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EFFECT OF BIOFERTILIZATION AND SOME SINAI'S FLORA EXTRACTS ON THE VEGETATIVE GROWTH AND YIELD OF BROAD BEAN

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ABSTRACT

Broad bean plants (*Vicia faba* L.) were cultivated in two field experiments at the Experimental Station of the Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt, during 2018/2019 and 2019/2020 seasons under North Sinai condition, to study the effect of soil application of some biofertilizers and foliar spray with some wild plant extracts on broad bean plants. Biofertilizer treatments contained combination of Arbuscular Mycorrhizal Fungi (AMF) + *Trichoderma harzianum* + *Rhizobium leguminosarum*. Three wild plant extracts treatments; i.e., Qeysoom Gebeli (*Achillea fragrantissima* L.), Harmal (*Peganum harmala* L.) and Mitnaan (*Thymelaea hirsute* L.) were sprayed on plants. Treatments were arranged in a randomized complete block design with three replicates in split plot system. The results indicated that biofertilizers had significant effected of all studied traits in both seasons. Foliar spraying of *Thymelaea hirsute* extract had the highest stimulation effects on spores count, root colonization, mycorrhizal status, and weight of non-active nodules, broad bean plant growth characters; i.e. stem length, number of branches per plant, leaf area, and shoot fresh and dry weight as well as both of fresh and dry weight of roots. Both of *Thymelaea hirsute* and *Achillea fragrantissima* extracts combined with biofertilizer treatment had significant effects on yield and its components (pod length, number of pods per plant, average pod weight total yield per plant, and weight of seeds per pod) in both seasons. The combination between *Thymelaea hirsute* and biofertilizer was the superior interaction treatment of this study.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), Biofertilizer, Broadbean, Plant growth, Yield, Sinai's Flora extracts

INTRODUCTION

Broad bean (*Vicia faba* L.) makes an important contribution to the diet of people in many countries. It represents a very interesting class of food crops due to its high protein content (about 30%) (Saad and El-Kholy, 2001 and Nawar *et al.,* 2010). It can grow successfully in different soil types and is an important crop for soil improvement and used as a break crop in cereal rotation to keep the soil fertile and productive through nitrogen fixation (Metwally *et al.,* 2011).It is the most important legume crop for human and livestock in Egypt and many parts of the world (Metwally *et al.,* 2011; Eman *et al.,* 2017).

Biofertilizer can transform important nutrient elements from unusable to usable form by biological processes leading to cultivation (Hedge *et al*., 1999).They improve nitrogen use efficiency, increase photosynthesis and the efficiency of solubilizing nutrients such as phosphorus and iron in soil and organic matter; enhance the production of plant hormones, induce systemic resistance mechanisms and induce roots in hydroponics (Tonfack *et al.,* 2009 andKapri *et al.,* 2010). AMF colonizes the roots of plants and radiates hyphae to the surrounding soil to supplement the root function of the host (Jansa *et al.,* 2011). AMF plays an important role in improving N_2 fixation by providing an environment conducive to rhizobia infecting plant roots (Mohammadi *et al.,* 2012).

Trichoderma spp. is a genus of fungi that occurs in many parts of the world and is widely used due to its numerous positive effects on plant growth and disease resistance; i. e., it is widely used as a biofertilizer and biopesticide (Alkadious and Abbas, 2012). To avoid intensifying the use of chemical fertilizers that can cause irreversible environmental damage, including salinity of the soil and eutrophication of local lakes and rivers due to manure runoff, AMF, *Trichoderma*, *Rhizobium* and rhizobacteria are considered effective biofertilizers (Singh *et al.,* 2016).

In recent years, AMF and *Trichoderma* also provide benefits unrelated to the quality and quantity of crop yields, including improved soil structure, increased soil microbiome biodiversity, soil carbon sequestration, tolerance to biotic and abiotic loads, etc., which are maintained during periods of vegetation (Martínez-Medina *et al.* and 2009; Bitterlich *et al.,* 2018). One of the well-known beneficial effects of *Trichoderma* on plants is to dissolve P through acidification, chelation or redox activity to improve the utilization of P by plants. The benefits of AMF and *Trichoderma* on crop growth have been fully evaluated (Szczałba *et al.,* 2019). In addition, Biofertilizers, products that contain living cells from different types of microorganisms have been used in an integrated nutritional system. Biofertilizers based on *Trichoderma* have also been reported to improve the absorption of N, P and K (Amaresan *et al.,* 2020).

Recently, many studies indicated that, many wild plant extracts are natural sources of many products and has stimulatory effects on bean plants. Mekki (2014) studied cytogenetic effects of crude extracts of *Peganum harmala* and their effects on *Vicia faba* plants and found that the highest concentration of ethanol extract resulted in the lowest MI and the highest values of plant height and the number of branches per plant. Contrarily, shoo *et al*. (2013) found that application of aqueous extract of whole *P. harmala* plant greatly suppressed growth of wheat and lettuce seedling, (Shao *et al*., 2013).

Therefore, the objectives of the present study are to investigate the effect of bio fertilizers, three wild plant extracts at different concentrations and their interactions on Broadbean yield and yield components.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm, Faculty of Environmental Agricultural Sciences, Arish University, Egypt, during the winter seasons of 2018/2019 and 2019/2020 to study the effect of soil application with biofertilization (combination of AMF + *Trichoderma harzianum* + *Rhizobium leguminosarum*) and spraying with some Sinai's Flora extracts; i.e,. Qeysoom Gebeli (*Achillea fragrantissima* L.), Harmal (*Peganum harmala* L.) and Mitnaan (*Thymelaea hirsute* L.) on growth and yield of broad bean plants in loamy sand soil. Seeds of broad bean cv."Luzde otono" imported from Fito Semillas Co., Turkey, were cultivated Irrigation water analyses is shown in Table 1 analysis were carried out according to (Jackson (1958)) .Soil physical and chemical properties were done according Piper (1947) (Table 2). One third of organic used fertilizers were added during soil preparation and the other two- thirds were divided into 20 equal portions and added twice weekly beginning 25 days after sowing. plant were irrigated by drip irrigation system, two dripper lines were used (8m length for each),on a plot of 0.9 m wide, so, plant area was 14.4m^2. Seeds were sown 15th October in both seasons. One set of plants were kept untreated to act as control. Foliar sprays as applied using a hand pressure sprayer. Triton-B emulsifier at a rate of 0.1% was used at 1.5 ml. 5 liter^{-1} extract as a surfactant. Other normal agricultural practices for broad bean production were done as recommended in North Sinai region.

One dripper line (7.2 m^2) was used for growth parameters and the other line was used for yield determination. The treatments were arranged randomly in a randomized complete block design RCBD (split plot design) with three replications. Treatments of factor A (biofertilizer) were randomly arranged in the main plots, while treatments of factor B (wild plant extracts) were randomly arranged in the sub-plots.

According to **Piper (1947)**

The following are details for the two factors under study:

Biofertilizer treatments:

Broad bean seeds (*Vicia faba* Lcv."Luzde otono" imported from Fito Semillas Co., Turkey. were surface sterilized with 2% sodium hypochlorite for 10 min, subsequently rinsed with sterilized distilled water.

