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OCCURRENCE OF PYTHIUM ROT OF CHINESE CABBAGE IN EGYPT AND ITS BIOCONTROL MEASURES

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Abstract: In Egypt, Chinese cabbage *Brassica rapa* var. *pekinensis* is a recently introduced as a winter crop grown throughout the country along the Nile valley as well as in new reclaimed lands. Pythium rot of Chinese cabbage was detected during the cultivation season 200⅝006 at four governorates throughout the north side of Egypt. Isolation trails revealed that *Pythium ultimum* was the causal organism of disease incidence. The cultivar of Chinese cabbage Napa (green) showed higher susceptibility to infection than Michihli cv. (red). *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* isolated from the rhizosphere of healthy Chinese cabbage could inhibit the *in vitro* growth of *P. ultimum* at different degrees. Under greenhouse and field trails, applying of biocontrol agents as a combination of soil mixing plus root dipping method was generally more effective than each method applied individually for suppressing Pythium rot incidence followed by soil mixing and root dipping methods. The applied bioagents could be arranged according to their activity for suppressing the disease incidence as follows: *T. harzainum*, *B. subtilis*, *T. viride* and *P. fluorescens*, respectively. The use of biocontrol agents as soil mixing and root dipping treatments could provide additional protection against crop loss due to Pythium rot disease.

Key words: Chinese cabbage, Pythium rot, *Trichoderma harzainum*, *Bacillus subtilis*, *T. viride*, *Pseudomonas fluorescens*, biological control

INTRODUCTION

Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*) is a leafy vegetable crop which has a major economic importance in many countries including Egypt. It belongs to the family brassicacea which includes a number of vegetable crops, including cabbage, cauliflower, broccoli, Brussels sprouts and kohlrabi.

In Egypt, chinese cabbage is recently introduced as a winter crop grown throughout the country along the Nile valley as well as in new reclaimed lands. Symptoms of Pythium rot disease as basal and crown rot of chinese cabbage were detected and first recorded during the cultivation season 2005/2006 at experimental agricultural station of National Research Center in Nobaria province, Beheira governorate, Egypt (El-Mohamedy and El-Mougy 2008). *Pythium ultimum* is ubiquitous soilborne pathogen which causes damping–off and root rot diseases on many plant species such as cabbage, carrot, cucumber, melon, turfgrass, cotton, wheat and others (Abdelzaher 2001). Annual tremendous economic loss due to the infection with *P. ultimum* were reported (Abdelzaher 2003; Tanina *et al.* 2004). *Pythium* spp. could be controlled by steaming the soil which is applied in a small scale. Although, fungicides could supply a good suppression of Pythium diseases, they have a hazardous effect to the environment. Attributed to the management strategy followed by the farmers as an unwise intensive use of fungicides, the chemical control could not give a satisfactory control of root rot disease because many Pythium species turned resistant to most of these chemicals or showed a lesser effect against the fungal oospores (Ichitani *et al.* 1994). Therefore, other ways to control pathogenic species of Pythium are needed. An investigation of this disease is considered important especially in view of its wide prevalence in Egypt particularly in sandy soils. Currently, increasing attention has been paid to biological control through the use of antagonistic microorganisms such as fungi and bacteria as an alternative to fungicides (Boehm and Hoitink 1992; Gravel *et al*. 2004). In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms. Many biological control agents such as *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp. suppress Pythium diseases largely through the biosynthesis of antibiotics or other Pythium–inhibiting substances (Moller *et al.* 2003; Carisse *et al.* 2003). Abdel-Kader (1997) reported that *T. harzianum* introduced to the soil was able to significantly reduce root rot incidence of bean plants more than the fungicide Rizolex-T. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Whipps and Lumsden 2001; McLean *et al*. 2004). Elad *et al*. (1980) observed that the application of wheat bran colonized by *T. harzianum* to soil infested with *R. solani* and *Sclerotium rolfsii* reduced the incidence

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of root diseases caused by these pathogens in beans. As for antagonistic bacteria, Kim *et al.* (1997) found that seed treatment with *Bacillus* spp. actively controlled three fungal root diseases of wheat and *Pseudomonas cepacia* or *Pseudomonas fluorescens* applied to pea seeds acted as a biological control agents against Pythium damping-off and Aphanomyces root rot and were able to reduce disease incidence (Parke *et al.* 1991; King and Parke 1993). In addition *Bacillus cereus* has been proven to have beneficial effects on crop health including enhancement of soybean yield, and suppression of damping-off of tomato (Smith *et al.* 1999) and alfalfa (Kazmar *et al.* 2000). Extensive laboratory testing demonstrated a powerful suppression of damping-off of alfalfa by diverse strains of *B. cereus*, which confirmed preliminary testing under field conditions (Handelsman *et al.* 1990; Kazmar *et al.* 2000). Furthermore, Georgakkopoulos *et al*. (2002) found that better biocontrol in cucumber was achieved when *B. subtilis* and *P. fluorescens* were applied by drenching or by coating seed in peat carrier. Pseudomonas antagonists were superior to Bacillus antagonists for controlling Pythium root rot on cucumber and sugar beets. The application of *Actinoplanes* spp. to infested by *P. ultimum* soil as soil mixing 5 days prior to replanting geranium or poinsettia seedlings reduced root rot severity and increased plant stand compared to non-treated plants after 6 weeks and it was as effective as the fungicide metalaxyl for reducing Pythium root rot (Filonow and Dole 1999).

