogen fertilizer from the recommended amount in the absence of root nodules.

Keywords: soybean; seeds bio-inoculation; biofertilizer; N fertilizer; nitrogen level; yield; oil yield.

INTRODUCTION

The soybean _Glycine max_ is one of the most important legume crops in the world and Egypt. Seeds are characterized by a high protein content, reaching about 40%. Soybean plays a significant role in human nutrition directly, through its use in products that are manufactured and consumed directly or indirectly, as it is a basic component in the production of animal and poultry feed. Soybean is also used in many other industries, including cosmetics and medicine. It is also considered an important oil crop used in the oil extraction industry, with an oil content of about 20% in its seeds.

Soybean cultivation in Egypt began in 1972 with an area not exceeding three thousand faddan with an average yield of 300 kg/fed. This area has increased and decreased over the years due to competition from other summer crops. However, starting from 2019 (29.5 thousand faddan with an average of 1.23 tons/fed), the cultivated area began to increase, as it reached 49 thousand faddan in the 2021 with an average of 1.28 tons/fed, and 88.3 thousand faddan with an average yield of 1.32 ton/fed in the 2022 (MALR, 2022), which reflects the state's interest in increasing soybean production due to its high price globally.

Egypt suffers from a severe shortage of soybeans it consumes annually. Egypt produces approximately 63 thousand tons, while its annual consumption is around 4.2 million tons in 2021 (FAO, 2022). This leads to large imports, to cover this shortage, which increases the burden on foreign exchange and negatively impacts the country's food security. Most of Egypt's soybean imports come from the United States (around 70% of total imports, FAO, 2022), which costs Egypt a lot of foreign currency. Therefore, we must focus on improving and increasing soybean production to reduce dependence on imports and conserve foreign currency.

Several reasons or problems limit soybean cultivation in Egypt, including: the high costs of soybean cultivation, especially fertilizer and pest control; competition with other summer crops; low yields and profits per unit area and the difficulty of root nodule bacteria (*Bradyrhizobium*) being present, their ineffectiveness with Rhizobium inoculation due to the high pH of Egyptian soil.

Research centers have worked to improve older soybean cultivars and develop new early maturing and high yielding cultivars, thus increasing farmers' economic returns and reducing production costs. Among these new cultivars are Giza 111, Giza 21, Giza 22, Giza 35, Giza 82, and Giza 83. These early cultivars are resistant to the cotton leafworm, and their average yield ranges between 1.3 and 1.7 tons /fed. El-Douby *et al.* (2002); El Haggan & Mekkei (2014) and El-Karamity *et al.* (2015) indicated significant differences in the yield and its components for several of those cultivars.

Mineral fertilization is one of the main reasons for the high cost of soybean production, given its high price. As a result of the absence of root nodule bacteria,

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farmers resort to fertilizing the soybean crop with a large amount of mineral nitrogen fertilizer (144 kg/ha) due to the important role of the nitrogen element for plants, as it enters chlorophyll synthesis, increasing vegetative growth, and enhancing protein content in seeds. El-Douby & Shams El-Din (1997); Allam (2005) and Nawar & Abdel-Galil (2008), where the trend of their results refers to the increasing mineral nitrogen fertilization improving yield and its components with rising prices for mineral fertilizers, including nitrogen fertilizers, farmers' profitability is significantly impacted. Furthermore, mineral fertilizers have numerous negative effects on the soil and environment, increasing pollution. Therefore, scientists are exploring ways to reduce reliance on mineral fertilizers. The use of alternatives to mineral fertilizers, such as the use of biofertilizers, is a method to improve the efficiency of atmospheric nitrogen fixation by other nitrogen-fixing bacteria in what is known as clean agriculture or environmental friendly agriculture (Singh *et al*., 1999; Palm *et al*., 2001 and Korobko *et al*., 2024). Also, Din *et al*. (2019) concluded that using *Aspergillus niger* and *Azotobacter* to inoculate seeds could provide a sustainable alternative to expensive and environmentally harmful chemical fertilizers, offering a more eco-friendly and cost-effective solution through biofertilizers.

Among the biofertilizers that can be used to fix atmospheric nitrogen are those containing microorganisms such as bacteria that are found in soils with a high pH (alkaline soil) and live freely in the soil (non-symbiotically), such as *Azotobacter*, *Azospirillum*, and *Bacillus polymyxa* (Herridge *et al*., 2008). These bacteria fix atmospheric nitrogen, making it available to plants grown in these soils. This reduces the need for chemical (mineral) nitrogen fertilizers, lowers production costs and contributes to reducing soil and environmental pollution. Buttery *et al.* (1992) and Purcell & King (1996) showed that environmental factors such as soil moisture, compaction, pH, temperature, pests, and diseases can limit biological nitrogen fixation (Jnawali *et al.,* 2015).

