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Research Article

Anti-fungal and bio-control properties of chitinolytic bacteria against safflower *Fusarium* root rot

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Abstract: A total of 78 rhizobacterial strains were isolated from 48 rhizospheric soil and root samples, collected from safflower *Carthamus tinctorius* L. fields located in different regions of Iran. The chitinolytic activity was measured in the presence of colloidal chitin as the sole carbon source. Eleven isolates were identified as chitinolytic bacteria, based on the formation of a clearly visible zone on the growth media. Four isolates including EM9, ES41, ES7 and ER13 exhibited the highest chitin degradation activity based on a clear zone diameter of more than 10 mm. According to a ribotyping analysis, EM9, ES41, and ES7 isolates were identified as *Bacillus cereus* and ER13 was found to be *Pantoea agglomerans*. In a dual-culture assay, morphogenic changes such as severely collapsed hyphae, decreased hyphal diameter with condensation and granulation of cytoplasm and highly rolled with formation of big clamydoconida in anomalous sporodochia -like structures were also observed using light microscope. Under greenhouse conditions, the application of selected chitinolytic isolates, i.e., EM9, ES41, ES7 and ER13, on safflower seeds significantly reduced seedling damping-off caused by *Fusarium solani*. In addition, the results revealed that root and shoot dry weight in infected plants that were treated with EM9 isolate suspension, increased by 14 and 22%, respectively.

Keywords: *Bacillus cereus*, Biological control, *Carthamus tinctorius*

Introduction

Phytopathogenic microorganisms affecting plant health and vigor are known to be a major threat to food production worldwide. Application of synthetic agrochemicals such as fungicides, although controls plant diseases, has become a growing concern about food safety (Compant *et al.,* 2005). Increasing use of agrochemicals causes several health and environmental hazards such as the development of pathogen resistance to the chemicals, environmental problems, and harms non-target organisms such livestock and, ultimately, humans. Biological control of plant fungal diseases has been an effective tool in the management of diseases to produce healthy foods and safe environments (Ahmed *et al.,* 2014). Gram-negative bacteria such as *Pseudomonas* sp., *Erwinia* sp., and *Agrobacterium* sp. represent the majority of bacteria considered to be used in biological control of pathogenic fungi. However, *Bacillus* genus, as a Gram-positive genus, offer greater advantages as a *Bacillus* species form endospores that are resistant to heat, radiation, organic solvents, and other adverse environmental factors. Production of metabolites

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such as siderophores, antibiotics, and lytic enzymes by rhizobacteria all contribute to bio-control mechanisms of soil-borne plant pathogens (Chet *et al.,* 1990). One of the most popular bacterial bio-control agents, *Bacillus subtilis*, was reported to be a chitinolytic species (Chang *et al.,* 2003).

Chitin is a linear polymer of *N*-acetyl glucosamine (NAG), and the second most abundant polysaccharide in nature, which serves as a fibrous strengthening element in the fungal cell wall. This structural element is selectively degraded by the chitinolytic organisms and used as a carbon source for their growth and multiplication. Bacterial genera *Achromobacter, Bacillus, Chromobacterium, Pseudomonas*, *Vibrio, Aeromonas* (Kamil *et al.,* 2007), *Enterobacteriaceae* family, and *Streptomyces* (Karthik *et al.,* 2015; Orlandelli *et al.,* 2015) have been found to be able to produce chitinase. The anti-fungal properties of chitinolytic soil bacteria may enable them to compete successfully for space and nutrition with fungi. Chitin is an important constituent of most fungal cell walls and the production of chitinase is a part of the lytic system that enables the bacteria to use living hyphae. Moreover chitinases inhibit fungal spore germination and germ tube elongation (Akocaka *et al.,* 2015).