The purpose of the present work was designed to survey the incidence of Pythium rot of Chinese cabbage throughout the north side of Egypt, isolation and identification of the causal fungus and evaluate its pathogenic ability. The effectiveness of rhizospheric bioagents against Pythium rot disease incidence on Chinese cabbage caused by *Pythium ultimum* under greenhouse and field conditions was also studied.

MATERIALS AND METHODS

Survey of Pythium rot disease of Chinese cabbage

Survey study on the incidence of Pythium rot disease of Chinese cabbage (basal rot and crown rot) was carried out during the 2005/2006 season. Four Governorates, *i.e.* Giza, Kalubeia, Ismaelia and Beheira (Nobaria province) were selected for survey where Chinese cabbage is widely spread especially in the new reclaimed lands. Plants of Chinese cabbage [*Brassica rapa* L. subsp. *pekinensis* cv. Napa (green) and *Brassica rapa* L. subsp. *chinensis* cv. Michihli (red)] showing disease symptoms were recorded. Hundred plants were examined in five farms at each surveyed governorate, and the percentage of basal and crown rot infection was recorded by counting the number of infected plants in relation to the total number of examined plants.

Isolation and identification of the causal organism (s)

Samples of Chinese cabbage plants showing typical basal and root rot symptoms were collected from surveyed fields. The root and basal parts of plant samples were rinsed in ethanol (5% v:v) for 30 sec, washed with tap water to remove the adhering soil particles, followed by sterile tap water, dried between two sheets of sterilized filter paper, and then cut to small pieces (2 sq cm each) and placed into Petri dishes containing sterilized media. The media used were Martin's medium (Allen 1961); VP3 selective medium (Ali-Shtayeh *et al*. 1986) and Pythium selective agar medium (Davison and McKay 1998). All plates were incubated at 20±2°C for 4–6 days until fungal colonies appearance. Single hyphal tip method was followed to pick up the margin of the colony and transferred to PDA or cornmeal agar slants for identification.

Identification of *Pythium* spp. was made using cultural and morphological characteristics such as sporangial shape and sexual organs with the aid of a light microscope according to Gilman (1957) and Barnett and Hunter (1972).

Pathogenicity test

Eight isolates of *P. ultimum* were evaluated for their pathogenic ability against two cultivars of Chinese cabbage plants *Brassica rapa* L. subsp. *pekinensis* cv. Napa (green) and *Brassica rapa* var. *chinensis* cv. Michihli (red)) in pot experiments under greenhouse conditions. The experiment were carried out in autoclaved (121°C for two hours) clay loamy soil (50% sand, 40% clay and 10% silt) artificially infested with the tested fungal isolates. Fungal mass production used for soil infestation was obtained by growing the tested isolates on sand-barley medium. This natural medium was prepared by mixing sand and barley (1:1, w:w and 40% water), then the mixture in glass bottles with cotton plugs was sterilized three times (1 hr each time) at 121°C. The autoclaved medium was then inoculated individually with a 5-mm disk of each tested fungal isolate grown and incubated at 20±2°C for 2 weeks. Soils were infested individually at a ratio of 5% (w:w) with tested pathogenic fungal cultures and mixed thoroughly to ensure equal distribution of fungal inoculum, then filled in plastic pots (25 cm-diameter) and irrigated every second day for 1 week before sowing. A set of pots were also amended with the same rate of sand barley medium free of fungal inoculum and reserved as control treatment. Surface sterilized Chinese cabbage seeds of either cultivars (using 3% sodium hypochlorite for 5 min, then picked up and air-dried) were planted in both infested and non-infested soils, five seeds per pot and ten replicates (pots). The average percentage of pre- and postemergence damping-off incidence was recorded after 15 and 30 days from sowing, respectively. All of described procedures were repeated three times and the average percentages were calculated.

Disease assessment

Percentages of damping-off incidence at the preemergence and postemergence stages were calculated in relation to the number of seeds sown. Meanwhile, the percentage of damping-off incidence at the postemergence stage was calculated as the number of diseased plants relative to the number of emerging seedlings. At the end of the experiment, cabbage plants were carefully pulled out from pots after being flooded with water in order to leave the root system undamaged. Plant roots showing rot lesions in addition to the visual root rot symptoms on the shoot system were considered diseased plants. Isolations from infected germinated cabbage seeds at

the preemergence stage as well as infected plants at the postemergence stage were carried out. Undeveloped, germinated seeds which were picked up from the soil, and the diseased cabbage plants were both water washed and surface sterilized with 3% sodium hypochlorite and then subjected to reisolation trials for the causal pathogens. The fungus obtained was compared with that used for soil infestation to prove its identity.

Rhizosphere studies

Natural microorganisms expected to act as antagonists against the target pathogen could be associated with the plant rhizosphere. To prove this, isolations of different bacteria and fungi from plant rhizoshere were carried out. Selected apparently healthy plants during the previously mentioned survey throughout natural infested fields with Chinese cabbage Pythium rot were subjected to isolation trails. Isolation of different bacterial and fungal populations following the method developed by Louw and Weblely (1959) for studying the microflora of the root region was used. They were purified and stored at 5°C till needed. All bacterial and fungal isolates were tested for their inhibitory effect against the tested pathogen under *in vitro* conditions.