Many studies have shown that the use of biofertilizers can enhance plant growth and improve crop productivity by promoting soil biological activity and improving nutrient availability to plants. Inoculated maize plants with a mixture of *Azotobacter* and *Azospirillum* bacteria increased plant height (Gholami *et al*., 2008). Havlin *et al*. (2005) explained that the increased growth of plants is due to the role of Azotobacter in fixing atmospheric nitrogen, which is used in building cells and chlorophyll, thus increasing plant growth. Hungria and Nogueira (2013) noted that soybean yield increased by 16-19% with inoculation with *Rhizobia* and *Azospirillum*. According to Zafar *et al*. (2011), the application of biofertilizers in soil improves plant nutrient absorption, stimulates cell division, and increases leaf cell size, resulting in greater leaf area.

Therefore, the use of such biofertilizers with soybean crop can play an important role in encouraging farmers to cultivate this crop in Egypt. This research aimed to study the effect of biofertilizers and nitrogen fertilization levels on the yield and its components of three soybean cultivars.

MATERIAL AND METHODS

Two field experiments were conducted at the Agricultural Research Station, Alexandria University, Egypt, where sowing was performed on the 20th of May in 2022and the 15th of May in 2023 summer seasons to study the effect of nitrogen fertilization levels and seed bio-inoculation on yield and its components of three soybean cultivars. The trial was conducted on a field characterized by clay soil (62% clay, 20% silt and 17.5% sand), of pH 8.36, EC of 2.23 dS m^{-1} and the available macro-elements were N = 12.0 ppm, P = 9.6 ppm and K = 32.8 ppm.

Seeds of the three investigated soybean cultivars, Giza 35, Giza 111 and Giza 83, were sown in a split-split-plot experiment with three replicates. The main plots were allocated to the three cultivars; subplots were devoted to nitrogen levels (48 kg N/ha as starter dose at planting, 96 kg N/ha split into two equal doses and applied at planting and 21 days after sowing (DAS), 144 kg N/ha split into three equal doses and applied at planting, 21 just after thinning and 36 (DAS) and sub-sub plots were assigned to treatment of seeds with bio-fertilizer or without. Treatment with bio-inoculant was at the rate of 300 g per 35 kg seeds, before sowing, using Arabic gum solution (16%) as an adhesive agent. Each of the experimental plots included 5 ridges, 3 meters long and 0.7 meters apart, resulting in an experimental plot area of 10.5 m^2. Four seeds were sown in hills 0.2 m apart on both sides of the ridge, then thinned to 2 plants/hill at 21 days after sowing. The previous crop was canola in both seasons. A single dose of 54 kg P_2O_5/ha was applied during seed bed preparation, in the form of calcium superphosphate (15.5 P_2O_5). Other cultural practices, including irrigation, weed control and pest control, were performed as recommended.

The applied bio-fertilizer was supplied by the General Authority of the Agricultural Budget Fund (GAABF) and was prepared as a mixture of the three strains, i.e. *Azotobacter*, *Azosprillum* and *Bacillus polymyxa*.

At harvest, five plants were taken randomly from the guarded ridge of each plot and data on plant height (cm), number of branches/plant and number of pods/plant were recorded. After air drying, the number of seeds/pod, the 100-seed weight (g) and seed yield/plant (g), as an average of five plants, were also recorded. Seed yield/ha was calculated after harvesting the entire guarded ridges (kg) then converted to t/ha, while harvest index was calculated according to the following formula:

Harvest index = Seed yield (kg) /Biological yield (seed + straw) (kg).

Seed oil percentage was determined in seed samples for each sub sub-sub plot using Soxhlet extractor according to A.O.A.C. (2000). Oil yield / ha. was calculated according to the following formula:

Seed yield/ha X oil %.

Statistical analysis:

Data were statistically analyzed according to Gomez and Gomez (1984) using the SAS (2007) 9.1.3 software for each year separately. Significance was declared at $p<0.05$ and the least significant difference (L.S.D.) procedure was used for comparison of means of the studied treatments.

RESULTS AND DISCUSSION

Main effect of the studied factors:

The general trend of the cultivar effect was non-significant for all studied traits, except number of pods and seed yield/ plant in the first season, where, the cultivar Giza 35 reflected superiority over Giza 111 and Giza 83, with number of 48.37 pods/plant and seed yield 18.1 g/plant, respectively (Tables 1&2). This result may be due to the difference in genetic makeup among the studied cultivars. These results were similar to El-Karamity *et al*. (2015), who indicated the superiority of the Giza 35 cultivar for the number of pods/plant and seed yield/plant. There were insignificant differences among the studied cultivars during the first season, for the four traits: plant height, number of branches per plant, number of seeds per pod and seed index.

