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ORIGINAL ARTICLE

Improvement of growth and nutritional quality of *Moringa oleifera* using different biofertilizers

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Abstract *Moringa* seeds were cultivated in polyethylene bags (1 kg capacity) filled with clay loamy soil. Bags were treated with microorganisms using three methods of inoculation i.e. soil inoculation (single or mixed cultures); leaf inoculation (single culture), and soil and leaf inoculation (mixed inoculation). Plants were harvested after 3 months of cultivation. Shoot and root lengths, shoot and root dry weights, leaves fresh and dry weights, vitamin C g/g fresh leaf, protein g/g leaves dry weight and mineral contents (Mg, P, K, Zn, Mn, Fe and Cu) were recorded. Biofertilization by different inoculation methods increased most of the parameters tested. The highest records of shoot and root lengths, and shoot and root dry weights were obtained with soil inoculation with mixed cultures of (*Azotobacter chroococcum* and *Saccharomyces cerevisiae*) and (*Azot. chroococcum* and *Bacillus circulans*). The same trend in respect of Vitamin C was obtained. But, the highest protein contents (g/g dry weight leaves) were obtained with soil inoculation with (*Azot. chroococcum* and *B. circulans*), (*Bacillus megatherium*) and (*Azot. chroococcum* and *S. cerevisiae*), which gave 0.73, 0.59 and 0.58 g protein/g leaves dry weight respectively. Generally, soil inoculation with either *B. megatherium*, *B. circulans*, (*Azot. chroococcum* and *Pseudomonas fluorescens*), (*Azot. chroococcum* and *B. circulans*), *Azot. chroococcum*, and (*Azospirillum brazilense* and *B. megatherium*) gave the highest records of Mg, P, K, Zn, Mn, Fe and Cu respectively.

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Introduction

Moringa oleifera Lamarck is a species of the monogeneric family Moringaceae (order: Brassicales), that includes 13 species of trees and shrubs (Fahey, 2005). *M. oleifera* is indigenous to Northwest India (Ramachandran et al., 1980) but, at present it is widely distributed in the tropics throughout the Pacific region (Aregheore, 2002), West Africa (Freiberger et al., 1998; Lockett et al., 2000), as well as Central America and the Caribbean (Ramachandran et al., 1980; Foidl et al., 1999). It is a typical multipurpose tree of significant economic importance

because of its several industrial and medicinal applications and various products to be used as food and feed which can be derived from its leaves and fruits (Ramachandran et al., 1980).

Leaves of *Moringa* represent an important source of nutrients for rural populations (Gupta et al., 1989; Lockett et al., 2000). Most reports indicate that *Moringa* leaves are rich in protein and present an amino acid composition, which is suitable for human and animal nutrition (Gupta et al., 1989; Makkar and Becker, 1996; Freiberger et al., 1998). Fahey (2005), referred to *Moringa* as a nutrient dense food source because of its high nutritional value of leaves, pods and seeds. 100 g of *Moringa* leaves contain four times more vitamin A than the same quantity of carrots, four times of calcium in a cup of milk, iron more than 100 g of spinach, seven times of vitamin C in 100 g of oranges and three times of potassium in 100 g of bananas. The protein quality of *Moringa* leaves also rivals that of milk and eggs (Fahey, 2005). Therefore, *Moringa* is a relatively good source of vitamins, minerals and essential amino acids and could be considered as a good alternative to be used to help alleviate the micronutrient malnutrition at household as well as national levels. The micronutrients of major concern include vitamin A deficiency (VAD), iron deficiency and iodine deficiency, with zinc being recently added (Labadarios et al., 1995; Kuhnlein, 2003).

According to (Fuglie, 2001), *Moringa* has gained popularity as a source of nutrition that can feed the needy, and save lives as well. *Moringa* leaves or leaf powder can be used successfully as a complex food to nourish small children, pregnant women and nursing mothers as a treatment for malnutrition, because it has significant quantities of vitamins A, B, C, calcium, iron and protein (Ramachandran et al., 1980).

This plant has been well documented for its medicinal importance for a long time (Abdulkarim et al., 2005).

High biomass productions of *Moringa* of over 100 t of dry matter/ha can be achieved under intensive farming conditions (Foidl et al., 1999) and in the last decade large-scale cultivation has been initiated (Makkar and Becker, 1996). Therefore, there is a need to make proper methods to enhance *Moringa* cultivation in Egypt, and to enhance its nutritional quality to be used for different purposes.

Because of the high rate of micronutrient deficiencies that persist in Egypt with the most variable groups especially women and children, the production and the quality of *Moringa* was the objective of this paper, along with:

(1) Determining the effect of various microbial inoculants as biofertilizers used in different methods of inoculation on growth parameters and nutrient contents of *Moringa* leaves.
(2) Highlighting the use of different biofertilizers on *Moringa* vegetables toward an end goal of micronutrient intervention, although the research may inform and include the planning of intervention strategies between the type of biofertilizers and the purpose of *Moringa* cultivation and usage.

