BIOCONTROL OF CERTAIN SOILBORNE DISEASES AND PROMOTION OF GROWTH OF CAPSICUM ANNUUM USING BIOFUNGICIDES

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Abstract

Colored pepper (Capsicum annuum L.) has great economic importance as a food vegetable crop in Egypt and all over the world. This crop is prone to infection with soilborne fungal pathogens such as Rhizoctonia solani, Fusarium solani and Macrophomina phaseolina. These mycopathogens were isolated from diseased pepper seedlings, identified; their virulence was confirmed in the greenhouse. Eight bacterial isolates mainly; (Bacillus subtilis and Pseudomonas fluorescens), and many fungal isolates mainly, (Trichoderma harzianum and T. viride), were isolated from the rhizosphere soil of pepper. They caused appreciable In vitro inhibition of the radial growth of the 3 pathogens in dual culture technique, in percentages ranging from (71-79%) and (80-87%), respectively. On infestation of pepper soil with these bioagents and the 3 pathogens separately in the greenhouse, they caused In vivo reduction of disease symptoms of pepper compared with the pathogens infested and non-infested control soils. In addition, they caused significant improvement of pepper growth compared with the control soil, however, promotion exerted by B. subtilis and T. harzianum was more than that of P. fluorescens and T. viride. These promoting activities could be attributed to the production of metabolites such as growth hormones; solubilization of phosphates and improvement of nutrient uptake. This is the first record of promoting the growth of pepper in greenhouse by B. subtilis and T. harzianum in Egypt. Thus these bioagents could be formulated then applied in the future in pepper fields of this country as safe, effective, ecofriendly biofungicides to control soilborne pathogens and also could be used as biofertilizers to promote the growth and productivity of this crop.

Key words: Capsicum annuum; Soilborne pathogens; Fungal diseases; Biocontrol; Biofungicides; Biofertilizers.

Introduction

Vegetable crops are used all over the world as sources of nutrients and fibers in the human diet. Pepper, Capsicum sp. belongs to the Solanaceae family and is divided into two main groups, pungent and non-pungent, which are also called hot and sweet pepper, respectively. Sweet pepper includes more than one cultivar used in greenhouse production, such as hybrids which have bell-shaped (Capsicum annuum L.) (Zayed et al., 2013). In Egypt, sweet pepper (Capsicum annuum L.) is one of the most common and preferable vegetable crop used for local market and exportation. High cash crops such as sweet pepper is very important in Egyptian and world agriculture due to its high profit and nutritional values for human health (Fawzy et al., 2012).

Soilborne pathogens are considered to be one of the major problems in agricultural production throughout the world, causing reduction in yield and quality of crops. Damping-off; root rot; charcoal rot and wilt of vegetables caused by R. solani, F. solani, M. phaseolina, F. oxysporum, Sclerotium rolfsii, Alternaria solani and Pythium spp., are the most deleterious diseases (Steinkellner et al., 2008).

Generally, phytopathogenic fungi are chemically controlled using synthetic fungicides, however, the use of these is increasingly restricted due to their harmful effects on human health and the environment (Harris et al., 2001). The increasing demand of production, regulations on the use of synthetic fungicides and development of pathogens resistant to these chemicals employed, justifies the search for alternative control strategies such as biological control.

Antagonistic bacteria are mainly soil inhabitants and could be developed into biofungicides for the management of damping-off, root and many soilborne diseases of different crops (Khabbaz & Abbasi, 2014). Many strains of Bacillus bacteria have been found to be potential biocontrol agents against fungal pathogens. Recently, results of Torres et al., (2016) study showed that B. subtilis, B. amyloliquefaciens significantly inhibited the growth of pathogenic M. phaseolina by two different mechanisms namely; lipopeptide synthesis and competition among microorganisms.

P. cepacia or P. fluorescens applied to pea seeds act as biological control agents against Pythium damping-off and Aphanomyces root rot, and were able to reduce diseases incidence (King & Parke, 1993). The mechanisms suggested to be involved in their biocontrol activities were antibiotics production; competition for nutrients; mycoparasitism and improvement of plant growth (Hjeljord et al., 2001)

Both of T. viride and T. harzianum were reported in many previous studies as good antagonists for inhibiting growth of several soil and seed borne plant pathogens (McLean et al., 2004; Poddar et al., 2004). In the study of Hussain et al., (2013), T. harzianum was highly antagonistic towards many soilborne pathogens such as; R. solani, M. phaseolina, F. oxysporum, F. solani and Pythium spp., as it showed a strong inhibitory effect on their growth and mycelial development. Sharon et al. (2001) demonstrated the possible role of chitinolytic and/or glucanases enzymes in the biocontrol exhibited by Trichoderma spp.

