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Physiological effects of organic and bio fertilizers on borage (*Borago officinalis* L.) plants

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Abstract

A pot experiment was carried out at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during 2021/2022 and 2022/2023 growing seasons to elucidate the impact of filter mud as organic manure, microorganisms as biofertilizers and their interactions on herb dry weight, flowers dry weight, seed yield, fixed oil % as well as the content of alkaloids in the herb, flowers and seeds of borage (*Borago officinalis* L.) plants. Filter mud was added at four levels (0, 70, 140 and 210 g/plant) compared with the check treatments. Microorganisms as biofertilizers used were eight treatments as follows: 0 (uninoculated plants), *Azotobacter chroococcum* (Az), *Bacillus circulans* (B.c), arbuscular mycorrhiza fungi (*Glomus mosseae* and *Glomus fasciculatum)* (AM), Az + B.c., Az + AM, B.c + AM and Az + B.c + AM. The obtained results indicated that the addition of filter mud with all levels resulted in a significant increase in all studied aspects, except for the low one (70 g/plant), mostly. Clearly, in the most cases, the tested parameters were gradually significantly augmented with increasing the levels of filter mud. Therefore, the filter mud when used at high level (210 g/plant) proved to be more effective in elevating the examined characteristics. At the same time, the inoculation with the tested useful microorganisms either single or in combination led to a significant increase in the studied traits, except *Azotobacter* (Az) and or *Bacillus* (B.c) treatments, mostly. It is obvious from data that the best results of all traits were detected when the inoculation with the triple combined treatment (Az + B.c + AM), followed by the double combined one (B.c + A.M), mostly. The interaction between the two studied factors on the examined parameters was significant effected. In addition, most combined treatments significantly increased the studied traits. Apparently, supplying the plants with the filter mud at high level (210 g/plant) plus the inoculation with the triple combined treatment (Az + B.c + AM), followed by the double combined one (B.c + AM) were the most effective treatments in augmenting these aspects, in many cases.

Keywords: borage, *Borago officinalis*, filter mud, *Azotobacter chroococcum*, *Bacillus circulans*, mycorrhiza fungi.

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1. Introduction

Borage (*Borago officinalis* L.) plant belongs to Boraginaceae Family, it is an annual flowering herb. It is one of the most important medicinal plants and native to Mediterranean region and has naturalized in many other countries. Borage is known as bee plant or bee bread (El-Hafid *et al*., 2002), it produces plenty of seeds (Stary and Jirasck, 1975; Simon *et al*., 1984). From borage seeds, oil was extracted which contained 20-30% gamma linolenic acid (GLA), whereas is an important essential fatty acid. Also, borage is rich in fatty acids namely, oleic and palmitic acids, besides to contains minerals, saponins, tannins and mucilage (Bianco *et al*., 1998; Velasco and Goffman, 1999). The leaves, flowers and seeds of borage contained many alkaloid compounds (Dodson and Stermitz, 1986). Borage has to be utilized in medicinal purposes like, treat urinary infections cold, bronchitis, in compresses to treat skin rashes and rheumatic conditions (Stodola and Volak, 1992) as well as borage is very useful as mild diuretic, anti-inflammatory, demulcent and diaphoretic properties. Utilizing organic and bio-fertilizers have become better agricultural practices for human and environment. Organic manures are positive alternative to mineral fertilizers for improving the structure of soil (Douda *et al.*, 2008). Moreover, organic manures contain plant growth regulators namely IAA and GA as well as macronutrient, and micronutrients which useful for microorganisms (Natarajan, 2007; Sreenivasa *et al.*, 2010). Filter mud is one of organic fertilizers, it is local organic manure, it contains high amounts of plant nutrients and organic matter, which play on a vital role in enhancing the plant growth and productivity. The increments in herb weight, flower weight and seed yield due to using organic manures which have been studied by El Houssini (2009), Fouad (2017), Awad (2019) on borage plants. An augment in fixed oil and alkaloids content on borage (Fouad 2017), elevate in oil content of sunflower (Kalkhoran *et al*., 2013; Manjunatha *et al.*, 2009; Rasool *et al*. 2015), increase in alkaloids content of periwinkle (Hassan, 2012) and on tobacco (Kurt and Ayan, 2014), as well as augment in fixed oil of roselle (Ahmed *et al*., 1998; Mahmoud, 2022), resulting from adding organic manures. Using bio-fertilizers (useful microorganisms) led to enhance soil fertility by elevating the number of microorganisms and accelerate certain microbial processes in rhizosphere zone. These microbiological processes capable to convert unavailable forms of nutrients to available forms (Alaa El-Din, 1982; Rao, 1981). In Egyptian soils, applying biofertilizers can able reduced pH which led to an augment in availability of trace elements consequently improve in the growth of plant (Mahfouz and Sharaf-Eldin, 2007). Biofertilizers as useful microorganisms are another way to achieve sustainable agriculture which act a vital role in providing the requirements of nutrients for plants (Bhardwaj *et al*., 2014). Biofertilizers have become decades that are divided into many microorganisms like, N-fixing bacteria, P-solubilizing microorganisms, K-solubilizing microorganisms and S-solubilizing microorganisms. The genus of Azotobader (*Az. chroococcum*) is considered the most common species of N-fixing bacteria inhabiting many soils around the world (Mahato *et al*., 2009). The most common type of free living heterotrophic and non-symbiotic N-fixing bacteria found in neutral or alkaline soils (Wani *et al*., 2016). These organisms provide a lot of N to non-leguminous plants (Aasfar *et al*., 2021), whereas N is converted to ammonia via them, then absorbed by plants (Prajapati *et al*., 2008). The improvement in herb dry weight due to the inoculation with N-fixing bacteria was reported by El-Houssini (2009) on borage, Hasan and Rabie (2019) on basil. An increase in flower yield as a result of applying N-fixing bacteria had been revealed by El- Houssini (2009) on borage. Seed yield was augmented due to the inoculation of N-fixing bacteria indicated on borage (Dessouky, 2002; El-Houssini, 2009; Hendawy and El-Gengaihi, 2010; Zayed *et al*., 2004) on black cumin (Safwat and Badran, 2002; Valadabadi and Farahani, 2011). The positive impact of N-fixing bacteria on fixed oil and alkaloids content was obtained by Hamad (2007) on guar, regarding fixed oil, meanwhile, by El-Houssini (2009) on borage, Saleh *et al*. (1998) on datura and, also Hassan *et al*. (2012) on lupine, concerning alkaloids content. As for the favourable influence of *Bacillus circulans* bacteria, it is a type of potassium solubilizing microorganisms that is silicate solubilizing bacteria which can improve K availability to crops from insoluble K sources, consequently raising crop yield and lowering the use of chemical fertilizers (Khalil *et al*., 2018; Padhan *et al*., 2019). The beneficial role of *Bacillus circulans* on elevating herb weight and seed yield have been examined by Gharib *et al*. (2008) on marjoram, regarding herb weight and by Darzi *et al*. (2012) and Mohammed *et al*. (2012) on anise plants, concerning seed yield. The most important microorganisms used as biofertilizers are mycorrhiza. It is more effective in the soil with both lower fertility and nutrient content. The inoculation with mycorrhiza fungus (*Glomus mosseae*) could improve the root system of plant and elevate the absorption of water and nutrient elements for the plant growth (Kumari, 2003; Waller *et al*., 2005). Mycorrhiza fungi play an important role in augment the root absorption surface area, consequently allowed the plants to use water and nutrient in the soil more efficiently (Roesty *et al*., 2006). The capability of mycorrhiza on enhancing the growth were described on datura (Abdel-Azeem, 1998; Saleh *et al*., 1998) on basil, Hasan and Rabie (2019) on periwinkle (Hassan, 2012; Sepehri *et al*., 2015) on guar (El-Sawah, 2021). An augment in seed yield due to the inoculation with mycorrhiza was emphasized by Ghorbani (2019) and El-Sawah (2021) on guar. The objective of this work was to evaluate the impact of filter mud as organic manure and biofertilizers as microorganisms (*Azotobacter chroococcum*, *Bacillus circulans* and mycorrhiza fungi), as well as, their interactions on herb dry weight, flowers dry weight, seed yield, fixed oil % and the contents of alkaloids in the herb, flowers and seeds of borage (*Borago officinalis* L.) plants to find out the most suitable treatment for enhancing these traits

