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Bean (*Phaseolus vulgaris,* L.) Damping-off Caused by *Pythium ultimum* var. *ultimum* and its Possible Control by *Pythium oligandrum*

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The effectiveness of *Pythium oligandrum* as a bio-control agent against *P. ultimum* var. *ultimum* was investigated to avoid harmful fungicides effects. Three isolates of *P. oligandrum* (MS_{15}, MS_{19} and MS_{31}) were evaluated against the plant pathogenic *P. ultimum* Trow var. *ultimum* the causative agent of *Phaseolus vulgaris* damping-off. *Pythium* species were identified according to their respective morphologically and sequencing of rDNA-ITS fragments including the 5.8 S rDNA to confirm the species identification. The three isolates of *P. oligandrum* reduced the mycelial growth of *P. ultimum* var. *ultimum* with 64.44, 65.56 and 66.76% during mycoparasitism on PDA plates while the millipored sterilized filtrates of *P. oligandrum* isolates reduce the pathogenic mycelial growth by 16.67, 17.78 and 18.89% respectively. Scanning electron microscope (SEM) of the strongest antagonistic isolate (MS_{31}) interaction revealed coiling, haustorial branches, infection pegs and penetration of *P. oligandrum* to *P. ultimum* var. *ultimum* hyphae. On the other hand, *P. oligandrum* isolates improved emergence of *Phaseolus vulgaris* seedling from 0-100% in damping-off disease using agar bottles and from 13.33 - 86.66% during pot experiments compared with the control.

Key words: Biological control, Damping-off, *Phaseolus vulgaris, P. oligandrum, P. ultimum var. ultimum.*

Common bean (*Phaseolus vulgaris* L.) is one of the most widely cultivated food legume species in the world[1]. Root rot diseases of *Phaseolus vulgaris* are widespread in the world and are often considered as a major constraint to production reducing both yield and quality[2]. *Pythium* as causal agent for *Phaseolus vulgaris* root rots have been cited as being among the major causes leading to bean yield losses in several countries[3,4]. The use of chemical fungicides controlling such diseases could led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide. Biological methods are needed for plant protection which are less dependent on chemicals and are more environmentally friendly[5,18].

Pythium is a complex genus spread worldwide containing over 200 described species with a broad host range and occupying a variety of terrestrial and aquatic ecological habitats[6-8]. *P. oligandrum* has received much attention as a potential biocontrol agent for damping-off diseases especially those caused by *P. ultimum* var. *ultimum*[7,9]. *P. oligandrum* use for biological control of *P. ultimum* var. *ultimum* has been previously

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reported[10,11]. There are at least 3 modes of *P. oligandrum* action on plant pathogens are known as mycoparasitism mediated by intimate hyphae interactions, antibiosis and enhancement of plant[12].

In Egypt, No attempts had been made to evaluate *P. oligandrum* as a biocontrol agent against *P. ultimum* var. *ultimum* the causal agent of damping-off of *Phaseolus vulgaris* seedlings. The present work was therefore, aimed at studying the occurrence of mycoparasitic *Pythium oligandrum* in soil rhizosphere of some plants cultivated in El-Minia governorate and evaluating the possibility of their use in the biocontrol of bean seedling damping-off caused by *P. ultimum* var. *ultimum*.

MATERIALS AND METHODS

Fungal isolates

Three different methods were used for isolation the biocontrol agent *P. oligandrum* during the survey of *Pythium* species associated with the rhizosphere soil of some crop and vegetable plants in El-Minia Governorate, Egypt in the period of 2006-2008. These methods including direct isolation from the soil (DIS), Soil serial dilution method (SSDM) and Zoospores baiting technique (ZBT) with NARM plates medium according to Elnaghy *et al.*[8].

For isolation of the pathogen, diseased roots and seedlings of *Phaseolus vulgaris* were rinsed in 50% ethanol (v/v) for 30 second followed by sterile distilled water, blotted with sterile filter paper and then transferred to selective medium (NARM)[13]. All plates were incubated at 20°C and examined daily. Obtained colonies were then purified and subjected to identification[8].

Identification of *Pythium* species

Morphological identification of isolated *Pythium* species was carried out using the two keys of Plaats-Niterink and Dick[6,15]. The method described by Senda *et al.*,[13] was adapted to obtain the required fungal structure. For molecular Identification DNA extraction and amplification were done using polymerase chain reaction (PCR) technique. The sequencing of rDNA-ITS fragments including the 5.8 S rDNA was analyzed to confirm the species identification as mentioned by Elnaghy *et al.* [8].

