

Rhizo-microbiota are among the pillars for proper canola (*Brassica napus* L.) and sesame (*Sesamum indicum* L.) production in marginal soil

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ABSTRACT

A field experiments were conducted at Agricultural Research Station, Ismailia Governorate, Egypt, through agricultural growing seasons (2015-2016, winter season) and (2016 summer season) to evaluate some biological treatments by mixture of bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*), mixture of fungi (*Pleurotus columbinus* and *Trichoderma harzianum*) and mixture of cyanobacteria (*Wolleea saccata* and *Spirulina platensis*) individually or in combination on growth and yield of canola (*Brassica napus* L.) and sesame (*Sesamum indicum* L.) plants grown in sandy soil, in addition to studies on their effects on some soil properties. Generally, the mixture of bacteria and cyanobacteria was more effective in increasing growth parameters of canola and sesame plants compared to other treatments. Inoculation of canola and sesame plants by mixture of bacteria and cyanobacteria gave significant enhancement in chlorophyll a, b and carotenoids production. Biological activities in terms of dehydrogenase, CO₂ and root colonization were significantly affected by the biological treatments. The previous determination recorded the highest values in combination between mixture of bacteria and cyanobacteria. The mixture of bacteria and cyanobacteria gave the highest significant values of seed yield kg fed⁻¹, weight of 1000 seed and oil percentage for canola and sesame plants. Generally, the results showed that the mixture of bacteria was better than either mixture of fungi or cyanobacteria treatments. Biological treatments enhanced the physical properties of soil, the highest increased values of soil aggregation, percentage of total porosity, water holding pores (28.8 – 0.19 μ), and fine capillary pores (<0.19μ) were in inoculation with the mixture of bacteria, fungi and cyanobacteria followed by mixture of fungi and cyanobacteria, while the lowest values obtained from the separate mixture of each one. Meanwhile, the macro pores (>28.8μ) significantly decreased at the same previous treatments. Bulk density showed no differences between treatments.

Keywords: Sandy soil, bacteria, fungi, cyanobacteria, canola, sesame, productivity

Introduction

Canola (*Brassica napus* L.) and sesame (*Sesamum indicum* L.) are the most important oil seed crops and could be cultivated under harsh environment such as salinity and drought in the newly reclaimed sandy soils. Canola production is the third largest oilseed crops, produces nearly 14.7 % of total vegetable edible oil in the world (Naderifar and Daneshian, 2012; Arabi *et al.*, 2017). Canola contains greater nitrogen in seeds and plant tissues than most grain crops (Megawer and Mahfouz, 2010).

Sesame is widely growing in tropical and sub-tropical regions of Asia, Africa, South and North America (Jaisingh *et al.*, 2016). Sesame oil has an excellent nutritional, medicinal, cosmetic and cooking quality which named queen of oils (Sabannavar and Lakshman, 2008). It is a versatile crop having diversified application because of being rich in minerals (Ca, P, K, etc), vitamin E and containing 42 – 45 % oil, 20 % protein, 14 – 20 % carbohydrate, 2-3 % fiber and about 80 % unsaturated fatty acids like oleic and linoleic acids (Jaisingh *et al.*, 2016).

In Egypt, sesame is considered as a food crop rather than oil seed crop because most of its production is used for snacks, confectionery, bakery products, tehana and halawa purposes (Boghdady *et al.*, 2012; Mahrous *et al.*, 2015).

Plant growth promoting rhizobacteria (PGPR) can significantly enhance plant growth and represent a mutually helpful for plant- microbe interaction. They reinforce plant either directly by

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production of various phytohormones, enzymes and solubilized minerals in the soil or indirectly by inhibiting phytopathogens (Naderifar and Daneshian, 2012; Dey *et al.*, 2014; EL-Tapey *et al.*, 2019). Plant growth promoting rhizobacteria (PGPR) could be deployed in agricultural production systems to alleviate biotic and abiotic stresses and produce sustainable environmentally friendly management tools (Vejan *et al.*, 2016).

Among various PGPR approaches, *Bacillus* species deem likely candidates due to their broad-spectrum antagonistic activity against phytopathogens, production of long-lived and stress-tolerant spores, secondary metabolites, lytic enzymes, resistance to adverse environments, and plant growth promotion (Vessey, 2003; Chandrasekaran and Chun 2016). *Bacillus subtilis* plays a significant role in improving plant growth and tolerance to both biotic and abiotic stresses. *Bacillus subtilis* can also solubilize soil P, enhance nitrogen fixation, and produce siderophores that promote its growth and suppresses the growth of pathogens. *Bacillus subtilis* enhances stress tolerance in their plant hosts by inducing the expression of stress-response genes, phytohormones, and stress-related. These strains have exhibited a variety of plant growth promotion effects on many crop species (Hashem *et al.*, 2019).

Azotobacter chroococcum is regarded as free-living aerobic nitrogen fixation. It synthesizes and secretes considerable amount of biologically active substance like B-vitamins, gibberellins and hetero-auxins which enhance the growth of plant and reinforce their tolerance to pathogenic diseases (Van loon 2007). The indole acetic acid (IAA) produced by *Azotobacter* is colonizing the seed or root surface and proposed to act in conjunction with endogenous surfaces to stimulate cell proliferation, elongation and also enhance the host's uptake of minerals (Jnawali *et al.*, 2015).

Pseudomonas type of bacteria is especially important because of its expanded distribution in soil and its ability to colonize the rhizosphere of many plants and to produce a great range of metabolites. *Pseudomonas* groups produce large amounts of phytohormones particularly IAA to stimulate growth, cytokines, isopentenyl adenosine, and ribose zeatin (Yong-Soon *et al.*, 2015).

