Abd-El-Khair et al. Bulletin of the National Research Centre (2019) 43:19
https://doi.org/10.1186/s42269-019-0062-5

Bulletin of the National Research Centre

RESEARCH Open Access

Field application of *Trichoderma* spp. combined with thiophanate-methyl for controlling *Fusarium solani* and *Fusarium oxysporum* in dry bean

H. Abd-El-Khair[1], I. E. Elshahawy[1*] and H. E. Karima Haggag[2]

Abstract

Background: Damping-off and root rot/wilt diseases caused by the soil-borne fungi *Fusarium solani* and *F. oxysporum* are a serious problem of dry bean productions in Egypt. This study examines the potential of controlling these diseases biologically by using three *Trichoderma* isolates, compatible with the fungicide thiophanatemethyl, i.e., *T. harzianum*, *T. viride*, and *T. virens*. Soil application with inoculants containing these isolates employed either alone or in combination with seed coating with thiophanate-methyl was applied.

Results: Under greenhouse and field conditions, all treatments significantly reduced the incidence of damping-off and root rot/wilt diseases and increased the percentage of survival plants. These treatments increased vegetative growth parameters and yield components of the survival dry bean plants compared with untreated control. Soil application with *Trichoderma* isolates combined with thiophanate-methyl seed treatments was more effective than using both of them individually. Meanwhile, *T. virens* + thiophanate-methyl was the most effective treatment. The tested treatments stimulated systemic defense responses in dry bean plants by activating defense enzymes including peroxidase, polyphenoloxidase, and chitinase.

Conclusions: Based on the obtained results, compatible isolates of *Trichoderma* spp. as soil treatment combined with thiophanate-methyl as seed treatment may have potential to develop a new biofungicide for integrated management of damping-off and root rot/wilt diseases in dry bean.

Keywords: *Trichoderma* spp., Compatible isolates, Thiophanate-methyl, Damping-off and root rot/wilt , Dry bean

Background

Dry bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for human consumption in the world. Several soil-borne fungi attacked bean plants causing damping-off and root rot/wilt diseases. These diseases were commonly attributed to *Fusarium solani* (Mart.) Sacc. in complex with *Rhizoctonia solani* and *F. oxysporum* (Abdel-Kader 1997; Estevez de Jensen et al. 1999, 2002). The disease incidence of damping-off and bean root rot has risen with the increased acreage of this crop, the shortening of crop rotation intervals, and the use of highly susceptible cultivars. Yield losses in several infested areas approach 50% (Estevez de Jensen et al. 2002). Biocontrol treatment with certain strains of *Trichoderma* spp. can be used for controlling several soil-borne diseases such as damping-off, root-rot, and wilt (El-Nagdi and Abd-El-Khair 2014; Woo et al. 2014; Elshahawy et al. 2017a, 2017b). Biological control methods are safe and environmentally friendly pesticide alternatives in agriculture application (Elshahawy et al. 2017c). But biological control alone is sometimes un-sufficient to eradicate or reduce the propagules of the pathogens. Therefore, integration methods between biocontrol agents and the fungicides could be effective. The tolerance of several biocontrol agents to fungicides was studied by many workers over the world (Howell et al. 1997; Wang et al. 2005; Howell

* Correspondence: ibrahim_nrc@yahoo.com
[1]Plant Pathology Department, Agricultural and Biological Research Division, National Research Centre, Giza, Egypt
Full list of author information is available at the end of the article

2007; Vasundara et al. 2015; Khalequzzaman 2016). In a previous study, we investigated in vitro the compatibility of ten *Trichoderma* spp. isolates (three of *T. harzianum*, three of *T. viride*, one of *T. virens*, and three of *Trichoderma* spp.) with seven fungicides viz., carbendazim, flutolanil, mancozeb, metalaxyl M + mancozeb, pencycuron, thiram + tolclofos-methyl, and thiophanate-methyl (Elshahawya et al. 2016). The results of our investigations revealed that each of flutolanil, pencycuron, and thiophanate-methyl when separately combined with *Trichoderma* spp. isolates reduced the growth of the tested soil-borne pathogens in the ranges of 22.2–100%, 43.7–100%, and 50.4–100%, compared to the reduction of 0.0–21.1%, 0.0–18.9%, and 15.6–18.9% resulted by the same fungicides when used alone, respectively. Our results suggested that the fungicides-*Trichoderma* spp. isolate combination may be effective in controlling soil-borne pathogenic fungi than individual treatment and reduced the amount of fungicide used. The results of our investigations revealed that *Trichoderma* spp. were highly compatible with thiophanate-methyl. Therefore, this work is amid to study the effects of *Trichoderma* spp. combined with thiophanate-methyl for controlling damping-off and root rot/wilt diseases of bean caused by *F. solani* and *F. oxysporum* in a greenhouse and in field experiments.