Preparation of biofertilizers agent

Different sources of biofertilizers (AMF + *T. harzianum* + *R. leguminosarum*) were used to study thies effect. For isolating these biofertilizer microbial agents were carried out in the Microbiology laboratory at Faculty of Science, Arish University, followed by testing the activity of these biofertilizers as follows:

AMF

Source, Preparation and multiplication of AMF inoculum

Firstly, AMF were isolated from rhizosphere of different medicinal plants *(Coriandrum sativum, Vicia faba, Lactuca sativa, Allium cepa, Pisum sativum* and *Cucurbita pepo)* grown in soils of North Sinai Governorate, Egypt. A rhizospheric soil sample of each plant species was collected to a depth of up to 20 cm and analyze the AMF community (spore counts and percent of root colonization). Three samples were extracted from 100 g of each plant soil sample by wet sieving and decanting. through a series of wire meshes, having a mesh size of1000-32µm sieves. (Gerdemann and Nicolson 1963) modified from INVAM (2020).

Spores were examined to detect different morphological properties such as spore shape, color, longest dimension, wall thickness, and hyphae. Some hyphae may be attached to suspensor-like cells. Stained spores with Meltzer's reagent were then mounted in poly-vinyl alcohol-lactic acid-glycerol (PVLG) to make permanent slides. Spores slides were examined through microscope with differential interference contrast (INVAM 2020).

Increasing the inoculum AMF by Trapping culture

To increase the AMF propagules, the mixtures of all identified AMF spores were multiplied on Sudan grass plant (*Sorghum sudanenses* Pers.) as a recommended trap plant in an autoclaved soil for a period of 4 months to increase spore counts in sterilized plastic pots having a sterilized sandy clay soil in the greenhouse of Botany and Microbiology Dept., Faculty of Science, Arish University, Egypt (Ravolanirina *et al.,* 1987). During transplantation, AMF inoculum was added at the rate of 50 g of inoculum AMF/seedling which contained (spores and colonized Sudan grass root fragments), were transferred to the experimental field and incorporated into the soil at a depth of 2-3 cm below Broad bean plants according to Menge and Timmer (1982).

AMF colonization in Broad bean

Fine roots were collected from soil at 30th day of transplanting, at flowering stages and yield stages, washed with tap water, cut into 0.5–1.0 cm long pieces, and fixed solution till investigation processing. In total, 100 root segments were cleared in 10% KOH, heated for 10 min at 80–90°C in a water bath, rinsed twice with distilled water, and placed with diluted HCl (1 N) for 5min. The

Table 1: Chemical properties of irrigation water

pH	EC ($dS.m^{-1}$)	Soluble ions ($meq.l^{-1}$)							
		Cations				Anions			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	CO_2^{--}	SO_4^{--}
First season (2018/2019)									
7.55	5.93	20.50	16.80	18.50	0.24	45.92	2.90	-	7.22
Second season (2019/2020)									
7.60	6.00	21.00	17.00	18.80	0.25	46.75	2.97	-	7.28

Table 2: Physical and chemical properties of investigated soil profile (0-30cm) of cultivated area

Properties	First season (2018/2019)	Second season (2019/2020)
Particles size distribution (%)		
Soil texture	Loamy sand	Loamy sand
Chemical properties (Soluble ions (in 1:5 soil water extract)		
Ca^{+}($meq.l^{-1}$)	3.90	3.90
Mg^{+}($meq.l^{-1}$)	3.62	3.43
Na^{+}($meq.l^{-1}$)	2.54	2.59
K^{+}($meq.l^{-1}$)	0.34	0.32
CO_3 ($meq.l^{-1}$)	-	-
HCO_3 ($meq.l^{-1}$)	4.30	4.40
Cl^{-} ($meq.l^{-1}$)	4.70	4.35
SO_4 ($meq.l^{-1}$)	1.50	1.45
EC(dSm-1) in 1:5 water extract)	0.08	1.02
pH(in1:2.5Soil water suspension extract)	8.10	8.13
Organic matter %	0.153	0.171
$CaCo_3$ %	9.2	9.5

Table 3: Main phyto-constituents of tested plant extracts

Qeysoom Gebeli Achillea fragrantissima		**Harmal *Peganum harmala***		**Mitnaan *Thymelaea hirsute***	
Family : Compositae		**Family:** Zygophyllaceae		**Family:** Thymelaeaceae	
Compound	%	Compound	%	Compound	%
Santolina triene	0.97	2-Acetyl-Thiazole	1.3	1-Heptene	0.17
α-Pinene	0.43	Santolina alcohol	2.0	Heptane	28.34
4-Methyl-2-pentenolide	0.12	cis-Dihydro-rose oxide	1.8	Hexamethylcyclotrisi-loxane	0.52
Camphene	1.18	1-Octen-ol	0.7	Iso menthone	0.48
β-Pinene	0.70	trans-Dihydro-Rose oxide	0.4	Cyclohexaneethanol	0.33
Myrcene	0.98	n-Octanol	1.1	β -citronello	0.96
Yomogi alcohol	1.41	Methyl butanoate,3-methyl-3-butenyl	2.9	Citronellyl formate	9.98
Methyl 2-oxohexanoate	0.09	3-Decanone	1.1	Citrol	1.87
p-Cymene	1.62	Camphor	2.7	Dodecamethylcyclo-hexasiloxane	1.36
Limonene	0.35	Benzene acetonitrile	1.3	α -copaene	1.45
β-Phellandrene	0.23	1-Dodecene	0.7	β -Bourbonene	2.43
Artemisia ketone	49.53	α -Terpinen-7-al	2.2	trans- β -caryophyllene	3.25
cis-Sabinene hydrate	0.26	Dihydro carveol acetate	1.0	α -Muurolene	0.47

Artemisia alcohol	1.21	Terpinyl acetate	0.9	α – Gurjunene	0.41
2,4-Dimethyl-2,4-heptadienal	0.13	Eugenol	17.2	Germacrene-D	12.98
trans-Sabinene hydrate	0.30	Decanoic acid	1.0	α -Humulene	0.95
Epoxyterpinolene	0.22	n-Undecanol	2.3	α -amorphene	1.49
Camphor	14.77	Methyleugenol	0.3	δ -selinen	0.31
cis-Chrysenthemol	0.14	(E)-Methyl isoeugenol	0.6	Aromadendrene	0.36
Lavandulol	0.45	Methyl p-tert-buthylphenil acetate	0.8	5-Methyl-2-N-methylphenylamino-2-Thiazoline	0.43
Borneol	0.83	β-Curcumene	1.9	δ -cadinene	2.55
Terpinen-4-ol	0.34	2E,4E-Dodecandienal	2.1	α –agarofuran	0.26
4-Methylacetophenone	0.12	(Z)-Nerolidol	1.6	Y-Eudesmol	11.81
Cryptone	1.18	Dodecanoic acid	5.9	Cyclopentasiloxane, decamethyl	2.59
p-Cymen-8-ol	0.68	Spathulenol	2.3	Neryl acetate	0.56
α-Terpineol	0.16	1-Esadecene	1.6	linalyl acetate	0.19
Cuminal	0.13	Cubenol	2.0	Morphin silyliert	0.83
Carvotanacetone	0.19	β-Acorenolo	2.9	Tetradecamethyl-heptasiloxane	11.83
Cuminol	0.21	Cedr-8,15-en-10-olo	4.1	Dodecamethyl-hexasiloxane	0.82
3-Oxo-p-menth-1-en-7-al	0.22	α-Eudesmol	1.6		
trans-Cadina-1(6),4-diene	0.31	n-Tetradecanol	12.3		
α-Curcumene	0.55	epi-α-Bisabololo	3.0		
δ-Amorphene	0.17	Ciperotundone	3.0		
(E)-Nerolidol	0.60	Eptadecane	1.2		
Caryophyllene oxide	0.22	Calamenen-10-one	0.5		
α-Bisabolol oxide B	2.62	Longifolol	2.7		
α-Bisabolol	11.20	Farnesale	1.0		
		Amorpha-4,9-diene	0.3		
Mansi *et al.,* (2019)		Apostolico *et al.,* (2016)		Kadri *et al.,* (2011)	

cleared root segments of Broad bean plants were then stained with 0.05% trypan blue stain in lactophenol and left in water bath for 5–10 min at 80–90°C (Trouvelot *et al.,* 1986 and Rajapakse & Miller 1994). The percentage of AMF colonization in root was calculated by the gridline intersect method (Giovannetti and Mosse1980) according to the following equation:

$$\text{AMF colonization (\%)} = \frac{\text{(Total number of root segments colonized)}}{\text{(Total number of root segments studied)}} \text{ X } 100$$