Safflower (*Carthamus tinctorius* L.) belonging to the Asteraceae family is an important oil crop cultivated in arid and semi-arid regions of the world and especially in Isfahan Province, producing about 25% of the total safflower production in Iran. Safflower oil consists of 75-80% linoleic acid that plays an important role in human nutrition (Sharifnabi and Saeidi, 2004). *Fusarium solani* (Mart.) Sacc. is a common phytopathogenic fungus causing seedling damp-off and root rot in many crops such as chili, potato, beans, and safflower (Bell *et al.,* 1998). The root rot disease is an important soil-borne disease in safflower fields in Iran, responsible for an extensive commercial loss by reducing both quality and quantity (Sharifnabi and Saeidi, 2004; Orlandelli *et al.,* 2015). Among the diseases caused by several fungal pathogens, the root rot caused by *Fusarium solani* is a major concern in many safflower-growing areas in Iran. In order to control the disease, several attempts have been made such as the cultivation disease-resistant cultivars, employment of biologic control tactics and application of native antagonists, all as eco-friendly approaches (Orlandelli *et al.,* 2015; Vasebi *et al.,* 2015).

The aim of the present study was to evaluate the *in vitro* and *in vivo* anti-fungal efficacy of chitinolytic rhizobacteria to control *Fusarium solani.* The study also investigated the effects of chitinolytic rhizobacteria on *Fusarium* root rot in safflower grown under greenhouse conditions.

Materials and Methods

Isolation of chitinolytic bacteria

In order to isolate the chitinolytic bacteria, 48 root and rhizospheric soil samples were collected from different safflower (*Carthamus tinctorius* L.) fields located in different parts of Isfahan Province, Iran. The rhizobacteria were isolated and purified according to methods described by Mahmoudi *et al.* (2011). The chitinolytic activity of each isolate was measured on minimal salt medium containing a colloidal chitin as the sole carbon and energy source (Kamil *et al.,* 2007). The medium consisted of 0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.1% NH_4Cl, 0.05% NaCl, 0.05% yeast extract, 1.5% agar, and 1.0% (w/v) colloidal chitin. The colloidal chitin was prepared as Kang *et al.* (1999) from practical grade of crab shell chitin (Sigma Co.). The plates were incubated in darkness at 27 °C for 10 days. Colonies showing clearance zones against a creamy background were considered as chitinase-producing bacteria. The bacterium that showed a distinct clearance zone on repeated subculture was selected for further studies. The clear zone diameters were measured (mm) and used to indicate the chitinase activity. For each isolate, three replicates were taken and the experiment was repeated twice.

Identification of chitinase producing rhizobacteria

The 16S rRNA gene of each isolate was amplified by PCR, using the universal primers

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pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AGGAGGTGATCCAGCCGCA-3′), as described by Mahmoudi *et al.* (2011). The PCR products were purified and directly sequenced by a BigDye Terminator and ABI Prism 3700 Genetic Analyzer (Macrogene, World Meridian Venture Center, Korea). The gene sequencing of 16S rRNA was aligned with the reference sequence published on the NCBI database using the BLAST algorithm. Additionally, some phenotypic characteristics of bacterial isolates were determined according to Bergey's Manual of Systemic Bacteriology (Bergey and Holt, 2000).

Anti-fungal activity of rhizobacteria

The anti-fungal activity was examined *in vitro* by inhibiting the growth of phyto-pathogenic fungus, *Fusarium solani* (source culture- isolated from safflower, IAU, Isfahan Branch, Iran), on the PDA medium. The fungal inoculum, (5 mm-diameter agar disc punched out with sterilized cork borer from an active growing fungal culture) was placed at the center of a Petri-dish. The bacterial isolates were streaked in 3 cm away from one side of the fungal inoculated plates. The Petri plates were incubated at 27 ºC for ten days, until the fungal mycelium reach to the plate margin away from the bacterial colony. The diameters of the fungal colony towards and away from the bacterial colony were measured and the growth inhibition percentage was calculated as follows (Idris *et al.,* 2007):

$$\text{Inhibition percentage} = \frac{(R-r)}{R} \times 100$$

where r is the half diameter of the fungal colony toward the bacterial colony and R denotes the half diameter of fungal colony away from the bacterial colony.

Hyphae abnormalities of antagonistic assay: light microscopy

In an antagonistic assay, the effects of chitinolytic bacteria on growth and morphological properties of pathogen fungus were evaluated. Following a ten-day dual culture incubation period, a sample of mycelium was taken from the periphery of the inhibited growing zone on the PDA medium. The samples were fixed in lacto-phenol-cotton blue stain and observed under the microscope (Nikon YS100, Japan) at 400-x magnification to examine the morphological abnormalities. Samples from control plates without impressed bacteria were also stained and observed. Photographs were taken with the help of a computer-attached Canon Color Camera.

