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Microbial Impact on Growth and Yield of *Hibiscus sabdariffa* L. and Sandy Soil Fertility

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ROSELLE plant is a valuable medicinal crop in arid and semi-arid regions. The use of microorganisms to enhance crop production is more favorable than chemical fertilizers attributable to food safety. A field experiment was implemented to inspect the impact of the bacterial mixture (*Bacillus subtilis* (BSR-8) and *Pseudomonas flourescens* (PSR-11)), *Pleurotus ostreatus* and mycorrhizeen® individually and /or in combination on the growth and yield of roselle plant and their impact on newly sandy soil. The tested bioagents significantly increased the growth and yield of roselle plant in comparable to the untreated plants. Also, inoculation increased soil dehydrogenase activity, root colonization and photosynthetic pigments. A significant enhancement in soil fertility properties occurred, where the soil NPK availability improved. The NPK concentrations and uptake increased in calyx and shoot in response to bioagents. Soil organic matter content and soil aggregates increased while EC and pH decreased. Generally, the application of microbial mixtures modified physio-chemical soil properties and consequently reflected on roselle yield production.

Keywords: Roselle, *Bacillus subtilis* (BSR-8), *Pseudomonas flourescens* (PSR-11), *Pleurotus ostreatus*, mycrrhizae

Introduction

Roselle (*Hibiscus sabdariffa* L.) is a tropical plant that belongs to family Malvaceae, and known in Egypt as Karkade. It is probably native of West Africa and widely distributed to other places of the world. The roselle calyces comprise of organic acids (tartaric, oxalic, malic, ascorbic and succinic acids), glucose, ß-carotene and lycopne, in addition to the anthocyanins delphinidin and cyandin. Roselle has also many medicinal properties. Moreover, the seeds contain sterols, including 3.2% ergosterol (Hashem et al. 2017). Agriculture is one of the human practices that contribute to increasing chemical pollutants as a consequence of excessive use of synthetic chemical fertilizers and pesticides, which cause further environmental devastations with possible risks to human health. Towards a sustainable agricultural perspective, crops produced needed to be equipped with resistance to disease, salt, drought, heavy metal stress, and better nutritional value (EL-Tapey et al. 2019). Bacteria known as plant growth promoting rhizobacteria (PGPR) are the most auspicious. PGPR may be used to enhance plant health and promote plant growth rate without environmental corruption (Vejan et al. 2016). Inoculating squash with salt-tolerant PGPR strains has a positive effect on nutrients uptake, growth characteristics and yield and yield components as well as fruits quality (Abdel-Rahman et al. 2021).

Soil fertility has deteriorated recently as a result of current intensive agricultural methods and cultivation of comprehensive crops by the farmers. The most important discipline for obtaining intense crop yield amongst resource-

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poor farmers is soil infertility particularly in developing nations. Therefore, preservation of soil fertility and quality is required to stabilize or improve the declining crop production levels (Sindhu and Sharma, 2020). Soils of Egypt are mostly sandy with low fertility and relatively low water holding capacity and are poor in mineral nutrients (El-Tapey et al. 2019).

Microorganisms present in soil cause mineralization and solubilization of nutrients through biogeochemical cycling and affect the chemical and biological properties of the soil leading to improved soil fertility. Thus, PGPR may act directly by increasing the acquisition of plant nutrients or by enhancing hormone levels on the plant surface, or may act indirectly by suppressing the diseases caused by the bacterial/ fungal plant pathogens (Sindhu and Sharma, 2020). Different physical and chemical properties of rhizospheric soil as soil pH, water potential, partial pressure of O_2 and release of root exudates were found to impact the survival, persistence and establishment of the supplemented PGPR strains in the rhizosphere of plants (Sindhu and Sharma, 2020). They can maximize plant nutrient uptake, increase plant growth, confer resistance to abiotic stress, and suppress disease. These living microorganisms are dynamic and potentially selfsustaining, reducing the need for repeated applications, and can avoid the problem of pests and pathogens evolving resistance to the treatments (Chaparro et al., 2012).

Uptake of nutrients such as phosphorus, nitrogen, zinc, iron, copper, potassium, magnesium, sulfur and other ions, is usually escalated by mycorrhizal inoculation of plants (Sembok et al., 2015 and Abdelhameid, 2020). Based on studies on roselle plant, 6 and 19% increase in leaf nitrogen and phosphorus contents with mycorrhizal hyphaes inoculation have been recorded (Mohammadpour-Vashvaei et al., 2015). In this respect, Taha et al. (2017) showed that the use of *Pleurotus columbinus* in association with *Azolla pinnata*, *Anabaena azollae* and *Azotobacter* spp. enhanced wheat productivity and soil fertility.

This study was conducted aspiring to impede the excessive use of chemical fertilizers. Some beneficial microbiota was used as a safe biological approach to reveal their effect on growth and yield parameters of roselle plant, in addition to their effect on physico-chemical soil properties.

Materials and Methods

Preparation of biocontrol agents

Bacillus subtilis (BSR-8), *Pseudomonas fluorescens* (PSR-11), *Pleurotus ostreatus* and mycorrhizal fungi were kindly obtained from Soils, Water and Environmental Research Institute (SWERI), Agriculture Research Center (ARC), Giza, Egypt.

Preparation of bacterial strains

Pseudomonas fluorescens and *Bacillus subtilis* strains were cultured individually in King'sand nutrient broth medium respectively in 250-ml flasks and placed on a rotary shaker at 120 rpm for 48 hr at 28±1°C. Then, the cell suspension of each strain was adjusted to provide 107 $CFUml^{-1}$. A mixture of the two bacterial strains was applied before planting as seed coating using Arabic gum, the boost addition of bacterial mixture was added after 30 days of planting as soil treatment at a rate of 20 L fed^{-1}.

Preparation of Pleurotus ostreatus

Glass bottles containing sterilized PD broth were inoculated with *Pleurotus ostreatus*. The bottles were incubated on a shaker at 250-350 rpm for 7 days at 25°C. The contents of the bottles were homogenized in a blender for 1 min and applied before planting as seed coating using Arabic gum, and the boost addition of bacterial mixture was added after 30 days of planting as soil treatment at a rate of 20 L fed^{-1}.

Preparation of mycorrhizal fungi

Mycorrhizeen® (is a commercial product containing mycorrhizal spores) was kindly obtained from Soil, Water and Environmental Research Institute (SWERI), Agriculture Research Center (ARC), Giza, Egypt. Mycorrhizeen® was added as a recommended dose of 10 gm mycorrhizeen®/ seed.