Pseudomonas fluorescens is a bacterium that belongs to the plant growth promoting rhizobacteria (PGPR) which is very influential to increase root surface area, increase nutrient uptake, improve plant production and inhibit the growth of pathogens, in addition its capability to dominate the rhizosphere, its ability to degrade large amounts of organic and inorganic compounds, interaction with plants and association in the rhizosphere (Mantelin and Touraine, 2004 ; Palleroni and Moore, 2004).

Trichoderma is a genus of fungi that is present in all soils. It has plant growth promotion and soil environmental improvement abilities (Lorito *et al.*, 2010). During the colonization in plant roots, the mycelia of *Trichoderma* make twine around the plant roots to form an aspersorium-like structure, then penetrate the root epidermis layer and survive for a long time between the plant cells of the epidermis and the cortex, having a direct promotional act on the growth of seedlings and nutrient uptake in the rhizosphere (López-Bucio *et al.*, 2015).

Pleurotus columbinus is a good source of organic matter and rich in macro and micro elements for plants, which helps to increase the soil biological activity (EL-Khadrawy, 2018).

Cyanobacteria are among the most important constituents of soil microflora and are found in significant numbers in different ecosystems (Pandey *et al.*, 2004). The main contribution of cyanobacteria is making porous soil and produce adhesive substances, excretion of phytohormones (auxin, gibberellins, etc.), vitamins, amino acids, improving the water holding capacity of soil through their characteristic jelly structure, and availability of soil phosphate by excretion of organic acids (Deepali *et al.*, 2020). Cyanobacteria have also been recognized as important agents in the stabilization of soil surfaces through the production of extracellular polysaccharides which are chief agents of aggregate formation and stabilization. The significance of polysaccharides in soil aggregation may be the direct result of binding soil particles into micro aggregates (Dhar *et al.*, 2015). The biomass of cyanobacteria is one of the beneficial bio-fertilizers which improves soil characteristics such as water holding capacity and amelioration of mineral nutrients (Singh *et al.*, 2014; Zarezadeh *et al.*, 2020).

Many cyanobacteria e.g., *Nostoc mucorum*, *Nostoc humifusum*, *Anabaena oryzae* and *Wolleea* sp. are capable of using atmospheric nitrogen as a source of nitrogen (nitrogen fixation) which may reduce the amount of mineral nitrogen fertilizers used in agriculture (Hegazi *et al.*, 2010; Singh *et al.*, 2014). *Spirulina platensis* can be used as a rich source of macro- and micronutrients for plants such as vitamins, amino acids, polypeptides, phytohormones (gibberellins, auxins, cytokinins), antioxidants and compounds with antibacterial and antifungal properties (Bhowmik *et al.*, 2010; Osman *et al.*, 2016; Nawrocka *et al.*, 2017).

Exopolysaccharides that released from some microorganisms such as *Azotobacter chroococcum*, cyanobacteria and, *Pleurotus columbinus* played an important role in fertility status and enhancing the formation of soil micro-aggregates and contribute to building up soil physical structure and consequently increase water storage in sandy soil under dry farming (Ashraf *et al.*, 2013; Taha *et al.*, 2017; EL-Tapey *et al.*, 2019).

This research investigated the effects of some microorganisms on canola and sesame plants and some physical properties of newly reclaimed sandy soil.

Materials and Methods

1. Microorganisms used:

1.1. Bacterial inoculants.

Bacillus subtilis and *Pseudomonas fluorescens* were kindly supplied from Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Ministry of Agriculture, Giza, while *Azotobacter* was isolated, purified and identified as mentioned by EL-Tapey *et al.* (2019). *Bacillus subtilis* was grown in nutrient broth medium as in (Difco Manual 1985) to reach 10^8 cfu /ml, *Pseudomonas fluorescens* was grown in king's medium (king *et al.*, 1954) to reach 10^8 cfu /ml and *Azotobacter* grown in modified Ashby' medium (Abd El Malek and Ishac, 1968)) to reach 10^7 - 10^8 cfu/ ml.

1.2. Cyanobacteria inoculants.

Wolleea saccata and *Spirulina platensis* were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC). *Wolleea saccata* strain was grown and propagated on BG11medium (Rippika *et al.* 1979), while *Spirulina platensis* was grown on Zarrouk medium (Zarrouk, 1966). The cultures were incubated in growth chamber under continuous illumination at 2000 lux to obtain mass culture of 1g dry weight per liter at log phase stage.

1.3. Fungal inoculants.

Trichoderma harzianum was obtained from Plant Pathology Research Institute, Agricultural Research Center, Giza. *Trichoderma harzianum* was refreshed from spore suspensions in flasks containing 50 ml of yeast extract dextrose broth (Barnett and Hunter1972). The white rot fungus *Pleurotus columbinus* was obtained from the Microbiology Department, Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC). *Pleurotus columbinus* was grown on potato dextrose medium, in a rotary shaker at 200 rpm for 7 days (Martin, 1950). After propagation, the growth and its medium was put in an electric mixer, then filtered and kept at 4 °C till field application.

2. Agricultural practices.

A field experiments was conducted in a sandy textured soil under sprinkler irrigation system, at Ismailia Agricultural Research Station, Ismailia Governorate, and cultivated with canola (*Brassica napus* L.) c.v Bactol as a winter crop and sesame (*Sesamum indicum* L.) c.v shandwil as a summer crop during the agricultural growing seasons of (2015–2016) and (2016) respectively. The experiments were laid in complete randomize plot design with three replicates. The area of each plot was 18 m² (6 x 3 m). The factors examined were three types of microorganism inoculation as individual or in combination as follows:

1. Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.
2. Fungi**: *Pleurotus columbines* and *Trichoderma harzianum*.
3. Cyanobacteria ***: *Wolleea saccata* and *Spirulina platensis*.
4. Mixture of Bacteria* +Fungi**.
5. Mixture of Bacteria* + Cyanobacteria ***.
6. Mixture of Fungi** + Cyanobacteria ***.
7. Mixture of Bacteria* +Fungi** + Cyanobacteria ***.
8. Control : with NPK fertilizers as recommended by Ministry of Agriculture

Calcium super-phosphate (15.5 % P₂O₅) was added at a rate of 100 kg fed⁻¹ during soil preparation and potassium sulphate (48 % K₂O) as 50 kg fed⁻¹ added immediately before 1st irrigation.

