Compatibility of biological control agents with fungicides against root rot diseases of wheat

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ABSTRACT

Root - rot diseases are considered the most serious diseases of wheat in Egypt, causing a considerable loss seed germination, plant stand and grain yield. The present study aims to evaluate the fungicidal activity of four chemical fungicides and two biological control agents. Four fungicides (i.e. triticonazole, carbendazim, carboxin + thiram and metalaxyl M + fludioxonil) and two biological control agents (i.e. *Trichoderma harzianum* and *Bacillus subtilis*) were evaluated separately and their compatibility against two pathogenic fungi, *Rhizoctonia solani* and *Fusarium graminearum* that causing root rot diseases of wheat. The *in vitro* studies showed that the fungicidal activity of the tested fungicides against *R. solani* mycelial growth was descendingly arranged as follows; metalaxyl M + fludioxonil > carbendazim > triticonazole > carboxin + thiram, while it was metalaxyl M + fludioxonil > triticonazole > carbendazim > carboxin + thiram against *F. graminearum* mycelial growth. In greenhouse trials, the fungicides were applied at 1.5 and 3.0 g kg^{-1} seeds as seed treatment, while biological control agents were applied at 4.5 and 9.0 x 10^6 spores mL^{-1} water as soil treatment. The results showed that all the treatments significantly reduced disease incidence and increased emergence and plant stands and the biological control agents were the inferior treatments. Generally, the most effective treatments for controlling root rot diseases were metalaxyl M + fludioxonil followed by carbendazim, triticonazole, while the lowest were carboxin + thiram, *B. subtilis* and *T. harzianum* as compared with the control treatment. Moreover, the results concluded that the efficiency of *T. harzianum* and *B. subtilis* in controlling *R. solani* and *F. graminearum* was obviously enhanced by their compatibility with low rates of the tested fungicides. In conclusion, the biological strategy which used throughout integrated pest management could be improved by using fungicides at low rates.

Keywords: Wheat, Root rot; *Rhizoctonia solani*; *Fusarium graminearum*; Fungicides and Biological control agents.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in Egypt, where it is used as a basic food grains for humans or as a main source of straw fodder for animal feeding. However, it attacked by many pathogenic organisms under Egyptian conditions. Root - rot diseases are considered the most serious diseases of wheat in Egypt, causing a considerable loss seed germination, plant stand and grain yield. Several soil- borne fungi such as *Rhizoctonia solani* and *Fusarium graminearum* cause wheat root rot by invading and colonizing in the root of wheat plants and enter in tissues of wheat seedling (El-Kholy, 1999 and Asran and El-Eraky-Amal, 2011). The fungus *R. solani* causes wheat damping off and root rot diseases (Moubarak and Abdal-Monaim, 2011) and caused localized patchy and stunted areas in the field (Ogoshi *et al*., 1990). Both *F. graminearum* and *F. culmorum* are also main soil- borne pathogens that cause root rot, foot rot, stem rot, crown rot and head blight in wheat (Nourozian *et al*., 2006) which resulted in considerable reduction in agricultural sector economy (Bentley *et al*., 2006).

Several fungicides either separately as triticonazole (Burlakoti *et al*., 2010; Kumar *et al*., 2017) and carbendazim (Sriraj *et al*., 2014; Kumar *et al*., 2017; Manasa *et al*., 2017; Masiello *et al*., 2019) or premixed as carboxin + thiram (Akgul and Erkilic, 2016; El-Sayed-Sahar and Abdel-Monaim, 2017; Koycu, 2018) and fludioxonil + metalaxyl (McMullen and Bradley, 2005; Miguel *et al*., 2015; Akgul and Erkilic, 2016) have been used as seed treatments to control root rot diseases. Application of fungicides is one of the most effective and widely recommended methods of disease control. However, continuous use of such chemical fungicides is not economical and eco-friendly. Also, the repeated use of such fungicides may develop resistant strains of the pathogens (Harman *et al*., 2004). Therefore, biological control agents can be widely used as alternative tools for controlling root rot diseases. For example, *Trichoderma* spp. and *Bacillus subtilis* were found to exhibit significant action against wheat root rot diseases caused by *R. solani* or *F. graminearum* (Moubarak and Abdel-Monaim, 2011; El-Ballat, 2017 and Ishtiaq *et al*., 2019).