Biochemical activities of the bioagents

The ability of bio-agents to solubilize phosphate was determined according to Nguyen et al. (1992). Indole acetic acid was estimated according to the method of Glickmann and Dessaux (1994). Gibberellic acid was identified using the method of Udagwa and Kinoshita (1961). Total carbohydrate content was determined as glucose by the phenol sulphuric acid method (Dubois et al., 1956).One ml of the sugar solution was mixed with 1 ml of 5% redistilled phenol solution and then 5 ml sulphuric acid (AR). After cooling by standing for 5 min at room temperature, each tube was shaken and placed in water bath at 30 °C for

2 min. The produced yellow orange color was determined calorimetrically at 490 nm.

Agricultural practices

Seed treatments

Seeds of a bright cultivar (Light Red Sepals c.v.) of roselle (*Hibiscus sabdariffa* L.) were kindly obtained from Horticultural Research Institute, Agricultural Research Center, Giza. Seeds were surface disinfected by immersing in sodium hypochlorite (1%) for 2 min, and washed several times with sterilized water, then soaked for one hour before planting in the tested treatments as follows: (1)- Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens* (BM), (2)- *Pleurotus ostreatus* (PO), (3)- Mycorrhizeen® (M), (4)- BM+ PO, (5)- BM + M, (6)- PO + M, (7)- BM + (PO) + (M), (8)-uninoulated control.

Field experiment

A field experiment was carried out during the summer seasons of 2018/2019 to study the effect of the previous inoculation treatments on growth and yield of roselle plant and soil fertility in a newly reclaimed sand textured soil at Ismailia Agricultural Research Station, Ismailia Governorate located between 30° 36-46.88= to 30° 37- 11.79=Northing and 32° 14-26.66= to 32° 14-51.62= Easting and elevation three meters from the sea level (EL-Tapey et al., 2019) and was watered by sprinkling. The soil was classified as Typic Torriorthents, sandy, mixed, thermic, very deep according to USDA (2014).The experimental area was divided into equal plots of 3 x 3 m. Plots were arranged in split-plot design with three replicates for each treatment. The experimental unit consisted seven ridges 3 meter in length and 60 cm apart (plot area = 3 x 3.5 = 10.5 m^2). The distance between plants was 50 cm.

Before planting, all plots were received phosphorus as calcium super-phosphate (15.5 % P_2O_5) at a rate of 100 kg P_2O_5 fed^{-1} during soil preparation. Potassium was added in the form of potassium sulphate (48 % K_2O) at a rate of 60 kg K_2O fed^{-1} once before 1st irrigation. Nitrogen was added as ammonium sulphate (20.5 % N) at rate of 70 kg N fed^{-1} in three equal doses i.e., 1/3 at cultivation time as based fertilization, 1/3 at stem initiation stage and the remaining was applied at bud initiation before flowering stage.

Plant sampling and determination

Three replicates of each treatment were uprooted after 60 days of planting to determine dehydrogenase activity according to Skujins (1976). The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined quantitatively as described by Metzner et al. (1965). Microbial root colonization was observed using the method described by Bilal et al. (1993). Ten centimeters of cleaned fresh root samples were chopped off and any soil particles were eliminated, then immersed with 2 ml of 2.3.5-triphenyl tetrazolium chloride solution (TTC) in test tubes. Tubes were then incubated in dark at 30 ºC for 24 hr. After immersing roots in TTC, samples were left to dry in room temperature. Colonization was examined under light microscope where plant tissues and tetrazolium reduction were visible as red color due to reduction of tetrazolium by bacterial cells. The percentage of colonization was calculated as follows:

$$\text{Colonization length} = \text{Root length} - \text{Non-colonized length}$$

$$\text{Colonization \%} = \text{Colonization length} \times 100 \,\text{Root length}$$

Plant samples were collected at harvest stage after 160 days from each plot. Shoot length (cm), number of branches, number of calyces, fresh and dry shoot yields (ton $fed.^{-1}$), fresh and dry calyx yields (ton fed^{-1}) and seed yield (ton fed^{-1}) were calculated.

Total N, P and K were determined in the digested plant solution. Total nitrogen (N) form (NO_3^- and NH_4^+) was determined according to Markus et al. (1985) using micro kejldahl. Total phosphorus (P) was extracted by ammonium bicarbonate according to Soltanpour (1991) and determined by (ICP-Plasma JY). Total potassium (K) was determined by using flame photometer according to Jackson (1973).

Soil sampling and determinations

Soil samples were randomly collected to determine some physical and chemical characteristics for all plots after harvest of roselle plant. Particle size distribution and contents of organic matter and calcium carbonate were determined according to Burt (2004). Soil aggregate size distribution (%) was carried out according to Rouiller et al. (1972). The soil aggregate percentage was calculated as the total differences between each fraction and its control except the last three fractions which are 0.25 – 0.125, 0.125 – 0.063 and < 0.063 mm according to El-Tapey et al. (2019). Data of representative entail soil samples are presented in Table 1.

All chemical properties were carried out in the soil saturation extract, i.e., electrical conductivity

(EC), soluble (Ca^{++}, Mg^{++}, Na^{+}, K^{+}) and anions ($CO_3^{=}$, HCO_3^{-}. Cl^{-}, $SO_4^{=}$) except soluble sulfateanion ($SO_4^{=}$) which was calculated by subtraction total anions from total cations. The saturation percentage was determined during preparation the paste, while, soil reaction (pH) was measured in the soil paste. These procedures were emphasized according to Burt (2004). The exchangeable sodium percentage (ESP) values were calculated from sodium adsorption ratio (SAR) determined by concentrations of soluble Na, Ca and Mg according to Richared (1954).

$$SAR=Na/\sqrt{((Ca+Mg)/2)}$$

$$ESP=\frac{100(-0.0126+0.01475\ SAR)}{1+(-0.0126+0.01475\ SAR)}$$

Available nitrogen N, P and K were determined according to the previous laboratory devices.

Statistical analysis

Data were subjected to statistical analysis according to Snedecor and Cochran (1981). Mean values were compared at a level of $p< 0.05$ by using least significance difference (L.S.D) test. SPSS (v. 24, IBM Inc., Chicago, ll, USA) and Costat (v. 6.400 CoHort software., California, USA) were used for data statistical analysis.

Results

Biochemical activities of the bioagents

The two strains appeared their ability to successfully solubilize inorganic phosphate, showing a greater potency with *P. fluorescens*. The production of gibberellic acid, indole acetic acid and total carbohydrates was tested with each of *B. subtilis*, *P. fluorescens* and *P. ostreatus*. Data in Tables 2 and 3 indicate that, *P. ostreatus* showed the maximum levels (192, 76 and 179.16 µg/ml, respectively). *P. fluorescens* was higher than *B. subtilis* in the production of both gibberellic acid and total carbohydrates. While, *Bacillus subtilis* was higher in IAA production.

