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A COMPARATIVE RESPONSE OF *Borago officinalis* L. PLANT TO THE BIO., CHEMICAL FERTILIZATION AND ADINOSINE-TRI-PHOSPHATE (ATP) TREATMENTS

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ABSTRACT

Two field experiments were carried out in a private farm in Abou-Zaabal at Kalubia Governorate, during two successive seasons: 1998-99 and 1999-2000. Borage plants (*Borago officinallis* L.) were fertilized with ammonium sulphate at 0, 150 and 200 kg/fed, and nitrobein (as a biofertilizer) at the rate of 0 and 5 kg/fed [Part I], or with calcium super-phosphate at 0 and 200 kg/fed, phosphorin (as a biofertilizer) with the rate of 10 kg/ fed, and sprayed with adinosine - .ri-phosphate (ATP) [Part II].

Data on plant height, number of both leaves and branches, dry weight of leaves and weight of seed yield were recorded at different stages of growth. Also, seed chemical constituents as total carbohydrates, crude protein and fixed oil, beside the fractions of the fatty acids were determined in the two seasons.

Nitrogen bio-and chemical fertilization increased the plant growth characters in the different stages compared with the control. Biofertilizer treatment was the most effective one. Also, the treatments increased the total carbohydrates, crude protein and fixed oil in seeds.

The percentage of unsaturated fatty acids in seeds increased by nitrogen fertilization (bio and chemical) and the ratio between unsaturated and saturated increased by about 3 to 4 times. Also nitrobein gave the highest values.

Using various sources of phosphorus (bio and chemical) as well as ATP effectively improved the plant growth characters and the chemical constituents of seeds. ATP seemed to be the most effective among the treatments.

These treatments raised the ratio of unsaturated to saturated fatty acids. However, some of them were undetected in phosphorin treatment.

Key words: *adenosine-tri-phosphate, bio-fertilizer, borage, Borago officinallis, growth constituents, nitrobein, nitrogen, phosphorin, phosphorus.*

1. INTRODUCTION

Borage plant (*Borago officinallis* L.) family Boraginaceae, is a herbaceous annual indigenous to the Mediterranean region, native to Europe and North Africa, where it has spread to other parts like Asia minor and North America.

It has long been grown in gardens as a medicinal herb, and as an excellent source of nectar for bees. The flowers and leaves of the plant are used medicinally, besides the ornamental values.

Current interest exists in borage as a seed crop, which contains a high percentage of gamma linolenic acid (GLA) [Craig and Bhathy, 1964], an unusual fatty acid and prostaglandin [Traitler *et al.*, 1984, Cutting 1985, and Jorgensen 1988]. rostaglandins are involved in regulating many metabolic functions in mammalian systems (White *et al.*, 1978; and Willis, 1981). Linolenic acid (LA) is the precursor of jasmonic acid (JA) and its methyl jasmonate (MeJA) which may delay senescence and acts as a growth regulator (Creelman and Mullet, 1997).

The constituents in seeds include tannins, saponins, mucilage, silicic acid and minerals. Also, borage oil has anti-inflammatory, with mild diuretic, diaphoretic and demulcent properties. It is also used as a good general tonic. In herbal medicine, it is used in infusions for urinary infection colds, bronchitis and rheumatic conditions. Externally, it is used in compresses for skin rashes (Stodola and Volak, 1992).

Fixed oils and fats are widely distributed and occur in both vegetative, reproductive organs and seeds. As lipids, and fats form an essential component of biological membranes (Trease and Evans, 1998). Saturated fatty acids are low in borage oil, its physical and chemical characteristics are similar to those for commercial vegetable oils (Oderinde *et al.,* 1990). Borage seeds, the richest known plant source of linoleic acid, contains (9% to 31%) lipids, of which (31% to 61%) is linoleic acid, whereas the rest of the major fatty acids are palmitic acid (12% to 18%), stearic acid (8% to 12%) and oleic acid (13% to 40%), (Silou *et al.,* 1999). The borage seeds also contain moderately high amounts of other minerals (Ca, Mg, Na, Mn, Fe and Cu) which made them potentially useful as food supplements; (Olaofe, 1994).

Nowadays, it has become necessary to search for untraditional fertilizers as substitutes for chemical nitrogen and phosphorous ones. Remarkable effects of untraditional fertilizers, especially the biofertilizers have been reported on growth and yield of potato. Imam and Badawy, (1978) found that treating seeds with *Azotobacter chrococcum* increased plant growth and yield and produced compounds detrimental to pathogens or that act as plant growth regulators. Azotobacters do synthesize stimulatory compounds such as gibberellins, cytokinins and indole acetic acid, which stimulate the plant cell expansion (Martin, 1982). The production of biologically active substances by the bacteria was the principal factor responsible for plant growth promotion.

Phosphorus plays an important role in many enzyme reactions depending on phosphorylation and energy conservation and transfer for a wide range of biochemical processes [Walker, 1980; Stevenson, 1986; and Marshner and Cakmak, 1986].

Phosphorus nutrition is doubly critical because the total supply of phosphorus in most soils is low and is not readily available for the plant use. Most of the basically adenosine-tri-phosphate (ATP) generating pathways, *i.e.* photophosphorylation, glycolysis, TCC-Cycle, and oxidative phosphorylation are restricted [Lyons and Breidenbach (1990) and Ortiz (1991).

In addition, synthesis of bioconstituents, minerals uptake, translocation and retention processes are dependent on the adenosine tri-phosphate (ATP) supply (Mengel and Kirkly, 1982).

Besides the involvement of ATP in the system of gene expression and function, it is also directly involved in gene (DNA) structure (Dashek, 1997). The AMP (the hydrolytic derivative of ATP) is the main precursor of cytokinins (Jameson, 1994).

The aim of this investigation was to determine the individual effects of nitrogen levels against biofertilizer nitrogen (Nitrobein). Also, adenosine-tri-phosphate (ATP) as a foliar spray, effect against phosphorus and biophosphorus fertilizer (Phosphorien), on plant growth, flowering, seed yield and chemical constituents of *Borago officinallis* plant were investigated.