As for the main effect of nitrogen levels, number of pods/plant, seed index, seed yield/plant, seed yield/ha, oil yield / ha and harvest index were significantly affected by N levels during the two growing seasons (Tables 1&2). The results indicated that the highest nitrogen level (144 kg N/ha) was superior to lower N levels for those traits, except for harvest index, where the lower nitrogen level (48 kg N/ha) resulted in the highest harvest index. The increase in the number of pods/plant may be due to the important role of nitrogen, as it leads to increase vegetative growth and improves the plant's potential to produce a greater number of pods with seeds (El-Douby & Shams El-Din, 1997 and Badran, 2003).

Table 1. Plant height, no. of branches/plant, no. of pods/plant, no. of seeds/pod and seed index means as affected by cultivars, nitrogen levels and seed bio-inoculation during 2022 and 2023 seasons

Treatments	Plant height (cm)		Number of branches/plant		Number of pods/plant		Number of seeds/pod		Seed index (g)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Cultivars:										
Giza 35	113.11	122.06	5.46	5.25	48.37	45.31	2.42	2.50	15.24	15.07
Giza 111	111.85	121.85	5.13	5.17	37.34	44.01	2.44	2.56	15.56	14.97
Giza 83	107.30	121.91	5.13	4.56	36.87	42.58	2.42	2.32	14.55	15.00
L.S.D. (0.05)	N.S.[1]	N.S.	N.S.	N.S.	6.06	N.S.	N.S.	N.S.	N.S.	N.S.
Nitrogen Levels (kg N / ha.):										
48	107.92	117.72	5.02	4.17	27.74	31.15	2.32	2.33	14.33	14.20
96	110.67	123.78	5.33	4.81	43.01	47.09	2.32	2.37	15.01	15.12
144	113.67	124.32	5.37	6.00	51.83	53.66	2.56	2.58	16.00	15.72
L.S.D. (0.05)	N.S.	N.S.	N.S.	N.S.	3.57	7.19	N.S.	N.S.	0.93	0.82
Seed bio-inoculation										
Without	109.99	121.23	4.94	4.52	34.05	37.75	2.41	2.36	14.62	14.64
With	111.52	122.65	5.54	5.46	47.67	50.18	2.44	2.49	15.61	15.38
L.S.D. (0.05)	N.S.	N.S.	N.S.	N.S.	2.06	4.87	N.S.	N.S.	0.62	0.51

N.S.: not significant at 0.05 level of probability.

Table 2. Seed yield/plant, oil seed %, seed yield/ha, oil yield/ha and harvest index mean as affected by cultivars, nitrogen levels and Seed bio inoculation in 2022 and 2023 seasons

Treatments	Seed yield/ plant (g)		Oil seed (%)		Seed yield / ha(t)		Oil yield / ha (t)		Harvest index (%)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Cultivars:										
Giza 35	18.10	17.01	20.83	20.13	3.93	4.05	0.82	0.81	34.15	33.33
Giza 111	14.35	16.41	20.28	20.06	3.90	3.95	0.76	0.82	33.45	32.41
Giza 83	13.28	15.92	20.56	20.08	3.48	3.93	0.71	0.79	32.99	32.41
L.S.D. (0.05)	2.96	N.S.*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Nitrogen Levels (kg N / ha.):										
48	9.69	10.96	20.28	20.18	2.85	2.90	0.58	0.58	36.43	35.20
96	15.90	17.54	20.84	19.88	3.99	4.28	0.81	0.87	32.90	32.57
144	20.14	20.83	20.55	20.21	4.46	4.75	0.91	0.95	31.27	30.37
L.S.D. (0.05)	1.96	2.07	N.S.	N.S.	0.39	0.46	0.07	0.09.	3.32	3.7
Seed bio inoculation										
Without	12.24	13.75	20.37	20.11	3.45	3.64	0.70	0.70	32.54	32.09
With	18.25	19.15	20.74	20.07	4.09	4.31	0.82	0.87	34.52	33.34
L.S.D. (0.05)	1.09	1.5	N.S.	N.S.	0.23	0.29	0.03	0.06	N.S.	N.S.

*N.S.: not significant at 0.05 level of probability.

Also, an increase of seed index trait may be due to an increase in the process of photosynthesis, in which nitrogen plays an important role through the formation of chlorophyll and an increase in leaf area index. The increase in plant yield and hectare yield is a result of an increase in the traits contributing to the yield. The same results were recorded by El-Douby & Shams El-Din (1997); Badran (2003); Allam (2005); Hussein & El-Melegy (2005) and El-Karamity *et al*. (2015).

Concerning the overall effect of seed bio-inoculation, number of pods /plant, seed index, plant yield, seed yield/ha, and oil yield/ha were affected significantly when seeds were inoculated with bio-fertilizer, which gave the highest values for these traits compared to no bio-inoculation as shown in Tables (1&2). These results could be due to the role played by these bacteria in fixing nitrogen, maximizing its use efficiency and increasing its availability in the soil, thus increasing the vegetative growth of the plants, which leads to an increase in the yield components and seed yield in turn**.** Havlin *et al*. (2005); Zafar *et al*. (2011) and Hungria & Nogueira (2013) reported similar results.