Materials and methods

Plant materials and fungicide

Bean seeds cv. Giza 4 was obtained from the Agricultural Research Centre (ARC), Egypt. The fungicide Thiophanate-methyl (Topsin – M 70% WG) was obtained from the Central Agricultural Pesticides Laboratory (CAPL), ARC, Egypt.

Pathogens

The soil-borne pathogens viz. *F. solani* and *F. oxysporum* which are considered the most virulent pathogens causing damping-off and root-rot/wilt diseases were obtained from the Plant Pathology Department, National Research Centre (NRC), Egypt. Pathogenic ability of these isolates toward dry bean was confirmed in a previous study (Abd-El-Khair and El-Gamal Nadia 2011). Fungal mass production used for soil infestation in greenhouse experiment was obtained by growing the tested isolates on sand-barley medium. This natural medium was prepared by mixing sand and barley (1:1, *w*:*w* and 40% water); then the mixture in glass bottles with cotton plugs was sterilized. The autoclaved medium was then inoculated individually with a 5-mm disk of each tested fungal isolate and incubated at 25 ± 2 °C for 2 weeks (Singleton et al. 1992).

Trichoderma spp.

Three isolates of *Trichoderma harzianum*, *T. viride*, and *T. virens* obtained from the Plant Pathology Department, National Research Centre, Egypt were used in this study. The compatibility of these isolates with thiophanate-methyl was confirmed in the previous study (Elshahawya et al. 2016). Fungal mass production used for soil treatment in greenhouse and field experiments was obtained by growing the tested isolates on sand-corn medium. This natural medium was prepared by mixing sand and corn (1:1, *w*:*w* and 40% water); then the mixture in glass bottles with cotton plugs was sterilized. The autoclaved medium was then inoculated individually with a 5-mm disk of each tested fungal isolate and incubated at 25 ± 2 °C for 2 weeks.

Greenhouse experiment

The experiment was conducted to assess the effect of soil treatment with each of *Trichoderma harzianum*, *T. viride*, and *T. virens*, combined with seed coating with thiophanate-methyl on pre- and post-emergence damping-off and root rot/wilt of bean caused by *F. solani* and *F. oxysporum* in pots. These experiments were carried out in potted soils under greenhouse conditions located at the Pest Rearing Department, CAPL, ARC, Giza, Egypt, following the soil infestation technique. Autoclaved sandy loamy soil was used. Formalin-sterilized plastic pots (30-cm diameter) were filled with 5 kg of autoclaved sandy loamy soil infested with the inocula of each of *F. solani* and *F. oxysporum* at the rate of 4% (*w*:*w*). The inoculum of each of *Trichoderma harzianum*, *T. viride*, and *T. virens* was added (at the rate of 1% *w*/*w*) to the infested soil 1 week later. Un-amended infested pots were used as control. One week later, dry bean seeds were sown. Bean seeds cv. Giza 4 were surface disinfected in 3% NaOCl for 3 min, washed three times in sterile distilled water, and then left for 30 min at room temperature before treating with the desired fungicide. Bean seeds were then soaked for 15 min in 1% methyl cellulose (MC) solution at the rate of 3 ml per 100 seeds. Thereafter, bean seeds were removed and placed in plastic bags and coated with thiophanate-methyl at the rate of 1 g per 1 kg of seeds. Bags were inflated with air and shaken vigorously. Thereafter, seeds were directly planted in the infested-potted soil.

Five bean seeds cv. Giza 4 were sown in each pot. Three pots were used as replicates per each treatment as well as for the control (Abd-El-Khair et al. 2010).

The treatments were as follows:

Experiment I

1. *F. solani* + *T. harzianum* + thiophanate-methyl
2. *F. solani* + *T. viride* + thiophanate-methyl
3. *F. solani* + *T. virens* + thiophanate-methyl
4. *F. solani* + *T. harzianum*
5. *F. solani* + *T. viride*

6. *F. solani* + *T. virens*
7. *F. solani* + thiophanate-methyl
8. *F. solani* only

Experiment II

1. *F. oxysporum* + *T. harzianum* + thiophanate-methyl
2. *F. oxysporum* + *T. viride* + thiophanate-methyl
3. *F. oxysporum* + *T. virens* + thiophanate-methyl
4. *F. oxysporum* + *T. harzianum*
5. *F. oxysporum* + *T. viride*
6. *F. oxysporum* + *T. virens*
7. *F. oxysporum* + thiophanate-methyl
8. *F. oxysporum* only

Data recorded

The incidence of pre- and post-emergence damping-off was recorded after 15 and 30 days of sowing, and the incidence of root rot/wilt was recorded up to 60 days of sowing. The percentages of survival bean plant and the growth parameters of bean such as averages of root length (cm), shoot length (cm), leaves number per plant, plant fresh weight (g), and plant dry weight (g) were recorded.