2. MATERIALS AND METHODS

This study included two parts:

Part I: Effect of Bio and chemical nitrogenous fertilization treatments.

Part II: Effect of Bio and chemical phosphorus fertilization and adino-sine-tri-phosphate treatments.

Seeds of *Borago officinallis* L. obtained from Medicinal and Aromatic Plants Research Section, Ministry of Agriculture, Giza, were directly sown on the 15th of September in the two seasons in the field.

Physical and chemical parameters of the experimental soil analysis are shown in Table (1).

Table (1): Physical and chemical analysis of the experimental soil.

Physical properties		Chemical parameters	
Soil type	: (clay)	p.H	: 7.5
Coarse sand	: 3.35%	Available Nitrogen	:25.2 ppm
Fine sand	: 28.8%	Available P_2O_5	: 115 ppm
Silt	: 26.75%	Available K_2O	: 180 ppm
Clay	: 43.68%		

Seeds were sown in plots (3 x 3.5 m), in hills on one side of 70 cm distance, between rows and 30 cm between each hill (3 seeds / hill). After seedling emergence, thinning was carried out twice, one seedling/ hill was left.

2.1.The treatments were as follows

2.1.1. Ammonium sulphate [$(NH_4)_2SO_4$], 20.5% N, at the rate of 0,150 and 200 kg/fed, against Nitrobein as Biofertilizer containing *Azotobacter chrococcum* bacteria, (produced by the Ministry of

Agriculture, Giza, Egypt). Ammonium sulphate was applied at two doses at one month interval, the first one was applied after 15 days from the final thinning. While "Nitrobein" was added as a presowing covering agent of seeds at the rate of 5 kg/fed.

2.1.2. Calcium superphosphate 15.5% P_2O_5 at 200 kg/fed, against "Phosphorin" as a Biofertilizer containing *Bacillus megatherium* bacteria (produced by the Ministry of Agriculture, Giza, Egypt) were used. Adenosine – tri- phosphate (ATP). P_2O_5 was added during the preparation of soil. Also, phosphorin was mixed with the soil at the rate of 10 kg/fed before planting. Meanwhile, ATP was sprayed twice (at two true leaf stage and 30 days afterwards).

The treatments of each part were arranged in plots, as a complete randomized design, 50 plants each in three replicates.

The data were recorded at vegetative, flower budding and flowering stages (90, 120 and 150 days from planting, respectively). Seeds were collected 4 weeks later (harvest date).

The recorded data were: plant height (cm), number of leaves/ plant, number of branches / plant, dry weight of leaves (g/plant) weight of seeds (g/plant). The data were subjected to the statistical analyses according to Gommez and Gommez (1984).

Samples were taken to determine the total carbohydrates and crude protein content in leaves and seeds according to (Herbert *et al.*, 1971) and A. O. A. C. (1975) respectively. Total lipids in seeds were determined at the harvesting date. The percentage of nitrogen was converted to percentage of crude protein by multiplying by (6.25).

The methyl esters prepared from oil samples and standard materials were analyzed by a Pye Unicum (GLC) equipped with a dual Flame Ionization Detector. The separation of fatty acid methyl esters was conducted with a column SP – 2310, 55% cyanopropyl phenyl silicon (1.5 x 4.0 mm). Column was used with temperatures program of 70°C to 190°C / min. the injector and detector temperatures were maintained at 250°C and 300°C, respectively. The pressure of carrier gas (nitrogen) was 18 kg/cm^2. The relative percentage of each compound was calculated according to the peak area by Varian 4370 integrator. Fatty acids were identified by matching their retention times (Rt) against those of the authentic samples as described by Kleiman *et al.* (1964).

3. RESULTS AND DISCUSSION

3.1. Effect of bio and chemical nitrogenous fertilization

3.1.1. Effect on growth characters

Nitrogen fertilization had positive significant effects on plant height, number of leaves and branches per plant, as well as dry weight of leaves, and seed yield. (Table 2) These parameters generally increased. Also Nitrobein (nitrogen biofertilizer) was the most effective treatment in stimulating the elongation of stem, increasing both the number of leaves and branches per plant, as well as the dry weight of leaves. It could also be recognized that the seed yield / plant showed the same response to nitrobein.

Ammonium sulphate (A.S) at 200 kg/fed was the second beneficial treatment. This trend was observed in the different stages of growth at the two seasons.

These results agree with those obtained by Zaied (1984) on soapwort plants; Jacoub (1995) on basil; Soliman (1997) on black cumin; Naguib *et al.,* (1998) on dill; and Abd El-Kawy (1999) on geranium plants. They found that treating plants with azotobactar as a bio fertilizer increased plant growth.

3.1.2. Effect on chemical constituents

3.1.2.1. Total carbohydrates content in leaves and seeds

3.1.2.1.1. Leaves

The data shown in Table (3) indicate that N-fertilization treatments, regardless of its nature (bio or chemical), increased the percentage of total carbohydrates in the leaves. The highest values in all stages of growth (20.51% to 30.22%) were recorded for nitrobein. These values were generally higher than those recorded for the two levels of N, compared with the control at the same stage (13.79% to 17.51%, respective).

The above mentioned results are in harmony with those obtained by Hellaly (1977) on *Hyoscyamus muticus*; Zerbe and Wild (1980) on *Sinapsis alba* ; El – Swaefy (1996) on *Mentha piperita*, Ramadan (1996) on guar; and Abd El-Kawy (1999) on geranium plants.

Table (2): Effect of bio - and chemical nitrogenous fertilization at different growth stages on the growth characters of *Borago officinallis* L. plants, during 1998/1999 and 1999/2000 seasons.