Two-way interaction effect:

With respect to the interaction between cultivars and seed bio-inoculation, the results reflected a significant effect on plant yield in both seasons and seed index in the first season (Table 3 and Fig. 1). Generally, bio-treated cultivars seeds showed higher means compared to non-treated. Giza 35 gave the highest mean values for plant yield in both seasons with averages of 21.03 and 19.47 respectively, followed by Giza 111(17.25 g); while Giza 83 had the lowest means (14.46 and 18.98 g) in the first and second seasons, respectively. Concerning seed index trait, inoculated seed of cultivars with bio-fertilizer led to an increase in seed weight except Giza 83, resulting in higher seed index values compared to non-treated seeds. The most responsive cultivars were Giza 111 and Giza 35 (16.22 and 15.62 g), while Giza 83 had the lowest average seed index of 14.98 g as shown in Table (3) and Fig. (2).

The interaction between nitrogen levels and seed bio-inoculation recorded significant effects for pods number, seed index, in the two season and seed yield/ha (in the second season). It was also found that increasing nitrogen levels combined with bio-inoculation increased the averages of those traits. Number of pods/plant gave the highest averages (57.80 and 60.61) as shown in Fig. (3); seed index (16.56 and 16.25 g) in Fig. (4), and seed yield which gave 4.81 ton/ha, as illustrated in Fig. (5). Hence, the treatment of 144 kg N/ha. With seeds treated by bio-fertilizer gave the best results as illustrated previously; in contrast, the lowest averages were found with the treatment of 48 kg N/ha without seed bio treatment, as clarified in Table (4).

Table 3 Seed yield/plant and seed index mean as affected by cultivars x seed bio -inoculation interaction in 2022 and 2023 seasons

Cultivars	Seed bio inoculation	Seed yield /plant (g)		Seed index(g)	
		2022	2023	2022	2023
Giza35	Without	15.16	14.54	14.85	14.75
	With	21.03	19.47	15.62	15.38
Giza111	Without	11.45	13.83	14.89	14.62
	With	17.25	18.99	16.22	15.32
Giza83	Without	10.10	12.87	14.11	14.56
	With	14.46	18.98	14.98	15.44
	L.S.D. (0.05)	1.88	2.6	1.07	N.S.[1]

N.S.: not significant at 0.05 level of probability.

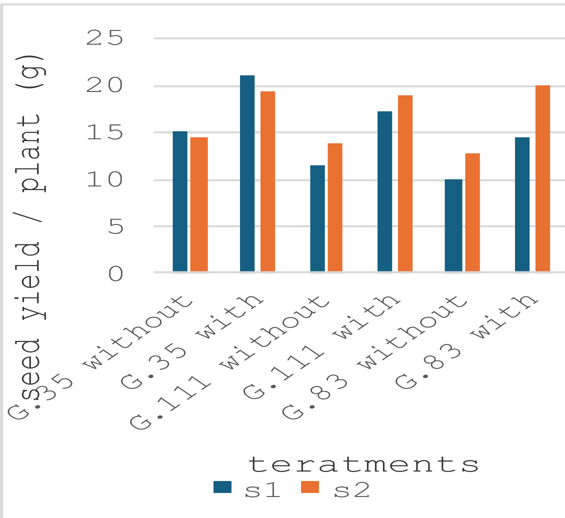


Fig. 1 effect of cultivars x Seed bio inoculation on seed yield/plant in 2022 (s1) and 2023 (s2) seasons

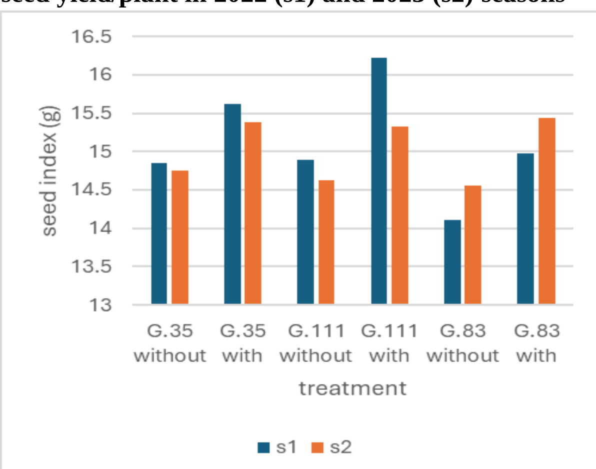
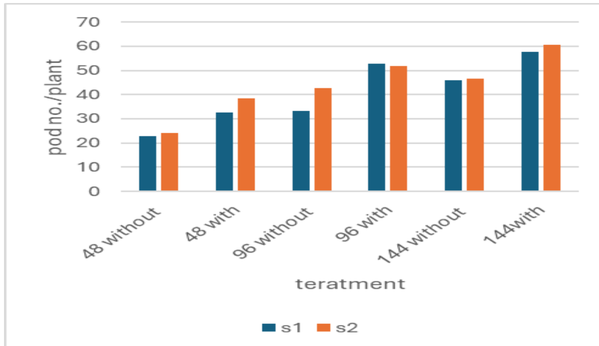