Antagonistic activity of *P. oligandrum* against the pathogenic *P. ultimum* var. *ultimum*

Sterilized PDA plates were inoculated by 7-mm discs cutting from the margins of each of the pathogenic strains and biocontrol fungus at a distance of 4 cm from each other. Three replicates were maintained per each treatment and the plates were incubated at 25°C for 3-5 days. Percentages of reduction in mycelial growth of the pathogenic *P. ultimum* was calculated and recorded in relation with control[14].

Effect of *P. oligandrum* filtrate on *P. ultimum* var. *ultimum* growth

Sterilized filtrate of 5-dayes old cultures of *P. oligandrum* isolates grown on potato dextrose broth (PDB) were tested against *P. ultimum* var. *ultimum*. Tested filtrate was obtained by either autoclaving or using Millipore filter of 0.22 µ. Equal volumes of both sterilized filtrates and sterilized double strength PDA medium were mixed (1:1 v/v) by gently rotating just before pouring in Petri plates. Discs of 3 day old culture of the pathogenic *P. ultimum* var. *ultimum* were placed in the middle of these plates and incubated at 25°C. Data were recorded when mycelial growth covers all surface of the control plates. Percentages of growth reduction due to antagonism were calculated[19].

Interaction between *P. oligandrum* and *P. ultimum* var. *ultimum* using SEM

Mycelial strips were taken from the contact zone between the two colonies of *P. oligandrum* and *P. ultimum* var. *ultimum* grown on CMA for three days at 25°C and then fixed in 2.5% gluteraldehyde-Sodium phosphate buffer (0.13 M, Ph 7.2) overnight. The specimens were washed three times with buffer and then post fixed in osmium tetra oxide for 2 hour, washed again with buffer and dehydrated using ascending series of ethanol level (30 - 100%) for 15 min at each step. the specimens were dried at critical point drier and coated with Platinum to examined using scanning electron microscope (JEOL JSM-6380 LA) operating at 20 kV [16].

Pathogenicity of *P. ultimum* var. *ultimum* and its possible control measure

In agar bottles assay

Pathogenicity and possible control of *P. ultimum* var. *ultimum* using *P. oligandrum* was investigated[17]. *Phaseolus vulgaris* seeds were surface sterilized and germinated to form radicals

and plumules for 3 days at 25°C then three viable seeds were grown onto 2% WA in 250 ml Erlenmeyer flask. Each flask inoculated by three 5-mm discs of *P. ultimum* var. *ultimum* and *P. oligandrum* separately to evaluate their pathogenicity and combined to evaluate the biocontrol activity of *P. oligandrum* (MS_{15}, MS_{19} and MS_{31}). Inoculation discs were cut from the margins of 5-days old colonies grown on CMA medium and were placed between *Phaseolus* seedlings under aseptic conditions. Inoculated flasks were incubated in a growth cabinet at 25ºC with 12 h photoperiod (91 µmol $m^{-2}S^{-1}$). Damping-off was determined as the difference between seedlings emergence in non-inoculated controls and inoculated one[17].

In soil pots assay

P. ultimum var. *ultimum* inoculum was performed[17]. Five grams of grass blade leaf segments (0.5 cm × 1 cm) and 2 gm glucose were moistened by adding 10 ml distilled water in 250 ml Erlenmeyer flask. After autoclaving, each flask was inoculated with three discs of *P. ultimum* var. *ultimum* culture then inoculated at 25ºC for 10 days. Inoculum concentration of 2.5%, was obtained by mixing thoroughly with 1gm of colonized grass leaf segments in the Erlenmeyer flask with 50 gm of oven-dried (70-80ºC for 2 days) loam sandy soil (LSS) using a sterilized mortar. Two and half gm of this mixture was added to 97.5 gm of sterilized soil (LSS). Inoculated soil was placed in plastic pots and exterior sterilized seeds of *Phaseolus vulgaris* were sowed after viability test as three seeds in each pot. The experiments were carried out in a growth cabinet and Damping-off was determined as mentioned above.