R. *leguminosarum*

Source, Preparation and multiplication of *R. leguminosarum* inoculum

R. leguminosarum strain used in this work was isolated from fresh surface-sterilized nodules present in the roots of broad bean plants. It was isolated from different locations in North Sinai, Egypt. The bacterial isolate selected from a collection of native rhizobia strains based on its ability to improve the growth of broad bean plants in vitro at Botany and Microbiology Dept., Faculty of Science, Arish University, Egypt. For inoculant preparation, the bacteria were grown on yeast mannitol agar media. Before sowed, the seeds of broad bean plant were soaked with *R. leguminosarum* for 30 min (Somasegaran and Hoben 1985).

T. harzianum

Source, Preparation and multiplication of *T. harzianum* inoculum

T. harzianum was firstly isolated from different locations in North Sinai soils and then grown on potato dextrose agar medium at 30°C for 7 days at Botany and Microbiology Dep., Faculty of Science, Arish University, Egypt, after incubation, spores were harvested in sterilized distilled water as previously described by (Rubio *et al.,* 2014

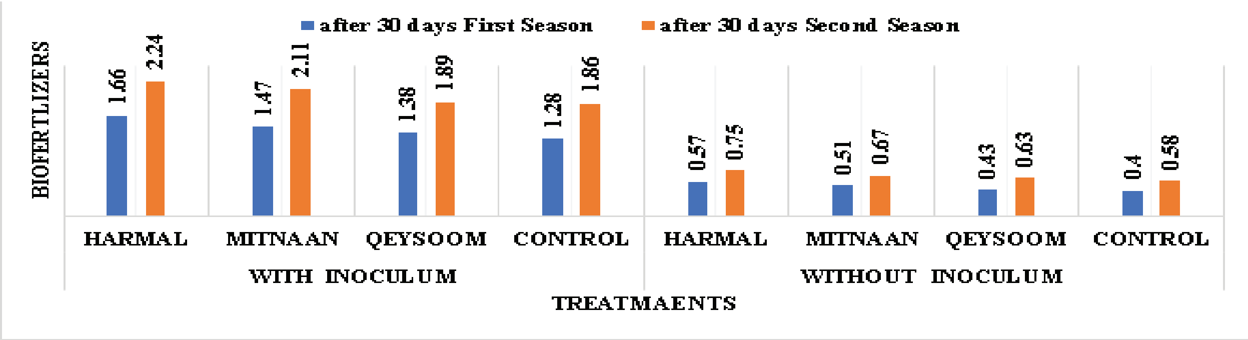
Table 4: Isolation of Biofertilizers from different plant species in North Sinai

Plant species	Family	Biofertilizers			
		AMF		T. harzianum	R. leguminosarum
			Root colonization (%)		
Coriander					
(*Coriandrum sativum* L.)	Apiaceae	18	13.00	-	-
Broad bean					
(*Vicia faba* L.)	Fabaceae	30	21.00	+	+
Lettuce					
(*Lactuca sativa* L.)	Asteraceae	25	19.00	+	-
Onion					
(*Allium cepa* L.)	Liliaceae	26	19.00	+	-
Pea					
(*Pisum sativum* L.)	Fabaceae	34	22.00	+	+
Squash					
(*Cucurbita pepo* L)	Cucurbitaceae	13	9.00	-	-
Average		24.33	17.17		

Table 5: Effect of biofertilizers treatments and some Sinai's Flora extracts on the spore counts and total root colonization (%) in Broadbean rhizosphere

Plant ext.	Spore count									Root Colonization's (%)								
	after 30 days			Flowering stage			Yield stage			after 30 days			Flowering stage			Yield stage		
	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean
First Season																		
Thymelaea hirsute	157.0 a	23.67e	90.33a	312.30a	44.33e	178.30a	420.7a	65.33e	243.00a	45.01a	13.57d	29.29a	85.47a	37.01d	61.24a	64.98a	25.95e	45.46a
Achillea fragrantissima	124.0 b	19.00ef	71.50b	290.00b	39.67ef	164.8 b	348.30b	54.6e	201.50b	42.85a	12.12de	27.49a	76.45b	31.25e	53.85b	55.35b	19.20f	37.27b
Peganum harmala	114.3 c	15.00f	64.67c	260.00c	31.33fg	145.7c	312.30c	40.33f	176.30c	36.00b	10.96ef	23.48b	73.77b	26.25f	50.01bc	51.45c	16.80f	34.12b
Control	63.67 d	14.33f	39.00d	204.70d	26.67g	115.7d	280.70d	29.33f	155.00d	29.58c	8.867f	19.23c	65.78c	25.13f	45.45c	44.42d	12.37g	28.40c
Mean	114.75 a	18.00b		266.75a	35.50b		340.50a	47.42b		38.36a	11.38b		75.37a	29.91b		54.05a	18.58b	
Second Season																		
Thymelaea hirsute	175.30 a	31.67e	103.50a	328.7a	49.33d	189.01a	522.0a	73.67e	297.80a	48.65a	14.65e	31.65a	87.50a	39.82e	63.66a	69.13a	27.86e	48.49a
Achillea fragrantissima	138.00 b	23.67ef	80.83b	311.30b	42.00d	176.7b	398.30b	59.00f	228.70b	44.55b	13.64e	29.10a	79.13b	35.04f	57.08b	58.75b	22.16f	40.46b
Peganum harmala	122.00 c	19.00f	70.50b	305.00b	40.00de	172.5b	372.30c	45.33g	208.80c	40.11c	11.52ef	25.81b	75.15c	29.38g	52.26c	53.25c	17.83g	35.54c
Control	66.67d	16.00f	41.33c	215.70c	32.33e	124.0c	306.00d	34.00h	170.00d	33.55d	9.600f	21.57c	69.74d	20.97h	45.35d	48.04d	15.71g	31.88d
Mean	125.50 a	22.58b		290.17a	40.92b		399.67a	53.00b		41.71a	12.35b		77.88a	31.30b		57.29a	20.89b	