The antagonistic bacterial isolates were identified according to Bradbury (1986); Lelliott and Stead (1987); Schaad (1988), whereas identification process described by Gilman (1957) and Barnett and Hunter (1972) was used for antagonistic fungal isolates.

***In vitro* antagonism**

The antagonistic ability against studied *P. ultimum* isolate of different microorganisms isolated from rhizospheric soil of healthy Chinese cabbage plants was evaluated on PDA plates. Three isolates of each *T. harzianum* (TH), *T. viride* (TV), *B. subtilis* (BS) and two isolates of *P. fluorescens* (PF) were tested. Antagonistic fungal and bacterial growth was first prepared. Ten ml of each individual bacterial isolate tested was grown for 48 h on nutrient broth medium and poured into flasks containing sterilized PDA medium before its solidifying and rotated gently to ensure equal distribution of bacterial growth, then poured into 9 cm-diameter Petri dishes. Inoculated plates were incubated for 48 h at 28±2°C. As for fungal growth, a 5-mm disk of the antagonistic fungi was transferred individually to the centre of a PDA plate then incubated at 25±2°C. The incubation period was 7 days for both antagonistic agents. Interactions between *P. ultimum* and biocontrol agents (TH, TV, BS and PF) were assayed using dull culture technique after Ferreira *et al.* (1999) on potato dextrose agar medium. A 5-mm disk of each antagonistic fungal or bacterial growth culture was placed onto PDA, 10 mm from the edge of the Petri dish. Another disk of the same diameter of *P. ultimum* growth culture was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with a culture disk of either a pathogenic or antagonistic culture alone at the same conditions. Both experimental and control dishes were arranged in a completely randomized design, with five replicates per treatment. All inoculated Petri dishes were incubated at 25±2°C and the fungal growth diameter away from and towards the antagonist agent was measured after the pathogenic fungal growth in the control treatment had reached the edge of the Petri dish. This test was repeated three times and the inhibition was calculated as the percentage reduction in colony diameter growth compared with the control.

Greenhouse experiment

The evaluation of bioagents against Pythium rot incidence caused by *P. ultimum* was carried out under greenhouse conditions at NRC. Napa (green), a highly susceptible cultivar of Chinse cabbage was used. Two isolates of each *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens* were tested. The used microorganisms inocula were prepared as follows:

Fungal mass production of either pathogenic or antagonistic fungi used for soil infestation was obtained by growing the tested isolates on sand-barley medium as mentioned above. As for spore suspension of antagonistic fungi, the tested bioagents individually grew on PDA medium at 25±2°C until the abundant heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula and then transferred to sterilized distilled water and filtered through nylon mesh. All spore suspensions were adjusted with sterile water to give a spore concentration of 10^6–10^7/ml. Meanwhile, bacterial bioagents were grown individually for 48 h in nutrient broth medium, and then cells were harvested by centrifugation. Bacterial isolates were resuspended in sterile distilled water and the concentration adjusted to give 10^9–10^{10} cells/ml.

The pot experiment was carried out using a clay loamy soil artificially infested with the pathogen. Fungal mass production of the pathogenic fungus used for soil infestation was obtained by growing the tested isolates on sand-barley medium as mentioned above. Soils were infested individually at a ratio of 5% (w:w) with pathogenic fungal culture and mixed thoroughly to ensure equal distribution of fungal inoculum, and then filled in plastic pots (25 cm-diameter) and irrigated every second day for 1 week before transplanting. Healthy transplants, 21 days old, of Chinese cabbage cv. Napa grown in autoclaved peat moss soil, obtained from Vegetable Research Department, National Research Center, Egypt were used.

Different applied treatments for biocontrol evaluation were performed as follows:

– root dipping (RD) method.
– soil infestation (SI) method (SI).
– root dipping (RD) plus soil infestation (SI) method.

Root dipping (RD) method

Roots of Chinese cabbage transplants were immersed for 30 min in either fungal or bacterial suspension supplemented with 1% (w/v) carboxymethyl cellulose (CMC) as adhesive polymers, then planted in pots containing infested soil with the pathogenic fungus.

Soil infestation (SI) method

Untreated Chinese cabbage transplants were planted in pots containing soil previously artificially infested with the pathogenic fungus followed by infestation with either

fungal or bacterial bioagents. The fungal inocula of *T. harzianum* or *T. viride* were added to the soil at the rate of 5% w:w, meanwhile the bacterial inocula were added to the soil as cell suspension at the rate of 50 ml/pot. Infested soils were carefully mixed thoroughly to ensure equal distribution of the added inocula one week before transplanting.

Root dipping (RD) plus soil infestation (SI)

Immersed Chinese cabbage transplants roots for 30 min in either fungal or bacterial suspension supplemented with 1% (w/v) carboxymethyl cellulose (CMC) were planted in pots containing soil infested with both pathogenic fungus and fungal bioagents inocula as stated earlier, while bacterial inocula were added to the soil as a cell suspension (10^9–10^{10} cells/ml) at the rate of 50 ml/pot. Ten replicates (pots) with five transplants were used for each particular treatment. Control treatment was untreated transplants planted in pots containing soil artificially infested with the pathogenic fungus only. The average percentage of Pythium rot incidence was recorded up to 45 days from transplanting (the experimental period).