An alternative way to increasing the crop yield is by using biofertilizers besides chemical ones. These include...
substances obtained from living organisms and microbial sources (Chen, 2006). Biofertilizers have many different benefits such as; increased access of nutrients to plants, providing growth-promoting factors for plants and effective recycling of solid wastes (Chen, 2006; Das et al., 2007). Addition of biofertilizers had a major effect on vegetative growth characters of sweet pepper (Berova & Karanatsidis, 2009; Berova et al., 2010); total yield (Berova & Karanatsidis, 2008; Bogevska et al., 2009) and quality of sweet pepper plants (Ghonicame & Shafeek, 2005; Reyes et al., 2008). Accordingly, root infecting fungi can be controlled by pelleting seeds with biocontrol agents as a safe and effective method; in addition plant growth could be also promoted more by this method (Ramzan et al., 2016).

The aims of the current work are; to use the bacterial and fungal bioagents as safe, effective and eco-friendly biofungicides to control the major soilborne pathogens of colored pepper in Egypt. In addition to their use as biofertilizers to promote the growth and productivity of pepper for the first time in this country. Thus we could displace using the deleterious fungicides and chemical fertilizers in the fields of pepper.

Materials and Methods

Isolation and identification of the fungal pathogens of pepper plant: Naturally diseased pepper seedlings exhibiting typical symptoms of damping off, root and charcoal rot diseases were collected from pepper fields of El-Munofia governorate—Egypt at November 2014. The mycopathogens were isolated from root pieces of pepper on Potato dextrose agar medium (PDA) according to (Rashad et al., 2012). R. solani was identified as described by Sneh et al. (1991); F. solani (Nelson et al., 1983) and M. phaseolina (Barnett & Hunter, 1972).

Isolation and identification of the rhizosphere bacteria and mycoflora of pepper: Apparently healthy pepper plants with their intact roots and adhering soil were collected from pepper fields, and then bacterial and fungal antagonists were isolated from the rhizosphere soil of these pepper seedlings by serial dilution following the methods of Lodha & Webster (1990); Aneja & Sharma (2010).

Rose Bengal medium was used for isolation of fungi, whereas, soil extract agar medium (Gibbons & Rokas, 2013) and King’s medium B (Schaad, 1980) were used for isolating rhizosphere bacteria. Developed bacterial isolates were identified according to Faiy & Hayward (1983); Holt & Krieg (1994); Cappuccino & Sherman (1996). However, fungal colonies were inspected microscopically and only Trichoderma spp., were selected and transferred to Gliotoxin fermentation agar medium (GFM) (Mukherjee et al., 2012). Colony morphology on petri dishes and microscopical studies on slide culture according to Leahy & Colwell (1990), were adopted for identification of Trichoderma spp. Isolates were compared to a taxonomic key for the genus Trichoderma (Rifai, 1969).

Pathogenicity assays of the mycopathogens against pepper plant in the greenhouse

Preparation of pathogens inocula: Inocula of R. solani, F. solani and M. phaseolina isolates were prepared by growing each isolate on PDA medium for 5 d. Flasks containing autoclaved corn sand meal medium supplemented with 0.2% peptone solution (Abd El-Moity, 1985) were inoculated separately with equal disks 0.5 cm of each isolate, and then inoculated flasks were incubated at 25°C. After 15 d incubation, inoculum concentration of each isolate was adjusted to contain 5 x 10⁶ cfu/g by adding only sterilized corn sand meal medium and mixed through.

Soil infestation: Inocula of R. solani, F. solani and M. phaseolina isolates (5 x 10⁶ cfu/g) were added separately to soil in pots at the rate of 10 g/kg soil. Each pot was sowed with 5 seeds of pepper (var. Bunji), separately. Pots containing non-infested soil but autoclaved corn sand meal served as control. Ten pots were used for each treatment.

