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Ameliorating Growth Performance and Active Compounds of Moringa Plant by Integrated Nutrients Management

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ABSTRACT

Two pot experiments were carried out during growth seasons of 2016 and 2017 at the experimental station of faculty of agriculture, Benha University. The investigation target was to find out the effect of using plant growth promoting rhizobacteria either PGPR-group (A) containing 7×10^9 cell suspension of each (*Azo. lipoferium* D178, *B. megaterium* ATCC14581 and *B. circulans* ATCC4513) or PGPR-group (B) containing 7×10^9 cell suspension of each (*A. chroococcum* EMCCN1458, *B. megaterium* ATCC14581 and *B. circulans* ATCC4513) individually or in combination with mineral fertilization rates (25, 50, 75 and100%) NPK on the vegetative growth characteristics and chemical constituents of *Moringa oleifera* plant at 75 and 150 DAS. The combination of plant growth promoting rhizobacteria PGPR group (A) and inorganic fertilizers treatments gave the highest values of dehydrogenase (DHA), alkaline phosphatase (ALP) and nitrogenase (N_2-ase) activities as compared with each one individually. In all treatments, enzymes activities were increased to reach the maximum values after 90 DAS. The highest values of enzymes activity recorded for treatment which inoculated with PGPR group (A) and amended with ¾ of mineral fertilizers followed by treatment which inoculated with PGPR group (B) amended with ¾ mineral fertilizers. Vegetative growth measurements, photosynthetic pigments, promoting endogenous phytohormones, total phenolics, flavonoids and ascorbic acid contents as well as the highest values of antioxidants activity were recorded for treatment that inoculated with PGPR group (A) amended with ¾ of mineral fertilizers followed by treatment that inoculated with PGPR group (B) amended with ¾ mineral fertilizers, respectively compared to mineral fertilization. Abscisic acid was reduced with different applied treatments as compared to the control, but the reduction was more obviously clear for treatment of PGPR group (B) amended with ½ mineral fertilizers. While, salicylic acid was increased with the different tested treatments compared with the control and reached its maximum values with PGPR group (B) amended with ¾ mineral fertilizers and PGPR group (A) with ¾ mineral fertilizers treatments, respectively.

Keywords: *Moringa oleifera*, Rhizobacteria, PGPR, Nitrogenase, Phosphatase, Dehydrogenase, Vegetative growth, Phytohormones and Antioxidants activity

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INTRODUCTION

Moringa oleifera is the most widely cultivated specie of family Moringaceae, order Brassicales according to Fahey (2005).

All organs of the moringa plant are edible but the root which is used as a condiment in the same way as horseradish contains the alkaloid spirochin, a potentially fatal nerve paralyzing agent as concluded by Foidl *et al.*, (2001) and Orwa *et al.*, (2009). The entire plant is of high protein, vitamins, mineral and carbohydrate contents, high value of nutrition to both of humans and livestock. Seeds have high oil content (42%) which edible and with medicinal uses according to Foidl *et al.*, (2001) and Abdulkarim *et al.*, (2005). It has naturalized in many locations in the tropics and widely cultivated in Africa Fahey (2005).

With the increase of population pressure, cost, side effects and development of resistance to allopathic drugs for infectious diseases, uses of medicine of plant sources for a wide variety of human ailments are increasing. So, large scale production of medicinal plants using modern cultivation technologies is being practiced across Asian countries to meet the high demand of such plants. The pests and diseases of plant are hampering growth and quality of medicinal plants. In addition, excessive use of pesticides may degrade the medicinal plant products quality. Thereby, the development of innovative technologies for medicinal plants cultivation is required as reported by Egamberdieva *et al.*, (2013).

Plant growth promoting rhizobacteria (PGPR) are bacteria colonizing plant rhizosphere which enhancing plant growth during many mechanisms i.e., Nitrogen fixation, Phosphate solubilization, quorum sensing and so on as mentioned by Bhattacharya and Jha (2012). PGPR offer several ways for replacing chemicals fertilization, pesticides and so on therefore, increasing quality has significantly resulted in their increases demand.

The medicinal plants quality i.e., active compounds content is largely influenced by abiotic as well as biotic rhizosphere factors. The rhizosphere microbes play important roles in improving medicinal plants value. The roles of microbes for plant growth, availability of nutrients, diseases resistance as well as yield quality of medicinal plants are investigated. Inoculation with PGPR and AM fungi represents a sustainable technology for enhancing quantity and quality of the components of medicinal plants. However, selection and inoculation with efficient bacteria or fungi for a particular plant is necessary for medicinal plants cultivation as reported by Egamberdieva *et al.*, (2013).

The objective of this study is to evaluate and understand the mechanisms of plant growth promotion by rhizobacteria as well as ameliorate growth performances and active compounds of moringa plant using bioinoculants of PGPR combined with mineral fertilizer levels. Hence, integrated nutrients management program of fertilization for moringa production and its active compounds could be achieved.

MATERIALS AND METHODS

Bacterial strains and plants

The PGPR inocula (*Azotobacter chroococcum* EMCCN1458, *Bacillus megaterium*, *Bacillus circulans* and *Azospirillum. lipoferium* D178 were obtained from Microbial Resource Center (MIRCEN) Cairo, Egypt.

Moringa oleifera seeds were obtained from Agricultural National Research Center, Giza, Egypt.

Experimental design: Pot experiments were carried out on the 5th of May to 5th of October, during the two growing successive seasons of 2016 and 2017 in Greenhouse at Faculty of Agriculture, Benha University, Qalyoubia, Egypt.