Field experiment

The field experiment was carried out during two successive seasons 2006/2007 and 2007/2008 in a field located at experimental agricultural research station of the National Research Center at Nobaria province, Behiera Governorate. Chinese cabbage in this field was heavily damaged by Pythium rot during the last five seasons.

Biocontrol measures for controlling Pythium rot disease were evaluated using the bioagents *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens* performed as root dipping (RD), soil infestation (SI) and root dipping (RD) plus soil infestation (SI). Fungal inocula grown on sand-barley medium, as stated earlier, were used as soil treatment. One week before planting, inoculum of each of fungal isolate was incorporated individually in 20 cm of the top soil at planting row sites at the rate of 120 g/m^2 after Abdel-Kader (1997), while bacterial inocula were added to the soil also at the same in time a form of cell suspension (10^9–10^{10} cells/ml) at the rate of 10 l/m^2. Chinese cabbage transplant roots were immersed 30 min before planting in each of *Trichoderma* spp. isolate spore suspension at concentration of 10^6–10^7 spore/ml or bacterial isolate cell suspension at concentration of 10^9–10^{10} cells/ml prepared as mentioned before. A field experiment, consisted of plots (7x6 m), each comprising of 9 rows and 12 site/row, and was conducted in a Complete Randomized Block Design with five replicates (plots) for each treatment as well as control. Chinese cabbage cv. Napa were planted in all treatments at the rate of one transplant/site. Plots received the traditional agricultural practices. Observations of Pythium rot infection were made up to 60 days, and the average percent of disease incidence was calculated.

Statistical analysis

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between *P. ultimum* isolates in their ability to infect cabbage plants. Also, the antagonistic inhibitory effect on the linear growth of pathogenic fungus *in vitro* as well as differences in the activity of bioagents against Pythium rot incidence were tested in greenhouse and field trails. A general linear model option of the analysis system SAS (SAS Institute Inc. 1996) was used to perform the ANOVA. Duncan's multiple range test at $p \leq 0.05$ level was used for means separation (Winer 1971).

RESULTS AND DISCUSSION

Survey of Pythium rot disease of Chinese cabbage

Survey study on Chinese cabbage (basal rot and crown rot) disease was preformed during 2005/2006 season at four Governorates, i.e. Giza, Kalubeia, Ismaelia and Beheira (Nobaria province) where Chinese cabbage is widely spread. Plants of Chinese cabbage [*Brassica rapa* var. *pekinensis* cv. Napa (green) and *Brassica rapa* var. *chinensis* cv. Michihli (red)] showing diseases symptoms were recorded. Data in table 1 show the high Pythium rot incidence 38.0 and 33.6% for Napa (green) and Michihli (red) cultivars, respectively, at Beheira governorate followed by Ismaelia, Kalubeia and Giza governrate in respective order. On the other hand the mean value of recorded Pythium rot incidence throughout the surveyed governorates for the cultivar variety Napa (green) was represented by 31.1%, while Michihli cv. (red) was recorded at the same locations as 25.9%. This observation leads to the conclution that the cultivar variety Napa (green) showed more susceptibility to infection by Pythium rot than Michihli cv, (red). In this regards, Pythium rot on Chinese cabbage has been reported by many investigators (Moller *et al*. 2003; Tanina *et al*. 2004; Tojo *et al*. 2001; 2005).

Table 1. Survey of average Pythium rot infection of Chinese cabbage plants at different locations throughout the North side of Egypt

Location (governorate)	Pythium rot infection	
	Chinese cabbage cvs.	
	Napa (green)	Michihli (red)
Giza	25.0*	21.0*
Kalubeia	28.8	23.2
Ismaelia	33.0	25.2
Beheira	38.0	33.6
Mean	31.1	25.9

*each figure represent the average recorded percentage of diseased Chinese cabbage at different surveyed locations belonging to the cited governorate

Agrios (1997) stated that the disease threshold in the field caused by soil borne plant pathogens is highly variable (depending on, e.g., the soil, climate conditions, crop species and variety, to the infesting phytopathogen. In this regard, high soil moisture levels are a key factor for the soil-borne pathogen *Pythium* to become a problem. It is classed as a "water mould" of the fungal class Oomycetes. *Pythium* generally infects root tips first and moves upward. Frequently, the outer part of the rotted root will slip off, leaving the inner core as a string. Once the root becomes infected, another environmental factor comes into play. When roots are subjected to cool temperatures, especially at the plant threshold of temperature

tolerance (less than 16°C), the membranes in the root cells lose some structural integrity and become "leaky". Low, poorly drained portions of fields can develop damping off problems, especially in wet seasons. This is why *Pythium* damping off can be such a common problem during cool, wet spring weather. The surveyed area in the present study located at the north side of Egypt where the climate is cool and the average air temperature ranges between 12–16°C with rainfall during the winter season, conditions were favorable for spreading the Pythium disease especially in susceptible host crops. Furthermore, sometimes older plants become infected with *Pythium* through small feeder roots. From there, the infection spreads into the taproot. A soft, grey to brownish-black surface rot up to the soil surface or slightly beyond, is characteristic of the seedling disease caused by *Pythium*. The outer root tissue "sloughs off" leaving the central core when the root is slipped between two fingers. Infected plants appear stunted and pale yellow-green above ground.