In-vitro antagonistic potential of the rhizosphere bacteria and Trichoderma isolates against the pepper pathogens

Rhizosphere bacteria: According to Sivanantham et al. (2013), bacterial isolates were streaked separately as thick bands on opposite edges of PDA plates. 4 mm diameter disc of each tested fungal pathogen was cut from of an actively growing culture by a sterile cork borer and then placed onto the center of these plates. The petri dishes were sealed using parafilm, incubated at 28-30°C in dark for 2-3 d. Mycelial disc of each pathogen only placed at center of PDA plates was maintained as control. The antagonistic potential of the bacterial isolates were recorded according to percentage of inhibition of radial growth of the fungal pathogens, compared with the control plates. This assay was conducted in five replicates for each isolate and repeated twice.

Rhizosphere Trichoderma spp.: Dual culture assay was conducted according to the methods of Sibounyawng et al. (2009a); Charoenporn et al. (2010). The selected mycoflora (T. harzianum, T. viride) and the 3 pathogens were cultured separately on PDA at 30-32°C for seven d. An agar plug of each pathogen was placed separately on one side of the PDA plate opposite to an agar plug of each tested Trichoderma isolate. Plates inoculated with a single plug of each pathogen only served as control, plates were then incubated at 30-32°C for 14 d. Five replicates were used for each fungal antagonist. Data were collected regarding pathogen colony diameter (cm). Percentage inhibition of pathogen radial growth was calculated using the following formula:

\[ \% \text{ inhibition of pathogen radial growth} = \frac{A- B}{A} \times 100 \]

where, A is the colony diameter of the pathogen on the control plate and B is the colony diameter of the pathogen when inoculated opposite to an antagonistic fungus. This experiment was repeated twice.
In the greenhouse, the selected bacteria and Trichoderma spp. against the pepper pathogens

**a. For bacterial antagonists**

According to the modified method of Gopalakrishnan *et al.* (2010), 8 treatments of the 2 selected bacterial isolates (*B. subtilis* and *P. fluorescens* with the 3 mycopathogens separately, and each bacterial isolate alone) were evaluated in the greenhouse for their *in vivo* antagonistic potential against the 3 pathogens. Pathogens inocula were mass produced separately on corn grains according to Gupta *et al.* (2002). Pot mixture (800 g) was prepared by mixing soil; sand and farm yard manure at 3:2:2 and filled in 8″ plastic pots followed by inoculation with each pathogen inoculum (200 g pot−1), inocula were mixed thoroughly with the pot mixture. 100 mL of water was added to each pot and then pots were covered with polythene sheets; the whole set-up was incubated at 32±2°C. One week later, pepper seeds (var. Bunjii) were surface sterilized with sodium hypochlorite solution (2.5% for 5 min), rinsed with sterilized water (4 times) and then allowed to sprout overnight in a Petri plate.

Sprouted seeds were transferred into the 2 bacterial isolates separately grown in nutrient broth medium (10⁸ cfu mL⁻¹) in presence of carboxymethyl cellulose for an hour, before being sown in pathogens infested soils (five treated seeds/pot). Doses of each bacterial isolate (5 mL per seedling) were applied twice separately (at 7 and 14 d after sowing) by soil drench method. Soils treated with fungal pathogens inocula only served as positive control; whereas, non-infested soil served as negative control. Ten pots were used for each set of treatments.

Growth parameters recognized include; numbers of germinating seeds, root length, shoot length, root fresh weight, shoot fresh weight and visual disease symptoms on pepper seedlings, which were compared with the positive and negative control seedlings. Disease incidences were recorded at day 21 after sowing.

**b. For Trichoderma isolates**

Preparation of pathogens inocula: *R. solani*, *F. solani* and *M. phaseolina* were grown on PDA medium at 25°C for 7 d in the dark. Inocula grown on crashed corn seeds were prepared according to Wong *et al.* (1984). Under aseptic conditions, the corn seeds were inoculated separately with four agar plugs (2 mm diameter each) cut from actively growing margins of each growing colony. Flasks were then incubated at 25°C for one week in the dark, shaken occasionally to ensure uniformity of colonization. Corn seeds free of inoculum and autoclaved twice served as control.