Plastic pots 30cm diameter were filled with a mix of 6 kg clay loam soil of pH 8.04, organic matter 3.5%, bulk density 1.36 g cm^{-3}, field capacity 52.6% and wilting point 16.95% as well as 100 g compost [herbal plant residues and cattle manure (50:50) are: pH 7.6, electrical conductivity (EC) 2.7 dS m^{-1}, organic matter values 31.7, N 1.9 and P 0.91% with a porosity 62.67%]. Prior to *Moringa oleifera* sowing, seeds were divided into three groups, two were coated by dipping seeds in mixture of 20% Arabic gum as an adhesive and inoculation with either PGPR group (A) which containing 7×10^9 cell suspension of each (*Azo. lipoferium* D178, *B. megaterium* ATCC14581 and *B. circulans* ATCC4513) or PGPR group (B) which containing 7×10^9 cell suspension for each of *A. chroococcum* EMCCN1458, *B. megaterium* ATCC14581 and *B. circulans* ATCC4513 for 30 min before sowing and the control (full dose of N, P and K mineral fertilizers only). Seeds were sown in each pot (2:3 seeds pot^{-1}) and nine experimental treatments with five replicates for each were arranged in a randomized complete block design as in the following setup (Table 1):

Table 1. Experimental design

Treatments	Description
T1	Control (full dose of mineral fertilizers i.e., N, P and K)
T2	Biofertilizer only (group A)
T3	Biofertilizer (group A) + ¼ dose of mineral fertilizers
T4	Biofertilizer (group A) + ½ dose of mineral fertilizers
T5	Biofertilizer (group A) + ¾ dose of mineral fertilizers
T6	Biofertilizer only (group B)
T7	Biofertilizer (group B) + ¼ dose of mineral fertilizers
T8	Biofertilizer (group B) + ½ dose of mineral fertilizers
T9	Biofertilizer (group B) + ¾ dose of mineral fertilizers

Moringa plant was weakly irrigated and the PGPR strains were applied at 30, 60 and 90 DAS. Chemical fertilizers were supplemented in pots with the assigned rats of the proposal experimental treatments; full recommended dose is 54 kg N / fed. as ammonium sulfate 20.5 % N, 25 kg P_2O_5 / fed. as calcium super phosphate 15.5 % P_2O_5 and 35 kg K_2O / fed. as potassium sulfate 48 % K_2O. It looks to be true that the activity of dehydrogenase (DHA), alkaline phosphatase (AlP) as well as nitrogenase (N_2-ase) were determined at 0, 30, 60, 90, 120 and 150 DAS. After 75 and 150 DAS, plant samples were collected for measuring different growth characteristics as well as chemical constituents.

Measurements of microbial enzymatic activity

Dehydrogenase and alkaline phosphatase activities were determined using spectrophotometer at 464 and 400 nm, respectively according to Schinner *et al.*, (1997). Whereas, nitrogenase activity was determined according to Okafor and MacRae (1973).

Morphological and chemical compositions measurement

Plant height (cm), stem diameter(cm), no. of leaves $plant^{-1}$, root, stems and leaves fresh as well as dry weights $plant^{-1}$, total dry weight and total leaf area $plant^{-1}$ were estimated at 75 and 150 (DAS) according to Deriaux *et al.,* (1973). Chlorophyll A, B as well as carotenoids colorimetrically measured in moringa leaves at 75 and 150 DAS using method of Wettestein (1957). Endogenous Phytohormones i.e., IAA, GA3, salicylic acid as well as abscisic acid were determined in moringa shoots at 150 DAS using high performance liquid chromatography as described by Koshioka *et al.*, (1983). Cytokinin was measured by HPLC as described by Nicander *et al.*, (1993). The antioxidants activity was determined according to method of Lee and Lee (2004).

Ascorbic acid content mg g^{-1} fresh weight was determined as described by A.O.A.C. (1990). Total phenolic compounds content was spectrophotometrically determined according to Shen *et al.*, (2009) and total flavonoids was estimated according to Mohdaly *et al.,* (2012) in moringa leaves at 75 and 150 DAS during 2017 season.

Statistical analysis

Results data were statistically analyzed and the means were compared using the Least Significant Difference test (L.S.D) at 5% level according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Soil microbial enzyme activities

Nitrogen fixation by *Rhizobacteria* is considered one of the most beneficial processes. Nitrogen is a vital nutrient for plant and it is not available due to the high energy required for breaking the triple bond between the two atoms. *Rhizobacteria* during nitrogen fixation were able to convert gaseous nitrogen (N_2) to ammonia (NH_3) that makes it available to the host plant, thereby supporting and enhancing plant growth Cain *et al.*, (2011).

Phosphorus is the second important mineral for plant. It exists in soil as mineral salts or incorporated into organic compounds. Despite phosphorus compounds being abundant in agricultural soils, the majority of them exist in an insoluble form Miller *et al.*,

(2010). Moreover, phosphate solubilization using plant associated bacteria considered one of the major mechanisms for promoting plant growth. This involves bacteria producing phosphatase and releasing organic acids into the soil, thereby solubilizing the phosphate complexes and converts them to orthophosphate that it is available to plant uptake.

The PGPR tend to have strong effects when applied with mineral fertilizers. The combination of PGPR and mineral fertilizers recorded higher values of DHA, ALP and N_2-ase activities compared with each one individually (Tables 2, 3 and 4). With all treatments, DHA, ALP as well as N_2-ase activities were increased from the beginning to reach the highest values after 90 DAS. That means, in the beginning microbial enzymes activity did not active enough and that may be attributed to small plant age which accompanied by a few root exudates. Moreover, PGPR strains need some time for colonizing plant root and attaining significant population on the host root system before being ready for beginning its activities. On the 90th DAS, the activity was reached its maximum values, since this may be attributed to enough nutrients from root exudates and root debris which represent nutritional substances to different soil microorganisms. This may be due to the effect of PGPR boost inoculation in supporting its colonization on plant root. After 90 DAS, such activities were decreased again, since this may be attributed to the nutrients shortage in the rhizosphere or the complete decomposed organic fertilizer or effects of rhizosphere on soil microbial enzymes activity. Moreover, intracellular enzymes activity was short lived, since they were degraded by protease unless they were adsorbed by clay or immobilized by humic molecules, Burns and Dick (2002).

