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Application of some bio-stimulants combined with mineral nitrogen fertilizer for enhancing growth, yield and quality of brussels sprouts

[1]Abou El- Magd, M.M., M. F. Zaki[1], S. A. Abou Sedera[2] and H. A. Hamouda[3]

[1]Vegetable Research Dept., [2]Agricultural Microbiology Dept., [3]Plant Nutrition Dept., Agricultural & Biological Research Division, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.

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ABSTRACT

Two field experiments were carried out at Nubaria, North West Egypt in winter seasons of 2016-2017 and 2017-2018 to evaluate the effect of three bio-stimulants, i. e. agrispon, azotobacter and microbin combined with two levels of mineral nitrogen fertilizer, i.e. 50 and 100 % of the recommended nitrogen fertilizer on the growth, nutrient content, yield and quality of brussels sprouts.

Results indicated that azotobacter treatment recorded the highest values of vegetative growth expressed as plant height, leaves number, stem diameter, sprouts number as well as fresh weight of leaves, stems, sprouts and total plant, N, P and K content of plant leaves and dry weight of leaves and stems. Lower values of these parameters were obtained by agrispon and the lowest by microbin. Similarly, the highest values of total yield were obtained by azotobacter treatment. Medium values were obtained by agrispon and microbin inoculated plants gave the lowest yield of sprouts. The high level of mineral nitrogen i.e., 100% recorded higher values of vegetative growth, N, P and K content of plant leaves, dry weight of leaves and stem and sprouts yield compared with 50% of the recommended mineral nitrogen. The combined effect of azotobacter with 100% mineral-N recorded the highest values of vegetative growth, N, P and K content of plant leaves, dry matter percentage and total sprouts yield. The lowest values of growth parameters, dry matter percentage and total yield were obtained by bicrobin combined with 50% nitrogen. Other interaction treatments ranged inbetween these two values.

Keywords: brussels sprouts, bio-stimulants, nitrogen fertilizer, growth, yield and quality

Introduction

Brussels sprouts crop is an untraditional vegetable crop in Egypt. It is cultivated in small areas most for research purposes. Great efforts may be paid in all cultivation and marketing programs for spreading the untraditional crops. Planting dates, cultivars, irrigation, fertilization and other growing programs of cruciferous crops in Egypt might be tried in growing brussels sprouts.

The producing areas of late brussels sprouts are restricted by the prevailing temperatures, which determine the suitable planting date, duration of the growing season and growth during this season and sprout formation. In Europe two partly overlapping sprout growing areas can be distinguished. A large area in north-western Europe and a smaller area near the Atlantic Ocean in southern England and western France and in north Italy. Outside Europe sprouts can be grown in parts of USA, Canada, Japan, Australia, South Africa, New Zealand, Chile, Argentina and Paraguay (Kronenberg, 1975). Dietary antioxidants, such as water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids, present in vegetables contribute both to the first and second defense lines against oxidative stress. As a result, they protect cells against oxidative damage, and may therefore prevent chronic diseases, such as cancer, cardiovascular disease. Diabetes-Brassica vegetables, which include different genus of cabbage, broccoli, cauliflower, brussels sprouts and kale, are consumed all over the world (Podsedek, 2007).

Brussels sprouts is a cruciferous crop rich in its mineral content and nutritional value. Sprouts contain also vitamins, provitamins, fats, proteins, amino acids and other anti-oxidant materials. Brussels sprout is an important vegetable species. Some trails were assigned in Rafah, North East Egypt dealing with cultivars and fertilization of brussels sprouts. Abou El-Magd, (2018) evaluated

Corresponding Author: Abou El- Magd, Vegetable Research Dept., Agricultural & Biological Research Division, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.

four cultivars in two planting dates (15 Oct. and 15 Nov.). Results indicated that plants of early half dwarf in the early planting date recorded higher values of total, marketable and unmarketable yields. In addition, higher yields of total sprouts, marketable and unmarketable yields were obtained by Roger cv. in the late planting. The present study was carried out in North, West Egypt to study the combined effects of three bio-stimulants and two mineral fertilization levels on the growth, N, P and K content, dry matter and yield of brussels sprouts.

Materials and Methods

Two field experiments were carried out in the farm of the National Research center at Nubaria, Beheira Governorate, North West Egypt during two winter seasons of 2016-2017 and 2017-2018.