Preparation of antagonistic Trichoderma inocula: Inocula of each fungal antagonist (*T. harzianum* and *T. viride*) were prepared on crashed corn seeds in the same way described before for pathogens inocula.

Soil infestation: Eight treatments of the fungal antagonists (*T. harzianum*, *T. viride* with the 3 pathogens separately, and each fungal isolate alone) were evaluated for *in vivo* antifungal potency in the greenhouse. In reference to Madbouly *et al.* (2014), ten free draining pots were used for each antagonistic treatment; each containing 3–4 Kg of non-sterile clay soil. One hundred g of crashed corn seeds infested with each pathogen inoculum were dispersed separately through the lower quarter of soil in each pot, and then left for 2 d. Two hundred g of corn seeds treated with inoculum of each antagonistic fungus (12.7×10⁸ cfu/g corn seed) were dispersed in the upper quarter of soil in each pot. After adding the antagonist's inocula, 5 pepper seeds (var. Bunjii) were sown in the upper quarter of soil in each pot and pots were watered daily. Pots containing soil treated with the pathogens only served as positive controls, whereas non treated soil served as negative controls. After 4–5 weeks, growth parameters of pepper seedlings were recorded as described before with the bacterial antagonists.

**Statistical analysis:** All treatments were replicated twice, data were recorded as mean ± SD (standard deviation) and subjected to analysis of variance (ANOVA) to analyze differences between applied treatments and disease incidence.

**Results**

**Isolation of fungal pathogens, rhizosphere bacteria and mycoflora of pepper:** Isolation of the fungal pathogens from roots of pepper plants showing typical symptoms of damping off, root and charcoal rot diseases respectively, led to the recovery of five fungal isolates in the isolation plates. These were identified mycologically according to the cultural; morphological and microscopical characteristics as; *R. solani*, *F. solani*, *F. oxysporum*, *F. sambucinum* and *M. phaseolina*.

Isolation of rhizosphere bacteria from healthy pepper plants led to the detection of 8 colony morphotypes in the soil extract agar and King’s B isolation plates, these were coded as; Pep1-Pep8. They were identified biochemically as *B. subtilis*, *B. amyloliquefaciens*, *P. putida*, *P. fluorescens*, *P. aeruginosa*, *Enterobacter cowanii*, *Azospirillum sp.*, and *Paeunibacillus polymyxa*.

About 9 different fungal colonies isolated from the rhizosphere of healthy pepper plants were observed in the PDA plates. *Trichoderma* spp., colonies were specifically selected and then identified by examining their shape; size and development of conidiophores, they were identified as *T. harzianum* and *T. viride*.

**Pathogenicity assay of the isolated fungal pathogens in the greenhouse:** *R. solani*, *F. solani* and *M. phaseolina* isolates showed high virulent activities against the pepper seedlings in the greenhouse compared with the non-infested control soil, as they caused disease symptoms in about 85-90% of these seedlings. The other two isolates of *F. oxysporum* and *F. sambucinum* caused wilting of 25% of the pepper seedlings only, thus were regarded as avirulent isolates and not-considered for further research.
In vitro antifungal activities of the rhizosphere bacteria and Trichoderma spp. against the pepper pathogens: Five bacterial isolates (Azospirillum sp., B. amyloliquefaciens, Paenibacillus polymyxa; P. aeruginosa and P. putida) showed weak antifungal potential against the 3 pathogens (Table 1), consequently were neglected from further research. On the other hand, three promising bacterial isolates namely; B. subtilis, Enterobacter cowanii and P. fluorescens appeared to be effective in suppressing pepper pathogens under In vitro conditions.

Enterobacter cowanii showed moderate antifungal potency against the 3 pathogens, it was mostly effective against F. solani as it surrounded its colony and prevented its radial spread (60% inhibition). However, B. subtilis and P. fluorescens were highly antagonistic against all pathogens causing inhibitory activities ranging from 71-79%.

On the other hand, both of T. harzianum and T. viride expressed high In vitro antifungal efficacies against R. solani, F. solani and M. phaseolina, as they inhibited their radial growth by 80-87% as clear in (Table 1). Both isolates surrounded the pathogen colonies in dual culture plates and caused their restricted spread.

In vivo antifungal potency of the bioagents against the pepper soilborne pathogens in the greenhouse: Results of In vivo effect of the soilborne pathogens on the growth parameters of pepper separately, and in combination with the bacterial, fungal bioagents in the greenhouse are shown in (Tables 2 and 3).