To develop an effective disease management program, the compatibility of potential biological control agents with low fungicide rates is essential (Wedajo, 2015). Several researchers stated that the combination of chemical fungicides with biological control agents including *Trichoderma* spp. (Jensen *et al*., 2002; Wang *et al*., 2005; Thoudam and Dutta, 2014; Wedajo, 2015; Dhanya *et al*., 2016) and *Bacillus subtilis* (Jensen *et al*., 2002; Basamma and Kulkarni, 2017; Rajkumar *et al*., 2018) may increase and enhance disease control rates and provide better management of soil- and seed-borne diseases.

Considering all these points the present study was conducted to evaluate the fungicidal activity of four chemical fungicides and two biological control agents when used separately or in combinations for controlling root rot diseases of wheat caused by *R. solani* and *F. graminearum*.

MATERIALS AND METHODS

Laboratory and greenhouse experiments were conducted at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Isolation and identification of the causal organisms:

Pure isolates of *Rhizoctonia solani* and *Fusarium graminearum*, obtained from wheat roots showing typical symptoms of root rot disease. Transverse sections of the wheat roots collected from the field were surface sterilized by 0.5 % sodium hypochlorite for 1 min., rinsed in sterilized distilled water and drained on paper towels. They were transferred on PDA medium in Petri dishes, each contained 3 pieces and replicated 3 times. The dishes were incubated at 27 °C for 5 days. The growing fungi were subcultured on PDA slants. Single spore technique was used for purification of the isolated fungi. The isolated fungi were identified in Department of Botany, Branch of Plant Pathology, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Experimental materials:

The wheat (*Triticum aestivum* L.) seeds, (Gemiza 9 cv.) were obtained from the Wheat Research Department, Agriculture Research Center, Ministry of Agriculture and Land Reclamation.

***Fungicides and Biological control agents*:**

Four chemical fungicides, triticonazole (Premis 2.5 % F.S.), carbendazim (Kemazed 50 % W.P.), carboxin + thiram (Vitavax 75 %W.P.) and metalaxyl M + fludioxonil (Maxim XL 3.5 % F.S.) and two biological control agents, Plant-guard (The fungus *Trichoderma harzianum* each one cm^3 of the liquid contain 30 million organisms) and Rhizo- N (The bacterium *Bacillus subtilis* each one g powder contain 30 million organisms) were evaluated against the two pathogenic fungi.

***In vitro* sensitivity tests:**

Sensitivity of *R. solani* and *F. graminearum* to the tested fungicides was evaluated according to Frisina and Benson (1988). The fungicides were diluted in sterile distilled water, then added to cooled PDA medium at concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, and 100.0 μg a.i. mL^{-1} for each fungicide. The poisoned media were poured in plates (9 cm diameter), other plates containing fungicide – free medium were used as control treatment. The medium was seeded by 0.4 cm diameter disk, removed from 7-day-old culture of the fungus, on solidified medium and incubated for 7 days at 25 °C. Each treatment was replicated four times. Growth on the fungicides amended medium was determined by measuring the colony diameter (cm). The percentage of growth inhibition was calculated relative to the control treatment. The concentration giving 50 % linear growth inhibition (EC_{50}) was determined by regression analysis of the log probit transformed data (Finney, 1971).

Pot experiments:

Soil infestation:

To prepare inocula required for the test, the isolates of *R. solani* and *F. graminearum* were grown in 500 ml conical flask contained barley grains medium. The used soil (clay loamy), was air dried, sieved and packed in some polyethylene bags which were steamed in an autoclave until a temperature of 100 °C (1.3-1.4 pressure) was reached and then holding the temperature at 90-110 °C (1.1-1.4 pressure) for one hour (Knudsen and Bin, 1990). The inoculum of each fungus was incorporated into the autoclaved soil at the level of 5 % W/W and thoroughly mixed. Plastic pots (20 cm in diameter), which were previously sterilized by 5 % formalin solution, were filled with infested soil, except control without infestation (the same amount of the sterile barely grains without fungus). All pots were daily irrigated for 7 days to stimulate the fungal growth and to ensure its distribution within the soil before cultivation.