In the same order, data in Tables (2, 3 and 4) show that all applied treatments which inoculated with biofertilizer PGPR group (A) exerted higher values of the estimated microbial enzymes activity than that inoculated with PGPR group (B). These results may be due to the effect of *Azospirillum* strain which has specific mechanisms for interacting with roots and evenly colonizing of the root interior. This endophytic living could mean that the N_2 fixed may have been principally transferred by direct and rapid transfer as occurs in legume nodules, rather than through the death and mineralization of semi-symbiotic diazotrophs James (2000). Moreover, endophytic bacteria gain mechanical protection by host, protection against high light intensities, low soil pH and they have favorable, suboptimal oxygen conditions Kallio (1978). Kohler *et al.,* (2007) concluded that the roles of inoculation are proliferating of microorganism as well as improving the microbiological activities in the rhizosphere. Similarly, Chauhan *et al.,* (2017) showed that microbial community is the main component of ecosystem which plays critical role in the nutrient biochemical transformation such as N_2 fixation. Thereby, N becomes available to plant growth for several years without N fertilization due to biological fixation. This process catalyzed by N_2-ase is necessary to maintain fertility in several ecosystems. The ability of fixing N_2 is widely distributed among many bacterial groups in several ecosystems.

In few words, the highest values of the three enzymes activity were recorded for treatment of inoculating with PGPR group (A) and amended with ¾ of mineral fertilizers followed by treatment that inoculated with PGPR group (B) and amended with ¾ mineral fertilizers.

Table 2. Effect of applied treatments on dehydrogenase activity in cultivated soil with moringa.

Treatments	Dehydrogenase activity (µg TPF g^{-1} dw h^{-1}) DAS											
	0	30	60	90	120	150	0	30	60	90	120	150
	1st season (2016)						2nd season (2017)					
T1	12	17	20	32	33	26	10	14	22	31	24	18
T2	15	18	44	52	49	22	12	17	41	46	44	31
T3	17	22	57	69	57	52	12	26	59	65	51	22
T4	17	28	64	84	72	36	14	31	71	77	63	34
T5	16	36	78	96	73	47	12	42	85	91	48	39
T6	13	18	25	41	38	22	13	25	46	41	36	25
T7	15	21	34	47	44	37	14	22	42	45	44	31
T8	15	25	56	62	46	31	12	36	55	67	51	37
T9	13	29	63	77	59	52	16	39	65	71	62	27

T1 to T9 see Table 1

Table 3. Effect of applied treatments on alkaline phosphatase activity in cultivated soil with moringa.

Treatments	Alkaline phosphatase (µg ρNP g^{-1} h^{-1}) DAS											
	0	30	60	90	120	150	0	30	60	90	120	150
	1st season (2016)						2nd season (2017)					
T1	7.2	15.4	21.7	32.9	28.4	17.6	5.4	12.3	19.7	28.0	22.5	13.6
T2	8.4	12.6	19.0	33.4	30.7	21.4	7.6	14.5	23.5	35.6	31.0	16.4
T3	7.3	15.3	24.0	38.3	32.5	18.8	6.8	16.2	26.3	39.4	27.6	18.0
T4	5.5	16.7	29.6	42.0	39.1	22.5	8.2	15.7	28.8	38.6	26.8	22.6
T5	6.6	19.4	35.8	48.2	41.3	29.9	7.1	20.4	38.5	44.3	36.5	27.4
T6	8.0	11.1	17.3	26.0	35.0	21.3	12.6	15.8	19.9	27.5	21.3	13.2
T7	4.0	10.7	15.1	28.0	33.6	22.7	9.8	18.5	17.6	33.0	18.6	16.3
T8	6.6	9.3	19.3	32.2	25.5	14.6	10.6	19.3	25.5	38.7	15.0	11.6
T9	7.3	13.8	24.5	37.6	27.2	19.5	7.3	16.8	29.9	35.5	25.4	19.8

T1 to T9 see Table 1

Table 4. Effect of applied treatments on nitrogenase activity in cultivated soil with moringa.

Treatments	Nitrogenase (µL C_2H_4 g^{-1} dw h^{-1}) DAS											
	0	30	60	90	120	150	0	30	60	90	120	150
	1st season (2016)						2nd season (2017)					
T1	12.4	24.9	31.6	33.8	27.6	21.4	10.5	22.5	37.7	49.7	55.5	37.7
T2	15.1	27.5	39.8	48.9	38.9	33.2	11.8	25.8	38.3	44.4	41.7	32.3
T3	14.6	31.3	47.5	57.6	44.4	42.8	15.9	29.0	43.5	57.7	27.2	25.9
T4	13.7	38.7	52.3	63.3	37.1	35.4	9.3	32.5	44.6	73.0	62.5	33.0
T5	19.4	45.2	68.9	87.1	71.3	62.3	12.2	39.3	59.7	84.5	77.4	42.2
T6	8.8	16.1	37.0	42.5	31.0	22.8	18.7	21.6	32.3	44.4	38.9	27.5
T7	10.5	27.8	39.4	55.7	53.4	28.6	14.5	22.2	36.2	47.8	29.0	33.7
T8	13.9	36.4	48.2	59.9	69.3	35.2	14.2	28.8	35.7	52.2	48.4	31.8
T9	16.4	39.2	55.5	73.4	77.9	42.8	9.7	31.4	47.5	61.4	32.2	39.8

T1 to T9 see Table 1

Vegetative growth parameters

In present study, the effects of mineral and biofertilizers on moringa growth performance have been studied. The following morphological characteristics as plant height, stem diameter, no. of leaves $plant^{-1}$, total leaf area $plant^{-1}$, root, stem and leaves fresh as well as dry weights of moringa plant at 75 and 150 DAS were recorded in Tables (5 and 6).

The obtained results revealed that individual inoculation with bioinoculants recorded the lowest values of the most vegetative growth characteristics. All treatments that amended with NPK mineral fertilizers levels either individually or combined with bioinoculants gave higher records for all of the tested parameters than that inoculated with bioinoculants solely. The highest values of vegetative growth characteristics were recorded with using inoculation by PGPR group (A) combined with ¾ dose of mineral fertilizers during the two growing seasons.