Characters / Treatments	Plant height (cm)						No. of leaves / plant					
	1st stage		2ND Stage		3rd Stage		1st stage		2ND Stage		3rd Stage	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
O	18.32	19.17	24.47	25.03	34.25	37.48	8.56	8.98	19.72	26.94	58.62	67.76
Nitrobein	31.33	32.25	42.29	44.17	55.26	57.76	16.39	18.65	49.17	55.95	162.68	183.8
A. S. (150)	20.44	21.71	27.58	29.75	38.62	40.44	9.00	9.31	25.33	27.93	98.13	111.72
A. S. (200)	26.54	27.43	35.83	37.22	50.28	53.17	9.17	10.22	27.17	28.23	110.24	115.67
L.S.D. at 5%	1.84	1.96	2.66	2.87	3.34	3.76	1.52	1.77	2.07	2.45	4.78	4.96

Characters / Treatments	No. of branches / plant						Dry weight of leaves (g)						Seed yield/ plant (g)	
	1st stage		2ND Stage		3rd Stage		1st stage		2ND Stage		3rd Stage		At harvest date	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
O	5.12	6.54	9.66	10.98	13.32	15.14	1.54	1.88	3.62	3.74	6.16	7.52	3.55	3.67
Nitrobein	16.51	17.39	25.43	27.11	32.14	36.36	9.65	10.31	16.31	18.11	26.6	27.81	7.85	7.96
A. S. (150)	7.44	8.11	12.27	14.42	16.74	18.17	2.33	2.68	4.66	5.36	9.32	10.76	3.87	3.95
A. S. (200)	12.62	13.41	18.93	21.56	28.39	29.55	3.25	3.89	6.58	7.79	13.27	15.56	4.59	4.89
L.S.D. at 5%	1.63	1.88	2.63	3.11	3.53	4.12	0.51	0.77	2.18	2.79	3.33	3.79	1.32	1.59

* A. S. = Ammonium sulphate
* 1st Stage = Vegetative growth stage
* 2nd Stage = Flower budding growth stage
* 3rd Stage = Flowering growth stage

Table (3): Effect of bio- and chemical nitrogenous fertilization at different growth stages on total carbohydrate and crude protein in leaves of *Borago officinallis*, L. plants, during 1998/1999 and 1999/2000 seasons.

Component / Treatment	Total carbohydrates (%) in leaves						Total crude protein (%) in leaves					
	1st Stage		2nd Stage		3rd Stage		1st Stage		2nd Stage		3rd Stage	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
0	13.79	13.85	17.39	17.51	15.69	15.78	21.15	21.56	25.55	26.11	23.38	24.07
Nitrobein	20.51	21.27	28.92	30.22	25.77	26.91	28.42	28.77	32.42	32.72	30.08	30.46
A. S. (150)	13.99	14.12	17.79	18.03	15.99	16.18	24.56	24.89	29.33	29.66	27.32	27.67
A. S. (200)	15.36	15.67	20.45	21.06	18.04	18.51	26.67	26.88	30.42	30.81	28.47	28.85
L. S. D. at 5%	1.21	1.47	2.18	2.65	1.82	2.21	1.64	1.87	3.21	3.62	2.48	2.76

* A. S. = Ammonium sulphate * 1st Stage = Vegetative growth stage * 2nd Stage = Flower budding growth stage * 3rd Stage = Flowering growth stage

3.1.2.1.2. Seeds

The results showed that Nitrobein gave the greatest value of carbohydrates (19.62%), followed by $[(NH_4)_2So_4)$ at 200 kg/fed treatment (11.14%), compared with (5.78%) for the control plants (Table 4).

These results are in agreement with those obtained by Oloaf and Sanni (1988) on some food seeds; El-Mogy (1993) on *Lupinus termis*; and Helal and Khalil (1997) on periwinkle.

These results may be due to the improvement of growth which led to the formation of carbohydrates in seeds, as well as the increase in the synthesized metabolites which in turn, improves seed production.

3.1.2.2. Crude protein content in leaves and seeds

3.1.2.2.1. Leaves

Data shown in Table (3) reveal that crude protein percentage in leaves of borage plants increased as a result of using different sources of nitrogen in comparison with the control. The highest values were obtained from the plants which received Nitrobein, at different growth stages. The greatest values were 32.42% and 32.72% in both seasons at the flower budding growth stage compared with 25.55% and 26.11, respectively in the control plants. These results are in agreement with those obtained by Helaly (1977) on *Hyoscyamus muticus;* Zerbe and Wild (1980) on *Sinopsis alba* plants.

3.1.2.2.2. Seeds

Crude protein in seeds followed the same trend in response to the N fertilization. Table (4) show that the values generally increased, the highest value was from plants received Nitrobein (36.32%) (2.851 g/plant), followed by the treatment of $[(NH4)_2SO_4]$ at 200 kg/fed (34.96%), (1.605 g/plant). These results were observed in both seasons and are in agreement with those obtained by Jacoub (1995) on sweet basil plants; and Shalan (2001) on *Legenaria siceraria* seeds.

3.1.2.3. Fixed oil content in seeds

The results recorded in Table (4) show that fixed oil percentage significantly increased by Nitrobein, A. S. at 150 and 200 kg/ fed in comparison with the control. The best results were obtained with Nitrobein application as a source of bio-nitrogen fertilizer with 28.54%; but dressing A.S. (150 kg/fed) gave the lowest improvement with

Table (4): Effect of bio- and chemical nitrogenous fertilization on total carbohydrate, crude protein and fixed oil contents in seeds of *Borago officinallis,* L. plants at the harvest date (3[rd] stage), during 1998-1999 and 1999/2000 seasons.

Components / Treatments	Total carbohydrate content				Crude protein content				Fixed oil content			
	Percentage		G/plant		Percentage		G/plant		Percentage		ml/plant	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
0	5.78	6.21	0.276	0.301	26.11	27.44	0.927	1.007	11.28	11.57	0.401	0.425
Nitrobein	19.62	19.98	1.542	1.591	36.32	36.86	2.851	2.934	28.54	29.21	2.241	2.328
A. S. (150)	9.62	9.84	0.373	0.389	33.74	34.28	1.306	1.354	15.76	16.23	0.611	0.642
A. S. (200)	11.14	11.59	0.511	0.567	34.96	35.19	1.605	1.721	21.35	22.17	0.981	1.084
L. S. D. at 5%	2.31	2.74	0.141	0.189	3.62	3.88	0.125	0.167	1.23	1.44	0.164	0.182

* A. S. = Ammonium sulphate

Table (6): Effect of bio- and chemical phosphorus fertilization at different growth stages on the growth characters of *Borago officinallis* L. plants, during 1998/1999 and 1999/2000 seasons.