Dehydrogenase activity

The existence of microorganisms was reflected in the dehydrogenase activity present in the rhizoplane. This was adverted to data in Fig. 1 which explicate that inoculation of *B. subtilis*, *P. fluorescens, P. ostreatus* together with mycorrhizae had the greatest effect (149.71 µg TPF/g dry soil), followed by inoculation with the bacterial mixture plus *P. ostreatus* (123.5 µg TPF/g dry soil), while the control had the least record (77.47 µg TPF/g dry soil).

TABLE 1. Some chemical and physical properties of experimental soil

pH	7.86	N	12.01
SP	21.5	P	2.23
EC (dSm^{-1})	0.91	K 70.1	
Soluble cations ($mmol_c\ Kg^{-1}$)		Particle size distribution (%)	
Ca^{++}	4.42	Coarsesand	82.03
Mg^{++}	1.56	Finesand	11.09
Na^{+}	3.36	Totalsand	93.12
K^{+}	0.046	Silt	2.20
Soluble anions ($mmol_c\ Kg^{-1}$)		Clay	4.68
$^{=}CO_3$	0.00	Textural class	Sandy
HCO_3^{-}	1.90	Dry sieving aggregates (size distribution%)	
Cl^{-}	5.06		
$SO_4^{=}$	1.66	10–1mm	5.26
SAR	1.94	1-0.5mm	22.51
ESP	1.58	0.5–0.25mm	35.92
O.M ($g\ kg^{-1}$)	4.2	0.25 – 0.125 mm16.71	
$CaCO_3$ ($g\ kg^{-1}$)	26.1	0.125 – 0.063 mm 7.93	
Available nutrients ($mgkg^{-1}$)		< 0.063 mm11.67	

SP: Saturation percentage, SAR: sodium adsorption ratio, ESP: exchangeable sodium percentage.

TABLE 2. Plant growth promoting and antifungal properties of *B. subtilis* (BSR-8) and *P. fluorescens* (PSR-11)

Bioagent	Phosphate solubilization	G.A.† (µg/ml)	IAA ‡(µg/ml)	Total carb. § (µg/ml)
B. subtilis	+	32	8.15	159.43
P. fluorescens	+++	22.09	13.09	155.7

G.A.†: Gibberellic acid, IAA‡: Indole acetic acid, Total carb.§: Total carbohydrates

TABLE 3. Plant growth promoting (PGRP) and antifungal properties of *Pleurotus ostreauts*

Bioagent	G.A.† (µg/ml)	IAA‡(µg/ml)	Total carb. § (µg/ml)	Total ph.¶ (mg/ g dry weight)
P. ostreauts	192	76	179.16	14

G.A.†: Gibberellic acid, IAA‡: Indole acetic acid, Total carb.§: Total carbohydrates, Total ph.:Total phenol.

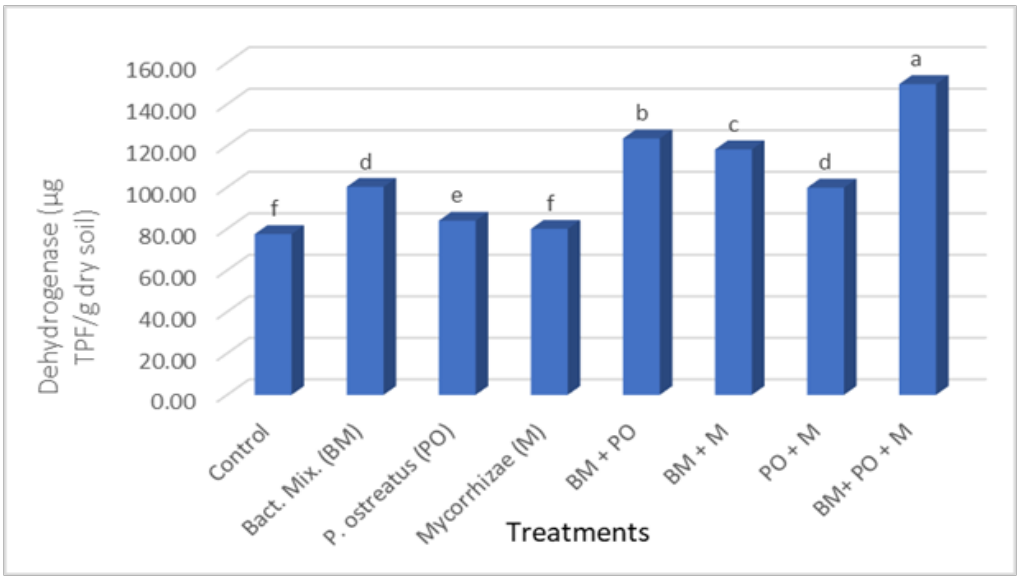

Fig. 1. Biological influence on dehydrogenase activity of roselle plant under field conditions. Data are expressed as a mean of three replicates. Significant between treatments was determined by Duncan's Multiple Range Test (DMRT). Statistical significance was considered as p< 0.05

Root colonization

As recognized in Fig. 2 microbial root colonization was highly affected by the supplemented treatments. Inoculation of the bacterial mixture of *B. subtilis* and *P. flourescens* individually (treatments 2) in addition to *P. ostreatus* and mycorrhiza (treatment 7) marked the uppermost values with 90 % colonization, followed by treatments 5 and 6 with 80 %, contrary to the control which showed only 60 %.

Photosynthetic pigments

The implementation of *B. subtilis*, *P. flourescens*, *P.ostreatus* and mychorrizae as seed treatment and soil incorporation individually and in mixtures, significantly increased chlorophyll a content but was not significant in response to chlorophyll b and carotenoids. This was clearly demonstrated in Fig. 3, where implementation of all microbes resulted in the highest chlorophyll a content with 1 mg/ g dry weight while, the control had the least value of 0.52 mg/g dry weight.