Also, similar data evidently confirm the stimulatory and significant effects of different applied treatments upon dry matter production and its accumulation in moringa shoot. In general, data in Tables (5 and 6) not only being a direct result for the vigorous growth obtained with bioinoculants and mineral fertilizers treatments but also considered as indicator for expectable high vegetative yield of moringa plant.

In this respect, Dobbelaere *et al.*, (2003) found that the inoculation with *Azospirillum sp*. resulted on increasing root and aerial parts dry weights of the PGPR inoculated plant.

Inoculation seeds of several crops and ornamental plants with mixture of PGPR before planting enhanced growth characteristics Zehnder *et al.*, (2001).

Many symbiotic PGPR and free living rhizobacterial species are found for IAA and GA_3 production in the soil rhizosphere and thereby, play significant roles in increasing root surface area as well as root tips number in several plants Han and Lee (2006).

Several studies indicated the positive effects of microorganisms on medicinal plants growth and performance improvements. In addition, for nitrogen fixing, *Azospirillum spp*. improving root growth during generation of stimulating compounds and this resulting in increasing both water and nutrients uptake thereby, increasing plant general performance as well Tilak *et al.*, (2005). Kloepper *et al.*, (2007) indicated that *Azospirillum spp*., *Azotobacter spp*. as well as *Pseudomonas spp*. are important growth stimulating bacteria which in addition for biological fixing of N_2 and solubilizing of phosphate in the soil, considerably affect plant growth regulators i.e., auxins and gibberellins as well as cytokinins, thereby improving plant performance.

Table 5. Effect of bio and chemical fertilization on some morphological characteristics of *Moringa oleifera* L. plant at 75 DAS during 1st and 2nd seasons.

Treatments	Plant height (cm)	Stem diameter (cm)	No. of leaves $plant^{-1}$	Root fresh weight g $plant^{-1}$	Stem fresh weight g $plant^{-1}$	Leaves fresh weight g $plant^{-1}$	Stem dry weight g $plant^{-1}$	Leaves dry weight g $plant^{-1}$	Root dry weight g $plant^{-1}$	Total dry weight g $plant^{-1}$	Total leaf area $plant^{-1}$ (cm^2)
	1st season (2016)										
T1	108.85	0.83	13.61	18.29	33.03	12.48	6.89	2.95	4.11	14.60	172.52
T2	87.47	0.72	12.33	14.64	25.50	10.93	5.48	2.66	2.89	11.03	147.80
T3	99.62	0.76	13.21	18.14	27.53	11.64	5.74	2.79	4.41	12.94	153.63
T4	111.25	0.84	13.72	21.12	41.42	15.50	8.26	3.60	5.01	16.22	181.33
T5	147.65	0.86	14.74	23.81	45.98	17.92	9.36	4.17	6.33	19.86	224.06
T6	81.41	0.64	11.74	11.15	25.31	9.69	7.36	2.23	2.67	12.26	136.12
T7	95.87	0.91	12.66	14.33	27.87	10.27	5.43	2.84	4.13	12.40	155.19
T8	104.63	0.80	13.80	18.12	34.68	11.52	7.83	2.58	3.77	14.18	168.32
T9	102.68	0.93	14.18	19.17	35.62	14.84	5.81	3.63	4.45	13.89	187.26
L.S.D. 0.05	4.53	0.11	0.69	2. 58	3.84	2.06	1.91	0.47	1.84	1.22	8.56
	2nd season (2017)										
T1	116.97	0.74	13.48	22.02	28.26	12.11	7.41	3.40	5.59	16.40	167.17
T2	107.24	0.63	10.37	17.92	22.71	10.82	6.93	2.88	3.51	13.32	144.58
T3	103.17	0.76	11.19	22.32	26.78	12.81	7.34	3.02	4.34	14.70	170.14
T4	108.78	0.77	13.89	23.30	33.73	15.38	8.78	3.84	6.09	18.71	182.47
T5	119.41	1.05	14.45	26.54	47.50	16.99	11.38	4.44	7.02	22.84	197.23
T6	73.94	0.60	10.56	13.86	22.22	8.16	6.02	2.46	2.85	11.33	158.70
T7	105.32	0.72	12.26	15.60	36.19	12.75	7.89	3.14	3.04	14.07	145.19
T8	119.61	0.95	14.11	16.88	38.14	13.24	8.78	3.17	3.40	15.35	173.42
T9	127.82	0.89	16.92	23.04	39.29	13.33	8.67	3.32	4.57	16.56	196.04
L.S.D. 0.05	3.73	0.09	1.13	3. 41	4.47	1.52	0.96	0.38	1.07	0.91	10.82

T1 to T9 see Table 1

Table 6. Effect of bio and chemical fertilization on some morphological characteristics of *Moringa oleifera* L. plant at 150 DAS during 1st and 2nd seasons.

