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Integration of *Paenibacillus polymyxa* and *Rhizophagus intraradices* for Controlling Root Rot Disease of Chickpea

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Abstract

The recent study investigates the biocontrol potential of *Paenibacillus polymyxa* and *Rhizophagus intraradices* (formerly *Glomus intraradices*), both individually and in combination, for controlling root rot and damping off diseases caused by *Rhizoctonia solani* of two cultivars of chickpea (Giza 3 and Giza 195) under greenhouse and field conditions at two locations, i.e., Giza and Etai El-Baroud. Preliminary results indicate that both *P. polymyxa* and *R. intraradices* significantly enhanced the proportion of surviving plants and lowered the incidence of damping off before and after emergence. The combined treatment exhibited the highest efficacy, suggesting synergistic effects that enhance plant defense mechanisms. There was an increase in the activity of peroxidase, polyphenol oxidase, and β-1,3 glucanase enzymes, correlating with elevated phenolic compound contents. On the other hand, there is an increase in the contents of micro and macro elements, total flavonoids, and proline. Moreover, there was a stimulatory effect on crop parameters and seed yield (kg/feddan) for the two cultivars in both locations.

Keywords: *Chickpea; Rhizoctonia solani; Paenibacillus polymyxa; Rhizophagus intraradices; Biological Control*

Introduction

After beans and soybeans, chickpea (*Cicer arietinum* L.) is the most significant legume crop grown worldwide [1]. Chickpea is a valued crop (due to its high protein content of about 40% of its weight) providing nutritious food for an expanding world population. Furthermore, the grain chickpea has possible health benefits, such as a lower risk of cancer, diabetes, and cardiovascular diseases [2]. In Egypt, the area under cultivation of chickpea dropped from 1650 hectares in 2020 to 850 hectares in 2023, and the imported value in 2023 was $ 44178000 [3].

Additionally, biotic and abiotic stress have a significant impact on chickpea yield. Of biotic factors, root rot pathogens emerge as very economically important worldwide. The phytopathogen *R. solani* causes root rot, and damping-off diseases in legume production regions worldwide [4,5]. This pathogen is difficult to control due to its soil-borne and saprophytic nature, as well as its wide host range, it causes huge yield losses in field crops every year [6]. Although certain chemicals are helpful for controlling *R. solani*, they are costly and unfriendly to the environment. Therefore, researchers around the world have changed their focus and concentrated on more eco-friendly methods for controlling plant diseases. Biological control is one

of the most important among these methods, with the advantages of greater public acceptance and reduced environmental impact [6,7].

Plant growth-promoting rhizobacteria (PGPR) are becoming a promising and eco-friendly approach to reduce the use of synthetic agrochemicals and promote plant growth [8]. *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) is a PGPR with a broad host plant range. It can form endospores and produce various antibiotics, *P. polymyxa* is considered a useful biocontrol agent commercially. Its tolerance of fungicides means that this bacterium can be used in tandem with current control methods [9]. Strains of *P. polymyxa* can fix atmospheric nitrogen, solubilize phosphate, and generate phytohormones, making them useful as efficient biofertilizers in commercial agriculture [10].

On the other hand, arbuscular mycorrhizal fungi (AMF) have gained as much attention as potential eco-friendly biocontrol agents because of antagonistic interactions with soil-borne plant pathogens [11] Isolates of *Rhizophagus intraradices* (formerly *Glomus intraradices*) [12] are known to form associations with roots of higher plants and stimulate the uptake of nutrients, and growth parameters under various environmental conditions. In the meantime, they supply plants with some measure of immunity known as Mycorrhiza-Induced Resistance (MIR), which is effective against the majority of soil-borne diseases [13].

However, *Paenibacillus* is a mycorrhiza helper bacterium (MHB) that promotes the germination of spores, growth of mycelium, colonization of roots, and biocontrol of soil-borne diseases, also promotes the functioning of arbuscular mycorrhizal symbiosis resulting in improving nutrients availability in the soil and uptake by plants [14]. So, the synergistic effects of PGPR and mycorrhizal fungi have attracted considerable attention in the past 25 years, due to their positive impacts on biocontrol efficiency and crop productivity [15-18].

Aim of the Study

This work aims to evaluate the utilization of *P. polymyxa* and *R. intraradices*, individually or in combination, for controlling root rot disease in chickpea under greenhouse and field conditions concerning growth parameters and yield.

Materials and Methods

Plant materials

Chickpea seeds (*Cicer arietinum* L.) cultivars (Giza 3 and Giza 195) were obtained from the Legume Res. Dept., Field Crops Res Inst., ARC, Giza, Egypt.