Greenhouse experiment

Safflower (*Carthamus tinctorius* L.) seeds, cultivar 'Koseh', were obtained from a local seed agency. The local land race of Koseh, which is widely grown in Isfahan province, is classified as susceptible genotype to *Fusarium* root rot disease (Sharifnabi and Saeidi 2004). Fifty milliliter of each selected chitinolytic bacterial inoculums (strains EM9, ES41, ES7 and ER13) containing 2×10^7 cfu ml^{-1} were added to 50 g surface disinfected safflower seeds in Erlenmeyer flask and were shaken at 180 rpm for 12 h. Extra suspension was drained off and the seeds were air-dried.

Fungal propagules were prepared by growing the fungus *Fusarium solani* on PDA medium for 7 days at 27 °C. A 200g of 24h water soaking wheat grains were added in Erlenmeyer flask and autoclaved for 15 min. Four disks of agar from the margin of actively growing colony of *F. solani* was introduced into the flasks and stored for 4 weeks at 27 °C. The flasks were shaking vigorously for 1 min each 5 days to give equal biological material. One well colonized wheat grain served as a fungus propagul in further experiment.

Polypropylene pots (20 cm diameter) were filled with autoclaved sandy loam soil, and then five propagules of fungus were laid in each pot at the depth of 5 cm and left for five days. Five safflower coated seeds were sown in each pot and placed in greenhouse at 28 ± 2 °C and relative humidity of 60%. The pots were randomly arranged with four replicates. The pots infested with the pathogen but not

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treated with bacteria, served as a control. After two weeks, the seedling damping-off percentage was assessed by counting the non-emergent seeds. Five-week-old seedlings were sampled and analyzed for root dry weight, shoot dry weight and root rot disease assessment. To evaluate disease severity, samples were taken to the laboratory and washed. The crowns and roots were split longitudinally with a sharp knife. The severity of root rot in each plant was then assessed. The disease severity index (DSI) is a visual scale and varies from 0 to 5 where 0 represents no visible symptoms and 5 represents total necrosis.

Statistical analyses

Statistical analyses of the data were performed by analysis variance (ANOVA) using the SPSS commercial statistical package (SPSS, Version 11.5 for Windows) and significant differences among the means were determined at $P \leq 0.05$, using the Least Significant Difference (LSD).

Results

Screening and identification of chitinolytic rhizobacteria

Among 75 bacterial strains isolated from 48 rhizospheric soil and root samples, 11 isolates showed chitinolytic activity with different clear zone size (Table 1). Four isolates (EM9, ES41, ES7 and ER13) out of 11 isolates, showed the widest inhibition zones with a diameter more than 10 mm. The identification of bacterial species was performed by phenotypic tests (Table 1) as well as PCR amplification of 16S rRNA gene, using universal primers. The 16S rRNA sequences were compared with those found in the GenBank database (http://www.ncbi.nlm. nih.gov). A more than 98% similarity with the identified species in the GenBank database and phenotypical properties were used for species identification.

BLAST analysis using these partial 16S rRNA sequences revealed that theses bacterial isolates belonged to six species from four different genera. Seven isolates including EM9, EM28, ES41, ES7, ES5, ES31 and ER17 showed a high similarity with *Bacillus* species, which among them, the EM9, ES41 and ES7 isolates with higher chitinolytic activity were referred to *Bacillus cereus* species based on the sequences alignment and phenotypical tests (Table 1). Three isolates, ES5, ES31 and EM28 with moderated chitin degradation activity and one isolate, ER17, were identified as *B. thuringiensis and B. subtilis*, respectively. ER13 and EM40 isolates showed 99% similarity with *Pantoea agglomerans* species, which has been found to degrade colloidal chitin. Two isolates, ER1 and EF12, with moderate chitinolytic activity, were known as *Arthrobacter* sp. and *Pseudomonas fluorescens*, respectively (Table 1).

Antifungal activity and hyphae abnormalities

The *in vitro* anti-fungal activities of chitinolytic bacterial isolates against phyto-pathogenic fungus, *Fusarium solani*, were measured by a percent radial growth inhibition. According to the results, EM9 and ES7 isolates had the highest effect on test fungus growth, where the largest inhibition zones were observed (Table 1).