Treatments	Plant height (cm)	Stem diameter (cm)	No. of leaves Plant^{-1}	Root fresh weight g plant^{-1}	Stem fresh weight g plant^{-1}	Leaves fresh weight g plant^{-1}	Stem dry weight g plant^{-1}	Leaves dry weight g plant^{-1}	Root dry weight g plant^{-1}	Total dry weight g plant^{-1}	Total leaf area plant^{-1} (cm^2)
					1st season (2016)						
T1	140.16	1.31	39. 76	41. 76	44.57	30. 42	10.17	7. 57	6. 23	23.97	445.30
T2	118.78	1. 15	32. 13	31. 63	42.05	21. 36	7.94	6. 19	5. 59	19.72	339.71
T3	122.05	1. 35	35. 73	37. 59	43. 46	28. 68	8. 23	6. 58	5.88	20.69	366.87
T4	142.40	1. 43	41.63	35.77	56. 93	32.83	10. 84	7. 96	7.62	26.42	477.16
T5	157. 43	1.58	47. 43	40.92	57.88	33. 55	11. 50	8.91	8. 14	28.55	584.06
T6	126.30	1.17	28.62	24. 37	50. 61	22.20	8.66	6. 98	6.36	22.00	283.42
T7	136. 13	1. 25	43. 36	28. 06	52.53	26. 69	8. 17	7. 73	6. 50	22.40	451.15
T8	134. 83	1.36	47.30	35.70	51. 17	27.52	9.24	8.05	7. 40	24.69	478.34
T9	154. 35	1.30	51.60	36. 16	55. 28	31.41	10. 01	8. 84	7. 22	26.07	532.40
L.S.D. 0.05	7.92	0.08	2.14	5. 67	4.15	3.11	2.02	1.07	0.77	2.20	46.85
					2nd season (2017)						
T1	130. 75	1.63	34.02	30.15	44. 35	28. 44	8. 52	6.21	7. 40	22.13	376.57
T2	120.34	1.32	32.30	26. 02	42. 65	22.93	8. 40	6. 16	6. 09	20.65	364.18
T3	142.18	1. 45	41. 13	26.27	43.64	26. 62	9. 29	7. 79	7. 82	24.90	339.20
T4	161.13	1. 74	46.39	38. 38	54. 03	33.80	9.77	7. 93	8.43	26.13	503.07
T5	154. 03	1.70	49. 63	40. 45	59.81	34.08	10. 60	8. 72	8. 61	27.93	557.31
T6	132.70	1. 24	27.68	33. 82	52.70	21. 39	7.70	6. 13	5. 47	19.30	225.47
T7	146. 64	1.41	37.66	36. 53	52. 95	24. 51	8. 35	6. 25	6.85	21.45	441.36
T8	149. 93	1. 52	43.63	37. 67	54.17	27.12	9. 91	6. 74	7. 70	24.35	458.30
T9	153. 76	1.50	45. 53	38.19	57. 68	31.23	10.21	7. 07	8. 93	26.21	460.53
L.S.D. .05	6.40	0.06	1.94	4. 15	3.88	2.23	1.87	0.89	1.12	1.72	33.20

T1 to T9 see Table 1

Azotobacter bacteria are able to produce antifungal compounds which fighting plant diseases as well as improving viability and plantlets germination, thereby improving overall of plant growth Chen (2006). Sanches Govin *et al.,* (2005) observed that biological fertilizers application improved the shoots performance of *Calendula officinallis* L. as well as *Matricaria recutita* L. plants. Youssef *et al.,* (2004) found that plant height, dry and wet weight of the shoots were increased in *Salvia officinalis* L due to the biological fertilizers application. In the same manner, favourable results were observed due to the effects of *Azospirillum* and *Azotobacter* as well as phosphate solubilizing bacteria on *Majorana hortensis* medicinal plant. PGPR were known for promoting plant growth and seedlings emergency Minorsky (2008). Similarly, Mahfouz and Sharaf-Eldin (2007) found that application of biofertilizer, which was a mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus megaterium* combined with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth of fennel plants as compared to chemical fertilizer treatments only.

Bacillus and Pseudomonas were identified for having critical roles in cell elongation, escalating ACC deaminase activity and promoting plant growth Sgroy *et al.,* (2009).

Arab *et al.,* (2015) recorded that the combined treatment of biofertilizer with different NPK (0, 25, 50, 100%) NPK (full dose of NPK fertilizer =150:75:50 mg kg^{-1} pot^{-1}) fertilizer rates significantly increased the morphological traits of marigold plants. Moreover, Said-Al Ahl *et al.,* (2015) concluded that the combined treatment between biofertilizers and N at 60 kg N fed^{-1}. gave the largest values of plant height, branches number, fresh as well as dry weight of *Anethum graveolens* L. plant.

Phytochemical constituents

Photosynthetic pigments

Data in Table (7) reveal that moringa plant inoculated with PGPR group (A) and amended with ¾ of mineral fertilizers, followed by treatment that inoculated with PGPR group (B) and amended with ¾ mineral fertilizers gave the highest leaves content of chlorophyll a, b, chl. a+b as well as carotenoids at 75 and 150 DAS comparison to the other applied treatments and the control (mineral fertilizers) treatments. Herein, it was clear that the most effective treatment led to maintain the highest content of photosynthetic pigments is recorded with using inoculation of PGPR group (A) combined with ¾ dose of mineral fertilizers during the both seasons.

Therefore, it could be concluded that increasing leaf area as well as increment of dry matter accumulation Tables (5 and 6) as well as photosynthetic pigments Table (7) in leaves of moringa plants reverse the stimulating effect of these treatments on the photosynthetic efficiency process, thereby more photosynthates being created as well as enhancing minerals translocation from root to leaves. Results of photosynthetic pigment traits as affected by using bioinoculants of PGPR is coincided to Arab *et al.,* (2015) where they found that the combined treatment of biofertilizer with different NPK (0, 25, 50, 100%) NPK (full dose of NPK fertilizer = 150:75:50 mg kg^{-1} pot^{-1}) fertilizer rates significantly increased the chlorophyll index of marigold plant. Said-Al Ahl *et al.,* (2015) reported that using the biofertilizers increased chlorophyll A, B as well as total carotenoids content of *Anethum graveolens* L. plant.

Table 7. Effect of bio and chemical fertilization on photosynthetic pigments (mg g^{-1} fr. wt.) content of *Moringa oleifera* L. leaves at 75 and 150 DAS during 1st and 2nd seasons.