Fig. 2. effect of cultivars x Seed bio inoculation on seed index in 2022 (s1) and 2023 (s2) seasons



on pod no./plant in 2022 (s1) and 2023 (s2) seasons

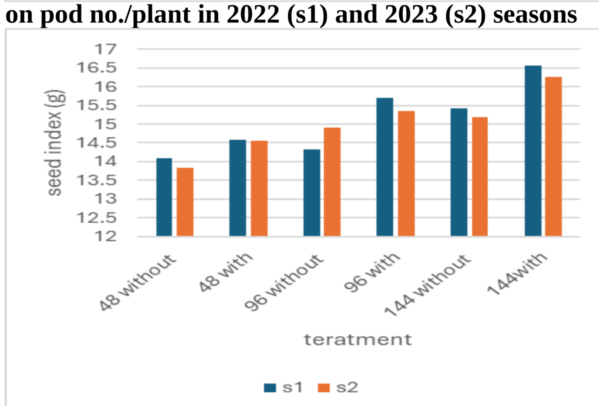


Fig.4. effect of nitrogen levels x Seed bio inoculation on seed index in 2022 (s1) and 2023 (s2) seasons

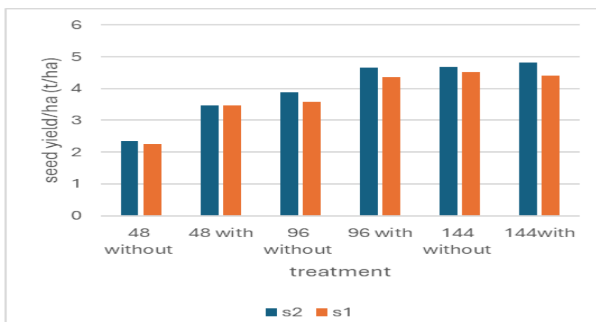


Fig .5. effect of nitrogen levels x Seed bio inoculation on seed yield/ha in 2022 (s1) and 2023 (s2) season

Table 4. of pods/plant, seed index and seed yield/ha mean as affected by nitrogen levels x seed bio-inoculation interaction in 2022 and 2023 seasons

Nitrogen level (kg N/ha.)	Seed bio inoculation	Number of pods/plant		Seed index (g)		Seed yield/ha (t)	
		2022	2023	2022	2023	2022	2023
48	Without	22.95	24.02	14.10	13.84	2.25	2.34
	With	32.53	38.29	14.57	14.56	3.46	3.46
96	Without	33.34	42.53	14.32	14.91	3.58	3.89
	With	52.68	51.65	15.70	15.34	4.37	4.66
144	Without	45.85	46.70	15.43	15.19	4.52	4.68
	With	57.80	60.61	16.56	16.25	4.40	4.81
	L.S.D. (0.05)	3.56	8.43	1.07	0.89	N.S.*	0.51

*N.S.: not significant at 0.05 level of probability.

Table 5. of branches/plant, no. of pods/plant and seed index means as affected by cultivars x nitrogen levels seeds bio-inoculation interaction in 2022 and 2023 seasons

Cultivars	Nitrogen level (kg N/ha.)	Seed bio-inoculation	Number of branches/plant (2022)	Number of branches/plant (2023)	Number of pods/pla nt (2022)	Number of pods/plan t (2023)	Seed index (g) 2022	Seed index (g) 2023
Giza 35	48	Without	4.89	3.5	26.73	25.19	14.53	13.93
		With	5.22	5.33	34.99	36.1	14.63	14.47
	96	Without	5.11	4.67	49.02	46.11	14.3	15.16
		With	6.11	5.0	63.31	53.17	16.13	15.52
	144	Without	5.11	6.0	49.89	47.7	15.73	15.16
		With	6.33	7.0	66.3	63.58	16.1	16.15
Giza 111	48	Without	5.0	4.5	21.21	26.86	14.07	13.63
		With	5.22	4.5	32.22	39.76	14.7	14.82
	96	Without	4.89	5.0	30.68	38.5	15.13	14.76
		With	5.22	4.67	49.18	48.89	16.2	15.02
	144	Without	4.89	5.67	42.18	48.57	15.47	15.48
		With	5.56	6.67	48.54	61.48	17.77	16.13
Giza 83	48	Without	4.22	3.0	20.9	20.0	13.7	13.95
		With	5.56	4.17	30.38	39.0	14.37	14.38
	96	Without	5.33	4.0	20.33	42.98	13.53	14.81
		With	5.33	5.5	45.56	52.88	14.77	15.47
	144	Without	5.0	4.33	45.48	43.82	14.61	14.92
		With	5.33	6.33	58.56	56.78	15.8	16.48
		L.S.D(0.05)	N.S.[1]	1.92	6.17	6.17	1.85	1.54

N.S.: not significant at 0.05 level of probability.