Seeds of brussels sprouts (*Brassica oleracea* Var. Gemmifera L.) were imported from Takii Company in Japan.

Random soil samples were collected before planting from the top layer (0-30 cm depth) for physical and chemical analysis .Soil analysis is presented in Table (1).

Table 1: Physical and chemical properties of the experimental soil during the two seasons of 2016/2017 and 2017/2018.

A. Physical properties

Season	Sand %	Clay %	Silt %	Soil texture
2016/2017	91.20	5.10	3.70	Sandy
2017/2018	92.33	4.78	2.95	Sandy

B. Chemical properties

Season	E.C. (mmohs/cm^3)	pH	OM (%)	$CaCo_3$ (%)	Cations (Meq./L)				Anions (Meq./L)			
					Ca^{++}	Mg^{++}	Na^+	K^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{--}
2016/2017	0.30	7.80	0.30	10.2	1.00	0.20	0.80	0.39	Nil	1.00	1.00	0.39
2017/2018	0.50	7.88	0.50	10.8	1.20	0.26	0.82	0.43	Nil	1.10	1.20	0.41

Seedlings:

Nursery foam trays 208 eyes were filled with media consisting of sand and peat moss 1:1 by volume. Seeds were drilled in the trays one in every eye. Trays were sprayed with irrigation water and arranged together in six vertical layers, then wrapped by plastic sheets and kept three days till seed emergence. Trays were arranged individually on the nursery tables. Recommendations of growing cruciferous transplants were followed till 35 days age and then trays were moved to the open field.

Field preparation:

Ditches 20 cm width and 20 cm depth were built under the locations of drip irrigation lines. Compost manure (5 m3/fed.) was carefully mixed with the recommended dose of calcium super-phosphate and spread along the ditches and covered with sand. Drip irrigation system was built. Irrigation lines were spread over the ditches and irrigation took place three days before planting. Seedlings were transplanted with their peat moss cubes one besides every irrigation eye (50 cm apart). Plot area was 22.5 m2 contains three rows each 10 m. long and 75 cm. width. Drip irrigation lines (transplants rows) were 75 cm. apart. Fertilization program was carefully prepared taking the experimental treatments in consideration. Recommendations of growing cruciferous plants including irrigation and pests, diseases and weeds control programs were followed all over the growth season.

Experimental treatments:

A. Bio-stimulant treatments:

Three bio-stimulant treatments were adopted as follows:-

1) – Microbin 2) – Agrispon 3) – Azotobacter

B. Mineral nitrogen treatments:

Two mineral nitrogen levels:-

1) - 50% of the recommended mineral nitrogen.
2) -100% of the recommended mineral nitrogen. The recommended dose of mineral N was 90 N units/ fed.

Experimental design:

Every experiment included six treatments which were the combinations of three bio-stimulant treatments and two mineral nitrogen levels with three replicates. Experimental design was split-plot design in which bio-stimulant treatments were assigned in the main plots and nitrogen levels were arranged in the sub-plots.

Statistical analysis:

Data of the experiment was arranged and statistically analyzed using M static (M.S.) software (freed 1988). The comparison among means of the different treatments was determined, as illustrated by **Snedecor and Cochran (1982).**

Data recorded:

I. Vegetative growth:

Three plants of every experimental plot were collected and the following measurements were recorded:
1. Plant height (cm).
2. Leaves number/ plant.
3. Stem length (cm).
4. Stem diameter (cm).
5. Leaves fresh weight (g/ plant).
6. Stems fresh weight (g/ plant).
7. Sprouts fresh weight (g/ plant).
8. Total plant fresh weight (g/ plant).

II. Chemical analyses

a) - Nutrients cotent:

Nitrogen, phosphorus and potassium as plant nutrients were determined in plant leaves. The dried leaves samples were wet digested with H2SO4 and analyzed for N, P and K as referred by Cottenie *et al*. (1982). Nitrogen was determined by the modified micro Kjeldah method. Phosphorous was determined colorimetrically by NH4-Metavanidate method. Potassium was estimated Flame-Photometrically.

b)- Dry matter percentage of leaves, stems and sprouts:

Plant organs were separated and a random sample of 100 g of leaves, stems and sprouts of every plot was collected and oven dried at 70^0C. Dry weight of every sample was recorded:
1. Leaves dry weight (%).
2. Stems dry weight (%).