Materials and methods

Microorganisms used

Azotobacter chroococcum, Azospirillum brazilense, Bacillus megatherium, *Bacillus circulans, Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were obtained from the Unit of Biofertilizers, Fac., Agric., Ain Shams University.

Seeds of Moringa

Seeds of *M. oleifera* were kindly obtained from Desert Research Center.

Soil used

A clay loamy soil was collected, mixed and passed through a 2 mm sieve in order to give uniform plant growth media. Soil was mechanically analyzed and the physical and chemical analyses are given in Table 1. The organic matter of the soil was 2.06, while the total nitrogen gave % 0.24.

Experimentation

A pot experiment was designed in a completely randomized indoor experiment design. The experiment was conducted in a plastic house covered with 200 μm thick polyethylene at the Unit of Biofertilizers, Faculty of agriculture, Ain Shames University. Cultivation was in 10×20 cm (in diameter) plastic bags filled with 1 kg soil. Seeds of *M. oleifera* were planted at the rate of one/bag and then watered when necessary. Healthy seedlings were divided into 4 groups and after 10 and 45 days of cultivation the seedlings were treated with 5 ml of either biofertilizer (10^8 cfu/ml) as follows:

(1) Control (without microbial inoculants).
(2) Soil inoculation (single or mixed inoculants).
(3) Leaf inoculation (single inoculants).
(4) Soil and leaf inoculation (mixed inoculants).

Ten replicates were made for each treatment. Plants were kept under the above mentioned conditions for 3 months (from June to September 2010). At the end of the experiment, samples of shoots and roots were collected to monitor the effect of inoculation on growth parameters and mineral contents of *M. oleifera*.

Parameters measured

The following parameters were determined: shoot length (cm) and root length (cm) were measured, dry weights of shoots and roots (g/plant) were recorded after oven drying at 70 °C until reaching a constant weight. Leaves fresh and dry weights were recorded. Total vitamin C, protein and mineral contents (Mg, P, K, Mn, Zn, Fe and Cu) were also recorded.

Chemical analysis

Total Mg, P, K, Zn, Mn, Fe and Cu content in *M. oleifera* leaves were determined by the method described by Jackson (1973).

Nitrogenase activity measurement.

Nitrogenase activity of all strains was measured by acetylene-reduction assay according to Mdlica et al. (1985).

Determination of phosphate solubilizing efficiency

Nutrient broth was inoculated with standard inocula of either tested strain (contained 35×10^7 cfu/ml), then 10 ml culture

Table 1 Physiochemical analysis of soils used in the greenhouse experiment.

Physical analysis						Chemical analysis															
Sand %	Silt %	Clay %	Soil Texture	pH	E.C. mmhos cm^{-1}	$CaCO_3$	Cations (mg L^{-1})				Anions (mg L^{-1})			Macroelements (ppm)			Microelements (ppm)			
							Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$SO4^{-}$	Cl^{-}	CO_3^{-}	N	P	K	Fe	Cu	Zn	Mn
30	36	34	Clay Loam	7.3	0.4	–	0.44	0.38	0.25	0.03	0.41	0.32	0.22	12	1	100	10.8	0.02	0.4	0.4

was transferred to the modified Buntt and Rovera medium with shaking at 100 rpm/30 °C for 7 days. The shaken cultures were centrifuged at 7500 rpm/10 min. The supernatant was examined for phosphate solubilization activity according to Jackson (1973).

Potassium mobilization efficiency

Nutrient broth was inoculated with standard inocula of either tested strain (contained 35×10^7 cfu/ml), then 10 ml culture was transferred to the modified Alexandrove's medium with shaking at 100 rpm/30 °C for 7 days. The shaken cultures were used to determine soluble K using flame photometer as described by Jackson (1973).

Indole acetic acid (IAA) and cytokinin production activity

The IAA was quantified using the colorimetric technique using Salkowski reagent as described by Glickmann and Dessaux (1995).

The cytokinin like substances were quantified according to the method described by Fletcher and Cullagh (1971).

Quantitative determination of ascorbic acid

Ascorbic acid was determined following a reported indophenols titration method (Anwar et al., 1990). Samples were homogenized in metaphosphoric acid solution and extracted. Vitamin C was titrated against 2,6-dichlorophenol-indophenol solution in the presence of formaldehyde to a pink end point.

Statistical analysis

Plant chemical analysis was statistically analyzed using One-way ANOVA and post hoc-LSD tests (the least significant difference) (SPSS Inc., program 2009) at 0.05 and 0.01 levels of probability (Snedecor and Cochran, 1982). The correlation coefficient was estimated among the physiological parameters. Discriminant analysis was used to classify several observations in these groups (Härdle and Simar, 2007).