Source of the pathogen and inoculum preparation

The fungus *R. solani* with accession number MW926319, was isolated from naturally infected chickpea plants, showing damping off symptoms, cultivated in Kafr El-Sheikh governorate [19]. The cultures were maintained on malt extract agar slants under a phosphate buffer (pH 6.5) at 4 ± 0.5°C [20]. The inoculum of *R. solani* was prepared according to [21].

Biotic agents:

A. Bacterial isolate: *Paenibacillus polymyxa* (isolate 9D14), was previously isolated and identified using the Biology system, and the inoculum was prepared according to [17].

B. Mycorrhizal inoculum: *Rhizophagus intraradices* with accession number MW410779 was kindly obtained from the Mycology and Plant Dis. Survey Dept., Plant Path laboratory. Res. Inst. ARC, Giza, Egypt. The isolate was previously identified using fatty acid methyl ester profiles [22]. The AMF spores were collected and isolated using the wet sieves method [23]. The AM fungus *R. intraradices* was grown for three months in a multispore pot culture containing a mixture of autoclaved Holland peat moss, vermiculite, clay, and sand 2:1:1:1 (w/w) with Sudan grass as a host plant. The inoculum was sieved through a 500-µm mesh and mixed with 1% methylcellulose as coating material [24]. The microbial inoculum is at a rate of nearly 300 spores/gm to ensure a mean of more than 30 spores on the surface of chickpea seeds [25].

Seed and soil treatments:

A. Seed biopriming: Healthy uniformity seeds of chickpea (cv. Giza 3 and Giza 195) were surface disinfested according to [17]. For a single treatment, 720 ml broth (1.9×10^9 cfu /mL) of *P. polymyxa* was used per one kg of the neutralized carrier material (dried peat moss and vermiculite (1:1 w/w) that was milled to pass through 200 µm mesh sieves). In the case of mixed treatment, 720 mL of the bacterial broth culture was mixed with one kg of *R. intraradices* inoculum as prepared previously. Healthy chickpea seeds were coated with the *P. polymyxa* and *R. intraradices* either solely or in combination, using 1% methylcellulose (as a sticker) fifteen hours before sowing time. For the control treatment the seeds were coated with peat moss and vermiculite-based formulation, Then, the coated seeds were placed on a screen cloth to dry.

B. Fungicide treatment: Chickpea seeds were treated with Rizolex-T 50% WP (20% Tolclophos-methyl and 30% Thiram), Sumitomo Chemical Company Ltd. at the recommended dose (3 g/kg).

Greenhouse experiments

The trials were carried out in the greenhouse of Plant Pathol. Res. Inst., ARC, Giza. Sterilized plastic pots (25 cm in diameter) with a 5% formalin solution were filled with steamed sandy clay soil 1:2 (v/v). Soil infestation was established by incorporating R. solani inoculum into the soil at a 2% soil weight rate. The disinfested soil was supplemented with sterilized uninoculated ground sorghum grains at the same rate as the control. To activate fungal growth, the infested soil was well-mixed and watered every two days a week before planting. During seeding, each pot received five grams of *Mesorhizobium ciceri* (rhizobium formulation) that was obtained from the Biofertilizer Production Unit at the ARC in Giza, Egypt. Five pre-treated chickpea seeds were seeded in each pot, and the pots were directly irrigated. For every treatment, twelve replicated pots were utilized in a Completely randomized design (CRD). During the experiment all pots were irrigated as needed, fertigated every 15 days to near-field capacity using a 0.1% 15:15:15 (N: P: K) fertilizer solution.

The treatments were as follows: 1) *P. polymyxa*, 2) *R. intraradices,* 3) *P. polymyxa* + *R. intraradices*, 4) Rizolex-T, 5) Untreated control (*R. solani*-infested soil), 6) Untreated healthy control (non-infested soil).

Sixty days after seeding, the colonization by AMF was determined as described by [26]. Twelve plants (four replicates, each of three plants) were uprooted. Roots and shoots were placed in paper bags and oven-dried at 70°C for 48 h. The dried samples were prepared for wet digestion as described by [27]. The digests were then analyzed for the measurement of nitrogen (N), phosphorus (P), and potassium (K) [28]; copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) [29].

Disease assessment

Disease incidence (DI) % was determined by recording pre- and post-emergence damping-off, and the percentages of surviving plants 15, 30, and 45 days after sowing, respectively. Reduction or increase % over the infected control was calculated as the following formula:

$$\text{Reduction or increasing \%} = \frac{\text{Disease incidence (DI) of infected control} - \text{DI of treatment}}{\text{DI of infected control}} x100$$

The plants were scored for disease severity through a 0-5 numerical rating scale for the degree of damage [30], and the disease index of root rot was calculated using the following equation:

$$\text{Disease Index} = \frac{\Sigma\,(FV)}{NX}\ x\ 100$$

Where: F=number of roots tested in each grade; V= degree of damage (0-5); N=total number of tested plants, and X= the highest degree of infection (5).