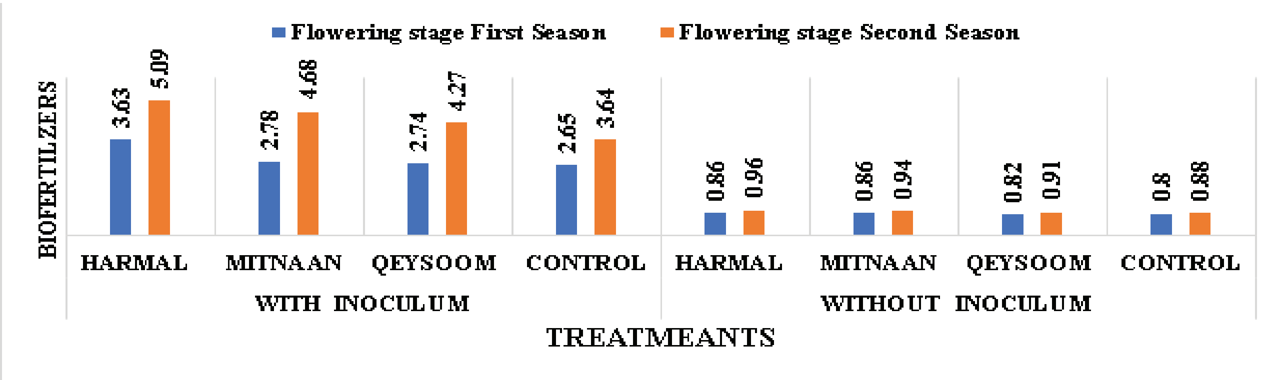
Values having the same alphabetical letter (s) did not significantly differ at 0.05 level of probability according to Duncan's multiple range test.



Trichoderma with biofertilizer (x*10^5 c.f.u/g soil)

Trichoderma with out biofertilizer (x*10^2 c.f.u/g soil)

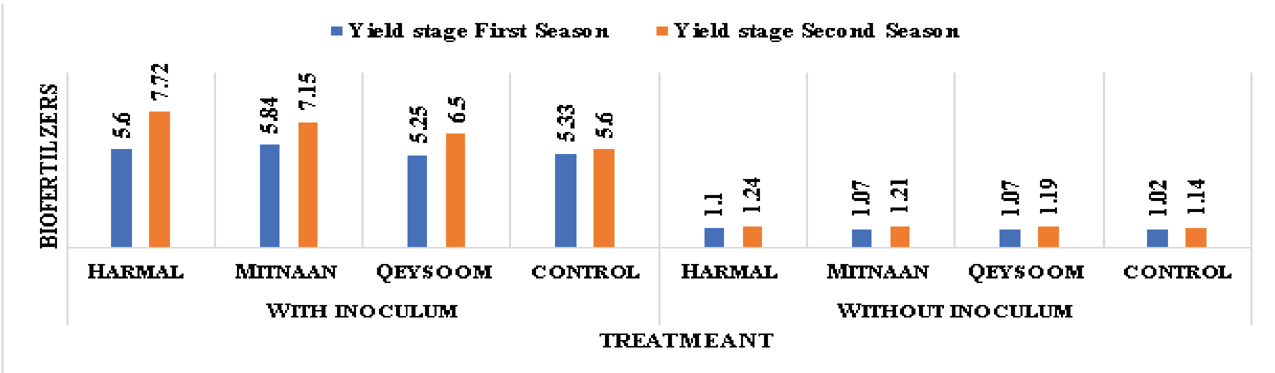
Figure. 1: Effect of Biofertilizers and plant extracts on T. harzianum formations after 30 days



Trichoderma with biofertilizer (x*10^6 c.f.u/g soil)

Trichoderma with out biofertilizer (x*10^2 c.f.u/g soil)

Fig.ure 2: Effect of Biofertilizers and plant extracts on T. harzianum formations at flowering stage.



Trichoderma with biofertilizer (x*10^7 c.f.u/g soil)

Trichoderma with out biofertilizer (x*10^2 c.f.u/g soil)

Figure. 3: Effect of Biofertilizers and plant extracts on T. harzianum formations at yield stage.

and Metwally & Al-Amri 2020).The biofertilizer of *Trichoderma*was irrigated with water in the experimental area for 3 days in the morning, and then once every 2–3 days according to the soil humidity (Ji *et al.,* 2020).

Sinai's flora aqueous extracts

Plant materials

Arial parts of Sinai's flora *Peganum harmala* L., *Achillea fragrantissima* L. and *Thymelaea hirsute* L. were collected from the western parts of North Sinai (Biir Lahfn, Nakhl, Al-Jafajafa, Wadi Al-Maghara, Ain Al-Jdirat and Al-Qassima), Egypt. Plants were identified and classified by plant protection Department, Faculty of Environmental Agricultural Sciences, Arish University, Egypt.

Extract preparation

The aquatic extracts were done from the aerial parts of Sinai's flora *P. harmala*, *A. fragrantissima* and *T. hirsuta* according to Al-Mizrakchi (1998). The stock solutions of the aqueous extracts as well as the different concentrations (5, 10 and 15%) were preparedin August 2018, according to the method described by Abdel-Salam *et al.* (2009) and Mekki (2014).

Selected plants were separately shade dried, finely powdered using a blender. Each finely powdered of sample *i.e.* 50, 100 and 150 g were placed in a flask with 1000 ml of distillated water, then the mixture was filtered twice, first through cheese-cloth (50% cotton and 50% polyester) and then through filter paper (Whatman No. 2). The final concentration of the prepared *P. harmala*, *A. fragrantissima* and *T. hirsuta* were 5, 10 and 15% as total solids. The

Table 6: Effect of biofertilizerstreatments and some Sinai's Flora extracts on the formation of Mycorrhizal status in Broadbean rhizosphere

Plant ext.	Mycorrhizal Status																	
	Hyphae						Vesicles						Arbuscules					
	Flowering stage			Yield stage			Flowering stage			Yield stage			Flowering stage			Yield stage		
	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean
First Season																		
Thymelaea hirsute	85.47 a	37.01d	61.24 a	64.98 a	25.95 e	45.46 a	55.03 a	20.82ef	37.92 a	43.92 a	13.25 e	28.59a	49.67a	12.08ef	30.88 a	37.92 a	9.00 e	23.46 a
Achillea fragrantissima	76.45 b	31.25 e	53.85 b	55.35 b	19.20 f	37.27 b	49.22 b	24.28d	36.75ab	40.52 b	13.23 e	26.88b	45.83 b	15.55d	30.69 a	34.67 b	8.70 e	21.68b
Peganum harmala	73.77 b	26.25 f	50.01bc	51.45c	16.80 f	34.12 b	47.53bc	22.74de	35.14bc	38.68 c	13.50 e	26.09b	44.62 b	14.07de	29.34 a	32.38 c	9.42 e	20.90b
Control	65.78 c	25.13 f	45.45c	44.42 d	12.37g	28.40 c	46.31 c	19.25f	32.78 c	37.31d	8.78 f	23.05c	41.33 c	10.32 f	25.83 b	30.17 d	5.97f	18.07c
Mean	75.37 a	29.91 b		54.05 a	18.58 b		49.52 a	21.77 b		40.11 a	12.19 b		45.36 a	13.05 b		33.78 a	8.27 b	
Second Season																		
Thymelaea hirsute	87.50 a	39.82 e	63.66 a	69.13 a	27.86 e	48.49 a	60.78 a	22.58g	41.68 a	45.32 a	15.15 d	30.23a	55.57 a	14.13 f	34.85 a	40.45 a	13.40 e	26.93 a
Achillea fragrantissima	79.13b	35.04 f	57.08b	58.75 b	22.16 f	40.46b	54.00 b	26.25e	40.13b	42.55 b	15.33 d	28.94ab	48.20b	18.78d	33.49b	36.88b	12.40 e	24.64b
Peganum harmala	75.15 c	29.38g	52.26 c	53.25 c	17.83 g	35.54 c	52.47 c	24.50f	38.48 c	41.40 b	15.43 d	28.42 b	47.53b	17.13 e	32.33 c	35.12 c	13.75 e	24.43b
Control	69.74d	20.97 h	45.35d	48.04 d	15.71 g	31.88d	48.60 d	20.45h	34.53d	38.63 c	9.23 g	23.92 c	46.45 c	12.03g	29.24d	32.45d	7.93 f	20.19c
Mean	77.88a	31.30b		57.29a	20.89b		53.96a	23.45b		41.98 a	13.78 b		49.44a	15.52b		36.23a	11.87b	

Values having the same alphabetical letter (s) did not significantly differ at 0.05 level of probability according to Duncan's multiple range test.