III. Total sprouts yield:

Sprouts of every plant were handly picked at the green mature stage and the following data was recorded:
1. Total yield (ton /fed).
2. Yield increasing (%)

Results and Discussion

A. Effect of bio-stimulants

1. Vegetative growth:

Data presented in Table (2) show clearly that bio-stimulants treatment enhanced the vegetative growth of brussels sprouts plants expressed as plant height, leaves number, stem height and stem diameter, sprouts number and mean weight of the sprout per plant as well as fresh weight of leaves, stems, sprouts and total plant. Vegetative growth parameters differed statistically according to bio-stimulant treatments. Brussels sprouts plants treated with azotobacter recorded the highest values of vegetative growth compared with agrispon and microbin. Vegetative growth expressed as plant height, leaves number, stem diameter, sprouts number and mean weight of the sprout per plant as well as fresh weight of leaves, stems, sprouts and total plant recorded their highest values by azotobacter followed by agrispon treatment. The lowest values of vegetative growth of brussels sprouts plants were obtained by microbin treated plants. Differences in stem height and sprouts number between these treatments were not significant. Treating brussels sprouts plants with azotobacter or agrispon led to significant increases in the vegetative growth expressed as plant height, leaf numbers and stem diameter as well as fresh weight of leaves, stems, sprouts and total plant. On the other hand, increases in stem height and sprouts number per plant were not significant. Increases in the vegetative growth characters due to azotobacter treatment were higher than those due to agrispon in comparison .The lowest values were those of microbin treatment.

These results were similar in the two seasons of the experiment. Ertan Yildirim *et al*. (2011); Zaki *et al*., 2012; Abou El-Magd *et al*., 2014; Abou El-Magd *et al*., 2015; Hanaa, 2016 on boccoli and Abou El-Magd *et al*., 2018 on chinese cabbage reported that bio-fertilization increased vegetative growth of the inoculated plants. Many investigators reported that bio-fertilization increased growth of broccoli plants and other cruciferous vegetables crops (Chaterjee *et al*., 2005 on broccoli; Singh and Singh, 2005 on cauliflower). Similar results were recorded by Wange and Kale (2004) on broccoli since they reported the growth response of broccoli to 12 different treatments of bio-fertilizers (Azotobacter and Azospirillum) and N level (100, 125 and 150 kg/ha). Manivannan and Singh (2004) demonstrated that the effect of bio-fertilizers namely *Azospirillum* sp. and *Azotobacter* sp. applied at 5 and 10% on the growth and yield of sprouting broccoli gave the maximum vegetative growth in plants applied with 5% *Azotobacter* sp. Hanaa (2016) reported that the increase in vegetative growth due to bio-fertilization could be attributed to the role of inoculation by micro-organisms in reducing soil acidity by secreting organic acids such as acetic, propionic, succinic and fumaric acids. These secretions resulted in conversion of some insoluble nutrient into available soluble forms (Singh and Kapoor, 1999 and Osman, 2007). In addition, the increase of vegetative growth by the inoculated plants might be attributed to secretion of some growth promoters such as gibberellins, auxins and cytokines by these micro-organisms (Ibrahim and Abdel-Aziz, 1977 and Abdel- Latif *et al*., 2001).

II. Chemical contents

a) Nutrients content:

N, P and K percentage in the leaf tissues were statistically influenced by the application of the different bio-stimulants (Table 3). Higher N, P and k percentages were recorded in the leaves of the plants treated by azotobacter. Lower N, P and K percentages were recorded in the leaves of the plants treated with microbin. The lowest percentages of N, P and K were recorded by agrispon. Azotobacter treatment created higher availability of nutrients around the root system. These conditions facilitates water and nutrients uptake from the soil solution. Translocation of water and nutrients to the aerial parts became faster. Consequently, N, P and K concentrations became higher in the leaf tissues.

b) Dry matter percentage:

Dry matter percentage in leaves and stems of brussels sprouts varied between bio-stimulants treatments (Table 2). Dry matter percentage of leaves and stems followed the same trend of the fresh weight. Azotobacter inoculated plants recorded the highest values of dry matter percentage followed by those of agrispon treatment. The lowest values of dry matter percentage were recorded in leaves and stems by microbin treatment. Results indicated that dry matter accumulation in leaves and stems was higher when the plants were inoculated by azotobacter. These results were similar and true in the two seasons. Zaki *et al*., 2012; Abou El-Magd *et al*., 2014; Hanaa, 2016 on broccoli and Abou El-Magd *et al*., 2018 on chinese cabbage reported that bio-fertilization increased dry matter content of broccoli plants.