Field experiments

Naturally infested soil with *F. solani* and *F. oxysporum* at El-Kantar El-Khairiya, Kalubeiya Governorate, Egypt, was chosen for estimating the efficiency of soil treatment with *Tricoderma* spp. combined with seed coating with thiophanate-methyl for controlling pre- and post-emergence damping-off and root rot/wilt of bean plants under field conditions. Field experiments were conducted during tow growth season of 2015 and 2016. The experiments were conducted with a completely randomized design (CRD) with eight treatments (*T. harzianum* + thiophanate-methyl, *T. viride* + thiophanate-methyl, *T. virens* + thiophanate-methyl, *T. harzianum*, *T. viride*, *T. virens*, thiophanate-methyl, and control) each with four experimental units. Experimental unit consisted of four rows. The dimensions of each row were 2 m in length, 50 cm in length, and 30 cm in height. The inoculum of each *Tricoderma* spp., freshly prepared, was incorporated to the soil at the rate of 300 g formulation/m length of the row. Therefore, a cavity of 15 cm in depth was made on the surface of each row. Then the inoculum was added to this cavity and then recovered with the soil and immediately irrigated. One week after incorporation, thiophanate-methyl coated and non-coated bean seeds were sown in holes at the rate of two seeds per hole. Irrigation and fertilization were conducted as generally recommended for bean production regimes.

Data recorded

Damping-off and root rot/wilt incidence

The incidence of pre- and post-emergence damping-off was recorded after 15 and 30 days of sowing, and the incidence of root rot/wilt was recorded up to 60 days of sowing.

Determination of enzyme activities

The effect of soil treatment with *Tricoderma* spp. combined with seed coating with thiophanate-methyl on the activities of the defense enzymes of peroxidase, polyphenoloxidase, and chitinase of bean plants was estimated at 60 days after planting under field conditions. To extract the enzyme, bean leaf samples (g) were homogenized with 0.2 M Tris HCl buffer (pH 7.8) at 0 °C containing 14 m M B-mercaptoethanol at the rate of 1/3 *w*/*v*. The extracts were obtained by filtering off the debris with a clean cloth and centrifuging at 3000 rpm for 15 min. The supernatants were recovered and kept in a tube in an ice bath until assayed. The supernatant was used to determine the activity of enzymes by using UV spectrophotometer. Peroxidase activity was assayed with guaiacol as the hydrogen donor as described by Hammerschmidt et al. (1984), and peroxidase activity was expressed as the increase in absorbance at 470 nm/g fresh weight/min according to the method described by Lee (1973). Polyphenoloxidase enzyme activity was determined by measuring the rate of quinone formation as a result of oxidizing 3,4-dihydroxyphenylalanine (DOPA), and polyphenoloxidase activity was expressed as the increase in absorbance at 475 nm/g fresh weight/min according to the method described by Bashan et al. (1985). The determination of chitinase enzyme was carried out using colloidal chitin as substrate and dinitrosalicylic acid (DNS) as a reagent to measure reducing sugars according to the method described by Monreal and Reese (1969). Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released/g fresh weight/60 min at 540 nm.

Growth parameter and yield components

The vegetative parameters viz., shoot fresh weight (g), and number of branches/plants as well as yield parameters viz., pod number, pod weight (g), weight of 100 seeds (g), and seeds weight (g) were recorded at harvest time.

Statistical analysis

Data were subjected to analysis of variance using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA. Means of values were compared by the Least Significant Difference (LSD) test at $P \leq 0.05$ level of significance (Steel and Torrie 1980).