Results and discussion

Assessment of enzymatic activity expression and some metabolic activities of selected strains

Considerable variations were generally recorded among the tested strains regarding their capabilities for nitrogenase activity, P solubilization, K mobilization, IAA, gibberellins and cytokinin production. Data in Table 2 show that *Azot. chroococcum, B. circulans, Azos. brazilense* were able to fix atmospheric nitrogen, and the high nitrogenase activities were obtained with *Azot. chroococcum* (133.9 µmol C_2H_4/ml/h). *B. megatherium* and *B. circulans* showed capabilities for P solubilization (77.6 and 7.44 ppm respectively). Only *B. circulans* was able to mobilize K (8.85 ppm). *P. fluorescens* and *Azos. brazilense* were able to produce IAA (6.25 and 4.7 mg/liter respectively) while *P. fluorescens, Azos. brazilense* and *S. cerevisiae* were able to produce cytokinins (2.695, 2.88 and 3.518 µg/ml respectively). The gibberellins production was recorded only in *Azos. brazilense* and *S. cerevisiae* (12.5 and 9.5 µg/ml respectively).

Table 2 Assessment of some metabolic activities of the selected strains.

Activities	Nitrogenase μmole C_2H_4 (ml/h)	P. Solubilizing (ppm)	K. Mobilizing (ppm)	Plant hormone IAA (mg/litter)	Plant hormone Cyto. (μg/ml)	Plant hormone Gb. (μg/ml)
Microorganism						
Azotobacter chroococcum	133.9	–	–	–	–	–
Bacillus megatherium	–	77.6 (86.2%)	–	–	–	–
Bacillus circulans	0.11	7.44	8.85 (99.4%)	–	–	–
Pseudomonas fluorescens	–	–	–	6.25	2.695	–
Azospirillum brazilense	0.608	–	–	4.7	2.88	12.5
Saccharomyces cerevisiae	–	–	–	–	3.518	9.5

Table 3 Effect of different types of biofertilizers on growth parameters of *Moringa* cultivated on pots for three months (from June to September 2010).

Treatments	Shoot length (Cm/plant)	Root length (Cm/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Soil inoculation				
Azotobacter chroococcum	110.80	11.40	3.34	0.63
Azospirillum brazilense	120.80	13.00	8.62	0.97
Bacillus megatherium	105.80	19.60	3.61	1.08
Bacillus circulans	103.20	21.60	4.62	0.86
Pseudomonas fluorescens	90.40	8.20	1.68	0.30
Saccharomyces cerevisiae	89.20	16.80	3.23	0.94
Azot. chroococcum and *P. fluorescens*	82.50	19.75	3.55	0.84
Azot. chroococcum and *B. megatherium*	94.75	21.00	3.29	0.61
Azot. chroococcum and *B. circulans*	133.50	24.25	11.50	1.82
Azot. chroococcum and *S. cerevisiae*	102.00	16.50	2.57	1.41
Azos. brazilense and *P. fluorescens*	120.25	22.50	4.79	1.65
Azos. brazilense and *B. megatherium*	107.50	20.00	4.69	1.44
Azos. brazilense and *B. circulens*	130.00	20.25	9.64	1.80
Azos. brazilense and *S. cerevisiae*	148.25	21.00	10.28	1.75
Leaf inoculation				
S. cerevisiae	85.6	6.00	2.67	0.54
Soil and leaves inoculation				
Azot. chroococcum (soil) and *S. cerevisiae* (leaf)	121.25	12.50	8.40	1.51
Azos. brazilense (soil) and *S. cerevisiae* (leaf)	124.50	19.00	7.69	1.81
Control	100.8	16.2	3.057	0.684
LSD 5%	31.54	7.84	5.586	1.882

Effect of biofertilizers on M. oleifera

Plant growth parameters

The response of *Moringa* seedlings to microbial inoculation is presented in Table 3. Seedlings inoculated with (*Azot. chroococcum* and *B. circulans*) as soil inoculants gave significant increases in shoot length, root length and shoot dry weight when compared with control (133.50 cm/plant, 24.25 cm/plant and 11.50 g/plant respectively). While (*Azos. brazilense* and *S.* cerevisiae) as soil inoculants showed significant increases in shoot length and dry weight when compared to control (148.25 cm/plant and 10.28 g/plant respectively). Seedlings treated with (*Azos. brazilense* and *B. circulans*) showed significant increase in shoot dry weight compared to control (9.64 g/plant).

Vitamin C contents in leaves

It is obvious from data recorded in Table 4 that in general, there was no significant increase in total vitamin C contents g/g fresh leaves, but when we compared the effect of different microbial inoculants used in different inoculation type on plant leaves fresh weight (g/plant) to control, the data showed that most of the treatments gave a significant increase in plant leaves fresh weight when compared to control. Seedlings treated with (*Azos. brazilense*and *S.* cerevisiae) used as soil inoculants, [*Azos. brazilense* and *S. cerevisiae* (leaf)] used as soil and leaf inoculants and seedlings treated with *B. circulans* used as soil inoculants gave the highest increase in leaves fresh weight compared to control (8.785, 8.141 and 7.6 (g) fresh leaves/plant respectively).