Characters / Treatments	Plant height (cm)						No. of leaves / plant					
	1[st] stage		2[nd] Stage		3[rd] Stage		1[st] stage		2[nd] Stage		3[rd] Stage	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
O	18.32	19.17	24.47	25.03	34.25	37.48	8.56	8.98	19.72	26.94	58.62	67.76
ATP (50ppm)	28.12	29.34	37.96	39.55	59.37	61.32	13.54	15.33	46.[illegible]2	45.99	192.48	223.96
Phosph	24.19	25.36	33.65	35.28	49.66	50.66	11.72	12.11	35.16	36.33	140.64	145.32
S. P. (200)	22.07	23.72	30.79	32.72	46.81	48.51	10.42	11.24	31.26	33.72	125.14	134.88
L.S.D. at 5%	1.52	1.69	2.25	2.39	2.89	2.95	1.27	1.42	1.88	2.12	3.67	4.21

Characters / Treatments	No. of branches / plant						Dry weight of leaves (g)						Seed yield/ plant (g)	
	1[st] stage		2[nd] Stage		3[rd] Stage		1[st] stage		2[nd] Stage		3[rd] Stage		At harvest date	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
O	5.12	6.54	9.66	10.98	13.32	15.14	1.54	1.88	3.62	3.74	6.16	7.52	3.55	3.67
ATP (50ppm)	16.71	17.32	24.21	25.98	38.69	46.22	7.49	8.11	14.98	16.22	36.96	38.44	10.32	10.76
Phosph	14.31	14.92	21.47	22.57	31.54	33.19	5.66	6.21	11.32	12.44	22.64	24.84	6.80	6.92
S. P. (200)	9.54	10.33	14.31	17.27	21.46	23.41	4.48	4.94	8.96	9.88	17.22	19.76	5.52	5.79
L.S.D. at 5%	1.44	1.69	2.25	2.89	2.88	3.51	0.48	0.66	1.77	1.96	2.76	3.14	1.25	1.42

* A.T.P. = Adinosine Tri Phosphate
* Phosph. = Phosphorein
* S. P. = Super phosphate

* 1[st] Stage = Vegetative growth stage
* 2[nd] Stage = Flower budding growth stage
* 3[rd] Stage = Flowering growth stage

21.35%, compared with 11.28% from the control. These results hold true in the two seasons, and are in harmony with those of Nour El-Dine *et al.,* (1983) on safflower seeds; Ahmed and Zaid (1993) on fenugreek; Patil *et al.,* (1997) on bottle gourd seeds; Soliman (1997); and Mohamed (1998) on black cumin seeds; and Shalan (2001) on *Legenaria siceraria* seeds.

3.1.2.4. Fatty acid fraction

The relative percentages of fatty acids extracted from brage seeds treated with bio and chemical nitrogen fertilizers are presented in Table (5) and Fig. (1).

Eight identified saturated fatty acids were grouped into three classes, *i.e.*, major fatty acids (more than 10%), minor fatty acids (less than 10%), and traces one (less than 1%). Some of the fatty acids which are below 0.1% have been labeled by the symbol(*). Borage fixed oil of seeds contains Palmitic, Palmitoleic, Oleic, Linoleic and δ- Linolenic acids as main major components, (Wertensip *et al.,* 1990).

Accordingly, in all treatments the major saturated fatty acid was Palmitic which ranged from (10.96%) in the plant treated with Nitrobein to (19.50%) with A.S. at 150 kg/fed, and (12.58%) with A.S. at 200 kg/fed, compared with (33.72%) for control. The same effect was shown with other saturated fatty acids, *i.e.*, Arachidic and Behenic which were classified as major saturated fatty acids. The results showed a great decrease in response to bio and chemical nitrogenous fertilization treatment.

In the case of the unsaturated fatty acids, the results in Table (5) show that Oleic and Linolieic acids were the major components and increased by all treatments compared with control. It ranged from 28.21% for the control to 35.23% at Nitrobein in the case of Linoleic acid.

Concerning δ- Linoleic acid, the data show a sharp increase with all treatments. It is classified as a minor unsaturated fatty acid at the control treatment 1.3%, and with A.S. at 150 kg/fed 4.25%. While it is considered as a major unsaturated fatty acid with the other treatments, having the maximum percentage (21.66%) by the Nitrobein application.

In addition, from the data recorded in Table (5) it may be concluded that Nitrobein treatment resulted in the highest value of total unsaturated fatty acids (80.63%) compared with (38.71%) for the

Table (5): Effect of bio- and chemical nitrogenous fertilization on fatty acids fraction in the fixed oil of *Borago officinallis* L. seeds

Treatment / *Components %*		Control	N	A. S. (150)	A. S. (200)
Caproic	C6: 0	*	*	0.18	0.10
Caprylic	C8: 0	*	0.51	*	*
Capric	C10: 0	0.45	0.64	*	0.10
Lauric	C12: 0	0.59	0.56	0.37	*
Myristic	C14: 0	0.75	*	0.30	*
Palmitic	C16:0	33.72	10.96	19.50	12.58
Palmitoleic	C16: 1	*	3.97	*	0.10
Oleic	C18: 1	9.08	15.88	21.66	20.60
Linoleic	C18:2	28.21	35.23	32.58	31.81
δ - Linolenic	C18: 3	1.30	21.66	4.25	16.85
Linolenic	C18: 3	0.12	0.67	0.11	0.13
Arachidic	C18:3	12.38	4.39	7.78	6.00
Eicosan-12-enoate	C20:1	*	3.22	4.97	*
Behenic	C22:0	11.79	2.28	7.64	11.70
Total identified		98.39	99.97	99.34	99.97
Total unsaturated (TU)		38.71	80.63	63.52	69.49
Total saturated (TS)		59.68	19.34	35.77	30.48
TU / TS		0.65	4.17	1.78	2.28