2. Materials and methods

2.1 Experimental site and treatments description

The present investigation was conducted at the Experimental Farm, Faculty of Agriculture Al-Azhar University, Assiut, Egypt during the two consecutive seasons of 2021/2022 and 2022 /2023 to study the influence of filter mud as organic manure and the inoculation with beneficial microorganisms as biofertilizers (*Azotobacter chroococcum* (AZ), *Bacillus circulans* (B.c) and arbuscular mycorrhiza (AM) as well as, their interactions on herb dry weight, flowers dry weight, seed yield / plant, and some chemical constituents of borage (*Borago officinalis* L.) plants. A split plot design with 3 replicates was carried out in this experiment, whereas, filter mud treatments were the main plots (A) included 4 levels and the examined microorganism treatments were considered the sub-plots (B) contained 8 treatments. Therefore, the interaction treatments (A×B) involved 32 treatments. Borage seeds were sown on 19th Sep. in the nursery beds for the two seasons. After 45 days from sowing (November 3st), the growing seedlings (average 12-15 cm height and 0.27-0.30 cm. diameter) were transplanted into a container (23 × 23 × 29 cm) of both seasons. This container was filled with 20 Kg sandy soil containing one seedling. The physical and chemical characteristics of the used soil were estimated according to Jackson (1973) and are in Table (1). The utilized filter mud (main plots, A) was obtained from Sugar and Integrated Industries Company, Naga Hammadi, Egypt. Such manure was added to the soil before transplanting the seedlings at 4 levels (0, 70, 140 and 210 g / seedling). The chemical properties of filter mud were determined according to Black *et al.* (1965) and are listed in Table (2).

Table (1): The physical and chemical properties the applied soil (average of the two seasons).

Characters		pH	EC	Ca%	Mg%	CO_3%	Cl%	N%	P%	K%
Texture	Sandy	8.7	4.6	0.2	0.12	0.252	3.98	0.030	0.024	0.07
Sand%	77.52									
Clay%	12.48									
Silt%	10.0									

Table (2): The chemical analysis of the used filter mud (average of both seasons).

