

REVIEW ARTICLE

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Biological control of *Phaseolus vulgaris* and *Pisum sativum* root rot disease using *Trichoderma* species

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Abstract

Background: Root rot pathogens reported to cause considerable losses in both the quality and productivity of common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.). It is an aggressive crop disease with detrimental economic influence caused by *Fusarium solani* and *Rhizoctonia solani* among other soil-borne fungal pathogens. Destructive plant diseases such as root rot have been managed in the last decades using synthetic pesticides.

Main body: Seeking of economical and eco-friendly alternatives to combat aggressive soil-borne fungal pathogens that cause significant yield losses is urgently needed. *Trichoderma* emerged as promising antagonist that inhibits pathogens including those inducing root rot disease. Detailed studies for managing common bean and pea root rot disease using different *Trichoderma* species (*T. harzianum*, *T. hamatum*, *T. viride*, *T. koningii*, *T. asperellum*, *T. atroviridae*, *T. lignorum*, *T. virens*, *T. longibrachiatum*, *T. cerinum*, and *T. album*) were reported both in vitro and in vivo with promotion of plant growth and induction of systemic defense. The wide scale application of selected metabolites produced by *Trichoderma* spp. to induce host resistance and/or to promote crop yield, may represent a powerful tool for the implementation of integrated pest management strategies.

Conclusions: Biological management of common bean and pea root rot-inducing pathogens using various species of the *Trichoderma* fungus might have taken place during the recent years. *Trichoderma* species and their secondary metabolites are useful in the development of protection against root rot to bestow high-yielding common bean and pea crops.

Keywords: *Phaseolus vulgaris*, *Pisum sativum*, Root rot, *Trichoderma* spp., Secondary metabolites, Biological control

Background

Soil-borne fungal diseases which occurring worldwide can destroy agricultural crops and cause significant yield losses. Fungal pathogens are considered a potent cause of soil-borne plant diseases (Strange and Scott 2005) and more than 1200 fungal species were implicated in plant diseases or crop failure in various major crops (Consolo et al. 2012). The common bean (*Phaseolus vulgaris* L.) is considered a strategic crop, especially in South America, Africa, and Asia (Torres

et al. 2009). Common bean represents a great source of carbohydrates, proteins, minerals, vitamins, and fibers in the human diet (Broughton et al. 2003). Common bean and the pea (*Pisum sativum* L.) plants are continually exposed to diverse root rot pathogens with main disease symptoms of reddish-brown lesions on the hypocotyl and tap roots, vascular discoloration, foliar chlorosis and wilt, and seedling death (Ronquillo-López et al. 2010). Root rot disease in different regions of the world may be caused by several fungal pathogens and fungal-like organisms including *Fusarium solani*, *Rhizoctonia solani*, *Pythium* spp., and *Fusarium oxysporum* (Ronquillo-López et al. 2010).

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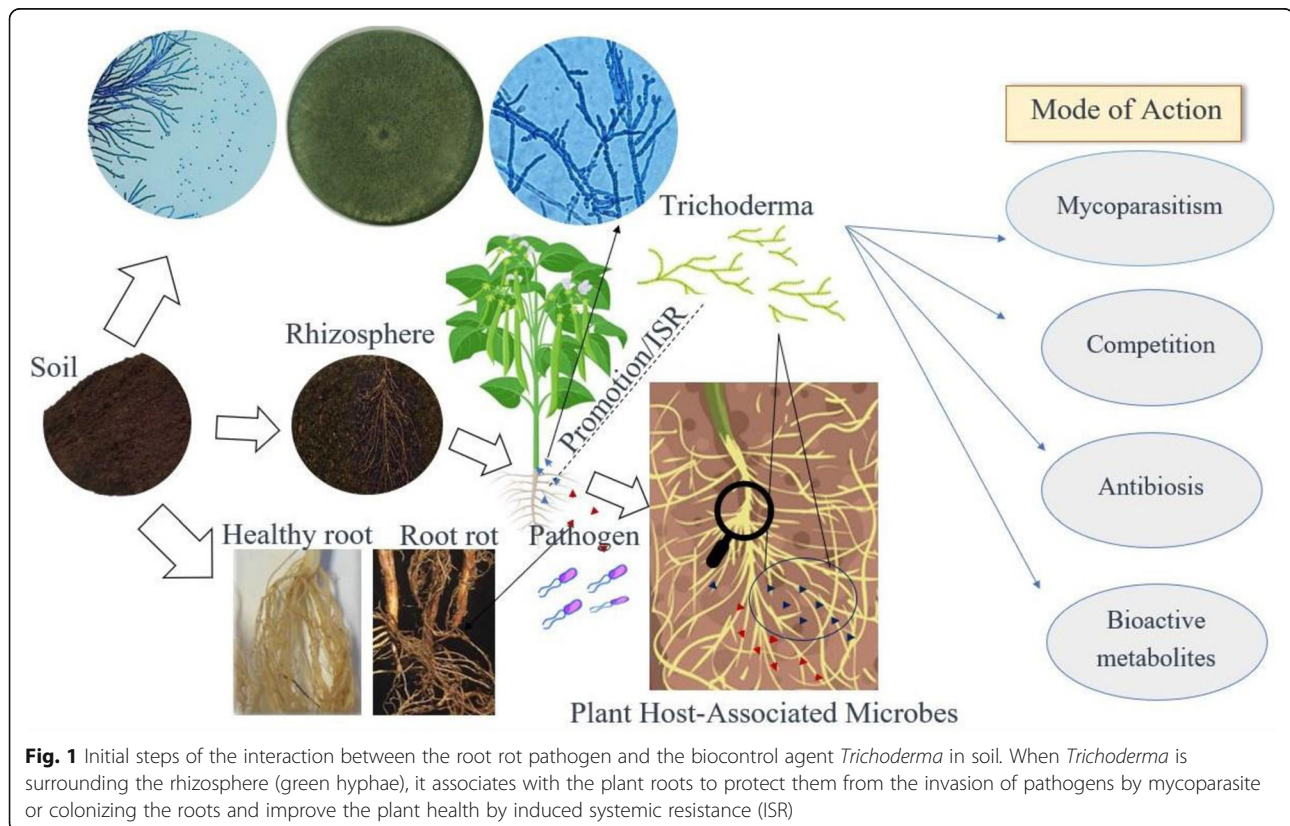
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Fusarium solani is one of the most aggressive pathogens that limits the productivity of common bean and is considered the most damaging *Fusarium* species (Toghueo et al. 2016). In some developing countries, root rot disease caused by *Fusarium* can wipe out the entire crop (Ongom et al. 2012). Similarly, *R. solani* has the potential to reduce the productivity and cause severe damage up to 94% disease incidence in the susceptible varieties and 39% in the resistant cultivars, causing a reduction of the root system in length and weight (Farrag 2011). Additionally, root rot pathogens can interact and increase disease severity, e.g., *Fusarium* spp. and *Rhizoctonia* spp. can form a high synergistic relationship on bean plants. *F. solani* is known to form thick-walled chlamydospores that can survive in soil in the absence of a host plant. Also, *R. solani* survives in soil for a long time through formation of sclerotia or fungal mycelium.