Results

Greenhouse experiments

Effect of Trichoderma spp. and thiophanate-methyl on disease incidence

Data presented in Table 1 show that all treatments highly reduced damping-off and root rot/wilt incidence and increased survival plants of dry bean compared to untreated plants. Dry bean seeds coated with thiophanate-methyl combined with *Trichoderma* spp. soil treatment reduced damping-off and root rot/wilt incidence more than individual treatment. *Trichoderma* spp. were more effective to reduce damping-off and root rot/wilt than thiophanate-methyl. On the other hand, the combination of thiophanate-methyl + *T. virens*, thiophanate-methyl + *T. harzianum*, and thiophanate-methyl + *T. viride* recorded almost the same protection against to any of the tested fungi. In the case of *Fusarium solani*, the combined treatments recorded the lowest percentages of damping-off (by the range of 0.0 and 7.1%), in comparison with 60.0 and 18.1% for the control. Root rot was also reduced to 7.1% in comparison with 33.3% for the control. In the case of *Fusarium oxysporum*, the combined treatments recorded the lowest percentages of damping-off (by the range of 7.1 and 7.7%), in comparison with 53.3 and 28.6% for the control. Wilt incidence was also reduced to the same range in comparison with 42.9% for the control.

Effect of Trichoderma spp. and thiophanate-methyl on vegetative growth parameters

In the present study, *Trichoderma* spp. (*T. harzianum*, *T. viride*, and *T. virens*) and chemical fungicide (thiophanate-methyl) individually or in combination recorded significant highly increased vegetative growth parameters of bean plants in the presence of pathogenic fungi (Table 2). *Trichoderma* spp. combined with thiophanate-methyl increased vegetative growth parameters of bean plants more than using any of them individually in terms of root length (cm), shoot length (cm), number of leaves/plant, plant fresh weight (g), and plant dry weight (g). Also, *Trichoderma* spp. gave vegetative growth parameters higher than the usage of chemical fungicide. Generally, the combination of thiophanate-methyl + *T. virens*, thiophanate-methyl + *T. harzianum*, and thiophanate-methyl + *T. viride* recorded almost the same vegetative growth parameters of bean plants in infested soil with each of *F. solani* or *F. oxysporum*.

Table 1 Effect of soil application with *Trichoderma* spp. and seed coating with thiophanate-methyl employed either alone or in combination on damping-off, root rot/wilt, and survival dry bean plants grown in soil artificially infested with each of *Fusarium solani* and *Fusarium oxysporum* under greenhouse conditions

Treatment	*Fusarium solani*			
	Damping-off (%)		Root rot (%)	Survival plants (%)
	Pre-emergence	Post-emergence		
Thiophanate-methyl + *T. harzianum*	6.7 e	7.1 e	7.1 e	85.8 b
Thiophanate-methyl + *T. viride*	6.7 e	7.1 e	7.1 e	85.8 b
Thiophanate-methyl + *T. virens*	6.7 e	0.0 f	7.1 e	92.9 a
T. harzianum	20.0 c	8.3 c	8.3 c	83.4 d
T. viride	13.3 d	7.7 d	7.7 d	84.6 c
T. virens	20.0 c	8.3 c	8.3 c	83.4 d
Thiophanate-methyl	26.7 b	16.7 b	9.1 b	72.8 e
Pathogen only	60.0 a	18.1 a	33.3 a	50.0 f
Treatment	*Fusarium oxysporum*			
	Damping-off (%)		Wilt (%)	Survival plants (%)
	Pre-emergence	Post-emergence		
Thiophanate-methyl + *T. harzianum*	13.3 d	7.7 e	7.7 d	84.4 b
Thiophanate-methyl + *T. viride*	13.3 d	7.7 e	7.7 d	84.4 b
Thiophanate-methyl + *T. virens*	6.7 e	7.1 f	7.1 e	85.8 a
T. harzianum	20.0 c	9.1 c	8.3 c	75.0 d
T. viride	20.0 c	8.3 d	8.3 c	83.4 c
T. virens	20.0 c	8.3 d	8.3 c	83.4 c
Thiophanate-methyl	26.7 b	16.7 b	18.2 b	72.7 e
Pathogen only	53.3 a	28.6 a	42.9 a	28.5 f

The same letter after means in each column is not significantly different according to Duncan's multiple range test ($P = 0.05$)

Table 2 Effect of soil application with *Trichoderma* spp. and seed coating with thiophanate-methyl employed either alone or in combination on vegetative growth parameter of dry bean plants grown in soil artificially infested with each of *Fusarium solani* and *Fusarium oxysporum* under greenhouse conditions