Characteristics	Value	Characteristics	Value
Total nitrogen %	2.85	Cu ppm	193
Total phosphorus %	2.3	E.C. (ds/m)	3.5
Total potassium%	0.75	Organic matter %	35.0
Zn ppm	110	C : N Ratio	8.3
Mn ppm	295	Organic carbon %	23.6
Fe ppm	4560	pH	7.3

Beneficial microorganisms were used as biofertilizer treatments (sub-plots, B) compared with the control (uninoculated plants), *Azotobacter Chroococcum* (Az), *Bacillus circulans* (B.c), arbuscular mycorrhiza (AM), Az + B.c, Az + AM, B.c + AM and Az + B.c + AM were used in this experiment. These microorganisms were obtained from Department of Agricultural Microbiology, Centre of National Research, Egypt. *Azotobacter chroococcum* (Az) as N-fixing bacteria 8 $\times 10^7$ cfu), *Bacillus circulans*. (B.C) as potassium solubilizing bacteria 7×10^9 cfu) and mycorrhiza fungi (AM fungi, *Glomus mosseae* NRC 31 and *Glomus fasciculatum* NRC 15) 275 spores /g media ″peat, vermiculite and perlite". These inoculants were applied at 10 ml/plant in case of Az and B.c, as well as AM fungi at 10 g / plant, twice times at 2 weeks interval starting November 18th during both seasons. The inoculants bacteria were added to the soil surface beside the plant, while the inoculants of mycorrhiza fungi were poured under each plant and then plants were irrigated immediately. All other agricultural practices were performed as usual. The end of this work (the second week of May), in the two seasons. Data were recorded as follows: herb dry weight (g) / plant, flower dry weight (g) / plant, seed yield (g) / plant and fixed oil % in the seeds, was determined by soxhlet apparatus according to A.O.A.C. (1990). At the same time alkaloids content (mg /g) in the dried herb, flowers and seeds were determined according to Pearson (1976). The obtained data were tabulated and statistically analyzed according to MSTAT (1986) using the L.S.D. test at 5% (Mead *et al*., 1993).

3. Results and Discussion

3.1 Herb dry weight (g)/plant

Shown data in Table (3) revealed that fertilizing borage plants with filter mud as organic manure positively affected on herb dry weight / plant during the two growing seasons. It could be noticed that such aspect was significantly increased by applying all levels of filter mud, except for the lowest one (70 g/plant) in the two seasons, as compared with the control treatment. It is obvious from data that such trait was gradually significantly augmented with increasing the levels of filter mud, during both seasons. Thus, the heaviest herb dry weight /plant was obtained due to the use of filter mud at the high level (210 g/plant) which augmented it by 24.8 and by 31.0 % over control in the first and the second seasons, respectively. The beneficial role of organic manure on increasing herb weight was also studied by El Houssini (2009), Fouad (2017), Awad (2019) on borage plants. Concerning to biofertilizer treatments, data in Table (3) exhibited that herb dry weight /plant of borage was significantly responded to the inoculation with the three examined microorganisms (*Az. coreoccum, B. circulans* and mycorrhiza), during the two seasons. Clearly, inoculating borage plants with these microorganisms either single or together led to a significant elevate in herb dry weight /plant, except for the inoculation, with *Azotobacter* and *Bacillus* each alone, in both seasons, in relative to uninoculated ones. Apparently, higher values of such aspect were detected by treating the plants with the combined treatments of either the three or two microorganisms, mostly, than those observed by individual ones, during the two growing seasons. Obviously, the highest values of herb dry weight were given by the inoculation with the triple combined treatment (*Azotobacter* + *Bacillus* + mycorrhiza) as ranged 39.1 and 40.1% over the check treatment, during the two successive seasons, respectively. The stimulating effect of *Azotober chorcorcum* on herb weight was also discussed by El-Houssini (2009) on borage, by Gharib *et al*. (2008) on marjoram concerning *Bacillus circulans* and by Abdel-Azeem (1998), Saleh *et al*. (1998) on datura, on periwinkle (Sepehri *et al*., 2015).

Table (3): Effect of filter mud, biofertilizers and their interactions on herb dry weight (g/plant) during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	93.3	105.6	116.3	125.1	110.1	88.4	93.6	110.5	116.7	102.3
Azotobacter chroococcum (Az)	99.7	107.8	117.7	127.2	113.1	97.5	100.0	113.0	121.5	108.0
Bacillus circulans (B.c)	103.3	113.1	120.7	130.3	116.9	100.1	104.2	109.4	131.4	111.3
Mycorrhiza (AM)	111.5	125.2	126.7	139.3	125.7	103.3	106.6	126.3	134.6	117.7
Az + B.c	116.8	117.2	129.4	133.2	124.1	100.6	110.6	130.2	129.8	117.8
Az + AM	120.2	126.9	125.9	141.3	128.6	109.3	118.2	129.6	135.5	123.2
B.c + AM	124.6	129.6	134.6	152.3	135.3	113.4	126.3	137.4	157.7	133.7
AZ + B.c + AM	129.4	141.3	168.8	173.3	153.2	121.8	127.4	158.6	165.3	143.3
Mean (A)	112.4	120.8	130.0	140.3		104.3	110.9	126.9	136.6	
LSD 0.05 A	8.8					7.6				
LSD 0.05 B	12.9					9.6				
LSD 0.05 A*B	25.8					19.2				

As for the interaction, it was statistically significant effect on herb dry weight /

plant of borage, during the two consecutive seasons. Obviously, the use of most combined treatments resulted in a significant augment in herb dry weight /plant comparing to untreated plants, during the two seasons. It appears that plants grown in filter mud at the high level (210 g/plant) plus the inoculation with the triple combined treatment (Az + B.c + AM), followed by the moderate level of filter mud (140 g/plant) + the inoculation with (Az + B.c + AM) then filter mud at the high level with the inoculation of double combined (B.c + AM) proved higher effect in increasing herb dry weight/plant than those revealed by other combined treatments during the two seasons, as clearly shown in Table (3).