Table 7: Effect of biofertilizers treatments and some Sinai's Flora extracts on the Weight of nodulation of *Rhizobium leguminosarumin* Broadbean rhizosphere

	Weight of active nodules						Weight of non-active nodules						Weight of all nodules					
	after 30 days			Flowering stage			after 30 days			Flowering stage			after 30 days			Flowering stage		
	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	With-out Bio.	Mean
First Season																		
Thymelaea hirsute	0.697 bc	0.153d	0.425bc	1.300a	0.270c	0.785a	0.403a	0.107b	0.255a	0.643a	0.683a	0.663a	1.100bc	0.260d	0.680ab	1.943a	0.913d	1.428a
Achillea fragrantissima	1.023 a	0.170d	0.597a	1.060b	0.327c	0.693a	0.370a	0.0567b	0.213a	0.537b	0.557b	0.547b	1.393a	0.227d	0.810 a	1.597b	0.863d	1.230b
Peganum harmala	0.790 b	0.187d	0.488b	1.070b	0.330c	0.700a	0.393a	0.063b	0.228a	0.483b	0.403b	0.443b	1.183b	0.250d	0.717ab	1.553bc	0.813d	1.183b
Control	0.657 c	0.103d	0.380c	1.037b	0.227c	0.632a	0.360a	0.087b	0.223a	0.403c	0.393c	0.398c	1.017c	0.190d	0.603 b	1.440c	0.630e	1.035c
Mean	0.792 a	0.153b		1.117a	0.288b		0.382a	0.078b		0.517a	0.509b		1.173a	0.232b		1.633a	0.805b	
Second Season																		
Thymelaea hirsute	0.770 c	0.173d	0.472bc	1.313a	0.280c	0.797a	0.417a	0.123b	0.270a	0.680a	0.153e	0.417a	1.187c	0.297e	0.742 a	1.993a	0.433d	1.213a
Achillea fragrantissima	1.017 a	0.177d	0.597a	1.177ab	0.313c	0.745a	0.433a	0.110b	0.2712a	0.563b	0.160e	0.362ab	1.450a	0.287e	0.868ab	1.740b	0.473d	1.107ab
Peganum harmala	0.880 b	0.193d	0.537ab	1.067b	0.383c	0.725a	0.410a	0.077b	0.243a	0.500c	0.177e	0.338b	1.290b	0.270e	0.780ab	1.567c	0.560d	1.063ab
Control	0.703 c	0.120d	0.412c	1.087b	0.257c	0.672a	0.387a	0.103b	0.245a	0.417d	0.133e	0.275c	1.090d	0.223e	0.657 b	1.503c	0.390d	0.947b
Mean	0.842 a	0.166b		1.161a	0.308b		0.412a	0.103b		0.54a	0.156b		1.254a	0.269b		1.701a	0.464b	

Values having the same alphabetical letter (s) did not significantly differ at 0.05 level of probability according to Duncan's multiple range test.

Table 8: Effect of biofertilizer treatments and some Sinai's Flora extracts on vegetative growth parameters of Broadbean plants

Plant ext.	Stem length (cm)			Number of branches .plant^{-1}			Number of leaves.plant^{-1}			Leaf area (dcm^2.Plant^{-1})		
	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean
First Season												
Thymelaea hirsute	128.30 a	96.27 bc	112.29A	14.45 a	12.25 b	13.35A	149.44 b	132.37 c	140.91AB	163.72 a	155.32 ab	159.52A
Achillea fragrantissima	113.19ab	98.45abc	105.82B	13.44 ab	11.67 c	12.56B	162.38 a	137.51 c	149.95A	147.45 b	142.19 b	144.82AB
Peganum harmala	90.34 c	80.07 d	85.21C	8.43 d	7.70 de	8.07C	109.61 d	93.67 e	101.64B	139.62bc	135.11 bc	137.37B
Control	83.27 cd	66.25 e	74.76D	7.75 de	5.69 e	6.72D	97.59 de	77.61 f	87.60 C	134.55bc	132.15 c	133.35B
Mean	103.78A	85.26B		11.02A	9.33B		129.76A	110.29B		146.34A	141.19A	
Second Season												
Thymelaea hirsute	132.62 a	101.94 c	117.28A	12.94a	10.82b	11.88A	146.71ab	124.09c	135.40B	164.11a	153.23ab	158.67A
Achillea fragrantissima	119.43b	108.71bc	114.07A	11.85ab	9.64c	10.75B	156.54a	140.11b	148.33A	161.29a	149.62b	155.46A
Peganum harmala	89.59 d	82.42 e	86.01B	9.61c	8.69cd	9.15C	114.33cd	76.68e	95.51C	151.33ab	147.63b	149.48AB
Control	85.22 de	65.17 f	75.20C	8.38cd	7.61d	8.00D	107.62d	68.54f	88.08D	136.88c	133.22c	135.05B
Mean	106.72A	89.56B		10.695A	9.190B		131.30A	102.36B		153.40A	145.93B	

Values having the same alphabetical letter(s) did not significantly differ at 0.05 level of probability according to Duncan's multiple range test.

Table 9: Effect of biofertilizer treatments and some Sinai's Flora extracts on vegetative growth parameters of Broadbean plants

Plant ext.	Shoot fresh weight (g)			Shoot dry weight (g)			Root fresh weight (g)			Root dry weight (g)		
	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean
First Season												
Thymelaea hirsute	1062.6a	980.4b	1021.50A	292.6a	268.1b	280.35A	109.22a	83.11bc	96.17A	43.71a	27.22cd	35.47A
Achillea fragrantissima	1043.2ab	970.2bc	1006.70AB	290.2a	279.1ab	284.65A	97.44ab	77.93bcd	87.69AB	39.62ab	29.19c	34.41AB
Peganum harmala	823.5c	833.5c	828.50B	243.6c	238.4c	241.00B	91.72abc	69.28cd	80.50B	37.33b	26.92d	32.13B
Control	678.5d	488.5e	583.50C	177.1d	137.5e	157.30C	88.34bc	54.22d	71.28C	35.48bc	23.68e	29.58C
Mean	901.95A	818.15B		248.10A	233.55B		96.68A	71.14B		39.04A	26.75B	
Second Season												
Thymelaea hirsute	1089.6a	1008.5b	1049.05A	298.5a	274.7b	286.60A	121.01a	107.24b	114.13A	51.39a	38.11b	44.75A
Achillea fragrantissima	1081.3a	1005.3b	1043.30A	292.5a	284.7ab	288.60A	115.47ab	95.56bc	105.52AB	46.21ab	34.92bc	40.57B
Peganum harmala	854.2d	848.2d	851.20B	248.8c	229.0d	238.90B	101.66b	88.42d	95.04B	38.37b	28.55c	33.46C
Control	706.2e	399.4f	552.80C	189.6e	146.8f	168.20C	92.11c	65.95e	79.03C	37.81b	24.49d	31.15D
Mean	932.83A	815.35B		255.40A	235.75B		107.56A	89.29B		43.445A	31.518B	

Values having the same alphabetical letter(s) did not significantly differ at 0.05 level of probability according to Duncan's multiple range test.

