Pak. J. Bot., 57(1): 359-371, 2025. DOI: http://dx.doi.org/10.30848/PJB2025-1(33)

BIOLOGICAL CONTROL OF CUCUMBER (*CUCUMUS SATIVUS* L.) AND RIDGE GOURD (*LUFFA DETANGULA* L. (ROXB.)) DAMPING-OFF BY FUNGAL AND BACTERIAL ANTAGONIST

GHULAM SARWAR SHAH[1], OWAIS IQBAL[2,4*], NASIR AHMED RAJPUT[3], MUHAMMAD ALI KHANZADA[1], JAMAL UDDIN HAJANO[2] AND ABDUL MUBEEN LODHI[1]

[1]Department of Plant Protection, Sindh Agriculture University Tandojam, Pakistan-70060
[2]Department of Plant Pathology, Sindh Agriculture University Tandojam, Pakistan-70060
[3] Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan-38000
[4]Key Laboratory of Agro-Biodiversity and Pest Management of Education Ministry of China, Yunnan Agricultural University, Kunming, China-650000
**Corresponding author's email: owais.iqbal.918@gmail.com*

Abstract

Chemical fungicides are easily accessible in the market to manage damping-off diseases; however, their negative impacts on crop physiology, including photosynthesis, environmental factors, and human health are worth mentioning. Recently, biological control received considerable attention as an alternative to the use of synthetic agrochemicals. In the present study, we evaluate nine *Bacillus* strains, two species of *Trichoderma* (*Trichoderma harzianum* and *Trichoderma polysporum*), and one *Paecilomyces variotii* against two strains (Pa04 and Pa11) of *Pythium aphanidermatum* under laboratory conditions. The results revealed that two *Bacillus* strains, including OI07 (39.3% and 40%) and OI15 (33.7% and 31%) exhibited the highest growth inhibition zones (GIZs) against both fungal isolates compared to other *Bacillus* strains. The dual culture test exhibited that *T. harzianum* was the most efficient fungal biocontrol agent, exhibiting the highest inhibition percentage (34.6% and 33.1%) against both strains, followed by *T. polysporum* (31.7% and 32.2%) and *P. variotii* (24.4% and 26.2%). Both bacterial strains and all fungal biocontrol agents (BCAs) were also employed alone or in combination against two strains (Pa04 and Pa11) of *P. aphanidermatum* in a pot experiment. The results of the pot experiment indicated that the combination of *T. harzianum* and *Bacillus* at ($1x10^5$ cfu per ml) was the most successful in suppressing pre- and post-emergence damping-off and also resulted in maximum seed germination at the same concentration. Furthermore, the plants treated with *T. harzianum* and *Bacillus* strains exhibited the highest plant measurements, such as root or shoot length and weight. This was followed by the *T. polysporum* and *Bacillus* strains treatment. In comparison, the treatment of *P. variotii* and *Bacillus* strains was found to be moderately effective at low, medium, and higher doses. In summary, the combination of *T. harzianum* with *Bacillus* is most powerful approach to reduce the damping-off disease incidence in both vegetables crop.

Key words: Oomycetes, *Pythium aphanidermatum*, Biocontrol agents, *Bacillus*, *Trichoderma*, *Paecilomyces*.

Introduction

Cucumis sativus L. and *Luffa acutangula* L. (Roxb.) commonly known as cucumber and ridge gourd, are members of the Cucurbitaceae family, which consists of 825 species in 118 genera (Ali *et al*., 2019; Ghaffar *et al*., 2021). It is cultivated extensively in greenhouses and open fields across the globe to meet the demand for fresh produce. Growing these vegetables in greenhouses provides a well-regulated environment, enabling the production of high-quality fruits throughout the year and avoiding the use of harmful pesticides (Punja *et al*., 2019). Globally, cucumber is the fourth most important vegetable crop, following onion, tomato, and cabbage (Raza *et al*., 2022). The presence of soil-borne pathogens can cause significant harm to crops, resulting in substantial economic losses across various regions. *Pythium* is a group of fungus-like organisms that belong to the kingdom Straminopila, phylum Oomycota, and comprises approximately 200 species (Ho, 2018). Among the many issues affecting greenhouse or field crops, fruits, vegetables, and ornamental plants globally, *Pythium* spp. is one of the most severe problems, especially in warm temperatures (Molin *et al*., 2021; Rushford *et al*., 2022). *Pythium* species are known to be the causative agents of damping-off and root rot diseases in tomatoes and cucurbits, which results in billions of dollars of loss globally each year (Muthukumar *et al*., 2016; Syed *et al*., 2020; Iqbal *et al*., 2023a).

Moreover, *P. aphanidermatum* is a significant threat to crops in many vegetable-growing countries, causing damping-off during pre and post-harvest stages resulting in substantial losses (Sayed *et al*., 2021; Mahmoud and Abdalla, 2021). Cucumbers and ridge gourd for instance, have been affected by this disease and developed up to 75% yield losses (Al-Mawaali *et al*., 2018; Karunasinghe *et al*., 2020). To control *Pythium* damping-off, several techniques have been employed in hot areas. These include soil mulching using polythene plastic sheets for 3-7 weeks to reduce the *Pythium* population in the soil. Additionally, bio-fumigation has also been found to be effective, albeit is less commonly used (Deadman *et al*., 2006; Ziedan, 2022). Some chemical fungicides are available in the market for controlling damping-off and rot root diseases, including Captan, Hymexazol, and Mefenoxam (Weiland *et al*., 2014). Nevertheless, the utilization of chemical fungicides can pose detrimental effects on human health as well as the environment and crop physiology (Ons *et al*., 2020). The application of bio-control agents, specifically plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF), is emerging as a novel and alternative approach to combating plant diseases, offering an alternative to conventional agro-pesticides (Al-Sadi *et al*., 2015; Abbas *et al*., 2019; Pandit *et al*., 2022). PGPR and PGPF actively participate in intricate biological control mechanisms and can act as effective biocontrol

agents (BCAs) that promote and enhance plant health (Bent, 2006; Iqbal *et al*., 2023b). These BCAs produce different types of antibiotics and promote systemic acquired resistance in the host against many phytopathogens, which reduces the yields by 80-90% (Boro *et al*., 2022; Lahlali *et al*., 2022). *Bacillus*, *Pseudomonas*, *Trichoderma*, and *Paecilomyces* are among the major types of biocontrol agents that are commonly used in this regard (Singh *et al*., 2021). Numerous studies have suggested that these beneficial microorganisms, known as BCAs, have the potential to enhance agricultural productivity and promote sustainability. Several species of *Bacillus*, *Trichoderma*, and *Paecilomyces* are utilized to combat a range of cucumber plant diseases, such as *Fusarium* wilt, powdery mildew, grey mold, damping-off, root and crown rot (Khan *et al*., 2004; Intana *et al*., 2008; Huang *et al*., 2012; Ma *et al*., 2022). These diseases are caused by different aggressive and destructive phytopathogens, *viz*., *P. aphanidermatum* (Ni and Punja, 2019), *Fusarium oxysporum* (Han *et al*., 2019), *Rhizoctonia solani* (Srivastava, 2021), *Botrytis cinerea* (Aoki *et al*., 2020), *Podosphaera xanthii* (Sarhan *et al*., 2020), and *Phytophthora melonis* (Hashemi *et al*., 2019). These fungal and biocontrol agents also enhance the growth promotion and yield of crops by reducing aggressive and destructive pathogens in the field (Younes *et al*., 2023). The aim of the present study was to assess the efficacy of PGPR and PGPF against *P. aphanidermatum* causing damping-off disease in cucumber and ridge gourd, both in laboratory and greenhouse conditions.

Material and Method

Survey and sample collections: Different cucumber and ridge gourd fields in the Hyderabad district of Sindh, Pakistan were selected to gather disease samples. The procedure involved soil collection from the rhizosphere of two infected vegetable plants at a 10 cm depth to isolate the pathogen. A total of 10 different infected fields of both vegetables were visited, and 2 samples from each field were collected in the plastic bags. The soil samples were promptly transported to the laboratory, along with a location tag.

Preparation of corn meal agar (CMA) medium: *Pythium aphanidermatum* was isolated using corn meal agar (CMA) medium. The medium was prepared by dissolving sixty grams (g) of maize seed and 20g of agar agar into 1 liter of distilled water and then autoclaved at 121ºC for 20 minutes. The medium was poured into a sterilized Petri plates and allowed to solidify for isolation.

Preparation on Luria-Bertani broth (LB) medium: The LB medium was prepared with the help of the following chemicals: 10g of Tryptone, 10g of yeast extract, 10g of NaCl, 20g of agar agar, and 1 liter distilled sterilized water. The materials were thoroughly mixed using a sterilized pipette and autoclaved at 121°C for 20 minutes.

Isolation, identification & purification of *Pythium aphanidermatum*: The rhizosphere soil sample collected from infected vegetables plants and brought to the laboratory for the isolation of pathogen. After that, a 5 gram soil from each sample was mixed in the distilled sterilized water (ddH_2O) in a plastic bag. Each bag contained potato plugs which were 12mm in diameter and 60mm in length. The 5 mm potato tubers were surface disinfected and cut into plugs using a sterilized Cork borer before being incubated at 25ºC for 24 hours, earlier to planting (Stanghellini *et al*., 1983). After the incubation period, the potato plugs underwent a rinse using tap water, cut into small pieces, and placed on CMA-containing plates amended with 5 µl of streptomycin and penicillin. Five pieces were placed on each petri plate. In addition, the plates were incubated at (30±2ºC) for 48-72 hours. The newly formed colonies underwent purification through a series of transfers using hyphal tips and were then kept on fresh CMA medium plates (Al-Sa'di *et al*., 2007). The description and taxonomy of *P. aphanidermatum* such as sporangial and oogonial traits were confirmed with previously reported literature (Dick, 1990; Lodhi *et al*., 2013; Ashwathi *et al*., 2017).

Isolates of fungal antagonistic: For the *In vitro* and greenhouse study, we collected three different fungal biocontrols agents including, *Trichoderma harzianum*, *T. polysporum* and *Paecilomyces variotii* from the Plant Disease Diagnosis & Research lab, Department of Plant Protection, Sindh Agriculture University, Tando Jam.