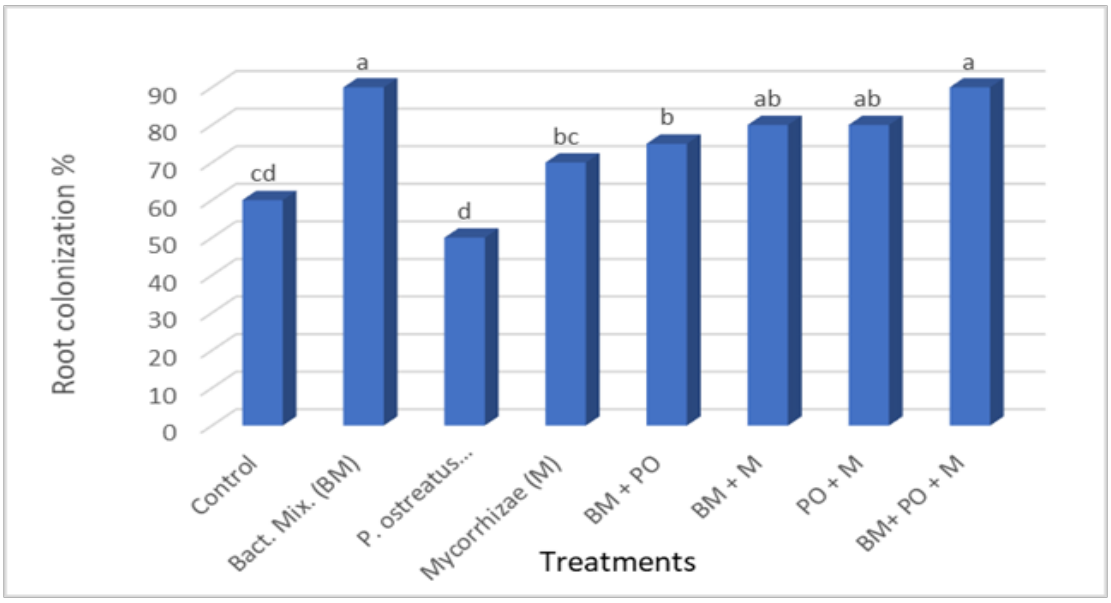

Fig. 2. Root colonization % of roselle plantas affected by some microbiota under field condition. Data are expressed as a mean of three replicates. Significant between treatments was determined by Duncan's Multiple Range Test (DMRT). Statistical significance was considered as P< 0.05

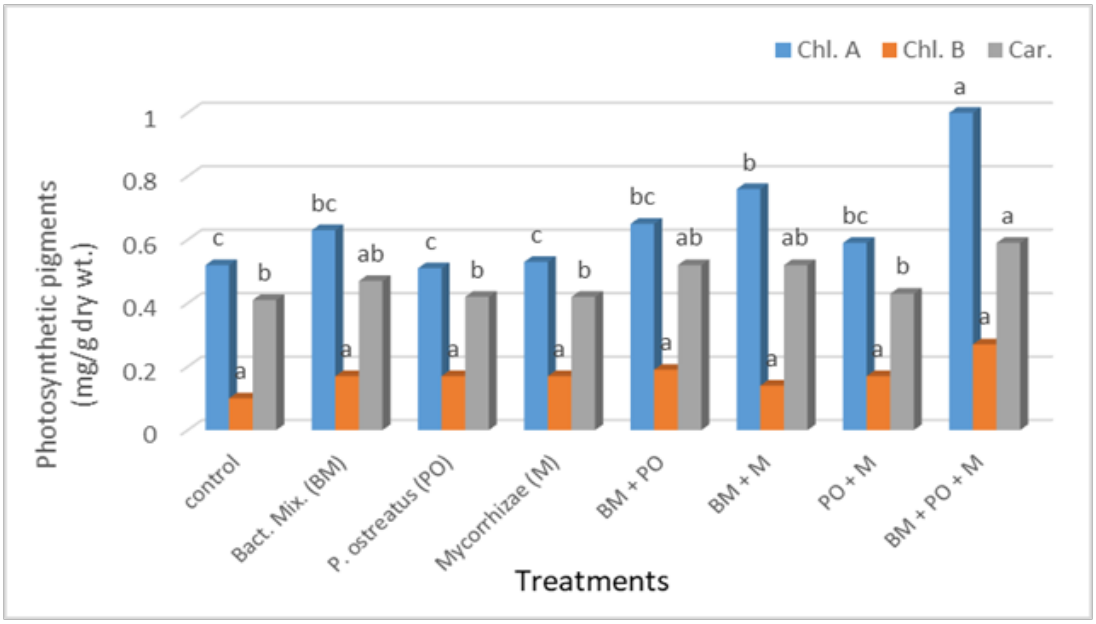

Fig. 3. The impact of some microbiota on photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) of roselle plant under field condition. Data are expressed as a mean of three replicates. Significant between treatments was determined by Duncan's Multiple Range Test (DMRT). Statistical significance was considered as P< 0.05

Growth parameters

Shoot length, branches and calyces' number were greatly influenced by inoculation with the different bioagents compared with un-inoculated one. Table 4 showed that, the treatment of bacterial mixture (BM), i.e., *Bacillus subtilis* and *Pseudomonas fluorescens*, *Pleurotus ostreauts* (PO) and mycorrhiza (M) gave the highest values of shoot height, branches and calyces' number being, 205.2 cm/plant, 40.3 branch/plant and 125.3 calyx/plant, respectively. Meanwhile, the lowest values of branches and calyxes number were

31.7 branch/plant and 103.7 calyx/plant obtained under *Pleurotus ostreatus* treatment compared with the control. The inoculation treatments of the bacterial mixture (BM) + mycorrhiza (M) and bacterial mixture (BM) + *Pleurotus ostreatus* (PO) ranked in between for shoot height, branches and calyxes number. However, the *Pleurotus ostreatus* treatment gave a high effect on shoot length.

Yield parameters

Manipulation of beneficial microbiota showed highly significant effects on yield parameters. It was clear from Table 5 that, the superior effect in yield production was achieved by the mixture of bioagents as general trend which positively reflected in fresh and dry weights of shoot, calyx and seed air dried weight. The dry weights of shoots, calyx and seedsof the previous treatment were, 3.44, 0.99 and 1.26 ton/ feddan, respectively. The mix of mycorrhiza with mixture bacteria gave the better values of calyx and seed parameters than mycorrhiza with *pleurotus ostreatus*. The individual treatments of P. ostreatus and mycorrhiza did not give significant results, while mixing each of them with the microbiota under study gave significant results. Inoculation treatments could be arranged descendingly due to their impacts on yield production as follows: bacterial mixture + *Pleurotus ostreatus*+ mycorrhiza <bacterial mixture + mycorrhiza <bacterial mixture + *Pleurotus ostreatus*<bacterial mixture <*Pleurotus ostreatus*+ mycorrhiza <mycorrhiza <*Pleurotus ostreatus*.