Various methods have been examined to manage root rot, particularly fungicide application (Al-Askar and Rashad 2010). However, abuse of these compounds can cause inhibition of pollinators, and beneficial soil microbial communities, and the emergence of fungicide-resistant pathogens. Additionally, the accumulation of these toxic compounds has detrimental effects on human health and the environment. Disease management using biocontrol agents represents a potential alternative to synthetic fungicides and some of the most promising

biocontrol agents belong to the genera *Trichoderma*. They have a variety of mechanisms to colonize various ecological niches through a broad antagonist spectrum, antibiotic, and mycoparasitism activity, and promote plant growth (Benítez et al. 2004). *Trichoderma*-based formulations are the most successful bio-fungicides used in integrated pest management, with more than 60% of the registered bio-fungicides based on *Trichoderma* species (Verma et al. 2007). They are currently used as commercial biological control agents against fungal and fungal-like root rot pathogens such as *R. solani*, *Pythium* spp., and *Fusarium* spp. (Saba et al. 2012). Several studies investigated the antagonistic activity of *Trichoderma* spp. against *F. solani* and *R. solani* to assess their efficacy as biocontrol agents and to elucidate their antagonistic mechanisms. Through various in vitro and in vivo assays, the antagonistic capacity with various mechanisms of *Trichoderma* spp. was explained (Fig. 1) including mycoparasitism (Fig. 2), induction of plant resistance, inhibitory metabolite production (antibiosis), plant growth stimulation, competition for nutrient and space, and production of lytic enzymes (Bastakoti et al. 2017). Their inhibition ability against root rot pathogens varied depended on both the specific *Trichoderma* species and pathogen (Singh et al. 2016).

This present review summarizes the recent studies that investigated the antagonistic ability of *Trichoderma* spp.



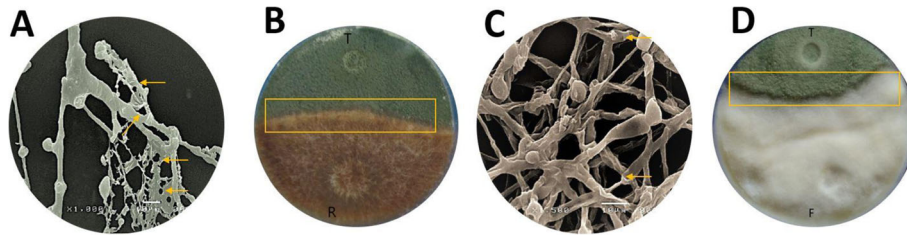


Fig. 2 Mycoparasitism of *Trichoderma* species: **A** SEM photomicrographs indicate the holes (yellow arrows) caused by the interaction between *Trichoderma harzianum* and root rot pathogen (*Rhizoctonia solani*) after 4 days of incubation, (magnification 500 ×; bar = 10 μm). **B** Dual culture assay shows the *T. harzianum* interact with *R. solani* in vitro. **C** Hyphal interaction of *Trichoderma asperellum* and a root rot pathogen (*Fusarium solani*) after 5 days of incubation, yellow arrows show the coiling of hyphae (magnification 1000 ×; scale bar = 10 μm). **D** Antagonism of *T. asperellum* strains against *F. solani* following a confrontation test

against fungal pathogens, particularly *F. solani* and *R. solani* that cause root rot disease in *P. vulgaris* and *P. sativum* crops. Moreover, describing the factors affecting *Trichoderma* efficiency. Finally, future prospects concerning the key factors needed for the development of reliable screening techniques for the prediction of biocontrol efficiency of a given isolate.

