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Evaluation of biofertilization on antimicrobial activity and phytochemical profile of *Moringa olifera* plant

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Abstract:

Pot experiment was carried out during May 2019 at private farm in el Manyial, Cairo, Egypt. The investigation target was to find out the difference between using plant growth promoting rhizobacteria (*B. cereus*, *Streptomyces chibaensis*, *B. megaterium*, *B. polymyxa*) either individual or in combination with each other and mineral fertilization on antimicrobial activity and some phytochemical constituents of *Moringa olifera* leaves. The highest values of antimicrobial activity recorded for mineral fertilization on treatment followed by mixed biofertilization treatment (GP4) (*B. megaterium*, *B. polymyxa*) against seven foodborne pathogens *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Rhizopus stolonifer* and *Botrytis cinera*, as compared with the control treatment. Biofertilization treatment (*B. megaterium*, *B. polymyxa*) recorded increasing over control in (GP10) by 160.8% and decreasing of chemical fertilization by 5.41% for polyphenol content, also, the antioxidant content shown increasing over the control (GP10) by 81.1% and decreasing of chemical fertilization (GP9) by 5.6% as well as vitamin C and A were increased with mineral and biofertilizer (GP4) treatments after 55 days. Biofertilization in (GP4) recorded increasing over control group (GP10) by 48.89%, 27.08% and 58% for vitamin A, C and chlorophyll respectively. Ethyl acetate extract was found to be most potent extract than ethanol and water against all tested microorganism.

Keywords: *Moringa olifera*, PGPR, antimicrobial activity, biofertilizers.

1. Introduction

Foodborne diseases are a global problem with significant impact on human health. Deteriorated food produces defects like change in color, odor, texture and appearance [16]. Bacteria, moulds, and yeasts are three types of microorganisms that cause food to spoil. Typical foodborne illnesses such as diarrhoea, fever, stomach cramps, and dysentery are caused by these germs. [20]. Mycotoxins are poisons produced by fungus that contaminate food and feed. [65]. Kidney and Liver damage in animals are acute diseases associated with mycotoxins [22].

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Plants have long been an important source of natural compounds for human health. [69]. Plants having recognised antibacterial characteristics can be quite useful in the treatment of diseases [16].

Various extraction procedures, such as water, methanol, ethanol, n-hexane, chloroform, butanol and acetone, have been used to study the potential antimicrobial activity of a variety of plant species

The antimicrobial activity of these extracts has been tested against a variety of pathogen isolates. [59,66].

Several studies have shown that plant extracts could be a possible source of antibacterial agents for treating typical infections caused by bacteria including *H. pylori*, *S. aureus*, *E. coli*, and others.

Several mechanisms of action have also been proposed for the chemical components that may be present in these plant extracts, such as flavonoids (Rutin and Quercetin), tannins, and others by [6, 66, 7].

All components of medicinal plants, such as the root, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil, have been used to treat a variety of ailments, including inflammation and viral diseases, as well as cardiovascular, gastrointestinal, haematological, and hepatorenal disorders [11].

Moringa (*Moringa olifera* Lam.) is a monogenoeric species belonging to the family:

The Moringaceae Order (Brassicales) contains 13 species of trees and shrubs belonging to the old world tropics [29]. *Moringa olifera* is a plant that is native to northwest India. [52], but at present it is widely distributed in the tropics throughout the pacific region [15], west Africa [55,38] as well as central America and the caribbean [52, 30]. It's primarily grown in the tropics and subtropics. It is applied in the food, pharmaceutical, and industrial industries. Moringa leaves have been reported to be a good source of natural antioxidants and antimicrobials, as well as a rich source of carotene, protein, vitamin C, calcium, and potassium; thus, the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids helps to extend the shelf life of fat-containing foods. [23, 62]. For a long time, this plant's medical value has been widely documented [1]. Moringa biomass yields of over a pound of dry matter can be reached under intense farming settings [30], and large-scale cultivation has begun in the previous decade [2]. As a result, strategies to improve Moringa growing in Egypt and its nutritional content so that it may be used for various reasons are needed. Plant growth promoting rhizobacteria (PGPR) are a type of bacteria found in the rhizosphere that colonise plant roots (rhizosphere) and promote plant growth [31,10]. The

effects of PGPR on plant growth, such as better germination, increased yield, and disease resistance against a wide range of pathogens via inducing systemic resistance in plants, have been widely investigated [54]. Chemical fertilizers are one of the problems that human societies face today because of the pollution they cause in the environment and the long-term effects they have on human health, so using biological fertilizers can help reduce chemical fertilizer use. The aim of work was to evaluate of biofertilization on antimicrobial activity and phytochemical profile of *Moringa olifera* plant.

2. Material and methods

2.1 Seeds of *Moringa*

Seeds purchased from Moringa Unit, National Research Center, Dokki, Egypt.