Treatment	Average vegetative growth parameters of bean plants				
	Fusarium solani				
	Root length (cm)	Shoot length (cm)	No. of leaves/plant	Plant fresh weight (g)	Plant dry weight (g)
Thiophanate-methyl + *T. harzianum*	17.2 b	43.7 b	6.7 b	15.2 b	2.43 c
Thiophanate-methyl + *T. viride*	16.5 c	44.0 b	8.3 a	14.1 c	3.43 a
Thiophanate-methyl + *T. virens*	19.7 a	45.2 a	6.8 b	16.4 a	2.98 b
T. harzianum	15.5 e	43.0 c	5.7 d	12.5 e	2.38 e
T. viride	14.8 f	41.2 d	5.7 d	11.6 f	1.95 f
T. virens	16.0 d	39.8 e	6.2 c	12.7 d	2.39 d
Thiophanate-methyl	14.0 g	38.7 f	5.5 e	11.5 g	1.94 g
Pathogen only	13.7 h	33.3 g	4.8 f	10.8 h	1.55 h
Treatment	Average vegetative growth parameters of bean plants				
	Fusarium oxysporum				
	Root length (cm)	Shoot length (cm)	No. of leaves /plant	Plant fresh weight (g)	Plant dry weight (g)
Thiophanate-methyl + *T. harzianum*	21.1 b	43.7 a	7.7 a	37.7 b	5.35 a
Thiophanate-methyl + *T. viride*	20.8 c	41.2 c	7.3 b	38.1 a	4.89 c
Thiophanate-methyl + *T. virens*	21.3 a	42.2 b	7.7 a	36.6 c	5.17 b
T. harzianum	18.7 e	42.0 b	6.5 d	25.1 f	4.63 d
T. viride	18.8 e	42.2 b	6.8 c	30.3 e	4.05 f
T. virens	20.2 d	41.2 c	6.8 c	32.5 d	4.41 e
Thiophanate-methyl	16.8 f	40.7 c	6.2 e	24.1 g	2.63 g
Pathogen only	15.7 g	30.0 d	5.2 f	14.8 h	2.24 h

The same letter after means in each column is not significantly different according to Duncan's multiple range test ($P = 0.05$)

Field experiments

Effect of Trichoderma spp. and thiophanate-methyl on disease incidence

The efficacy of soil treatment with *Trichoderma* spp. and seed coating with thiophanate-methyl individually and/or combined against damping-off and root rot/wilt diseases of dry bean were evaluated under field conditions. Data in Table 3 clearly demonstrate that all treatments significantly reduced damping-off and root rot/wilt severity compared with the control. Dry bean seed coated with thiophanate-methyl together with soil treatment with *Trichoderma* spp. were more effective than using either alone. Also, the obtained data show that soil treatment with any of *Trichoderma* spp. (*T. harzianum*, *T. viride*, and *T. virens*) was almost similar to seed coated with thiophanate-methyl in reducing damping-off and root rot/wilt. Thiophanate-methyl + *T. virens* recorded the highest redaction of damping-off and root rot/wilt in both seasons, whereas recorded 92.6% and 93.0% survival plants compared with 68.2 and 66.0% in control plants in both seasons, respectively. In the contrary, the individual treatment with each of *T. harzianum*, *T. viride*, *T. virens*, and thiophanate-methyl were recorded the lowest ones in both seasons (85.2, 85.5, 86.4, and 85.6% survival plants in first seasons and 85.9, 87.6, 84.4, and 83.2% in second season, respectively).

Effect of Trichoderma spp. and thiophanate-methyl on growth parameters and yield components

The effect of soil treatment with each of *Trichoderma* spp. (*T. harzianum*, *T. viride*, and *T. virens*) and seed coated with the chemical fungicide (thiophanate-methyl) individually and/or combined on growth parameters and yield components of dry bean under field conditions during seasons 2015 and 2016 were studied. Data in Table 4 indicate that the combination between *Trichoderma* spp. and thiophanate-methyl significantly improved dry bean growth and yield components more than used individually. Also, soil treated with any of *Trichoderma* spp. were more effective in this respect than seed coated with the chemical fungicide. The combinations between thiophanate-methyl + *T. virens* were the most effective treatments; they recorded the highest fresh weight of shoot (28.8 and 45.6 g) and number of branches (4.2 and 5.2 branch/plant) in both seasons, respectively. Also, this treatment recorded the

Table 3 Effect of soil application with *Trichoderma* spp. and seed coating with thiophanate-methyl employed either alone or in combination on damping-off, root rot/wilt, and survival dry bean plants grown under field conditions