From these data it could be concluded that some treatments gave total vitamin C per plant higher than the control

Table 4 Effect of biofertilizers on vitamin C content of *Moringa* leaves.

Treatments	g vitamin C/g fresh weight leaves	Leaves (g) fresh weight/plant	Total vitamin C g/plant
Soil inoculation			
Azotobacter chroococcum	0.351	3.12	1.10
Azospirillum brazilense	0.256	6.375	1.63
Bacillus megatherium	0.12	6.42	0.77
Bacillus circulans	0.333	7.6	2.53
Pseudomonas fluorescens	0.288	2.5	0.72
Saccharomyces cerevisiae	0.516	4.78	2.47
Azot. chroococcum and *P. fluorescens*	0.80	4.75	3.80
Azot. chroococcum and *B megatherium*	0.352	2.938	1.03
Azot. chroococcum and *B circulans*	0.784	6.734	5.28
Azot. chroococcum and *S. cerevisiae*	0.448	5.04	2.26
Azos. brazilense and *P. fluorescens*	0.36	3.5	1.26
Azos. brazilense and *B. megatherium*	0.512	4.25	2.18
Azos. brazilense and *B. circulens*	0.584	5.563	3.25
Azos. brazilense and *S. cerevisiae*	0.568	0.785	4.99
Leaf inoculation			
S. cerevisiae	0.44	4.08	1.80
Soil and leaves inoculation			
Azot. chroococcum (soil) and *S. cerevisiae* (leaf)	0.304	5.161	1.57
Azos. brazilense (soil) and *S. cerevisiae* (leaf)	0.448	8.141	3.65
Control	0.816	3.25	2.65
LSD 5%	0.0456	3.63	–

Table 5 Effect of biofertilizers on the protein content of *Moringa* leaves.

Treatments	g protein/g dry weight leaves	Leaves dry weight (g)/plant	Total protein (g)/plant
Soil inoculation			
Azotobacter chroococcum	0.249	0.949	0.24
Azospirillum brazilense	0.41	0.893	0.37
Bacillus megatherium	0.433	1.352	0.59
Bacillus circulans	0.271	1.848	0.50
Pseudomonas fluorescens	0.315	0.405	0.13
Saccharomyces cerevisiae	0.213	1.42	0.30
Azot. chroococcum and *P. fluorescens*	0.216	1.025	0.22
Azot. chroococcum and *B. megatherium*	0.241	0.563	0.14
Azot. chroococcum and *B. circulans*	0.2	1.716	0.34
Azot. chroococcum and *S. cerevisiae*	0.232	1.166	0.27
Azos. brazilense and *P. fluorescens*	0.258	1.218	0.31
Azos. brazilense and *B. megatherium*	0.299	0.912	0.27
Azos. brazilense and *B. circulens*	0.221	1.593	0.35
Azos. brazilense and *S. cerevisiae*	0.331	1.757	0.58
Leaf inoculation			
S. cerevisiae	0.194	1.364	0.26
Soil and leaves inoculation			
Azot. chroococcum (soil) and *S. cerevisiae* (leaf)	0.258	1.740	0.45
Azos. brazilense (soil) and *S. cerevisiae* (leaf)	0.258	1.898	0.49
Control	0.235	0.616	0.14
LSD 5%	0.0285	0.8854	–

as a final data and these treatments are (*Azot. chroococcum* and *B. circulans*), (*Azos. brazilense* and *S.* cerevisiae), (*Azot. chroococcum* and *P.* fluorescens) and (*Azos. brazilense* and *B.* circulans) as soil inoculants and (*Azos. brazilense* (soil) and *S. cerevisiae* (leaf) as a soil and leaf inoculants which gave (5.25, 4.99, 3.8, 3.65 and 3.25 g vitamin C/plant respectively).

Protein contents of plant leaves

Data recorded in Table 5 show that in general most of the treatments gave increases in total protein g/g leaves dry weight and the increases were significant in some treatments. The highest increase was obtained with *B. megatherium* and *Azos. brazilense* used as soil inoculants (0.433 and 0.41 g protein/g leaves dry weight respectively).