***Trichoderma* species against *Phaseolus vulgaris* root rot disease**

Several studies investigated the antagonistic activity of *Trichoderma* spp. against *F. solani* and *R. solani* to assess their efficacy as biocontrol agents and to elucidate their antagonistic mechanisms through various in vitro and in vivo assays and in open field trials. In a greenhouse condition study, *T. harzianum* was applied as a biocontrol agent against *R. solani* via 2 methods of applications; simultaneous application with the pathogen; and 1 month before the pathogen inoculation. Recorded data indicated that *T. harzianum* could only suppress the root rot disease induced by *R. solani* in the pre-inoculation treatment with no effect on the disease in the simultaneous application. Findings reported by Nasir Hussein et al. (2018), showed that *T. harzianum* treatment reduced the disease severity up to 49% than the infected (untreated) control treatment. Moreover, bean plants treated with *T. harzianum* were improved in growth parameters in comparison with untreated plants. This study indicated that the timing application of treatment with *T. harzianum* may have a significant effect against *R. solani*. This pathogen aggressively attacked the hypocotyl part of the bean plant causing infection. *T. harzianum* colonization of the hypocotyl enhances the plant functions to suppress *R. solani* pathogen (Nasir Hussein et al. 2018).

In another interesting study, the biocontrol capacity of 3 *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *Trichoderma* spp.) against *R. solani* was assessed both in vitro and in vivo experiments (Asad et al. 2014). Dual culture results showed that the 3 isolates inhibited

R. solani through parasitism, overgrowing the pathogen after 1 week of incubation. Also, water-soluble metabolites extracted from the tested isolates were high in antagonistic effect against *R. solani*, as shown with *T. asperellum*, which exhibited the highest rate of inhibition (74.4%), followed by *Trichoderma* spp. (70.0%) and *T. harzianum* (67.8%). In the in vivo assay, *Trichoderma* species were applied at 7 days before and after the pathogen's inoculation. Bean plants treated with *T. asperellum*, 1 week before pathogen inoculation, had the highest relative biomass (127%), followed by *T. harzianum* (126%), 1 week after pathogen inoculation compared to the untreated healthy control (Asad et al. 2014).

From these results, it could be established that the timing of *Trichoderma* species application is an important factor that limiting the efficiency. Simultaneous application of *Trichoderma* species with the pathogen was not effective in comparison to 1 week or even 1 month before the pathogen inoculation. Furthermore, evaluating the efficiency of biocontrol agent *T. harzianum* against *R. solani*, in the greenhouse and open field trials was achieved by Matloob and Juber (2013). They found that *T. harzianum* exhibited a high antagonistic activity against *R. solani* and notable improvement of plant growth parameters and yield components. The greenhouse results showed that *T. harzianum* reduced disease incidence (31.2%) and severity (18.8%) of bean root rot than the infected control treatment (*R. solani*), (100%) incidence and (80%) severity, and increased fresh and dry weights (3.25 and 0.305 g), than the infected control treatment (*R. solani*) (1.36 and 0.162 g). Besides, the field trials showed that *T. harzianum* reduced the incidence (50.0%) and severity (26.8%) of root rot than the infected control treatment (*R. solani*) (100 and 76.8%) of disease incidence and severity, respectively. Significant increment in dry weight and yield (487.3 g and 1.83 kg), respectively was also noted when *T. harzianum* was applied, in comparison to the infected control treatment (*R. solani*) (213.7 g and 0.61 kg) of dry weight and yield, respectively.

The great potential of *T. harzianum* as a biocontrol agent against *R. solani* was reported by Mayo et al. (2015) who evaluated the antagonistic ability of 23 *Trichoderma* isolates, using dual cultural technique and inhibitory metabolites assessment in vitro. The highest inhibition rate (72.77%) in the confrontation assay was produced by T021 isolate. Additionally, 5 *Trichoderma* isolates (T003, T004, T006, T020, and T022) had a high inhibition rate (86.7%). Fifteen *Trichoderma* isolates were selected based on the in vitro analysis to test in vivo against *R. solani*. Bean plants treated with isolate T019 alone had the highest growth, while the plants treated with both *Trichoderma* isolate T019 and *R. solani* had a similar growth to untreated plants. *Trichoderma* isolate T019 was identified, using internal transcribed spacer 1 (ITS1) sequence to be *T. harzianum*.