Field experiments

During the winter growing season of 2020-2021, experiments were done in fields with a history of Rhizoctonia root rot at Giza (28 October) and Etai El-Baroud (29 October) Agric. Res. stations located in Giza and El-Beheira Governorates to examine the impact of biotic treatments on damping-off disease control. The disinfected seeds received the same treatments as the greenhouse experiment. In a randomized block design (RBD) with four replicates, twenty plots were arranged. Each plot was 10.5m^2 and had five rows, each measuring 3.5 x 0.6m. One seed per hill was planted in hills spaced 20 cm apart on both sides of the row ridge. About 50 kg of moistened fine sandy soil was mixed with 800 grams of *Mesorhizobium ciceri* formulation and added to field soil (feddan). Other agricultural practices, such as irrigation and fertilization, were carried out following the Egyptian Ministry of Agriculture and Land Reclamation recommendations. The disease incidence (DI) percentage was calculated as previously described. At harvest, ten plants were taken randomly from the inner rows of each plot as a sample. Crop components were recorded, and seed yield (kg)/feddan was estimated.

Effect of biopriming chickpea seed with biotic treatments on the activity of oxidative enzymes, phenol content, total flavonoids, and proline

Chickpea plants were grown as previously described in the greenhouse experiment. The activities of peroxidase (PO) [31]; polyphenol oxidase (PPO) [32]; β-1,3 glucanase [33]; phenolic contents [34,35]; and total flavonoids [36] were determined in tissue extracts after fifteen days and after forty days for proline content [37].

Statistical analysis

The data obtained were analyzed using [38] computer statistical software (ASSISTAT) using two-way analysis of variance (ANOVA). The least significant difference (LSD) was used to compare the mean values at a significance level of $P \leq 0.05$.

Results

Greenhouse experiments

Impact of Rizolex-T, *P. polymyxa*, and *R. intraradices* alone and in combination on the occurrence of damping-off disease of chickpea plants grown in artificially infested soil by *R. solani*

Results indicated that treatments with *P. polymyxa* and *R. intraradices* alone and in combination significantly reduced the percentage of pre- and post-emergence damping off and increased the percentages of surviving plants for the two cultivars

G3 and G195 (Table 1). The most effective treatment was Rizolex-T followed by combined treatment with (*P. polymyxa* + *R. intraradices*) compared with control grown in artificially infested soil. Meanwhile, the results showed that all treatments decreased the disease index of root rot of *R. solani*; the lowest values were scored with Rizolex-T, followed by combined treatment with (*P. polymyxa* + R. *intraradices*), followed by *R. intraradices* and *P. polymyxa,* respectively, for the two cultivars.

Cultivars	Treatments	Damping- off				Survived plants %	Increasing over infected control %	Disease index
		Pre-emergence		Post-emergence				
		Incidence %	Reduction %	Incidence %	Reduction %			
Giza 3	*P. polymyxa*	8	78.9	8	42.8	84	75.0	40.5
	R. intraradices	12	68.4	8	42.8	80	66.6	42.5
	P. polymyxa + R. intraradices	8	78.9	6	57.1	86	79.2	39.6
	Rizolex-T	4	89.5	8	42.8	88	83.3	23.1
	Control (*R. solani*)	38	0.0	14	0.0	48	0.0	64.1
	Healthy control (non-infested soil)	0.0		0.0		100		0.0
Giza 195	*P. polymyxa*	4	88.8	10	37.5	86	79.2	40.4
	R. intraradices	8	77.7	10	37.5	82	70.8	43.2
	P. polymyxa + R. intraradices	8	77.7	4	75.0	88	83.3	38.9
	Rizolex-T	4	88.8	4	75.0	92	91.7	22.8
	Control (*R. solani*)	36	0.0	16	0.0	48	0.0	63.8
	Healthy control (non-infested soil)	0.0		0		100		0.0
L.S.D. (≤0.05)	Treatment (T)	0.4		0.6		0.5		
	Cultivar (C)	0.2		0.4		0.3		
	T x C	0.5		0.8		0.8		

Table 1: *Impact of Rizolex-T, P. polymyxa, and R. intraradices on the occurrence of damping-off disease of chickpea plants (cv. Giza 3 & Giza 195) grown in artificially infested soil by R. solani.*

Impact of Rizolex-T, *P. polymyxa*, and *R. intraradices* alone and in combination on the contents of macro and microelements of chickpea plants grown in artificially infested soil by *R. solani*

Table 2 shows that all bioagent treatments, whether applied individually or in combination, significantly enhanced the content of macro- and micronutrients of chickpea plants compared to the control grown in artificially infested soil. It can be

generalized that across both cultivars (Giza 3 and Giza 195), the combined treatment of *P. polymyxa* and *R. intraradices* resulted in the highest concentrations of phosphorus (P) and nitrogen (N), followed by *P. polymyxa* alone. Potassium (K) levels were highest in plants treated with the combined treatment, followed by *R. intraradices* alone. Additionally, the concentrations of basic micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were significantly increased by all treatments, with the most pronounced effect observed in the combined treatment.