Table 2: Effect of bio-stimulants on vegetative growth of Brussels sprouts plants during the two seasons of 2016/2017 and 2017/2018.

Bio-stimulants	Plant height (cm)	Leaves No./plant	Stem height (cm)	Sprouts No/plant	Sprouts Weight (g/plant)	Fresh weight (g / plant) Leaves	Stems	sprouts	Total	Dry weight (%) Leaves	Stems
					First season (2016/2017)						
Microbin	29.17	85.00	23.25	9.70	120.58	241.63	99.67	130.67	471.96	18.96	15.95
Agrispon	33.17	84.00	23.58	9.13	144.25	260.45	161.83	155.47	577.75	20.66	18.25
Azotobacter	35.75	89.83	24.00	9.82	163.88	264.70	199.17	168.05	631.92	20.71	24.02
L.S.D at 0.05	0.58	3.35	N.S.	N.S.	3.23	12.75	5.77	5.77	17.97	1.03	1.38
					Second season (2017/2018)						
Microbin	30.37	86.08	21.42	11.20	123.92	249.30	104.00	133.83	487.13	20.21	17.37
Agrispon	35.20	85.75	22.08	10.47	147.75	268.95	165.33	160.30	594.58	21.91	19.50
Azotobacter	34.87	91.58	22.50	11.15	166.55	274.04	203.50	173.03	650.57	21.96	24.77
L.S.D at 0.05	0.66	2.12	N.S.	N.S.	3.64	1.72	4.12	5.55	7.05	1.03	1.02

Table 3: Effect of bio-stimulants on sprouts yield, physical quality of sprouts and nutrients concentrations in the leaves of Brussels sprouts plants during two seasons of 2016/2017 and 2017/2018.

Bio-fertilizers	Sprouts yield (ton/fed.)	Increases (%)	Physical sprouts quality Stem diameter (cm)	Mean weight of sprout (g)	Nutrients concentration in leaves N (%)	P (%)	K (%)
				First season (2016/2017)			
Microbin	2.154	0.000	2.90	13.95	1.98	0.48	1.90
Agrispon	3.113	0.959	2.97	17.72	1.78	0.39	1.78
Azotobacter	3.224	1.070	3.12	18.50	2.12	0.52	1.95
L.S.D at 0.05	0.292	---	0.09	2.10	0.11	0.01	0.09
				Second season (2017/2018)			
Microbin	2.320	0.000	3.08	10.57	1.93	0.49	2.00
Agrispon	3.263	0.943	2.81	13.67	1.77	0.41	1.87
Azotobacter	3.540	1.220	3.15	14.31	2.03	0.51	2.12
L.S.D at 0.05	0.204	---	0.04	0.81	0.08	0.02	0.10

III. Total sprouts yield:

Data in Table (3) show clearly that the total yield of sprouts was significantly increased by bio-fertilization. Inoculating brussels sprouts plants by azotobacter, agrispon or microbin enhanced yield of sprouts. The highest values of total yield were obtained by azotobacter treatment. Medium values of total sprouts yield were obtained by agrispon. Microbin inoculated plants yielded the lowest sprouts yield. agrispon and azotobacter treatments outyielded those of microbin by 0.959, 1.070 ton/fed. in the first season and 0.943, 1.220 ton/ fed. in the second season. These increases amounted to 44.6, 49.7% and 40.6, 52.6% due to agrispon and azotobacter treatments, respectively compared with microbin in the two successive seasons of the experiment.