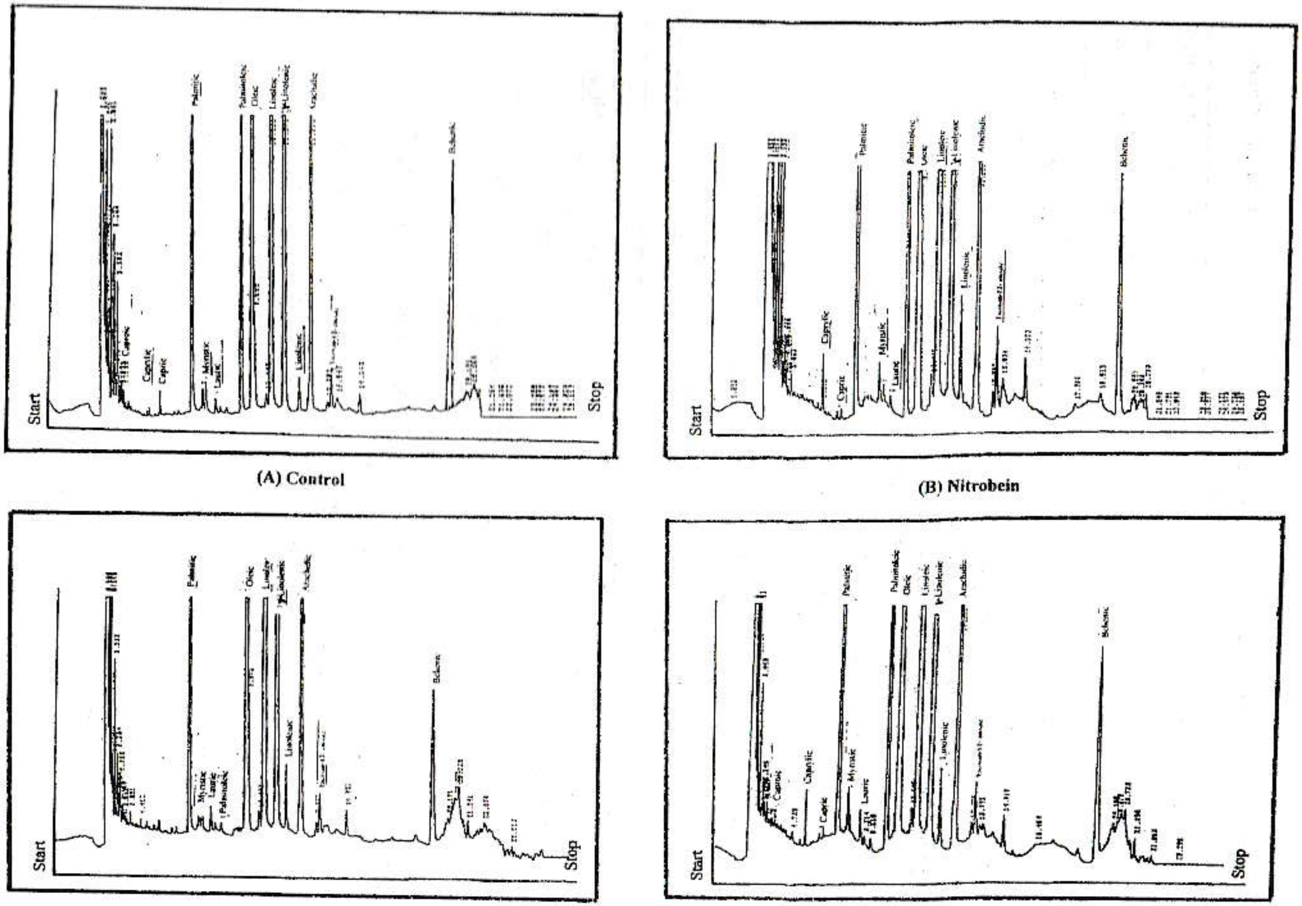
A.S. = Ammonium sulphate   TU / TS = Total Unsaturated / Total Saturated ratio

* = Below 0.1%

control. Also it led to a lower value of total saturated fatty acids (19.34%) compared with (59.68%) for the control.

Finally, Nitrobein treatment recorded a marked increasing effect on the ratio of TU/ TS gaining 4.17% compared with 0.65% for the control.

These results coincide with those obtained by Talaat and Youssef (1998) on *Borago officinalis*. However, the seed oil from other Boraginaceae family in general contains considerable amounts of other unsaturated fatty acids, beside δ- Linolenic acid. For example *Amblynotus repestris* reported by Tseveguren and Aitzetmuller (1996). The same observations were recorded by Hurtubise *et al.*, (1992) on



(A) Control

(B) Nitrobein

(C) A.S. (150)

(D) A.S. (200)

Fig. (1): Effect of bio-and chemical nitrogenous fertilization on the chromatograms of fatty acid fraction in the fixed oil of *Borago officinallis* seeds.

Lemna minor and Baljeet *et al.*, (1996) on the seeds of *Guizatia abyssinica* plants.

3.2.Effect of bio- and chemical phosphorus fertilization and A.T.P.

3.2.1. Effect on growth characters

The recorded data in Table (6) indicate that phosphorus treatments improved the estimated characters compared with those of untreated control. They showed also considerable differences among these treatments. The superiority was for exogenous ATP spraying application. It was therefore considered the most effective treatment for increasing growth characters.

It was remarked that, plant height, number of leaves and branches, as well as dry weight of leaves and seed yield per plant increased with Phosphorein treatment than dressing calcium super phosphate at 200 kg/fed. These were observed at the three stages of growth in both seasons of the experiment.

These results are in harmony with those of Mengel and Kirkly (1982) with ATP treatment; Martin (1982) with Bio-phosphorus nutrition; Saber and Kabesh (1988) with Biofertilizer phosphate treatments on lentil plants; Hauka *et al.*, (1990) on barley and tomato plants; and Shalan (2001) on *Langenaria siceraria* plants.

Meanwhile, dressing S.P. at 200 kg/ fed resulted in the least growth parameters and plant production. These results may be attributed to the major role of ATP in activating most processes in plant metabolism according to Mengel and Kirkby (1982).