***Fungicidal seed and biocontrol treatments*:**

This trial was carried out to investigate the efficiency of fungicides at two rates (as seed treatment) and biological agents at two concentrations (as soil treatment) separately and the compatibility of fungicides at the low rate with biological control agents for controlling the incidence of artificial infection with *Rhizoctonia* and *Fusarium* root rot on wheat growing in pots under greenhouse conditions. Seeds were treated with the tested fungicides (1.5 and 3.0 g product kg^{-1} seeds) according to Warhman *et al*., (1989) with some modification. The appropriate amounts of the tested fungicides were placed in plastic bags, seeds were added, then a few drops of 5 % animal glue solution were added and shaken by the hand until the seeds were uniformly covered by the chemical. Treated seeds were then dried at room temperature for 24 h before planting. Biological control treatments were carried out by adding spore suspension (10 mL pot^{-1}) of the biological agent, at concentrations of 4.5 and 9.0 × 10^6 spore mL^{-1} water on the surface of infested soil.

The experiments were designed as follows:

1- Fungicide- free seeds in non-infested soil.

2- Fungicide- free seeds in soil infested with *R. solani* or *F. graminearum*.

3- Fungicide- treated seeds in soil infested with *R. solani* or *F. graminearum*.

4-Fungicide- free seeds in soil infested with *R. solani* or *F. graminearum* and biological agent.

5-Fungicide -treated seeds in soil infested with *R. solani* or *F. graminearum* and biological agent.

Each pot was planted with 15 wheat seeds and each treatment was replicated four times. Pre- and post- emergence damping –off % were recorded after 14 and 42 days from sowing, respectively, and the survival plants were recorded at the end of the experiment.

RESULTS AND DISCUSSION

In vitro activity of the fungicides on the isolated fungi

The *in vitro* sensitivity of *R. solani* and *F. graminearum* to four fungicides was determined. The EC_{50} values of the tested fungicides are listed in Table (1). Based on the EC_{50} values, the isolates of *R. solani* and *F. graminearum* are considered to be more sensitive to metalaxyl M + fludioxonil (EC_{50} = 0.24 and 0.32 μg a.i. mL^{-1}, respectively) followed by carbendazim (EC_{50} = 0.59 and 1.763 μg a.i. mL^{-1}, respectively) and triticonazole (EC_{50} = 1.58 and 1.32 μg a.i. mL^{-1}, respectively). However, the EC_{50} values for carboxin + thiram were considerably higher (EC_{50} = 8.39 and 29.43 μg a.i. mL^{-1}, respectively), indicating less sensitivity of the fungi.

Table 1. EC_{50}* values (μg a.i. mL^{-1}) of the tested fungicides against mycelial growth of *Rhizoctonia solani* and *Fusarium graminearum* grown on PDA medium.

Fungicides	*Rhizoctonia solani*	*Fusarium graminearum*
Triticonazole	1.58	1.32
Carbendazim	0.59	1.763
Carboxin + thiram	8.39	29.43
Metalaxyl M + fludioxonil	0.24	0.32

EC_{50}* = Effective concentration as (μg a.i. mL^{-1}) that give 50 % inhibition of the fungal growth.

Data listed in Table (1) show the efficacies of the tested fungicides against both *R. solani* and *F. graminearum* under laboratory condition. The obtained results were previously confirmed by other researchers. For example, El-Kholy (1999) found that the EC_{50} values of triticonazole were 1.10 and 0.90 μg mL^{-1} against mycelial growth of *R. solani* and *F. graminearum*, respectively. Also, the EC_{50} values of triticonazole for isolates of *F. graminearum* ranged from 0.043 to 4.965 μg mL^{-1} (Burlakoti *et al*., 2010). Similarly, carbendazim effectively inhibited the mycelial growth and sclerotial production of *R. solani* at lower concentration (Sriraj *et al*., 2014; Kumar *et al*., 2017). Also, mycelial growth of *F. graminearum* was strongly inhibited by carbendazim (Ivic *et al*., 2011). Carboxin + thiram fungicide exhibited a moderate effect against the growth of *R. solani* and it was less effective against *F. graminearum* growth (Table 1). These findings were confirmed by El-Ballat (2017) who found that the EC_{50} values of carboxin + thiram to *R. solani* and *F. graminearum* were 4.50 and 54.0 μg mL^{-1}, respectively. On the other hand, the results in the same table showed high efficacies of metalaxyl M + fludioxonil against both *R. solani* and *F. graminearum* under laboratory condition. These results are in accordance with those obtained previously (McMullen and Bradley, 2005; Miguel *et al*., 2015; Akgul and Erkilic, 2016). Sameer (2018) found that *R. solani* and *F. graminearum* were sensitive to fludioxonil with EC_{50} values of 0.29 and 0.371 μg mL^{-1}, respectively.