The nitrogen fertilizers in the form of ammonium sulphate (20.5 % N) at a rate of 60 kg fed⁻¹ was added in three equal dose, i.e., 1/3 at cultivation time as based fertilization, 1/3 at stem initiation stage and the remaining 1/3 was applied at bud initiation before flowering stage in accordance with treatment variables. Another ordinary agricultural practice under sprinkler irrigation was applied. The normal agronomic practices of growing canola and sesame were practiced till harvest. Bacteria and fungi inocula were added at rates of 20 l fed⁻¹, while cyanobacteria was added at rate 30 l fed⁻¹.

3. Plants sampling and determination

Plant samples were taken after 75 days of planting to determine root length, shoot height, number of branches and shoot dry weight as vegetative parameters. Carotenoids, chlorophyll a and b were determined according to Saric *et al.* (1976). Biological activities such as dehydrogenase activity, CO₂ and root colonization were determined according to Skujins (1976), Pramer and Schmidt (1964) and Bilal *et al.* (1993), respectively. At harvest, canola and sesame plants were collected in each plot to determine total seed yield (kg fed⁻¹), numbers of pods plant⁻¹, 1000 seed weight (g) and oil content (%).

4. Soil determination

A representative soil sample was air-dried and ground to pass a 2 mm screen and kept for some chemical and physical analysis (Table1) according to the methods described by Page *et al.* (1982). Particle size distribution was carried out by pipette method according to Gee and Bander (1986). Soil samples from all plots after harvest for both seasons were used to determine soil aggregates size distribution (%) according to Rouiller *et al.* (1972) and the soil aggregate percentage was calculated as the total differences between each fraction and its control except the last two fractions which are 0.125 – 0.063 and < 0.063 mm. Bulk density was carried out using the core method according to Black (1982). Total soil porosity was calculated as described by Richards (1954). Pore size distribution was calculated from the soil moisture retention curve according to De-Leenheer and De-Boot (1965). Soil was classified as Typic Torriorthents, sandy, mixed, thermic, very deep according to USDA (2014).

Table 1: Some physical and chemical characteristics of experimental soil

pH: In suspension 1:2.5	8.23	Fine sand	24.46
Sp	20.6	Total sand	84.99
EC* (dS.m⁻¹)	0.86	Silt	9.82
Soluble cations** (meq.l⁻¹)		Clay	5.20
Ca⁺⁺	2.54	Textural class	Sand
Mg⁺⁺	1.26	Bulk Density (Mg.m⁻³)	1.73
Na⁺	4.20	Total Porosity (%)	39.4
K⁺	0.94	Pore size distribution (%)	
Soluble anions** (meq.l⁻¹)		Macro-pores (> 28.8 μ)	28.94
CO₃⁼	0.00	Micro-pores (<28.8 μ)	
HCO₃⁻	1.50	Water Holding Pores (28.8- 0.19μ)	8.66
Cl⁻	5.66	Fine Capillary Pores (<0.19)	1.76
SO₄⁼	1.78	Dry sieving aggregates size distribution (%)	
SAR	3.05	10 – 1 mm	4.06
ESP	3.15	1 - 0.5mm	21.50
CaCO₃ (%)	5.70	0.5 – 0.25mm	42.30
O.M (%)	0.37	0.25 – 0.125mm	15.51
Particle size distribution (%)		0.125 – 0.063mm	7.55
Coarse sand	60.20	< 0.063mm	9.08
EC* (dS.m⁻¹), soluble cations** and anions** (meq.l⁻¹) : In saturated paste extract			

5. Statistical analysis

Analysis of Variance (ANOVA) and least significant difference (LSD) were calculated according to Steal and Torrie (1983).

Results and Discussion

1. Effect of microorganisms on growth parameters of canola and sesame plants

Table 2 showed the root length, shoot height, number of branches and shoot dry weight in the studied canola and sesame plants after 75 days from planting under the effect of some biological treatments individually or in combination. Root length significantly increased under all biological treatments for canola and sesame plants except the individual treatment by fungi as compared to control. The highest root length of canola plant under the treatment of bacteria, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum* in combination with the cyanobacteria *Wolleea saccata* and *Spirulina platensis* was 33.2cm plant⁻¹, followed by individual bacteria treatment which was 30.2 cm plant⁻¹. For sesame plant, the mixture of bacteria and cyanobacteria was superior and gave the highest value of root length (27.3 cm plant⁻¹) followed by the mixture of all microbes and individual treatment of bacteria as compared to other treatments which reached to 22.2 and 21.2 cm plant⁻¹, respectively.

The combination treatment of bacteria and cyanobacteria exhibited significant increase in shoot height for canola and sesame plants compared to other treatments being 135.3 and 177.0 cm plant⁻¹ respectively, and increase percentage reached to 9.7 and 10.6 % over individual bacteria treatment for canola and sesame plants respectively, while the increase percentage reached to 41.6 and 32.4 % over individual cyanobacteria treatment for canola and sesame plants, respectively. No significant difference was observed for shoot height between individual bacterial treatment and the mixture of three types of microorganisms gave 123.3 and 126.7 cm plant⁻¹ respectively for canola, while sesame recorded 160.0 and 165.4 cm plant⁻¹, respectively.

For the number of branches per plant, the same attitude was occurred. The highest values for canola and sesame plants were 5.0 and 4.6 branches per plant that recorded under mixture of bacteria and cyanobacteria respectively, followed by the individual bacteria treatment which gave 4.6 and 4.4 branches per plant for the corresponding respective plants. No significant differences were occurred for canola and sesame branch numbers under individual fungi or cyanobacteria treatments as compared with control. On the other side, individual bacteria treatment recorded significant increase in the number of branches of canola and sesame plants than the individual treatments with fungi or cyanobacteria.

