Biocontrol Agents for Controlling Wheat Root Rot Disease under Greenhouse Conditions

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ABSTRACT

Background: The most important wheat diseases that caused by soil-borne fungi are the root-rot disease. The current investigation conducted with evaluation the efficacy of some bioagents, Bacillus subtilis, Pseudomonas fluorescens, Azospirillum brasilense, Trichoderma harzianum, commercial bioagent (Planta guard) and chitosan against the causal pathogenic organisms of wheat root rot disease under greenhouse conditions.

Methods: Wheat seedlings infected with root rot disease were subjected to the causal fungal isolation trails. In greenhouse, wheat grains were sown individually in pots containing artificially infested soil with the pathogenic fungi R. solani or F. graminearum. Furthermore, the tested bioagents and the fungicide Topsin-M 70 were applied to the infested soil before sowing.

Result: The isolated fungi were R. solani or F. graminearum had proved their pathogenic ability to induce root rot disease of wheat. In pot experiment, all applied treatments affect root rot incidence of grown wheat seedlings in artificially infested soils with disease incidents. In infested soils with root rot incidents, bacterial bioagents reduced root rot incidence by 84.5-93.6% and 28.4- 35.3% 66.6, respectively and by 43.7% for T. harzianum. Moderate effect was obtained by chitosan and planta guard treatments in soil infested with either pathogenic fungi.

Key words: Biocontrol, F. graminearum, R. solani, Root rot, Wheat.

INTRODUCTION

From cereal crops, wheat (Triticum aestivum L.) is one of the greatest vital crop worldwide and considered the first strategic food crop in Egypt. Wheat plants are infected with many diseases during the growth stages and storage periods causing quantitative and qualitative reduction in plant stand, produced yield and stored grains. Root rot of wheat is considered a serious problem causing high reduction in yield (peining et al., 1976). Fusarium graminearum, F. equiseti, F. solani, Drechslera halodes and Rhizoctonia solani were recorded as causal pathogens of wheat root-rot diseases (Hashem and Hamada, 2002; Atef, 2008; Asran and Eraky, 2011).

Also, cereal diseases such as seedlings blight, root rot and head blight caused by different Fusarium species, e.g. F. culmorum, F. graminearum, F. poae and F. avenaceum resulting in yield loss were reported (Fernandez and Jefferson, 2004; Nicholson et al., 2004).

Recently, the development of beneficial microorganisms is considered modern advantage for controlling plant diseases instead of using agrochemical fungicides which had negative effect on the environment and human health.

Bacillus subtilis B. megaterium, Trichoderma viride and T. harzianum applied as bioagents were effective for controlling root rot disease caused by F. graminearum, D. halodes and R. solani on wheat under greenhouse conditions (Moubark, 2011). Mousa et al (2013) recorded that the use of P. ûuorescens and B. subtilis as biocontrol agents were efficient against F. graminearum the causal of wheat root rot under greenhouse conditions.

The aim of present research was to evaluate the potential of some bioagents, T. harzianum B. subtilis and P. ûuorescens as well as the commercial bio-product Planta guard for controlling wheat root rot caused by R. solani and F. graminearum under greenhouse conditions.

MATERIALS AND METHODS

Tested materials

The used antagonistic bioagents, T. harzianum, B. subtilis, P. ûuorescens were kindly obtained from Culture Collection Unit, Plant Pathology Dept., National Research Centre, Egypt. Meanwhile, chitosan and Planta Guard were purchased from Al-Gamhoria Company Ltd. for chemicals and medicinal instruments, Cairo, Egypt.

Isolation, purification and identification of wheat root rot causal organisms

Samples of wheat seedlings showing root rot disease symptoms were collected from different fields at Kafr El-Dawar district, Alexandria governorate and subjected to
isolation trails. The appeared fungal colonies were purified using hyphal tip technique and identified according to their cultural, morphological and microscopically characters after Barnett and Hunter (1972) and Nelson et al. (1990). Pure cultures were maintained on PDA medium, Sigma-Aldrich at 4°C until use.