2.2 Soil texture used

Table (1): The chemical and mechanical analysis of soil

Chemical analysis	pH value	8.30
	Electrical conductivity (EC) at 25°C	0.38 ds/m
	Available N	1.44 ppm
	Saturated percentage (SP)%	12%
Soluble Cations and anions (meq/L)	Co ³⁻⁻	0.00
	HCo ³⁻⁻	1.31
	Cl ⁻	1.27
	SO ⁴⁻⁻	1.22
	Ca ⁺⁺	1.51
	Mg ⁺⁺	0.43
	Na ⁺	1.62
	K ⁺	0.04
Mechanical analysis	Coarse sand (%)	17.87
	Fine sand (%)	75.90
	Silt (%)	4.37
	Clay (%)	1.86
	Soil texture	Sand

2.3 Microorganisms used

1- Bioagents (PGPR): *Bacillus cereus*, *Bacillus megaterium*, *Bacillus polymyxa* and *Streptomyces chibaensis*

Were obtained from Microbiology Laboratory, Faculty of Women, Ain Shams University.

2- Food pathogen microorganisms

Staphylococcus aureus ATCC 6538, *Psuedomonas aeruginosa*, *E. coli* ATCC 10536 and *Salmonella typhimurium* ATCC 14028, were obtained from TCS Bioscience LTD, Botolph Claydon, Buckingham, MK 18 2LR, England. *Aspergillus niger*, *Rhizopus stolonifer* and *Botrytis cinera* were isolates and identified by The Regional Center for Mycology and Biotechnology, al Azhar University.

2.4 Chemical fertilizers

Three commercial fertilizers namely; urea (46%), super phosphate (15% P₂O₅) and potassium sulphate (48% K₂O), were used as nitrogen source, phosphate and potassium (NPK) respectively.

2.5 Cultural media: Two different media were used for the growth of the tested bioagents and food pathogen microorganisms.

- 1. Standard plate count agar (Himedia laboratories):** is a microbiological growth medium commonly used to assess total or viable bacterial growth [9].
- 2. Sabouraud Dextrose Agar (OXOID):** acidic pH medium for the isolation of dermatophytes, other fungi and yeasts (Sabouraud, 1910) [57].

2.6 Experimental technique

To remove all dirt particles, *Moringa olifera* seeds were thoroughly cleaned in water. Seeds were steeped in water for three days, with the water changed twice daily, before being planted in pots. At private Farm El Manyial, a completely randomised indoor experiment design was used to create the pot experiment. Cultivation was done during spring 2019 in pots with a diameter of 30 cm and a depth of 35 cm, filled with 1 kg of soil. *Moringa olifera* seeds were sown at a rate of three seeds per square foot and then watered as needed. Healthy seedlings were separated into four groups and inoculated with 5 ml (1.5x10⁶ CFU/ml) of each biofertilizer after 15 days from planting day and every 10 days for the duration of the experiment: as following:

1. Soil inoculation with single bioagents
2. Soil inoculation with mixed bioagents
3. Control (without microbial inoculants)

4. Soil inoculation with chemical fertilizers (NPK 19% 19% 19%)

Three replicates were made for each treatment. After 55 days samples of leaves were collected to monitor the effect of inoculation on antimicrobial effects and phytochemical content were recorded.

2.7 Screening for Synergism In vitro

Five PGPR isolates were screened *in vitro* for synergistic screening as the trials below shown. 5 µl drops of each isolate culture (10^7 CFU/ml) were streaked equidistantly on of the standard plate count medium plates between the two parallel streaks of the two tested isolates at distance of 20 mm from each other (center to center) as designed below. Plates were incubated at 37°C for 16- 20 hours. Control plates not inoculated with bacteria. Synergistic effect was assessed by the ability to each isolate to grow in a good manner in presence of the other one [72,73]. The positive synergistic used to inoculate the soil to investigate their efficacy of Plant Growth Promoting Rhizobacteria (PGPR) on growth parameters of *Moringa olifera* plants in pot experiment. **The trials in vitro:**

1- <i>Bacillus megaterium</i>	+	<i>Bacillus polymyxa</i>
2- <i>Bacillus megaterium</i>	+	<i>Streptomyces chibaensis</i>
3- <i>Bacillus megaterium</i>	+	<i>Bacillus subtilis</i>
4- <i>Bacillus cereus</i>	+	<i>Bacillus polymyxa</i>
5- <i>Bacillus cereus</i>	+	<i>Streptomyces chibaensis</i>
6- <i>Bacillus cereus</i>	+	<i>Bacillus subtilis</i>
7- <i>Bacillus subtilis</i>	+	<i>Bacillus polymyxa</i>
8- <i>Bacillus subtilis</i>	+	<i>Streptomyces chibaensis</i>
9- <i>Bacillus polymyxa</i>	+	<i>Streptomyces chibaensis</i>
10- <i>Bacillus megaterium</i>	+	<i>Bacillus cereus</i>

***Among all of possible PGPR combinations, only four cases of synergistic effect were detected; both effects were among the following**

1- <i>Bacillus cereus</i>	+	<i>Streptomyces chibaensis</i>	(GP1)
2- <i>Bacillus cereus</i>	+	<i>Bacillus megaterium</i>	(GP2)
3- <i>Bacillus cereus</i>	+	<i>Bacillus polymyxa</i>	(GP3)
4- <i>Bacillus megaterium</i>	+	<i>Bacillus polymyxa</i>	(GP4)

*** The single PGPR strains used in this experiment**

5- <i>Bacillus cereus</i>	(GP5)
6- <i>Bacillus polymyxa</i>	(GP6)
7- <i>Bacillus megaterium</i>	(GP7)

8- *Streptomyces chibaensis*

(GP8)

*Chemical treatment by (NPK 19% 19% 19%)

(GP9)

* Control group

(GP10)

2.8 Extract preparation:

After harvest 55 day, leaves from each treatment of ten groups were weighed as fresh, washed with tap water, sterile distilled water, oven dried, and ground into powder, then soaked in 1000 ml of water or organic solvents (70% ethyl alcohol, ethyl acetate) until exhaustion (1-3 times), in a warm water bath (up to 40°C), with frequent shaking from time to time. Under reduced temperature (40°C) and pressure, the extracts were filtered and concentrated. The extract was then kept in a dark glass bottle in the refrigerator at 4°C according to the methods described by [70, 19, 53].