Isolation and identification of the causal organism (s)

Pythium, *Rhizoctonia*, *Fusarium* and *Phytophthora* were isolated from Chinese cabbage plants showing typical basal and root rot symptoms, collected from different surveyed fields (Table 2). Tabulated data revealed that *P. ultimum* was a dominant fungus and its frequency was between 48.6–57.3 %, followed by a less frequent *R. solani*, *F. solani* and *Phytophthora* spp. in addition to other unidentified fungi, respectively. Several reports confirmed the isolation of *P. ultimum* from Chinese cabbage at high frequency (Kageyama *et al*. 1997; Tojo *et al*. 2001; Tanina *et al*. 2004). The high frequency of isolated *P. ultimum* in this study may be attributed to environmental conditions during winter cultivation season at the north side of Egypt. These environmental conditions could be most favourable for phycomycetes fungi. This explanation is similarly reported by Tojo *et al*. (2001), who stated that abundant soil moisture and low temperature are the two most important environmental factors that regulate the distribution of *P. ultimum.* Previous studies have shown that among several Pythium species that cause damping-off and root rot, *P. ultimum* is the most consistently virulent and the most frequently isolated (Georgakkopoulos *et al*. 2002). Also, Hendrix and Campbell (1973) noted that *P. ultimum* is widespread and important oomycetous plant pathogen causing seed decay, pre- and postemergence damping-off and root rot in many plant species.

Pathogenicity test

The pathogenic ability of eight isolates of *P. ultimum* to induce basal root rot of Chinese cabbage cv. Napa (green) and cv. Michihli (red) plants was tested in a pot experiment with artificially infested soil under greenhouse conditions (Table 3). Presented data show that all the tested fungal isolates were able to cause Pythium rot at different degrees on the two tested cultivars of Chinese cabbage

Table 2. Frequency of occurrence of fungi isolated from Chinese cabbage plants showing Pythium rot infection under field conditions in different governorates in Egypt

Location (governorate)	Frequency of isolated fungi				
	Pythium ultimum	*Rhizoctonia solani*	*Fusarium solani*	*Phytophthora* spp.	others (unidentified)
Giza	48.6*	22.7	17.3	10.5	1.1
Kalubeia	52.4	25.2	13.7	7.3	1.7
Ismaelia	55.2	18.5	13.5	9.8	3.1
Beheira	57.3	19.3	14.3	8.3	0.9

*each figure represents the percentage of isolates in relative to all isolated fungi

Table 3. Pathogenic ability of different *Pythium ultimum* isolates A to induce Pythium rot disease on Chinese cabbage under greenhouse conditions

Pythium isolates	Pythium rot incidence %			
	preemergence [B]		postemergence [C]	
	Chinese cabbage cultivars			
	Napa (Green)	Michihli (Red)	Napa (Green)	Michihli (Red)
P. ultimum (N_1)[D]	24 ef	22 ef	52.6 g	51.2 g
P. ultimum (N_2)	26 fg	24 ef	64.8 h	63.1 h
P. ultimum (G_1)	18 d	14 bc	36.5 d	27.9 bc
P. ultimum (G_2)	12 bc	10 b	31.8 c	24.4 bc
P. ultimum (K_1)	18 d	20 de	34.1 d	22.5 b
P. ultimum (K_2)	14 bc	12 bc	30.2 c	20.4 b
P. ultimum (I_1)	26 fg	20 de	45.9 f	37.5 de
P. ultimum (I_2)	10 b	8 b	35.5 d	30.4 c
Control	0 a	0 a	0 a	0 a

[A] soil artificially infested with the root rot pathogens at the rate of 5% w:w
[B] values represent the incidence of infection rated as percentage of emerged plants in relation to the number of seeds sown in soil artificially infested with pathogenic fungus
[C] values represent the incidence of infection rated as percentage of infected plants in relation to the number of emerged plants in soil artificially infested with pathogenic fungus
[D] the letters N, G, Q and I are codes of location sites: Nobaria, Giza, Kalubeia and Ismaelia governorates
Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)

at both pre-and postemergence stages. *P. ultimum* isolate N_2 caused a significantly high disease incidence recorded as 26 & 24% and 64.8 & 63. % at pre-, and postemergence stages of Chinese cabbage cvs. Napa (green) and Michihli (red), respectively. Meanwhile, *P. ultimum* isolate N_1 caused lesser disease incidence recorded as 24 & 22% and 52.6 & 51.2% followed by isolate I_1 (26 & 20%) and (45.9 & 37.5%) at pre-, and postemergence stages of Chinese cabbage cvs. Napa and Michihli, respectively. The other tested Pythium isolates caused root infection ranging between 10–18 & 8–20% and 30.2–36.5% & 22.5–30.4% in respective order, at pre-, and postemergence stages of Chinese cabbage cvs. Napa and Michihli.