To evaluate the efficacy of *P. oligandrum* against damping-off disease of *Phaseolus vulgaris* in artificially infested soil with *P. ultimum* var. *ultimum.* Mycelial mats of *P. oligandrum* were got from cultures developed in V-8 juice broth. Aliquots of 20 ml medium were transferred to sterilized Petri plates to inoculate with a single disc (0.5 cm diam) cut from *Pythium* colony developed on CMA. The plates were incubated in darkness for 10 days at 25ºC. Mycelial mats were cleaned with sterile distilled water and fragmented by tissue homogenizer. The preparation was utilized to outer garment of *Phaseolus vulgaris* seeds using identical volume (100 ml) of 3% carboxy methyl cellulose solution (CMC) and the preparation of mycelial mats of *P. oligandrum* (MS_{15}, MS_{19} and MS_{31}) were mixed and about 3 exterior sterilized seeds of *Phaseolus vulgaris* were supplemented. After leaving the seeds to soak for 2 min in the preparation, the seeds were taken and spread in the sterile opened Petri dishes to dry overnight in a refrigerator at 5ºC. All seeds were then utilized directly in damping-off experiments. Subsequently, coats from *P. oligandrum* isolates suspension at concentration 1000 propagules per ml were made around surface sterilized *Phaseolus vulgaris* seeds. Three coated seeds of *Phaseolus vulgaris* seeds were sown into *P. ultimum* var. *ultimum* infested soil at 5 mm depth from the soil surface found in free draining plastic pot (300 gm capacity, 13 cm diameter). In controls non-coated *Phaseolus vulgaris* seeds were sown into either *P. ultimum* var. *ultimum* infested soil or into *P. ultimum* var. *ultimum* free soil were also performed. All pots were placed in a growth cabinet as mentioned above. Numbers of *Phaseolus vulgaris* seeds, which damped-off was counted after 14 days from sowing. Experiments were performed with ten replicate pots per treatment[17].

Histology of infected *Phaseolus vulgaris* seedlings

Small parts of root and stem tissues of infected *Phaseolus vulgaris* seedling were fixed, washed and dehydrated as mentioned above in SEM experiment. After dehydration the tissues were cleared by toluene and embedding in paraffin wax to cut by microtome to making sectioning. These slides were stained with safranin and light green, then mounting using Canada balsam to examine under light microscope.

RESULTS

Isolation and identification of *Pythium* species showed the presence of three isolates of *P. oligandrum* (MS_{15}, MS_{19} and MS_{31}) obtained from the rhizosphere soil of *Raphanus sativus*, *Eruca sativa* and *Allium cepa*, respectively, during survey of *Pythium* species in El-Minia Governorate. These three isolates were identified morphologically as *P. oligandrum* Drechsler. In addition, the sequencing of rDNA for these isolates was closely related to Gen-Bank accession number of *P. oligandrum* (AY986954.1) with 100% similarity.

Results of etiological strain isolation from

the infected *Phaseolus vulgaris* seedling indicated that *P. ultimum* var. *ultimum* was the only fungus present in infected *Phaseolus vulgaris* tissues. Morphological identification and genetically criteria of this isolate was closely related with the characterized *P. ultimum* Trow var. *ultimum* (Gen-Bank accession number (AY598657.1) with 100% similarity.

Antagonistic activity of the three isolates of *P. oligandrum* (MS_{15}, MS_{19} and MS_{31}) against the pathogenic *P. ultimum* var. *ultimum* represented in table (1) showed that *P. oligandrum* isolates reduced the mycelial growth of *P. ultimum* var. *ultimum* and caused reduction percentage of 53.56, 37.78 and 38.89%, respectively, after 3 days of incubation. Moreover, the reduction in growth of the pathogenic *Pythium* increased by increasing the incubation time where the highest reduction in *P. ultimum* var. *ultimum* growth reached to 64.44, 65.56 and 66.76%, respectively, after 6 days of inoculation as a result of the antagonistic activity of *P. oligandrum* isolates (Fig. 1).

Table 1. The reduction in mycelial growth of the pathogenic *P. ultimum* var *ultimum* by *P. oligandrum* isolates

Incubation period	Antagonistic isolates	*P. ultimum* var. *ultimum* linear growth (mm)	Reduction
3 days	*P. oligandrum* MS_{15}	58[b]	35.56
	P. oligandrum MS_{19}	56[a]	37.78
	P. oligandrum MS_{31}	55[a]	38.89
	Control	90[c]	0.00
	LSD	1.63	
6 days	*P. oligandrum* MS_{15}	32[a]	64.44
	P. oligandrum MS_{19}	31[a]	65.56
	P. oligandrum MS_{31}	30[a]	66.67
	Control	90[b]	0.00
	LSD	2.31	

value within a column followed by the same latter are not significantly different according to Dancun's multiple range test (P≥0.05)

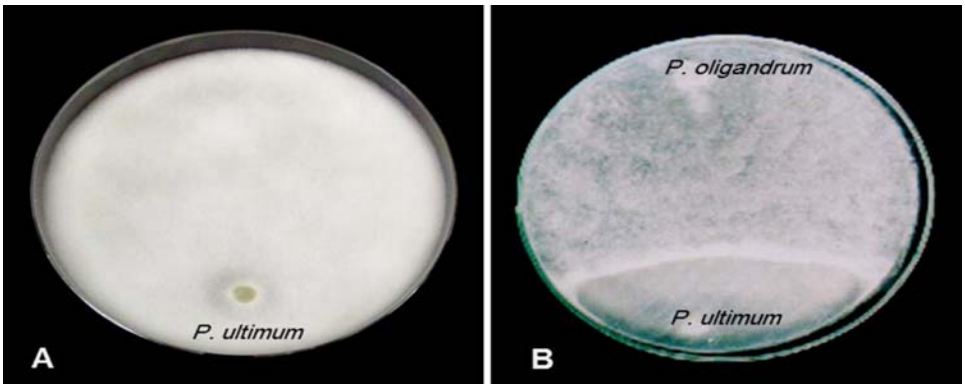

Fig. 1. Antagonistic study between *P. olgandrum* (MS31) and *P. ultimum* var *ultimum* (MS133) on PDA, at 25°C. (A) *P. ultimum var ultimum* control (B) Antigonism after 6 days of incubation