Treatment	Season 2015			
	Damping-off (%)		Root rot (%)	Survival plants (%)
	Pre-emergence	Post-emergence		
Thiophanate-methyl + *T. harzianum*	11.7 cd	5.7 b	3.8 b	90.5 ab
Thiophanate-methyl + *T. viride*	10.0 d	3.6 b	3.6 b	92.8 a
Thiophanate-methyl + *T. virens*	10.0 d	3.7 b	3.7 b	92.6 a
T. harzianum	21.7 b	6.4 b	8.4 b	85.2 b
T. viride	20.0 bc	6.3 b	8.2 b	85.5 b
T. virens	26.7 b	6.8 b	6.8 b	86.4 ab
Thiophanate-methyl	18.3 bcd	6.2 b	8.2 b	85.6 b
Control	36.3 a	13.4 a	18.4 a	68.2 c
Treatment	Season 2016			
	Damping-off (%)		Wilt (%)	Survival plants (%)
	Pre-emergence	Post-emergence		
Thiophanate-methyl + *T. harzianum*	8.3 cd	5.5 b	3.6 b	90.9 ab
Thiophanate-methyl + *T. viride*	8.3 cd	3.6 b	3.6 b	92.8 a
Thiophanate-methyl + *T. virens*	5.0 d	3.5 b	3.5 b	93.0 a
T. harzianum	18.3 bc	6.0 b	8.1 b	85.9 bc
T. viride	18.3 bc	6.2 b	6.2 b	87.6 abc
T. virens	25.0 b	6.8 b	6.8 b	84.4 bc
Thiophanate-methyl	21.7 b	8.4 ab	8.4 b	83.2 c
Control	40.0 a	13.7 a	19.8 a	66.5 d

The same letter after means in each column is not significantly different according to Duncan's multiple range test ($P = 0.05$)

highest yield components, i.e., number of pods (9.0 and 12.0 pods/plant), pod weight (3.35 and 3.25 g), weight of 100 seeds (44.14 and 50.50 g), and weight of seed (0.44 and 0.51 g) in both seasons, respectively. On the contrary, dry bean seed coated with thiophanate-methyl only was less effective in both seasons compared with the other treatments.

Effect of Trichoderma spp. and thiophanate-methyl on enzymatic activities in dry bean plants grown under field conditions

The effect of soil treatment with each of *Trichoderma* spp. (*T. harzianum*, *T. viride*, and *T. virens*) and seed coated with the chemical fungicide (thiophanate-methyl) individually and/or combined on enzyme activities of peroxidase, polyphenoloxidase, and chitinase in dry bean plants grown under field conditions during seasons 2015 and 2016 was studied. Data presented in Table 5 reveal that all treatments were pronounced in the induction of defense enzyme in comparison with the control. Data in Table 5 also reveal that the combination between *Trichoderma* spp. and thiophanate-methyl significantly induce defense enzymes in bean plants more than used individually. They induced high activation of peroxidase (by 83.3, 83.5, and 82.9% and 86.9, 86.4, and 85.7% increase over control), polyphenoloxidase (by 64.2, 63.9, and 65.6% and 65.3, 64.8, and 65.0% increase over control), and chitinase (by 92.4, 91.5, and 91.0% and 92.1, 91.5, and 91.0% increase over control) in both seasons, respectively. On the contrary, the individual treatment of *T. harzianum*, *T. viride*, *T. virens*, and thiophanate-methyl only occupied significantly the second in the induction of these enzymes compared with the control.

Discussion

In the present study, dry bean seeds coated with thiophanate-methyl combined with *Trichoderma* spp. soil treatment reduced damping-off and root rot/wilt incidence more than using any of them individually. Data also reveal that *Trichoderma* spp. were more effective to reduce damping-off and root rot/wilt than thiophanate-methyl. On the other hand, the combination of thiophanate-methyl + *T. virens*, thiophanate-methyl + *T. harzianum*, and thiophanate-methyl + *T. viride* recorded almost the same protection against to any of the tested fungi. It was suggested that the addition of *Trichoderma* spp. to the soil before sowing reduced the inoculums of the pathogens and bean seeds coated with

Table 4 Effect of soil application with *Trichoderma* spp. and seed coating with thiophanate-methyl employed either alone or in combination on growth parameters and yield components of dry bean grown under field condition