In vitro chitinolytic isolates' antagonistic test against fungal isolate showed the occurrence of hyphal abnormality. In microscopic examination, treated fungus showed severe anomalies in their hyphae-for instance, the diameter of hyphae increased, the cytoplasm showed granulation, condensation and started leaving the cell wall (Fig. 1a). Impressing fungus hyphae with antagonist bacteria were developed of bulbous anomalous structure in hyphal tips, especially in conidiogenous mycelia, and production of micro-conidia were inhibited (Fig. 1b, c). An interesting observation was the extreme production of big clamidoconidia in sporodochia-like structures (Fig 1d, e). Rolling and curling hyphae with a degradation of the cell wall were observed in some treatments (Fig. 1f, g).

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Table 1 Properties of chitinolytic bacteria isolated from safflower rhizosphere.

Strains	*rrs* sequencing and phenotypic identification	Gene bank accession No.	Gram	Colony and cell morphology	Chitinolytic activity (mm)[1]	Mycelial growth inhibition (%)[2]
EM9	*Bacillus cereus*	EU350369.1	+	White, rod shape, motile	15.0	57.3
ES41	"	DQ923487.1	+	As above	11.5	31.3
ES7	"	EU513393.1	+	As above	12.8	49.8
ER13	*Pantoae agglomerans*	AY996965.1	-	Yellow, rod, motile metallic green on EMB,	11.3	43.4
EF6	"	FJ544372.1	+	As above	8.8	27.6
EM28	*Bacillus thuringiensis*	JN896993.1	+	White, rod shape, motile	4.5	31.4
ES5	"	EF206345.1	+	As above	4.0	15.0
ES31	"	JN896992.1	+	As above	7.5	19.4
ER17	*Bacillus subtilis*	HF584927.1	+	Orange, rod, motile	8.5	28.4
ER1	*Arthrobacter* sp.	AF134184.1	+	White, like filamentous, non-motile	3.5	14.1
EF12	*Pseudomonas fluorescens*	U63902.1	-	Creamy white, rod, fluorescent on King-B, motile	6.0	29.2

[1] Chitin degrading activity of the bacteria was performed on chitin containing salt medium for ten days. The clear zone diameters around the bacterial colonies were measured (mm) and used to indicate the chitinolytic activity.
[2] Antagonistic activity of all selected rhizobacterial isolates against *Fusarium solani* was determined using dual culture technique on PDA and percentage of inhibition growth was assessed as Idris *et al.* (2007).

Suppression of safflower root rot disease by chitinolytic bacteria

In an *in vivo* assay, co-inoculation of safflower pots with *F. solani* and chitin degrading bacteria provided a substantial reduction in root rot disease (Table 2) compared to the inoculation of safflowers with *F. solani* alone. *Bacillus cereus*, strain EM9, caused a significant reduction in safflower damping-off and increased root and shoot dry weight compared to those plants that were inoculated with the pathogen alone as well as other tested bacteria. Control plants inoculated with *F. solani* but not treated by rhizobacteria showed up to 70% damping-off so that the majority of the plants were killed by the pathogen (Table 2).

An application of bacterial suspension, in the form of covering the seeds, decreased root rot symptoms in safflower seedlings. The average damping-off varied between 10 to 27%, depending on the bacterial strain. The disease level in the treated plants was significantly lower than that in the control treatment. In disease assessment, plants treated with *B. cereus* EM9 showed the highest root and shoot dry weight and the lowest disease symptoms compared to the other plants, and, therefore, rated No. 1 according to the disease severity index (DSI). In this assay, the ES41 strain was rated No. 2, and ES7 and ER13 were No. 3. Plants inoculated with *F. solani* but not treated with bacteria showed up to 70% disease incidence with the majority of plants completely stunted or dead and, hence, placed at No. 4, based on the disease severity scaling.