The effect of culture filtrates of *P. oligandrum* isolates on *P. ultimum* var. *ultimum* growth represented in Table (2) reveal that millipored sterilized culture filtrates of *P. oligandrum* isolates was more effective in reducing the mycelial growth of *P. ultimum var. ultimum* than in case of autoclaved cultured filtrate where the millipored filtrate of *P. oligandrum* isolates

Table 2. Effect of culture filtrates of *P. oligandrum* isolates on mycelial growth of *P. ultimum* var. *ultimum*

Incubation period	Antagonistic isolates	*P. ultimum* var. *ultimum* linear growth (mm)	Reduction
3 days	*P. oligandrum* MS_{15}	84[b]	10.00
	P. oligandrum MS_{19}	81[a]	10.00
	P. oligandrum MS_{31}	80[a]	11.11
	Control	90[c]	0.00
	LSD	3.52	
6 days	*P. oligandrum* MS_{15}	75[a]	16.67
	P. oligandrum MS_{19}	74[a]	17.78
	P. oligandrum MS_{31}	73[a]	18.89
	Control	90[b]	0.00
	LSD	3.12	

Value within a column followed by the same latter are not significantly different according to Dancun's multiple range test (P.0.05)

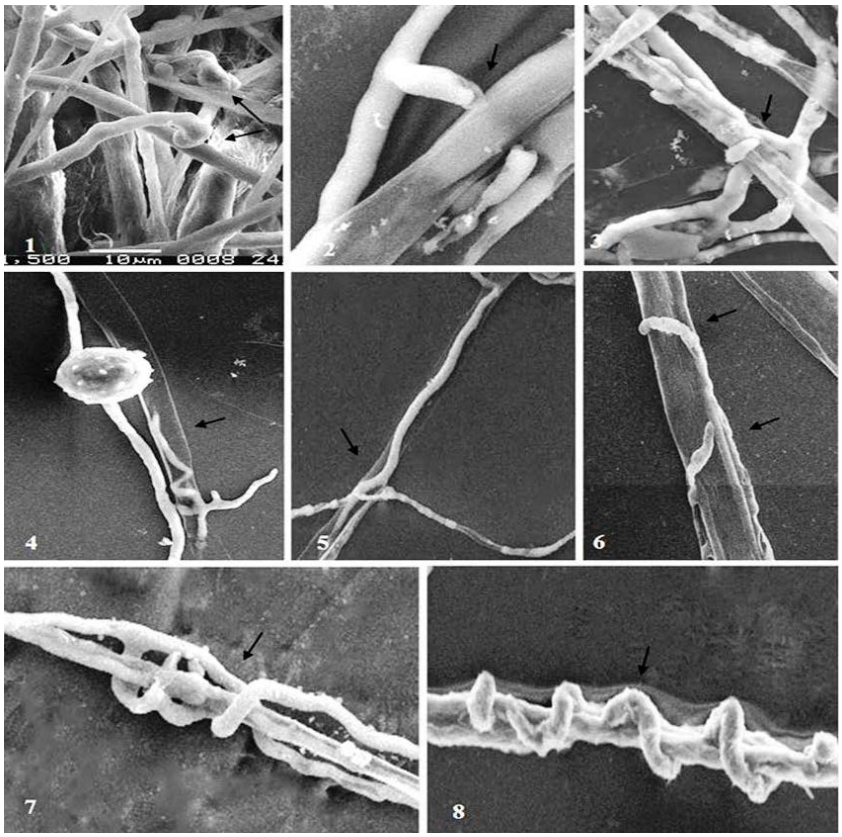

Fig. 2. Scanning electron micrographs of the mycoparasite *P. oligandrum* (MS31) hyphae interacting with hyphae of plant pathogens *P. ultimum* var. *ultimum* (MS133) on CMA: (1) Haustorial branches of *P. oligandrum* hyphae adhering to *P. ultimum* var. *ultimum* hyphae. (2 and 3) Infection pegs of *P. oligandrum*. (4 and 5) Hyphae of *P. oligandrum* grow inside hyphae of *P. ultimum* var. *ultimum*. (6) *P. oligandrum* hyphae form dense coils and tightly encircle hyphae of *P. ultimum* var. *ultimum*. (7and 8) *P. oligandrum* hypha is associated with marked collapse and loss of turgor of *P. ultimum* var. *ultimum* hyphae, where *P. oligandrum* twisted on hyphae of *P. ultimum* var. *ultimum.* Bar in photo (1) is applicable for all photos (10 µm).

inhabit the mycelial growth of *P. ultimum* var. *ultimum* by 16.67, 17.78 and 18.89%, respectively, compared with the control.