Treatments	At 75 DAS				At 150 DAS			
	Chlorophyll (mg g^{-1} fr. wt.)			Carotenoids	Chlorophyll (mg g^{-1} fr. wt.)			Carotenoids
	Chl. A	Chl. B	(A+B)		Chl. A	Chl. B	(A+B)	
				1st season (2016)				
T1	1. 24	0. 98	2.22	1. 81	1.17	0.67	1.84	1.67
T2	1. 13	0. 69	1.82	0.82	0.89	0.64	1.53	0.90
T3	1.16	0. 88	2.04	0.86	1.12	0.77	1.89	1.89
T4	1.66	0.75	2.41	1. 28	1.23	0.80	2.03	1.78
T5	1. 73	0.77	2.50	1. 51	1.53	0.93	2.46	1.81
T6	1.20	0.61	1.81	0.72	0.81	0.65	1.46	0.94
T7	1. 28	0. 58	1.86	0.89	0.90	0.67	1.57	1.72
T8	1. 14	0.80	1.94	1. 27	1. 21	0.66	1.87	0.98
T9	1. 67	0. 69	2.36	1. 42	1.40	0.71	2.11	1.83
				2nd season (2017)				
T1	1. 76	0.90	2.66	0.87	1.13	0.77	1.90	1.54
T2	1.24	0.85	2.09	0.84	1. 10	0.54	1.64	0.63
T3	1. 65	0.94	2.59	1.52	1.44	0.65	2.09	1. 85
T4	1. 87	0.84	2.71	0.92	1. 21	0.70	1.91	1. 06
T5	1. 94	0.76	2.70	0.95	1. 25	0.93	2.18	1. 56
T6	1. 43	0.98	2.41	0.80	1.05	0.72	1.77	0.62
T7	1. 57	0.92	2.49	1.27	1.29	0.63	1.92	1. 75
T8	1. 56	0.96	2.52	1.16	1. 70	0.62	2.32	0.92
T9	1. 92	0.93	2.85	0.89	1.32	0.64	1.96	1. 26

T1 to T9 see Table 1

Endogenous phytohormones

Data reported in Table (8) show that the highest values of endogenous phytohormones content were observed in inoculated plants with the PGPR group (A) amended with ¾ of mineral fertilizers and PGPR group (B) amended with ¾ of mineral fertilizers, followed by treatment that inoculated with PGPR group (A) amended with ½ of mineral fertilizers, respectively compared with the control (full dose of mineral fertilization).

Regarding auxins level, it was highly increased in moringa shoots with all bioinoculants treatments that amended with NPK mineral fertilizers levels compared with that of the control. In the same order, the treatments of PGPR group (B) amended with ¾ mineral fertilizers followed by PGPR group (A) with ¾ mineral fertilizers were the most effective treatments which were highly increasing auxins content compared with the control and the other applied treatments.

For gibberellins level, data clearly showed that the level of gibberellin like-substances in moringa shoots was increased with PGPR group (B) amended with ¾ mineral fertilizers and PGPR group (A) with ¾ mineral fertilizers as compared with other and control treatments.

Moreover, data in Table (8) clearly indicate that level of cytokinins was positively responded to the different applied treatments. Since the activity was the lowest in cases of the control. In this respect, the treatment of PGPR group (A) amended with ½ mineral fertilizers was the most effective treatments which highly increased cytokinins.

Obtained increment of endogenous phytohormones in moringa plants could interpret both of the obtained modifications in growth characteristics improvement Tables (5 and 6) and metabolical performance Table (7).

Also, data in Table (8) show that salicylic acid level increased with the different tested treatments compared with the control and reached its maximum values with PGPR group (B) amended with ¾ mineral fertilizers and PGPR group (A) with ¾ mineral fertilizers treatments, respectively.

In this respect, these results being of great interest for interpreting obtained vigorous growth of moringa plant as obvious in the present study.

Obtained results are in agreement with those mentioned by Kloepper *et al.,* (2007) and Sakr *et al.,* (2014) who indicated that *Azospirillum, Azotobacter* and *Pseudomonas* as well as *Bacillus* are the important growth stimulating bacteria in addition to biological fixing of N_2 and solubilizing soil phosphate, thereby affecting on plant growth regulators especially auxins and gibberellins as well as cytokinins resulting in improvement of the plant performance. Also, it is secreting indol-3-acetic acid, gibberellins and kinetin phytohormones Mali and Bodhankar (2009). The IAA stimulates cell division, shoot elongation, root proliferation, root hair number and formation of lateral and adventitious roots as well as floral buds and fruit development. Cytokinins affect root initiation and cell division as well as cell enlargement and increase root volume.

Azospirillum promotes plant growth by several mechanisms including N fixation, production of phytohormones (i.e., auxins, gibberlines and cytokinins), phosphate solubilization and mobilization, promoting root growth and enhancing water and minerals uptake as reported by Bashan and de-Bashan (2010); Purushothaman *et al.,* (1980); Bottini *et al.,* (1989); Crozier *et al.,* (1988) and Strzelczyk *et al.,* (1994).

Gibberellins promote cell division and elongation, root and root hair abundance. Subsequently, *Azospirillum* induces great root system growth, surface area and volume, leading to improve water and mineral uptake, resulting in enhancing foliage parameters and accumulation of dry matter and nutrients in shoots. Furthermore, *Azospirillum* produces polyamine cadaverine synthesized from lysine amino acid correlated with cell growth Cassa´n *et al.,* (2009). *Azospirillum lipoferum* produces gluconic organic acid which reduces pH of the medium to help in solubilizing

insoluble phosphate in rock phosphate Rodriguez *et al.*, (2004) and then released soluble phosphate in the soil is ready for uptake by plants.

Besides providing P to plants, the phosphate solubilizing bacteria also, augment growth of plants by N_2 fixation, synthesizing plant growth promotion substances as indole acetic and gibberellic acids Ding *et al.,* (2005) and Vikram *et al.,* (2007). Potassium dissolving bacteria bring available K into soil solution to benefit soil fertility and plant K uptake by excreting organic acids i.e., citric, oxalic, malic and succinic as well as tartaric. Acidolysis of the rhizospher helps in chelating silicon and aluminum cations bound to insoluble K bearing minerals Prajapati and Modi (2012) and Meena *et al.,* (2014). *Bacillus sp.* release siderophores, high affinity iron chelating compounds for scavenging insoluble iron from minerals and form soluble Fe complexes which can be taken up by active transport Tian *et al.,* (2009).