It is interesting to note that the green cultivar (Napa cv.) of Chinese cabbage showed higher susceptibility to Pythium rot infection than the red cultivar (Michihli cv.), at both pre- and postemergence growth stages. Moreover, the records of Pythium rot infection at postemergence stage was represented by higher percentages than that recorded at preemergence growth stage. This observation was true for both cultivars of Chinese cabbage. The presented results indicate that *P. ultimum* is the causal agent of Pythium rot of Chinese cabbage in Egypt. This was also reported by El-Mohamedy and El-Mougy (2008). They stated the first record of Pythium rot of Chinese cabbage in Egypt caused by *P. ultimum*. Also, the present findings are in agreement with those reported by Kikumoto (1987) and Tojo *et al*. (2005). *Pythium* spp. were reported to cause rot infection to cabbage and other brassicaceous plants in many countries. In Japan stem and crown root rot of Chinese cabbage caused by *P. ultimum* was named as Pythium rot (Kikumoto 1987). In Denmark Moller and Hockenhul (1997) noted that *P. tracheiphilum* causes leaf and head rot of maturing Chinese cabbage, with losses of 40 to 50 %. Furthermore, Pythium rot of Chinese cabbage was reported to be caused by *P. aphanidermatum* (Saha and Singh 1988). Similar rot diseases of other brassicaceous plants caused by Pythium species are also known as Pythium rot (Tanina *et al*. 2004).

***In vitro* antagonism**

The inhibitory effect of antagonistic fungi and bacteria *in vitro* was tested in this study antagonistic agents applied as growth culture disks. Percentages of the reduction in growth of *P. ultimum* (isolate N_2) in response to antagonistic agents are presented in Table 4. The presented data show that the growth of pathogenic fungus was significantly reduced by the inhibitory action produced by all antagonistic agents tested. The antagonistic fungi had a greater effect on the retardation of growth (80.0–100%) compared with the bacterial agents (72.2–88.8%). The inhibitory effect of *T. harzianum* (1 & 2) and *T. viride* (1 & 2) was significantly higher than of the isolate No. 3 of each. The antagonistic bacteria also showed the same trend.

Similar results were reported by many investigators (Andersen *et al.* 2003; Carisse *et al.* 2003; Leclère *et al.* 2005). They reported the inhibitory effect of antagonistic fungal and bacterial microorganisms such as *Trichoderma* spp., *B. subtilis* and *P. fluorescens* that cause a growth reduction of *P. ultimum* under *in vitro* conditions. The inhibition in growth of the pathogen could be attributed to antibiosis, hyperparasitism (We *et al*. 1986) or production of chitinase and *ß*-1,3 glucanase enzymes which degrade the cell wall leading to lysis of mycelium of the pathogen (Ahmed and Baker 1987).

Table 4. Growth reduction of *P. ultumum* (isolate N_2) [A] in response to the inhibitory effect of antagonistic agents [B] *in vitro*

Antagonistic agent	Linear growth	Growth reduction [C]
T. harzianum (1)	0.0 g	100
T. harzianum (2)	0.0 g	100
T. harzianum (3)	18.0 d	80.0
T. viride (1)	0.0 g	100
T. viride (2)	0.0. g	100
T. viride (3)	12.0 f	86.6
B. subtilis (1)	10.0 f	88.8
B. subtilis (2)	18.0 d	80.0
B. subtilis (3)	25.0 b	72.2
P. fluorescens (1)	16.0 de	82.2
P. fluorescens (2)	18.0 d	80.0
P. fluorescens (3)	21.0 c	76.6
Control	90.0 a	0

[A] *P. ultumum* isolate which proved itself to have the highest pathogenic ability to Chinese cabbage plants (Table 3)
[B] selected bioagents isolated from the rhizosphere of healthy Chinese cabbage plants
[C] values are percentages of reduction in growth of *P. ultumum* (isolate N_2) in the presence of bioagents calculated in relation to its growth in medium free of an antagonistic agent
Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)

Greenhouse experiment

The efficacy of highly antagonistic *in vitro* fungal and bacterial rhizispheric isolates was evaluated against Pythium rot pathogen in a pot experiment using soil artificially infested with the disease incitent under greenhouse conditions. Isolates No. 1 and 2 of each *T. harzainum*, *T. viride*, *B. subtilis* and *P. fluorescens* were tested. The incidence of pre- and postemergence Pythium rot of Chinese cabbage cultivars was presented in table 5. All tested biocontrol agents obviously significantly suppressed Pythium rot incidence on Chinese cabbage compared to the control treatment. Applying bio control agents as a combination of soil mixing plus root dipping method was generally most effective than each individually for suppressing Pythium rot incidence followed by soil mixing and root dipping methods. The high reduction in disease incidence of 91.6, 75.0 and 58.3 % was recorded for *T. harzainum* (1) applied as soil mixing plus root dipping, soil mixing and root dipping methods. The least reduction in disease incidence was recorded for *P. fluorescens* (2) application that amounted to 54.1, 37.5 and 33.3 %. Moderate effect in the same trend was observed concerning *B. subtilis*, *T. viride* approaches in respective order.