Three-way interaction effect:

The results showed a significant three-way interaction among the studied factors (cultivars, nitrogen levels, and seed bio-inoculation) for all studied traits, except plant height, seeds number per pod, and oil percentage. Concerning the number of branches per plant, it was only significant during the first season, that demonstrated that the three factors under study are not independent of each other.

1. Number of branches/ plant:

The results of Table (5) indicated that number of branches/plants was significantly affected by the three-way interaction in the second season only. Treatment means were divided into two groups with significant differences between them and insignificant differences within each group. The highest mean within the first group of treatments was 7 branches/plant, which was recorded with Giza 35 cultivar fertilized with 144 kg N/ha with seeds bio-inoculation. It was significantly equal to the means of Giza 111 cultivar fertilized with 144 kg N/ha with or without seeds bio inoculation, Giza 83 cultivar fertilized with 144 kg N/ha with seeds bio-inoculation, as well as when the three cultivars were fertilized with 96 kg N/ha with seeds bio-inoculation. While the second group included the rest of the treatments. Where, the cultivar Giza 83 which was fertilized with 48 kg/ha without seeds bio inoculation gave the lowest number of branches (3 branches/plant).

2. Pods number/plant:

Data in Table (5) reported that the number of pods/plant was significantly affected by the three-way interaction in both seasons. Giza 35 cultivar fertilized with 144 kg N/ha and treated with seed bio-inoculation gave the highest significantly higher mean with an increase of 46 and 44 pods/plant for the first and second seasons respectively, compared to the lowest no. of pods/plant of 20.33 for the first season and 20 for the second season, which was recorded with Giza 83 cultivar fertilized with 48 kg N/ha without seed bio-inoculation.

3. Seed index:

Results presented in Table (5) indicated that seed index was significantly affected by the interaction between the three factors during the two studied seasons. The results of the first season showed that the level of 144 kg N/ha for the cultivar Giza 35 and the level of 96 kg N/ha for the cultivar Giza 111 with seed bio-inoculation for both, gave the highest means with insignificant differences between them with means ranging between 16.1 and 17.77 g. While the lowest mean value (13.53 g) was recorded by the cultivar Giza 83 which fertilized with 96 kg N/ha and without seed bio-inoculation. As for the results of the second season, the levels of 144 and 96 kg N/ha with seed bio-inoculation for the three cultivars gave the highest means, which ranged between 15.02 and 16.48 g. These means did not differ significantly from the means of the two treatments: 144 kg N/ha without seed bio-inoculation with Giza 35 and Giza 111 cultivars and 96 kg N/ha seed bio-inoculation, Giza 35 cultivar. As for the lowest mean value, during the second season, the results reflected that the treatment 48 kg N/ha without seed bio-inoculation for Giza 111 cultivar was 13.63 g.

4. Seed yield / plant:

Seed yield/plant was significantly affected by the three-way interaction during both studied seasons. Giza 35 fertilized with 96 or 144 kg N/ha and inoculated with bio-fertilizer produced the highest seed yield / plant (24.78 and 25.90 g), respectively in the first season. However, the highest seed yield/plant (25.26 and 24.40 g) resulted from the same cultivar fertilized with 144 kg N/ha and inoculated with bio-fertilizer in the second season. While Giza 83 fertilized with 96 kg N/ha without seed bio-inoculation gave the lowest mean value (6.68 g) in the first season and (6.86 g) in the second season with 48 kg N/ha without seed bio-inoculation (Table 6).

5. Seed yield/ha:

Table (6) showed that the seed yield /ha. significantly affected by second order interaction in both seasons. The results reported that the level of 144 kg N/ha with or without seed bio-inoculation and the level of 96 kg N /ha with seed bio-inoculation were superior, with insignificant differences between them, as they had the highest means of seed yield/ha in the first season with the two cultivars Giza 35 and Giza 111, with means ranged from 4.52 to 4.83 t/ha, and gave the highest means in the second season with the three cultivars, with means ranged from 4.48 to 4.89t/ha. While the lowest means were recorded at the level of 48 kg N /ha without seed bio-inoculation with the three cultivars, (2.14 and 2.38 t/ha) in the first season and (2.09 and 2.36 t/ha) in the second season.