In addition to post-emergence damping-off symptoms of pepper in R. solani treated soil (germinated seeds become soft mushy and then disintegrated, slightly darkened water-soaked lesions become visible on stems of young seedlings), R. solani caused decrease in the number of germinating pepper seedlings (38), root and shoot length (4, 3.5 cm), fresh wt. of root and shoot (7, 4 g), compared with the non-treated control soil (50, 13, 20 cm- 21, 24.5 g, respectively). However, infestation of soil with R. solani and each of the bacterial bioagents separately (Table 2) caused significant reduction in disease symptoms and improvements in the same growth parameters of pepper (B. subtilis: 42-10, 8.5 cm- 17.5, 12.5 g and P. fluorescens: 39-8.5, 6.5 cm- 15.5, 10 g) compared with R. solani treated soil.

On treatment of pepper soil with R. solani and each of the fungal bioagents separately (Table 3), we observed complete absence of disease symptoms and major enhancements in the growth parameters of pepper compared with pathogen infested soil (T. harzianum: 50-12.5, 11 cm- 21.5, 15.5 g and T. viride: 48-11, 9.5 cm-20, 12 g) and with the non-treated control soil.

In pepper soil infested with F. solani and M. phaseolina only, they caused typical disease symptoms. Similarly, the same antagonistic potential of the bacterial and fungal bioagents were observed against both of F. solani and M. phaseolina respectively, as clear in (Tables 2 and 3). Infestation of soil with F. solani and M. phaseolina in presence of both of the bacterial and fungal bioagents separately, caused reduction of the virulence of these pathogens in addition to improvements of growth parameters of pepper.

Inoculation of soil with each of the bacterial bioagents only (Table 2) caused promotion of the growth parameters of pepper even more than the non-treated soil (B. subtilis: 50-14, 22 cm- 23.5, 26.5 g and P. fluorescens: 50-13, 20.5 cm- 22, 24.5 g). The growth promoting effect of B. subtilis was more than that of P. fluorescens. On the other hand, treatment of soil with each fungal bioagent only (Table 3) enhanced the growth of pepper significantly (T. harzianum: 50-15.5, 23 cm- 25.5, 27.5 g and T. viride: 50-14, 21 cm- 24, 22 g) compared with the control soil. T. harzianum exerted stimulating effect on the growth parameters of pepper seedlings more than that of T. viride. The bioagents and the pathogens were re-isolated from the upper and lower soil layers respectively, thus verifying Koch’s postulates.

Table 1. In vitro antifungal activities of the rhizosphere bacteria and Trichoderma isolates against R. solani, F. solani and M. phaseolina pathogens of pepper, using dual culture technique.

<table>
<thead>
<tr>
<th>Bacterial\fungal antagonists</th>
<th>% inhibition of mycelial radial growth</th>
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<tbody>
<tr>
<td></td>
<td>R. solani</td>
</tr>
<tr>
<td>Azospirillum sp.</td>
<td>29±0.02</td>
</tr>
<tr>
<td>B. amyloliquefaciens</td>
<td>27±0.10</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>74±0.02</td>
</tr>
<tr>
<td>Enterobacter cowanii</td>
<td>47±0.09</td>
</tr>
<tr>
<td>Paenibacillus polymyxa</td>
<td>32±0.07</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>20±0.16</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>71±0.0</td>
</tr>
<tr>
<td>P. putida</td>
<td>31±0.10</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>85±0.12</td>
</tr>
<tr>
<td>T. viride</td>
<td>81±0.65</td>
</tr>
</tbody>
</table>

* Results are averages of 5 replicates. ± mean SD (standard deviation); mean values followed by the same letter are not significantly different (p<0.05).
Discussion

Isolation of fungal pathogens from infected pepper seedlings led to the recovery of 5 fungal isolates, three of them only were selected according to their virulence in the greenhouse, mainly: *R. solani* (causal agent of damping-off disease) (Yang et al., 1992), *F. solani* (root rot) (Agrios, 1988) and *M. phaseolina* (known of causing charcoal rot disease) (Jana et al., 2005).