Table 10: Effect of biofertilizer treatments and some Sinai's Flora extracts on yield and its componentsof Broadbeen plants

Plant ext.	Pod length (cm)			Number of pods.plant^{-1}			Average pod weight (g)			Total yield (g.plant^{-1})			Seed weight (g. plant^{-1})		
	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean	With Bio.	Without Bio.	Mean
First Season															
Thymelaea hirsute	20.22a	19.62a	19.92A	18.12a	17.29ab	17.71A	26.65b	25.45bc	26.05A	506.53a	491.37ab	498.95A	276.19ab	263.56b	269.88AB
Achillea fragrantissima	19.45a	18.66ab	19.06A	17.62a	16.74b	17.18A	27.67a	26.31b	26.99A	489.48b	476.42b	482.95A	286.95a	271.18ab	279.07A
Peganum harmala	18.11ab	17.38b	17.75B	15.36c	12.33d	13.85B	24.93c	22.92d	23.93B	361.15c	352.31c	356.73B	233.66c	226.69c	230.18B
Control	17.09bc	16.49c	16.79C	10.62e	9.45f	10.04C	20.79e	17.45f	19.12C	254.52d	217.58e	236.05C	149.47d	135.49d	142.48C
Mean	18.72A	18.04A		15.43A	13.95B		25.01A	23.03B		402.92A	384.42B		236.57A	224.23B	
Second Season															
Thymelaea hirsute	21.22a	19.55ab	20.39A	17.89a	16.86ab	17.38A	27.92c	26.76d	27.34B	538.89a	507.45ab	523.17A	283.30ab	274.34b	278.82AB
Achillea fragrantissima	20.75a	18.88b	19.82A	17.02ab	16.11b	16.57B	31.02a	29.45b	30.24A	492.11b	482.23b	487.16B	291.75a	285.66a	288.71A
Peganum harmala	18.21b	17.32bc	17.77B	15.23bc	13.72c	14.48C	23.45e	20.03f	21.74C	349.42c	333.56c	341.49C	244.26bc	213.71c	228.99B
Control	17.74bc	17.04c	17.39B	11.16d	8.45e	9.81D	20.44f	17.11g	18.78D	270.35d	219.56e	244.96D	148.4d	136.56d	142.48C
Mean	19.48A	18.20B		15.33A	13.79B		25.71A	23.34B		412.69A	385.70B		241.93A	227.57B	

Values having the same alphabetical letter (s) did not significantly differ at 0.05 level of probability according to Duncan's multiple range test.

amount of obtained aqueous extracts were preserved in sterile dark bottles (500 ml) in a cool environment (4^{o} C) until used. The chemical constituents of the aqueous extracts of *P. harmala*, *A. fragrantissima* and *T. hirsuta* were investigated using Gas chromatography-mass GC/MS analysis spectrometry **(Table 3)**.

Extract applications

The tested plants of *P. harmala, A. fragrantissima* and *T. hirsuta* were applied as a foliar spray thrice by 15-day intervals, beginning to 30 days after sowing.

Plant measurements

Vegetative growth

Samples of five plants from each experimental unit were randomly taken at 75 days after sowing, for measuring the following characteristics: stem length, number of branches per plant, number of leaves, leaf area, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight.

Yield and its components

Green pods of each experimental unit were harvested at a proper maturity of consumption stage, counted, weighed and the following data were calculated: pod length, number of pods/plant, average pod weight(gm), total green pod yield/plant (gm) and seed weight/plant (gm).

Determination of biofertilizers in broad bean rhizosphere

AMF parameters

Spore count (spores/100gsoil) and Total root colonization (%)

Three soil and root samples were randomly collected at 30 days after sowing, at flowering, and at yield stage, respectively in two seasons.

Formation of AMF status (Hyphae, Vesicles and Arbuscules) in Broadbean rhizosphere:

Three root samples were randomly collected at flowering, and yield stage, respectively in two seasons were investigated.

***Rhizobium leguminosarum* parameters**

Weight of active nodules, Weight of non-active nodules and Weight of all nodules:

Three root samples were randomly collected at 30 and 45 days in two seasons.Each plant was washed by water to remove all the soil particles attached with roots to enhance of nodules. The number of root nodules was determined at 30 and 45 days after planting on 10 randomly selected plants per treatments.

***Trichoderma harzianum* parameters**

Formation and count of *T. harzianum* in Broad bean rhizosphere: Three soil samples were randomly collected at 30 days after sowing, flowering, and yield stage, respectively in two seasons.

Isolation of biofertilizer from different plants in North Sinai

Different locations of the Experimental Station of the

Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt, were used to study their content of AMF, *T. harzianum* and *R. leguminosarum* in roots and rhizosphere soils in different plants (table 4).

Data presented in Table 4 show that, three plants and 100 root fragments of every six plant species were analyzed per site. Direct observation of the soil and root samples under the microscope showed that the roots of all studied plants were containing AMF (spore count and root colonization), while some plants positive with *T. harzianum & R. leguminosarum* and negative with others. Lehmann *et al.,* (2012) found that the data on 320 different crops, including those in the families' *Asteraceae, Fabaceae, Cucurbitaceae, Pedaliaceae* and *Poaceae* from 39 published studies suggested that the new crops have not lost the ability to respond to AMF compared to their ancestors.

T. harzianum isolates were identified according to their phenotypic characteristics. The color and shape of the mycelia, shape of phialide, conidiophores arrangement, conidia shape and color are observed on PDA after 7d of incubation were observed under a microscope. *T. harzianum* have Colony color (yellowish green and dark green), Colony appearance (concentric circle), Conidiophore arrangement (compactly and loosely arranged), Conidia shape (globose to sub-globose), Conidia color (light green) and Phialide shape (flask- shaped) Ji *et al.,* (2020).

Depending on the frequency of occurrence, it was found that the most dominant species were the taxa: *Acaulospora laevis, Funneliformis mosseae, Glomus etunicatum, Glomus monosporum, Gigaspora margarita, Rhizophagus intraradics* and *Scutellospora spp.* Isolated AMF spores have been identified using keys suggested by (INVAM, 2020). Our findings are consistent with many authors have found that genus *Funneliformis and Glomus*are a predominantly distributed genus in the soil all over the world (Mansour, 2010; Yaseen *et al.,* 2016; Teixeira *et al.,* 2017 and Nafady *et al.,* 2018).

Several works emphasized the role of AMF in sustaining plant cover in semi-arid and arid ecosystems (Smith and Read 2008 and Brundrett 2009). Mahmoudi *et al.,* (2020) showed that the highest AMF root colonization belongs to the Fabaceae family in all the studied sites, always with higher values in the site inside each conserved area. The density of AMF spores isolated from the rhizosphere of the sampled plants varied between site to site. Also, the highest density of AMF spores found in the Fabaceae family in all the studied sites. These differences are particularly evident in arid and semi-arid soils with high organic matter turnover rates and low organic matter content (Mohammad *et al.,* 2003).