3.2 Flower dry weight (g)/plant

The presented data in Table (4) cleared that flower dry weight / plant of borage was positively responded to the application of filter mud as organic manure, during the two consecutive seasons. Obviously, the addition of filter mud at all levels, in both seasons, led to a significant increase in flower dry weight /plant, except for the lowest level (70 g/plant) of such manure, in the two seasons, as compared to unfertilized plants. As regard, increasing the levels of filter mud such parameter was gradually significantly elevated, during the two seasons. Therefore, the presence of filter mud as organic manure with the highest level (210 g/plant) produced the heaviest weight of dried flowers /plant reached 22.0 and 27.5% over the check treatment, during the two experimental seasons, respectively. The capability of organic manures on enhancing flower weight was described by many authors such as El Houssini (2009), Fouad (2017) and Awad (2019) on borage plants.

Table (4): Effect of filter mud, biofertilizers and their interactions on flower dry weight (g/plant) during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	37.3	38.2	40.0	43.9	39.8	30.2	35.7	40.2	42.5	37.2
Azotobacter chroococcum (Az)	40.5	42.4	42.8	44.1	42.4	37.9	39.2	42.3	44.3	40.9
Bacillus circulans (B.c)	42.1	45.2	46.1	47.5	45.2	39.7	40.7	44.7	47.7	43.2
Mycorrhiza (AM)	45.8	46.0	48.7	49.2	47.4	39.5	43.8	45.5	49.4	44.6
Az + B.c	42.6	45.7	50.4	54.8	48.4	38.8	45.1	48.7	50.9	45.9
Az + AM	45.9	47.4	52.5	55.4	50.3	43.7	45.7	50.8	53.3	48.4
B.c + AM	46.0	48.3	55.8	62.0	53.0	46.0	46.3	52.4	60.4	51.3
AZ + B.c + AM	48.8	53.3	64.3	68.6	58.7	47.3	48.2	59.2	64.7	54.9
Mean (A)	43.6	45.8	50.1	53.2		40.4	43.1	48.0	51.7	
LSD 0.05 A	2.8					3.2				
LSD 0.05 B	4.2					6.2				
LSD 0.05 A*B	8.4					12.4				

Concerning biofertilization treatments, flower dry weight /plant of borage was positively affected by the inoculation with the studied microorganisms, during the two consecutive seasons (Table 4). From the obtained data, it could be observed that treating borage plants with all treatments of the tested microorganisms either separately or in combination, in the two seasons, resulted in a significant increase in flower dry weight /plant, except for *Azotobacter* (AZ), in both seasons and, also *Bacillus* (B.c), in the second one, as compared to uninoculated ones. In most cases, the inoculation with the combined treatments either the two or three microorganisms gave higher values of such trait than those obtained by single ones, during both seasons. Moreover, the most effective treatment in augmenting flower dry weight /plant was detected by the triple combined treatment (Az + B.c + AM.) which increased it by 47.5 and by 46.2% over uninoculated ones, during both seasons, respectively. The simulative influence of *Az. chroococcum* on flower weight was also examined by Shaalan (2005) and El- Houssini (2009) on borage, by Amiri *et al.* (2022) on *Echium amoenum*, regarding *Bacillus circulans* and by Abdel-Azeem (1998) and Saleh *et al*. (1998) on datura, Hassan (2012) and Sepehri *et al*. (2015) on periwinkle. With respect to the interaction, the listed data in Table (4) pointed out that significant effect on flower dry weight /plant of borage, during the two growing seasons. Clearly, supplemented the plants with the most combined treatments caused a significant elevate of flower dry weight /plant, during the two consecutive seasons, compared to control plants. Apparently, the utilization of filter mud at the high level (210 g/plant) + inoculation with the triple combined treatment (Az + B.c + A.M), followed by such manure at the previous level + the double combined one (B.c + AM), then filter mud the moderate one (140 g/plant) with the triple combined one (Az + B.c + AM) proved to be more effective in augmenting flowers dry weight /plant than those noticed by other combination treatments, during the two successive seasons.

3.3 Seed yield (g/plant)

The obtained data in Table (5) indicated that supplying borage plants with filter mud positively affected seed yield/plant during the two experimental seasons. Obviously, the use of filter mud with all levels resulted in a significant augment in seed yield /plant, except for the lowest one of such manure (70 g/plant), during the two seasons, if compared with unfertilized plants. In this connection, such parameter was gradually significantly increased with augmenting the levels of filter mud, during both seasons. Thus, applying filter mud at the highest level (210 g/plant) produced the heaviest seed yield as ranged 27.9 and 34.4% over the check treatment, during both seasons, respectively. The efficiency of organic manures on elevating seed yield has to be revealed by El Houssini (2009), Fouad (2017) and Awad (2019) on borage plants. In this regard to biofertilization treatments, data proved that seed yield /plant of borage was significantly influenced by the inoculation with the three tested microorganisms, during the two growing seasons. It is clear from data that inoculating borage plants with these microorganisms either single or together led to a significant increase in seed yield /plant, except for *Azotobacter* treatment (Az), in both seasons and, also *Bacillus* (B.c), in the first one, as compared to uninoculated plants. Higher values of seed yield /plant were given by the application of the combined treatments either the two or three microorganisms, mostly, than those detected by individual ones, in both seasons. Apparently, the inoculation with the triple combined treatment (Az + B.c + AM) proved more effective in increasing such aspect than those obtained by other treatments and control, in both seasons. Numerically, this previous treatment elevated seed yield /plant by 46.4 and by 46.5% over uninoculated ones, during the two successive seasons, respectively, as clearly insured in Table (5).