Isolation and identification of *Bacillus* strains: To isolate *Bacillus* strains, a total of five rhizosphere soil samples from healthy cucumber and ridge gourd plants were randomly selected. The topsoil of the rhizosphere was removed, and a 10 cm depth of soil from both healthy vegetable's plants were collected and labeled. The samples were taken immediately to the laboratory, and soaked for 30 minutes. A final 10g soil was measured from each sample using a weighing machine and put into a 250 ml conical flask. Afterward, a volume of 90 ml sterile distilled water with 0.9% NaCl was added into the flask. The sample was properly shaken in the hot water bath at 37ºC with 200 rpm for 30 min. After completely mixed, the samples were divided into five 2ml sterilized tubes. To separate spore-forming bacteria (specifically *Bacillus*), the sample was subjected to heat at 80°C for a period of 10 minutes using a hot water bath. Following this, the sample was successively diluted 7 times. After serial dilution, 0.1 ml of the suspension was spread over a Luria broth (LB) medium that was previously sterilized and allowed to cool. The Petri plates were placed upside-down position in the incubator for overnight. The incubation temperature was set at 37°C, accordance with the procedure described by Mavingui *et al*., (1992). Colonies were visually inspected and selected based on their physical characteristics, with a preference for those exhibiting restricted, waxy growth (1-4 mm in diameter) and irregular edge extension. The selected colonies were streaked on LB media for further multiplication and purification (Kada *et al*., 1980; Errington & Jones, 1987). The *Bacillus* strains were stored for further study at -20°C in centrifuge tubes containing LB medium with 30% glycerol.

Antifungal activity of different fungal biocontrol agents against two strains of *P. aphanidermatum*: In this study, three biocontrol agents, namely *T. harzianum*, *T. polysporum*, and *P. variotii*, were evaluate for their promising biocontrol ability against two strains (Pa04 and Pa11) of *P. aphanidermatum* by dual assay plate method (Whipps, 1987). To perform the test, a (5 mm) agar disc was cut from 7-day-old pure cultures of each fungal biocontrol agent and placed on one side of PDA-containing plates. Also, a (5 mm) agar disc of 4-day-old pure culture of each strain of *P. aphanidermatum* was also placed on the opposite side of the same plate. Each biocontrol agent was tested separately in the same manner. A 5 mm agar disc of *P. aphanidermatum* was plated alone on one side as a control. The plates were incubated at a temperature of (27 $\pm$ 2ºC). The experiment consisted of 3 treatments with 6 replications. A straight line was drawn at the center on the backside of the Petri plates, and the colony growth (mm) was measured using a measuring scale every 24 hours until the petri plate was filled in any treatment. The mycelial growth inhibition percentage was calculated using a formula as given by (Madrigal & Melgarejo, 1995).

$$\text{Percent inhibition} = \frac{\text{Pathogen mycelial growth in check plate - Pathogen mycelial growth in treatment plate}}{\text{Pathogen mycelial growth in check plate}} \times 100$$

Screening of bacterial strains: A total of 31 bacterial strains were screened for their antagonistic activity on PDA medium against two isolates (Pa04 and Pa11) of *P. aphanidermatum* using dual assay plate method on PDA (Fokkema, 1973). The bacterial strains were purified on liquid LB medium. A filter paper was cut of 5 mm size and placed 3 pieces on PDA plate at 90º angles. A 0.3 µl actively grown bacterial suspension was swamped on two filter paper and also 0.3 µl distilled sterilized water (ddH_2O) was flooded on one filter paper for control. Two strains were tested in each plate. The plates were sealed with parafilm tape and incubated at 28±2ºC for two days as overturned position. The plates were examined on a daily basis and the growth inhibition zone (GIZs) of fungus was determined in diameter. Among all tested bacterial strains, only 9 strains showed antagonistic activity against tested pathogen.

Inhibition of *P. aphanidermatum* by bacterial strains: Among 31 bacterial strains, 9 bacterial strains were selected based on screening results, and evaluated for their antagonistic activity against *P. aphanidermatum* by dual assay method (Fokkema, 1973). The strains were grown as per the aforementioned method. Sterilized filter paper was cut into 5mm pieces and three pieces were placed on each plate at a 120° angle. A bacterial suspension and ddH_2O water were poured as described above. The experiment was arranged as the aforementioned screening method. After a period of 24 hours, a 5mm fungal disk obtained from a fresh culture of each strain was placed in the middle of the same bacterial suspension inoculated plate. The Petri plates were covered with Parafilm and placed upside-down position in an incubator at a temperature of 27± 2°C. This setup allowed for the pathogen hyphae to be controlled and grow close to the bacterial isolates. The diameter of the growth inhibition zone caused by the pathogen was measured. The experiment followed a completely randomized design (CRD) with six replications, and the entire experiment was repeated 3 times.

Pot experiment

Inoculum preparation: The *Bacillus* strains were cultured in LB broth tubes at 37 °C under a shaking bath at 200 rpm. Once the strains had developed, a 1ml suspension was mixed in 9ml of distilled sterilized water (ddH_2O) and the concentration was adjusted to colony forming units (1 x 10^3, 10^4, and 10^5 cfu/ml) (Szczech and Shoda, 2004). In the case of the *P. aphanidermatum* strains and PGPF suspension, a 4-day-old culture of the pathogen and 7-days old culture of PGPF were mixed with 10ml of distilled sterilized water (ddH_2O) and rubbed with an image brush to obtain a conidial solution. After that, the suspension was collected in a sterilized beaker and the inoculum density was adjusted to colony forming units (1 x 10^3, 10^4, and 10^5 cfu/ml) by adding water and using a hemocytometer (Yuan & Crawford, 1995).

Growth promotion activity of cucumber and ridge gourd plants under greenhouse conditions: Two *Bacillus* strains, which were responsible for the highest growth inhibition zone *In vitro*, and three PGPF were used in a pot experiment. A 70% ethanol was utilized for the surface sterilization of thermopole pots. Seeds of the cucumber variety (HCU-1170) and ridge gourd variety (LF-03) were raised in 8 cm diameter sterilized thermopole pots, containing 300 g mixture of sterilized peat moss and soil at a standard ratio of 3:1, until the seedlings had reached the true two-leaf stage. Next, the seedlings were transplanted to earthen pots comprising 4 kg of a mixture of soil at a standard ratio of 3:1. Before transferring the seedling in pots, conidial suspension of *P. aphanidermatum* strains (Pa04 and Pa11) were also added in the soil composition for check the pre-emergence damping-off. The experiment followed a Randomized Complete Block Design (RCBD) with 8 different treatments and 6 replications (Table 1). The pathogen suspension was inoculated again after two days of transplanting, and the application of PGPR and PGPF were performed simultaneously. After 9 days of sowing, the pre-emergence damping-off was recorded with the help of the following formula (Reddy *et al*., 1994):

$$\text{Pre-emergence \%} = \frac{\text{Number of sowing seeds - Number of emerged seedlings}}{100 \times 100} - 100 \text{ Germination rate \%}$$

Table 1. Details of treatments.

Sr#	Treatment details
T1	Pathogen + *Trichoderma harzianum* + *Bacillus* strains (10^3, 10^4 and 10^5 cfu/ml)
T2	Pathogen + *Trichoderma polysporum* + *Bacillus* strains (10^3, 10^4 and 10^5 cfu/ml)
T3	Pathogen + *Paecilomyces variotii* + *Bacillus* strains (10^3, 10^4 and 10^5 cfu/ml)
T4	Pathogen + *Trichoderma harzianum* (10^3, 10^4 and 10^5 cfu/ml)
T5	Pathogen + *Trichoderma polysporum* (10^3, 10^4 and 10^5 cfu/ml)
T6	Pathogen + *Paecilomyces variotii* (10^3, 10^4 and 10^5 cfu/ml)
T7	Pathogen + *Bacillus strains* (10^3, 10^4 and 10^5 cfu/ml)
T8	Control

Post-emergence damping-off was calculated by following formula (Reddy *et al*., 1994):

$$\text{Post-emergence \%} = \frac{\text{Number of infected seedlings}}{\text{Numbers of emerged seedlings}} \times 100$$

The germination percentage was recorded by following formula (Orchard, 1977):

$$\text{Seed germination} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sowing}} \times 100$$

The data of plant shoot, root length and weight were recorded separately.

DNA extraction: For the extraction of DNA, a CTAB method developed by Doyle and Doyle (1987) was utilized with some adjustments from the biocontrol agent culture and other microorganisms. To determine the DNA concentration and purity, the Li *et al*. (2006) method was used for performing a Nano-drop. Furthermore, a 1% agarose gel was utilized to analyse the DNA concentration and purity by running the samples for 30 minutes.

PCR based detection: In the PCR based detection, two primers, ITS1 and ITS4 were evaluated to amplify a specific sequence region (White *et al*., 1990). The PCR reactions were conducted with a fixed amount of reagents, including 1.5 µl of each primer, 7 µl of master mix, and 0.5 µl of Platinum Taq-polymerase, in a total volume of 12.5 µl of reaction. An automated thermal cycler was employed to conduct the PCR amplification with a protocol consisting of an initial denaturation at 96°C for 9 min, followed by 40 cycles of denaturation at 96 °C for 30 seconds and annealing at 53°C for 1 minutes. The final extension was carried out at 72°C for 7 min. The amplified products were detected on a 1.5% agarose gel containing ethidium bromide (Li *et al*., 2006).

Characterization of the strains: The manufacturer's recommendations (Bio Product) were followed in sequencing the PCR-amplified products that were positive. A bioEdit v7.2 version software was used for the analysis to attain 16S rDNA sequences (Hall, 1999), and (NCBI) blast tool was utilized for comparison with retrieved ones. After that, the sequence was uploaded to MEGA-7 software and aligned with the help of ClustalW program (Kumar *et al*., 2016). A phylogenetic tree was constructed with the help of the neighbour joining method with 1000 bootstrap value and Tamura 3-parameter model (Kong *et al*., 2000).

Table 2. *In vitro* broad range antagonistic activity of *Bacillus* strains on mycelial growth of two strains of *P. aphanidermatum*.