Greenhouse pot experiments

Separately effect of the tested fungicides and bioagents on damping-off diseases caused by R. solani and F. graminearum.

The effects of the tested fungicides applied at 1.5 and 3.0 g kg^{-1} seeds (as seed treatment) and biological control agents applied at 4.5 and 9.0 × 10^6 spores mL^{-1} (as soil treatment) on pre- and post- emergence damping- off diseases of wheat caused by *R. solani* and *F. graminearum* are listed in Table (2). The incidence of pre- and post- emergence of untreated seeds and soil were 40.00 and 33.33 %, respectively, for *R. solani* and were 33.33 and 42.50 %, respectively, for *F. graminearum*.

Table 2. Separately effects of the tested fungicides and bioagents against *Rhizoctonia solani* and *Fusarium graminearum* on wheat growing under greenhouse conditions.

Treatments	Rate of application (g kg^{-1}seeds) or (spores mL^{-1} water)	*Rhizoctonia solani* average % of			*Fusarium graminearum* average % of		
		Pre-emergence damping -off	Post-emergence damping -off	Plant survivals	Pre-emergence damping -off	Post-emergence damping-off	Plant survivals
Control (1)*		5.00	3.51	91.67	5.00	3.51	91.67
Control (2)**		40.00	33.33	40.00	33.33	42.50	38.33
Triticonazole	1.50	21.67	12.77	68.33	16.67	18.00	68.33
	3.00	11.67	11.32	78.33	10.00	9.26	81.67
Carbendazim	1.50	20.00	12.50	70.00	16.67	22.00	65.00
	3.00	10.00	9.26	81.67	6.67	10.71	83.33
Carboxin +	1.50	25.00	17.78	61.67	25.00	28.89	53.33
thiram	3.00	16.67	14.00	71.67	16.67	20.00	66.67
Metalaxyl M +	1.50	13.33	11.54	76.67	10.00	12.96	78.33
fludioxonil	3.00	5.00	3.51	91.67	3.33	6.90	90.00
Trichoderma	4.5x10^6	31.67	29.27	48.33	28.33	32.56	48.33
harzianum	9.0x10^6	25.00	24.44	56.67	20.00	20.83	63.33
Bacillus subtilis	4.5x10^6	31.67	26.83	50.00	26.67	31.82	50.00
	9.0x10^6	20.00	20.83	63.33	18.33	18.37	66.67
L.S.D. at 5 %	Treatments (T.)	2.97	2.59	3.76	3.26	3.19	4.18
	Rates (R.)	1.88	2.04	2.17	1.88	1.67	3.26
	T. x R.	3.29	3.51	4.19	4.08	4.31	5.22

* Control (1) = Untreated seeds in non-infested (sterilized) soil

** Control (2) = Untreated seeds in soil infested with pathogenic fungus (*R. solani* or *F. graminearum*)

Results indicated that all the tested fungicides and biological control agents were able to control damping-off caused by both fungi, and fungicides were the superior treatments. Metalaxyl M + fludioxonil was the most effective fungicide tested, followed by carbendazim and triticonazole, but carboxin + thiram was the least effective. *Trichoderma harzianum* was inferior to *Bacillus subtilis* against *R. solani* and *F. graminearum*. It was noticed that increasing the application rate of the tested fungicides and bioagents resulted in enhancing their efficiencies against the pathogenic fungi with increasing the growing plants. Results showed that the high rate of triticonazole, carbendazim, carboxin + thiram, metalaxyl M + fludioxonil, *T. harzianum* and *B. subtilis* gave 78.33, 81.67, 71.67, 91.67, 56.67 and 63.33 % plant survivals, respectively, for *R. solani* and gave 81.67, 83.33, 66.67, 90.00, 63.33 and 66.67 % plant survivals, respectively, for *F. graminearum*. The L.S.D. values for treatments in Table (2) revealed that metalaxyl M + fludioxonil significantly raised the plant survivals over all fungicidal treatments. The statistical analysis showed that the difference between carbendazim and triticonazole was not significant. The results showed that carboxin + thiram fungicide was the least effective one. Also, there was no significant difference between carboxin + thiram and *B. subtilis* in case of *F. graminearum*, but there was significant in case of *R. solani*. The statistical analysis also showed that the difference between *B. subtilis* and *T. harzianum* was not significant in both pathogenic fungi, but significant only at higher concentration in case of *R. solani*. Also, the statistical analysis showed that the difference between the efficiency of the two applied rates or concentrations was highly significant in all treatments.