The results also showed that combined treatments (*P. polymyxa* + *R. intraradices*) improved the colonization percentage in the roots over the R. *intraradices* alone treatment (97% compared with 93% respectively for Giza 3 and 96% compared with 93% respectively for Giza 195).

Cultivars	Treatments	Macro elements mg/g dry weight			Micro elements ppm			
		N	P	K	Fe	Mn	Zn	Cu
Giza 3	*P. polymyxa*	2.85	0.343	2.68	331	125.2	28.1	7.2
	R. intraradices	2.68	0.287	2.74	350	121.1	28.6	7.7
	P. polymyxa + *R. intraradices*	3.08	0.353	3.1	521	129.0	28.8	8.2
	Rizolex-T	2.38	0.263	2.49	216	123.5	24.9	7.2
	Control (*R. solani*)	2.02	0.190	1.78	191	101.2	18.9	5.1
	Healthy control (non-infested soil)	2.45	0.233	2.36	230	122.3	25.8	7.3
Giza 195	*P. polymyxa*	2.89	0.310	2.61	485	101.6	27.7	10.1
	R. intraradices	2.69	0.283	2.79	469	119.6	30.5	14.7
	P. polymyxa + *R. intraradices*	3.36	0.318	3.09	607	138	35.4	16.4
	Rizolex-T	2.38	0.247	2.49	225	73.6	27.3	9.1
	Control (*R. solani*)	2.08	0.187	1.89	192	37.6	19.3	6.8
	Healthy control (non-infested soil)	2.45	0.220	2.36	221	42.2	27.1	8.6

Table 2: *Impact of Rizolex-T, P. polymyxa, and R. intraradices on the contents of micro and macro elements of chickpea plants (cv. Giza 3 & Giza 195) grown in artificially infested soil by R. solani.*

Impact of *P. polymyxa* and *R. intraradices* alone and in combination on the activity of enzymes, phenol, flavonoids, and proline of chickpea plants grown in artificially infested soil by *R. solani*

The activity of peroxidase, polyphenol oxidase, and β 1,3 glucanase enzymes

The effect of various treatments on the activity of defense-related enzymes in chickpea plants infected with *R. solani* is shown in table 3. Data showed that the activities of these enzymes were higher than the untreated control plants in infested soil with *R. solani*. Among all treatments, the highest activities of polyphenol oxidase and *β*-1,3 glucanase were achieved with combined treatment (*P. polymyxa* + *R. intraradices*), while peroxidase was high with *P. polymyxa* treatment and *R. intraradices* treatment for cultivar Giza 3 and with *R. intraradices* treatment in cultivar Giza 195.

Cultivars	Treatments	Peroxidase activity absorbance at 430 nm		Polyphenol oxidase activity absorbance at 495 nm		ß-1,3 glucanase activity µg glucose released min^{-1} mg^{-1}	
		Activity	Increasing over infected control %	Activity	Increasing over infected control %	Activity	Increasing over infected control %
Giza 3	*P. polymyxa*	1.548	188.2	0.035	34.6	1.283	35.1
	R. intraradices	1.539	186.6	0.045	73.1	1.415	48.9
	P. polymyxa + *R. intraradices*	1.334	148.4	0.052	100	1.545	62.6
	Control (*R. solani*)	0.537	0.0	0.026	0.0	0.95	0.0
	Healthy control (non-infested soil)	0.521		0.012		1.204	
Giza 195	*P. polymyxa*	1.188	36.4	0.044	22.2	1.032	13.4
	R. intraradices	1.781	104.4	0.046	27.7	1.087	19.4
	P. polymyxa + *R. intraradices*	1.567	79.9	0.061	70.6	1.275	40.1
	Control (*R. solani*)	0.871	0.0	0.036	0.0	0.91	0.0
	Healthy control (non-infested soil)	1.011		0.035		1.039	

Table 3: *Impact of P. polymyxa and R. intraradices on the activity of oxidative enzymes of chickpea plants (cv. Giza 3 & Giza 195) grown in artificially infested soil by R. solani.*

Phenolic contents

The total phenol content in all treatments was greater than that of the control grown in artificially infested soil (infected plants). Table 4 recorded the highest value with *R. intraradices* treatment in cultivar Giza 3 and with *P. polymyxa* treatment in cultivar Giza 195 as 82.9% and 41.6% increase over untreated control, respectively. For free phenol content, the maximum increase was shown with *R. intraradices* treatment for the Giza 3 cultivar and with combined treatment (*P. polymyxa* + *R. intraradices*) for the Giza 195 cultivar as a 150% and 130% increase over control, respectively.