2.9 Parameters measures

1- Antimicrobial susceptibility test:

The antibacterial properties of *Moringa olifera* leaf extracts against food pathogen microorganisms were determined using the agar well diffusion technique. 100 µl of extract were soaked in plate wells using sterile cotton swabs from standardizing inoculum (1.5×10^6 CFU/ml) that were made for each bacterial, mould strain and streak onto the target medium. For bacteria, the plates were incubated at 35-37°C for 18-24 hours, while for fungi; they were incubated at 22-24°C for 3-5 days. The diameter of the inhibition zone surrounding the well (including the well diameter) in millimeters was used to measure antimicrobial activity (mm). In the control wells, distilled water or solvents were used. The tests were carried out in threes according to [3, 25].

2- Phytochemical (analysis).

The prepared extracts were subjected to standard preliminary phytochemical analysis for the presence of total polyphenol, total chlorophyll, antioxidant percent, vitamin A and C at the Faculty of Agriculture, Ain Shams University's Central laboratory.

I. Quantitative estimation of the total polyphenol contents.

The Folin–Ciocalteu Colorimetric Method was used to evaluate the total amounts of phenolic compounds present in the natural extracts. [63, 28].

II. Estimation of Total chlorophyll for the most potent *Moringa* leaves extract

Total chlorophyll calorimetrically measured in *Moringa* leaves after 55 days using method of [42]

III. Determination of antioxidant activity using method of [18].**IV. Estimation of Vitamin C and Vitamin A**

The Association of Official Analytical Chemists (AOAC) official methods No., 967.21 and 980.45 [12, 13] were used to determine the vitamin C (ascorbic acid) and vitamin A (b-carotene) respectively.

2.10 Statistical analysis

Using the Costat software program Version 6.303 (2004), the data were subjected to a suitable statistical analysis of variance according to [64]. After validating the error homogeneity, the data was subjected to a combined analysis of variance. Duncan's multiple range tests were used to assess differences in means at the 0.05 probability level.

3. Results

Table (2): The effect of plant growth promoting rhizobacteria (PGPR) on antibacterial activity of *Moringa olifera* leaves extracts growing in sand soil against foodborne pathogens bacteria after 55 days cultivation

Food pathogen	<i>Staph aureus</i>				<i>Salmonella typhimurium</i>				<i>E.coli</i>				<i>Pseudomonas aeruginosa</i>			
	Inhibition zone (mm)															
Solvents Treatment	Aqueous	Ethanol	ethyl acetate	Mean	Aque-ous	Ethanol	ethyl acetate	Mean	Aque-ous	Ethanol	ethyl acetate	mean	Aque-ous	ethanol	ethyl acetate	mean
GP (1)	6.0 J	10.0H	14.0 DE	10.0 D	7.0 IJ	6.0 J	16.0 D	9.67 F	6.0 H	12.0 E	15.0 D	11.00 D	0 J	10.0 I	12.0 GH	7.33 D
GP (2)	6.0 J	11.0 GH	18.0 C	11.67C	6.0 J	9.0GH	19.0 BC	11.33 CD	6.0 H	12.0 E	17.0 C	11.66 CD	0 J	11.0 HI	15.0 DE	8.67 C
GP (3)	7.0 IJ	10.0 H	13.0 EF	10.0 D	7.0 IJ	8.0 HI	19.0 BC	11.33 CD	7.0 H	11.0 EF	16.0 CD	11.33 CD	0 J	13.0 FG	15.0 DE	9.33B C
GP (4)	8.0 I	15.0 D	21.0 B	14.67 B	8.0 HI	12.0 F	20.0 B	13.33 B	7.0 H	15.0 D	23.0 A	15.0 B	0 J	16.0 CD	22.0 A	12.6 A
GP (5)	6.0 J	12.0 FG	15.0 D	11.00 C	6.0 J	8.0 HI	18.0 C	10.67 E	6.0 H	11.0 EF	17.0 C	11.33 CD	0 J	10.0 I	10.0 I	6.67 D
GP (6)	6.0 J	11.0G H	14.0 DE	10.33 DE	7.0 IJ	8.0 HI	18.0 C	11.0 DE	6.0 H	10.0 FG	19.0 B	11.67 CD	0 J	10.0 I	11.0 HI	7.00 D
GP (7)	7.0 IJ	11.0G H	15.0D	11.00 C	7.0 IJ	9.0 GH	19.0 BC	11.66 C	6.0 H	11.0 EF	19.0 B	12.00 C	0 J	12.0 GH	17.0 BC	9.67B
GP (8)	7.0 IJ	11.0G H	14.0DE	10.67 DE	6.0 J	10.0G	16.0 D	10.67 E	6.0 H	9.0 G	16.0 CD	10.33 DE	0 J	13.0 FG	14.0 EF	9.00B C
GP (9)	10.0 H	17.0C	24.0A	17.0 A	10.0G	15.0DE	22.0 A	15.67 A	10.0 FG	17.0 C	22.0 A	16.33 A	0 J	18.0 B	22.0 A	13.3 A
GP (10)	7.0 IJ	8.0I	11.0G H	8.67 F	6.0 J	6.0 J	14.0 E	8.67 G	6.0 H	9.0 G	15.0 D	10.00 E	0 J	10.0 I	10.0 I	6.67 D
Mean	7.0 c	11.6 b	15.9 a		7.0 c	9.1 b	18.1 a		6.6 c	11.7 b	17.9 a		0 C	12.3 B	14.8 A	

Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Key: Gp(1): *B.cereus* +*Streptomyces* GP(2): *B.megatrium*+*B.cereus* GP(3): *B.polymyxa*+*B.cereus* GP(4): *B.polymyxa*+ *B.megatrium*
 GP(5): *B.cereus* GP(6): *B.polymyxa* GP(7): *B.megatrium* GP(8):*Streptomyces* GP(9): chemical fertilizers group. GP(10): Control group

Table (3): The effect of plant growth promoting rhizobacteria (PGPR) on antifungal activity of *Moringa olifera* leaves extracts growing in sand soil against foodborne pathogens fungi after 55 days cultivation

Food pathogen	<i>Aspergillus niger</i>				<i>Rhizopus stolonifer</i>				<i>Botrytis cinerea</i>			
	Inhibition zone (mm)											
Solvents treatment	Aqueous	Ethanol	ethyl acetate	Mean	Aqueous	Ethanol	ethyl acetate	Mean	Aqueous	ethanol	ethyl acetate	Mean
GP (1)	8.0 cd	6.0 ef	7.0 de	7.0 D	6.0 fg	7.0 ef	9.0 cd	7.33 D	6.0 gh	6.0 gh	10.0 cd	7.33 BC
GP (2)	9.0 bc	8.0 cd	8.0 cd	8.33 BC	7.0 ef	8.0 de	11.0 ab	8.66 BC	7.0 fg	6.0 gh	9.0 de	7.33 BC
GP (3)	6.0 ef	6.0 ef	6.0 ef	6.0 E	6.0 fg	5.0 g	10.0 bc	7.0 DE	6.0 gh	6.0 gh	8.0 ef	6.67 C
GP (4)	9.0 bc	8.0 cd	10.0 ab	9.0 B	7.0 ef	7.0 ef	12.0 a	8.66 BC	7.0 fg	8.0 ef	13.0 a	9.33 A
GP (5)	6.0 ef	8.0 cd	8.0 cd	7.33 D	6.0 fg	6.0 fg	10.0 bc	7.33 D	6.0 gh	6.0 gh	11.0 bc	7.66 BC
GP (6)	6.0 ef	7.0 de	9.0 bc	7.33 D	6.0 fg	7.0 ef	7.0 ef	6.66 DE	6.0 gh	7.0 fg	10.0 cd	7.67 BC
GP (7)	6.0 ef	8.0 cd	10.0 ab	8.0 BC	7.0 ef	9.0 cd	11.0 ab	9.0 B	6.0 gh	7.0 fg	12.0 ab	8.33 AB
GP (8)	6.0 ef	8.0 cd	7.0 de	7.0 D	6.0 fg	9.0 cd	8.0 de	7.66 D	6.0 gh	6.0 gg	10.0 cd	7.33 BC
GP (9)	9.0 bc	11.0 a	11.0 a	10.3 A	9.0 cd	11.0 ab	11.0 ab	10.3 A	7.0 fg	6.0 gh	10.0 cd	7.67 BC
GP (10)	8.0 cd	6.0 ef	7.0 de	7.0 D	8.0 de	6.0 fg	6.0 fg	6.66 DE	7.0 fg	6.0 gh	9.0 de	7.33 BC
Mean	7.3 B	7.6 B	8.3 A		6.8 C	7.5 B	9.5 A		6.4 B	6.4 B	10.2 A	

Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Key: Gp(1): *B.cereus* +*Streptomyces* Gp(2): *B.megatrium*+*B.cereus* Gp(3): *B.polymyxa*+*B.cereus* Gp(4): *B.polymyxa*+ *B.megatrium* Gp(5): *B.cereus*
 Gp(6): *B.polymyxa* Gp(7): *B.megatrium* Gp(8):*Streptomyces* Gp(9): chemical fertilizers Gp(10): Control group

Table (4): The effect of plant growth-promoting rhizobacteria (PGPR) on the phytochemical character of Moringa leaves cultivated on pots for 55 days in sand soil

Groups	Total polyphenols µg/ml	Total chlorophyll (mg/ml)	Antioxidants %	Vit.A mg/100gm	Vit. C Mg/100gm
GP 1	249.50 ^g	38.30 ^e	50.80 ^e	4.16 ^g	155.00 ^e
GP 2	320.00 ^c	51.00 ^b	71.78 ^{bc}	5.11 ^c	177.00 ^b
GP 3	253.30 ^g	37.90 ^e	53.50 ^e	4.33 ^f	161.00 ^d
GP 4	328.70 ^b	52.93 ^{ab}	73.10 ^b	5.21 ^b	183.00 ^a
GP 5	269.20 ^f	39.60 ^e	57.30 ^d	4.23 ^g	169.00 ^c
GP 6	278.50 ^e	43.50 ^d	67.50 ^c	4.83 ^d	167.00 ^c
GP 7	315.90 ^{cd}	50.19 ^{bc}	73.00 ^b	5.17 ^{bc}	182.00 ^{ab}
GP 8	309.80 ^d	48.00 ^c	66.60 ^c	4.48 ^e	173.00 ^{bc}
GP 9	346.50 ^a	55.13 ^a	77.20 ^a	6.31 ^a	189.00 ^a
GP 10	126.00 ^h	33.50 ^f	40.30 ^f	3.50 ^h	144.00 ^f