Treatment	Season 2015					
	Shoot fresh weight (g)	No. of Branches/plant	No. of pods/plant	Pod weight (g)	Weight of 100 seeds (g)	Seed weight (g)
Thiophanate-methyl + *T. harzianum*	24.5 ab	3.6 ab	6.2 c	2.99 c	38.40 d	0.38 d
Thiophanate-methyl + *T. viride*	22.1 ab	3.4 abc	8.2 b	3.44 b	44.16 a	0.44 a
Thiophanate-methyl + *T. virens*	28.8 a	4.2 a	9.0 a	3.35 a	44.14 a	0.44 a
T. harzianum	14.0 cd	2.2 c	6.2 c	2.23 f	34.93 e	0.35 e
T. viride	20.4 bc	4.0 a	5.8 c	2.47 e	40.35 c	0.40 c
T. virens	21.5 ab	3.0 abc	5.8 c	2.85 d	43.41 b	0.43 b
Thiophanate-methyl	11.8 d	2.7 bc	2.9 d	1.88 g	31.22 f	0.31 f
Control	10.8 d	2.1 c	2.7 d	1.32 h	27.28 g	0.27 g
Treatment	Season 2016					
	Shoot fresh weight (g)	No. of Branches/plant	No. of pods/plant	Pod weight (g)	Weight of 100 seeds (g)	Seed weight (g)
Thiophanate-methyl + *T. harzianum*	32.8 b	4.6 ab	8.8 b	3.14 b	43.99 c	0.44 c
Thiophanate-methyl + *T. viride*	37.6 ab	5.2 a	9.2 b	3.07 c	50.11 b	0.50 b
Thiophanate-methyl + *T. virens*	45.6 a	5.2 a	12.0 a	3.25 a	50.50 a	0.51 a
T. harzianum	17.5 c	3.4 bcd	9.1 b	1.96 f	37.24 f	0.37 e
T. viride	18.3 c	3.0 cd	7.6 c	2.58 d	41.41 d	0.41 d
T. virens	23.4 c	4.0 abc	6.5 d	2.19 e	38.90 e	0.39 d
Thiophanate-methyl	14.4 c	2.8 cd	2.8 e	1.99 g	30.71 g	0.31 f
Control	14.0 c	2.0 d	2.6 e	1.42 h	28.56 h	0.29 g

The same letter after means in each column is not significantly different according to Duncan's multiple range test ($P = 0.05$)

thiophanate-methyl protect them from invasion by such pathogens. In the previous study, we found that *Trichoderma* spp. were highly compatible with thiophanate-methyl in vitro tests (Elshahawya et al. 2016). Also, the combined treatment of *Trichoderma* spp. and thiophanate-methyl highly reduced the growth of *F. solani* and *F. oxysporum* than individual treatment (Elshahawya et al. 2016). Hefnawy et al. (2014) found that *T. harzianum* and *T. koningii* combined with low dose of Rizolex T 50% increased the inhibition percentages of pathogenic fungi and may be beneficial in controlling soil-borne fungi. Bhale and Rajkonda (2015) reported that *T. viride*, *T. harzianum*, *T. koningii*, and *T. virens* were compatible to fungicides viz., Mancozeb and Captan at lower concentrations. Also, Wedajo (2015) reported that by increasing the fungicide concentrations to 400 ppm (sancozeb) and 600 ppm (curzate), *T. harzianum* and *T. viride* tolerate the fungicides 50% and slightly incompatible at higher concentrations of 800 and 1000 ppm, and completely inhibited beyond 1000 ppm, compared to the control for both fungicides. The obtained results are in agreement with those obtained by many workers. Chaparro et al. (2011) reported that *Trichoderma* spp. were able to survive in soils with remnants of fungicide molecules such as fungicides of Captan, thiabendazol, and the mixture Captan-Carboxin. Mahmood et al. (2015) reported that thiophanate-methyl combined with *Trichoderma* spp. gave adequate disease control of bean seedlings and can protecting bean seedling in the field. The use of *Trichoderma* spp. combined with some commercial fungicides was applied by many workers to obtain additive effects against soil-borne pathogens. Howell et al. (1997) reported that cotton seeds treated with *T. virens* plus metalaxyl gave seedling stands significantly greater than the untreated seeds as well as seeds treated with fungicide only. Wang et al. (2005) suggested that *Trichoderma* combined with fludioxonil could be applied into a disease management program for controlling of *Fusarium* root rot in coneflower. Howell (2007) indicated that *Trichoderma* spp. plus chloroneb produce the optimum combination treatment for damping-off control of cotton, followed by *T. virens* plus chloroneb plus metalaxyl. Khalequzzaman (2016) showed that seed treatment with Provax 200 (2.5 g/kg seed), followed by seed treatment with *T. harzianum* compost (1:5) gave the lowest foot and root rot disease incidence of lentil than the untreated control.