Cultivars	Treatments	Total phenol		Free phenols	
		Activity	Increasing over infected control %	Activity	Increasing over infected control %
Giza 3	*P. polymyxa*	4.795	18.5	3.434	48.4
	R. intraradices	7.405	82.9	5.785	150
	P. polymyxa + *R. intraradices*	4.881	20.6	3.406	47.2
	Control (*R. solani*)	4.048	0.0	2.314	0.0
	Healthy control (non-infested soil)	3.509		1.389	
Giza 195	*P. polymyxa*	5.212	41.6	2.686	103.9
	R. intraradices	4.846	31.7	2.396	81.9
	P. polymyxa + *R. intraradices*	4.084	10.9	2.617	130
	Control (*R. solani*)	3.68	0.0	2.317	0.0
	Healthy control (non-infested soil)	3.184		1.43	

Table 4: *Impact of P. polymyxa and R. intraradices on the phenolic contents of chickpea plants (cv. Giza 3 & Giza 195) grown in artificially infested soil by R. solani.*

Total flavonoids and proline

Results in table 5 showed that total flavonoids and proline increased with all treatments over the infected control. The higher increases were observed with combined treatment (*P. polymyxa* + *R. intraradices*) followed by *R. intraradices treatment* for the two cultivars. Meanwhile, the lowest concentrations of total flavonoids were recorded in infected control plants and non-infected plants, while the lowest concentrations of proline were observed with *P. polymyxa,* infected control plants, and un-infected (healthy) control plants.

Cultivars	Treatments	Total flavonoid mg quercetin /g dry weight	Increasing over infected control %	Proline µmol g/ fresh weight	Increasing over infected control %
Giza 3	*P. polymyxa*	0.607	113.7	0.36	16.1
	R. intraradices	0.704	147.9	0.66	112.9
	P. polymyxa + *R. intraradices*	0.757	166.5	1.10	254.8
	Control (*R. solani*)	0.284	0.0	0.31	0.0
	Healthy control (non-infested soil)	0.296		0.36	
Giza 195	*P. polymyxa*	0.313	84.1	0.37	23.3
	R. intraradices	0.465	173.5	0.74	146.7
	P. polymyxa + *R. intraradices*	0.510	200.0	1.55	416.7
	Control (*R. solani*)	0.170	0.0	0.30	0.0
	Healthy control (non-infested soil)	0.11		0.36	

Table 5: *Impact of P. polymyxa and R. intraradices on the contents of total flavonoids and proline of chickpea plants (cv. Giza 3 & Giza 195) grown in artificially infested soil by R. solani.*

Field experiments

Impact of Rizolex-T, *P. polymyxa,* and *R. intraradices* alone and in combination on the occurrence of damping-off disease of chickpea plants grown under field conditions

Generally, the application of *P. polymyxa* and *R. intraradices* alone or in combination caused a significant decrease in pre- and post-emergence damping off and an increase in surviving plants over the control of the two cultivars at Giza and Etai El-Baroud (Table 6). Results also showed that the most effective treatments in decreasing pre-emergence damping off and increasing the survived plants were Rizolex-T and the combined treatment (*P. polymyxa* + *R. intraradices*), followed by *R. intraradices* and *P. polymyxa* treatments. For the post-emergence damping off, the reduction varies with the different treatments, taking into consideration that all treatments were more effective than the untreated control treatment.

Cultivars	Treatments	Damping-off				Survived plants %	Increasing over infected control %
		Pre-emergence		Post-emergence			
		Incidence %	Reduction %	Incidence %	Reduction %		
Giza 3	*P. polymyxa*	12	52.9	3.7	22.3	84.3	20.9
	R. intraradices	9.2	63.9	2.5	47.3	88.3	26.6
	P. polymyxa + *R. intraradices*	6.1	75.9	2.3	52.7	91.6	31.4
	Rizolex-T	4.9	80.6	3.1	36.1	92.0	31.9
	Untreated control	25.5	0.0	4.8	0.0	69.7	0.0
Giza 195	*P. polymyxa*	11.7	63.8	2.0	44.4	86.3	34.8
	R. intraradices	9.6	70.3	1.7	51.9	88.7	38.4
	P. polymyxa + *R. intraradices*	7.3	77.4	2.4	33.3	90.3	26.3
	Rizolex-T	4.9	84.8	2.1	40.8	93.0	45.3
	Untreated control	32.4	0.0	3.6	0.0	64	0.0
L.S.D ≤0.05	Treatment (T)	2.1		2.4		6.0	
	Cultivar (C)	1.2		1.5		3.8	
	T x C	4.2		4.2		8.5	