Our present results are in agreement with those obtained previously. For example, El-

Kholy (1999) found that triticonazole was effective in reducing the *Rhizoctonia* and *Fusarium* root rot incidence on wheat plants under greenhouse conditions and increased emergence and plant stands. Burlakoti *et al*. (2010) suggested that triticonazole has the potential to control *Fusarium* diseases in field conditions if applied in a timely manner. Carbendazim was effective against *R. solani* at the lowest concentration by maximum inhibiting the mycelial growth and sclerotia formation (Sriraj *et al*., 2014; Kumar *et al*., 2017). Also, carbendazim has been widely used to control *Fusarium* diseases on several crops including wheat, showing a great capability to prevent *Fusarium* infections (Manasa *et al*., 2017; Mannai *et al*., 2018). In field trials, triazole and benzimidazole carbamate fungicides were confirmed to be effective to reduce *F. graminearum* contamination (Masiello *et al*., 2019). Carboxin + thiram, was among fungicides tested by El-Ballat (2017) who found that this fungicide was effective against *Rhizoctonia* and *Fusarium* root rot of wheat. Fludioxonil used alone or in mixture with metalaxyl M, has been largely used for cereals seed treatments, showing a high efficacy against *Rhizoctonia* and *Fusarium* root rot diseases (Miguel *et al*., 2015; Soovali *et al*., 2017). Akgul and Erkilic (2016) found that tebuconazole, carboxin + thiram and metalaxyl M + fludioxonil used as seed treatments had significant effects on *Fusarium* foot rot disease of wheat by penetrate wheat seeds and can be transported from the seeds to the shoots.

A large number of plant diseases have been successfully controlled through fungal and bacterial antagonists (El-Ballat, 2017; Ishtiaq *et al*., 2019). El-Sayed-Sahar and Abdel-Monaim (2017) reported that biological control agents significantly reduced the disease severity of root rot diseases caused by *Rhizoctonia* and *Fusarium*. They found that *B. subtilis* was the most effective bioagents followed by *T. harzianum*. The biological control agents secreted enzymes and secondary metabolites in wheat plants and induced systemic disease resistance against *R. solani* and *F. graminearum* (Ishtiaq *et al*., 2019).

Combined effect of T. harzianum and fungicides on wheat damping-off diseases caused by R. solani and F. graminearum.

Results in Table (3) reveal that *T. harzianum* moderately controlled the pathogens and then increased the percent of plant survivals which recorded 48.33 % for both pathogenic fungi when 4.5 × 10^6 spores mL^{-1} of *T. harzianum* were used. However, when *T. harzianum* was applied at 9.0 × 10^6 spores mL^{-1}, the percent of plant survivals were 56.67 and 63.33 % with *R. solani* and *F. graminearum*, respectively.

The combined fungicidal activity of *T. harzianum* at 4.5 and 9.0 × 10^6 spores mL^{-1} with the tested fungicides at 1.5 g product kg^{-1} seeds against pre- and post-emergence damping off diseases caused by *R. solani* and *F. graminearum* was evaluated. Results in Table (3) indicate that application of fungicides with *T. harzianum* gave considerable control for *R. solani* and *F. graminearum* and increased the percent of plant survivals. The results indicated that *T. harzianum* (9.0 × 10^6 spores mL^{-1}) with metalaxyl M + fludioxonil (1.5 g kg^{-1} seeds) gave a high percentage of plant survivals. Application of *T. harzianum* at high concentration with carbendazim (1.5 g kg^{-1} seeds) improved the activity of *T. harzianum* and did not improve the activity of carbendazim. *T. harzianum* at 9.0 × 10^6 spores mL^{-1} and the fungicide carboxin + thiram at 1.5 g kg^{-1} seeds gave 56.67 and 61.67 % plant survivals, respectively for controlling *R. solani* and gave 63.33 and 53.33 % plant survivals, respectively, for controlling *F. graminearum* when they were used alone. However, application of *T. harzianum* with carboxin + thiram at the same rates increased plant survivals to 73.33 and 66.67 % for controlling *R. solani* and *F. graminearum*, respectively. The statistical analysis showed that the application of fungicide with *T. harzianum* significantly differed from that of bioagent applied separately. The results indicated that application of *T. harzianum* as soil treatment with fungicides as seed treatment improved the antagonistic action against pre- and post- emergence damping off.