Interaction between the strongest isolates of *P. oligandrum* (MS_{31}) and *P. ultimum* var. *ultimum* using SEM were provided in figure (2). The characterization of coiling, haustorial branches, infection pegs and penetration of *P. oligandrum* to *P. ultimum* var. *ultimum* hyphae were showed in contact area between the two Pythia. *P. oligandrum* attacked *P. ultimum* var. *ultimum* by entangling its hyphae with either thin haustorial branches or infection pegs leading finally to its total destruction. These characters of mycoparasitism were showed clearly at early stages of contact region between the two colonies of *P. oligandrum* and *P. ultimum* var. *ultimum*.

Pathogenicity of *P. ultimum* var. *ultimum* and its possible control measure by *P. oligandrum* isolates (MS_{15}, MS_{19} and MS_{31}) in both agar bottles and soil pots assay were tabulated in table (3). It was found that, the three isolates of *P. oligandrum* were non-pathogenic to *Phaseolus vulgaris* seedlings while *P. ultimum* var. *ultimum* was highly pathogenic one causing 100% and 83.3% seedling damping-off when the pathogen and biocontrol were cultured with seedlings alone in both 2%WA and soil pots, respectively, on the other hand, the application of the bio-control isolates of *P. oligandrum* (MS_{15}, MS_{19} and MS_{31}) resulted in considerable enhancement in seedlings emergency of *Phaseolus vulgaris* from 0 – 96.6% in 2% WA assay and from 16.66 – 86.66% in soil treatments. The suppuration of damping off in *Phaseolus vulgaris* by the three isolates of *P. oligandrum* ranged from 86.66% - 96.66% in agar bottles and from 80 - 86.66% in soil pots compared with the control. Moreover, *P. oligandrum* isolate (MS_{31}) was the most effective isolate in all treatments.

Histological studies of infected *Phaseolus vulgaris* seedlings with the pathogenic *P. ultimum* var. *ultimum* showed that, *P. ultimum* var. *ultimum* hyphae grew abundantly at the root surface and rapid ingress throughout the root system. By 3 days post-inoculation, hyphae of *P. ultimum* var. *ultimum* were visible in the epidermis and inside cortical cells until reach to Phylum and medulla. In the following days, the infected cell layers of the cortical area were lysised and destroyed (Fig. 3).

Table 3. Effect of *P. oligandrum* isolates on *Phaseolus vulgaris* pre-emergence damping-off disease grown in 2% water agar in pots containing loam sandy soil

Treatments	*Phaseolus vulgaris* seedlings					
	2% water agar			Soil pots		
	No. of Survival	Inhibition (%)	Survival (%)	No. of Survival	Inhibition (%)	Survival (%)
Control (No *Pythium*)	30^{c}	0.0	100	30^{c}	0.0	100
P. ultimum var. *ultimum*MS_{133}	0.0^{a}	100	0.0	5^{a}	83.33	16.66
P. oligandrum MS_{15}	30^{c}	0.0	100	30^{c}	0.0	100
P. oligandrum MS_{19}	30^{c}	0.0	100	30^{c}	0.0	100
P. oligandrum MS_{31}	30^{c}	0.0	100	30^{c}	0.0	100
P. ultimum var. *ultimum* MS_{133} + *P. oligandrum* MS_{15}	26^{b}	13.33	86.66	24^{b}	20.0	80.0
P. ultimum var. *ultimum* MS_{133} + *P. oligandrum* MS_{19}	29^{c}	3.33	96.66	25^{b}	16.66	83.33
P. ultimum var. *ultimum* MS_{133} + *P. oligandrum* MS_{31}	29^{c}	3.33	96.66	25^{b}	13.33	86.66
LSD	1.49			2.06		

*Value within a column followed by the same latter are not significantly different according to Dancun·s multiple range test ($P \geq 0.05$).