Regarding shoot dry weight, the highest values were 53.8 and 62.3 g plant⁻¹ for canola and sesame plants, respectively under the mixture of bacteria and cyanobacteria, followed by individual bacteria treatment which recorded 47.5 and 55.6 g plant⁻¹ for canola and sesame, respectively. The dry weight of shoot recorded no significant differences under individual fungi treatment which reached 26.7 and 34.2 g plant⁻¹ for canola and sesame plants, respectively as compared with control.

Adesemoye and Klopper (2009) conclude several plant growth promoting (PGP) mechanisms of PGPR such as modification and increased branches in root hair, root development, improvement in germination of seeds, increase in leaf area per plant, shoot and root weights, release of certain phytohormones, augmented nutrients and water uptake by plants, chlorophyll content, hydraulic activity, protein content, and nutrient uptake-including phosphorus and nitrogen, increased biomass of the plants with more vigor growth and better carbohydrate accumulation.

A beneficial effect of *Azotobacter* on growth of various plants was reported by Nasaruddin (2014) who asserted that *Azotobacter* plays an important role in the nitrogen cycle in nature as it possesses a variety of metabolic functions. Besides, playing role in nitrogen fixation and producing siderophores, *Azotobacter* has the capacity to produce vitamins such as thiamine and riboflavin, and plant hormones viz., indole acetic acid (IAA), gibberellins and cytokines. *Azotobacter chroococcum* improved the plant growth by enhancing seed germination, increasing root development and advancing the root architecture by inhibiting pathogenic microorganisms around the root systems of plants (Revillas *et al.*, 2000; Mali and Bodhankar 2009; Abd El-Fattah *et al.*, 2013).

On the other side, fluorescent *Pseudomonas* is considered the most promising group of plant growth promoting rhizobacteria. It produces secondary metabolites such as antibiotics, phytohormones, volatile compounds hydrogen cyanide, siderophores and produced of indole - 3 - acetic acid (Sivasakthi *et al.*, 2013). In the same trend, Egamberdieva (2015) analyzed the plant growth promoting bacteria for their growth-stimulating effects on two wheat cultivars. The results showed *Pseudomonas* sp. and *Pseudomonas fluorescens* were able to colonize the rhizosphere of both wheat cultivars and significantly stimulated the shoot and root lengths and dry weight of plant. Also, *Azotobacter* in combination with *Pseudomonas fluorescens* had been shown to increase the plant height, dry weight,

and yield of maize up to 30 % over the control (Gholami *et al.*, 2009). In the same trend, Rashi *et al.*, 2016 reported that sesame plant treated with *Azotobacter* spp., *Pseudomonas* spp. and *Bacillus* spp. enhanced growth of seedlings, increased shoot length and number of branches.

On the other side, *Spirulina* is rich in organic nitrogenous components like amino acids which enhanced plant height, number of leaves, and increased plant biomass (Yassen *et al.*, 2018; Godlewska *et al.*, 2019). Mógor *et al.* (2018) showed also that the enzymatic hydrolysates of *Spirulina platensis* had a cytokinin-like effect which effectively promoted lettuce growth.

Table 2: Impact of some microorganisms on growth parameters of canola and sesame plants through growing seasons.

Treatments	Root length (cm plant ⁻¹)	Shoot height (cm plant ⁻¹)	Number of branches plant ⁻¹	Shoot dry weight (g plant ⁻¹)
Canola (2015–2016 : Winter season)				
Bacteria*	30.2	123.3	4.6	47.5
Fungi**	20.2	85.5	3.3	26.7
Cyanobacteria ***	23.3	95.5	3.6	30.6
Bacteria* +Fungi**	27.1	116.4	4.3	40.7
Bacteria* + Cyanobacteria ***	33.2	135.3	5.0	53.8
Fungi** + Cyanobacteria ***	27.4	113.2	4.0	37.5
Bacteria* +Fungi** + Cyanobacteria ***	29.7	126.7	4.4	45.4
Control	19.7	83.8	3.3	24.5
L.S.D. 5 %	3.4	8.0	1.1	4.0
Sesame (2016 : Summer season)				
Bacteria*	21.2	160.0	4.4	55.6
Fungi**	15.4	121.5	3.3	34.2
Cyanobacteria ***	16.8	133.6	3.3	40.6
Bacteria* +Fungi**	19.5	146.3	4.3	52.4
Bacteria* + Cyanobacteria ***	27.3	177.0	4.6	62.3
Fungi** + Cyanobacteria ***	20.8	151.0	3.6	47.5
Bacteria* +Fungi** + Cyanobacteria ***	22.2	165.4	4.3	53.6
Control	15.5	115.0	3.0	33.5
L.S.D. 5 %	3.3	9.4	1.0	4.2

Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.

Fungi:** *Pleurotus columbines* and *Trichoderma harzianum*.

Cyanobacteria *:** *Wolleea saccata* and *Spirulina platensis*

2. Effect of microorganisms on chlorophyll and carotenoids contents of canola and sesame plants.

Table 3 represents the effect of biological treatments on chlorophyll a, chlorophyll b, and carotenoids in leaves of canola and sesame plants after 75 days from planting. The highest values of these parameters of canola plants were 2.084, 0.803 and 1.113 mg g⁻¹ obtained under the mixture of bacteria and cyanobacteria respectively, while sesame plants were 1.763, 0.633 and 1.164 mg g⁻¹, respectively. The mixture of bacteria, fungi and cyanobacteria treatment came in the second order after the mixture of bacteria and cyanobacteria for chlorophyll a, chlorophyll b and carotenoids contents in canola and sesame plants which reached to 1.902, 0.746 and 0.993 mg g⁻¹ respectively for canola plants, while for sesame plant gave 1.673, 0.587 and 1.073 mg g⁻¹ under the previous treatment, respectively. The mixture of three types of the microorganisms recorded the nearly result from the individual bacteria treatment with both plants. Bacteria treatment alone was better than the treatment of mixture of bacteria and fungi, while mixture of fungi and cyanobacteria treatment was better results than individual fungi treatment or cyanobacteria treatment with regard to chlorophyll and carotenoid contents for canola and sesame plants as generally trend. The lowest values were obtained under control and individual fungi treatment. *Azospirillum* and *Azotobacter* improved the chlorophyll contents of plant leaves. Increase in leaf chlorophyll contents led to increase level of photosynthesis which correlated with increased amount of carbohydrates which ultimately led to the increase in plant height and number of branches per plant and ultimately seed yield (Nosheen *et al.*, 2019).