Table 6A: *Giza agricultural research station.*

Cultivars	Treatments	Damping-off				Survived plants %	Increasing over infected control %
		Pre-emergence		Post-emergence			
		Incidence %	Reduction %	Incidence %	Reduction %		
Giza 3	*P. polymyxa*	8.3	74.7	1.3	44.1	90.4	39.3
	R. intraradices	6.9	78.8	2.1	13.8	91.0	40.2
	P. polymyxa + *R. intraradices*	5.7	82.5	1.6	33.3	92.7	42.8
	Rizolex-T	4.53	86.1	2.0	16.6	93.4	43.9
	Untreated control	32.7	0.0	2.4	0.0	64.9	0.0
Giza 195	*P. polymyxa*	10	67.9	2.2	18.5	87.8	44.4
	R. intraradices	7.9	74.8	2.0	25.9	90.1	36.2
	P. polymyxa + *R. intraradices*	5.2	83.3	1.6	40.7	93.2	40.9
	Rizolex-T	4.3	86.1	1.5	44.4	94.2	42.5
	Untreated control	31.2	0.0	2.7	0.0	66.1	0.0
L.S.D ≤0.05	Treatment (T)	2.9		1.6		5.1	
	Cultivar (C)	1.8		1.0		3.2	
	T x C	4.0		2.2		7.2	

Table 6B: *Etai El-Baroud agricultural research station.*

Table 6: *Impact of Rizolex-T, P. polymyxa, and R. intraradices on the occurrence of damping-off disease of chickpea plants (cv. Giza 3 & Giza 195) under field conditions.*

Impact of Rizolex-T, *P. polymyxa,* and *R. intraradices* alone and in combination on some crop parameters and yield of chickpea plants under natural infection

The application of *P. polymyxa* and *R. intraradices*, either alone or in combination, significantly improved various growth parameters and yield of chickpea plants across two locations, while all growth parameters were significantly reduced in

control (Table 7). Results revealed that the plant height increased significantly with *P. polymyxa* treatment over the other treatments in two locations, while all treatments resulted in a higher number of branches compared with the untreated control. The number of capsules per plant and seed weight per plant significantly increased with the combined treatment (*P. polymyxa* + *R. intraradices*) and *R. intraradices* alone treatment. Additionally, the combined treatment showed a remarkable increase in hundred-seed weight over untreated control. Overall, chickpea seed yield responded positively to the application of combined treatment (*P. polymyxa* + *R. intraradices*) followed by *R. intraradices* alone treatment. Meanwhile, *P. polymyxa* alone recorded the lowest value of all treatments except the plant height; it was still more than untreated control plants. Notably, no significant differences were observed between the two cultivars.

Cultivars	Treatments	Plant height (cm)	Number of branches/ plant	Number of capsules/ plant	Seed weight/ plant (g)	100 -seed weight (g)	Seed yield (kg/fed)
Giza 3	*P. polymyxa*	70.5	2.5	29.3	10.6	18.4	994.2
	R. intraradices	63.8	2.8	31.8	13.2	20.0	1075.0
	P. polymyxa + *R. intraradices*	67.3	2.5	31.3	14.5	20.9	1204.3
	Rizolex-T	68.3	2.5	34.5	15.1	20.6	1325.8
	Untreated control	49	1.0	17.0	4.2	15.2	530.8
Giza 195	*P. polymyxa*	68	2.8	33.1	12.4	21.7	1190.8
	R. intraradices	65.5	2.8	35.8	16.7	22.8	1222.5
	P. polymyxa + *R. intraradices*	64.3	2.5	35.5	16.8	23.5	1283.0
	Rizolex-T	65.5	2.8	40.0	17.4	24.1	1305.0
	Untreated control	52	1.3	19.8	6.9	18.6	624.8
L.S.D ≤0.05	Treatment (T)	2.0	0.6	2.2	0.5	1.0	36.3
	Cultivar (C)	1.2	0.4	1.4	0.3	0.6	22.9
	T x C	2.8	0.8	3.1	0.7	1.4	51.3