Table (5): Effect of filter mud, biofertilizers and their interactions on seed weight (g/plant) during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	14.7	16.7	16.9	18.2	16.6	13.7	14.5	16.0	17.8	15.5
Azotobacter chroococcum (Az)	16.1	17.1	17.0	19.7	17.5	14.9	15.0	17.0	18.8	16.4
Bacillus circulans (B.c)	16.0	16.2	18.9	19.8	17.7	15.0	17.5	18.3	19.7	17.6
Mycorrhiza (AM)	17.3	17.6	19.1	21.5	18.9	14.8	17.6	19.3	20.5	18.1
Az + B.c	17.2	18.9	19.8	20.9	19.2	15.3	17.7	20.2	21.5	18.7
Az + AM	16.9	19.1	20.9	21.1	19.5	17.4	18.0	20.8	22.0	19.6
B.c + AM	19.1	19.1	22.0	25.5	21.4	16.5	17.5	21.2	24.6	20.0
AZ + B.c + AM	20.5	21.0	26.2	29.3	24.3	20.0	21.1	22.5	27.0	22.7
Mean (A)	17.2	18.2	20.1	22.0		16.0	17.4	19.4	21.5	
LSD 0.05 A	1.7					1.9				
LSD 0.05 B	2.0					1.9				
LSD 0.05 A*B	4.0					3.7				

The enhancement in seed yield due to the inoculation with *Azotobacter chroococcum* was also reported by Dessouky (2002), Zayed *et al*. (2004), Hendawy and El-Gengaihi (2010), and El-Houssini (2009) on borage, Safwat and Badran (2002), and Valadabadi and Farahani (2011) on black cumin, by Darzi *et al*. (2012) and Mohammed *et al*. (2012) on anise plants, regarding *Bacillus circulans* by Ghorbani (2019) and El-Sawah (2021) on guar, Dawood *et al.* (2019) on flax, Abou El-Ghait *et al.* (2021) and Moghith, 2021) on chia concerning mycorrhiza. It is evident from the given data in Table (5) that seed yield of borage /plant had significant effect by the interaction treatments, during the two consecutive seasons. Clearly, the most combined treatments led to a significant increase in seed yield /plant, compared to untreated plants, in both seasons. In this connection, plants grown in filter mud at the high level (210 g/plant) + the inoculation with the triple combined treatment (Az + B.c + A.M), followed by the same previous level of filter mud (210 g/plant) with the double combined treatment (B.c + A.M) were the most effective treatments in augmenting seed yield/plant than those revealed by other combination treatments, during the two experimental seasons.

3.4 Fixed oil percentage

As shown in Table (6), fixed oil % of borage seeds were positively responded to supplement the plants with filter mud as organic manure, during the two successive seasons. Apparently, the addition of filter mud at all levels resulted in a significant increase in fixed oil %, except for the lowest one (70 g/plant), in both seasons, as compared to the check treatment. Significant differences among all levels of filter mud were noticed, both seasons, except for between the low and moderate levels of such manure, in the second season. Obviously, the highest values of fixed oil % were given by utilizing filter mud at the high level (210 g/plant) which augmented it by 6.2 and by 5.7% over unfertilized ones, during the two growing seasons, respectively. The augment in fixed oil as a result of applying organic manures have to be proved by Manjunatha *et al.* (2009), Kalkhoran *et al*. (2013), and Rasool *et al*. (2015) on sunflower, and on roselle (Ahmed *et al.*, 1998; Mahmoud, 2022).

Table (6): Effect of filter mud, biofertilizers and their interactions on fixed oil % during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	27.99	28.17	29.24	29.87	28.82	26.99	28.70	29.04	29.40	28.54
Azotobacter chroococcum (Az)	28.69	28.90	29.76	30.00	29.34	27.93	29.00	29.35	29.96	29.06
Bacillus circulans (B.c)	28.67	29.02	29.80	30.26	29.44	28.94	29.31	29.44	30.19	29.47
Mycorrhiza (AM)	29.11	29.64	30.56	30.80	30.02	29.48	29.64	29.86	30.56	29.88
Az + B.c	28.72	29.10	29.91	30.58	29.58	29.09	29.41	29.33	30.85	29.67
Az + AM	29.15	29.70	30.18	30.93	29.99	29.52	29.76	29.78	31.00	30.01
B.c + AM	29.86	30.02	30.69	31.95	30.63	29.66	30.20	30.24	31.18	30.32
AZ + B.c + AM	30.02	30.94	31.78	32.33	31.27	30.25	30.40	31.37	31.91	30.98
Mean (A)	29.03	29.44	30.24	30.84		28.98	29.55	29.80	30.63	
LSD 0.05 A	0.56					0.59				
LSD 0.05 B	0.63					0.61				
LSD 0.05 A*B	1.26					1.23				