Increasing of biofertilizers in soil

After isolation and identification of AMF from different plants, we used these spores and increased it by taping plant. The inoculum potential of AMF in soil as spores have been increased by growing Sudan grass plant as a recommended trap plant for a period of 4 months to increase spore density. The two elements related to the inoculum potential of AMF namely; spore density and root colonization rate were used. Data in Table 4 indicated that average spore count was 24.33 spores/100 g dry soil and 17.17 % average of root colonization. This result is considered very low because these soils are very poor in nutrients. After 4 months from using Sudan grass plant, the spore counts reached 211 spores/100 g dry soil and 81.00 % of root colonization. These results were in harmony with those found by (Yaseen *et al.,* 2016 and Nafady *et al.,* 2018).

After 3–5 days of growing, *R.leguminosarum* inoculant was suspended in sterilized 0.8% NaCl and then transferred to a sterilized mix of peat and vermiculite. Colony-forming units (CFU) were adjusted to 10^9/ c.f.u /ml

T. harzianum was grown in 500 ml conical flasks containing potato dextrose broth for 8 days. The cultures were then filtered through Whatman no. 1 filter paper and the mycelial mat was macerated using a blender for 1 min and mixed with 250 ml of 0.1 M $MgSO_4.7H_2O$ solution. *T. harzianum* was determined using a hemocytometer and inoculated by soaked up Broad bean seeds of 500 ml of spore suspension (2×10^4 c.f.u /ml).

Statistical analysis

Statistical analysis of the obtained data was carried out according to statistical analysis of variance in a randomized complete block design RCBD (a split plot design) with three replications, according to Snedecor and Cochran (1980). Duncan's multiple range tests was used for comparison among means (Duncan, 1958).

RESULTS AND DISCUSSION

Effect of inoculum with biofertilizers treatments and some Sinai's Flora extracts on the spore counts and total root colonization's (%) in broad bean rhizosphere

Data in Table 5 show that application of biofertilizers had significant effected of all studied traits (spore counts and total root colonization's (%)) at all sampling dates in both seasons. the highest values were recorded with soil application with biofertilizers, the highest values of spoor count was at yield stage, while the highest value of root colonization was recorded at flowering stage in both seasons.

As regard to the effect of plant extracts, there was significant effect on the previous studied treats. The highest value of all treats were recorded with foliar spray with *Thymelaea hirsute* in all stage for both spore count and root colonization's% in both seasons.

Results indicated that the higher number of spores count and total root colonization's (%) increased by combination of biofertilizers and plant extracts compared to control and treatments without biofertilizers and plant extracts in all stages *T. hirsute* combination with biofertilizer showed the best plant extract's among all plant extracts in all stages of growth at two seasons compared with the same plant alone without biofertilizer and control.

This result was agreed with several reports (Lovelock *et al.,* 2003 and Pereira *et al.,* 2019) which observed a part from the rate of spore formation and the number of AMF spores in a soil also depends on the rates of spore germination and degradation of AMF are obligate biotrophs, the number of spores and propagules tends to be higher in the rhizosphere than in the bulk soil and higher in the rhizosphere of plants with a higher intensity of AMF colonization. Mahmoudi *et al.,* (2020) observed that the mycorrhizal frequency and spore density, varied between plants in the same site and, for each plant, between sites and evidenced a positive effect of mycorrhized plants on soil microbial activity. Metwally (2020) showed that the highest colonization rate was recorded in onion roots dually inoculated with AMF and *Trichoderma viride*, followed by those singly inoculated with AM fungi.

Martínez-Medina *et al.,* (2009) found that the presence of *T. harzianum* significantly increased AMF colonization, except in the treatment with *G. mosseae*. AM fungal colonization was affected by the saprophytic fungi, for instance, *Trichoderma*; these effects were variable, depending on the inherent characteristic of both agents (Martínez-Medina *et al.,* 2011), and they found thatdescribed a synergistic effect on AM root colonization in melon plants because of *T. harzianum* and *Glomus constrictum* or *Glomus intraradices* inoculation. While, Enhanced AMF root colonization by the *Trichoderma* inoculation in black pepper was shown by Umadevi *et al.* (2017).

Effect of inoculum with bio fertilizer treatments and some Sinai's Flora extracts on the formation of Mycorrhizal status in broad bean rhizosphere

Data recorded in Table 6 show that application of biofertilizers had significant effects on all studied traits (Hyphae, Mycorrhiza Status and Arbuscules) at all sampling data in both seasons. the highest values were recorded with soil application with biofertilizers, the highest values of Hyphae, Mycorrhiza Status and Arbuscules were at flowering stage in both seasons.

As regard to the effect of plant extracts, there were significant effects on the previous studied treats. The highest values of all traits were recorded with foliar spray with *Thymelaea hirsute* in all stages for both Hyphae, Mycorrhiza Status and Arbuscules in both seasons.

Data in Table 6 indicated that the combination of Biofertilizers and different Sinai's Flora extracts significantly increased the formation of hyphae, vesicles and arbuscules compared with the same treatments as a single at all stages were used in two seasons. Mycorrhizal status increased by the time of growth in Broadbean plants. The combination of *T. hirsute* with biofertilizer showed the best among all stages of growth at two seasons compared with the same treatment alone without biofertilizer and controltreatment.

These results are in line with Mahmoudi *et al.,* (2020) who showed that the all the structures characteristic of root colonization by AMF (intracellular aseptate hyphae, vesicles and arbuscules) were observed. AMF status and sporulation increased when the development of the mycelium begins to be restricted by nutrients and is a highly carbon-demanding process. This may explain why AMF status increased in the rhizosphere varies between plant species and for the same plant species between sites (Sghir *et al.,* 2013 and Al-Areqi *et al.,* 2013). *T. harzianum* significantly increased root colonization by *G. intraradices, G. constrictum* and *G. claroideum*, reaching values significantly higher than the most effective AMF inoculated alone (*G. mosseae*). Similar interactions have been proposed for other saprophytic fungi (Fracchia *et al.,* 1998 and Fracchia *et al.,* 2000)

Effect of inoculum with biofertilizer treatments and some Sinai's Flora extracts on the Weight of nodulation of *R. leguminosarum* in Broadbean rhizosphere

Data in Table 7 show that application of biofertilizers had significant effects on all studied traits (weight of active nodules, weight of non-active nodules and weight of total nodules) at all sampling dates in both seasons. the highest values were recorded with soil application with biofertilizers, the highest value of these traits was recorded at flowering stage in both seasons.

As regard to the effect of plant extracts, there was significant effect on the previous studied traits. The highest values of all treats were recorded with foliar spray with *Thymelaea hirsute* in all traits except weight of active nodules and weight of total nodules at 30 days stage that had highest value with chillea fragrantissima in both seasons.

The combination between Sinai's Flora extracts and biofertilizers are better than using them separately. The combination between biofertilizers and *A. fragrantissima* increased significantly the weight of active nodules and weight of total nodules after 30 days while,the combination between biofertilizers and *T. hirsute* increased significantly weight of active nodules, weight of non-active nodules and weight of total nodules at flowering stage (Table 7).

These results coincided with Rudresh *et al.,* (2005) and Yobo *et al.,* (2011) showed *Trichoderma* mixed with *Bacillus spp.* or *Rhizobium spp.* greatly promoted growth of bean and chickpea plants. Woo *et al.,* (2014) who found that the capability to respond to AMF and *Trichoderma* are beneficial for plant growth in legume plants. These results agree with Dubova *et al.,* (2015) and Pereira *et al.,* (2019) observed that the applying *Rhizobium laguerreae* with or without AMF in legume crops significantly increased number of nodules/plants. AMF inoculation improved the nodule number, dry weights of nodules, and increase in biomass of nodules (Oruru *et al.,* 2017).