Many investigators indicated that bio-fertilization led to significant increases in the total yield of cruciferous plants (Zaki *et al*., 2012; Abou El-Magd *et al*., 2014; Hanaa, 2016 on broccoli and Abou El-Magd *et al*., 2018 on chinese cabbage). Total sprouts yield was positively enhanced by bio-stimulants. The highest and significant value of total sprouts yield was obtained by azotobacter treatment. These results might be due to the high content of N, P and K. in plant Leaf which was the highest with azotobacter treatment followed by microbin and agrispon in a descending order. Total yield of sprouts was parallel with the leaf content of N, P and K. In addition, total sprouts yield followed the same trend of the vegetative growth and dry matter accumulation. These increases may be due to the role of azotobacter, microbin, and agrispon in producing GA3, IAA and cytokinins within the plant tissues .These compounds act as growth promoters which stimulate vegetative growth and dry matter accumulation. Hanaa, 2016 reported that Bio-fertilization induced nutrients uptake enhancement in treatment (azotobacter, agrispon and microbin). This enhancement may be due to many reasons such as root system efficiency improvement, rhizosphere pH reduction and increase nutrients dissolving and availability in the soil which encouraged photosynthetic activity of the treated plants. Enhancing photosynthesis activity caused more different metabolic substances production. Consequently, more dry matter accumulation in plant tissues leading to higher sprouts yield.

These results come along with those obtained by Ertan Yildirim *et al*. (2011) who reported that root inoculations of broccoli plants with *Bacillus cereus*, *Brevibacillus reuszeri*, and *Rhizobium rubi* increased total heads yield compared to the non-inoculated plants. Zaki *et al*. (2012) mentioned that higher main, secondary and total heads yields were obtained by the plants inoculated by *Bacillus circulans*; *Bacillus megaterium* and *Azotobacter chroococcum* compared with the control. Abou El-Magd *et al*. (2013) showed that heads yield was significantly increased by phosphorine application as compared with the check. Also, Abou El-Magd *et al*. (2014) recorded that broccoli plants treated with bio-nitrogen had higher main head yield than non-inoculated plants Singh *et al*. (2014) mentioned that inoculating seedlings with Azospirillum + Azotobacter significantly increased total heads yield of broccoli compared to other treatments. In addition, Abou El-Magd *et al*., 2018 came to similar results. They reported that chinese cabbage yield was enhanced by bio-fertilization.

B. Effect of mineral nitrogen:

1. Vegetative growth:

Data presented in Table (4) showed that the increase in nitrogen level caused significant increases in the vegetative growth of brussels sprouts plants. Increasing nitrogen level from 50% to 100% increased plant growth expressed as plant height, leaves number, stem height and stem diameter as well as fresh weights of leaves, stems, sprouts and total plant. These increases are true and consistent in the two seasons of the experiment. Zaki, *et al*, 2016 working on broccoli came to similar results. Other studies reported that mineral nitrogenous fertilization increased plant growth (Zaki *et al*., 2012; Saad *et al.,* 2013; Zaki *et al*., 2012; Hanaa, 2016 and Abou El-Magd *et al*., 2018). Increases in the vegetative growth by nitrogen applications might be due to its role in photosynthesis. Nitrogen is a main component of cell protoplasm. It is also the principal component of protein molecule. It is clear that nitrogen is important in protein synthesis and plays an important role in the cell enlargement and cell division and consequently on plant growth. Nitrogen also plays a great role in photosynthesis and in turn in the condensation of its products through plant tissues.

II-Chemical content

a) Nutrients content:

N, P and K percentages in leaf tissues were enhanced by the nitrogenous fertilization (Table 5). Higher N, P and K concentrations in leaf tissues were recorded by the high level of nitrogenous fertilization (100% of the recommended dose). The low level of nitrogenous fertilization recorded lower N, P and K concentrations in the leaf tissues. These results might be due to the high availability of nitrogen in the soil solution which enabled plants higher absorption of soil solution (water and nutrients). The higher absorption and translocation rate of water and nutrients increased N, P and K concentrations in the leaf tissues. In addition N, P and K are in a balance state in the soil solution. So that, the increase in nitrogen availability, translocation and concentration in the leaf tissues was accompanied with increases in P and K concentrations. The resulting state created favorable conditions for increasing N, P and K concentrations in the leaf tissues.

b) Dry matter percentage:

Dry matter accumulation was increased in the leaves and stems of brussels sprouts plants by nitrogenous fertilization (Table 4). Higher percentage of dry matter was recorded when the plants received 100% of the recommended mineral nitrogen. Low dry matter accumulation resulted by the low level of nitrogenous fertilization. These results might be due to the resulting increase in N, P and K concentrations in the leaf tissues which led to higher photosynthetic activity. These favorable conditions increased net assimilation rate and relative growth rate leading to higher photosynthetic products and higher carbohydrates, proteins and fats. Consequently higher dry matter accumulation might be expected. Many investigators came to similar results (Zaki *et al*., 2012; Zaki *et al*., 2016; Hanaa, 2016 and Abou El-Magd *et al*., 2018).