In respect to biofertilization treatments, data in Table (6) emphasized that fixed oil % of borage was significantly affected by the inoculation with the three studied microorganisms, during the two consecutive seasons. Clearly, adding all microorganisms either separately or in combination led to a significant augment in fixed oil%, except for Azotobacter (Az) treatment, in both seasons and also *Bacillus* (B.c) alone, in the first season, as compared to uninoculated plants. Higher values of such trait were as observed due to the inoculation with the triple or double inoculants, mostly, than those detected by single ones, during both seasons. Moreover, the application of the triple combined treatments (Az + B.c + AM) proved to be more effective in elevating fixed oil % as reached 8.5 and 8.5% over untreated plants, during the two successive seasons, respectively. The primitive influence of *Azotobacter chroococcum* on fixed oil was also obtained on by El-Houssini (2009) on borage; Hamad (2007) on guar; by Mohamed *et al.* (2020), Abd El-moneim (2023) on *Nigella sativa* concerning *Bacillus circulans* and on sunflower (Abdallah *et al.*, 2013), on flax Dawood *et al.* (2019), Abou El-Ghait *et al.* (2021), Moghith (2021) on chia regarding mycorrhiza. It worthy mention that the interaction between the two studied factors on fixed oil % of borage had significant effect, during the two seasons (Table 6). At the same time, such aspect was significantly increased resulting from applying the most combined treatment, compared to untreated ones, in the first season. In this concern, the use of all combined treatments, in the second season, led to a significant augment in fixed oil %, except for 0 filter mud with *Azotobacter* (Az) treatment, as compared to control. Apparently, supplying the plants with filter mud at the high level (210 g/plant) with the triple combined treatment (Az + B.c + AM), followed by the same level of such manure + the double combined one (B.c + AM), then the moderate level of filter mud (140 g/plant) plus the triple combined one (Az + B.c + AM) were the most effective treatments in augmenting fixed oil% than those revealed by other combined treatments.

3.5 Alkaloids content in the herb

The given results in Table (7) exhibited that filter mud as organic manure was the main effect on alkaloids content in borage herb had statistically significant effect, in the two seasons. It is obvious that fertilizing the plants with all levels of filter mud resulted in a significant increase in alkaloids content of herb, except for the low one (70 g/plant) of such manure, during the two growing seasons. However, the differences among all levels of filter mud were significant, in both seasons, except for between the low and moderate levels, in the first season. Clearly, the highest values of such parameter were detected from plants grown in organic conditions at the high level (210 g/plant) as ranged from 23.0 to 31.5 % over the check treatment, during the two consecutive seasons, respectively. In relation to biofertilization treatments, the obtained data in Table (7) showed that alkaloids content in borage herb was positively responded to the inoculation with the tested microorganisms as biofertilizers, during the two experimental seasons. Apparently, such trait was significantly augmented, in the two seasons, due to inoculating the plants with the three microorganisms either single or in combination, except for *Azotobacter* (Az) treatment or *Bacillus* (B.c) alone, in the first season, as compared to uninoculated plants.

Table (7): Effect of filter mud, biofertilizers and their interactions on alkaloids content (mg/g d.w.) of herb during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	0.241	0.240	0.256	0.273	0.254	0.208	0.222	0.245	0.258	0.233
Azotobacter chroococcum (Az)	0.262	0.250	0.280	0.297	0.275	0.217	0.240	0.268	0.277	0.250
Bacillus circulans (B.c)	0.259	0.279	0.294	0.302	0.284	0.223	0.246	0.273	0.283	0.257
Mycorrhiza (AM)	0.266	0.288	0.311	0.333	0.299	0.228	0.250	0.282	0.298	0.264
Az + B.c	0.243	0.280	0.284	0.311	0.278	0.222	0.241	0.280	0.315	0.265
Az + AM	0.280	0.285	0.296	0.358	0.302	0.232	0.270	0.288	0.328	0.280
B.c + AM	0.278	0.283	0.309	0.364	0.309	0.256	0.273	0.290	0.330	0.290
AZ + B.c + AM	0.291	0.296	0.312	0.371	0.318	0.273	0.277	0.313	0.350	0.303
Mean (A)	0.265	0.275	0.293	0.326		0.232	0.253	0.280	0.305	
LSD 0.05 A	0.024					0.023				
LSD 0.05 B	0.019					0.029				
LSD 0.05 A*B	0.037					0.058				

It could be noticed that the application of combined treatments either double or triple inoculants gave higher values of alkaloids content of herb, mostly, than those revealed by individual ones, during the two successive seasons. Moreover, the inoculation with the triple combined treatment (Az + B.c + AM), followed by the double combined (B.c + AM), then (Az + AM) which proved more effective in increasing alkaloids content in the herb than those obtained by other treatments and control, in both seasons. Numerically, these above-mentioned superior treatments elevated such aspect by 25.2, 21.7 and by 18.9 % in the first season and by 30.0, 24.5 and by 20.2 % in the second season, over uninoculated plants, respectively. As for the interaction, gave significant effect on alkaloids content in borage herb, during the two experimental seasons. Obviously, supplemented the plants with the most combined treatment resulted a significant elevate in such trait, comparing to untreated plants, during both seasons. In this concern, the addition of filter mud at the high level (210 g/plant) plus the inoculation with the triple combined treatment (Az + B.c + AM), followed by the double combined one (B.c + AM) then with the double combined one (Az + AM) were the most effective treatments in augmenting alkaloids content of herb than those observed by other combination treatments, during the two growing seasons, as clearly indicated in Table (7).

3.6 Alkaloids content in the flowers

Data in Table (8) pointed out that alkaloids content in borage flowers, which significantly affected by the application of filter mud, during the two seasons. It could be noticed that the presence of plants under organic conditions and treated with all levels led to a significant increase in alkaloids content of flowers, except for the low level (70 g/plant), in both seasons, if compared with unfertilized ones. By augmenting filter mud levels, such aspect was gradually significantly increased, in the two seasons. Thus, the use of filter mud at the high level (210 g/plant) registered the highest values of alkaloids content in the flowers as ranged from 22.3 to 27.6 % over the check treatment, during two successive seasons, respectively. On the other hand, inoculating borage plants with the tested microorganisms positively affected alkaloids content of borage flowers, during both seasons (Table 8). From the listed data it could be noticed that the inoculation with the three microorganisms either separately or together led to a significant increase in alkaloids content of flowers, except for the treatment of *Azotobacter* (Az), in both seasons, as compared to uninoculated ones.