The obtained results indicated that low rates of triticonazole, carboxin + thiram and metalaxyl M + fludioxonil could control diseases of wheat, caused by *R. solani* and *F. graminearum*, in the presence of *T. harzianum* which is resistant to these fungicides. Previous results proposed that application of fungicide may metabolically weaken the pathogen and make it vulnerable to potent antagonists (Viji *et al*., 1997). Naar and Kecskes (1998) reported that the tolerant bioagents exhibited greater antagonism with the addition of fungicides. Also, the application of fungicides with bioagents would provide similar disease suppression as achieved with higher fungicide use (Monte, 2001). *Trichoderma* has the ability to degrade xenobiotic compounds and can live in environments with residues of fungicides (Chaparro *et al*., 2011). Tapwal *et al*., (2012)

Table 3. Greenhouse activity of *Trichoderma harzianum* on *Rhizoctonia solani* and *Fusarium graminearum* in the presence of different fungicides.

Fungicides at low rate (1.5 g kg^{-1}seeds)	Separated fungicide			Fungicide + *Trichoderma harzianum* at 4.5 × 10^6 spores mL^{-1}			Fungicide + *Trichoderma harzianum* at 9.0 × 10^6 spores mL^{-1}		
	average % of			average % of			average % of		
	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals
Control (1)*	5.00	3.51	91.67	-	-	-	-	-	-
Control (2)** *R. solani*	40.00	33.33	40.00	31.67	29.27	48.33	25.00	24.44	56.67
Triticonazole	21.67	12.77	68.33	20.00	12.50	70.00	18.33	10.20	73.33
Carbendazim	20.00	12.50	70.00	20.00	12.50	70.00	18.33	12.24	71.67
Carboxin + thiram	25.00	17.78	61.67	21.67	14.89	66.67	18.33	10.20	73.33
Metalaxyl M + fludioxonil	13.33	11.54	76.67	10.00	7.41	83.33	8.33	5.45	86.67

L.S.D. at 5 %	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals
Treatments (T.)	3.13	2.86	3.55
Rates (R.)	1.89	1.67	2.18
T. x R.	4.23	4.08	4.66

Fungicides at low rate (1.5 g kg^{-1}seeds)	Separated fungicide Pre-emergence damping -off	Separated fungicide Post-emergence damping -off	Separated fungicide Plant survivals	4.5 × 10^6 Pre-emergence damping -off	4.5 × 10^6 Post-emergence damping -off	4.5 × 10^6 Plant survivals	9.0 × 10^6 Pre-emergence damping -off	9.0 × 10^6 Post-emergence damping -off	9.0 × 10^6 Plant survivals
Control (1)*	5.00	3.51	91.67	-	-	-	-	-	-
Control (2)** *F.graminearum*	33.33	42.50	38.33	28.33	32.56	48.33	20.00	20.83	63.33
Triticonazole	16.67	18.00	68.33	15.00	15.69	71.67	15.00	11.67	75.00
Carbendazim	16.67	22.00	65.00	18.33	20.41	65.00	16.67	18.00	68.33
Carboxin + thiram	25.00	28.89	53.33	20.00	25.00	60.00	18.33	18.36	66.67
Metalaxyl M + fludioxonil	10.00	12.96	78.33	6.67	10.71	83.33	5.00	5.26	90.00

L.S.D. at 5 %	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals
Treatments (T.)	3.41	3.39	4.19
Rates (R.)	2.00	1.89	2.58
T. x R.	4.37	4.05	5.04

* Control (1) = Untreated seeds in non-infested (sterilized) soil

** Control (2) = Untreated seeds in soil infested with pathogenic fungus (*R. solani* or *F. graminearum*)

suggested that fungicides can be compatible with *Trichoderma* to control soil-borne plant pathogens. *Trichoderma* spp. were found to be compatible with triazole fungicides such as triticonazole (Thoudam and Dutta, 2014), thiram (Bagwan, 2010), carboxin (Rubayat and Alam Bhuiyan, 2012), carboxin + thiram (Jensen *et al*., 2002) and fludioxonil (Wang *et al*., 2005) for controlling root rot diseases. Wang *et al*. (2005) suggested that fludioxonil and *Trichoderma* could be integrated into a disease management program for *Fusarium* root rot.

Results in Table (3) also indicated that application of *T. harzianum* with carbendazim enhanced the activity of *T. harzianum* and did not improve the fungicide. This result was confirmed by (Rubayat and Alam Bhuiyan, 2012; Tapwal *et al*., 2012; Thoudam and Dutta, 2014) who found that *Trichoderma* was incompatible with carbendazim.