Pathogenic ability of isolated wheat root rot causal organisms

Pathogenic ability of the isolated fungi was evaluated in pot experiment under greenhouse conditions. The inoculum of either *R. solani* or *F. graminearum* previously prepared as growth suspension (10⁶ cfu/mL) was added individually to pots containing loamy soil at the rate of 2L/pot, then mixed thoroughly. Another set of un-infested soil was left as control treatment. The infested and control soils were sown with 40 surface sterilized wheat grains cv. Misr 1. Five pots were used as replicates for each treatment. Disease symptoms of root rot appearance of wheat seedlings was observed and recorded 14-21 days after emergence.

**Greenhouse experiments**

At 2019 growing season, pot experiment (30cm diameter) containing loamy soil was conducted at greenhouse belong to plant pathology Dept., NRC, Egypt for evaluating the efficacy of some bioagents against the incidence of wheat root rot. The two pathogenic fungal inoculum weper were prepared as growth suspension (10⁶ cfu/mL) and used for soil infestation as previously mentioned. Then after the tested bioagents were also used as growth suspension (10⁶ cfu/mL), meanwhile the commercial bio-product materials, planta guard (10⁴ cfu/mL) and chitosan 2%/L were used at concentration of 1 ml/L. Both bioagents and other biotic materials used as soil drench at the rate of 2L/pot one week before wheat grains sowing. Untreated set of pots containing loamy soil was used for control treatment. The fungicide Topsin-M 70% was used for comparison as soil drench at the rate of 2g/L. Surface sterilized forty wheat grains were sown in each pot. Five pots were used for each treatment and control as well. Observation for root rot incidence was detected and recorded. After 60 days of sowing (the experimental period) accumulated percentage of disease incidence was calculated.

**Statistical analysis**

The obtained data were subjected to IBM SPSS software version 14.0. Analysis of variance was determined and the mean values were compared by Duncan’s multiple range test at P <0.05.

**RESULTS AND DISCUSSION**

Pathogenic ability of isolated wheat root rot causal organisms

The tested isolated two pathogens *R. solani* and *F. graminearum* proved their pathogenic ability to infect wheat seedlings causing root rot disease incidence. Data presented in Table (1) revealed that the recorded root rot incidence were 82.2 and 74.6% for wheat seedlings grown in infested soil with *R. solani* and *F. graminearum*, respectively. Meanwhile, un-infested control treatment showed no disease symptoms. In this concern, it was reported that wheat suffered mainly by *Rhizoctonia* species causing root rot worldwide (Demirci, 1998; Hashem and Hamada, 2002; Atef, 2008; Tunali et al., 2008; Hamada et al., 2011; Guo et al., 2012). Furthermore, in all regions worldwide where cereal crops are cultivated, it was reported that seedling blight, root rot and head blight diseases caused by various soilborne pathogenic fungi, *Fusarium* spp. and *R. solani* (Fernandez and Jefferson, 2004; Nicholson et al., 2004; Asran and Eraky, 2011). Also, Nourozian et al. (2006) stated that *F. graminearum* and *F. culmorum* are predominant and cause root rot, food rot, crown rot, stem rot and head blight in wheat.

**Greenhouse experiment**

Presented data in Table (2) and Fig (1) showed records for wheat seedlings root rot incidence and their reduction at each used biotic treatment and fungicide as well. Data in Table (1) revealed significant effect of all applied biotic and fungicide treatments against the incidence of root rot disease. *Rhizoctonia solani* and *F. graminearum* caused root rot infection ranged between 14.6-30.52% and 17.50-59.50% at applied biotic treatments, respectively. The fungicide treatment recorded 10.0 and 3.0% root rot incidence at treatment of *R. solani* and *F. graminearum*, compared with 92.0 and 83.17% at untreated control, respectively. On the other hand, illustrated data in Fig (1)

**Table 1:** Pathogenic ability of isolated fungi to induce root rot disease of wheat under greenhouse conditions.

| Soil infestation | Root rot incidence % |
|------------------|----------------------|
| *Rhizoctonia solani* | 82.2 ± 5.11 b |
| *Fusarium graminearum* | 74.6 ± 5.41ab |
| Control | 0.00 ± 0.00 a |

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05.