Noorieh *et al.* (2013) reported that PGPR species like *Azotobacter* and *Pseudomonas* increased the physiological and biochemical traits of *Brassica napus* L. under normal and stress condition. The

same attitude was observed by Heidari and Golpayegani (2012) who suggested that PGPR inoculation enhanced photosynthetic pigments of basil (*Ocimum basilicum* L.). On the other side, application of *Spirulina* extract gave a positive effect of carotene and chlorophyll a and b as well as increased amount of carbohydrates (Yassen *et al.*, 2018).

Table 3: Impact of microorganisms on chlorophyll and carotenoids contents of canola and sesame plants through growing seasons.

Treatments	Chlorophyll a mg g ⁻¹	Chlorophyll b mg g ⁻¹	Carotenoids mg g ⁻¹
Canola (2015–2016 : Winter season)			
Bacteria*	1.883	0.720	0.973
Fungi**	1.421	0.576	0.621
Cyanobacteria ***	1.701	0.573	0.763
Bacteria* +Fungi**	1.793	0.622	0.971
Bacteria* + Cyanobacteria ***	2.084	0.803	1.113
Fungi** + Cyanobacteria ***	1.821	0.674	0.873
Bacteria* +Fungi** + Cyanobacteria ***	1.902	0.746	0.993
Control	1.373	0.556	0.537
L.S.D. 5 %	0.140	0.083	0.075
Sesame (2016 : Summer season)			
Bacteria*	1.625	0.562	1.055
Fungi**	1.465	0.464	0.610
Cyanobacteria ***	1.523	0.472	0.694
Bacteria* +Fungi**	1.587	0.514	0.899
Bacteria* + Cyanobacteria ***	1.763	0.633	1.164
Fungi** + Cyanobacteria ***	1.634	0.490	0.792
Bacteria* +Fungi** + Cyanobacteria ***	1.673	0.587	1.073
Control	1.361	0.410	0.542
L.S.D. 5 %	0.099	0.053	0.087

Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.

Fungi:** *Pleurotus columbines* and *Trichoderma harzianum*.

Cyanobacteria *:** *Wollea saccata* and *Spirulina platensis*.

3. Effect of microorganisms on biological parameters of canola and sesame plants.

Table 4 revealed the positive action of biological treatments on dehydrogenase activity, carbon dioxide and percentage of root colonization for canola and sesame plants after 75 days from planting. All bio treatments had a positive role and increasing of these biological parameters.

The mixture of bacteria and cyanobacteria was more preferable and gave the highest values of dehydrogenase activity for canola and sesame plants which recorded 340.6 and 253.3 μgTPFg⁻¹ soil, respectively. On the other hand, no significant difference was observed for dehydrogenase activity between the individual bacterial treatment and the mixture of bacteria, fungi and cyanobacteria which gave 285.0 and 287.3 μgTPFg⁻¹ soil respectively for canola plants, while for sesame plants were 213.3 and 220.4 μgTPFg⁻¹ soil, respectively.

The individual bacteria treatment gave the higher values than the mixture of bacteria and fungi which were 285.0 and 243.3 μgTPFg⁻¹ soil respectively for canola, and the increase percentage reached 17% while, for sesame plants recorded 213.3 and 190.2 μgTPFg⁻¹ soil respectively, and the increase percentage reached 12%. The mixture of fungi and cyanobacteria treatment gave better results for dehydrogenase activity than individual fungi treatment which were 173.2 and 146.3 μgTPFg⁻¹ soil respectively for canola, while the values for sesame plants were 174.3 and 126.4 μgTPFg⁻¹ soil, respectively. Individual bacteria treatment was more supportive than individual fungi or cyanobacteria treatment and gave the highest dehydrogenase activity for both plants. Regarding to carbon dioxide, inoculation with bacteria and cyanobacteria led to significant increase of carbon dioxide which gave 241.3 and 207.2 mg 100 g⁻¹ soil for canola and sesame plants, respectively. The mixture of bacteria, fungi and cyanobacteria recorded the second increase of carbon dioxide for canola and sesame which recorded 196.2 and 191.9 mg 100 g soil⁻¹, respectively. The individual bacteria treatment was the most effective on carbon dioxide compared to individual cyanobacteria or individual fungi treatments and recorded 183.1 and 176.1 mg 100 g⁻¹ soil for canola and sesame, respectively.

Root colonization was also potentiated by inoculation with bacteria and cyanobacteria which gave 81.1% and 76.6% for canola and sesame plants, respectively. Individual treatment of bacteria gave significant increase of root colonization which gave 76.3 % and 70.1% for canola and sesame plants, respectively compared to other individual treatments and control.

The increase in dehydrogenase enzyme activity was attributed to the intense activity of microflora as a mixture of biomass than each individual one. The highest increase in microbial respiration was recorded with the mixture of microorganism in the soil (Massoud, 2005). Significant enhancement in the soil microbiological parameters such as dehydrogenase activity, alkaline phosphatase and microbial biomass were obtained in selected combinations of bacteria and cyanobacteria (Nain *et al.*, 2010).