III. Total sprouts yield:

Data in Table (5) showed that mineral nitrogen fertilization significantly enhanced total sprouts yield of brussels sprouts crop. Increasing mineral nitrogen applications from 50% to 100% of the recommended dose resulted higher sprouts yield increases. Application of the full recommended dose increased total sprouts yield up to 1.810 and 1.892 in the first and second seasons, respectively compared with 50% level. These increases resemble 101.1% and 90.3% in the two respective seasons.

Many investigators reported that increasing nitrogenous applications increased total yield of cruciferous crops. Abou El-Magd *et al*., 2010 and Saad *et al*. (2013) reported similar results. In addition, Abou El-Magd *et al*., 2015; Zaki *et al*., 2016; Hanaa, 2016 and Hanaa *et al*., 2016 on broccoli crop and Abou El Magd *et al*., 2018 on chinese cabbage reported that mineral nitrogen applications increased total yield of the tested crops. The increased levels in nitrogen fertilizer corresponds increases in N, P and K concentration in the leaf tissues. Consequently, higher photosynthetic activities, net assimilation rate and relative growth rate reflected increases in vegetative growth of brussels sprouts plants. Increases in the total yield might also be due to more nutrients availability that improves the absorption of nutrients and water, also increased photosynthetic surfaces by increasing leaves number and leaf area as well as fresh weight and chlorophyll content which allows better photosynthetic capacity and dry matter accumulation .Since the total yield of sprouts is the summation of all these increases, higher yields were obtained by the high levels of nitrogen fertilizer.

C. Effect of the interaction

1. Vegetative growth:

Interaction of bio and mineral nitrogenous fertilization affected vegetative growth of brussels sprouts (Table 6). The combined effect of bio-stimulants and mineral nitrogenous rates enhanced vegetative growth expressed as plant height, leaves number, stem height, stem diameter, and fresh weight of leaves, stems, sprouts and total plant. The highest values of these parameters were obtained by the combined effect of azotobacter and 100% recommended dose of mineral nitrogen fertilizer. On the other hand, the lowest values of growth parameters were obtained by the combined effect of microbin with 50% mineral nitrogen. The other interaction treatments ranged in between these two

Table 4: Effect of N fertilization on vegetative growth of Brussels sprouts plants during two seasons of 2016/2017 and 2017/2018.

Nitrogen rates (%)	Plant height (cm)	Leave No./ plant	Stem height (cm)	Sprouts No/ plant	Sprouts Weight (g/plant)	Fresh weight (g / plant) Leaves	Fresh weight Stems	Fresh weight sprouts	Fresh weight Total	Dry weight % Leaves	Dry weight % Stems
					First season (2016/2017)						
50	30.78	77.78	24.67	6.61	82.11	187.85	143.67	86.46	417.97	21.46	18.37
100	34.61	94.78	22.56	12.49	203.70	323.34	163.44	216.33	703.12	18.76	20.44
L.S.D at 0.05	1.05	2.72	1.08	1.84	5.56	10.31	4.87	2.06	9.84	0.86	0.67
					Second season (2017/2018)						
50	31.48	79.75	22.94	7.89	85.50	196.90	147.72	92.00	436.62	22.71	19.28
100	35.48	95.86	21.06	13.99	206.64	331.28	167.50	219.44	718.23	20.01	21.81
L.S.D at 0.05	0.67	0.97	0.51	0.41	2.19	1.67	3.38	2.83	6.01	0.86	0.69

Table 5: Effect of mineral nitrogen levels on sprouts yield, physical quality of sprouts and nutrients concentrations in the leaves of Brussels sprouts plants during the two seasons of 2016/2017 and 2017/2018.

Nitrogen rates (%)	Sprouts yield (ton/fed.)	Increases (%)	Physical quality of sprouts Stem Dia. (cm)	Physical quality of sprouts Mean weight of sprout (g)	Nutrients concentration N (%)	Nutrients concentration P (%)	Nutrients concentration K (%)
				First season (2016/2017)			
50	1.790	0.000	2.70	14.39	1.88	0.51	1.82
100	3.871	1.810	3.29	19.06	2.04	0.41	1.93
L.S.D at 0.05	0.297	0.00	0.03	1.31	0.06	0.02	0.08
				Second season (2017/2018)			
50	2.095	0.000	2.79	10.77	1.80	0.49	1.82
100	3.987	1.892	3.23	14.93	2.02	0.45	2.17
L.S.D at 0.05	0.076	0.00	0.06	0.56	0.14	0.01	0.10

Table 6: Effect of the interaction (Bio-nitrogen x Nitrogen level) on vegetative growth of Brussels sprouts plants during two seasons of 2016/2017- and 6017/2018.