Antioxidants content and free radicals scavenging activity

Total phenolics and flavonoids content

Results in Table (9) indicated that total phenolics and flavonoids were influenced by fertilizer source and fertilization rates. It was observed that the treatment of inoculation with PGPR group (A) or group (B) and amended with ¾ of mineral fertilizers increased total phenolics and flavonoids content in *Moringa oleifera* L. leaves at 75 and 150 DAS during 2017 season. Total phenolics and flavonoids were enhanced with treatment that inoculated with PGPR group (A) and amended with ¾ of mineral fertilizers followed by treatment that inoculated with PGPR group (B) and amended with ¾ mineral fertilizers respectively, compared to mineral fertilization. It was apparent that treatment inoculated with PGPR group (A) and amended with ¾ of mineral fertilizers, where it was observed that total phenolics and flavonoids had the highest values (total phenolics was 46.12 and total flavonoids was 29.26 mg g^{-1} dry wt.). The present results suggested that the application of treatment inoculated with PGPR and amended with mineral fertilizers rates can enhance *Moringa oleifera* L. non-enzymatic antioxidants content. Obtained results were in harmony with those mentioned by Arab *et al.,* (2015) where they concluded that the combined treatment of biofertilizer with NPK fertilizer rates significantly increased the flavonoids content of *Calendula officinalis* L. plant.

Table 8. Effect of bio and chemical fertilization on endogenous phytohormones of *Moringa oleifera* L. plant at 150 DAS during 2nd season.

Treatments	Promoters							Inhibitors		Salicylic acid	
	Gibberellins µg g^{-1} fr.wt.	Auxins µg g^{-1} fr. wt.			Cytokinins µg g^{-1} fr.wt.	Total promoters µg g^{-1}fr.wt.	% relative to control	Abscisic acid µg g^{-1} fr.wt.	% relative to control	µg g^{-1} fr.wt.	% relative to control
		3 indole acetic acid	Indole butyric acid	Total µg g^{-1} fr.wt.							
T1	85.82	11.98	11.92	23.90	150.71	260.43	100.00	3.30	100.00	8.24	100.00
T2	111.91	7.82	30.03	37.85	218.36	368.12	141.35	1.51	45.76	4.89	59.34
T3	103.20	17.19	28.81	46.00	349.94	499.14	191.66	2.08	63.03	13.57	164.68
T4	112.50	12.76	6.30	19.06	398.35	519.91	199.64	2.31	70.00	18.91	229.49
T5	133.01	37.52	60.71	98.23	315.81	547.05	210.06	2.77	83.94	25.77	312.74
T6	128.79	28.15	42.11	70.26	182.26	381.31	146.42	1.60	48.48	17.46	211.89
T7	132.80	33.93	59.64	93.57	161.06	387.43	148.77	1.78	53.94	13.75	166.87
T8	131.62	26.34	59.47	85.81	288.73	506.16	194.36	1.26	38.18	14.08	170.87
T9	147.35	44.84	95.06	139.90	253.96	541.21	207.81	2.19	66.36	34.14	414.32

T1 to T9 see Table 1

Table 9. Effect of bio and chemicals fertilization on some non-enzymatic antioxidants and free radicals scavenging activity of *Moringa oleifera* L. leaves at 75 and 150 DAS during 2nd season.

Treatments	At 75 DAS				At 150 DAS			
	Total phenolics mg g^{-1} dry wt.	Total flavonoids mg g^{-1} dry wt.	Ascorbic acid mg g^{-1} fr.wt.	DPPH scavenging activity µg ml^{-1}	Total phenolics mg g^{-1} dry wt.	Total flavonoids mg g^{-1} dry wt.	Ascorbic acid mg g^{-1} fr.wt.	DPPH scavenging activity µg ml^{-1}
T1	41.78	27.28	1.40	18.16	44.54	28.15	2.49	19.41
T2	36.77	24.59	2.36	17.74	39.22	27.89	2.54	18.30
T3	38.03	25.86	2.12	18.57	40.18	26.47	2.08	20.54
T4	43.45	27.43	2.27	19.64	45.34	28.17	3.02	21.92
T5	46.12	29.26	1.18	22.85	45.91	32.28	3.62	22.13
T6	33.94	21.32	2.51	16.64	41.50	25.09	2.32	19.24
T7	34.28	25.66	1.70	16.89	39.83	27.90	2.44	21.08
T8	35.85	24.07	1.08	19.08	45.70	28.83	2.90	20.77
T9	42.22	23.97	1.67	20.76	44.12	26.46	3.18	21.60

T1 to T9 see Table 1 DPPH: 1, 1-Diphenyl-2-picrylhydrazyl

Said-Al Ahl *et al.,* (2015) Showed that treatments of 60 kg N fed^{-1} combined with biofertilizer gave the highest total flavonoids content in fertilized dill plants.

Ascorbic acid content

Data presented in Table (9) clearly indicated that the effect of different applied treatments on ascorbic acid content in *Moringa oleifera* L at 75 and 150 DAS during 2017 season. It was observed that ascorbic acid content was maximized with treatments of PGPR group (B) solely at 75 DAS meanwhile, its highest value was reached using treatment inoculated with PGPR group (A) and amended with ¾ of mineral fertilizers at 150 DAS. In contrast, the reduction in ascorbic acid content under high mineral fertilization rates was mentioned in other studies Seung and Adel (2000); Hassan *et al.,* (2005) and Toor *et al.,* (2006).