TABLE 4. Some growth parameters of roselle plant (*Hibiscus sabdariffa* L.) under different microbiota inoculation

Treatments	Shoot length (cm/ plant)	(No of branches per plant)	No of calyxes
Control	173.0 d	29.3 f	102.3 e
Bacterial mixture † (BM)	198.8 b	36.7 bc	114.3 c
P. ostreauts(PO)	200.5 b	31.7 ef	103.7 de
Mycorrhizae (M)	196.9 b	33.3 de	105.3 d
BM + PO	189.5 c	37.3 b	115.7 c
BM + M	196.3 b	39.0 ab	120.0 b
PO + M	186.6 c	34.7 cd	113.7 c
BM + PO + M	205.2 a	40.3 a	125.3 a

Bacterial mixture †(BM):*Bacillus subtilis* and *Pseudomonas fluorescens*.

TABLE 5. Some yield parameters of roselle plant (*Hibiscus sabdariffa* L.) under microbiota inoculation

Treatments	Shoot yield (ton/ feddan)		Calyx yield (ton/ feddan)		Seed yield (ton/ feddan) air dried
	Fresh	Dry	Fresh	Dry	
Control	6.81 e	1.36 e	2.20 d	0.23 g	0.33 d
Bact. Mix. † (BM)	7.81 de	2.17 cd	3.60 b	0.72 c	1.01 b
P. ostreauts(PO)	9.45 bc	1.92 de	2.60 d	0.29 f	0.34 d
Mycorrhizae (M)	9.58 bc	2.28 cd	2.70 cd	0.33 e	0.38 d
BM + PO	10.42 b	3.29 ab	3.87 ab	0.79 b	1.09 ab
BM + M	8.53 cd	2.51 cd	3.97 ab	0.81 b	1.18 ab
PO + M	10.21 b	2.69 bc	3.40 bc	0.57 d	0.64 c
BM + PO + M	13.36 a	3.44 a	4.53 a	0.99 a	1.26 a

Bact. Mix. †(BM): Bacterial mixture of *Bacillus subtilis* and *Pseudomonas fluorescens*.

Plant analysis

Macronutrient concentrations and uptake in roselle calyx

Mineral content is an essential component of the nutritive values of roselle calyx. Table 6 displayed the significant difference among treatments. It was conspicuous that, inoculation with microbial mixture enhanced N, P and K concentrations and uptake, these values were, 2.71, 0.37 and 2.09 %, and 26.85, 3.71 and 20.70 kg.fed^{-1}, respectively. Also, data implied that the inoculation with individual bacterial mixture or in combination with other bioagents gave the highest levels of nitrogen and potassium, while mycorrhiza gave the highest level of phosphorus. The lowest values of N, P and K concentrations and uptake were obtained under *Pleurotus ostreatus* treatment as compared with the control. The treatments of bacterial mixture + mycorrhiza and bacterial mixture + *Pleurotus ostreatus* ranked between N and K concentrations and uptake.

Macronutrient concentrations and uptake in roselle shoots

Data in Table 7 explicated the potency of beneficial microorganisms in increasing the mineral content in shoot of roselle plant. The highest values of N, P and K concentrations and uptake were 1.81, 0.37 and 1.97 % and 62.52, 12.9 and 67.48 kg/feddan obtained from the supplementation of all microbes, respectively, followed by bacterial mixture + mycorrhiza treatment. Meanwhile, the respective corresponding lowest values of N, P and K concentrations and uptake were0.97, 0.05 and 0.53 % and 18.62, 1.01 and 10.13 Kg/feddan obtained from *Pleurotus ostreatus* treatment as compared to the control.

Soil analysis

Microbiota inoculation on soil fertility statues after roselle plant (Hibiscus sabdariffa) cultivation

Data in Table 8 showed the availability of nitrogen, phosphorus and potassium (NPK-macronutrients, mg.kg^{-1} soil). The available nitrogen and potassium significantly increased under the mixture treatments than the individual one. Available phosphorus concentrations significantly increased in the presence of mycorrhiza in the mixed treatment compared with control. It is worth noting that, the individual mycorrhiza treatment gave the best value than the mixture treatment of bacterial mixture and *Pleurotus ostreatus*. Mostly, the differences between microbiota treatment were not significant while they were significant as compared with control. However, the highest values of N, P and K were, 15.57, 4.16 and 76.15 (mg.kg^{-1} soil) obtained under the mixture treatment of bacterial mixture, *Pleurotus ostreatus* and mycorrhiza, respectively. The lowest values of available N and K were 12.98 and 72.24 (mg.kg^{-1} soil), respectively, obtained under mycorrhiza and were 2.36 (mg.kg^{-1} soil) for available P obtained under *Pleurotus ostreatus*.

TABLE 6. Macronutrient concentrations and uptake of roselle plant calyx (*Hibiscus sabdariffa* L.) under different microbiota inoculation.

Treatments	Nitrogen (N)		Phosphorus (P)		Potassium (K)	
	Concentration (%)	Uptake (Kg/feddan)	Concentration (%)	Uptake (Kg/feddan)	Concentration (%)	Uptake (Kg/feddan)
Control	1.17 c	2.72 d	0.04 e	0.08 f	1.47 d	3.39 f
Bact. Mix.† (BM)	1.63 b	11.73 b	0.16 d	1.14 de	1.66 bc	11.96 c
P. ostreauts (PO)	1.17 c	3.38 d	0.07 e	0.20 f	1.49 d	4.33 ef
Mycorrhizae (M)	1.40 bc	4.62 d	0.23 bc	0.75 e	1.49 d	4.93 e
BM + PO	1.68 b	13.30 b	0.19 cd	1.52 cd	1.68 b	13.27 b
BM + M	1.73 b	14.02 b	0.37 a	2.97 b	1.79 b	14.51 b
PO + M	1.43 bc	8.16 c	0.28 b	1.60 c	1.53 cd	8.72 d
BM + PO + M	2.71 a	26.85 a	0.37 a	3.71 a	2.09 a	20.70 a

Bact. Mix. † (BM): Bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas fluorescens* (PSR-11).

TABLE 7. Macronutrient concentrations and uptake of roselle plant shoots (*Hibiscus sabdariffa* L.) under different microbiota inoculation

Treatments	Nitrogen (N)		Phosphorus (P)		Potassium (K)	
	Concentration (%)	Uptake (kg/feddan)	Concentration (%)	Uptake (kg/feddan)	Concentration (%)	Uptake (kg/feddan)
Control	0.93 d	12.70 f	0.04 d	0.48 c	0.53 e	7.27 e
Bact. Mix.†(BM)	1.19 c	25.85 de	0.07 cd	1.51 c	0.69 d	14.96 cd
P. ostreauts (PO)	0.97 d	18.62 ef	0.05 d	1.01 c	0.53 e	10.13 de
Mycorrhizae (M)	1.03 cd	23.38 de	0.12 c	2.77 c	0.56 e	12.70 cde
BM + PO	1.21 c	39.28 b	0.09 cd	2.89 c	0.87 c	28.53 b
BM + M	1.49 b	37.48 bc	0.35 a	8.95 b	1.04 b	26.00 b
PO + M	1.07 cd	28.60 cd	0.28 b	7.70 b	0.67 d	18.19 c
BM + PO + M	1.81 a	62.52 a	0.37 a	12.99 a	1.97 a	67.48 a

Bact. Mix. † (BM): Bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas fluorescens* (PSR-11).