Means followed by the same letter are not significantly different ($P \leq 0.05$) according to

Duncan' s multiple range test

GP(1): *B.cereus* +*Streptomyces*

GP(2): *B.megatrium*+*B.cereus*

GP(3):

B.polymyxa+*B.cereus*

GP(4): *B.polymyxa*+ *B.megatrium*

GP(5): *B.cereus*

GP(6): *B.polymyxa*

GP(7): *.megatrium*

GP(8):*Streptomyces*

GP(9): chemical

fertilizers group.

GP(10): Control group

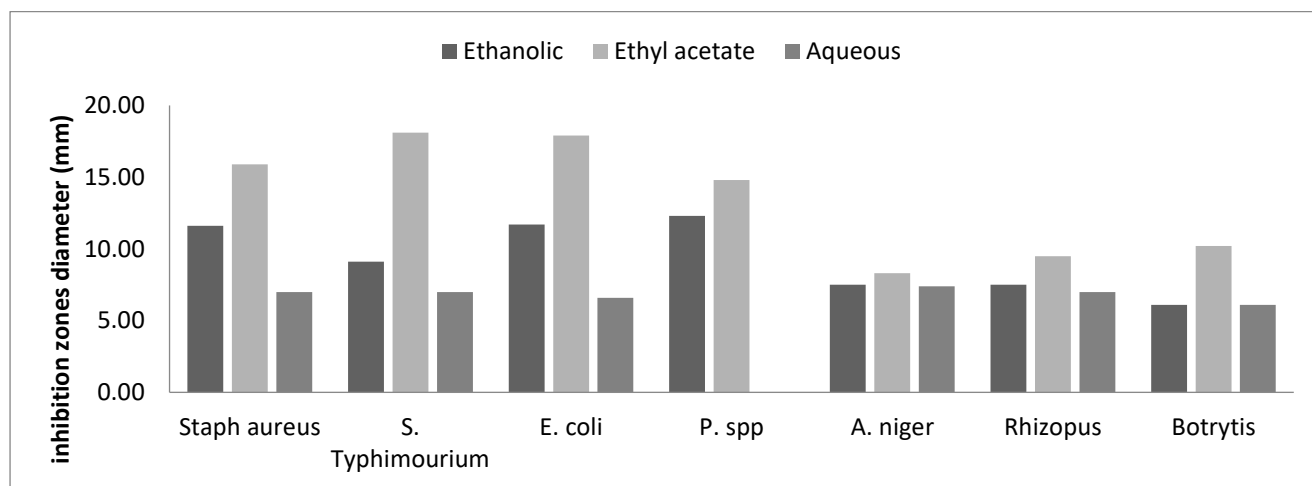


Figure (1): The mean value of inhibition zones diameter of microorganisms from Moringa leaves extracts.

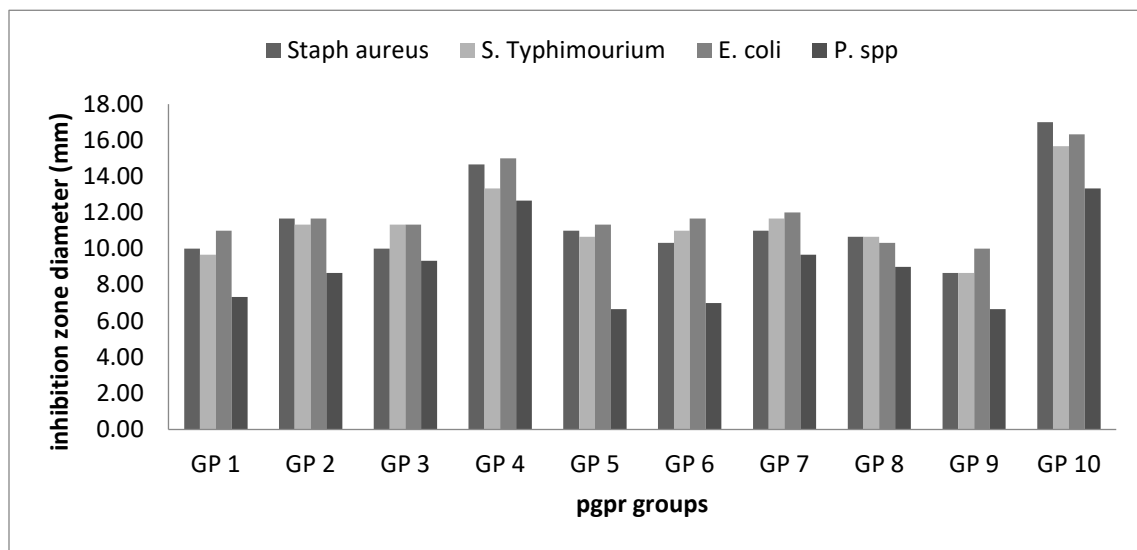


Figure (2): The mean value of inhibition zones diameter of food pathogen bacteria from PGPR groups