Inoculation with PGPR enhanced the production of total phenolics, flavonoids and ascorbic acid contents in moringa plant (Table 9). The increase in secondary metabolites production under inoculation with PGPR in the present study go on line with Weibel *et al.,* (2000)

and Ibrahim and Jaafar (2011) where they reported that plants grown under inoculation with PGPR conditions have higher micronutrients content than conventionally grown plants . Considering the fact that some of chemical reactions in cells involve minor elements, either directly or indirectly, this could explain why biofertilized plants exhibited higher production of secondary metabolites Bimova and Pokluda (2009). Also, Heaton (2001) reported that the type and value of fertilizer as well as the level of application directly influence the level of nutrients available in plants and indirectly influence plant physiology and the biosynthesis of secondary compounds (phytonutrients) in plant.

In general, increasing total phenolics, flavonoids and ascorbic acid content with different applied treatments considered as a direct result of increasing both photosynthesis rate and efficiency. Also, that was preceded with large photosynthetic area Tables (5 and 6) and high concentration of photosynthetic pigments Table (7) under the application of biofertilizer and mineral fertilizer treatments.

The DPPH scavenging activity

Data in Table (9) indicated the effects of fertilizer source and rates of mineral fertilizers on DPPH scavenging activity. Data clearly indicated that treatments inoculated with PGPR group (A) and amended with ¾ of mineral fertilizers and the DPPH antioxidant activity recorded the highest value being 22.85 µg ml^{-1} followed by treatment inoculated with PGPR group (B) and amended with ¾ mineral fertilizers, and the least was the inoculation with PGPR group (B) solely treatment being (16.64 µg ml^{-1}).

Results imply that the usage of biofertilizer can enhance the radical scavenging activity in moringa plant and reduce the usage of mineral fertilizers rates. It must be noted that the DPPH assay principally measuring the activity of watersoluble antioxidants Frankel *et al.*, (1994).The combination of phenolics and ascorbic acid produced a synergistic effect on DPPH radical scavenging activity Murakami *et al.*, (2003). Other studies reported the increase of total phenolics, flavonoids, ascorbic acid content and DPPH scavenging activity with usage of bioinoculants of PGPR and mineral fertilizers levels Bimova and Pokluda (2009) and Ibrahim *et al.*, (2013).

CONCLUSION

Based on these findings it can be concluded that, as response to microbial enzymatic activities, the highest values of vegetative growth, photosynthetic pigments, endogenous phytohormones, total phenolics and flavonoids were obtained for inoculated treatment with *Azo. lipoferium* D178, *B. megaterium* and *B. circulans* (group A) amended with ¾ of mineral fertilizers. Moreover, using ¾ of the recommended mineral fertilizers in combination with biofertilizers had high growth and yield of moringa plant without significant differences when using the highest treatment (full dose of mineral fertilizers). Using of biofertilization decreases the environmental pollution that caused by excessive application of mineral fertilizers.

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تحسين النمو والمركبات الفعالة في نبات المورينجا بإستخدام نظام الإدارة المتكاملة للمغذيات

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أجريت تجربتي أصص في موسمي النمو ٢٠١٦ و ٢٠١٧ بمزرعة قسم النبات الزراعي - كلية الزراعة بمشتهر- جامعة بنها بهدف دراسة تأثير إستخدام مجموعتين منفردتين من البكتريا المشجعة لنمو النبات PGPR وهما المجموعة (أ) وتتكون من (*Azo. lipoferium* D178, *B. megaterium* ATCC14581 and *B. circulans* ATCC4513) والمجموعة (ب) وتتكون من (*A. chroococcum* EMCCN1458, *B. megaterium* ATCC14581 and *B. circulans* ATCC4513) وكذلك عند تدعيم كل منها بثلاث معدلات من الأسمدة المعدنية (NPK) ٠.٢٥ ، ٠.٥٠ ، ٠.٧٥ % من المعدل الموصى به هذا بالإضافة إلى الكنترول (جرعة كاملة من الأزوت والفوسفور والبوتاسيوم) علي خصائص النمو الخضري والمكونات الكيميائية لنبات المورينجا أوليفيرا عند عمر ٧٥ و١٥٠ يوم من الزراعة خلال موسمي الدراسة للحد من الإستخدام المفرط للأسمدة الكيماوية فى الإنتاج النباتى. تم الحصول علي أعلي معدل لنشاط إنزيمات الديهيدروجينيز والفوسفاتيز القلوي والنيتروجينيز عند الخلط بين البكتريا الجذرية المحفزة لنشاط النبات والتسميد المعدني، لوحظ زيادة في معدل نشاط هذه الإنزيمات حتي وصل النشاط أقصاه عند ٩٠ يوم من الزراعة وذلك عند إستخدام اللقاح الحيوي المجموعة (أ) مع إضافة ٤/٣ الجرعة من التسميد المعدني يليه إستخدام اللقاح الحيوي المجموعة (ب) مع إضافة ٤/٣ الجرعة من التسميد المعدني. إستجابة للنشاط الإنزيمي فقد سُجل أعلي معدل من القياسات النباتية الخضرية والمحتوى من صبغات البناء الضوئى والهرمونات النباتية المنشطة وكذلك المركبات الفينولية والفلافونيدات الكلية وحامض الأسكوربيك هذا بالإضافة إلى أعلي قيم فى نشاط مضادات الأكسدة عند إستخدام التلقيح الحيوي المجموعة (أ) مع إضافة ٤/٣ الجرعة المستخدمة من التسميد المعدني متبوعة بإستخدام اللقاح الحيوي المجموعة (ب) مع إضافة ٤/٣ الجرعة المستخدمة من التسميد المعدني. كما ظهر إنخفاض في معدل إنتاج حامض الأبسيسيك والذى كان واضحاً مع إستخدام المعاملة الحيوية المجموعة (ب) مضافاً إليها ٢/١ جرعة التسميد المعدني، أما المحتوى من حامض الساليسيلك فظهر زيادة في معدل الإنتاج وذلك عند المقارنة بالكنترول وكانت هذه الزيادة واضحة عند إستخدام اللقاح الحيوي المجموعة (ب) مع٤/٣ الجرعة من التسميد المعدني يليها إستخدام المجموعة (أ) مع٤/٣ الجرعة المستخدمة من التسميد المعدني علي التوالي.