TABLE 8. Impact of microbiota inoculation on available macronutrients in soil after roselle (*Hibiscus sabdarriffa* L.) plant cultivation

Treatments	Available macronutrient concentrations(mg/kg)		
	Nitrogen (N)	Phosphorus (P)	Potassium (K)
Control	12.01 f	2.23 d	70.1 d
Bact. Mix. † (BM)	13.65 d	2.51 c	73.01 c
*P. ostreaut*s (PO)	13.29 e	2.36 cd	72.62 c
Mycorrhizae (M)	12.98 e	3.3 b	72.24 c
BM + PO	14.96 b	2.6 c	75.22 ab
BM + M	14.84 b	4.01 a	75.83 a
PO + M	14.32 c	3.92 a	73.52 bc
BM + PO + M	15.57 a	4.16 a	76.15 a

Bact. Mix. † (BM): Bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas fluorescens* (PSR-11).

Microbiota inoculation on soil pH, EC and soluble ions after roselle plant cultivation

Data presented in Table 9 showed that soil pH and EC values slightly decreased under microbiota treatments. The most effective treatment that decreased pH values obtained from the treatment of bacterial mixture (BM) i.e., *B. subtilis* and *P. fluorescens, P. ostreauts* (PO) and *mycorrhiza* (M), followed by the treatment BM + M. Values of EC (ds m^{-1}) were significantly decreased under all treatments of microbial inoculation except the individual mycorrhizal treatment which recorded non-significant result. The lowest values of pH and EC were 7.79 and 0.79 (ds m^{-1}) obtained under the inoculation by BM + PO + M, respectively.

However, soil salinity which obtained from the electrical conductivity values (EC) in saturated soil paste extract often expresses soluble ions, especially total cations, which took the same trend of EC values. Concerning to soluble cations, data presented in Table 9 showed that the soluble calcium ion (Ca^{++}), magnesium (Mg^{++}), sodium (Na^{+}) and potassium (K^{+}) were significant decreased under all inoculation treatment except the individual treatments of *P. ostreatus* and mycorrhiza which recorded an insignificant decrease. Referring to anions, no considerable changes in HCO_3^- was detected. While, all the studied microbial inoculation led to significant decrease in soil soluble Cl^-

except individual mycorrhizal inoculation which recorded insignificant decrease. The $SO_4^=$ values were significantly decreased in response to all microbial inoculation treatments as compared with control. The lowest values of Cl^- and $SO_4^=$ were 5.06 and 0.83 mmole Kg^{-1}obtained under BM+ PO + M treatment, respectively.

Microbiota inoculation on soil chemical properties

Results in Table 10 exhibited that, the SAR and ESP values significantly decreased relative to all microbial inoculation treatments. The lowest SAR and ESP values were 1.08 and 1.21 obtained by BM + PO treatment, respectively, followed by 1.69 and 1.22 obtained by BM + PO + M treatment. The individual mycorrhizal inoculation achieved the highest values which were 1.84 and 1.43. Generally, the inoculation treatments could be arranged descending due to their impact on SAR and ESP values as follows: Control> M> PO> BM>PO +M >BM +M > BM + PO +M> BM + PO.Concerning organic matter percent, no significant differences were observed, so no clear trend was found between treatments. $CaCO_3$ % values were significantly decreased as a result of all microbial inoculation treatments. The treatment of BM + PO + M showed the lowest $CaCO_3$ content which was 1.74 %. While, the highest value was 2.41 % obtained by *P. ostreatus*. The results of $CaCO_3$ content due to inoculation treatments by BM + PO and BM + M were found in between.

Microbiota inoculation and soil aggregates after roselleplant cultivation

The studied aggregate categories are the following diameter (mm): 10-1, 1-0.5, 0.5-0.25, 0.25-0.125, 0.125-0.063 and 0.063. They are designated as follows: very large, large, medium, sub-medium, small, very small and extremely small. Data in Table 11 showed that, the individual treatment of bacterial mixture, i.e.*B. subtilis* and *P. fluorescens* gave the highest increase values of aggregates percent than *P. ostreatus* or mycorrhiza treatments. Meanwhile, the inoculation of bacterial mixture, *P. ostreatus* and mycorrhiza gave the best significant result among all treatment which was 9.07%. Generally, the values of all diameters under individual *P. ostreatus* or mycorrhiza treatments showed insignificant increases. The highest values of dry sieving aggregates size distribution % were occurred for those of 0.5-0.25 mm than other fractions and the lowest were those having diameters 1-10 and 0.125 -0.063 mm.

TABLE 9. Impact of microbial inoculation on soil pH, EC and soluble ions after roselle (*Hibiscus sabdariffa* L.) plant cultivation

Treatments	pH	EC (ds/m)	Ca^{++}	Mg^{++}	Na^+	K^+	$CO_3^=$	HCO_3^-	Cl^-	$SO_4^=$
Control	7.86	0.9 a	3.30 a	2.44 a	3.34 b	0.20 a	0	2.0 b	1.5 a	5.78 a
Bact. Mix. † (BM)	7.78	0.57 d	1.60 d	1.25 c	2.73 c	0.08 c	0	2.0b	1.0 b	2.66 d
P. ostreauts (PO)	7.83	0.64 c	1.65 d	0.85 d	3.70 a	0.18 ab	0	1.5 c	1.0 b	3.88 b
Mycorrhizae (M)	7.82	0.66 b	2.20 b	1.25 c	3.10 b	0.18 ab	0	1.5 c	1.0 b	4.23 b
BM + PO	7.70	0.55 e	1.85 c	1.44 b	1.80 f	0.10 c	0	1.5 c	0.5 c	3.19 c
BM + M	7.68	0.55 e	1.65 d	1.25 c	2.45 d	0.15 b	0	2.5 a	0.5 c	2.50 e
PO + M	7.99	0.57 d	2.20 b	1.25 c	2.10 e	0.17 ab	0	2.0 b	1.5 a	2.22 e
BM + PO + M	7.62	0.4 f	1.65 d	0.85 d	1.40 g	0.10 c	0	2.0 b	1.0 b	1.00 f

Bact. Mix. † (BM): Bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas fluorescens* (PSR-11).