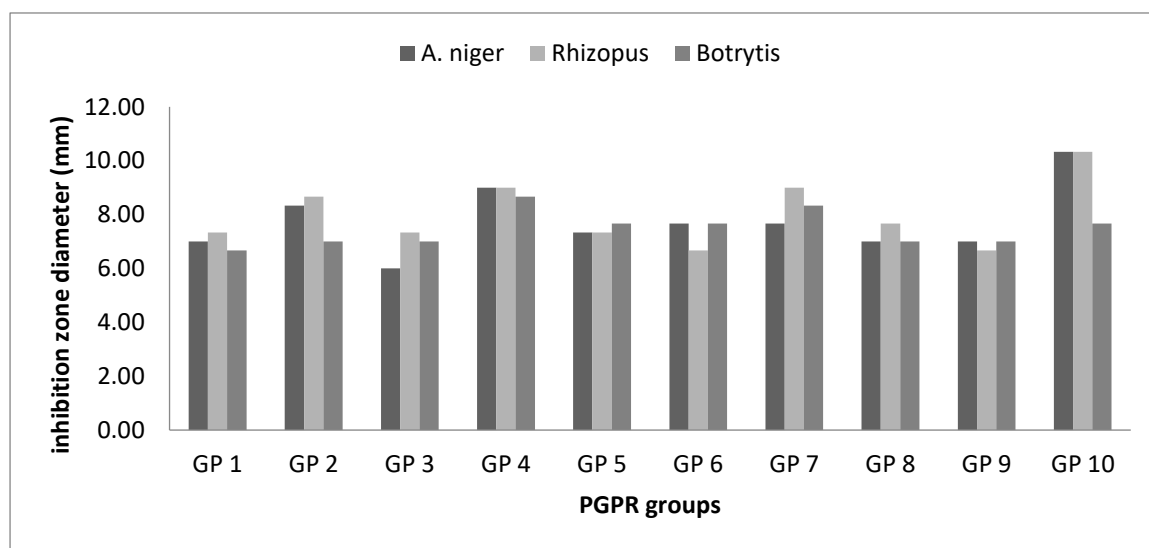


Figure (3): The mean value of inhibition zones diameter of food pathogen fungi and PGPR groups

The effect of plant growth promoting rhizobacteria (PGPR) on antibacterial activity of *Moringa olifera* leaves extracts growing in sand soil against foodborne pathogens after 55 days cultivation.

From the results in **Table (2)** and **fig. (1 and 2)**, it is markedly showed that ethyl acetate was found to be the strongest solvent and demonstrate the maximum inhibitory effect compared with ethanol and water against all the tested bacteria in all treatments used. It was noticed also, that applications of *Moringa olifera* seeds with mineral fertilizers and PGPR either individually or in mix formulations treatments gave the best results than the control treatments (GP10) in increasing the antibacterial activity of *Moringa* leaves extract against the tested bacteria. The most effective treatments (large zone of inhibition) were obtained with the application of mineral fertilization (GP9) followed by mix formulation treatments of PGPR (*B. polymyxa* + *B. megaterium*) or (GP4) treatment against *Staph. aureus*, *Salmonella typhimurium*, *E.coli* and *Pseudomonas aeruginosa*. These treatments recorded 17.0, 15.67, 16.33 and 13.3 mm for mineral fertilizers (GP9) while recorded 14.67, 13.33, 15.0 and 12.6 mm for (GP4) treatments against the tested bacteria respectively. The results also showed that the application of PGPR mix formulation treatment (*B. polymyxa* + *B. megatrium*) (GP4) were increased antimicrobial activity of *Moringa olifera* leaf extracts than application of PGPR as single formulation treatment ((*B. polymyxa* or *B. megatrium*). *Pseudomonas aeruginosa* proved to be the most difficult to inhibition by aqueous extract in all treatments of *Moringa olifera* plant extracts. This result may be due to permeability barrier provided by the cell wall or to the membrane accumulation mechanism [74].

The effect of plant growth promoting rhizobacteria (PGPR) on antifungal activity of *Moringa olifera* leaves extracts growing in sand soil against foodborne pathogens after 55 days cultivation.

As shown in **Table (3)** and **Fig. (1 and 3)** all plant extracts have no to moderate antifungal activity on the selected fungal species as compared to their antibacterial effect. Maximum enhancement of antifungal activity of moringa leaf extracts was seen in mineral fertilization treatments (GP9) followed by the mix formulation treatments of PGPR (*B. polymyxa* + *B. megatrium*) or (GP4) against *Aspergillus* and *Rhizopus* when compared with the control treatment (GP10). Their values recorded 10.3 and 10.3 mm for mineral fertilization treatments (GP9) while recorded 9.0 and 8.66 for (GP4) treatments against the tested fungi respectively. Results also showed that the mix formulation treatment (*B. polymyxa* + *B. megatrium*) represented the highest antifungal activity (with significant difference) of *moringa olifera* leaf extract against *Botrytis* when compared with mineral fertilizer treatment (GP9) and control (GP10).

The effect of plant growth promoting rhizobacteria (PGPR) on phytochemical content of *Moringa olifera* leaves extracts growing in sand soil after 55 days cultivation.