Another, *T. harzianum* isolate (RU01) was tested in vitro against *F. solani*, using the dual culture technique. Obtained results indicated that RU01 isolate inhibited the mycelial growth of *F. solani* through overgrowing after 3-4 days of incubation (Abeyasinghe 2012). Surprisingly, *F. solani* was able to over grow the *Trichoderma* mycelia afterward. There was a reduction in conidia spore production than the control treatment. In a greenhouse experiment, disease parameters (lesion diameter and disease severity) were lower in treated seedlings than the infected (untreated) plants. It also promoted *P. vulgaris* growth by increasing both the length and weight of fresh root (Abeyasinghe 2012). This study combined the in vitro and greenhouse application of *T. harzianum* RU01 to assess its antagonistic capacity and confirm its ability to combat root rot in common bean. The antagonistic activity of *T. harzianum* RU01 was explained by parasitism in this research.

Moreover, endophytic *Trichoderma* spp. were also reported to be used as biocontrol agents against soil-borne pathogenic fungi. Toghueo et al. (2016) isolated endophytic *Trichoderma* spp. from the bark of almond (*Terminalia catappa*) and the isolates with the highest potential of antagonistic effect against *F. solani* were identified through ITS-5.8S rRNA region sequencing as *T. atroviridae* and *Trichoderma* spp. The antagonistic activity of these 2 endophytes was evaluated in vitro. The results of the dual culture technique between *F. solani* and the 2 endophytes showed that *F. solani* was inhibited at 90% by *T. atroviridae* and 86.99% by *Trichoderma* species. *T. atroviridae* was more aggressive than *Trichoderma* spp. against *F. solani* in spore to spore confrontation assessment at (34.36 and 22.68%) after 24 h, respectively. Volatile compounds produced by both isolates had similar inhibition effect against *F. solani*, but the non-volatile components of *T. atroviridae* scored higher mycelial growth inhibition of *F. solani* than *Trichoderma* species. *P. vulgaris* seed germination was

promoted to 100% in the first day of germination using 2×10^5 conidia/ml of *T. atroviridae* and promoted to 77.09% at 8×10^5 conidia/ml, in the presence of *F. solani* compared to (57%) germination in the presence of *F. solani* alone. Bean seedling germinated from seeds treated with *T. atroviridae* had a lower disease severity and incidence (11.42 and 40%), respectively, than the non-treated plants. This research interrupted the biocontrol ability of *Trichoderma* spp. with the established mechanisms from earlier studies (i.e., parasitism, nutrient and space competition, and antibiosis) and explored other modes of action related to non-volatile and volatile metabolites production. Obtained results of both in vitro and in vivo indicated that *T. atroviridae*, as a biocontrol agent, had a potential effect against *F. solani*.

In the open field experimental trials and in vitro assays, 4 *Trichoderma* species (*T. hamatum*, *T. harzianum*, *T. album*, and *T. viride*) were investigated as biocontrol agents against *F. solani* and *R. solani* (Abd-El-Khair et al. 2010). Each of the previous *Trichoderma* species showed an antagonistic capacity in vitro, with *T. hamatum* that had the highest inhibitory effect (reducing mycelial growth) against both *R. solani* and *F. solani*. In the field application, common bean plants treated with *T. album* and *T. viride* had 0% of root rot. In addition, all the tested species induced defense responses than the control treatment. The inhibition effect against *R. solani* and *F. solani* may be due to the accumulation of enzymes such as chitinase, peroxidase, and polyphenol oxidase. So, accumulation and upregulation of antioxidant enzymes in the host plant might play an essential act in protection against pathogens (Ketta 2015). *T. viride* exhibited the highest activity of chitinase enzyme (430%), followed by *T. album* (174%), *T. harzianum* (150%), and *T. hamatum* (132%). However, *T. harzianum* increased the activity of polyphenol oxidase enzyme in bean plants (255%), followed by *T. viride* (108%), *T. hamatum* (103%), and *T. album* (58%). The range of peroxidase enzymatic activity was 26-124% with *Trichoderma* treatment application. Moreover, there was a positive correlation between the macro- and micronutrients concentration and enzyme activity, i.e., chitinase, peroxidase, and polyphenol oxidase in the bean plants treated with all *Trichoderma* species which resulted in higher growth and yield parameters (Abd-El-Khair et al. 2010).

Several studies investigated the biocontrol ability of specific *Trichoderma* species against root rot fungal pathogens. One of the most studied *Trichoderma* species was *T. harzianum*, which showed an efficacy against *F. solani* and *R. solani*. A proteomic study of the effect of *T. harzianum* ALL 42, isolated from Brazilian soil, on growth promotion of *P. vulgaris* and inducing its defense mechanisms against *F. solani* and *R. solani*, was