**Table 2:** Effect of some bioagents on wheat root rot incidence in artificially infested soil with disease pathogens under greenhouse conditions.

| Treatment               | Wheat root rot disease % |
|-------------------------|--------------------------|
|                         | *Rhizoctonia solani*     | *Fusarium graminearum* |
| *Bacillus subtilis*     | 5.80 ± 0.42 f            | 55.60 ± 1.04 b |
| *Pseudomonas fluorescentes* | 13.33 ± 0.55 f         | 59.50 ± 2.16 b |
| *Azospirillum brasilense* | 14.20 ± 0.35 de        | 53.77 ± 3.11 c |
| Chitosan                | 20.50 ± 2.04 d          | 24.20 ± 1.38 e |
| *Trichoderma harzianum* | 30.52 ± 1.24 c          | 46.81 ± 3.06 cd |
| Planta Guard            | 14.60 ± 0.00 de         | 17.50 ± 0.75 de |
| Topsis-M 70%            | 10.00 ± 0.52 e          | 3.00 ± 0.27 g |
| Untreated control       | 92.00 ± 2.32 a          | 83.17 ± 1.43 a |

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05.
showed that B. subtilis, P. fluorescens and A. brasilense have superior effect against disease incidence compared with T. harzianum. They revealed announced reduction in disease incidence calculated as 93.6, 85.5, 84.5% and 33.1, 28.4, 35.3% as well as 66.8 and 43.7% for grown wheat seedling in infested soil with R. solani and F. graminearum, in relevant respective order. Meanwhile, moderate effect was observed at chitosan treatment whereas it could reduce disease incidence by 77.7 and 70.9% at soil infested with R. solani and F. graminearum, respectively. High reduction 86.3% of root rot was recorded for planta guard (the commercial bio-product containing 10 spore suspension of T. harzianum) at infested soil with R. solani and 78.9% at F. graminearum infested soil. At fungicide treatment high reduction was observed in disease incidence in soil infested with R. solani and F. graminearum calculated as 89.1 and 96.3%, respectively. The stated results were found in a harmony with previous reports of several investigators. Several researchers recorded that biological control agents are sustainable and effective bio-inputs that could be used for plant diseases. Trichoderma harzianum and Pseudomonas fluorescens gave maximum disease control of sunflower collar rot (Gandhi et al., 2017). Also, Bacillus subtilis, P. fluorescens, Trichoderma and Gliocladium were used beneficially against Fusarium oxysporum f. sp. Lycopersici of tomato wilt and F. graminearum of wheat (Moussa et al., 2013; Estefania and Ljiga, 2018; Fitrainingisil et al., 2019). Recognition of certain rhizobacteria can trigger a systemic resistance reaction that renders the host less susceptible to subsequent infection by virulent agents (Bhutani et al., 2018; Herve et al., 2019; Fidous et al., 2019). Furthermore, Trichoderma spp. are considered within the greatest recorded fungi for biological approach for controlling plant pathogens. These antagonists produce various enzymes such as chitin-lytic enzyme, which implicated as an agent sharing in their ability as biocontrol action (Cherif and Benhamou, 1990; Lorito et al., 1993; Lima et al., 1997). Atef (2008) reported that application of either T. harzianum or B. subtilis individually or in combination was effective in control root rot of wheat caused by R. solani under in vitro and greenhouse trails. Moreover, it was reported that wheat grain dressing with antagonistic bacteria, including Pseudomonas and Bacillus, have essential effect for controlling F. graminearum and F. culmorum the causal agents of seedling blight disease (Johansson et al., 2003; Khan et al., 2006). Also, reduction of anthracnose symptoms on strawberry plants previously inoculated with A. brasilense was observed (Tortora et al., 2011). In the present greenhouse study chitosan treatment found to have suppressing effect against the both pathogens causing root rot disease incidence. Chitosan as byproduct was reported to have inhibition efficacy against various plant pathogens either as direct effect against pathogenic microorganisms (Allan and Hadwiger, 1979). Whereas the toxic effect as mode of action of Chitosan has generally suppress the growth of plant pathogenic fungi through its connection with the ultrastructural changes in the fungal hyphae (Lafl-amme et al., 1999). Also, chitosan treatment has indirect effect through enhancing plant resistance to the all parts of plants, e.g. seeds (Benhamou et al., 1994); fruits (Benhamou, 2004) and leaves (Trotel-Aziz et al., 2006). Referring to the obtained results in present work, it is clearly to note that application of vital bioagents or commercial bio-products considered safe and promising for controlling such root rot diseases.

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