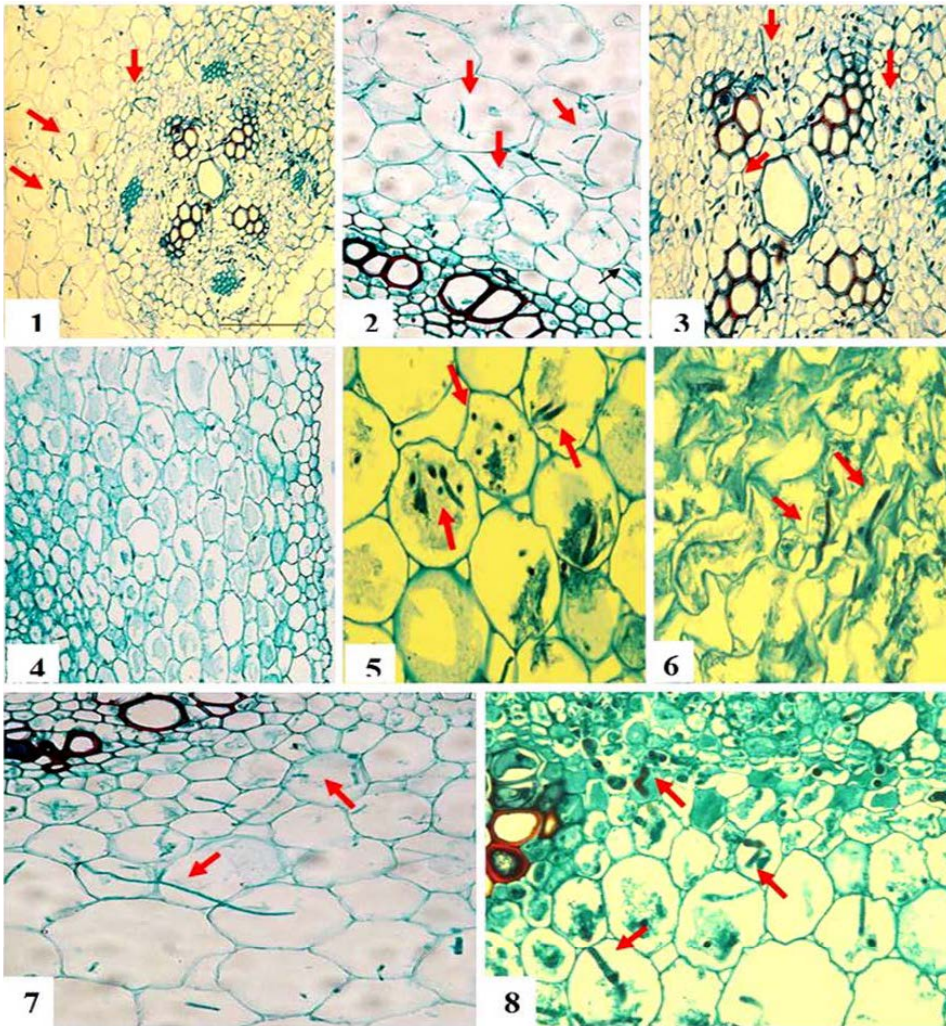

Fig. 3. Light micrographs of *Phaseolus vulgaris* root and steam colonized by *P. ultimum* var. *ultimum* (MS133), 3 days after inoculation. 1&2- Abundant hyphae of the *P. ultimum* in the epidermis and the cortex. 3- Colonizing hyphae appear with strongly light green-stained in phylum and medulla. 4- Cortical free of hyphae (control). 5- Hyphae of *P. ultimum* var. *ultimum* (MS133) inside cortical area inside cortical cells 3 days after inoculation. 6- Lysis of root cortical cells 7 days after inoculation. 7&8 - *Phaseolus vulgaris* steam tissues colonized by *P. ultimum* var. *ultimum* (MS133), abundant multiplication of hyphae of *P. ultimum* var. *ultimum* in cortex. Bar in photo 1 is applicable for all photos = 20 µm.

DISCUSSION

Root damping-off diseases are widely disturbed all over the world and are considered as a major problem to bean cultivators, as they reduce significantly the bean yield[20]. Several control measures of that disease are available, including fungicidal control, enhancement of genetic resistance which may lead to deteriorating human health[5,20]. In Egypt, there is no effective control method to eliminate damping-off of many crop and vegetable plants caused by *P. ultimum* var. *ultimum* although, that pathogenic is widely distributed throughout the country[8,9,17,18]. However, fungicides are regularly used by the farmers to management such diseases without obvious results[17,18,20]. *P. ultimum* var. *ultimum* was isolated in this study as the causal agent of *Phaseolus vulgaris* damping-off and the pathogenicity test concerned with the pathogenic isolate of *P. ultimum* var.

ultimum MS_{133} which it was highly virulent to *Phaseolus vulgaris* seedlings causing 100% damping off. On contrast, the three isolates of *P. oligandrum* ($MS_{15,}$ $MS_{19,}$ and MS_{31}**)** were found to be non-pathogenic. This result was in accordance with the result of Nzungize *et al.*,[20,23]. Histological studies were performed to detect mode of parasitism through which pathogen cause the disease. The penetration and colonization of *P. ultimum* var. *ultimum* hyphae to the epidermal and cortical cells until reach to phylum plant root and stem of *Phaseolus vulgaris* seedling was obvious and showed the effects of this pathogen to lysis the cell layers of cortical area leading to damping-off. These results agree with many worker recorded that *Pythium* growth was mainly root intracellular[12, 16, 22].