This observation could be attributed to the high introduced inoculm density throughout soil mixing and root coating with the tested bioagents. It is expected that when the introduced antagonists established in the rhizosphere

Table 5. Activity of various antagonistic agents on Pythium rot incidence of Chinese cabbage [A] under greenhouse conditions

Antagonistic agent [B]	Disease incidence [C]					
	soil mixing [D]		root dipping [D]		soil mixing + root dipping [D]	
	infection	reduction	infection	reduction	infection	reduction
T. harzianum (1)	12.0 fg	75.0	20.0 de	58.3	4.0 i	91.6
T. harzianum (2)	20.0 de	58.3	24.0 d	50.0	10.0 g	79.1
T. viride (1)	22.0 d	54.1	30.0 b	37.5	10.0 g	79.1
T. viride (2)	26.0 cd	45.8	32.0 b	33.3	14.0 f	70.8
B. subtilis (1)	16.0 ef	66.6	22.0 d	54.1	8.0 h	83.3
B. subtilis (2)	18.0 e	62.5	22.0 d	54.1	6.0 hi	87.5
P. fluorescens (1)	28.0 bc	41.6	30.0 b	37.5	18.0 e	62.5
P. fluorescens (2)	30.0 b	37.5	32.0 b	33.3	22.0 d	54.1
Untreated control	48.0 a					

[A] Napa (green) the highly susceptible cultivar variety of Chinese cabbage was used
[B] microorganisms isolated from the rhizosphere of healthy Chinese cabbage which proved to have high antagonistic effect against the pathogenic fungus *in vitro*
[C] values represent the incidence of infection rated as percentage of infected plants in relation to the number of transplanted plants in soil artificially infested with pathogenic fungus for different approaches of bioagents treatments
[D] different approaches of bioagents treatments
Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)

of the root court of Chinese cabbage transplants, then it could compete with the target pathogen and suppress its ability to infect the host plant. The use of *Trichoderma* spp., *B. subtilis* and *P. fluorescens* for controlling Pythium rot diseases is reported by many workers (Harman 2000; Georgakkopoulos *et al*. 2002). Moreover, the reduction in Pythium rot incidence in soil mixed with bacteria may probably be due to competition as bacteria may compete with germinated oospores of the pathogen for soluble carbon and nitrogen sources supplied by root exudates that stimulate oospore germination and by eliminating these sources (Weller 1988).

Presented data revealed that the applied bioagents could be arranged according to their activity for suppressing disease incidence as follows: *T. harzainum*, *B. subtilis*, *T. viride* and *P. fluorescens*, respectively. It is interesting to note that one isolate (No.1) of each bioagent applied showed superior effect than the other (No. 2).

Therefore, only one isolate of each tested bioagents that showed higher effect on disease suppression was selected and applied under field conditions.

Field experiment

The field trial preformed during two successive growing seasons 2006/2007 and 2007/2008 in heavily naturally infested field with Pythium rot pathogen was located in the experimental research station of National Research Center at Nobaria province to evaluate the efficacy of the bioagents *T. harzianum, B. subtilis* and *P. fluorescens* to control Pythium rot of Chinese cabbage Napa cv. The data presented in Table 6 also show a similar trend of disease reduction recorded in a pot experiment. All applied treatments varied in their effect on disease incidence. The application of *T. harzianum* was the most superior treatment for reducing Pythium rot disease in the two growing seasons. It could reduce the disease incidence by 78.9, 73.7, 62.9% and 74.0, 66.8, 62.0% in applied treatments of soil mixing plus root dipping, soil mixing and root dipping methods preformed at the two growing seasons, respectively. Meanwhile, *P. fluorescens* applied in different methods showed the least disease reduction compared with the other applied bioagents, although it significantly reduced the disease incidence.

Many investigators observed that some soils tend to suppress soilborne diseases. Beneficial soil microorganisms are responsible for this disease suppression. Loper (1988) reported that *P. fluorescens* strain 3551, isolated from cotton rhizosphere soil, protected cotton from seed colonization and preemergence damping-off caused by *P. ultimum.* Also, the fluorescent *Pseudomonas* sp. DSS73 was originally isolated from the rhizoplane of sugar beet seedlings as a biosurfactant-producing strain capable of inhibiting the growth of the rootpathogenic microfungi *R. solani* and *P. ultimum* (Nielsen *et al.* 1998).

According to Harman (2000) natural factors limiting the number of soilborne pathogens occur through a combination of antagonism by other soil fungi and bacteria, natural release of antibiotics from other bacteria and fungi, and by competitive exclusion of habitat in the root zone or rhizosphere. Numerous other fungi and bacteria are being evaluated for effectiveness in biological control of soil-borne diseases. Roots are continually growing, so fungi and bacteria, such as *Trichoderma* and *Bacillus subtilis*, that are applied as seed treatments and grow along with the roots are most promising. The fungi, *Trichoderma* spp., have been extensively studied and a particularly virulent strain is available for biocontrol of seedling diseases. *Trichoderma* is attracted to other fungi and then excretes a chitinase enzyme that degrades the cell walls of many fungi. Similar results were recorded by many instigators serving with various crops (Gravel *et al*. 2004; Rankin and Paulitz 1994; Georgakopoulos *et al*. 2002; Nwaga *et al.* 2007). Previous studies have shown that *T .harzianum* and *B. subtilis* can effectively protect many plant species against Pythium root rot diseases for example on poinsettia (Boehm and Hoitink 1992), on cu-