3.2.2. Effect on chemical constituents

3.2.2.1. Total carbohydrate content in leaves and seeds

3.2.2.1.1.Leaves

Data in Table (7) show the total carbohydrates % in the leaves at different growth stages. It can be emphasized that foliar application of ATP at 50 ppm resulted in the highest value of total carbohydrates especially at flower budding (24.61%). However, in the first season, dressing application of S.P. at 200 Kg/fed led to 18.77% of carbohydrates. On the other hand, Phosphorein treatment gave the synthesis of the least value (15.25%) even less than the control (17.39%). These results were confirmed in the second season. They followed a similar trend as those obtained by Zerbe and Wild (1980) on

Sinapsis alba; El-Swaefy (1996) on *Mentha piperita* and Abd El-Kawy (1999) on geranium.

3.2.2.1.2. Seeds

Data in Table (8) reveal that the greatest value of carbohydrates in seeds resulted from ATP treatment (14.38% and 14.87% in both seasons), followed by S.P.(200) which resulted in (10.78% and 10.96%, respectively), the least value of carbohydrates in seeds was with Phosphorien application (6.33% and 7.71%) compared with the control (5.78 and 6.21%).

These findings are in accordance with the observations of Bishr and Makarim (1984) on *Ammi majus* seeds; Oloafe and Sanni (1988) on some food seeds, El-Mogy (1993) on *Lupinus termis* seeds.

These results might be due to the improvement of growth which leads to the formation of carbohydrates in seeds of plants as it was observed in the synthesized metabolites which in turn improves the seed production.

3.2.2.2. Crude protein content in leaves and seeds

3.2.2.2.1. Leaves

Data recorded in Table (7) indicate that ATP application resulted in the highest percentage of crude protein at the flower budding growth stage (33.26% and 33.67% in both seasons), followed by the Phosphorein treatment (31.07% and 31.82%, respectively). The lowest increasing values were recorded with S.P. (200) dressing application (28.24% and 28.76%) compared with the control treatments (25.55% and 26.11% in both seasons). These results are in agreement with those obtained by Helaly (1977) on *Hyoscyamus muticus*; Zerbe and Wild (1980) on *Sinapsis alba*.

3.2.2.2.2. Seeds

Regarding the crude protein in the seeds, data presented in Table(8) indicate that the content of crude protein increased as a result of using different sources of phosphorus in comparion with the control. The highest value was obtained from ATP application (4.155 g/plant), followed by the Phosphorein treatment (2.541 g/plant), the least content recorded with S. P. (200) (1.689 g/ plant), compared with 0.927g/plant) for the control treatment. These results were obvious in both seasons

Table (7): Effect of bio- and chemical phosphorus fertilization at different growth stages on total carbohydrates and crude protein in the leaves of *Borago officinallis* L. plants, during 1998/1999 and 1999/2000 seasons.

Component / Treatment	Total carbohydrates (%) in leaves						Total crude protein (%) in leaves					
	1st Stage		2nd Stage		3rd Stage		1st Stage		2nd Stage		3rd Stage	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
0	13.79	13.85	17.39	17.51	15.69	15.78	21.15	21.56	25.55	26.11	23.38	24.07
A. T. P. (50ppm)	18.11	18.35	24.61	25.04	22.17	22.53	30.62	30.78	33.26	33.67	37.24	37.77
Phosph.	12.69	12.82	15.25	15.51	14.04	14.23	27.26	27.87	31.07	31.82	28.43	28.97
S. P. (200)	14.49	14.66	18.77	19.09	16.74	16.99	22.45	22.89	28.24	28.76	26.75	26.96
L. S. D. at 5%	1.14	1.25	2.05	2.26	1.71	1.88	1.45	1.62	2.89	3.33	2.33	2.48

* A. T. P. = Adinosine Tri phosphate
* Phosph. = Phosphorein
* S. P. = Super Phosphate
* 1st Stage = Vegetative growth stage
* 2nd Stage = Flower budding growth stage
* 3rd Stage = Flowering growth stage

Table (8): Effect of bio- and chemical phosphorus fertilization on total carbohydrates, crude protein and fixed oil contents in seeds of *Borago officinallis* L. plants at the harvest date (3rd stage), during 1998/1999 and 1999/2000 seasons.

Components / Treatments	Total carbohydrate content				Crude protein content				Fixed oil content			
	Percentage		G/plant		Percentage		G/plant		Percentage		ml/plant	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
0	5.78	6.21	0.276	0.301	26.11	27.44	0.927	1.007	11.28	11.57	0.401	0.425
A. T. P. (50ppm)	14.38	14.87	1.484	1.499	40.27	41.15	4.155	4.428	37.44	38.17	3.864	4.107
Phosph.	6.33	7.71	0.431	0.534	35.36	36.87	2.541	2.689	32.76	33.25	2.228	2.301
S. P. (200)	10.78	10.96	0.595	0.635	30.43	31.62	1.689	1.831	25.31	25.89	1.397	1.499
L. S. D. at 5%	2.11	2.45	0.096	0.126	3.34	3.56	0.088	0.098	1.17	1.38	0.166	0.198

* A. T. P. = Adenosine Tri phosphate
* Phosph. = Phosphorien
* S. P. = Super phosphate

and are in agreement with those obtained by Ige *et al.,* (1984) on soybean seed; Paul and Southgate (1985) on some food seeds; Jacoub (1995) on sweet basil seeds; and Shalan (2001) on *Lagenaria siceraria* seeds.

3.2.2.3. Fixed oil content in seeds

Data in Table (8) reveal that fixed oil percentage and content significantly increased with all treatments. ATP at 50 ppm may be considered the most effective treatment for increasing fixed oil % and yield/ plant. It resulted in 37.44% of fixed oil (3.864 ml/ plant). The least yield was found with dressing calcium superphosphate at 200 kg/ fed (1.397 ml/plant), compared with the control (0.401 ml/plant). The results had the same trend in the two seasons. These results are in close agreement with those reported by Marshner and Cakmak (1986) on cotton seeds; Wasudevan *et al.* (1996) on sunflower seeds; Baljeet *et al.,* (1996) on seeds of *Guizotia abyssinica*; Patil *et al.* (1997); and Shalan (2001) on bottle gourd seeds of (*Lagenaria siceraria)* plants.