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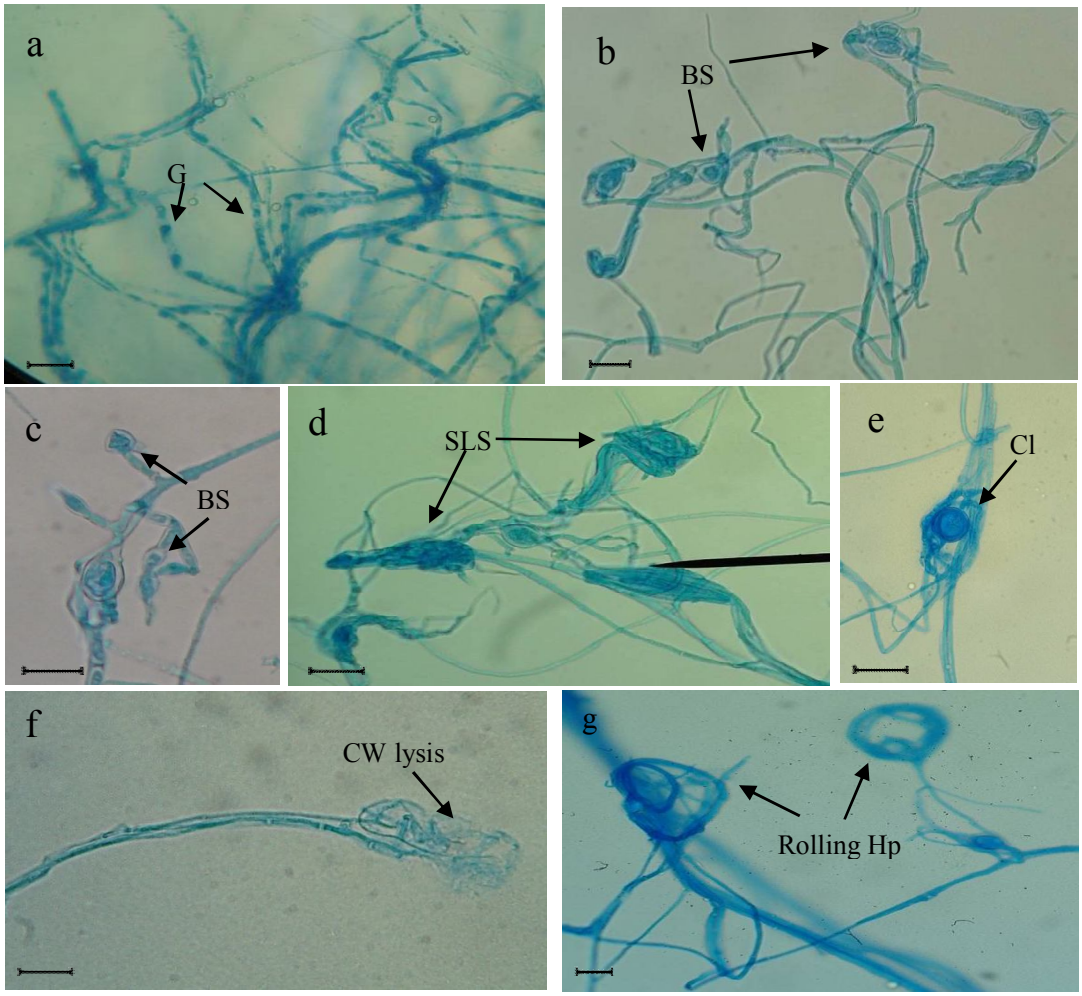

Figure 1 Effects of chitin degrading bacteria on morphological properties of *Fusarium solani* 7 days after incubation at 27 ± 1 °C. Mycelial growth and conidiation of *F. solani* affected by bacterial antagonists. Impressing fungus with chitinolytic bacteria showing anomalies in the hyphae, mycelia become wrinkled with condensed and granulated cytoplasm (GC) [a]; bulbous anomalous structure (BS) and inhibition of tip growth of hyphae [b,c]; highly produced of sporodochia like structure (SLS) on fungus thallus with big clamidoconidia (Cl) [d, e] abnormal hyphae (Hp) were rolled and in some cases cell wall (CW) was degraded [f, g]. Bar 10 µm.