Table (8): Effect of filter mud, biofertilizers and their interactions on alkaloids content (mg/g d.w.) of flowers during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	0.253	0.248	0.271	0.304	0.269	0.214	0.233	0.257	0.271	0.244
Azotobacter chroococcum (Az)	0.270	0.277	0.290	0.307	0.286	0.234	0.263	0.275	0.296	0.267
Bacillus circulans (B.c)	0.268	0.280	0.302	0.319	0.292	0.252	0.265	0.292	0.294	0.276
Mycorrhiza (AM)	0.280	0.292	0.310	0.342	0.306	0.251	0.272	0.284	0.292	0.275
Az + B.c	0.280	0.292	0.306	0.320	0.300	0.247	0.268	0.285	0.327	0.282
Az + AM	0.293	0.300	0.317	0.365	0.319	0.248	0.271	0.291	0.350	0.290
B.c + AM	0.284	0.305	0.328	0.376	0.324	0.269	0.273	0.294	0.359	0.299
AZ + B.c + AM	0.293	0.312	0.342	0.384	0.333	0.281	0.285	0.339	0.362	0.317
Mean (A)	0.278	0.288	0.308	0.340		0.250	0.266	0.290	0.319	
LSD 0.05 A	0.020					0.018				
LSD 0.05 B	0.023					0.027				
LSD 0.05 A*B	0.045					0.054				

Clearly, higher values of such aspect were detected due to supplying the double or triple combined treatments, more than those revealed by single ones, in both seasons. Moreover, the highest values of alkaloids content in the flowers were obtained resulting from the triple combined treatment (Az + B.c + AM), followed by the double combined one (B.c + AM), then the other double combined one (Az + AM), comparing to other treatments and control, in the two seasons. Numerically, the three previous superior treatments increased alkaloids content of flowers by 23.8, 20.4 and by 18.6 %, in the first season and by 29.9, 22.5 and by 18.9 %, in the second one, over control. Accordingly, the data in Table (8) cleared that alkaloids content in borage flowers was positively responded to the interaction treatments in both seasons it seems to be that such parameter was significantly increased as a result of applying the combined treatments, as compared to untreated plants, during the two experimental seasons. Apparently, supplying the plants with the high level of filter mud (210 g/plant) + the inoculation with the triple combined treatments (Az + B.c + AM), followed by the double combined one (B.c + AM), then the double combined one (AZ + AM), then the use of such manure at the moderate level (140 g/plant) plus the inoculation with the triple combined treatments (Az + B.c + AM) proved more effective in elevating alkaloids content than those observed by other combination treatments, during the two consecutive seasons.

3.7 Alkaloids content in the seeds

The recorded data in Table (9) emphasized that the utilization of filter mud as organic manure significantly affected alkaloids content in borage seeds, during the two experimental seasons. Apparently, adding filter mud at all levels, in both seasons, led to a significant augment in alkaloid of seeds, except for the low one (70 g/plant), in the second season, compared to untreated plants. However, such trait was gradually significantly increased with augmenting the levels of filter mud, in both seasons. Therefore, plants grown in organic conditions at the high level (210 g/plant) gave the highest value of alkaloids content of seeds reached 23.3 and 28.4 % over unfertilized ones, during the two seasons, respectively. The important role of organic manures in elevating alkaloids content have been explored by Hassan (2012) on periwinkle, Kurt and Ayan (2014) on tobacco, Fouad (2017) on borage plants.

Table (9): Effect of filter mud, biofertilizers and their interactions on alkaloids content (mg/g d.w.) of seeds during 2021/2022 and 2022/2023 seasons.

Biofertilization treatments (B)	Filter mud g/plant (A)									
	First season					Second season				
	Control	70	140	210	Mean (B)	Control	70	140	210	Mean (B)
Control	0.275	0.300	0.310	0.323	0.302	0.260	0.288	0.312	0.361	0.305
Azotobacter chroococcum (Az)	0.292	0.317	0.335	0.342	0.322	0.268	0.293	0.345	0.366	0.318
Bacillus circulans (B.c)	0.285	0.334	0.353	0.341	0.328	0.286	0.305	0.342	0.369	0.326
Mycorrhiza (AM)	0.310	0.347	0.354	0.366	0.344	0.313	0.313	0.351	0.359	0.334
Az + B.c	0.305	0.333	0.344	0.391	0.343	0.310	0.330	0.340	0.380	0.340
Az + AM	0.331	0.345	0.356	0.414	0.361	0.309	0.321	0.341	0.385	0.339
B.c + AM	0.330	0.350	0.373	0.430	0.371	0.307	0.331	0.357	0.392	0.347
AZ + B.c + AM	0.343	0.352	0.392	0.439	0.381	0.311	0.335	0.389	0.425	0.365
Mean (A)	0.309	0.335	0.355	0.381		0.296	0.315	0.347	0.380	
LSD 0.05 A	0.017					0.022				
LSD 0.05 B	0.026					0.027				
LSD 0.05 A*B	0.052					0.055				

Regarding biofertilization treatments, the presented results in Table (9) proved that the application of the tested microorganisms as biofertilizers positively influenced alkaloids content in borage seeds, during the two successive seasons. It appears that the inoculation with the three microorganisms either single or together resulted a significant increase in such aspect, except for