No#	Isolates	Antagonistic activity against Pa04 and Pa11
01.	OI01	---
02.	OI02	---
03.	OI03	+
04.	OI04	---
05.	OI05	---
06.	OI06	---
07.	OI07	++++
08.	OI08	---
09.	OI09	---
10.	OI10	---
11.	OI11	+
12.	OI12	---
13.	OI13	---
14.	OI14	---
15.	OI15	++++
16.	OI16	---
17.	OI17	---
18.	OI18	+
19.	OI19	---
20.	OI20	---
21.	OI21	++
22.	OI22	---
23.	OI23	---
24.	OI24	---
25.	OI25	+++
26.	OI26	---
27.	OI27	---
28.	OI28	++
29.	OI29	---
30.	OI30	---
31.	OI31	+++

Bacterial strains against *Pythium aphanidermatum* were determined using plate culture method on PDA medium under laboratory conditions. The width of growth inhibition zone GIZ is as follows. Three --- represents 0 mm growth inhibition, one + represents 1-10 mm, two ++ represents 11-20 mm, three +++ represents 21-30 mm and four ++++ represents 31-40 mm growth inhibition.

Results

Morphological characters: The pathogen grown in isolation displayed a copious amount of white aerial mycelium lacking a distinct pattern. The mycelium was non-septate and formed filamentous inflated sporangia. The pathogen also formed globose oogonia with a smooth surface and a diameter of 23-25 µm and its antheridia were intercalary in position and had a bell shape.

Molecular characterization: In phylogenetic analysis, we included 14 closest sequences of *P. aphanidermatum* revealed in the BLAST search along with representative sequences of other members of clade A, namely *Pythium deliense, P. monospermum, P. adhaerens*, and *P. porphyrae* (Levesque and de Cock, 2004). In the ITS sequence analysis, our two sequences of *P. aphanidermatum*

(OQ6442511 cucumber) and (OR4379861 ridge gourd) were found to be 99.8% identical to the rest of the GenBank sequences of *P. aphanidermatum* we used, except for MN8186551 and MK3112531, which showed 99.3% and 99.5% sequence homology to our isolate, respectively. Moreover, our isolates showed only 98.7% and 98.8% sequence similarity with *P. deliense* MH0178561 and OP5975201, respectively. The other members of clade A, such as *P. monospermum* ON3129721, *P. adhaerens* AY5986192, and *P. porphyrae* MF9781641, were distantly related to our isolates, showing only 93.6%, 90.6%, and 91.1% sequence homology (Fig. 1).

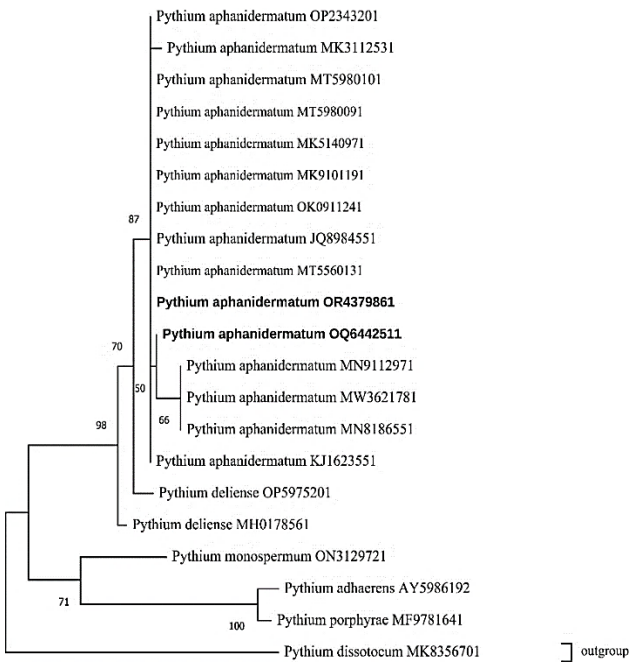

Fig. 1. Phylogenetic tree yielded through Maximum Likelihood method and Tamura 3-parameter model by using MEGA X. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the 21 taxa analyzed. The tree with the highest log likelihood (-1545.81) is shown. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. *Pythium dissotocum* a closely related species used as an out group. The entry with arrow yielded during the present study.

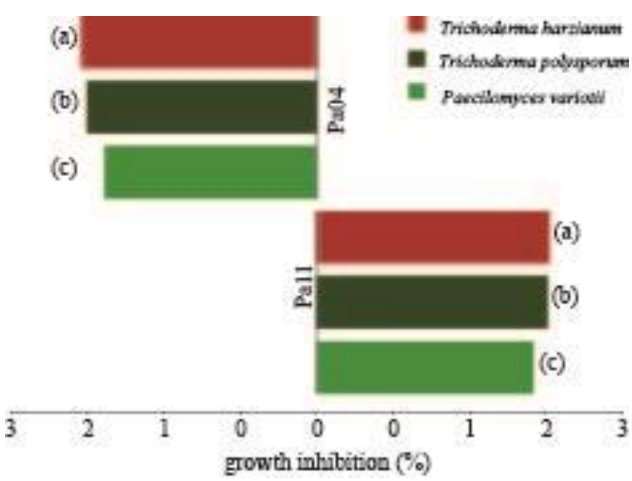

Fig. 2. *In vitro* effect of different biocontrol agents on mycelium growth of two strains of *P. aphanidermatum*. The different letters (above bars) indicate significant difference at the p<0.05 level.

Antifungal activity of different PGPF on mycelium growth of *P. aphanidermatum* strains *In vitro*: Three different fungal bio-control agents, namely *T. harzianum, T. polysporum* and *P. variotii* were evaluated *In vitro* against both strains using the dual assay plate method. The tested fungal bio-control agents greatly inhibited the mycelial growth of both strains of *P. aphanidermatum*. Among all the tested bio-control agents, both *Trichoderma* species showed highly antifungal activity on the mycelial growth of both strains as compared to *P. variotii*. The results revealed that the maximum inhibition percentage (34.6% and 33.1%) was recorded with *T. harzianum*, followed by *T. polysporum* (31.7% and 32.2%) and *P. variotii* (24.4% and 26.2%) against both strains (Pa04 and Pa11) in the dual culture test (Fig. 2).

***In vitro* screening of 31 bacterial strains on mycelium growth of *P. aphanidermatum*:** In the present study 31 bacterial strains were screened for their potential antagonistic activity against both strains of *P. aphanidermatum* on a potato dextrose agar (PDA) medium using the dual culture technique (Nakkeeran *et al*., 2006). Among these, only 9 strains (OI03, OI07, OI11, OI15, OI18, OI21, OI25, OI28, and OI31) were showed antagonistic activity against tested pathogen. The other 22 strains did not show any antifungal or antagonistic activity against *P. aphanidermatum* strains (Table. 2).

***In vitro* broad range antagonistic activity of *Bacillus* strains:** Based on screening results, out of 31 strains, only 9 *Bacillus* strains were evaluated for their broad range antagonistic activity on mycelial growth of (Pa04 and Pa11) under *In vitro* conditions. Among them, two strains namely, OI07 and OI15 showed the highest growth inhibition zones (GIZs) compared to the other strains. The GIZs produced by OI07 (39.3% and 40%) and OI15 (33.7% and 31%) were particularly noteworthy, demonstrating their strong inhibitory effects on the tested pathogen. In addition, OI31 (26.30% and 24.31%), OI25 (23.10% and 19%), OI21 (19.70% and 19.99%) and OI28 (16.50% and 20.22%) also exhibited significant GIZs of Pa04 and Pa11, respectively (Fig. 3). The other three *Bacillus* strains (OI03, OI11 and OI18) were not found more effective against tested pathogen.

Effect of antagonistic fungi and bacteria against *P. aphanidermatum* on growth parameters of cucumber and ridge gourd plants in soil infestation method

Pot experiment: In this study, all biocontrol agents and two bacterial strains which showed highly antagonistic activity against the damping-off pathogen *P. aphanidermatum*. A pot experiment was conducted using artificially-infested soil with disease incidence under greenhouse conditions (El-Mohamedy & El-Mougy, 2009). The results showed that, the application of all tested PGPF and PGPR, either alone or in combination, significantly reduced both pre- and post-emergence damping-off disease and enhanced cucumber and ridge gourd seed germination compared to the control (Fig. 4a & b). The combination of *T. harzianum* + *Bacillus* strains was

found to be most effective, producing maximum seed germination at a higher concentration of (1 x 10^5 cfu/ml), followed by *T. polysporum* + *Bacillus* strains at the same concentration in the soil infestation method. When applied alone at a higher concentration of (1 x 10^5 cfu/ml), *T. harzianum* and *Bacillus* strains showed the maximum suppression of *Pythium* incidence and enhanced seed germination, followed by *T. polysporum*. However, the application of *P. variotii* alone or in combination with *Bacillus* strains was the least effective in enhancing seed germination at all used concentrations (Fig. 5).

Root and shoot length of cucumber: The application of antagonistic biocontrol agents not only reduced the infection of the damping-off pathogen but also stimulated plant growth. Control plants showed the minimum root length (12.23 cm) and shoot length (22.21 cm). Among the treated plants, the highest root length (27.33 cm, 27.02 cm, 26.59 cm) was observed in those treated with *T. harzianum* + *Bacillus* strains, followed by *T. polysporum* + *Bacillus* strains (27.01 cm, 26.01 cm, 21.11 cm) and *P. variotii* + *Bacillus* strains (24.77 cm, 24.68 cm, 19 cm) at high, medium and lower doses (Fig. 6a). Similarly, the highest shoot length (38.16 cm, 37.78 cm, 35.34 cm) was recorded in plants treated with *T. harzianum* + *Bacillus* strains, followed by *T. polysporum* + *Bacillus* strains (37.99 cm, 37.31 cm, 32.44 cm) and *P. variotii* + *Bacillus* strains (37.93 cm, 37.22 cm, 30.37 cm) at high, medium and lower doses, respectively (Fig. 6b).