3.2.2.4. Fatty acid fraction

The relative percentages of fatty acids extracted from oil seeds of *Borago officinalis* plants treated with Bio and chemical phosphorus fertilizer are recorded in Table (9) and Fig. (2).In all the treatments the major saturated fatty acids were polmitic. It ranged from 10.7% with foliar ATP treatment at 50 ppm (the least value) to 15.19% and 16.23% for the Phosphorein and S. P. (200) kg/fed applications, respectively, against 33.72% for the control (the highest recorded value). Some results were found with arachidic and behenic acids (as saturated fatty acids) which were classified as major fatty acids (more than 10%) were found in the control (12.38% and 11.79%), comparing with ATP, Phosphorein and S. P. (200) treatments. They were 4.13%, 4.58% and 5.48% respectively. These are considered as minor fatty acids (less than 10%). Moreover, there were traces of saturated fatty acids (less than 1%) *i.e.* Caprylic, Capric, Lauric and Myristic acids.

In case of the unsaturated fatty acids, the results obtained showed that Oleic and Linoleic acids were found as a major group (more than 10%) which increased greatly with phosphorein treatment (21.8% for Oleic acid). As far as linoleic acid, its value was 36.44 % at ATP

Table (9): Effect of bio-and chemical phosphorus fertilization on fatty acid fraction in the fixed oil of *Borago officinallis* L. seeds.

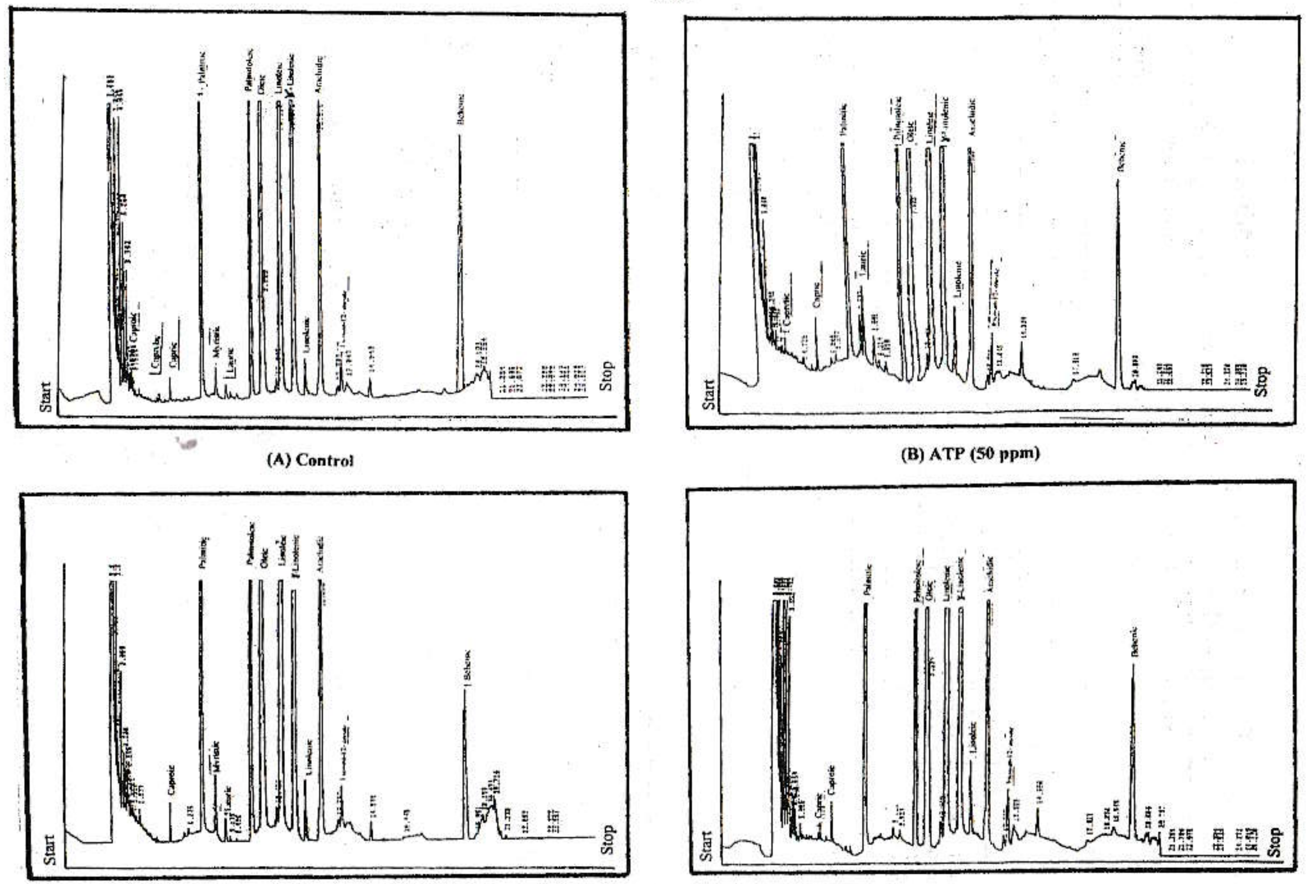
Components %	Treatment	Control 0	A. T. P. (50 ppm)	Phosphorein	S. P. (200)
Caproic	C6: 0	*	*	*	*
Caprylic	C8: 0	*	0.22	*	0.35
Capric	C10: 0	0.45	0.14	*	0.25
Lauric	C12: 0	0.59	*	*	*
Myristic	C14: 0	0.75	*	*	*
Palmitic	C16:0	33.72	10.7	15.19	16.23
Palmitoleic	C16:0	*	3.86	*	2.25
Oleic	C18: 1	9.08	16.13	21.80	15.56
Linoleic	C18: 2	28.21	36.44	35.90	34.24
δ - Linolenic	C18: 3	1.30	23.18	21.90	19.14
Linolenic	C18: 3	0.12	0.68	*	0.47
Arachidic	C20: 0	12.38	4.13	4.58	5.48
Eicosan-12-enoate	C20:1	*	2.84	*	2.15
Behenic	C22: 0	11.79	1.64	*	1.88
Total identified		98.39	99.96	99.37	98.00
Total unsaturated (TU)		38.71	83.13	79.60	73.81
Total saturated (TS)		59.68	16.83	19.77	24.19
TU / TS		0.65	4.94	4.03	3.05

A. T. P. = Adenosine Tri phosphate S. P. = Super phosphate
TU/ TS = Total unsaturated / Total Saturated Ratio * = Below 0.1%

treatment, compared with 9.08% and 28.21% for the control respectively.