Considering the variety response, it was found that the two cultivars Giza 35 and Giza 111 with bio-inoculation of seeds with a low level of nitrogen led to a significant increase estimated at 1.33 and 0.92 t/ha for Giza 35 in the 2022 and 2023 seasons, respectively and 1.29 and 1.12 t/ha for the 2022 and 2023 seasons for Giza 111. This significant increase continued as the yield/ha reached (4.61 and 4.65 t/ha for Giza 35) and (4.69 and 4.75 t/ha for Giza 111) with nitrogen level up to 96 kg N/ha and no significant differences were shown with the increase obtained with increasing nitrogen level up to 144 kg N/ha, while bio-inoculation of Giza 83 seeds led to a significant increase in the seed yield/ha under the low level of N (48 kg N/ha) the increase was estimated at 1.02 and 1.5 t/ha in 2022 and 2023. Further application of N to 96 or 144 kg N/ha, with or without bio-inoculation, did not result in significant increases in seed yield/ha in that cultivar.

6. Oil yield/ha.:

The results in Table (6) showed that the oil yield/ha was significantly affected by the interaction between the three studied factors in both seasons. The results of the first season indicated that Giza 35 cultivar fertilized by 144 kg N/ha with or without seed bio-inoculation had the highest significant mean values (1.02 and 1.04 t/ha), while the two cultivars Giza 35 and Giza 111 had the lowest means (0.43 and 0.40 t/ha), respectively, at the level of 48 kg N/ha without seed bio-inoculation.

Table 6. seed yield/plant, seed yield/ha and oil yield/ha. means as affected by cultivars x nitrogen levels x seed bio inoculation interaction in 2022 and 2023 seasons

Cultivars	Nitrogen level (kg N/ha.)	Seed bio-inoculation	Seed yield/plant(g)		Seed yield/ha. (t)		Oil yield/ha (t)	
			2022	2023	2022	2023	2022	2023
Giza 35	48	Without	9.43	8.63	2.14	2.57	0.43	0.53
		With	12.42	12.85	3.47	3.49	0.69	0.70
	96	Without	17.01	17.20	3.82	4.00	0.83	0.85
		With	24.78	20.30	4.61	4.65	0.92	0.93
	144	Without	19.04	17.79	4.80	4.75	1.04	0.91
		With	25.90	25.26	4.72	4.83	1.02	0.95
Giza 111	48	Without	7.24	9.10	2.22	2.09	0.40	0.41
		With	11.49	14.50	3.51	3.21	0.76	0.64
	96	Without	11.26	13.98	3.63	3.93	0.67	0.80
		With	19.33	18.06	4.69	4.75	0.94	0.98
	144	Without	15.84	18.42	4.83	4.82	0.89	0.97
		With	20.93	24.40	4.52	4.89	0.90	0.98
Giza 83	48	Without	6.95	6.86	2.38	2.36	0.56	0.48
		With	10.59	13.80	3.40	3.86	0.62	0.72
	96	Without	6.68	15.66	3.30	3.72	0.72	0.74
		With	16.33	20.12	3.80	4.58	0.78	0.93
	144	Without	16.67	16.08	3.94	4.48	0.79	0.92
		With	22.45	23.02	3.97	4.72	0.79	0.96
		L.S.D (0.05)	3.26	4.51	0.69	0.87	0.10	0.18

N.S.: not significant at 0.05 level of probability.

Table 7. Harvest index means as affected by cultivars x nitrogen levels x seed bio-inoculation interaction in 2022 and 2023 seasons

Cultivars	Nitrogen level (kg N/ha.)	Seed bio-inoculation	Harvest index (%)	
			2022	2023
Giza 35	48	Without	36.74	35.48
		With	37.86	36.37
	96	Without	34.29	32.22
		With	34.41	34.89
	144	Without	28.93	29.85
		With	32.69	31.14
Giza 111	48	Without	36.16	33.69
		With	38.6	35.58
	96	Without	31.14	32.20
		With	35.04	32.42
	144	Without	29.37	29.96
		With	30.40	30.60
Giza 83	48	Without	33.55	34.06
		With	35.67	36.04
	96	Without	30.49	31.19
		With	32.02	32.51
	144	Without	32.23	30.17
		With	34.00	30.47
		L.S.D (0.05)	6.52	N.S.*

*N.S.: not significant at 0.05 level of probability.

The results of the second season reflected that the three cultivars fertilized by level 144 or 96 with or without seed bio-inoculation had the highest means with insignificant differences between them with a mean ranging from 0.80 to 0.98 t/ha, while the lowest means ranged from 0.41 to 0.53 t/ha, which were recorded at the level 48 kg N/ha without seed bio inoculation with any of the three cultivars.

7. Harvest index:

Harvest index was significantly affected by the three factors interaction during the first season only. The results showed that the harvest index for plants fertilized by 48 kg N/ha with or without seed bio-inoculation, for the three cultivars, recorded the highest mean values with insignificant differences between them, with a mean ranging from 33.55% to 38.6%. While the lowest mean value for harvest index trait was recorded (28.93%) with Giza 35 plants fertilized with 144 kg N/ha without bio-inoculation, as shown in Table (7).