Root and shoot weight of cucumber: The application of bio-control agents successfully suppressed pathogen activity in the treated plant. The minimum root weight (2.65 g) and shoot weight (7.66 g) were recorded in inoculated-untreated plants. The maximum root weight (7.77 g, 7.21 g, 7.02 g) of plants was found in inoculated-treated plants with *T. harzianum* + *Bacillus* strains treatment, followed by *T. polysporum* + *Bacillus* strains (7.23 g, 6.43 g, 6.34 g), and *P. variotii* + *Bacillus* strains (6.55 g, 6.32 g, 6.21 g) at high, medium and lower concentration of cfu (Fig. 7a). The *T. harzianum* + *Bacillus* strains treated plants gave significantly the highest shoot weight (16.22 g, 16.17 g, 15.55 g) followed by *T. polysporum* + *Bacillus* strains (16.01 g, 15.22 g, 15.02 g), and *P. variotii* + *Bacillus* strains (15.79 g, 14.85 g, 13.97 g) at all three doses (Fig. 7b).

Root and shoot length of ridge gourd: The utilization of opposing biocontrol agents not only decreased the occurrence of damping-off pathogen infection that was intentionally introduced but also enhanced the growth of the plants. The minimum root length was observed in control plants, which gave root length (17 cm) and shoot length (16.71 cm). Whereas, the maximum root length (30.33 cm, 29.08 cm, 27.77cm) was observed in those treated with *T. harzianum* + *Bacillus* strains, followed by *T. polysporum* + *Bacillus* strains (28.91cm, 27.79 cm, 25.41 cm) and *P. variotii* + *Bacillus* strains (27 cm, 26.13 cm, 24.61 cm) at maximum, medium and lower doses (Fig. 8a). Similarly, the highest shoot length (27.29 cm, 25.13 cm, 21.79 cm) was recorded in treated plants with *T. harzianum* + *Bacillus* strains, followed by *T. polysporum* + *Bacillus* strains (26.13 cm, 23.39 cm, 20.41 cm) and *P. variotii* + *Bacillus* strains (23.22 cm, 22.91 cm, 20 cm) at high, medium, and lower doses (Fig. 8b).

Root and shoot weight of ridge gourd: The implementation of biocontrol agents effectively inhibited the activity of pathogens in the treated plant. The lowest root weight (2.9 g) and shoot weight (0.31 g) were recorded in inoculated-untreated plants. The highest root weight (0.8 g, 0.72 g, 0.63 g) of plants were recorded in inoculated-treated plants with *T. harzianum* + *Bacillus* strains treatment, followed by *T. polysporum* + *Bacillus* strains (0.71 g, 0.65 g, 0.58 g), and *P. variotii* + *Bacillus* strains (0.63 g, 0.57 g, 0.51 g) at maximum, medium, and lowest concentration (Fig. 9a). The *T. harzianum* + *Bacillus* strains treated plants produced significantly the maximum shoot weight (5.1 g, 4.7 g, 4.5 g) followed by *T. polysporum* + *Bacillus* strains (4.8 g, 4.4 g, 4.2 g), and *P. variotii* + *Bacillus* strains (4.3 g, 4.1 g, 3.8 g) at all three doses (Fig. 9b).

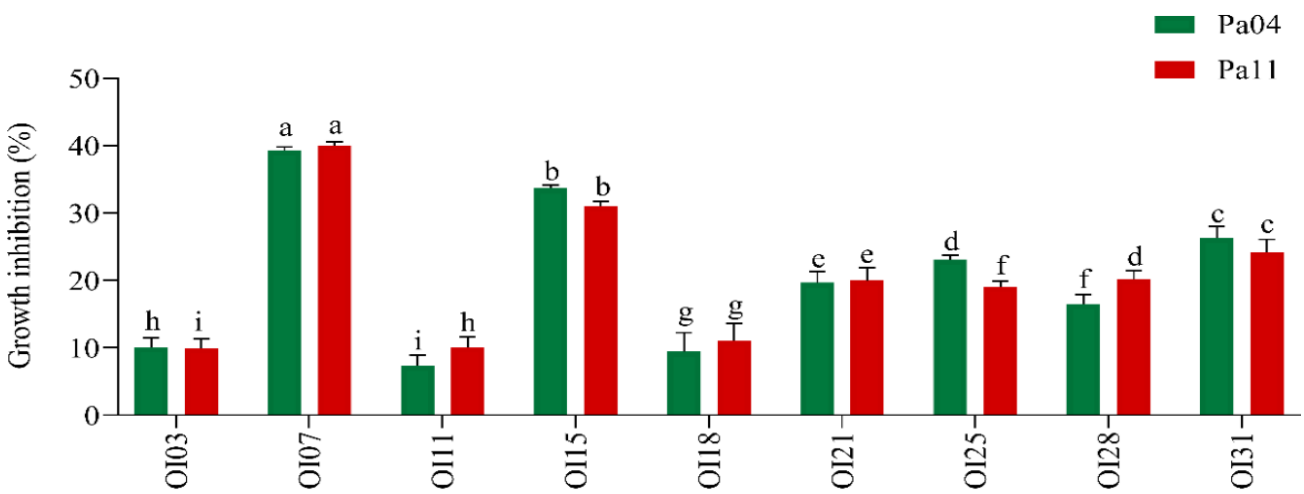

Fig. 3. Antagonistic activity of *Bacillus* strains against two strains of *P. aphanidermatum*. Error bar are representing the ± SD of six replications and different letters (above bars) indicate significant difference at the $p<0.05$ level.

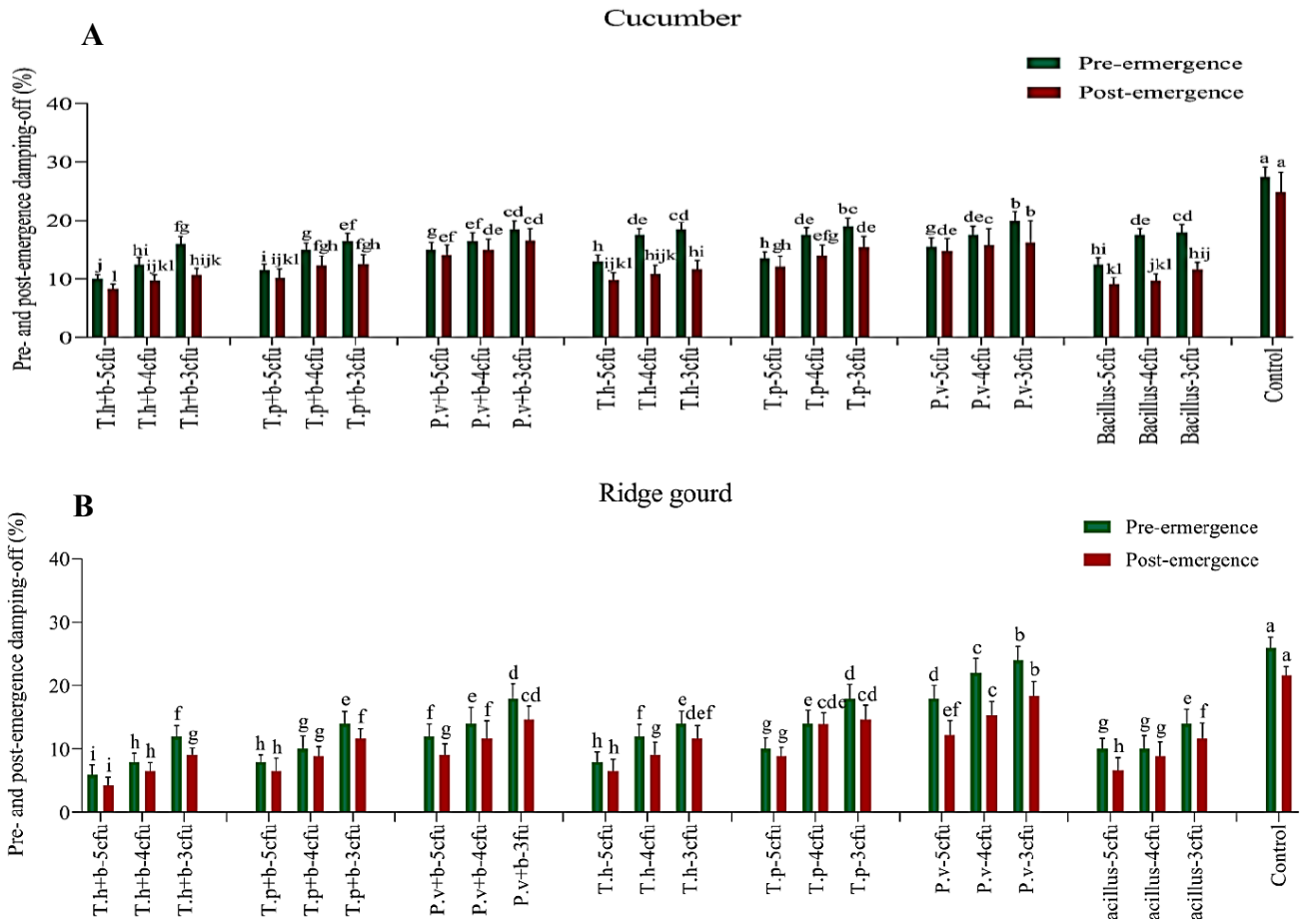

Fig. 4. Pre- and post-emergence damping-off (%) of cucumber and ridge gourd by soil infestation method. Error bar represent the ± standard deviation of six replications and different letters (above bars) indicate significant difference at the p<0.05 level.