Azotobacter (Az) treatment, in both seasons and, also Bacillus (B.c) one, in the second season, as compared to uninoculated ones. In most cases, inoculated plants with double or triple combined treatments registered higher values of alkaloids content in the seeds more than those proved by separately ones, in the two seasons. Obviously, the most effective treatments in augmenting such aspect were given by the inoculation with the triple combined treatment (Az + B.c + AM), followed by the double combined one (B.c + AM), then the double combined one (Az + AM) than those obtained by other treatments and control, during the two growing seasons. Numerically, these previous superior treatments elevated alkaloids content in the seeds by 26.2, 22.8 and by 19.5 %, in the first season and by 19.7, 13.8 and by 11.1% in the second one, over uninoculated plants. The beneficial role of *Azotobacter chroococcum* in enhancing alkaloids content was, also described by El-Houssini (2009) on borage, Saleh *et al*. (1998) on datura and, also Hassan *et al*. (2012) on lupine, by Hassan (2012) on periwinkle, regarding mycorrhiza. With respect to the interaction, plants grown in most combined treatments significantly increased alkaloids content in the seeds, as compared to untreated ones, in both seasons. Moreover, the addition of filter mud at the high level (210 g/plant) with the triple combined treatment (Az + B.c + AM) or with any the double combined treatments namely (B.c + AM), (Az + AM) or (Az+ B.C), and also the moderate level of such manure (140 g/plant) + the triple combined treatment (Az + B.c + AM) were more effective in increasing such trait than those obtained by other combination treatments, as clearly shown in Table (9). From the obtained data, it could be discussed as follows: The enhancement in herb dry weight, flowers dry weight, seed yield, fixed oil % and alkaloids content in herb, flowers and seeds of borage due to the addition of filter mud as organic manure, might be attributed to the positive, biological and physiological roles of organic manures which were described by numerous authors like, Reynders and Vilassak (1982) proved that organic manures contain microorganisms namely, Azotobacter and Azospirillium which canable N-fixing and release phytohormones (IAA, GA and cytokinins) which can be augment nutrient absorption, growth and dry matter. Organic manures are the main source of nutritional elements like N, P, and S, as well as, contain high concentrations of B and Mo (Bohn *et al*. 1985). The same previous investigators showed that organic matter is a source of energy for Azotobacter growth. Organic manures are vital role in improve microbial biomass (Dhull *et al*. 2004). Also, Douda *et al*. (2008) indicated that organic manure has a positive alternative to mineral fertilizers for improving soil structure. Inoculating borage plants with the three examined microorganisms namely, *Azotobacter chroococcum*, *Bacillus circulans* and mycorrhiza either alone together resulted an enhance in the studied traits reflect the beneficial roles of these microorganisms which were explained by many authors for examples: As for *Azotobacter chroococcum* bacteria, it can be augment plant nutrient element, particularly N element which acts a major role in the building of amino acids and nucleic acids (Larimi *et al*., 2014; Soliman, 2008), and producing the plant hormones. In addition, it helps to secrete stimulates materials which could be helping grow the roots (Govedarica *et al*., 1997). Moreover, the ability to release phytohormones similar to GA3 and IAA that they can be promote the plant growth traits, nutrient absorption and photosynthesis. (Fayez *et al*., 1985). Concerning the useful role of *Bacillus circulans* bacteria, it is considered as K solubilizing microbes, which could be improve K availability to the plants and their absorption by plant roots and become environment friendly than those utilizing K chemical fertilizers (Padhan *et al*., 2019). *Bacillus circulans* play an important role in silicate solubilizing bacterium which can elevates K availability to crops from insoluble K sources, therefore, lead to enhance the crop yield and reduce the use of chemical fertilizers (Khalil *et al*. 2018; Padhan *et al*., 2019). With respect to the stimulative impact of mycorrhiza fungi, it is a group of soil microorganisms living in very intimate contact with the roots of most plant species. These fungi augmented the roots absorption from surface area which allowed the plants to use the water and nutrients in the soil more efficiency (Rosety *et al*., 2006) Arbuscular mycorrhizal (AM) fungi could be assist the plant in nutrients uptake like, N, P, Zn and Cu, as well as, sometimes of K (George, 2000; Neumann and George, 2005) besides to elevate plant dry weight (Lee and George, 2005). Early flowering and augmenting the number of flowers were observed from plants grown in AM colonization (Gaur and Adholeya, 2005; Nowak, 2004; Usha *et al*. 2005). Furthermore, glutamine synthetase, arginine and urease enzyme activity were influenced by mycorrhiza. It was noted that arginine and urease, which act critical role in the transfer of N from fungus mycelium into the roots of plant (Cruz *et al*., 2007). Mycorrhiza plays an important role in elevating P absorption via the plants by many mechanisms such as, dissolving P soil by organic acids and phosphate enzyme (Chen *et al*., 2001), mycorrhiza mycelium can increase the mobility of P and its strong desire for phosphate ions comparing to the roots of plant (Mukerji and Chamola, 2003) Mycorrhiza not only affecting the plant growth, but also can be influence the production of secondary metabolites in medicinal plants (Toussaint *et al*., 2007). From the revealed results, it could be recommended to supply the sandy soil of borage (*Borage officinalis* L.) plants with filter mud at 210 g/plant and inoculated the plants with biofertilizers namely, *Azotobacter chroococcum, Bacillus circulans* each at 10 m/plant and mycorrhiza fungi *Glomus mossene* NRC

31 and *Glomus fasciculatum* NRC15) at 10 g/plant to enhance herb dry weight, flowers dry weight, seed yield, fixed oil % in the seeds and the contents of alkaloids in the herb, flowers and seeds under the conditions of these studies.

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