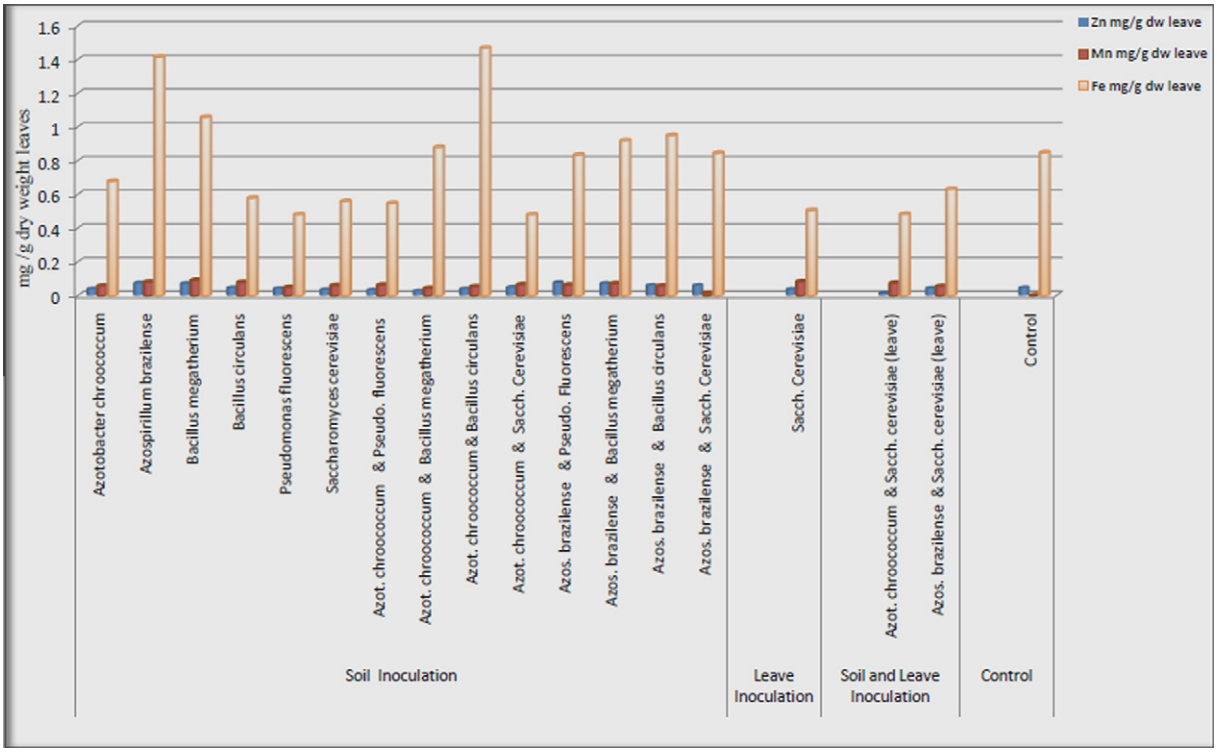

Figure 1 Effect of microbial biofertilizers on Zn, Mn and Fe contents of *Moringa* leaves (mg/g leaves dry weight).

Leaves dry weights per plant were higher in most treatments compared to control, and the increase was significant for some treatments. The treatments [(*Azos. brazilense* and *S.* cerevisiae (leaf)] which were used as soil and leaf inoculants and *B. circulans* used as soil inoculants gave the highest increase in leaves dry weight per plant (1.898 and 1.848 g leaves dry weight/plant respectively). From this data it could be concluded that all treatments gave an increase in total protein (g)/plant when compared to control except the treatment inoculated with *P. fluorescens* used as soil inoculants which gave total protein (g)/plant less than control (0.13 g protein/plant) and the treatment (*Azot. chroococcum* and *B.* megatherium) used as soil inoculants which gave total protein (g)/plant equal to the control (0.14 g protein/plant). The treatments which gave the highest increase in total protein (g)/plant were *B. megatherium* and (*Azos. brazilense* and *S.* cerevisiae) used as soil inoculants (0.59 and 0.58 g protein/plant respectively).

Effect of different inoculation methods on the mineral contents of Moringa leaves

Data recorded in Fig. 1 show that, *Moringa* seedlings treated with deferent microbial inoculants gave significant increases in leaves' Zn content (mg/g leaves dry weight) compared to control, all of them were used as soil inoculants, while the other treatments were less or equal to the control. The highest significant increase was recorded with seedlings treated with (*Azos. brazilense* and *P. fluorescens*) which gave (0.078 mg Zn/g leaves dry weight).

Fig. 1 shows that all treatments gave significant increases in leaves' Mn content (mg/g leaves dry weight) compared to control. As an exception, seedlings treated with (*Azos. brazilense* and *S.* cerevisiae) were used as soil inoculants. Seedlings treated with *B. megatherium* used as soil inoculants gave the highest significant increases (0.090 mg Mn/g leaves dry weight).

Four treatments gave significant increases in Fe content in leaves when compared to control, and the highest increases were recorded in seedlings treated with (*Azot. chroococcum* and *B. circulans*) as soil inoculants which gave (1.468 mg Fe/g leaves dry weight).

In general, from the data obtained in Fig. 2 significant differences between the different treatments used were recorded. Most of the treatments gave significant increases in total Mg (mg)/g leaves dry weight when compared to control. Inoculation with *B. megatherium* as soil inoculants gave the highest increase in Mg mg/g leaves dry weight (3.112 mg Mg/g leaves dry weight) compared to control.

When P content was compared to control some treatments showed increases in P contents. The best treatments were *B. megatherium*, (*Azos. brazilense* and *P. fluorescens*), *P. fluorescens* and (*Azos. brazilense* and *B. megatherium*) as soil inoculants which gave (25.750, 25.4, 24.725 and 23.55 mg P/g leaves dry weight respectively).