Bio-stimulants	Nitrogen rates (%)	Plant height (cm)	Leave No./ plant	Stem height (cm)	Sprouts No/ plant	Sprouts Weight (g/plant)	Fresh weight (g / plant) Leaves	Stems	Sprouts	Total	Dry weight (%) Leaves	Stems
First season (2016/2017)												
Microbin	**50**	27.00	80.67	23.17	5.50	60.17	203.07	88.00	60.17	351.23	20.68	15.00
	100	31.33	89.33	23.33	13.90	181.00	280.19	111.33	201.17	592.69	17.24	16.90
Agrispon	**50**	31.33	71.67	22.83	7.00	90.73	176.72	160.33	95.43	432.49	22.59	16.23
	100	35.00	96.33	24.33	11.27	197.77	344.17	163.33	215.50	723.00	18.73	20.27
Azotobacter	**50**	34.00	81.00	28.00	7.33	95.43	183.75	182.67	103.77	470.18	21.10	23.87
	100	37.50	98.67	20.00	12.30	232.33	345.66	215.67	232.33	793.66	20.32	24.17
L.S.D at $_{0.05}$		N.S.	4.31	0.96	0.55	4.16	16.44	7.44	7.44	23.16	1.32	N.S.
Second season (2017/2018)												
Microbin	**50**	28.03	83.08	21.00	7.00	63.33	211.57	93.17	65.83	370.57	21.93	16.25
	100	32.70	89.08	21.83	15.40	184.50	287.03	114.83	201.83	603.69	18.49	18.48
Agrispon	**50**	34.03	73.42	21.33	8.17	94.23	185.22	163.83	101.10	450.16	23.84	17.48
	100	36.37	98.08	22.83	12.77	201.27	352.67	166.83	219.50	739.00	19.98	21.52
Azotobacter	**50**	32.37	82.75	26.50	8.50	98.93	193.92	186.17	109.07	489.15	22.35	24.12
	100	37.37	100.42	18.50	13.80	234.17	354.16	220.83	237.00	811.99	21.57	25.42
L.S.D at $_{0.05}$		0.85	2.74	1.23	0.96	4.70	2.22	5.31	7.15	9.08	1.32	N.S.

interaction treatments. These results were similar in their trend in the two seasons. It was concluded that bio-stimulants and mineral rates act dependently on these parameters.

The combined effect of bio-stimulants and mineral nitrogen created favorable conditions for water and nutrients absorption. Consequently, increases in the leaf content of nutrients. These increases enhanced photosynthetic activity, net assimilation rate and relative growth rate reflecting higher vegetative growth in brussels sprouts plants. Zaki *et al*., (2016) came to similar results with broccoli crop. Generally, they reported that, the highest values of vegetative growth of broccoli plants such as plant height, leaves number as well as fresh and dry weight of leaves, stems and apical heads were obtained by inoculating plants with microbin when fertilized by 50% N recommended dose + one foliar spray of amino magnical. Other interaction treatments ranged in-between these two treatments. Hanaa, 2016 reported that combined effect of bio-fertilization (*Azotobacter chrococcum*+ *Arbiscular mycrrohrzae*) with application of 75% mineral + 25% organic of the recommended fertilizer units produced the highest values of all vegetative growth characteristics of broccoli plants compared to the other interactions. Increased vegetative growth of broccoli plants may be referred to the combined effect of bio-fertilizer and 75% mineral + 25% organic of the recommended fertilizer units which maintains and promotes productivity by soil fertility adjustment, micro-organisms activity improvement, inducing more plant nutrient supply (Jen *et al*., 2008).