Three rhizosphere bacterial isolates showed considerable *In vitro* antifungal activities against the three fungal pathogens. *Enterobacter cowanii* showed moderate antifungal potential against *F. solani* only, thus was excluded from further research. On the other hand, *B. subtilis* and *P. fluorescens* had good antifungal potency against the three mycopathogens in accordance with results of Tan et al. (2013); Rajeswari, (2015). This *In vitro* antifungal potency was attributed by Zarrin et al. (2009) to the production of inhibitory substances, antifungal antibiotics, cell wall degrading enzymes and siderophores released by the bacteria into the culture media.

Many species of rhizosphere fungi were observed in the PDA isolation plates but *Trichoderma* isolates (identified as *T. harzianum* and *T. viride*) only were selected, because of being potent biocontrol agents of many fungal pathogens (Mokhtar & Dehimat, 2014; Naglot et al., 2015). In the current study, *T. harzianum* and *T. viride* showed appreciable *In vitro* antifungal potential against the 3 pathogens, they overgrow and prevented them from radial spread. These observed activities might be attributed to more than one mechanism such as: mycoparasitism (as they overgrow the pathogens) and production of antifungal antibiotics (direct antagonism), in accordance with Zeilingier & Omann (2007); Vinale et al. (2008).

In the greenhouse, treatment of soil with the fungal pathogens and the bacterial bioagents (*B. subtilis* and *P. fluorescens*) reduced the virulence of these pathogens and improved the growth parameters of pepper.
compared with soil infested with the pathogens only. These In vivo antifungal potential could be attributed to the production of antifungal antibiotics, cell wall degrading enzymes, and/or Fe-chelating siderophores. In a previous study, Ahimou et al. (2000) attributed the antifungal potential of Bacillus sp. to its ability to synthesize a wide variety of antifungal lipopeptides such as classes of surfactin, iturin and fengicin which were able to modify hydrophobicity of bacterial surfaces, consequently their adhesion to fungal mycelia.

The growth promotion of pepper seedlings by B. subtilis and P. fluorescens was explained before by Weller, (2007) as rhizobacteria are aggressive root colonizers so may enhance plant growth by producing metabolites, incorporating root exudates and competing with other soil microbes. In later studies, they attributed these promoting activities to ACC deaminase and phosphate solubilization (Shaharoona et al., 2008) and production of siderophores (Braud et al., 2009).

On infestation of pepper soil with T. harzianum and T. viride together with the fungal pathogens separately, we observed considerable reduction of disease symptoms and pronounced improvement in the growth parameters of pepper. Howell, (2003) referred the strong antifungal potential of T. harzianum to the production of chitinolytic and glucanolytic enzymes, which break down chitin and β-glucan polysaccharides responsible for fungal cell wall rigidity.

The use of chemical fertilizers to enhance soil fertility and crop productivity have many disadvantages. Trichoderma have the ability to promote plant growth directly through solubilization of phosphates, minerals such as: Fe, Mn and Mg which have important role in plant growth, and indirectly through control of the rhizosphere root pathogens. Vinale et al. (2008) added that the enhanced plant growth by T. harzianum was due to the production of secondary metabolites as auxin like compounds, which cause development of the root system and exploration of a larger volume of the soil.

In accordance with our results, the protection exerted by T. harzianum against the fungal pathogens was more than B. subtilis, this difference might be due to several modes of actions exerted by Trichoderma spp., as they are well-known producers of cell wall-degrading enzymes and antibiotics. The antagonistic T. viride and P. fluorescens occupied significantly the second degree of reducing soilborne pathogens in accordance with Abdel-Kader et al. (2012).

This is the first time to record the In vivo growth promoting activities of B. subtilis and T. harzianum on pepper plant in Egypt. Our future prospectus is to mass produce and then formulate these bioagents to be used as safe, ecofriendly biofungicides against pepper soilborne pathogens, and at the same time as effective biofertilizers to promote the productivity of this crop in the fields of Egypt.

Conclusion

B. subtilis and T. harzianum could be used as potent biofungicides to reduce the incidence of major soilborne fungal pathogens of colored pepper, moreover, they may be applied as biofertilizers to promote the growth and productivity of this crop. In the future, both bioagents could be applied on a wide scale in the pepper fields of Egypt, hence displace the use of deleterious, environmentally non-safe synthetic fungicides and chemical fertilizers.

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