The K content in *Moringa* leaves showed significant increases in some treatments when compared to control. Seedlings treated with *B. megatherium* used as soil inoculants gave the highest significant increase in K content in leaves (12.050 mg K/g leaves dry weight).

Data recorded in Table 6 show that, there were three treatments that recorded Cu in the detection methods. These treatments were *Azot. chroococcum, Azos. brazilense* and *B. megatherium* and all of them recorded 0.003 mg Cu/g leaves dry weight.

Canonical discriminant analysis

From data recorded in this study we classified the treatments into groups according to their similarity to each other Fig. 3.

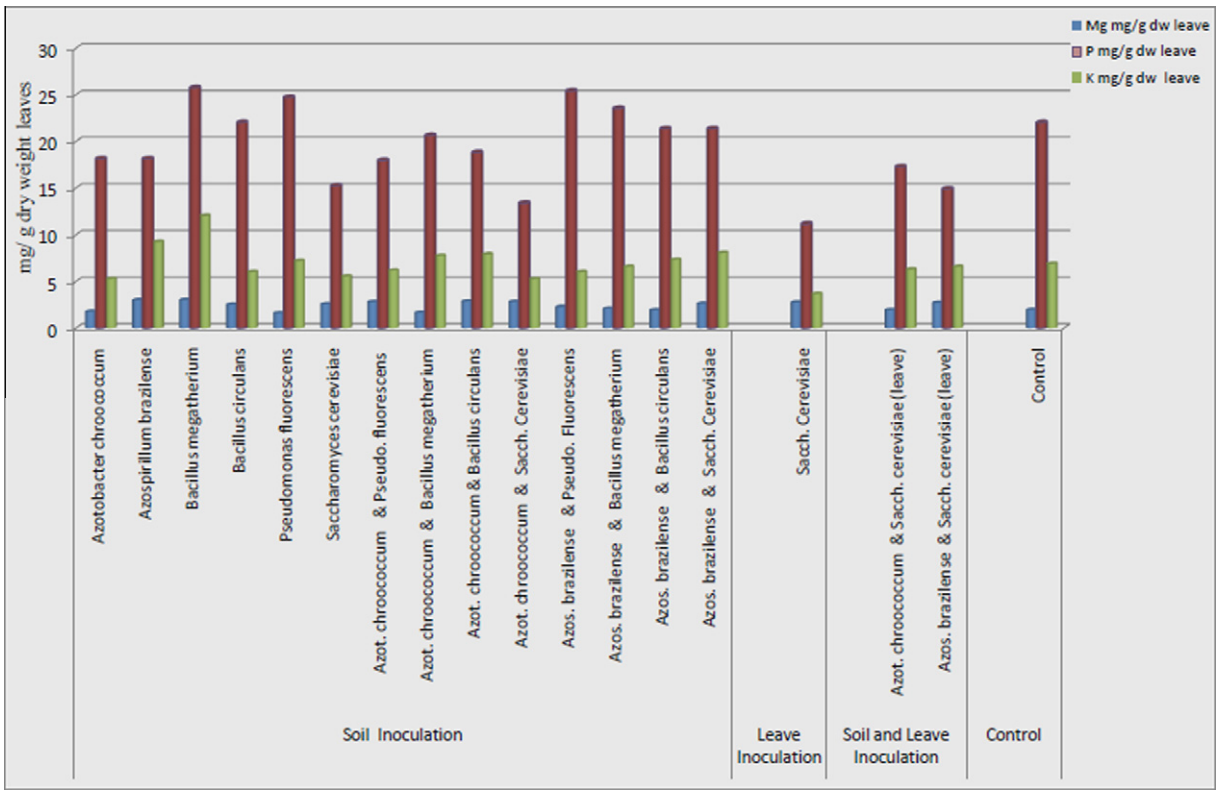

Figure 2 Effect of microbial biofertilizers on Mg, P and K contents of *Moringa* leaves (mg/g leaves dry weight).

Table 6 Effect of inoculation with different biofertilizers on the copper content of *Moringa* leaves cultivated on pots for three months.

Treatments	Cu mg/g dry weight leaves
Soil inoculation	
Azotobacter chroococcum	0.003
Azospirillum brazilense	0.003
Bacillus megatherium	0.003
Bacillus circulans	ND
Pseudomonas fluorescens	ND
Saccharomyces cerevisiae	ND
Azot. chroococcum and *P. fluorescens*	ND
Azot. chroococcum and *B. megatherium*	ND
Azot. chroococcum and *B. circulans*	ND
Azot. chroococcum and *S. cerevisiae*	ND
Azos. brazilense and *P. fluorescens*	ND
Azos. brazilense and *B. megatherium*	ND
Azos. brazilense and *B. circulens*	ND
Azos. brazilense and *S. cerevisiae*	ND
Leaf inoculation	
S. cerevisiae	ND
Soil and leaves inoculation	
Azot. chroococcum (soil) and *S. cerevisiae* (leaf)	ND
Azos. brazilense (soil) and *S. cerevisiae* (leaf)	ND
Control	ND

ND = not detected

The treatments (10, 16, 18) which are (*Azot. chroococcum* and *B. megatherium*), (*Azos. brazilense* and *B. circulans*) and (*Azos. brazilense* and *S. cerevisiae* (soil)) were the treatments showing most similarity to control.