Table 7A: *Giza agricultural research station.*

Cultivars	Treatments	Plant height (cm)	Number of branches/ plant	Number of capsules/ plant	Seed weight/ plant (g)	100 -seed weight (g)	Seed yield (kg/fed)
Giza 3	*P. polymyxa*	73.5	2.8	29.4	11.6	18.9	1005.8
	R. intraradices	67.3	2.8	32.2	13.5	20.8	1207.5
	P. polymyxa + *R. intraradices*	66.5	3.0	31.9	13.6	21.3	1259.3
	Rizolex-T	75.0	2.8	33.4	14.6	21.9	1295.8
	Untreated control	51.8	1.5	17.7	5.3	15.3	506.0
Giza 195	*P. polymyxa*	74.5	2.5	33.0	14.6	21.8	1178.0
	R. intraradices	69.5	2.5	32.3	14.7	22.5	1418.0
	P. polymyxa + *R. intraradices*	73.3	2.5	33.3	15.1	22.8	1259.3
	Rizolex-T	72.0	2.8	33.0	15.3	23.3	1299.3
	Untreated control	55.0	1.3	19.5	7.3	18.9	648.3
L.S.D ≤ 0.05	Treatment (T)	2.8	0.5	5.2	0.7	0.3	25.7
	Cultivar (C)	1.8	0.3	3.3	0.5	0.5	23.0
	T x C	4.0	0.7	7.4	1.0	1.1	36.3

Table 7B: *Etai El-Baroud agricultural research station.*

Table 7: *Impact of Rizolex-T, P. polymyxa, and R. intraradices on some crop parameters of chickpea plants (cv. Giza 3 and Giza 195) under natural infection at Giza and Etai El-Baroud Agricultural Research Stations during winter growing season 2020-2021.*

Discussion

Chickpea (*Cicer arietinum* L.) is an important pulse crop that is cultivated and consumed worldwide, mainly in Afro-Asian countries [39]. *Rhizoctonia solani* attacks chickpeas and causes seedling blight and root rot, which could be a significant barrier to the production of chickpeas [40]. The use of fungicides is often limited due to the risk of the development of resistant strains; also, some chemicals may be carcinogenic and have harmful effects on natural resources [41]. Biocontrol methods as an alternative have attracted much more attention and are becoming a promising and eco-friendly approach to reducing the use of synthetic agrochemicals. They also play an important role in managing plant disease; they likewise increase soil fertility [42].

According to the current investigation, treatments with *P. polymyxa* and *R. intraradices*, alone or combined, remarkably decrease damping-off and improve plant survival under greenhouse and field conditions. The most effective treatment was Rizolex-T, followed by the combined application of *R. intraradices* and *P. polymyxa*. Additionally, all treatments reduced the *R. solani* root rot disease index. The lowest disease severity was observed in plants treated with Rizolex-T, followed by the combined microbial treatment, across both cultivars (G 3 and G 195).

In this respect, *P. polymyxa* represents a fast-growing rhizosphere bacterium that also secretes an antimicrobial compound, that directly kills pathogens [43,44]. Meantime, it can produce antibiotic compounds like polymyxin that can suppress the growth of pathogens under field conditions [45] and secrete phenolic compounds that play a role as selective antimicrobials, forming a beneficial microbiome for the plant [45]. Additionally, it produces cell wall-degrading enzymes, including β-1,3-glucanases, cellulases, and chitinases [46], and triggers induced systemic resistance [47]. Additionally, it can create biofilms around the roots of different hosts, protecting them from invading fungi [48].

On the other hand, the AMF aids in the control of plant pathogens like *Rhizoctonia solani* [49] by competing with soilborne pathogens for nutrients and space, triggering plant defense mechanisms [50], producing antimicrobial compounds, and changing the microflora in the rhizosphere [51,52].

Although Rizolex-T is effective in controlling *R. solani* under greenhouse and field conditions, the combined treatment is effective in controlling the disease and increasing the survival of plants without any significant differences with the fungicide treatment.

Several works showed that the combined use of *G. intraradices* and the PGPR isolates inhibited pathogens more than the individual treatment did [53,54]. Dual and triple treatments (Mycorrhizeen, *P. polymyxa,* and *P. fluorescens*) increased the percentage of surviving plants and decreased the disease severity of *R. solani* in soybean plants [17].

As a result of the suppression of *R. solani* pathogenesis in chickpea seedlings, several biochemical changes have been associated with treated seeds with *P. polymyxa* and *R. intraradices* as individual treatments or combined treatments, such as an increase in the activities of peroxidase (PO), polyphenol oxidase (PPO), and β-1,3 glucanase enzymes, and an increase in total phenolic contents.

However, the increase in oxidase activity in plants was associated with enhanced plant resistance to infection by numerous pathogens [55,56]. Additionally, peroxidases contribute to forming phytoalexins and reactive oxygen species (ROS) with antifungal characteristics [57]. Also, the PPO enzyme is involved in the oxidation of polyphenols into quinones, which are

more toxic antibacterial substances, and the lignification of plant cells following microbial invasion [58]. The development of cell wall thickenings, which are typically accompanied by the deposition of lignin, is a defense response of phenol synthesis, which serves as a physical barrier to prevent the invasion of the pathogen and restricts the infection process. In addition, it suppresses disease development by inhibiting extracellular fungal enzymes [59].