conducted to elucidate the molecular mechanisms responsible for these interactions (Pereira et al. 2014). *T. harzianum* ALL 42 promoted the agronomical parameters of bean plants. Bean plants treated with *T. harzianum* ALL 42 showed a differential expression pattern for defense response genes that encoded chitinase, glucanase, lipoxygenase, and peroxidase compared to untreated plants, as well as treated plants with *F. solani* or *R. solani* alone. In addition, *T. harzianum* ALL 42 increased plant resistance to *R. solani* as they increased defense response genes encoding peroxidase and glucanase compared to bean plants treated with *R. solani* alone. The proteomic analysis results indicated that 33, 22, and 11% of the identified proteins related to metabolism, defense response, and oxidative stress response, respectively were found in the bean leaves. In addition, 17.2, 24.1, and 10.3% of the identified proteins related to metabolism, defense response, and oxidative stress response, respectively were found in the bean roots. This work aimed to elucidate the molecular mechanisms associated with inducing bean plant resistance and growth promotion when treated with *T. harzianum* ALL 42, but did not identify the exact main plant defense mechanism. However, the growth-promoting ability of *T. harzianum* ALL 42 was partially acting due to its ability to trigger expression of genes encoding defense response-related enzymes. This expression was minimal for bean plants treated with *T. harzianum* ALL 42 and *F. solani*. This study showed the higher ability of *T. harzianum* ALL 42 to promote the growth and induce the plant resistance in the presence of *R. solani* rather than *F. solani* (Keswani et al. 2014).

Another investigation achieved by Akrami et al. (2012) reported the protective effect of *T. harzianum* (T1) and *T. asperellum* (T2) individually and in combination in the bean seed pelleting against *F. solani*. It concluded that the reduction of root rot disease was (59.8%) after adding both *T. harzianum* (T1) and *T. asperellum* (T2) with concentration (3×10^8 conidia/ml) in 10% sugar suspension for seed pelleting. In contrast, the reduction rate was 53.5% after adding the above species in water suspension. Moreover, *T. harzianum* (T1) alone exhibited a high reduction of 53.4% than *T. asperellum* (T2), which scored (42.9%) under concentration of 6×10^8 conidia/ml in 10% sugar and water suspension as well. Muriungi et al. (2013) evaluated the efficacy of *T. viride* and *T. koningii* against *Fusarium* root rot of bean in vitro and in vivo. They investigated the sporulation ability of *Trichoderma* species on 3 types of carrier media (broken rice grains, vermiculite, and sorghum grains). In vitro studies showed that the inhibition percentage of *Fusarium* growth (1 week old after culturing) was closed to 100%, when *T. viride* was applied using sprinkling or equidistant methods described by Dhingra

and Sinclair (1986). In addition, a very poor growth of *Fusarium*, with 91% of growth reduction, was observed when *T. koningii* was applied using the same methods. In greenhouse trials, *T. viride* grown in broken rice grains exhibited 32% root rot severity, which was lower than the fungicide (metalaxyl 10% + imidacloprid 10% + carbendazim 10%) and un-infested control treatment. Similarly, *T. koningii* grown in broken rice grains resulted in (56%) severity (moderate) with non-differences with the fungicide. Interestingly, broken rice grain medium was the most effective one for both growth and survival of *Trichoderma* (spore concentration per gram of carrier) determined after 18 days of both *T. viride* and *T. koningii* with (2.5×10^7 and 1.4×10^7 conidia/g), respectively. From these results, it could be recommended that, adding of 10% sugar during the preparation of *Trichoderma* suspension for bean seed pelleting against *F. solani* increases the inhibition rate compared to water suspension. Moreover, carrier media are used for *Trichoderma* growth playing an important role in the improvement of its sporulation ability and consequently its efficacy against pathogenic fungi. So, the factors affecting *Trichoderma* efficiency could be concluded here as follows: (1) Timing of application (before or after the inoculation process of pathogenic fungus), (2) adding of carbohydrate source (10% sugar) during the preparation of *Trichoderma* suspension for bean seed pelleting is more effective than water suspension, and (3) using of broken rice grains carrier media for *Trichoderma* growth plays an important role in the improvement of its sporulation ability and consequently its efficacy against pathogenic fungi.

The field application of bio-fungicides alone in management of soil-borne fungal pathogens is not highly effective. Hence, combinations of bio- and synthetic fungicides alternatively could be effective against the fungal pathogens with reduction amount of the fungicide applied. For instance, the combination of *T. harzianum* with methyl bromide fumigant in reduced amount under field application was achieved by Barakat (2002) who found that the combination reduced the incidence of bean root rot and damping-off caused by *R. solani*. The study concluded that using either *T. harzianum* alone or methyl bromide in reduced amount was not highly effective in disease management of bean plants. An increment in vegetative parameters, fresh and dry weight and yield components were found with a sub-lethal dose of methyl bromide combined with *T. harzianum*. Yield components increased by 75%, when *T. harzianum* was combined with a reduced dose of methyl bromide compared to the control treatment, which was close to the full dose of the fumigant. Unfortunately, field application of *Trichoderma* spp. in management of soil-borne fungal pathogens is limited and not highly effective.