The other treatments were different from the control. According to the similarity to each others we classify the treatments into groups. The treatments (3, 11) [*Azos. brazilense* and (*Azot. chroococcum* and *B. circulans*)] and (6, 14) [*P. fluorescens* and (*Azos. brazilense* and *P. fluorescens*)] gave a group of similar results for most of the recorded parameters. While the treatments, (2, 12), (5, 15) and (8, 13, 17) which are [*Azot. chroococcum* and (*Azot. chroococcum* (soil) *S. cerevisiae* (leaf))], [*B. circulans* and (*Azos. brazilense* and *B. megatherium*)] and [*S. cerevisiae* (soil), (*Azot. chroococcum* and *S. cerevisiae* (soil)) and (*Azos. brazilense* (soil) and *S. cerevisiae* (leaf))] gave data similar to each other.

The treatment (4) *B. megatherium*, (7) *S. cerevisiae* (leaves) and (9) [*Azot. chroococcum* and *P. fluorescens*] gave distinct results from each other and from other treatments.

From this discriminate analysis we can conclude that inoculation with *B. megatherium* gave the best results for all data recorded.

Bivariate correlations between parameters recorded

From Table 7, it could be concluded that:

Leaves fresh weight (g) was positively correlated with leaves dry weight, shoot length (cm), shoot dry weight (g), Magnesium (mg)/g leaves dry weight at 0.01 level and with root length (cm), root dry weight (g), and Iron (mg)/g leaves dry weight at 0.05 level. The leaves dry weights were positively correlated with shoot length (cm) at 0.01 level and with shoot dry weight (g) and root dry weight (g) at 0.05 level.

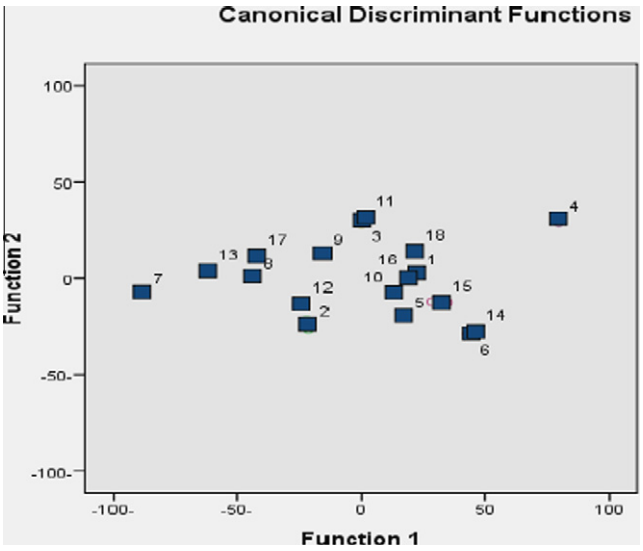

Figure 3 Canonical discriminant analysis of the applied treatments. 1 = control; 2 = *Azotobacter chroococcum*; 3 = *Azospirillum brazilense*; 4 = *Bacillus megatherium*; 5 = *Bacillus circulans*; 6 = *Pseudomonas fluorescens*; 7 = *Saccharomyces cerevisiae* (Leaves); 8 = *S. cerevisiae* (Soil); 9 = *Azot. chroococcum* and *P. fluorescens*.; 10 = *Azot. chroococcum* and *B. megatherium*; 11 = *Azot. chroococcum* and *B. circulans*; 12 = *Azot. chroococcum* and *S. cerevisiae* (leaves); 13 = *Azot. chroococcum* and *S. cerevisiae* (soil); 14 = *Azos. brazilense* and *P. fluorescens*; 15 = *Azos. brazilense* and *B. megatherium* ; 16 = *Azos. brazilense* and *B. circulans*; 17 = *Azos. brazilense* and *S. cerevisiae* (leaves); 18 = *Azos. brazilense* and *S. cerevisiae* (soil).

The shoot length (cm) positively correlated with root dry weight (g) at 0.01 level and with shoot dry weight (g) and iron (mg)/g dry weight leaves at 0.05 level. Root length (cm), was positively correlated with shoot dry weight (g), phosphorus contents (mg)/g leaves dry weight and vitamin C (g)/g leaf fresh weight at 0.05 level. Shoot dry weight (g), was positively correlated with root dry weight (g) and Iron (mg)/g leaves dry weight at 0.05 level. Root dry weight (g) has no correlation with other parameters.

Magnesium (mg)/g leaves dry weight was positively correlated with manganese and iron (mg)/g leaves dry weight at 0.01 level, and was negatively correlated with phosphorus (mg)/g leaves dry weight at 0.05 level. Phosphorus (mg)/g leaves dry weight was positively correlated with potassium, zinc (mg)/g leaves dry weights and with protein g/g leaves dry weight at 0.01 level.