Concerning δ - Linolenic acid, the data recorded a sharp increasing effect with all treatments used. The maximum percentage was of ATP foliar application (23.18%), followed by Phosphorein treatment (21.9%), and S. P. (200),: (19.14%), which was considered as a major unsaturated fatty acid, compared with the control (1.3%). It was ranged in minor group.

In addition, the data shown in Table (9) and Fig. (2) indicate that ATP application resulted in the highest value of total unsaturated fatty acids (83.13%) and the least value of total saturated ones (16.83%). This was followed by Phosphorein treatment with 79.6% and 19.77%,



(A) Control

(B) ATP (50 ppm)

(C) Phosphorein

(D) S.P. (200)

Fig.(2): Effect of bio-and chemical nitrogenous fertilization and ATP on the chromatograms of fatty acid fraction in the fixed oil of *Borago officinallis* seeds.

respectively, compared with the control which recorded 38.71% and 59.68%, respectively.

Finally, all treatments used had a marked increasing effect on the ratio of TU/TS which ranged from 4.94% at ATP foliar spraying treatment to 3.05% at S. P. (200), compared to the control with 0.65%.

These results are in agreement with those obtained by Talaat and Youssef (1998) on the fatty acid constituent of *Borago officinalis* oil seeds; Tsevegsuren and Aitzetmuller (1996) on the seed oils from other Boraginaceae genera *i.e.*, *Amblynotus repestris* seeds; Baljieet *et al.*, (1996) on seed of *Guizotia obyssinica* plants; and Hurtubise *et al.*, (1992) on *Lemna minor* seeds.

Recommendations

From these results, it may be noticed that the growth characters, seed yield per plant, fixed oil production and fatty acid components of *Borago officinallis* plant grown in Egypt, can be improved by the application of some effective, safe and of low cost treatments, *i.e.*, Bio-nitrogen fertilization (Nibrobein), Bio-phosphorus fertilization (Phosphorein), and physiological phosphorus source (Adenosine-Tri-Phosphate). These treatments could be recommended for increasing the productivity of Borago plants under the conditions of this work.

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مقارنة استجابة نبات خبز النحل للتسميد الحيوى والكيماوى النتروجينى والفوسفورى والأدينوزين تراى فوسفات على النمو والمحتوى الكيماوى

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ملخص

أجرى هذا البحث فى تجربتين حقليتين بمزرعة خاصة بأبو زعبل، محافظة القليوبية خلال موسمين متتاليين (١٩٩٨-١٩٩٩) و (١٩٩٩-٢٠٠٠). حيث عوملت نباتات خبز النحل بمستويات مختلفة من سماد سلفات الأمونيوم (صفر، ١٥٠، ٢٠٠ كجم/فدان). كما عوملت النباتات بالنتروجين الحيوى (النتروبين) بمعدل ٥كم / فدان (**التجربة الأولى**).

ومن ناحية أخرى أجرى تسميد النباتات بسماد سوبر فوسفات الكالسيوم بالجرعة الموصى بها وهى ٢٠٠ كجم/ فدان، كما لقحت بعض البذور باستخدام السماد الحيوى للفسفور (فسفورين) بمعدل ١٠ كجم/ فدان. هذا بالإضافة إلى رش النباتات بأدينوزين تراى فوسفات بمعدل ٥٠ جزء فى المليون(**التجربة الثانية**).

سجلت النتائج على طول النبات، عدد الأوراق والأفرع، الوزن الجاف للأوراق بالإضافة إلى محصول البذور خلال المراحل المختلفة لنمو النباتات. كذلك تم تقدير المحتوى الكيماوى فى الأوراق والبذور من الكربوهيدات الكلية والبروتين، ومحتوى البذور من الزيت الثابت، بالإضافة إلى فصل محتوى الزيت من الأحماض الدهنية المستخرج من البذور بواسطة التفريد الغازى الكروماتوجرافى : وكانت النتائج كما يلى

- أظهر التسميد الحيوى النتروجينى (نتروبين) تفوقاً كمصدر للتسميد النتروجينى مقارنة بالتسميد الكيماوى عند التسميد بسلفات الأمونيوم بمعدل ١٥٠، ٢٠٠كجم/ فدان رغم الزيادة الناتجة عنها مقارنة بالكونترول فيما يتعلق بصفات النمو الخضرى والتركيب الكيماوى وإنتاج الزيت.

- أدى استخدام أدينوزين تراى فوسفات بتركيز ٥٠ جزء فى المليون كمصدر لعنصر الفوسفور تفوقاً كبيراً عن النتائج المتحصل عليها عند استخدام التسميد الحيوى للفوسفور (فوسفورين) الذى جاء فى المرتبة الثانية من ناحية الأهمية، وكان كذلك أعلى من التسميد الكيماوى بسوبر فوسفات الكالسيوم بمعدل ٢٠٠ كجم/ فدان، فيما يتعلق بالصفات تحت الدراسة مقارنة بالكنترول.
- تشير النتائج المتحصل عليها بالنسبة للتفريد الغازى الكروماتوجرافى لمكونات الزيت الثابت من الأحماض الدهنية بالبذور أن رش النباتات بأدينوزين تراى فوسفات يعتبر أعلى وأفضل معاملة مقارنة بباقى المعاملات حيث ذادت نسبة الأحماض الدهنية غير المشبعة إلى الأحماض الدهنية المشبعة بحوالى ٣ إلى ٤ مرات. يلى تلك المعاملة التسميد الحيوى الفوسفورى حيث أعطى نتائج أفضل من التسميد الكيماوى.