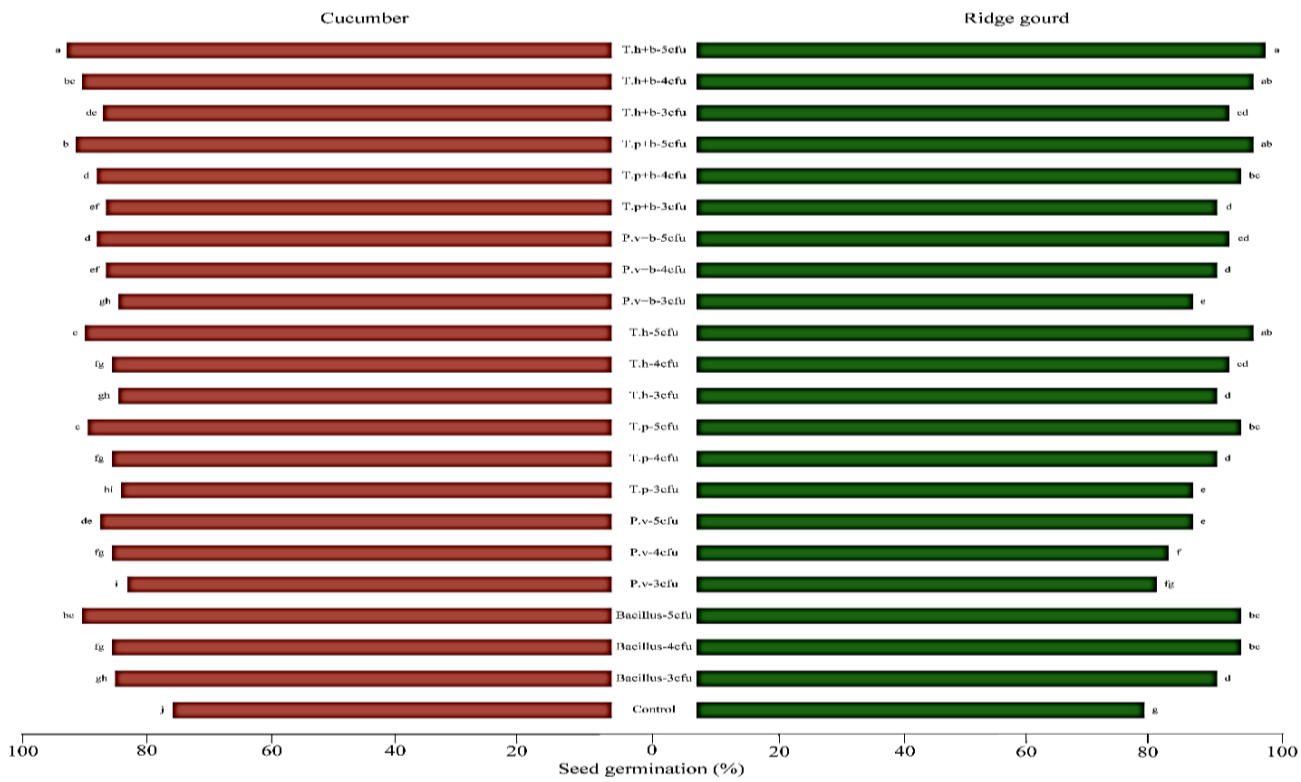

Fig. 5. Effect of fungal and bacterial antagonist on cucumber and ridge gourd seed germination. The red bars show cucumber seed germination %, while green bars represent ridge gourd seed germination %. The different letters (above bars) indicate significant difference at the p<0.05 level.

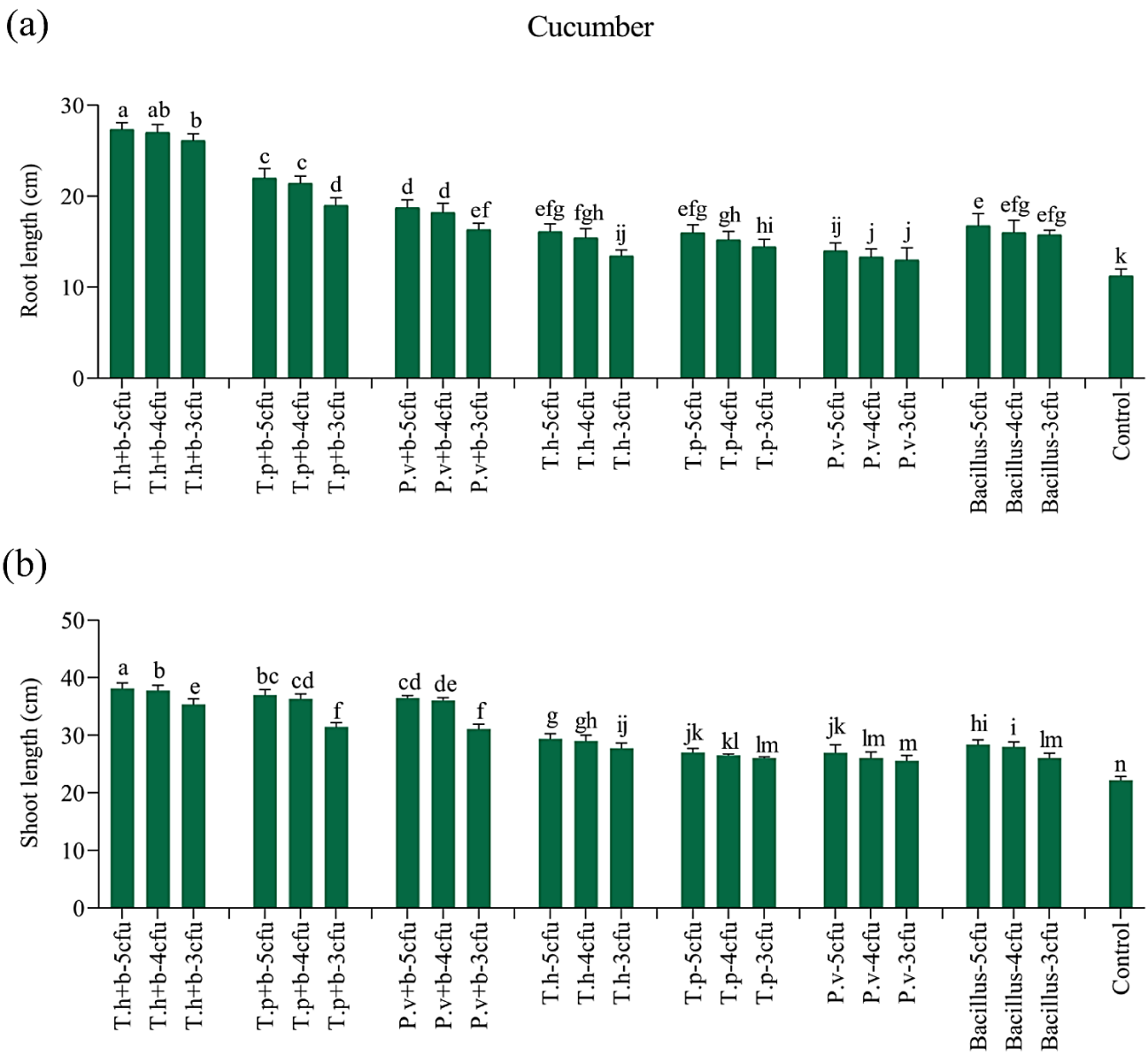

Fig. 6. Effect of fungal and bacterial antagonist on root and shoot length of cucumber. Error bar represents the mean ± SD of six replications. The different letters (above bars) indicate significant difference at the $p<0.05$ level.

Discussion

Soil-borne diseases pose numerous challenges to vegetable crop production worldwide. Among these diseases, damping-off caused by *P. aphanidermatum* is a significant threat to cucumber and ridge gourd production in both greenhouse and field conditions (Halo *et al*., 2018; Al-Mawaali *et al*., 2018). In the present study, two strains of *P. aphanidermatum* were isolated from rhizosphere soil of damping-off infected cucumber and ridge gourd vegetables. The isolated pathogen showed a copious amount of white aerial mycelium was non-septate. The pathogen also formed filamentous inflated sporangia and globose oogonia with a diameter of 23-25 µm. The morphological character was closely related to *P. aphanidermatum,* which was reported previously (Lodhi *et al*., 2013). Various approaches have been employed to prevent and treat this disease, such as using fungicide-treated seeds, crop rotation, resistant varieties, cultural techniques, biological control, and soil solarization (Van Bruggen *et al*., 2016). Furthermore, the use of plant growth promoting fungi (PGPF) and plant growth promoting rhizobacteria (PGPR) considered as BCAs is a relatively new technique for control this disease and managing the crop (Iqbal *et al*., 2024). BCAs play a critical role in controlling plant diseases and enhancing plant growth (Bent, 2006). Several researchers have reported that these BCAs are highly effective against various phytopathogens, including *P. aphanidermatum* (Ni & Punja, 2019), *Fusarium oxysporum* (Han *et al*., 2019), *Rhizoctonia solani* (Srivastava, 2021), *Botrytis cinerea* (Aoki *et al*., 2020), *Podosphaera xanthii* (Sarhan *et al*., 2020), and *Phytophthora melonis* (Hashemi *et al*., 2019). A previous study also demonstrated that *Trichoderma* species and *Bacillus* species produced antimicrobial metabolites, which are highly effective against a large number of fungal pathogens, including *P. aphanidermatum*, *F. oxysporum*, *Curvularia lunata*, *R. solani*, *Colletotrichum*

gloeosporiodes, and *Bipolaris sorokiniana* (Jeyaseelan *et al*., 2012; Dania *et al*., 2016). In the present study, we found interesting and promising results for controlling the damping-off disease pathogen *In vitro* or in greenhouse. Our results indicate that the application of biological control agents in combination is the most effective technique for controlling *P. aphanidermatum*. Overall, these findings suggest that the combination of *T. harzianum* and *Bacillus* strains could be a promising strategy for controlling damping-off disease and improving seed germination in cucumber crops. Similarly, Abd-El-Khair *et al*., (2018) demonstrated that the combined application of *T. harzianum* and *Bacillus* successfully decreased the incidence of *Pythium* damping-off disease in the field. However, the overall results of our study indicate that the *Bacillus* strain OI07 showed the highest antifungal antagonistic activity, followed by *T. harzianum*, and produced the maximum GIZs against *P. aphanidermatum In vitro*. Our study also found that the application of biocontrol agents (BCAs) in combination significantly reduced pre- and post-emergence damping-off and controlled the infection of *P. aphanidermatum* in cucumber and ridge gourd crops under pot experiments. These results agree with those recorded by Chen *et al*., (2015). However, current research indicates that when two biological control agents (BCAs), each of which has a separate basic mechanism, are used together enhance the growth promotion and biological activity of plants. *Trichoderma* species kill phytopathogens through mycoparasitism in plants. *Trichoderma* and *Bacillus* species produce many antibiotics and secrete compounds, which play a vital role against various pathogens and increase the growth and development of plants (Silva *et al*., 2019). Kamala & Indira (2011) observed different antibiotic mechanisms in *T. harzianum*, such as b-1, 3-glucanase activity, protease, cellulase, and chitinase, as well as volatile and non-volatile compounds. They obtained a 76.67 mm mycelial growth inhibition by dual assay plate. Ni & Punja, (2019) concluded that *Bacillus* spp.. and *Trichoderma* spp. produced a large number of bioactive antibiotic compounds against many pathogens and could control pre- and post-emergence damping-off disease in vegetable crops.