Microorganisms helped to release the fixed forms of nutrients and improve the carbon content in soil and multiplication and metabolic activities by their growth (Malik *et al.*, 2013; Ryazanova *et al.*, 2009). Colonization of roots by *Bacillus subtilis* is beneficial to both the bacterium and the host plant. Approximately 30% of the fixed carbon produced by plants is secreted through root exudates. Colonization of the roots by bacteria provided a nutrient source and in exchange, plants were the recipient of bacterial compounds and activities that stimulated plant growth and provided stress protection to their hosts. *Bacillus subtilis* formed a thin bio-film on the roots for long-term colonization of the rhizosphere. Chemotaxis was required for *B. subtilis* to locate and colonize young roots (Allard *et al.*, 2016).

Inoculation of plants with *Pseudomonas* bacteria affected plant development by reducing the uptake of toxic ions, inducing systemic resistance, producing phytohormones, increasing nutrient uptake and establishing root colonization (Chu *et al.*, 2019).

Cyanobacteria inoculation generally enhanced the soil biological activity in terms of increasing the total bacterial, total cyanobacterial counts, CO₂ evolution, dehydrogenase activity, nitrogenase and phosphatase activities (Mahmoud *et al.*, 2007). Inoculation by mixture of bacteria and cyanobacteria gave highest rate of root colonization of canola plants (EL-Tapey *et al.*, 2019).

Table 4: Impact of microorganisms on biological parameters of canola and sesame plants through growing seasons.

Treatments	Dehydrogenase µgTpF g ⁻¹ soil	CO ₂ mg100 g ⁻¹ soil	Colonization %
Canola (2015–2016 : Winter season)			
Bacteria*	285.0	183.1	76.3
Fungi**	146.3	100.0	41.4
Cyanobacteria ***	166.4	107.3	43.5
Bacteria* +Fungi**	243.3	168.5	67.2
Bacteria* + Cyanobacteria ***	340.6	241.3	81.1
Fungi** + Cyanobacteria ***	173.2	120.7	48.3
Bacteria* +Fungi** + Cyanobacteria ***	287.3	196.2	75.6
Control	123.5	91.3	37.3
L.S.D. 5 %	10.1	8.2	7.2
Sesame (2016 : Summer season)			
Bacteria*	213.3	176.1	70.1
Fungi**	126.4	87.2	40.4
Cyanobacteria ***	148.6	104.4	42.0
Bacteria* +Fungi**	190.2	163.6	63.3
Bacteria* + Cyanobacteria ***	253.3	207.2	76.6
Fungi** + Cyanobacteria a ***	174.3	136.1	47.0
Bacteria* +Fungi** + Cyanobacteria ***	220.4	191.9	70.0
Control	115.3	85.2	33.2
L.S.D. 5 %	9.4	8.07	7.0

Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.

Fungi:** *Pleurotus columbines* and *Trichoderma harzianum*.

Cyanobacteria *:** *Wollea saccata* and *Spirulina platensis*.

4. Effect of some microorganisms on yield parameters of canola and sesame plants.

Table 5 elucidated the effect of bio treatments on shoot height, number of pods per plant, weight of 1000 seed, seed yield and oil % for canola and sesame plants at harvest. The mixture of bacteria and

cyanobacteria was preferable and gave the significant highest values of shoot height were 145.4 and 210.0 cm plant⁻¹ for canola and sesame plants, respectively. The individual treatment of bacteria was superior as compared with individual treatments of fungi or cyanobacteria and recorded 136.0 and 181.0 cm plant⁻¹ for shoot height of canola and sesame plants, respectively. The application of individual treatment of bacteria led to increase percentage of shoot height reached to 18.2 and 34.0%, respectively for canola and sesame plants over individual treatments of fungi, while the increase percentage reached to 13.3 and 26.3% over individual treatment of cyanobacteria, respectively for canola and sesame plants. The control and individual treatment of fungi showed the lowest shoot height were 109.8 and 115.2 cm plant⁻¹, respectively for canola, while for sesame plant the values were 133.0 and 135.0 cm plant⁻¹, respectively. The mixture treatment of bacteria and cyanobacteria significantly enhanced the number of pods per plant being 177.3 and 103.0 pods / plant for canola and sesame plants, respectively. Individual treatment of bacteria gave significant increase in number of pods compared to individual treatment of cyanobacteria or fungi in both plants.

In addition, numbers of pods were higher under the mixture treatment of fungi and cyanobacteria which were 157.2 and 75.3 pods / plants, respectively than individual treatment of fungi which were 136.8 and 54.0 pods / plant for canola and sesame, respectively.

Regarding the weight of thousand seed, the highest significant value were 4.09 and 4.72 g plant⁻¹ obtained for the mixture of bacteria and cyanobacteria for canola and sesame plants, respectively. The individual bacteria treatment was superior compared to individual fungi or cyanobacteria treatments which gave 3.75 and 4.42 g for canola and sesame plants, respectively. The lowest biological treatment obtained was in the individual fungi treatment which recorded 3.03 and 3.93 g for canola and sesame plants, respectively.

Table 5: Impact of microorganisms on yield parameters of canola and sesame plants through growing seasons.