Generally, the results showed the importance of the three-way interaction among the three factors, which means that the effect of each factor is not independent of the other factor, i.e. the genotype is affected by the surrounding environmental conditions and the treatments it is treated with, and it responds to them differently depending on its genotype and its interaction with them, these results agree with the results of Lee *et al.* (1999); Danee *et al.* (2001); Abd Alla & Omran (2002); Pedersen & Lauer (2004); Malik *et al.* (2006); Iqbal *et al.* (2008); Ruhul Amin *et al.* (2009) and Khalil *et al*. (2012). Also, seed bio inoculation fixes nitrogen and maximizes the benefit from it and from the added mineral fertilizer, increases the absorption of nutrients and also increases the role of nitrogen in increasing vegetative growth, which results in an increase in the plant size, the leaf area index, and an increase in the products of the photosynthesis process, which, increasing the accumulation of seed components and an increase in its weight, and thus an increase seed index and increase plant's ability to produce pods having seeds. This leads to an increase in plant yield and the yield /ha. While the increase in oil yield /ha reflects an increase in seed yield didn't seed oil %, the harvest index was a result of the sharp increase in plant size (biomass of plants) with increase nitrogen level, which reduces the harvest index (Zerpa *et al*., 2013; Seneviratne *et al.,* 2016; Capatana *et al*., 2017; Prusinski *et al.*, 2020; Zilli *et al.*, 2021 and Ksiezak & Bojarszczuk, 2022).

CONCLUSION

Obtained results of the present study indicated a differential response of soybean cultivars to applied nitrogen levels and biofertilization with the mixture of non-symbiotic bacteria. To obtain a profitable seed yield/ha, both Giza 35 and Giza 111 require an application of 96 kg N/ha with biofertilization, since that treatment was statistically comparable to 144 kg N/ha with or without biofertilization. Hence, there was a saving of 33% of applied mineral nitrogen. On the other hand, Giza 83 requires only 48 kg N/ha with biofertilization to give high seed yield comparable to higher N levels. That may be attributed to its early maturing habit, compared to Giza 35 and Giza 111. This represents a saving of 66% of applied mineral N, which would be favorable for the farmer, resulting in a significant decrease in production costs, and in the meantime, preserves the environment from the harmful effects of pollution to the soil and water.

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الملخص العربي

تأثير تلقيح البذور حيوياً و مستويات التسميد النيتروجينى على المحصول و مكوناته لثلاثة اصناف فول صويا

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أُقيمت تجربتان حقليتان في محطة بحوث كلية الزراعة، جامعة الإسكندرية، مصر، فى الموسمين الصيفيين ٢٠٢٢ و٢٠٢٣ لدراسة تأثير التلقيح الحيوي للبذور (مع وبدون) ومستويات التسميد النيتروجيني (٤٨، ٩٦، و١٤٤ كجم /هكتار) على المحصول ومكوناته لثلاثة أصناف من فول الصويا (جيزة ٣٥، جيزة ١١١، وجيزه ٨٣). أظهرت النتائج وجود تفاعل ثلاثي معنوي بين العوامل تحت الدراسة لمعظم الصفات المقاسة في كلا الموسمين معامل الحصاد في الموسم الأول وعدد الأفرع/نبات في الموسم الثاني. أعطت النباتات المسمدة بمستويين ١٤٤ كجم نيتروجين/هكتار أو ٩٦ كجم نيتروجين/هكتار مع التلقيح الحيوي للبذور أعلى القيم المعنوية للصفات المتأثرة معنويا، وكان صنف جيزة ٣٥ هو الأكثر استجابة لهما. بينما كانت أقل القيم معنوية للصفات المتأثرة هي تلك التي تم الحصول عليها من مستوى النيتروجين ٤٨ كجم نيتروجين/هكتار بدون تلقيح بيولوجي للبذور، وكان أقل الأصناف مع هذه المعاملة هو صنف جيزة ٨٣ .أظهرت صفة معامل الحصاد اتجاهاً معاكساً للصفات الأخرى، حيث أدت زيادة مستوى التسميد النيتروجيني إلى انخفاض في معامل الحصاد في حين كان مستوى النيتروجين المنخفض بدون أو مع تلقيح البذور حيويا أعلى معامل حصاد في الموسم الأول. ولذا فقد تم استنتاج انه تحت ظروف هذه التجربة فقد أدى استخدام التلقيح الحيوى للبذور بالبكتريا المثبتة للنيتروجين غير التكافلية إلى تعظيم الأستفادة من السماد النيتروجينى مما أدى إلى توفير حوالى ٣٣٪ من السماد النيتروجين المعدنى (٤٨ كجم ن / هكتار) من الكمية الموصى بها فى حالة عدم تواجد العقد الجذرية.

الكلمات المفتاحية: فول الصويا؛ التلقيح الحيوي للبذور؛ التسميد الحيوي؛ سماد النيتروجين؛ مستوى النيتروجين؛ المحصول؛ محصول الزيت.