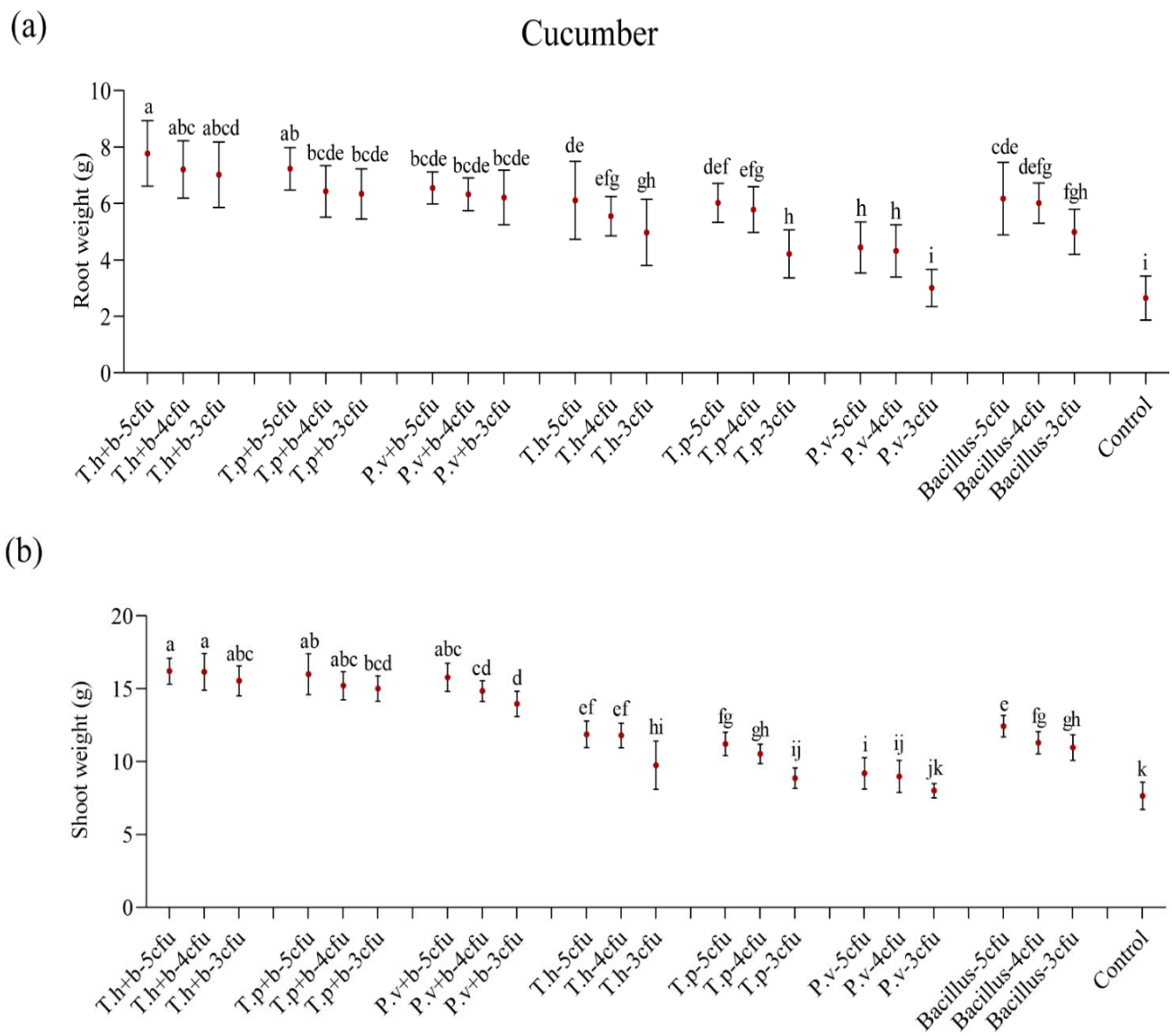

Fig. 7. Effect of fungal and bacterial antagonist on root and shoot weight of cucumber. Each dot represents the range of weight and error bars are showing the mean ± SD of six replications. The different letters (above bars) indicate significant difference at the $p<0.05$ level.

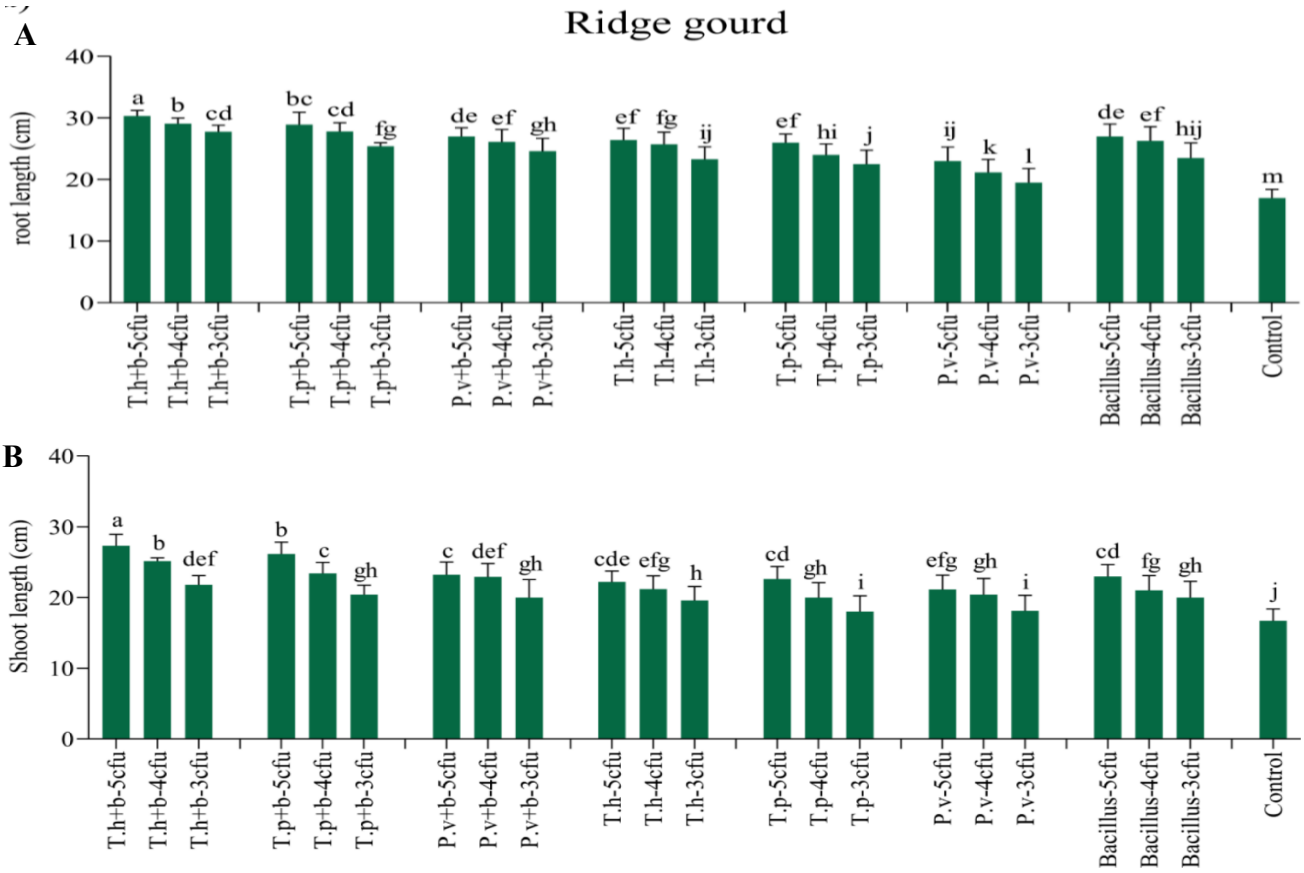

Fig. 8. Effect of fungal and bacterial antagonist on root and shoot length of ridge gourd. Error bar representing the mean ± SD of six replications and different letters (above bars) indicate significant difference at the $p<0.05$ level.