Data in **Table (4)** showed that the phytochemical content of *Moringa olifera* leaves fertilized with PGPR and mineral fertilizers behaviors were taken the same trends of antimicrobial activity where that the mineral fertilizers exhibited the highest effect on polyphenols, total chlorophyll, antioxidants, vitamin A, and C in leaves of *Moringa olifera* leaves followed by mix formulation treatments of PGPR (*B. polymyxa* + *B. Megaterium*) when compared with the control treatments (GP10). Polyphenol contents and antioxidant of values were recorded 346.5, 328.7 $\mu\text{g/ml}$, and 77.2, 73.0% for mineral fertilization (GP9) and biofertilization (GP4) treatments respectively. Biofertilization treatment as in (GP4) recorded increasing over control in (GP10) by 160.8% and decreasing of chemical fertilization by 5.41% for polyphenol content, also, the antioxidant content shown increasing over the control (GP10) by 81.1% and decreasing of chemical fertilization (GP9) by 5.6%. Data presented in Table (4) clearly indicated that as vitamin C and A were increased with mineral and biofertilizer (GP4) treatments after 55 days. Their highest values were reached using mineral fertilizer treatments followed by biofertilizers as in GP4 treatment. On the other hand biofertilization in (GP4) recorded increasing over control group (GP10) by 48.89%, 27.08% and 58% for vitamin A, C and chlorophyll respectively.

4. Discussion

Moringa olifera is regarded as one of the world's most beneficial trees because every portion of the tree can be utilized for food, medicine, or industrial purposes [29]. The use of biofertilizers or rhizosphere that promotes plant growth had a substantial impact on the growth and biomass of *Moringa olifera* plants. PGPR colonizes the plant rhizosphere and promotes plant growth through a variety of processes, including nitrogen fixation, phosphate solubilization, and nutrient availability and absorption [47]. In recent years, the usage of PGPR, either alone or in combination, has emerged as a viable alternative to chemical fertilizer, which has become one of the world's most pressing issues due to the pollution it causes. The amounts of harmful metals in phosphate fertilizers, as well as its impact on soil pollution, plant accumulation, and human health, are discussed. Due to its negative health effects, the introduction of numerous heavy metals into the human food chain via diverse agricultural products has received greater attention in recent years. Through the food chain, some potentially harmful metals and trace elements found in agricultural soils enter the human body easily. All of these elements are found in low concentrations in soils, and several are required for plants and animals. When large levels of important trace elements are present in the environment, they cause toxicity. [56]. Also, utilization of PGPR reduce the chemical fertilizer used and helping in achieving sustainability of forms [14,36,37]. PGPR has been shown to

have a favourable influence on plant growth and an antagonistic effect on plant pathogens in several studies. *Aicaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Brodyrhizobacteria*, *enterobacteria*, *serratia*, and *Streptomyces* are among the most successful rhizobacteria [67,41]. This study looked at the antibacterial and antifungal activities of *Moringa olifera* leaf extracts against the bacteria and fungi that were used. The current findings revealed that ethyl acetate extracts were more successful than ethanol extracts in inhibiting the growth of the investigated bacteria, whereas water extracts failed to do so. The polarity of the molecule being extracted by each solvent can be used to explain these findings [50]. Sharma *et al.*, (2011) [61] observed that ethanolic extracts were more efficient than aqueous extracts [49,2] found that the organic extracts showed that same or greater activity than the aqueous extracts. This may be due to that the most bioactive compounds from plants are generally soluble in polar solvents. These finding agree with that reported by [50,17] They confirmed that, when compared to other solvents such as methanol and ethanol, water is not a good solvent for extracting antimicrobial chemicals from medicinal plants. In addition, *Moringa olifera* leaf extracts had a higher level of antibacterial activity than their inhibitory impact against the tested fungus, according to the current data. This backs up other researchers' findings that pathogenic fungus is more resistant to plant extracts than harmful bacteria. [48,8,5] phenolic compounds could denature the enzymes responsible for spore germination or interfere with the amino acids involved in germination, phenolic compounds could denature the enzymes responsible for spore germination or interfere with the amino acids involved in germination, phenolic compounds could denature the enzymes responsible for spore germination, phenolic compounds could denature the enzymes responsible for spore germination, phenolic compounds could denature. The antibacterial and antifungal effect of soil fertilization with mineral and biofertilizers (PGPR) on *Moringa olifera* leaf extracts after 55 days of cultivation revealed that mineral fertilization (GP10) treatments followed by co-inoculation treatments (*B.polymyxa* +*B.megatrium*) or GP4 had the greatest inhibitory effect against the tested bacteria and fungi when compared to the control treatment. In comparison to PGPR treatments, which have an indirect influence on nutrient solubility in soil, mineral fertilizers were more soluble and quickly released nutrients for plant production. [4,43], where, Fertilizers cause serious environmental contamination notably in the agricultural soils. The dire necessity for increased food production has been more marked than ever before. Mineral fertilizers, which are indeed an important nutrient source used for enhanced food production, have unfortunately now become a 'necessary evil'. Excessive and continuous uses of nitrogen and phosphorous

fertilizers for decades have converted the agricultural soils into virtual chemical time bombs [24,46].