Combined effect of B. subtilis and fungicides on wheat damping-off diseases caused by R. solani and F. graminearum.

B. subtilis at 4.5 and 9.0 × 10^6 spores mL^{-1} (as soil treatment) and fungicide at 1.5 g kg^{-1} seeds (as seed treatment) were evaluated separately and in combinations for controlling pre- and post- emergence damping - off caused by *R. solani* and *F. graminearum*. Results in Table (4) reveal that the bioagent moderately controlled the pathogens and consequently increased the percent of plant survivals. The results in Table (4) indicated that when the bioagent *B. subtilis* was applied at 4.5 × 10^6 spores mL^{-1} for controlling the two pathogens *R. solani* and *F. graminearum*, it resulted in 50.00 % plant survivals for both pathogens, but when used at 9.0 × 10^6 spores mL^{-1}, the corresponding plant survivals rates were 63.33 and 66.67 %, respectively.

Table 4. Greenhouse activity of *Bacillus subtilis* on *Rhizoctonia solani* and *Fusarium graminearum* in the presence of different fungicides.

Fungicides at low rate (1.5 g kg^{-1}seeds)	Separated fungicide			Fungicide + *Bacillus subtilis* at					
				4.5 × 10^6 spores mL^{-1}			9.0 × 10^6 spores mL^{-1}		
	average % of			average % of			average % of		
	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals
Control (1)*	5.00	3.51	91.67	-	-	-	-	-	-
Control (2)** *R. solani*	40.00	33.33	40.00	31.67	26.83	50.00	20.00	20.83	63.33
Triticonazole	21.67	12.77	68.33	18.33	10.20	73.33	15.00	9.80	76.67
Carbendazim	20.00	12.50	70.00	16.67	8.00	76.67	11.67	7.54	81.67
Carboxin + thiram	25.00	17.78	61.67	20.00	14.58	68.33	18.33	10.20	73.33
Metalaxyl M + fludioxonil	13.33	11.54	76.67	8.33	7.27	85.00	5.00	5.26	90.00

L.S.D. at 5 %	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals
Treatments (T.)	3.16	2.74	4.89
Rates (R.)	1.98	1.60	3.39
T. x R.	4.52	4.33	5.79

Fungicides at low rate (1.5 g kg^{-1}seeds)	Separated fungicide: Pre-emergence damping -off	Separated fungicide: Post-emergence damping -off	Separated fungicide: Plant survivals	4.5 × 10^6: Pre-emergence damping -off	4.5 × 10^6: Post-emergence damping -off	4.5 × 10^6: Plant survivals	9.0 × 10^6: Pre-emergence damping -off	9.0 × 10^6: Post-emergence damping -off	9.0 × 10^6: Plant survivals
Control (1)*	5.00	3.51	91.67	-	-	-	-	-	-
Control (2)** *F.graminearum*	33.33	42.50	38.33	26.67	31.82	50.00	18.33	18.37	66.67
Triticonazole	16.67	18.00	68.33	13.33	15.38	73.33	10.00	9.26	81.67
Carbendazim	16.67	22.00	65.00	11.67	13.21	76.67	6.67	7.14	86.67
Carboxin + thiram	25.00	28.89	53.33	18.33	20.41	65.00	13.33	11.54	76.67
Metalaxyl M + fludioxonil	10.00	12.96	78.33	8.33	9.09	85.00	5.00	3.51	91.67

L.S.D. at 5 %	Pre-emergence damping -off	Post-emergence damping -off	Plant survivals
Treatments (T.)	3.22	3.19	4.31
Rates (R.)	2.03	2.07	2.53
T. x R.	4.09	4.16	5.17