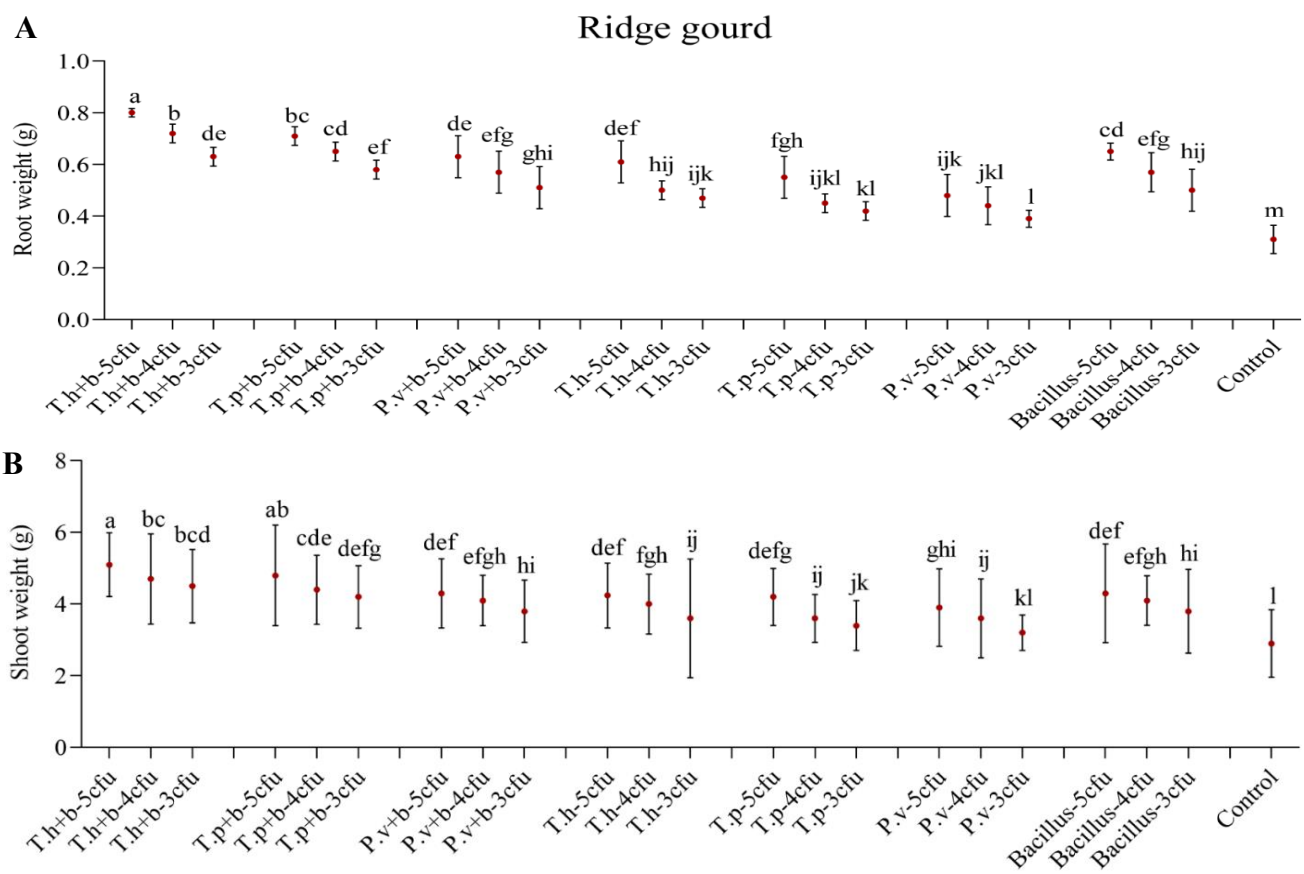

Fig. 9. Effect of fungal and bacterial antagonist on root and shoot weight of ridge gourd. Each dot represents the weight range, while error bars depict the standard deviation of six replications. The different letters (above bars) indicate significant difference at the $p<0.05$ level.

Conclusion

Our result suggested that, the treatment of fungal and bacterial antagonists alone or in combination was the most powerful tool for controlling *Pythium* damping-off disease in cucumber and ridge gourd plants. Furthermore, the application of these biocontrol agents reduces the pre- and post-emergence damping-off as well as enhances the seed germination.

Acknowledgements

The authors wish to express their gratitude to the Plant Disease Diagnosis & Research Lab, Department of Plant Protection, Sindh Agriculture University Tandojam, Pakistan, for providing the bioagents in this study. The authors also wish to express their deep gratitude to Dr. Muhammad Atiq, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan for his critical review of the manuscript.

References

Abbas, A., S.U. Khan, W.U. Khan, T.A. Saleh, M.H.U. Khan, S. Ullah, A. Ali and M. Ikram. 2019. Antagonist effects of strains of *Bacillus* spp. against *Rhizoctonia solani* for their protection against several plant diseases: Alternatives to chemical pesticides. *C.R. Biol.*, 342: 124-135.

Abd-El-Khair, H., K.H. Haggag and H.I. Seif El-Nasr. 2018. Field application of *Trichoderma harzianum* and *Bacillus subtilis* combined with *Rhizobium* for controlling *Fusarium* root rot in faba bean in organic farming. *Mid. East. J. Appl. Sci.*, 8: 865-873.

Ali, Q., M.R. Yaseen and M.T.I. Khan. 2019. Energy budgeting and greenhouse gas emission in cucumber under tunnel farming in Punjab, Pakistan. *Sci. Hort.,* 250: 168-173.

Al-Mawaali, Q., A.M. Al-Sadi, F.A. Al-Said, M.S. Rahman, I. Al-Zakwani, A. Ali, M. Al-Yahyai and M. Deadman. 2018. Effect of grafting on resistance to vine decline disease, yield and fruit quality in muskmelon cv. Sawadi. *J. Agric. Mar. Sci.*, 23: 2-10.

Al-Sadi, A.M., R.S. Al-Masoodi, M. Al-Ismaili and I.H. Al-Mahmooli. 2015. Population structure and development of resistance to hymexazol among *Fusarium solani* populations from date palm, citrus and cucumber. *J. Phytopathol.,* 163: 947-955.

Al-Sa'di, A. M., A. Drenth, M.L. Deadman, A.W.A.M. De Cock and E.A.B. Aitken. 2007. Molecular characterization and pathogenicity of *Pythium* species associated with damping-off in greenhouse cucumber (*Cucumis sativus*) in Oman. *Plant Pathol.*, 56: 140-149.

Aoki, Y., S. Haga and S. Suzuki. 2020. Direct antagonistic activity of chitinase produced by *Trichoderm*a sp. SANA20 as biological control agent for grey mould caused by *Botrytis cinerea*. *Cogent. Biol.*, 6: 1747903.

Ashwathi, S., C. Ushamalini, S. Parthasarathy and S. Nakkeeran. 2017. Morphological, pathogenic and molecular characterisation of *Pythium aphanidermatum*: A causal pathogen of coriander damping-off in India. *The Pharma Innovation.*, 6: 44-48.

Bent, E. 2006. Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In: (Eds.): Tuzun, S. and E. Bent. *Multigenic and Induced Systemic Resistance in Plants*. Springer, Boston, MA. pp. 225-258.

Boro, M., S. Sannyasi, D. Chettri and A.K. Verma. 2022. Microorganisms in biological control strategies to manage microbial plant pathogens: A review. *Arch. Microbiol.*, 204: 666**.** https://doi.org/10.1007/s00203-022-03279-w.

Chen, J.T., M.J. Lin and J.W. Huang. 2015. Efficacy of spent blewit mushroom compost and *Bacillus aryabhattai* combination on control of *Pythium* damping-off in cucumber. *J. Agric. Sci.*, 153: 1257-1266.

Dania, V.O., O.O. Fadina, M. Ayodele and P.L. Kumar. 2016. Evaluation of isolates of *Trichoderma*, *Pseudomonas* and *Bacillus* species as treatment for the control of post-harvest fungal rot disease of yam (*Dioscorea* spp.). *Arch. Phytopathol. Pflanzenschutz.*, 49: 456-470.

Deadman, M., H.A. Hasani and A.A. Sa'di. 2006. Solarization and biofumigation reduce *Pythium aphanidermatum* induced damping-off and enhance vegetative growth of greenhouse cucumber in Oman. *J. Plant Pathol.*, 88: 335-337.

Dick, M.W. 1990. Keys to *Pythium*. University of Reading Press. *Reading*. UK: Pp. 64.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, 19: 11-15

El-Mohamedy, R.S. and N.S. El-Mougy. 2009. Occurrence of *Pythium* rot of Chinese cabbage in Egypt and its biocontrol measures. *J. Plant Prot. Res.*, 49: 309-318.

Errington, J. and D. Jones. 1987. Cloning in *Bacillus subtilis* by transfection with bacteriophage vector ϕ105J27: isolation and preliminary characterization of transducing phages for 23 sporulation loci. *Microbiol.*, 133: 493-502.

Fokkema, N.J. 1973. The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. *Physiol. Plant Pathol.,* 3: 195-205.

Ghaffar, A., A.Q. Zehri, B.K. Solangi, Z.A. Mastoi, A. Wahab, J.K. Tareen, A. Nabi, M.K. Gola and M.A. Alizai. 2021. Effect of nitrogen application methods on the growth and yield of ridge gourd (*luffa acutangulla*). *Pak. J. Biotechnol.*, 18: 23-29.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic Acids Symposium Series*. 41(41): pp. 95-98.

Halo, B.A., R.A. Al-Yahyai and A.M. Al-Sadi. 2018. *Aspergillus terreus* inhibits growth and induces morphological abnormalities in *Pythium aphanidermatum* and suppresses *Pythium*-induced damping-off of cucumber. *Front. Microbiol.,* 9: 95.

Han, Y., J. Zhao, B. Zhang, Q. Shen, Q. Shang and P. Li. 2019. Effect of a novel antifungal peptide P852 on cell morphology and membrane permeability of *Fusarium oxysporum. Biochim. Biophys. Acta. Biomembr.*, 1861: 532-539.

Hashemi, L., A.R. Golparvar, M. Nasr Esfahani and M. Golabadi. 2019. Correlation between cucumber genotype and resistance to damping-off disease caused by *Phytophthora melonis*. *Biotechnol. Biotechnol. Equip.*, 33: 1494-1504.

Ho, H.H. 2018. The taxonomy and biology of *Phytophthora* and *Pythium*. *J. Bacteriol Mycol.*, 6: 40-45.

Huang, X., N. Zhang, X. Yong, X. Yang and Q. Shen. 2012. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. *Microbiol. Res.*, 167: 135-143.

Intana, W., P. Yenjit, T. Suwanno, S. Sattasakulchai, M. Suwanno and C. Chamswarng. 2008. Efficacy of antifungal metabolites of *Bacillus* spp. for controlling tomato damping-off caused by *Pythium aphanidermatum*. *Walailak. J. Sci. Tech.*, 5: 29-38.

Iqbal, O., C. Li and A.M. Lodhi. 2023a. Antagonistic *Pseudomonas*: alternative to chemical fungicides for the management of phytopathogens. In: (Eds.): Abd-Elsalam,

K.A. and M.A. Alghuthaymi. *Biofungicides: Eco-Safety and Future Trends: Types and Applications*, Volume 1. CRC Press, Taylor & Francis Group, USA. pp. 216-246. https://doi.org/10.1201/9781003287575.

Iqbal, O., C. Li, N.A. Rajput and A.M. Lodhi. 2023b. Management of phytopathogens by antagonistic *Bacillus* spp in tomato crop. *In*: F. Lops (ed.), *Tomato Cultivation and Consumption - Innovation, Sustainability and Health*. IntechOpen, London, U.K. doi: 10.5772/intechopen.112439.