Table 5 Effect of soil application with *Trichoderma* spp. and seed coating with thiophanate-methyl employed either alone or in combination on the activity of peroxidase, polyphenoloxidase, and chitinase enzymes of dry bean plants grown under field condition

Treatment	Season 2015		
	Peroxidase	Polyphenoloxidase	Chitinase
Thiophanate-methyl + *T. harzianum*	0.968 a	2.483 b	2.272 a
Thiophanate-methyl + *T. viride*	0.984 a	2.457 b	2.047 b
Thiophanate-methyl + *T. virens*	0.947 a	2.579 a	1.928 b
T. harzianum	0.721 bc	1.084 c	1.542 c
T. viride	0.649 b	1.062 c	1.313 d
T. virens	0.588 c	1.079 c	1.549 c
Thiophanate-methyl	0.581 c	1.061 c	1.244 d
Control	0.162 d	0.888 d	0.173 e
Treatment	Season 2016		
	Peroxidase	Polyphenoloxidase	Chitinase
Thiophanate-methyl + *T. harzianum*	0.976 a	2.594 a	2.118
Thiophanate-methyl + *T. viride*	0.939 ab	2.554 a	1.975
Thiophanate-methyl + *T. virens*	0.893 b	2.570 a	1.864
T. harzianum	0.721 c	1.069 c	1.481
T. viride	0.624 d	1.145 b	1.206
T. virens	0.490 e	1.097 bc	1.621
Thiophanate-methyl	0.650 d	1.077 c	1.287
Control	0.128 f	0.899 d	0.167

The same letter after means in each column is not significantly different according to Duncan's multiple range test ($P = 0.05$)

In the present study, under field conditions, data reveal that the combination between *Trichoderma* spp. and thiophanate-methyl significantly induce the defense enzymes in bean plants more than used individually. A positive correlation between the biocontrol activates of *Trichoderma* species isolates and enhancement of peroxidase, polyphenoloxidase, and chitinase enzymes in bean plants to resist infection with soil-borne pathogens. The reduction in bean damping-off and root-rot/wilt incidence may be due to an increase in the defense-related enzymes such as peroxidase, polyphenoloxidase, and chitinase. The oxidative enzymes play an important role in induced resistance by the oxidation of phenols to oxidized toxic products (quinine) which limit fungal activity. Peroxidases catalyze a number of reactions that fortify plant cell walls. These reactions include the incorporation of phenolics into cell walls and lignifications and suberization of plant cell walls. On the other hand, the chitinase enzymes play roles in plant defense against fungi by hydrolyzing their cell wall. The amount of them significantly increase and play the main role of defense reaction against fungal pathogen by degrading cell wall, because chitin is a major structural component of the cell walls of many pathogenic fungi. These results also are in agreement with those obtained by Abd-El-Khair et al. (2010) and Abd-El-Khair and El-Gamal Nadia (2011).

In the present study, under greenhouse conditions, *Trichoderma* spp. combined with thiophanate-methyl were increased vegetative growth parameters of bean plants more than using any of them individually in terms of root length (cm), shoot length (cm), number of leaves/plant, plant fresh weight (g), and plant dry weight (g). Also, under field conditions, *Trichoderma* spp. and thiophanate-methyl significantly improve the dry bean growth and yield components more than used individually. It is to be noted that growth promotion effect is one of the mechanisms of *Trichoderma* spp. exerted for the control of phytopathogenic diseases (Benítez et al. 2004; Sharma et al. 2012; Gajera et al. 2013). The capacity of *Trichoderma* spp. to promote growth results from the production of phytohormones that promote growth characteristics of the plants. Hexon et al. (2009) described induced production of three auxin-related compounds (indole-3-acetic acid, indole-3-acetaldehyde, and indole-3-ethanol) causing the development of *Arabidopsis* seedlings in response to inoculation with *T. virens* and *T. atroviride*. This was also supported by Harman et al. (2004) who found that *Trichoderma* spp. colonize root surfaces and penetrate the epidermis before producing or releasing a variety of compounds that induce localized or systemic resistance responses. Therefore, plants become protected from the pathogenic fungus, indicating induction of SAR in plants treated with the biological isolates.

Conclusion

In conclusion, the use of these compatible isolates of *Trichoderma* spp. as soil treatment combined with the fungicide (thiophanate-methyl) as seed treatment could be recommended for the control of damping-off and root rot/wilt of dry bean.

Acknowledgements
Not applicable.

Funding
Not applicable.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Plant Pathology Department, Agricultural and Biological Research Division, National Research Centre, Giza, Egypt. [2]Pest Rearing Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Dokki, Giza, Egypt.

Received: 13 June 2018 Accepted: 24 January 2019
Published online: 04 February 2019

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