Table 2 *In vivo* properties of chitinolytic rhizobacteria on safflower root rot disease caused by *Fusarium solani.*

Bacteria strains	*In vivo* assays[1]			
	Mean damping off (%)[2]	Mean aerial dry weight (g) ± SE[2]	Mean root dry weight (g) ± SE[2]	DSI[3]
Bacillus cereus EM9	10.5a	2.87 ± 0.3a	1.60 ± 0.6a	1
Bacillus cereus ES41	26.2b	1.90 ± 0.4c	0.86 ± 0.4b	2
Bacillus cereus ES7	18.6b	2.23 ± 0.2b	1.37 ± 0.4a	3
Pantoae agglomerans ER13	21.3b	2.56 ± 0.6ab	0.92 ± 0.5b	3
Control (no bacterial treatment)	70.5c	1.16 ± 0.5d	0.35 ± 0.6c	5

[1] Safflower seeds var. Kooseh were coated by bacterial antagonist suspension, planted into inoculated pots with *F. solani,* and incubated in greenhouse at 27 ± 1 °C.
[2] Means with the same letters in each column do not differ significantly ($p \leq 0.05$, least significant difference test).
[3] DSI: Disease Severity Index (see materials and methods).

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Discussion

Plant root environment (known as rhizosphere) consist of a wide range of root colonizing bacteria having the capacity to enhance plant growth and reduce the intensity of plant diseases by suppressing the growth of deleterious rhizosphere micro-organisms or by inducing systemic resistance. The isolation and screening of chitinolytic bacteria from root and rhizosphere of safflower plant has helped in the detection of chitin degrading rhizobacteria potential which act against damaging fungal root pathogens. In the present research, 11 bacterial strains, isolated from 48 root and rhizospheric soil samples, showed different abilities for chitinolytic activities and to inhibit *F. solani* growth in dual culture assay. The results revealed that the EM9 and ES7 isolates, with high chitinolytic activity, had greater radial growth inhibition against fungal pathogen. Chitin lytic enzyme production is not only a bio-control mechanism related to these bacteria but is also the most important mechanism (Chang *et al.,* 2003). It has been reported that there is high correlation between the anti-fungal activity and chitinase-producing ability of chitinolytic bacteria (Compant *et al.,* 2005). Furthermore, some bacterial isolates have the ability of producing antibiotics such as b-1, 3-glucanase and siderophore, which strengthen their antagonistic activities (Ghanbarzadeh *et al.,* 2016).

All isolated bacterial antagonists significantly inhibited *F. solani* growth after being grown for ten days on PDA. These isolates were classified in four genera based on 16s rRNA homology and phenotypic characteristics. Seven isolates were related to *Bacillus* sp., among which, three isolates were known as *B. cereus*, three isolates were known as *B. thuringiensis* and one isolate was known as *B. subtilis*. *Bacillus cereus* is a large, gram-positive, endospore-forming bacterium that is very common in soils and plants (Suryanto *et al.,* 2012). Chitinase production has been reported in different species of *Bacillus* such as *B. megaterium*, *B. circulans*, *B. cereus* (Huang, 2005), *B. subtilis* (Wang, 2006), *B. thuringiensis*. Other strains had low frequency and fell within the genera *Pantoea* (two strains), *Pseudomonas* (one strain), and *Arthrobacter* (one strain).

The main goal of this study was to evaluate the potential of antagonistic activities in chitinolytic isolates against *F. solani* on safflower seedling. Since chitinolytic bacteria have a strong ability to inhibit mycelia growth in *F. solani* under *in vitro* conditions, they were selected for this purpose. *Fusarium solani* caused root rot disease, characterized by grayish-dark red to black discoloration and dry rotting of infected roots. The results of *in vivo* test revealed that plants produced from inoculated seeds with rhizobacteria had lower seedling damping-off percentage. In addition, they showed a significant increase in root and shoot dry weight. By contrast, the damping-off in non-treated plants was recorded to be 70%. In disease severity (DSI) assessment, the least disease index score (No. 1) was attributed to plants treated with efficient chitin degrading bacterium i.e., the EM9 strain. The application of chitinolytic bacteria as bio-control agents for controlling some plant diseases such as tomato wilt caused by *F. oxysporum* f.sp. *lycopersici* (Hariprasad *et al.,* 2011), barley leaf strip caused by *Bipolaris sorokiniana* (Zhang and Yuen, 2000) and carnation wilt caused by *F. oxysporum* f. sp. *dianthi* (Ajit *et al.,* 2006) had been previously reported.