II. Chemical content

a) Nutrients content:

Higher values of N, P and K percentages in leaf tissues were obtained by the combined effect of bio-stimulants and mineral nitrogen (Table 7). The highest values of N, P and K percentages were obtained by the combined effect of azotobacter treatment and 100% recommended nitrogen. These values did not reach the level of significance for N and K in the first season. These increases might be due to the abundant nutrients in the soil solution. Consequently, the higher uptake of water and nutrients by the root system. Increases in N, P and k in plant leaves might also be due to the enhanced translocation of water and nutrients from the root system to the aerial parts. These favorable conditions increased the leaf content of N, P and K nutrients.

b) Dry matter percentage:

Data in Table (7) recorded significant increases in the dry weight percentage due to the interaction of bio-stimulants and mineral nitrogen fertilization. Leaves and stems dry matter percentage of brussels sprouts plants recorded their highest values by the combined effect of azotobacter treatment and 100% of the recommended mineral nitrogen. The lowest dry matter percentage was recorded by microbin combined with 50% of the recommended mineral nitrogen. Other interaction treatments ranged inbetween these two values. Increased vegetative growth of broccoli plants may be referred to the combined effect of bio-stimulants and 75% mineral + 25% organic of the recommended fertilizer units which maintains and promotes productivity by soil fertility adjustment, microorganisms activity improvement, inducing more plant nutrient supply (Jen *et al*., 2008).

III. Total sprouts yield:

Total yield of sprouts recorded its highest levels by the combined effect of azotobacter with 100% of the recommended level of mineral nitrogen (Table 7). The lowest sprouts yield was recorded by combining microbin with 50% mineral nitrogen level. Other interaction treatments yielded values of total yield ranging inbetween these two values. Results of total sprouts yield followed the same trend of vegetative growth, leaf nutrients content and dry matter accumulation. Since total yield is the function of the vegetative growth and dry matter accumulation, the expected results might be due to these parameters. These results are similar and true in the two seasons of the experiment. Azotobacter combined with 100%mineral N recorded an increase amounted to 3.349 and 3.35 ton/fed. This increase equals 409.4 and 330.8% compared with microbin with 50% mineral N.

Hanaa, 2016 came to similar results. She reported that the highest value of total heads yield of broccoli was recorded by application of 75% mineral + 25% organic of the recommended fertilizer units. Integrated application of biological, mineral and organic fertilizers led to the highest content of

Table 7: Effect of the interaction (Bio-stimulants x mineral N level) on sprouts yield, physical quality of sprouts and N, P and K concentrations in the leaves of Brussels sprouts plants during two seasons of 2016/2017 and 2017/2018.

Bio-fertilizers	Nitrogen rates (%)	Sprouts yield (ton/fed.)	Increases (%)	physical quality of sprouts: Stem Dia. (cm)	Mean weight of sprout (g)	Nutrients concentration in leaves: N (%)	P (%)	K (%)
				First season (2016/2017)				
Microbin	**50**	0.818	0.000	2.67	12.72	1.87	0.52	1.87
	100	3.489	2.671	3.13	15.18	2.10	0.44	1.93
Agrispon	**50**	2.270	2.452	2.60	15.28	1.73	0.39	1.70
	100	3.956	3.138	3.33	20.15	1.82	0.38	1.87
Azotobacter	**50**	2.280	1.462	2.83	15.16	2.03	0.63	1.90
	100	4.167	3.349	3.40	21.85	2.20	0.41	2.00
L.S.D at $_{0.05}$		0.376	0.000	0.11	N.S.	N.S.	0.01	N.S.
				Second season (2017/2018)				
Microbin	**50**	1.002	0.000	2.83	9.11	1.77	0.50	1.73
	100	3.639	2.637	3.33	12.03	2.10	0.48	2.27
Agrispon	**50**	2.520	1.518	2.68	11.56	1.73	0.37	1.67
	100	4.006	3.004	2.93	15.78	1.80	0.45	2.07
Azotobacter	**50**	2.763	1.761	2.87	11.65	1.90	0.60	2.07
	100	4.317	3.315	3.43	16.97	2.17	0.42	2.17
L.S.D at $_{0.05}$		0.263	0.000	0.06	N.S.	0.10	0.02	0.13

total, main and secondary heads yield compared to the other treatments, which may be due to the induced enhancement of soil fertility and nutrient supply. Sharma *et al*. (2008) found similar results. They recorded the highest values of apical and lateral heads weight of broccoli when applying Azotobacter with recommended practice (100% NPK+20 t/ ha of cow manure) compared to the recommended practice (100% NPK+20 t/ha of cow manure) without bio-fertilization.

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