***Trichoderma* species against *Pisum sativum* root rot disease**

Another common and valuable food source for millions of people worldwide is pea (*Pisum sativum* L.), the essential leguminous crop for both local consumptions as well as exportation. Although the high-yielding cultivars are produced, the average seed yield per unit is still insufficient due to the infection with many diseases (Hamid et al. 2012). Pea root rot and damping-off diseases caused by soil-borne pathogenic fungi, such as *F. solani* and *R. solani*, are the most severe seedling diseases that cause substantial losses, either in seed quality or in yield. Hamid et al. (2012) studied the efficiency assessment of *T. harzianum*, *T. viride*, *Pseudomonas fluorescens*, and *Gliocladium virens* against *F. solani*. In vitro results indicated that *T. harzianum* exhibited the highest inhibition percentage in dual culture investigation (78.60%), followed by *T. viride* (75.72%), *G. virens* (69.52%), and *P. fluorescens* (68.37%). In pot experiments, *T. harzianum* reduced the disease incidence and severity (21.30 and 10.94%), respectively, followed by *T. viride* (25.30 and 12.02%), *P. fluorescens* (29.28 and 14.98%), *G. virens* (38.64 and 17.58%), than the fungicide carbendazim 50 WP (14.64 and 4.98%). No difference occurred between *T. harzianum* and carbendazim in seed germination percentage (90.00 and 90.00%), and number of days needed for seed germination (7.26 ± 0.07 and 7.34 ± 0.10), respectively, followed by *T. viride* (86.00% and 7.32 ± 0.13), *P. fluorescens* (83.00% and 7.37 ± 0.08), *G. virens* (82.00% and 7.53 ± 0.08), than the control treatment (61.00% and 7.88 ± 0.08).

The efficacy of *T. harzianum*, *T. viride*, *G. virens*, and *P. fluorescens* on managing of pea root rot disease, caused by *F. solani* was evaluated by Mudasar et al. (2012). In field trials, *T. harzianum* reduced disease severity percentages (63.81%), followed by *T. viride* (60.44%), *P. fluorescens* (51.19%), *G. virens* (41.82%) than the fungicide carbendazim 50 WP (82.42%). No difference occurred between *T. harzianum* and the fungicide in seed germination percentage (80.00 and 80.00%), as well as the number of days needed for seed germination (6.92 ± 0.05 and 7.01 ± 0.08), respectively, followed by *T. viride* (75.00% and 6.94 ± 0.08), *P. fluorescent* (73.00% and 6.98 ± 0.07) and *G. virens* (70.00% and 6.98 ± 0.07), than the control treatment (59.00% and 7.21 ± 0.06).

Evaluation of seed treatment was investigated as well as soil application of *T. koningii* SMA-7 (Bio-1), RMA-8 (Bio-II), and JMA-11 (Bio-III), and *T. harzianum* SMA-4 (Bio-IV) by Kapoor et al. (2006). These treatments were mixed with farmyard manure against *F. solani*. In vitro investigations established that the maximum mycelial growth inhibition of *F. solani* was recorded (82.16%) with *T. harzianum* SMA-4 (Bio-IV), followed by *T. koningii* JMA-11 (Bio-III) at 80.60%, RMA-8 (Bio-II) at

75.19%, whereas SMA-7 (Bio-1) at 70.53%. In addition, they investigated the 4 bioagents of *Trichoderma*, in the form of soil and seed applications against *F. solani* under open field conditions. The most effective bioagent, through soil application, was *T. koningii* SMA-7 (Bio-1), which exhibited the least infection rate (3.46%) of root rot-wilt complex, followed by *T. harzianum* SMA-4 (Bio-IV) with 3.63%, *T. koningii* RMA-8 (Bio-II) with 9.49%, and *T. koningii* JMA-11 (Bio-III) with 23.13% than the control treatment (32.50%). Furthermore, the used bioagents were less effective through seed application than using a soil application. *T. koningii* RMA-8 (Bio-II) scored the least disease incidence (19.11%) of root rot-wilt complex, followed by *T. koningii* JMA-11 (Bio-III) with 25.03%, *T. harzianum* SMA-7 (Bio-1) with 31.62%, *T. koningii* SMA-4 (Bio-IV) with 33.97% compared to control treatment (32.50%).

Another important point, the efficiency of seed priming (polyethylene glycol-PEG-8000 in ration 30.2 g/100 ml), seed dressing (fungicide Rizolex-T 50% WP at a recommended dose of 3 g/kg seeds), and seed coating [seeds were immersed in 1% carboxymethylcellulose (CMC) for 30 min, then coated with an individual suspension (10^7 CFU/ml) of *Bacillus subtilis*, *Pseudomonas fluorescence* and 3×10^4 conidia/ml of *T. harzianum*]. Then, seed bio-priming (spore suspension of *T. harzianum*, as well as bacterial suspension of *B. subtilis*, *P. fluorescence* supplemented with 1% CMC solution, against pea root rot disease induced by *R. solani*, *F. solani*, *F. oxysporum*, *Sclerotium rolfsii*, and *Pythium* spp. Furthermore, plant growth and crop yield, under greenhouse and open field conditions, were examined during the 2 growing seasons 2005/06 and 2006/07 by El-Mohamedy and Abd El-Baky (2008). Interestingly, the results of greenhouse experiment indicated that the incidence of disease, at pre-emergence (15 days after sowing) and root rot (45 days after sowing), was suppressed by all types of seed treatments. Seed bio-priming with *T. harzianum* reduced the pre-emergence damping-off 15 days after sowing by *F. solani* (58.8%), *R. solani* (50%), and *S. rolfsii* (50%), followed by seed coating treatment, *F. solani* (29.4%), *R. solani* (30%), and *S. rolfsii* (28.5%), than the control treatment with a reduction 0% of all pathogens. The same trend was observed when applying the bio-priming seed treatment, which resulted in a reduction of *F. solani*, *R. solani*, and *S. rolfsii*, after 45 days from sowing, followed by seed coating treatment. Moreover, the results obtained from the field experiment showed that the disease incidence, in the pre-emergence (15 days after sowing) and root rot (45 and 60 days after sowing), was eliminated by types of seed treatments used over 2 seasons 2005/06 and 2006/07. In the first season 2005/06, seed bio-priming with *T. harzianum* resulted in a decrease in disease incidence, in the pre-emergence