Iqbal, O., R.N. Syed, N.A. Rajput, Y. Wang, A.M. Lodhi, R. Khan, S.M. Jibril, M. Atiq and C. Li. 2024. Antagonistic activity of two *Bacillus* strains against *Fusarium oxysporum* f. sp. *capsici* (FOC-1) causing *Fusarium* wilt and growth promotion activity of chili plant. *Front. Microbiol.,* 15: 1388439.

Jeyaseelan, E.C., S. Tharmila and K. Niranjan. 2012. Antagonistic activity of *Trichoderma* spp. and *Bacillus* spp. against *Pythium aphanidermatum* isolated from tomato damping off. *Arch. Appl. Sci. Res.*, 4: 1623-1627.

Kada, T., K. Hirano and Y. Shirasu. 1980. Screening of environmental chemical mutagens by the rec-assay system with *Bacillus subtilis*. In: (Eds.): de Serres, F.J. and A. Hollaender. *Chemical Mutagens.* Springer, Boston, MA. pp. 149-173. https://doi.org/10.1007/978-1-4613-3072-1_6

Kamala, T. and S. Indira. 2011. Evaluation of indigenous *Trichoderma* isolates from Manipur as biocontrol agent against *Pythium aphanidermatum* on common beans. *Biotech.*, 1: 217-225.

Karunasinghe, T.G., S.S.N. Maharachchikumbura, R. Velazhahan and A.M. Al-Sadi. 2020. Antagonistic activity of endophytic and rhizosphere fungi isolated from sea Purslane (*Sesuvium portulacastrum*) against *Pythium* damping-off of cucumber. *Plant Dis.*, 104: 2158-2167.

Khan, J., J.J. Ooka, S.A. Miller, L.V. Madden and H.A.J. Hoitink. 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora* crown rot and leaf blight. *Plant Dis.*, 88: 280-286.

Kong, W., X. Deng, Z. Liang and W. Tang. 2000. Cloning and sequencing of the citrus huanglongbing pathogen DNA. *Acta Hydrobiol. Sin*., 30: 71-75.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33: 1870-1874.

Lahlali, R., S. Ezrari, N. Radouane, J. Kenfaoui, Q. Esmaeel, H. El Hamss and E.A. Barka. 2022. Biological control of plant pathogens: A global perspective. *Microorganisms*, 10: 596.

Levesque, C.A. and A.W. De Cock. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycol. Res.*, 108: 1363-1383.

Li, W., J.S. Hartung and L. Levy. 2006. Quantitative real-time PCR for detection and identification of *Candidatus Liberibacter* species associated with citrus huanglongbing. *J. Microbiol. Methods*, 66: 104-115.

Lodhi, A.M., M.A. Khanzada, S. Shahzad, A. Ghaffar and C.A. Lévesque. 2013. Prevalence of *Pythium aphanidermatum* in agro-ecosystem of Sindh province of Pakistan. *Pak. J. Bot*., 45: 635-642.

Ma, T., C. Yang, F. Cai, L. Cui and Y. Wang. 2022. Optimizing fermentation of *Bacillus amyloliquefaciens* 3-5 and determining disease suppression and growth in cucumber (*Cucumis sativus*). *Biol. Control*, 176: 105070.

Madrigal, C. and P. Melgarejo. 1995. Morphological effects of *Epiccocum nigrum* and its antibiotic flavipin on Monilinia laxa. *Can. J. Bot.*, 73: 425-431.

Mahmoud, A.F. and O.A. Abdalla. 2021. Biological control of fungi associated with damping-off and root rot disease of cucumber (*Cucumis sativus* L.). *Arch. Phytopathol.*, 54: 870-885.

Mavingui, P., G. Laguerre, O. Berge and T. Heulin. 1992. Genetic and phenotypic diversity of *Bacillus polymyxa* in soil and in the wheat rhizosphere. *Appl. Environ. Microbiol.*, 58: 1894-1903.

Molin, C., N.R. Ribeiro, M.N. Matsumoto, N. Fernando Giasson, J. Brollo, B. Zanardo and J. Huzar-Novakowiski. 2021. Damping-off of soybean in southern Brazil can be associated with different species of *Globisporangium* spp. and *Pythium* spp. *Plant Pathol.*, 70: 1686-1694.

Muthukumar, A., R. Udhayakumar and R. Naveenkumar. 2016. Eco friendly management of damping-off of solanaceous crops caused by *Pythium* species. In: (Eds.): Kumar, P., V. Gupta, A. Tiwari and M. Kamle. C*urrent Trends in Plant Disease Diagnostics and Management Practices.* Springer; Cham, Switzerland. pp. 49-90.

Nakkeeran, S., K. Kavitha, G. Chandrasekar, P. Renukadevi and W.G.D. Fernando. 2006. Induction of plant defence compounds by *Pseudomonas chlororaphis* PA23 and *Bacillus subtilis* BSCBE4 in controlling damping-off of hot pepper caused by *Pythium aphanidermatum*. *Biocon. Sci. Technol.*, 16: 403-416.

Ni, L. and Z.K. Punja. 2019. Management of fungal diseases on cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) crops in greenhouses using *Bacillus subtilis*. *Bacilli and Agrobiotechnology: Phytostimulation and Biocontrol,* 2: 1-28.

Ons, L., D. Bylemans, K. Thevissen and B.P. Cammue. 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. *Microorganisms*, 8: 1930.

Orchard, T.J. 1977. Estimating the parameters of plant seedling emergence. *Seed Sci. Technol.*, 61-69.

Pandit, M.A., J. Kumar, S. Gulati, N. Bhandari, P. Mehta, R. Katyal, C.D. Rawat, V. Mishra and J. Kaur. 2022. Major biological control strategies for plant pathogens. *Pathogens*, 11: 273.

Punja, Z.K., A. Tirajoh, D. Collyer and L. Ni. 2019. Efficacy of *Bacillus subtilis* strain QST 713 (Rhapsody) against four major diseases of greenhouse cucumbers. *Crop Prot.*, 124: 104845.

Raza, A., J. Tabassum, M.S. Mubarik, S. Anwar, N. Zahra, Y. Sharif, M.B. Hafeez, C. Zhang, F.J. Corpas and H. Chen. 2022. Hydrogen sulfide: an emerging component against abiotic stress in plants. *Plant Biol.*, 24: 540-558.

Reddy, M.S., R.K. Hynes and G. Lazarovits. 1994. Relationship between *In vitro* growth inhibition of pathogens and suppression of pre-emergence damping-off and post-emergence root rot of white bean seedlings in the greenhouse by bacteria. *Can. J. Microbiol.*, 40: 113-119.

Rushford, C.A., R.L. North and G.L. Miller. 2022. Detection of *Pythium* spp. in golf course irrigation systems. *Plant Dis.*, 106: 46-56.

Sarhan, E.A., M.H. Abd-Elsyed and A.M. Ebrahiem. 2020. Biological control of cucumber powdery mildew (*Podosphaera xanthii*) (*Castagne*) under greenhouse conditions. *Egypt. J. Biol. Pest Cont.*, 30: 1-7.

Sayed, S.R, S.A. Abdelmohsen, H.M. Abdelzaher, M.A. Elnaghy, A.A. Mostafa, F.F. Al-Harbi and A.M. Abdelbacki. 2021. Myco-suppression analysis of soybean (*Glycine max*) damping-off caused by *Pythium aphanidermatum*. *Plants*, 10: 788.

Silva, R.N., V.N. Monteiro, A.S. Steindorff, E.V. Gomes, E.F. Noronha and C.J. Ulhoa. 2019. *Trichoderma*/pathogen/plant interaction in pre-harvest food security. *Fung. Biol.*, 123: 565-583.

Singh, S., R. Balodi, P.N. Meena and S. Singhal. 2021. Biocontrol activity of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia solani*. *Ind. Phytopathol.*, 74: 703-714.

Srivastava, A. 2021. Studies the effect of *Trichoderma harzianum* for biocontrol of cucumber (*Cucumis sativus* L.) and suppression of *Rhizoctonia solani*. *Plant Arc.*, 21: 2059-2062.

Stanghellini, M.E., L.J. Stowell, W.C. Kronland and P.V. Bretzel. 1983. Distribution of *Pythium aphanidermatum* in rhizosphere soil and factors affecting expression of the absolute inoculum potential. *Phytopathol.*, 73: 1463-1466.

Syed, R.N., A.M. Lodhi and S. Shahzad. 2020. Management of *Pythium* Diseases. In: (Eds.): Rai, M., K.A. Abd-Elsalam and A.P. Ingle. *Pythium Diagnosis, Diseases and Management*. CRC Press, Taylor & Francis Group, USA. pp.306-335.

Szczech, M. and M. Shoda. 2004. Biocontrol of *Rhizoctonia* damping-off of tomato by *Bacillus subtilis* combined with *Burkholderia cepacia*. *J. Phytopathol.*, 152: 549-556.

Van Bruggen, A.H., A. Gamliel and M.R. Finckh. 2016. Plant disease management in organic farming systems. *Pest Manag. Sci.*, 72: 30-44.

Weiland, J.E., L. Santamaria and N.J. Grünwald. 2014. Sensitivity of *Pythium irregulare, P. sylvaticum*, and *P. ultimum* from forest nurseries to mefenoxam and fosetyl-Al, and control of *Pythium* damping-off. *Plant Dis.*, 98: 937-942.

Whipps, J.M. 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. *New Phytol.*, 107: 127-142.

White, T.J., T.D. Bruns, S.B. Lee and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In: (Eds.): Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White. *PCR Protocols: A Guide to Methods and Applications*, Academic Press, New York, pp. 315-322.http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Younes, N.A., T.R. Anik, M.M. Rahman, A.A. Wardany, M.F. Dawood L.S.P. Tran, A.A.H. Abdel Latef and M.G. Mostofa. 2023. Effects of microbial biostimulants (*Trichoderma album* and *Bacillus megaterium*) on growth, quality attributes, and yield of onion under field conditions. *Heliyon*, 9: 1-11.

Yuan, W.M. and D.L. Crawford. 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Appl. Environ. Microbiol.*, 61: 3119-3128.

Ziedan, E.S.H. 2022. A review of the efficacy of biofumigation agents in the control of soil-borne plant diseases. *J. Plant Prot. Res.*, 62: 1-11.

(Received for publication 30 August 2023)