Table 6. Pythium rot incidence of Chinese cabbage [A] in response to biocontrol treatments under field conditions during two successive growing seasons 2006/2007 and 2007/2008

Antagonistic agent[B]	Disease incidence [C]					
	soil mixing [D]		root dipping [D]		soil mixing + root dipping [D]	
	infection	reduction	infection	reduction	infection	reduction
First season 2006/2007						
T. harzianum	8.3 f	73.7	11.6 e	62.9	6.6 g	78.9
T. viride	11.6 e	62.9	14.1 bc	54.9	8.3 f	73.4
B. subtilis	14.1 bc	54.9	15.8 bc	49.5	12.5 d	60.0
P. fluorescens	15.8 bc	49.5	16.6 bc	46.9	13.3 cd	57.5
Untreated control	31.6 a					
Second season 2007/2008						
T. harzianum	11.6 e	66.8	13.3 cd	62.0	9.1d f	74.0
T. viride	15.8 bc	54.8	16.6 bc	52.5	11.6 e	66.8
B. subtilis	16.6 bc	52.5	18.3 b	47.7	14.1 bc	59.7
P. fluorescens	17.5 b	50.0	19.1 b	45.4	15.8 bc	54.8
Untreated control	35.0 a					

[A] Napa (green) the highly susceptible cultivar variety of Chinses cabbage was used
[B] microorganisms isolated from the rhizosphere of healthy Chinese cabbage proved to have high antagonistic effect against the pathogenic fungus *in vitro*
[C] values represent the incidence of infection rated as percentage of infected plants in relation to the number of transplanted plants in soil artificially infested with pathogenic fungus at different approaches of bioagents treatments
[D] different approaches of bioagents treatments
Figures followed by the same letter in the same column are not significantly different ($p \leq 0.05$)

cumber and sugar beet (Elad and Chet 1987; Georgakopoulos *et al.* 2002) and on cauliflower (Abdelzaher 2003). Furthermore, Ahmed and Baker (1987) reported that the rhizosphere nutrients conversion of *T. harzianum* was directly correlated with its competitive saprophytic ability. Many works attributed the suppression of Pythium rot by *Bacillus subtilis* to the decreasing infection rather than antibiosis (Elad and Chet 1987). Klich *et al*. (1991) pointed out that *B. subtilis* produced peptidolipid compounds that have been shown to have antifungal properties but not all fungal species were sensitive to these compounds. The results of the present study could support the production of antifungal compounds that inhibit the growth of *P. ultimum* on PDA medium (Table 4). These reports support the idea that the use of a biofungicide made of one or many antagonistic microorganisms could become an interesting alternative to fungicides for controlling rot disease caused by Pythium species.

CONCLUSION

Damping off occurs when young seedlings or transplants are stressed and conditions, favor the pathogen growth. Avoiding seedling disease entirely could be achieved by delaying planting in warm season crops until soil temperatures are above 16°C at the four-inch depth, either under plastic mulch or bare ground. The use of biocontrol agents as soil mixing and root dipping treatments could provide additional protection against crop loss due to Pythium rot disease.

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POLISH SUMMARY

WYSTĘPOWANIE ZGNILIZNY KAPUSTY CHIŃSKIEJ WYWOŁYWANEJ PRZEZ *PYTHIUM* W EGIPCIE I SPOSOBY JEJ ZWALCZANIA

W Egipcie, kapusta chińska *Brassica rapa* var. *pekinensis* jest wprowadzoną ostatnio rośliną uprawną w całym kraju wzdłuż doliny Nilu, jak również na nowo pozyskanych terenach uprawnych. Zgnilizna kapusty chińskiej spowodowana przez *Pythium* była wykryta w sezonie uprawy 2005/2006 w czterech gubernatorstwach w północnej części Egiptu. Przeprowadzone izolacje wykazały, że czynnikiem sprawczym choroby był grzyb *Pythium ultimum*. Odmiana kapusty chińskiej Napa (zielona) wykazywała wyższą wrażliwość na infekcję niż odmiana Michihli (czerwona). *Trichoderma harzianum, T. viride, Bacillus subtilis* i *Pseudomonas fluorescents* wyosobnione z rizosfery zdrowych roślin kapusty chińskiej mogły inhibować *in vitro* w różnym stopniu wzrost *P. ultimum*. W doświadczeniach szklarniowych i polowych, zastosowanie czynników biologicznego zwalczania w kombinacji zmieszania ich z ziemią plus metoda moczenia korzeni w zawiesinie patogena było zwykle bardziej efektywne niż każda z metod stosowana oddzielnie, mniej skuteczne były metody mieszania z ziemią i metoda moczenia korzeni. Zastosowane czynniki biologicznego zwalczania można by uszeregować według ich skuteczności w ograniczaniu patogenów: *T .harzianum, B. subtilis, T. viride* i *P. fluorescent*. Zastosowanie czynników biologicznych zwalczania w formie mieszania z ziemią i moczenia korzeni mogło dodatkowo chronić plon przed ewentualnymi skutkami wystąpienia zgnilizny spowodowanej przez Pythium.