damping-off 15 days after sowing) with all pathogens (72.8%), root rot after 45 days (72.2%), and root rot after 60 days (67.6%), followed by seed coating treatment with 48.4, 46.3, and 43.2%, respectively, than the control treatment with 0% reduction in all stages. The same trend was observed during the following season 2006/07 when the bio-priming seed treatment resulted in the highest efficacy, followed by seed coating treatment. Moreover, during both seasons, bio-priming followed by seed coating with *T. harzianum* treatments stimulated plant height, the average number of leaves/plant, the average number of branches/plant and dry weight of shoots/plant, as well as yield improvement, i.e., the average number of pods/plants, average pod weight/plant and total yield of pea plants than the control treatment.

El-abd et al. (2013) studied the effect of biological seed treatments, such as priming with 1% carboxymethylcellulose, seed coating with (3×10^6 conidia/ml) of *T. harzianum*, and bio-priming 1% CMC mixed with (3×10^6 conidia/ml) of *T. harzianum*, against pea root rot and damping-off diseases, growth and yield of plants under different concentrations of phosphorus fertilization (0, 25, 50, and 75 kg P_2O_5 /feddan) (feddan = 4200 m²). The results of pre-emergence damping-off percentages indicated that the usage of bio-priming 1% CMC mixed with (3×10^6 conidia/ml) of *T. harzianum*, reduced the disease incidence to 4%, followed by seed coating and priming treatments with no differences with phosphorus amount (50 or 75 kg P_2O_5). A similar trend was obtained by root rot incidence. Disease reduction was achieved when seed bio-priming 1% CMC mixed with (3×10^6 conidia/ml) of *T. harzianum* treatment with (75 kg/fed P_2O_5). This treatment reduced post-emergence pea root rot to 4.25% after 40 days, and to 3% after 60 days. The second effective treatment was seed coating, followed by seed priming. These results indicate that the general increase in vegetative growth and yield, such as plant height, number of leaves/plant, fresh weight of leaves, pod yield, and green seed TSS with both increased levels of phosphorus fertilization and bio-priming 1% CMC, mixed with 3×10^6 conidia/ml of *T. harzianum* treatment was a direct result of the *T. harzianum* ability to management root rot disease and increase root mass.

Evaluation of different leaf extracts, chemicals, and 2 *Trichoderma* species (*T. harzianum* and *T. viride*), against the root rot of pea in a field trial, was investigated by Singh et al. (2014). *T. harzianum* and *T. viride* treatments were highly effective in reducing the root rot disease incidence with 18.94 and 19.52%, respectively, followed by drek (neem) *Melia azadirachta* seed extract (21.00%) and drek (neem) leaf extract (25.37%). All treatments were compared to fungicides carbendazim and (metalaxyl and mancozeb) as well as the control

treatment which scored root rot disease incidence (9.55, 8.56, and 38.96%), respectively. *T. harzianum* and *T. viride* improved plant height, the number of branches/plants, pod weight, the number of pods/plant, pod length, the number of grains/pod, and yield/ha. However, drek (neem) seed and leaf extracts were found to enhance growth and yield parameters, compared to the fungicides carbendazim and (metalaxyl and mancozeb) and control treatment.

Combination of *Trichoderma* species with beneficial bacteria such as *P. fluorescens*, *Rhizobium* sp., and *B. subtilis* under open field and greenhouse conditions was studied. Negi et al. (2014) evaluated the effects of pea seed treatment with *T. harzianum* and *T. virens*, individually or in combination with *P. fluorescens*, against *R. solani* and *F. solani* under field conditions. They found that *T. harzianum* reduced root rot disease severity in combination with *P. fluorescens*. The combination between *T. harzianum* and *P. fluorescens* as seed bio-priming recorded the least disease severity (20%), followed by *T. harzianum* + *P. fluorescens* + *T. virens* (30%), *P. fluorescens* (31.1%) than the untreated control treatment (49.4%). In addition, the combination of *T. harzianum* with *P. fluorescens* had a remarkable increase in planta, for instance, germination percentage, shoot, root and seedling lengths, vigor index, number of pods/plant, pod weight, number of grains/pod, and total yield/ha were recorded. These investigations revealed that the combination of *T. harzianum* with *P. fluorescens* was the most effective treatment in reducing disease severity percentages and increasing plant growth parameters, as well as yield components compared to *T. virens* or *T. harzianum* treatment alone.