Treatments	Shoot height (cm plant ⁻¹)	Number of pods plant ⁻¹	Weight of 1000 seed	Seed yield (Kg fed ⁻¹)	Oil %
Canola (2015-2016: Winter season)					
Bacteria*	136.0	167.7	3.75	1389.2	43.2
Fungi**	115.2	136.8	3.03	1295.5	34.4
Cyanobacteria ***	120.3	144.7	3.20	1303.7	35.3
Bacteria* +Fungi**	130.6	163.5	3.41	1334.3	4.20
Bacteria* + Cyanobacteria ***	145.4	177.3	4.09	1408.8	46.3
Fungi** + Cyanobacteria ***	125.2	157.2	3.32	1325.4	37.0
Bacteria* +Fungi** + Cyanobacteria ***	138.3	170.2	3.86	1384.0	42.0
Control	109.8	130.8	2.87	1280.2	34.2
L.S.D. 5 %	4.3	10.4	0.18	14.0	1.6
Sesame (2016: Summer season)					
Bacteria*	181.0	90.2	4.42	567.0	47.0
Fungi**	135.0	54.0	3.93	520.3	43.0
Cyanobacteria ***	143.3	64.3	4.12	518.4	43.2
Bacteria* +Fungi**	173.4	84.5	4.40	546.2	45.6
Bacteria* + Cyanobacteria ***	210.0	103.0	4.72	590.3	51.4
Fungi** + Cyanobacteria ***	160.5	75.3	4.21	541.4	45.4
Bacteria* +Fungi** + Cyanobacteria ***	180.3	94.0	4.47	562.6	47.3
Control	133.0	52.1	3.81	512.5	42.0
L.S.D. 5 %	7.6	9.06	0.19	9.4	2.1

Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.

Fungi:** *Pleurotus columbines* and *Trichoderma harzianum*.

Cyanobacteria *:** *Wollea saccata* and *Spirulina platensis*.

The mixture treatment of bacteria and cyanobacteria gave the highest results of canola and sesame seed yield which were 1408.8 and 590.3 kg fed⁻¹, respectively. No significant differences between the individual bacteria treatment and mixture of all microbes for canola and sesame seed yield. The respective corresponding values of the previous treatments were, 1389.2 and 1384.0 kg fed⁻¹ for canola plants, while 567.0 and 562.6 kg fed⁻¹ for sesame plants, respectively.

With respect to oil percentage in canola and sesame seeds, the results showed that the highest values were 46.3 and 51.4% for the mixture of bacteria and cyanobacteria followed by the individual

treatment of bacteria which were 43.2 and 47.0 % for canola and sesame, respectively. The lowest values of oil percentage in canola and sesame seeds were 34.2 and 42.0 % for control treatment.

Plant Growth Promoting Rhizobacteria (PGPR) as biofertilizers had positive effects on seed's yield of many crops and significant increase in all growth and yield parameters compared to untreated plant due to symbiotic N₂-fixing bacteria, phosphate and potassium releasing (Tsegaye *et al.*, 2017; Bechtaoui *et al.*, 2019). Application of PGPR improved leaf area index, dry matter, harvest index, yield and yield components, seed oil content and protein content in sesame (Nosheen *et al.*, 2011; Jahanm *et al.*, 2013).

According to Bhattacharyya and Jha, (2012) *Pseudomonas* spp. as plant growth promoting rhizobacteria produced hormones and anti-metabolites which promoted root growth, decomposed of organic matter which helped in soil mineralization there by increased availability of nutrients and improved crop yield. Farhan *et al.* (2010) reported increase in chlorophyll content, number of shoots, height of shoots, number of branches, number and area of leaf per plant, number of seeds per plant and percentage of oil for sesame seeds in presence of *Pseudomonas putida* and *Pseudomonas fluorescens*. Also inoculation with the N-fixing bacteria *Azospirillum* and *Azobacter* increased the sesame and canola seed yields and oil quality (Yasari *et al.*, 2008; Shakeri *et al.*, 2016).

In addition, cyanobacteria changed the soil microflora and improved soil fertility by producing growth-promoting agents, as well as increasing growth and yield of some crops, enhancing soil microbial activity and promoted nutrient content (Karthikeyan *et al.*, 2007; Shariatmadari *et al.*, 2015).

5. Impact of biological treatments on soil aggregates through growing seasons.

Table 6 showed that the distribution of stable aggregates was changed with the most treatments. The aggregate size distribution of the small soil diameter (0.20 - 0.125 mm), very small (0.125 – 0.063 mm) and extremely small (< 0.063 mm), were decreased, while the large fraction (10 – 1 mm), medium (1 – 0.5 mm) and sub medium (0.25 – 0.125), were increased under all treatments except individual treatment of bacteria treatment as compared with control.

Table 6: Effect of some microorganisms on soil aggregates size distribution through growing seasons.

Treatments	Dry sieving aggregates size distribution (%)						Aggregates (%)
	10 – 1 mm	1 - 0.5 mm	0.5 – 0.25 mm	0.25 – 0.125 Mm	0.125 – 0.063 mm	< 0.063 mm	
Canola (2015 – 2016 : Winter season)							
Bacteria*	4.08	21.53	42.33	15.60	7.55	8.91	0.17
Fungi**	4.71	22.30	43.10	14.88	6.80	8.21	1.62
Cyanobacteria ***	4.12	21.61	42.61	16.06	7.10	8.50	1.03
Bacteria* +Fungi**	6.11	24.00	45.75	10.74	5.70	7.70	3.23
Bacteria* + Cyanobacteria ***	5.63	23.61	44.20	12.15	6.50	7.91	2.22
Fungi** + Cyanobacteria ***	6.40	24.33	46.20	9.92	5.60	7.55	3.48
Bacteria* +Fungi** + Cyanobacteria ***	6.87	25.82	47.11	7.98	5.21	7.01	4.41
Control	4.06	21.50	42.30	15.51	7.55	9.08	0.00
L.S.D. 5 %	0.358	0.321	0.451	0.522	0.451	0.372	
Sesame (2016 : Summer season)							
Bacteria*	4.09	21.55	42.41	15.40	7.65	8.90	0.08
Fungi**	4.70	22.40	43.34	14.73	6.63	8.20	1.80
Cyanobacteria ***	4.20	21.66	42.81	15.65	7.22	8.46	0.95
Bacteria* +Fungi**	6.23	24.05	45.80	10.88	5.51	7.53	3.59
Bacteria* + Cyanobacteria ***	5.62	23.82	44.19	12.25	6.36	7.76	2.51
Fungi** + Cyanobacteria ***	6.55	24.61	46.40	9.68	5.44	7.32	3.87
Bacteria* +Fungi** + Cyanobacteria ***	6.91	26.10	47.30	7.58	5.10	7.01	4.52
Control	4.06	21.50	42.30	15.51	7.55	9.08	0.00
L.S.D. 5 %	0.408	.386	0.524	0.563	0.520	0.380	

Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.