Under greenhouse conditions, safflower root and shoot dry weight increased by 15% due to the application of the EM9 chitinolytic isolate on the seeds. The EM9 isolate was found to be an effective isolate in the inhibition of fungal growth and prevention of the root and crown rot disease. It is possible that an increase in plant growth might be due to the ability of this bacterium to produce growth-regulator hormones, siderophore or solubilize phosphate (Vasebi *et al.,* 2015). An increase in soybean growth due to the application of plant growth-promoting bacteria capable of producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, b-1,3-glucanase or siderophores, or

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those that were able to solubilize phosphorus *in vitro*, has been documented by a number of researchers (Compant *et al.,* 2005; Suryanto *et al.,* 2012).

In conclusion, the biological control of fungal diseases of plants is eco-friendly and is a potential component of integrated disease management. Microorganisms, which secret a complex of mycolytic enzymes, are considered to be possible biological control agents of plant diseases. The results of this research showed strong chitinolytic activity from three *Bacillus* isolates, among which *B. cereus* EM9 exhibited the most desirable qualities that lead us to consider the practical use of this organism in the control of *Fusarium* and other soil born fungi. Seed coat technique induced high reduction in percentage of *F. solani* infection and significantly improved the percentage of survival safflower plants. All chitinolytic isolates treatments reduced seedling damping-off and increased dry-weight of roots and aerial parts of infected plants. Promising researches on the use of antagonistic microorganisms and their products to control the fungal diseases of crop plants will likely lead to the development of safe, efficacious, and environment-friendly biological control strategies.

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ویژگی‌های ضدقارچی و بیوکنترلی باکتری‌های تجزیه‌کننده کیتین علیه پوسیدگی فوزاریومی ریشه گلرنگ

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چکیده: توانایی تجزیه کیتین ۷۸ سویه باکتریایی جدا شده از ۴۸ نمونه خاک و ریشه مزارع مختلف گلرنگ *Carthamus tinctorius* L. مورد بررسی قرار گفت. فعالیت تجزیه کنندگی کیتین باکتری‌ها روی محیط حاوی کیتین کلوئیدی و براساس تولید هاله شفاف اطراف کلونی‌های باکتری‌ها سنجیده شد. از میان باکتری‌های مورد مطالعه، یازده سویه باکتریایی توانستند کیتین موجود در محیط را با شدت‌های متفاوت تجزیه کرده و هاله شفاف اطراف کلونی‌های خود ایجاد کنند که در چهار جدایه EM9، ES41، ES7 و ER13 قطر هاله تولید شده بیش از ۱۰ میلی‌متر بود و توانایی بالاتری در تولید آنزیم‌های تجزیه‌کننده کیتین از خود نشان دادند. براساس توالی‌یابی ژن ۱۶sریبوزومی و برخی ویژگی‌های مورفولوژیک، سویه‌های EM9، ES41 و ES7 به‌عنوان گونه‌ی *Bacillus cereus* و سویه ER13 به‌عنوان *Pantoea agglomerans* تشخیص داده شدند. در آزمون ضدقارچی، این باکتری‌ها به خوبی از رشد میسلیوم‌های *Fusarium solani* جلوگیری کرده و در مطالعات میکروسکوپی، تغییرات مورفولوژیکی شامل پژمردگی و پیچیدگی شدید میسلیوم‌ها، کاهش قطر میسلیوم و گرانوله شدن سیتوپلاسم سلول‌ها، تشکیل کلامیدوسپورهای بزرگ و ساختارهای شبه اسپورودوخیوم در میسلیوم‌های قارچ هدف مشاهده شد. در مطالعات گلخانه‌ای، باکتری‌های قوی در تجزیه کیتین، سویه‌های EM9، ES41، ES7 و ER13، در روش پوشش‌دهی بذر به‌طور معنی‌دار از وقوع بوته‌میری و پوسیدگی ریشه و طوقه فوزاریومی روی گیاهان گلرنگ جلوگیری کردند. هم‌چنین وزن خشک ریشه و اندام‌های هوایی در گیاهان تیمار شده با سویه EM9، به‌ترتیب ۱۴ و ۲۲ درصد افزایش را نشان دادند.

واژگان کلیدی: *Bacillus cereus*، *Carthamus tinctoirus*، کنترل بیولوژیک

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