Table 10. Impact of some microbiota on soil SAR, ESP, CaCO3 and O.M. after roselle (*Hibiscus sabdariffa* L.) plant cultivation

Treatments	SAR	ESP	O.M (%)	$CaCO_3$(%)
Control	1.97 c	1.62 c	0.38 e	2.61 a
Bact. Mix.† (BM)	2.29 b	2.07 b	0.50 bc	1.96 d
P. ostreauts (PO)	3.31 a	3.49 a	0.41 de	2.41 b
Mycorrhizae (M)	2.36 b	2.17 b	0.39 e	2.36 b
BM + PO	1.40 de	0.80 de	0.52 b	1.95 d
BM + M	2.03 c	1.71 c	0.53 b	1.92 d
PO + M	1.60 d	1.09 d	0.45 cd	2.12 c
BM + PO + M	1.25 e	0.59 e	0.70 a	1.74 e

Bact. Mix. † (BM): Bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas fluorescens* (PSR-11).

TABLE 11. Effect of microbiota inoculation on soil aggregate size distribution after roselle (*Hibiscus sabdarriffaL.*) plant cultivation

Treatments	Dry sieving aggregates size distribution (%)						Aggregates (%)
	1 – 10 Mm	1 - 0.5 mm	0.5 – 0.25 mm	0.25 – 0.125 mm	0.125 – 0.063 mm	< 0.063 mm	
Control	5.26 h	22.51 c	35.92 g	16.71 a	7.93 a	11.67 a	0.00 g
Bact. Mix.† (BM)	7.01 d	24.11 b	37.23 de	15.52 c	6.01 d	10.12 d	4.66 d
P. ostreatus (PO)	5.61 f	22.81 c	36.79 ef	16.20 b	7.36 b	11.23 b	1.52 f
Mycorrhizae (M)	5.38 g	22.79 c	36.72 f	16.35 b	7.24 b	11.52 ab	1.20 f
BM + PO	7.40 b	24.66 ab	39.47 b	13.16 e	5.46 ef	9.85 de	7.84 b
BM + M	7.28 c	24.35 ab	38.01 c	14.91 d	5.82 de	9.63 e	5.95 c
PO + M	6.21 e	23.24 c	37.53 d	15.80 c	6.41 c	10.81 c	3.29 e
BM + PO + M	7.86 a	24.91 a	39.99 a	13.41 e	5.11 f	8.72 f	9.07 a

Bact. Mix. † (BM): Bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas fluorescens* (PSR-11).

Discussion

Generally,medicinal plants intervene in different systems of the organism providing several preventive and therapeutic actions, due to the presence of a diversity of nutritional and bioactive compounds. *H. sabdariffa* revealed the presence of several interesting compounds (Jabeur et al., 2017). Roselle plant is considered to be one of the important medicinal plants in Egypt under the name of Karkadeh, due to its natural-coloured materials, its extracts became a basic material in medicine, food and cosmetic industries according to the world return to nature (Ottai et al., 2004).

Response to the production of several plant growth promoting substances was reported to be positively affect plant growth and resistance. In the present work, the tested bioagents showed their ability to produce IAA. IAA may increase root growth and root length, resulting in greater root surface area, which enable the plant to access more nutrients from soil, while gibberellins may be involved in cell division and elongation, seed germination, stem elongation, flowering, fruit setting and may regulate root hair abundance and hence promote root growth (Shafi et al., 2017,Tsegaye et al., 2017 and Putrie et al., 2021). The tested bioagents successfully solubilized

phosphate in a form that the plant can absorb which is positively reflected on plant growth and yield and on soil fertility, similar results was reported by Gouda et al., 2018.

It was evident from the present data that seeds and soil treatment with the all the bacterial mixture showed the greatest bacterial colonization which in turn reflected on growth and yield and plant protection. Wang et al., (2018) reported that *B. subtilis* and *P. flourescens* effectively colonize plants and control diseases. A variety of bacterial traits, such as motility, chemotaxis to seed and root exudates, production of pili or fimbriae, production of specific cell surface components, capacity to use specific components of root exudates and protein secretion, and quorum sensing, contribute to the colonization process. PGPR move from the rhizosphere to root surfaces guided by chemotaxis and facilitated by flagella. Chemotaxis is an important competitive colonization trait (Suryadi et al., 2019).

In accordance with findings of the present study, Khalil et al. (2017) and Al-Sayed et al. (2020) reported that photosynthetic pigments increased in response to microbiota inoculation may result from enhancing plant growth and biomass production. Bioagents may stimulate chlorophyll synthesis through encouraging pyridoxal enzymes formation, that play role in α-amino levulinic synthetase as a primary compound in chlorophyll synthesis.

Vegetative growth parameters i.e., shoot length and number of branches recorded at harvest stage. Data recorded that, the mixture of bio-fertilizer inoculation was significantly increased growth parameters, which may be as a consequence of synthesis of phytohormones, N_2 fixation, synthesis of some enzymes that modulate the level of plant hormones (Gouda et al., 2018 and Sindhu and Sharma, 2020), solubilization of inorganic phosphate and minerlization of organic phosphate by mycorrhiza (Fallahi et al., 2016; Bagyaraj, 2018; Adeyemia et al., 2019; Ganzour et al., 2020) which makes phosphorus available for plant, the synthesis of growth promoting compounds like cytokinins, gibberellins and indole acetic acid (Mahfouz and Sharaf-Eldin, 2007;Vejan et al., 2016 and Tsegaye et al., 2017). These results were similar to Khalil et al. (2017) and Al-Sayed et al. (2020). Increase number of calyxes may be to increasing the number of branches.

Fresh and dry weight of shoot, calyx and air-dried seed weight were significantly increased under inoculation of combined bioagents comparing to individual ones and the control. This may be ascribed to the collective effect of bioagents i.e., production of amino acids, vitamins and growth promoting substances like indole acetic acid and gibberellic acid El Naim et al. (2017) secreted by these introduced beneficial microorganisms which resulted in enhanced nutrient uptake, translocation and synthesis of photosynthate assimilates and consequently increased plant growth characters. These results are supported by other published papers, Khalil et al. (2017) and Al-Sayed et al. (2020). It was clear that mycorrhiza yield production was not effective when used individually. This was in agreement with Sembok et al., 2015 which found that mycorrhizal spores were not effective in enhancing the above-soil vegetative growth or the yield of roselle calyxes. The chief role of mycorrhizal fungi in symbiosis is to provide mineral nutrients by inspecting the soil. The hyphae are more extended and thinner than roots or root hairs. So, they are more effectual in reaching soil interstices (Novero et al., 2008).