Because hydrolytic enzymes like β-1,3 glucanase can hydrolyze fungal cell walls, they aid in defense against invading fungal pathogens and induce the host root to create metabolites like terpenes and phenols that give the host tissue resistance against pathogen invasion [60].

Results showed that flavonoids were determined by all bioagent treatments greater than the untreated control, especially with mycorrhizal, either as a combined treatment (*P. polymyxa* + *R. intraradices*) or individual treatment. However, root exudates contain various chemical signals, including flavonoids, which are known to promote nodulation in legume plants. Released flavonoids in the rhizosphere can protect plants from biotic and abiotic threats [61] and play a role in auxin biosynthesis [62]. On the other hand, AMF significantly alters the metabolic profiles of host plants, leading to an increase in primary and secondary metabolites, including flavonoids [63]. It is concluded that AM fungi accelerated flavonoid synthesis in plants [64,65].

There is also a noticeable increase in proline content with *R. intraradices* alone or combined with *P. polymyxa* treatments. According to [66], proline is an essential regulator that enhances plant resistance to various abiotic stresses. Moreover, AMF can improve proline metabolism under low-temperature and low-nitrogen environments [67].

Results also conclude that single or co-inoculation with *P. polymyxa* and *R. intraradices* treatments increased the contents of micro and macro elements. In this respect, *P. polymyxa* promotes plant growth and directly increases soil iron absorption and phosphorus solubilization [46]. Nitrogen-fixing genes in *P. polymyxa* strains allow them to transform atmospheric nitrogen into ammonia, which is a beneficial source of nitrogen for plants [68, 69]. Furthermore, there is an increase in the host plant's phosphorus nutrition as a response to AMF colonization [69]. AMF can assist plants in acquiring macro- and micro-nutrients such as Cu, K, Mg, N, and Zn, particularly when present in soils in less soluble forms [70].

Furthermore, plant nutrients, i.e., nitrogen, phosphorus, potassium, calcium, magnesium, iron, and zinc, were enhanced by a combined inoculation of *G. mosseae* and *Bacillus subtilis* [71]. In addition, the dual inoculation of AMF and PGPR improved soil properties and nutrient acquisition that should be used as a biofertilizer to increase soil quality and production [69].

Once again, the high improvements in plant growth parameters and overall yield were observed with the combined treatment compared to untreated plants. Meanwhile, mycorrhizae and plant growth-promoting bacteria have a significant potential to minimize fertilizer use, improve soil fertility, increase crop yield, improve nutrient absorption, and decrease the environmental impact of mineral fertilizers [72]. [73] reported PGPR are associated with AMF to improve plant growth and productivity. A variety of plant growth stimulators are produced by *Paenibacillus polymyxa*, such as cytokinin [74] and auxin in the form of indole-3-acetic acid [75].

Also, AMF stimulates plant development and increases yield by secreting phytohormones and supplementing nutrients [76]. Additionally, *R. intraradices* enhance seedlings and the growth of roots, the uptake of nutrients, and overall parameters of growth under various environmental conditions [12].

However, interactions between bacteria and AMF primarily take place in the mycorrhizal hyphosphere; certain bacterial groups exhibit a stronger association with AMF hyphae than others, indicating specificity in their interactions [77]. Arthurson., *et al.* [78] reported that *Paenibacillus brasilensis* exhibits a higher degree of physical attachment to vital AMF hyphae irrespective of the fungal species. Hildebrandt, *et al.* [79] have demonstrated that *Glomus intraradices* can complete their life cycle in the absence of a host plant when accompanied by an isolate of *Paenibacillus.*

Meantime, *Paenibacillus* bacteria were isolated from the external mycelium of *G. intraradices* [80]. Additionally, a bacterial isolate from the mycorrhizosphere of Sorghum inoculated with *G. mosseae* was identified as *Paenibacillus* sp [53]. Moreover, when plants were co-inoculated with *Paenibacillus favisporus*, *G. intraradices* colonized soybean roots more effectively [81]. These data suggest that *Paenibacillus* species are widely spread in the mycorrhizosphere and might promote the growth of AMF symbiosis.

Conclusion

According to the current work, *Paenibacillus polymyxa* and *Rhizophagus intraradices,* both individually and in combination, have biocontrol potential for controlling root rot and damping off diseases caused by *Rhizoctonia solani* in chickpea. The combined treatment exhibited the highest efficacy, suggesting synergistic effects that enhance the activity of peroxidase, polyphenol oxidase, and β-1,3 glucanase enzymes, correlating with elevated phenolic compound, flavonoid, proline, macro, and microelement contents. Moreover, there is a stimulatory effect on crop parameters and seed yield.

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