Data in Figs.1, 2 and 3illustrate that the combination between biofertilizers and Sinai's Flora extract a significantly increased the formation and count of *T. harzianum*. The count of *T. harzianum* in the yield stage was the highest count among all stages specially in *P.*

harmala treatment combined with biofertilizers at 7.72 * 10^7 c.f.u./g soil followed by *T. hirsute* 7.15 * 10^7 c.f.u./g soil combined with biofertilizers. In this connection, Fracchia *et al.,* 1998 while stimulation of the germination of *T. harzianum* conidia by *G. intraradices*. Martínez -Medina *et al.,* (2009) found that the number of *T. harzianum* colonies recovered from the substrates co-inoculated with AMF decreased significantly compared with no AMF treatment, except in the case of inoculation with *G. intraradices.* AMF colonize plant roots and hyphae intothe surrounding soil, complementing the host's root functions(Jansa *et al.,* 2011).rhizobia with AMF and *Trichoderma* was considered beneficial for plant growth (Woo *et al.,* 2014). Similar results have been observed by Sharma *et al.* (2016) reported a synergistic effect with *Trichoderma* application on AM fungal colonization and host plant and found in this study where a noteworthy interaction between AM fungi and *T. viride* was observed.

Effect of biofertilizers treatments and some Sinai's Flora extracts on vegetative growth of faba bean plant

Data presented in Tables 8 and 9 show that broad bean plants treated with biofertilizers gave the highest increase in plant growth characters; i.e. is Stem length, number of branches per plant, number of leaves/plant and leaf area over the untreated plants in both seasons. This positive effect of biofertilizers treatments is on the same line with those obtained by Azarmi *et al.,* (2011) Pereira *et al.,* (2014), Mahato *et al.,* (2018) and Min and Win (2020) who found that the use of *Trichoderma* as biofertilizer increased the growth and yield of many crops biomass production such as beans, brinjal, cucumber, maize, and tomatoes compared with other treatments without fertilizer. Also, Agbo *et al.,* (2012) and Hossain & Akter (2020) observed that the combined between *Trichoderma* as biofertilizer with chemical fertilizer treatment gave significantly higher yield of all crops than control treatment.

It was evidently clear that most of the applied foliar treatments greatly improved all studied growth characters of broad bean plants with various significant degrees compared with those of control in both seasons. However, foliar spraying of *T. hirsute* extract had the highest stimulation effect on broad bean plant growth characters; i.e., Stem length, number of branches per plant number of leaves/plant, leaf area, fresh and dry weight of both shoots and roots/plant. However, foliar spraying of *A. fragrantissima* extract had the highest effect on number of leaves of broad bean plants as compared to control treatment, followed by foliar spraying of *P. harmala* in both seasons. These results war the same in both seasons. The previous results showed that it is highly possible that there are phytotoxins that contribute to the plant growth inhibitory properties of *P. harmala*. (Shao *et al.,* 2013) reported that application of 0.05 g/mL aqueous extract of whole *P. harmala* plant greatly suppressed growth of wheat and lettuce seedling, indicating the presence of active phytotoxins in this plant. Also, there were significant differences between plant extracts on vegetative growth, where *T. hirsute* recorded the highest value in most characters, this may be due to *T. hirsuta* extracts are rich sources of natural antioxidants which appears to be an alternative to synthetic antioxidants which reflected on growth of plants (Amari *et al.,* 2014 and Badawya *et al* 2019).

As for the interaction effect between plant extracts and biofertilizers on plant growth , the same data in Table 8 show that interaction between *T. hirsute* with biofertilizer was the best interaction treatment which increased stem length, number of branches/plant, leaf area, fresh and dry weight of both shoots and roots/plant. Woo *et al.,* (2014), Poveda *et al*. (2019), Szczałba *et al.(* 2019) and Metwally, (2020) reported that Co-inoculation of rhizobium*, T. harzianum* and AMF were beneficial for plant growth of legumes

Effect of biofertilizers treatments and some Sinai's Flora extracts on yield and its components of faba bean plant

Data in Table 10 show that adding biofertilizers treatments significantly increased the pod length, number of pods per plant, average pod weight total yield per plant, and weight, of seeds per pod in both seasons, respectively. These observations are similar to that recorded by Gomaa *et al.* (2002) and Abdel-Wahaband Said (2004), who stated that the combined inoculations overcome the single inoculation with rhizobia only. To improve bean production, the inoculation strategy must be based on the selection of effective biofertilizer strains for the conditions of cultivated areas. Agbo *et al.,* 2012 and Hossain & Akter (2020) observed that the combined between *Trichoderma* as biofertilizer with chemical fertilizer treatment gave significantly higher yield of all crops than control treatment.

Yield and its quality were improved through inoculation with the combination of AMF and *Trichoderma* followed by a separate inoculation of these fungi (Chandanie *et al.,* 2009; Nzanza *et al.,* 2012; Colla *et al.,* 2015a, and b; Metwally 2020).

As for pod length, number of pods per plant, average pod weight total yield per plant, and weight of seeds per pod were significantly increased with different plant extracts. *T. hirsute* and *A. fragrantissima* extracts significantly increased pod length, number of pods per plant, average pod weight, total yield (g per plant) and seed weight of broad bean plants *Vicia faba* L. compared with other treatments in both seasons. Control treatment significantly decreased all yield and its components of Broad bean plants *Vicia faba* L.

Interaction between adding biofertilizers treatments and spraying some Sinai's extracts significantly increased pod length, number of pods per plant, average pod weight total yield per plant, and weight of seeds per pod compared with control treatment in both seasons. The interaction between *T. hirsute* extract and adding biofertilizers treatment recorded a significantly increase in pod length, number of pods per plant, average pod weight, total yield per plant, and weight of seeds per pod in both seasons (Table 10).

These results are in accordance with the findings by Pereira *et al.,* (2019) who conclude that inoculation of faba bean with *R. laguerreae* significantly increased yield parameters, the inoculation with AMF also improved productivity parameters such as number of pods and weight of pods and seeds. Plants inoculated with *R. laguerreae* and co-inoculated with AMF presented higher rates of increased of grain yield (in terms of pod length, number of pods and seeds produced, and weight of pods and seeds per pod and yield (Tons/feddan). Also, Veselaj *et al.,* (2018) found that combined application of AMF with *R. leguminosarum* under saline conditions, resulted a higher yield than the same treatment separately and control. Ravikumar (2012) and Denton *et al.,* (2013) Improvement in grain yield, namely in the number of pods, seeds and weight of seeds, were reported in other studies with rhizobial inoculation in faba bean. And in other leguminous plants (Oliveira *et al.,* 2017; Youseif *et al.*, 2017).

CONCLUSION

The combined application of biofertilizers and some Sinai's Flora plant increased crop growth and yield of faba bean plant. These combinations also improved counts and the formation of AMF, *Rhizobium* and *Trichoderma spp., well as* increased the percentage of AMF colonization. The main advantages of the biofertilizers and foliar spray with some Sinai's Flora plants were reflected in positive impacts on plant quality and performance. Application of some Sinai's Flora alone or combined with biofertilizers increased several characters of broad bean plants and enhanced plant growth and yield and its components. The extracts of *Thymelaea hirsute* and *Achillea fragrantissima* plants are considered one of the promising extracts in the near future, which will be used in fertilizing many plants and trees as a substitute for chemical fertilizers.

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