The mycorrhizal fungi extend their filaments in soil and plant roots. This filamentous network stimulates bi-directional nutrient movement, where soil nutrients and water transfer to the plant and plant photosynthates move to the fungal network. Mycorrhizal symbiosis benefits the host plant by increasing the root system ability to absorb and translocate phosphorus using an extensive network of external hyphae (Bhandari and Garg, 2017, Zou et al., 2019). Mycorrhizal fungi act as biological control as they interact with plant pathogens and diminish disease incidence (Mazen et al., 2008, Talaat and Abdallah, 2008, Tripathi et al., 2017 and Bagyaraj, 2018). Narh Mensah et al. (2018) also found that *Pleurotus* spp. enhance the growth of tomato and wheat. N, P and K concentrations and uptake in calyx and shoot were highly significant increased as a result of inoculation by bacterial mixture, i.e., *Bacillus subtilis* and *Pseudomonas fluorescens*, *Pleurotus ostreauts* and Mycorrhiza. This increment may be assignable to the conversion of the unavailable forms of nutrients to available forms by the microbiota and consequently increasing absorption and translocation of these elements (El-Tapey et al., 2019). In addition, production of indole acetic acid and cytokinins was increased the surface area of root length through enhancing root hair branching with an eventual increase on nutrients uptake from the soil (Khalil et al.,2017 and Aly et al., 2020).

In respect to NPK soil availability, results revealed that the mixture of microbiota treatment gave significant increase in NPK than the individual one. The superiority of the mixed bacterial mixture, *P. ostreatus* and mycorrhiza may be due to improvement of the activity of microorganisms already present in the soil for nitrogen fixation, phosphorus and potassium (El-Tapey et al., 2019). A number of compounds like polysaccharides, peptides, lipids, etc. were extracellularly excreted (Mandel, 1999), these compounds possibly diffuse around soil particles and enhanced the macronutrients availability, also polysaccharides are made of fibber which can also entangle the soil particle and protect nutrients from leaching (Zain et al., 2018 and El-Tapey et al., 2019). Inoculation by mycorrhiza, either alone or mixed with another microbiota, resulted in significant phosphorus availability compared to control (Mazen et al, 2008). Biofertilizers have a big role in building up soil fertility. They provide (1) Excretion of growth – promoting substances, (2) increase in soil biomass after their death and decomposition (Taha et al., 2017).

Soil pH and EC were decreased in response to microbial inoculation as general trend. This may be resulted from the production of organic acids by bacteria during mineralization of organic materials (Youssef and Eissa, 2017). Microorganisms excrete a number of extracellular organic components which lead to adsorb both sodium and magnesium ions and consequently soil EC decrease (Zian et al. 2018). This result was in accordance with Al-Sayed (2020).

Results showed that, although there was a slight increase in OM values under the mixture treatments as compared with individual one, no significant differences were observed under all microbiota inoculation. This result is in harmony with Al-Sayed et al. (2020) who assumed that, increasing in vegetative growth and total plant biomass that might have resulted due to the enhancement of photosynthesis and better translocation and accumulation of nutrients. The corresponding to addition of these biofertilizers to the soil lead to increase the soil organic matter, which is consequently, increased the soil biological activity (Taha et al., 2017).

The exchangeable sodium percentage (ESP) values were taken the same trend for both sodium adsorption ration (SAR) and electrical conductivity (EC) values under all microbiota inoculation treatments, and this is considered a quite result since, the ESP values were calculated from SAR which determined by concentrations of soluble Na^{+}, Ca^{++} and Mg^{++} (Richared, 1954). However, both values of SAR and ESP don't reflect sodicity effect because its values were less than 13 and 15 (USDA, 2014), respectively.The studied microbiota significantly decreased $CaCO_3$ content. This due to dissolving and mobilization of $CaCO_3$ content which enhanced by acidic reaction of microbiota treatments and organic matter decomposition. This result is in agreement with the concepts of Kononov (1961), Emmerich et al. (1982), El-Tapey (1998) and Youssef and Eissa (2017).

The inoculation of microbiota mixture i.e., bacterial mixture (*B. subtilis* and *P. fluorescens*), *P. ostreatus* and mycorrhiza gave the highest significant values of aggregates size distribution than other treatments. As general trend, the inoculation of mixed microbiota was better than using them individually. This indicates that mixing of these microorganisms under study may increase the production of cement materials such as polysaccharides and thus increase soil aggregates. The superiority of the respective treatments may be due to improvement of microbial biomass (Zian et. al., 2018). Similar results are in agreement with Abo-Kora (2004) who mentioned that increasing aggregates stability of soil is associated with root growth of alfa alfa due to polysaccharides production in the rhizosphere. In addition, mixture microbiota treatment may enhance soil carbon which is crucial to form organic materials necessary to cement soil particles (Darwish et. al., 2012 and El-Tapey et. al., 2019). Aggregate is a naturally occurring cluster or group of soil particles in which the forces holding the particles together are much stronger than the forces between adjacent aggregates. Andrade et al., 1998 showed that more aggregates were formed when both the roots and the fungus were present. The hyphae on their own had less effect than roots on their own (Chotte, 2005). The class of 0.5-0.25 of dry sieving aggregates size distribution percent were higher than other fractions, while the lowest were those having diameter 1-10 and 0.125 -0.063 mm. This may be due to soil formation processes (Zian et.al., 2018 and El-Tapey et.al., 2019).

Conclusion

The excessive use of mineral fertilizers contributes to evolution of impermissible environmental and health hazards; therefore, eco-friendly microorganism can be successfully

exploited in modern agriculture without affecting the ecosystem. The outcome of this study indicated that application of the conglomeration of the bacterial mixture of *Bacillus subtilis* (BSR-8) and *Pseudomonas flourescens* (PSR-11), *Pleurotus ostreatus* and mycorrhizae positively affected the growth and yield of roselle plant than their individual application. It was apparent that the consortium of all the tested bioagents significantly increased soil dehydrogenase activity, root colonization and photosynthetic pigments. The impact of the examined bioagents on soil fertility was reflected on improving NPK availability, enhancing NPK concentration and uptake in both calyx and shoot, increasing soil aggregates and decreasing EC and PH. While, *P. ostreatus* showed insignificant effect when used individually. Thus, the inoculation of a mixture of beneficial microorganisms improved the physico-chemical soil properties.

Author contribution

This study was designed and implemented by authors,where all authors contributed in writing the manuscript, interpreting information presented and have read and agreed to the version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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