In comparison to *B. polymyxa* or *B. megatrium* as a single inoculant, co-inoculation of *B. megatrium* and *B. polymyxa* increased antibacterial and antifungal activities of moringa leaf extracts. This could be due to direct and indirect plant growth enhancements such as the creation of growth-promoting chemicals and the solubilization of minerals such as phosphorus [68, 35]. Also crucial in these activities is the synergistic link between these microorganisms in a dual compatible mixture. Inoculation of plants with selected PGPR can mobilize phosphorus from poorly accessible sources and improve plant nutrition, according to [45]. For *B. polymyxa* and *B. megatrium* in tomato plants, higher growth and phosphorus uptake have been documented [33]. The antibacterial effects of Moringa leaf extracts were similar to those reported by [39,34], who found antibacterial activity in *Moringa olifera* seed and leaf extracts. This could be owing to the presence of important phytochemicals like tannins, alkaloids, flavonoids, treponoids, and saponin [27,51, 26]. These phenolic compounds act directly on bacteria and impede growth by altering cell membrane production and/or critical enzyme synthesis. [44,40].

In this study, all fertilization methods increased total chlorophyll. This effect balances out the treatments' stimulating effect on the photosynthetic efficiency mechanism, leading in increased photosynthetic production and better mineral translocation from root to leaf. [58] The use of biofertilizers improved the chlorophyll A, B, and total carotenoid content of *Anethum graveolens* in plants, according to [32], who discovered that combining biofertilizers with varied NPK fertilizer rates considerably increased the chlorophyll index of marigold plants. The current study also found that all fertilization procedures increased vitamin C and A levels. The increase in secondary metabolite production under inoculation with PGPR go on line with [71,33] where they discovered that plants inoculated with PGPR have higher micronutrient content than plants produced traditionally. Because several chemical reactions in cells include minor elements, either directly or indirectly, biofertilizer plants produced more secondary metabolites.[21] found that the kind and value of fertilizer, as well as the rate at which it is applied, have a direct impact on the amount of nutrients available in plants, as well as an indirect impact on plant physiology and secondary metabolite (phytochemical) production. Based on the antioxidants analysis of *Moringa olifera* leaves the results of this experiment prove that all fertilization treatments detected an increase in antioxidant content compared with the control treatment. The combined treatment of PGPR which is (*B. polymyxa*

+ *B.megatrium*) enhances the antioxidant and defense enzyme activity. *Moringa olifera* plants can tolerate the diseases and stresses in a much better way with higher protein and phenolic content [60].

5. Conclusion

Based on these findings, it can be concluded that *Moringa olifera* leaf ethyl acetate extract has potent antifungal and antibacterial activity against the bacteria and fungi tested. When compared to a single formulation, mineral fertilization and combined application treatments (*B. polymyxa* + *B.megatrium*) could improve antifungal and antibacterial activity, total phenolic compounds, photosynthesis pigments, and antioxidant activity of *Moringa olifera* leaves extracts after 55 days of cultivation. *Moringa* leaves have varying nutrient quality depending on the microbial inoculants used and the type of inoculation used.

However, more research into the impact of various microbial inoculants on other moringa leaf components is required. Beneficial microorganisms could potentially be employed as an environmentally beneficial (eco- friendly) and long-term replacement for toxic pesticides used in pest control of plant diseases.

6. References

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الملخص العربي

تقييم المعاملات الحيوية على النشاط الكيميائي والمضاد للميكروبات لنبات المورينجا أوليفيرا

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الملخص العربي

أجريت تجربة الأصيص خلال شهر مايو 2019 بأحدى المزارع الخاصة بمنطقة المنيل - القاهرة ، مصر. الهدف من البحث هو معرفة الفرق بين استخدام البكتيريا الجذرية المعززة لنمو النبات (*B. cereus* ، *Streptomyces* sp. ، *B. megaterium* ، *B. polymyxa*) إما بصورة فردية أو مخلوطة وبين استخدام المواد الكيميائية على النشاط المضاد للميكروبات وبعض المكونات النباتية من أوراق المورينجا أوليفيرا. وقد سجلت أعلى قيمة للنشاط المضاد للميكروبات للنبات المعالج كيميائيا (مجموعة 9) متبوعاً بالنبات المعالج حيويًا (مجموعة 4) [*B. polymyxa* ، *B. megaterium*] وذلك ضد السبع ميكروبات المستخدمة في البحث *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Rhizopus stolonifer*, *Botrytis cinera* and وذلك مقارنة بمجموعه الكنترول (مجموعة 9). وسجلت نتائج التحليل لأوراق المورينجا المعاملة حيويًا بميكروبات (*B. polymyxa* ، *B. megaterium*) زياده في المحتوى البولي فينول عن مجموعة الكنترول (GP9) بنسبة 160.8% مع انخفاض عن مجموعه الاوراق المعاملة كيميائيا بنسبة 5.41%. وقد سجلت المواد المضادة للاكسدة زيادة عن مجموعة الكنترول (مجموعة 10) بنسبة بنسبة 81.1% مع انخفاض عن مجموعه الاوراق المعاملة كيميائيا (مجموعة 9) بنسبة 5.6% هذا بالإضافة الي زيادة في محتوى فيتامين C بنسبة 27.08% وفيتامين A بنسبه 48.89%. ومجموعه الكلوروفيل بنسبة 58% وذلك بعد 55 يوما من الزراعة. واثبتت المقارنة بين المستخلصات ان مستخلص الايثيل اسيتات هو الافضل تاثيرا ضد جميع الميكروبات المختبرة.