* Control (1) = Untreated seeds in non-infested (sterilized) soil

** Control (2) = Untreated seeds in soil infested with pathogenic fungus (*R. solani* or *F. graminearum*)

Results in Table (4) indicated that the usage of fungicides as seed treatments with *B. subtilis* as soil treatment considerably controlled *R. solani* and *F. graminearum* which resulted in increased plant survivals rates. For example, the usage of *B. subtilis* (9.0 × 10^6 spores mL^{-1}) with metalaxyl M + fludioxonil (1.5 g kg^{-1} seeds) gave high plant survivals rates. When *B. subtilis* at 9.0 × 10^6 spores mL^{-1} and carbendazim at 1.5 g kg^{-1} seeds were used separately, they gave 63.33 and 70.00 % plant survivals, respectively, for controlling *R. solani* and gave 66.67 and 65.00 % plant survivals, respectively, for controlling *F. graminearum*. However, application of *B. subtilis* with carbendazim at the same rates increased plant survivals to 81.67 and 86.67 % for controlling root rot diseases caused by *R. solani* and *F. graminearum*, respectively. These results indicated that *B. subtilis* was compatible with all fungicides. The results obtained are in agreement with those obtained by many investigators. Dheepa (2013) reported that *B. megaterium* was compatible with tebuconazole + trifloxystrobin at 2000 ppm. Also, *B. subtilis* was compatible with triazole fungicide at the same concentration (Zalte *et al.*, 2013). *B. subtilis* was compatible with carbendazim, hence it used as a component in integrated disease management (Basamma and Kulkarni, 2017; Rajkumar *et al.*, 2018). Jensen *et al*. (2002) evaluated the positive effects of *T. harzianum* and *B. subtilis* alone or when combined with carboxin + thiram as biological control agent treatments against the dry bean root rot pathogens. Silimela and Korsten (2001) reported that the efficiency of the bioagents could further be improved when it was applied with the recommended fungicides at their lower concentrations. Srinivas and Ramakrishnan (2002) reported that integration of bioagents and fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually.

CONCLUSION

This work shows that the biological strategy which used throughout integrated pest management (IPM) could be improved by using fungicides at low rates.

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مدى توافق المركبات الحيوية مع مبيدات الفطريات في مواجهة أمراض عفن الجذور في القمح

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الملخص العربي

تعتبر امراض عفن الجذر من الامراض الخطيرة على القمح وتتسبب في خفض نسبة إنبات البذرة واستقامة النبات ومحصول الحبوب. تم تقييم أربعة مبيدات فطرية (ترايتيكونازول وكاربندازيم وكاربكسين + ثيرام وميتالاكسيل + فلودايوكسونيل) واثنين من المركبات الحيوية (ترايكوديرما هارزيأنم وباسلس ساتليس) منفردين وكذلك توافقهم عند استخدامهم ضد فطري ريزوكتونيا سولاني وفيوزاريوم جرامينيرام المسببين لأمراض عفن الجذور في القمح. بناءً على قيم EC_{50} لمبيدات الفطريات أظهرت الدراسات المعملية أن كفاءة المبيدات المختبرة على نمو فطر ريزوكتونيا سولاني تناقصت على النحو الآتي ميتالاكسيل + فلودايوكسونيل > كاربندازيم > ترايتيكونازول > كاربكسين + ثيرام بينما كانت ميتالاكسيل + فلودايوكسونيل > ترايتيكونازول > كاربندازيم > كاربكسين + ثيرام ضد نمو فطر فيوزاريوم جرامينيرام. بالنسبة لتجارب الصوبة فأنه تم تطبيق مبيدات الفطريات بمعدلين 1.5 و3 جم/كجم بذور كمعاملات بذور بينما طبقت المركبات الحيوية بتركيزين $4.5x10^6$ و $9.0x10^6$ جرثومة / مل ماء كمعاملات تربة. النتائج أوضحت أن كل المعاملات سببت نقصًا في حدوث الأمراض وزيادة في أنبثاق وإقامة النباتات. المركبات الحيوية كانت أقل فاعلية من مبيدات الفطريات لمكافحة مرض عفن الجذور المتسبب عن الفطرين. عمومًا كانت أكثر المعاملات فعالية في المكافحة ميتالاكسيل + فلودايوكسونيل يليه كاربندازيم وترايتيكونازول بينما كانت كاربكسين + ثيرام وباسلس ساتليس وترايكوديرما هارزيأنم أقل فاعلية مقارنة بالكنترول. توافق ترايكوديرما هارزيأنم وباسلس ساتليس مع مبيدات الفطريات كافح موت البادرات قبل وبعد الأنبثاق المتسبب عن الفطرين كما أن كفاءة المركبات الحيوية في مكافحة ريزوكتونيا سولأني وفيوزاريوم جرامينيرام تحسنت بسبب توافقها مع المعدلات المنخفضة لمبيدات الفطريات.

الكلمات المفتاحية: القمح، عفن الجذور، ريزوكتونيا سولأني، فيوزاريوم جرامينيرام، مبيدات الفطريات، المركبات الحيوية.