Potassium (mg)/g leaves dry weight was positively correlated with iron, zinc (mg)/g leaves dry weight and with protein (g)/g leaves dry weight at 0.01 level, and it was negatively correlated with vitamin C (g)/g leaves fresh weight at 0.05 level. Zinc (mg)/g leaves dry weight was positively correlated with iron (mg)/g leaves dry weight and protein (g)/g leaves dry weight at 0.01 level. The copper (mg)/g leaves dry weight has not any correlation with all parameters.

Manganese (mg)/g dry weight leaves were negatively correlated with vitamin C contents g/g leaf fresh weight at 0.01 level. Iron (mg)/g leaves dry weight was positively correlated with protein (g)/g leaves dry weight at 0.05 level. Vitamin C (g)/g leaves fresh weight was negatively correlated with protein (g)/g leaves dry weight at 0.05 level.

Table 7 Bivariate correlations between parameters recorded.

	Leaf (g) f.w.	Leaf (g) d.w.	Shoot (cm)	Root (cm)	Shoot (g) d.w.	Root (g) d.w.	Mg	P	K	Zn	Cu	Mn	Fe	Vitamin C
Leaf d.w.	.726**													
Shoot cm	.483**	.518**												
Root cm	.292*	.184	.130											
Shoot d.w.	.349**	.333*	.323*	.321*										
Root d.w.	.332*	.329*	.461**	.246	.279*									
Mg	.491**	.227	.075	.058	.086	.185								
P	−.074	−.191	.086	.346*	.003	−.028−	−.329*							
K	.247	−.008	.144	.215	.145	.080	.243	.575**						
Zn	.189	−.049	.177	.054	−.004	.088	.261	.461**	.405**					
Cu	0	0	0	0	0	0	0	0	0	0				
Mn	.022	−.047	−.175	−.167	−.086	−.040	.362**	−.196	.006	.048	0			
Fe	.277*	.138	.272*	.254	.287*	.263	.349**	.225	.591**	.447**	0	−.032		
VitaminC	.094	.238	.100	.306*	.157	.162	.045	−.177	−.279*	−.206	0	−.564**	.134	
Protein	.169	−.171	.079	−.147	−.032	−.058	.231	.496**	.774**	.571**	0	.201	.319*	−.609**

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

Biologically active products, which called microbial inoculants, containing active strains of selective microorganisms like *Azot. chroococcum*, *Azos. brazilense*, *B. megatherium*, *B. circulans*, *P. fluorescens* and *S. cerevisiae*, either individually or in combinations, help in increasing the plant growth by biological nitrogen fixation, phosphate solubilization, potassium mobilization, nutrient uptake and plant growth promoting substances production. In the present study, the increase of growth may be attributed to an improved uptake of nitrogen, plant growth promoting substances and nutrient uptake in seedlings. Nitrogen fixing bacteria of the genera *Azospirillum* sp. and *Azotobacter* sp. have promoted plant growth of agronomically important field crops by 10–30% in the field experiment (Okon, 1985; Okon and Labandera-Gonzalez, 1994; Sumner, 1990). Similarly in the present study seedlings treated with bioinoculants showed better growth and root biomass compared to control. Growth may be attributed to an increase of root biomass, accumulation of nitrogen, and the production of gibberellins and cytokinin like substances (Tien et al., 1979) which promote the growth of seedlings. The total protein content was found to be maximum in seedlings inoculated with *Azos. brazilense* and *B. megatherium*. These results are in agreement with other findings of (Singh et al., 1999) by the greater supply of nitrogen for growing tissues. But not all such trials are successful and there are even cases where a decline in yield was associated with inoculation (Nguyen et al., 2002), this may reflect incompatibilities between bacterial strains and plant cultivars, as well as adequate soil-N for nutrition, as noted. It is also notable that a large number of different diazotrophic as well as non diazotrophic species may contribute to the beneficial effects on the growth and yield, such as *Azot. chroococcum, Azos. brazilense, B. megatherium, B. circulans, P. fluorescens* and *S. cerevisiae*. There is little evidence of clearly preferred combinations of plant and microbial species to obtain beneficial effects, although some studies have suggested variation in response based on genotype (Han and New, 1998).

Conclusions

This study can infer that under appropriate management, the use of more efficient biofertilizers leads to an increased growth and biomass of *M. oleifera*. The present study has clearly shown that the combined application of biofertilizers might play a significant role in improving the growth response and nutrient uptake of *M. oleifera* seedlings thereby producing good quality planting stock. These seedlings may perform better growth, survival and more biomass production in nutrient impoverished soil. From the present work, it could be concluded that biofertilizers have profound effects on the nutrient content of *M. oleifera* leaves. Different microbial inoculants and different types of inoculation result in a different nutrition quality for *Moringa* leaves. However, there is still a need to investigate the effects of different microbial inoculants on other components of *Moringa* leaves.

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