Biological control of damping-off diseases is complicated process as a result of the pathogens occurs surroundings the rhizosphere interface. The rhizosphere is typified by intense the microbial activity involving, a high population of microorganisms, rapid change in pH, salt concentrations, and osmotic and water potential[24]. The effective microorganisms of interest for biological control of fungal plant pathogens include some *Pythium* spp. as *P. oligandrum*. The biocontrol agent can protect the plant from fungal attacks through competition with the pathogen for nutrients, the production of antifungal metabolites, parasitism (Lysis of the pathogen) or through induction of plant resistance mechanisms[21,24]. The growth of *P. ultimum* var. *ultimum* was successfully inhibited by *P. oligandrum* isolates with growth reductions reach to 66.67% after 6 days of inoculation with *P. oligandrum* MS_{31}. SEM of contacting area between biocontrol agent (*P. oligandrum*) and pathogenic isolates (*P. ultimum* var. *ultimum*) showed that mycoparasitism was highly effective than biocontrol filtrate effect and *P. oligandrum* millipored sterilized filtrates showed more exhibiting activity compared autoclaved filtrates. This means that the effectiveness of filtrates was strongly affected by autoclaving and active biocontrol metabolites may be destroyed by heating[19]. *P. oligandrum* isolates ($MS_{15,}$ $MS_{19,}$ MS_{31}) parasitized hyphae of *P. ultimum* var. *ultimum* by entangling the hyphae of *P. ultimum* var. *ultimum* with thin haustorial branches or infection pegs, eventually leading to destruction, revealing the mode of action of antagonism between *P. oligandrum* and *P. ultimum* var. *ultimum*[11,20]. There were at least three known modes of *P. oligandrum* action on plant pathogens: mycoparasitism mediated by intimate hyphae interactions, antibiosis and enhancement of plant resistance due to protein metabolite (Oligandrin) production[11,12]. The coiling and penetration of *P. oligandrum* hyphae to *P. ultimum var. ultimum* hyphae observed in this study had frequently been reported[22, 20]. Additionally, the isolate MS31 of *P. oligandrum* was the most effective antagonistic one and reduced the disease severity of *P. ultimum* var. *ultimum* infected *Phaseolus vulgaris* to 96.66% in agar bottles and 86.66% in soil pots assay. These results confirm the ability of *P. oligandrum* to prevent the infection by *P. ultimum* var. *ultimum*[17, 23]. It can be concluded that *P. oligandrum* isolated in this study worked as mycoparasite and antifungal metabolites producer which suppress the plant parasitic *P. ultimum* var. *ultimum.*

The present work evaluated that *P. oligandrum* can effectively control mycelial growth and used as biocontrol agent against damping-off disease of *Phaseolus vulgaris* caused by *P. ultimum* var. *ultimum*. Further studies on the confirmation of the efficiency and importance of *P. oligandrum* against other root diseases are needed.

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REFERENCES

1. Broughton, W. J., Hernandez, Blair, G. M., Beebe, S., Gepts, P. and Vanderleyden, J. Beans (*Phaseolus* spp.) model food legumes. *Plant and Soil* 2003; **252**: 55–128.
2. Abawi G. S. and Widmer T. L. Impact of soil health management practices on soil borne pathogens, nematodes and root diseases of vegetable crops. *Appl. Soil Ecol*. 2000; **15**: 37-47.
3. Otsyula R. M., Buruchara R. A., Mahuku G. and Rubaihayo P. Inheritance and transfer of root rots (*Pythium*) resistance to bean genotypes. *Afr. Crop Sci. Soc*. 2003; **6**: 295-298.
4. Miklas N. P., Kelly D. J., Beebe E. S. and Blair W. M. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS

breeding. *Euphytica*, 2006; **147**: 105-131.
5. Patkowska E, Pi'ta D. and Pastucha A. Biological control of runner bean (*Phaseolus coccineus* L.) against soil-borne pathogenic fungi Polish Chitin Society. *Monograph* 2007; **12**:185-195.
6. Plaats-Niterink A. J. Monograph of the Genus *Pythium*. *Studies in Mycology* 1981; **21**: 1-244.
7. Patkowska, E. Effectiveness of grapefruit extract and *Pythium oligandrum* in the control of bean and peas pathogns. *J Plant Protect Res* 2006; **46**(1):15-28.
8. Elnaghy, M. A., Abdelzaher, H. M. A., Shoulkamy, M. A. and Sayed, S. R. Ecological Studies on *Pythium* Species Associated with Some Plants Rhizosphere in El-Minia, Egypt. *J Pure Appl Microbio* 2014; **8**(1): 195-204.
9. Abdelzaher, H. M. A., Elnaghy, M. A. and Fadl-Allah, E. M. Isolation of *Pythium oligandrum* from Egyptian soil and its mycoparasitic effect on *Pythium ultimum var. ultimum* the damping-off organism of wheat. *Mycopathol.* 1997; **139**: 97-106.
10. Deacon J. W. Studies on *Pythium oligandrum*, an aggressive parasite on other fungi. *Trans Br Mycol Soc* 1976; **66**: 383-391.
11. Vallance, J., Le Floch, G., Deniel, F., Barbier, G., Le´ vesque, C. A. and Rey, P. Influence of *Pythium oligandrum* biocontrol on fungal and oomycete population dynamics in the rhizosphere. *Appl Environ Microbiol* 2009; **75**: 4790-4800.
12. Orlikowski, L. B., Jaworska-Marosz, A. Infulence of *Pythium oligandrum* on population of *Fusarium oxysporum* f. sp. dianthi and development of *Fusarium* wilt of carnation. *Plant Prot. Sci*. 2002; **38**, (Special Issue 1): 209-211.
13. Senda, M., Kageyama, K., Suga, H. and Lévesque, A. Two new species of *Pythium*, *P. senticosum* and *P. takayamanum*, isolated from cool-temperate forest soil in Japan. *Mycologia* 2009; **101**(4): 439-448.
14. Patil A., Laddha A., Lunge A., Paikrao H. and Mahure S. *In vitro* antagonistic properties of selected Trichoderma species against tomato root rot causing *Pythium* species. *Internat J Sci, Environ and Technol*, 2012; **1**(4): 302 – 315.
15. Dick, M. W. Keys to *Pythium*. Reading: College of Estate Management 1990; 64 pp.
16. Benhamou, N., Rey, P., Picard, K. and Yves Tirilly. Ultrastructural and Cytochemical Aspects of the Interaction between the Mycoparasite *Pythium oligandrum* and Soil borne Plant Pathogens. *Biochem Cell Biol* 1999; **89**(6): 506-517.
17. Abdelzaher, H. M. A. Pre-emergence damping-off disease of maize caused by *Pythium ultimum* var. *ultimum* in Minia, Egypt. Annual Meeting of Phytopathological Society of Japan. Yamagata University Kojirakawa, Japan 2009; 26-28.
18. Gherbawy, Y. and Abdelzaher, H. M. A. Isolation of fungi from tomato rhizosphere and evaluation of the effect of some fungicides and biological agents on the production of cellulase enzymes by *Necteria haematococca* and *Pythium ultimum* var. *ultimum*. *Czech Mycol* 1999; **51**(2-3): 157-170.
19. Mostafa, A. A. Pathogenesis of *Aspergillus flavus* and *A. niger* on Tilapia sp. in Egypt. A study on the role of their mycotoxins and biological control of these fungi". *Ph.D. Thesis*, Fac. of Sci., Ain shams Univ. Cairo. Egypt, 2004; 56-57.
20. Nzungize, J. R., Lyumugabe, F., Busogoro, J. P. and Baudoin, J. P. *Pythium* root rot of common bean. biology and control methods. A review. *Biotechnol*. *Agron. Soc. Environ* 2012; **16**(3): 405-413.
21. Kinoshita T., Ichitani T. Hyphal interaction between a mycoparasite, *Pythium* o*ligandrum* and *P. ultimum* – light microscope observation of their interface in cucumber root. Nippon Kingakukia Kaiho1996; **37**: 1-7.
22. Le Floch, G., Benhamou, N., Salerno, M.I., Tirilly, Y., Rey, P. Early events in the atypical interaction between tomato root and the biocontrol fungus, *Pythium oligandrum*. *Plant Physiol Biochem* 2005; **43**, 1–11.
23. Nzungize, J., Gepts, P., Buruchara, R., Buah, S., Ragama, P., Busogoro, J. P. and Baudoin J. P. Pathogenic and molecular characterization of *Pythium* species inducing root rot symptoms of common bean in Rwanda. African *J Microbio Res* 2011; **5**(10): 1169-1181.
24. Handelsman, J. and Stabb, E. V. Biocontrol of soilborne plant pathogens. *Plant Cell* 1996; **8**: 1855-1869.