Trichoderma harzianum, *Rhizobium* sp., and *B. subtilis* were used as biological agents to manage pea root rot complex and improve plant growth parameters according to the study done by Muhanna et al. (2018). The results revealed that the root rot complex was caused by *R. solani*, *F. oxysporum*, *Thielaviopsis basicola*, and *S. sclerotiorum*. Field experiments indicated that all treatments decreased disease severity percentages. With *T. harzianum* at 50 and 100 ml (concentration 3×10^7 conidia/ml), disease severity was decreased (15.1 and 13.8%) and (17.8 and 15.6%), respectively, than the control treatment (22.2 and 20.5%). *Rhizobium* sp., at (50 and 100 ml), reduced the disease severity (16.9 and 13.1%) and (17.8 and 14.2%), respectively, followed by treatments with *B. subtilis* (15.4 and 13.3%) and (16.9 and 14.7%), than the control treatment. Obtained results showed that all the biocontrol agents increased the growth parameters when applied at 100 ml.

Rhizobium leguminosarum combined with *Trichoderma lignorum*, *T. longibrachiatum*, and *T. koningii* were used as biological agents against pea root rot

complex and improved plant growth parameters according to the study of Ketta et al. (2021). Greenhouse experiments indicated that combination of *R. leguminosarum* with *T. longibrachiatum* reduced the post-emergence damping-off (6.67%) and root rot (9.58%) caused by *R. solani*. Survived plants, nitrogen fixation, and yield parameters were also increased. Treatment of *R. leguminosarum* combined with *T. koningii* against *F. solani* reduced the post-emergence damping-off (6.67%) and root rot (13.06%).

The outcomes of using beneficial microbes such as *Trichoderma* fungi for management of root rot in the bean and pea have benefits for the plant health and production. For instance, they suppress pathogens, promoting plant growth, improving the availability of nutrient uptake, such as iron, nitrogen, and phosphorus, and enhancing host capacity under stressful conditions in the rhizosphere. Besides, the different mode of action of *Trichoderma* that includes competition for nutrients and space in the colonization sites, antibiosis and stimulation of plant immunity, and defense mechanisms. Secondary metabolites (SMs) produced by *Trichoderma* species are considered to be one of the most effective bioactive molecules in the biological control strategies. Microorganisms and plants, mainly produce these natural compounds from different pathways derived from acetyl coenzyme A, or amino acids, which have many biological activities related to the survival functions of the organism, such as competitive as an antifungal activity and auxin production as a symbiotic relationship. Beneficial microorganisms such as *Trichoderma* species can produce bioactive molecules that can participate in the interactive process, between plants and their invaded pathogens, with consequences for crop production. Biocontrol agents belonging to *Trichoderma* genus are well-known producers of SMs, i.e., mycotoxins, antibiotics, and pigments, which are suppressive compounds to soil-borne pathogens or microbial competitors.

Beside the abovementioned recommendations for improvement of *Trichoderma* efficiency, soil application treatments are more effective than using a seed application. In case of seed application treatment, bio-priming was an effective treatment compared to seed priming and seed coating. The combination of *Trichoderma* species with beneficial bacteria such as *P. fluorescens*, *Rhizobium* sp., and *B. subtilis* is recommended.

Future prospects

The greatest challenge in the field of biocontrol management of soil-borne pathogenic fungi is to insure a truly beneficial effect for the environment. Although, *Trichoderma* species are considered as biocontrol agents in integrated plant disease management, their biocontrol potential is yet to be limited to laboratory experiments.

Moreover, information concerning its utilization is limited and not distributed among farmers. Finally, genetic engineering and molecular tools are needed for improvement of biocontrol agents (BCAs) to be used efficiently against a wide range of soil-borne plant pathogens.

Conclusions

This review focused on the biological management of common bean and pea root rot-inducing pathogens, i.e., *R. solani* and *F. solani*, using different species of the *Trichoderma* fungus. Several strains of *Trichoderma* have been already registered as commercial biological control agents and some are currently used against root rot fungal pathogens. The wide scale application of selected metabolites produced by *Trichoderma* spp. to induce host resistance and/or to promote crop yield is still limited because of their low effectiveness in disease management compared to synthetic pesticides, and their performance can be adversely affected by a wide range of biotic and abiotic agents. Therefore, studying the characteristics of *Trichoderma* species, their interactions with pathogen/plant and biocontrol mechanisms can enhance the capability of *Trichoderma* spp.

Abbreviations

ITS: Internal transcribed spacer; rRNA: Ribosomal RNA; CMC: Carboxymethylcellulose; SMs: Secondary metabolites; BCAs: Biological control agents

Acknowledgements

Not applicable

Authors' contributions

OAH created the idea of the manuscript. HAK collected the literature and wrote the half of the manuscript. OAH collected the literature and wrote the second half of the manuscript. HAK and OAH revised the manuscript several times. HAK formatted the manuscript according to the journal guidelines. HAK is responsible for the correspondence. The authors read and approved the final manuscript.

Funding

This work was done without any supporting funds.

Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 5 March 2021 Accepted: 12 June 2021

Published online: 22 June 2021

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