Fungi:** *Pleurotus columbines* and *Trichoderma harzianum*.

Cyanobacteria *:** *Wollea saccata* and *Spirulina platensis*.

The highest values due to soil inoculation by the mixture of bacteria, fungi and cyanobacteria of the two cultivated seasons by canola and sesame compared with control. The inoculation treatments could be arranged descending due to their impact on sandy soil aggregates as follow: bacteria + fungi + cyanobacteria > fungi + cyanobacteria > bacteria + fungi > bacteria + cyanobacteria > fungi > cyanobacteria > bacteria > control.

The biological treatments enhanced the aggregation of the smaller soil fractions by cement materials like exopolysaccharides, that released from investigated microorganisms and consequently those smaller fractions were moved to the largest size distribution and the largest fractions were increased (Taha, 2017). Zian (2018) and El-Tapey *et al.* (2019) mentioned that, increasing aggregate stability of soil is associated with root growth which due to polysaccharides produced in the rhizosphere. Ashraf *et al.* (2013) reported that exopolysaccharides released from some microorganisms played a role in fertility and enhanced the formation of soil micro-aggregates and contributed to build up soil physical structure, regulated nutrients and water flow from rhizosphere soil to the plant.

6. Impacts of microorganism treatments on soil physical properties

6.1. Bulk density:

Table 7 showed that the values of bulk density decreased under all inoculation treatments whether mixed or single of them. The highest significant decrease was obtained under the mixture of microbiota inoculation than the individual ones. The mixture of bacteria, fungi and cyanobacteria gave 7.42 and 7.51% over control for soil cultivated by canola and sesame, respectively.

Table 7: Effects of microorganisms on bulk density, total porosity and pore size distribution through growing seasons.

Treatments	Bulk Density (Mg.m ⁻³)	Total Porosity %	Pore size distribution %		
			Macro-pores Drainable pores (> 28.8 μ)	Micro-pores (<28.8 μ)	
				Water Holding Pores (28.8-0.19μ)	Fine Capillary Pores (<0.19 μ)
Canola (2015 – 2016 : Winter season)					
Bacteria*	1.72	39.41	28.94	8.67	1.77
Fungi**	1.71	40.50	27.85	9.50	1.92
Cyanobacteria ***	1.73	40.01	27.01	8.80	1.85
Bacteria* +Fungi**	1.67	41.52	26.20	11.30	2.35
Bacteria* + Cyanobacteria ***	1.69	41.77	26.80	10.22	2.00
Fungi** + Cyanobacteria ***	1.65	42.90	26.00	11.85	2.46
Bacteria* +Fungi** + Cyanobacteria ***	1.62	43.60	25.11	12.20	2.88
Control	1.75	39.40	28.94	8.66	1.76
L.S.D. 5 %	0.291	0.485	0.460	0.414	0.404
Sesame (2016 : Summer season)					
Bacteria*	1.75	39.40	28.93	8.65	1.76
Fungi**	1.71	40.72	27.51	9.59	1.99
Cyanobacteria ***	1.72	40.31	27.10	9.01	1.88
Bacteria* +Fungi**	1.66	42.10	26.03	11.51	2.40
Bacteria* + Cyanobacteria ***	1.69	41.81	26.64	10.45	2.11
Fungi** + Cyanobacteria ***	1.63	43.70	25.56	11.63	2.51
Bacteria* +Fungi** + Cyanobacteria ***	1.60	44.01	24.78	12.50	2.95
Control	1.73	39.40	28.94	8.66	1.76
L.S.D. 5 %	0.283	0.470	0.381	0.461	0.424

Bacteria*: *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*.

Fungi:** *Pleurotus columbines* and *Trichoderma harzianum*.

Cyanobacteria *:** *Wolleea saccata* and *Spirulina platensis*.

6.2. Total porosity:

Total porosity percentages increased in all inoculation treatments except the single mixture of bacteria. The mixture of bacteria, fungi and cyanobacteria significantly increased followed by fungi and cyanobacteria, especially at second season after sesame cultivation, which were 43.60 and 42.90% respectively for soil cultivated by canola, while for soil cultivated by sesame plant, gave 44.01 and

43.10 %, respectively. Meanwhile, the lowest increases were obtained under bacteria treatment for soil cultivated by canola and sesame.

6.3. Pore size distribution percentage:

The macro-pores ($>28.8\mu$) which represented drainable pores was significant decreased under the mixture treatments than the single ones, while the micro-pores ($<28.8\mu$), i.e., water holding pores ($28.8 - 0.19 \mu$) and fine capillary pores ($< 0.19\mu$) took the opposite trend where it increased. In addition, bacteria treatment showed no change for pore size distribution percentage. The superior treatment was bacteria, fungi and cyanobacteria for first season of canola and second season of sesame. This was due to improving soil aggregation through production of cement organic material by microbiota and enhancing soil structure. Increasing the apparent soil volume led to decrease in bulk density (Abd EL-Hamid *et al.*, 2013). In addition, a thin coat of humate as organic material under biofertilizers helped in increasing of total soil porosity and micro-pores ($< 28.8\mu$) and lowered loss of water from the soil by leaching or deep percolation (El-Tapey *et al.*, 2019). Cyanobacteria increased soil stability and reduced erosion by water and wind, increased surface moisture by holding large amounts of water, fixed carbon and nitrogen, and increased soil fertility. In arid and semiarid environments, cyanobacteria were one of the most important microorganisms forming biological soil crust communities (Chamizo *et al.*, 2018).

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