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Evaluate The Efficiency Of Trichoderma Harzianum To Protect Seed And Seedlings Of Wheat Against The Damping-Off Pathogen

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Abstract

Current study aimed to evaluate the ability of the biocontrol agent Ttichoderma harzianum to protect seed and seedlings of wheat from the infection by Fusarium graminearum pathogen. The pathogenic fungus was isolated from infected spikes of wheat collected during 2019 growing season, while an isolate of T. harzianum was obtained from plant pathology laboratory of Faculty of Agriculture, University of Kufa. The pathogenicity test was carried out on the pathogenic fungus in lab and in pots to examine its pathogenicity to the seed and seedlings of wheat. T. harzianum was also tested for its ability to inhibit F. graminearum and protect wheat seeds. Results showed that F. graminearum was reduced the germination percentage sharply as it reached 0.00% in comparison with control treatment which gave about 86%. The pathogenic fungus also increased seed rot up to 100% compare to 13.33 in control. T. harzianum showed high ability to inhibit the radial growth of F. graminearum when it recorded 58.33%. While, the pathogenic fungus was reduced the germination of wheat seeds in pots to 46.33 compare to 83.33 in control. T. harzianum was increased the germination percentage and reduced seed rot in petri plates and pots and can be used as an efficient bio-agent to protect seeds and seedlings of wheat from the infection by F. graminearum.

Key words: Ttichoderma harzianum, bio-control agent, inhibition, Fusarium graminearum, wheat, pathogenicity.

1. Introduction

Wheat (Triticum aestivum L.) is an important cereal crop that occupies the first place among grain crops globally in terms of cultivated area, productivity and consumption (Giraldo et al. 2019). It is a herbal plant belongs to Poaceae family and has a great nutritional and economic importance as wheat flour contains 76.31g/100g carbohydrates (mainly starch), 10.33g/100g proteins, 2.70g/100g fibers and different proportions of minerals and lipids (Gomez et al. 2020).

Wheat crop is subjected to many plant pathogens during different stages of its growth particularly seeds rot and damping off diseases that caused by soil-borne fungi (Lamichhane et al. 2017), which leads to direct economic losses represented by seed and seedlings damages or death, while the indirect effect consisting of the cost of replanting and lower productivity due to the delay in sawing dates (Horst 2013). Fusarium spp. can cause several diseases to wide range of plant hosts including damping-off. Abdul Jalill and Numan (2016) observed that Fusarium graminearum caused 100% of seed damping-off in two wheat varieties, this fungus attacks seed and seedlings of wheat and reduced its germination in semi-tropical regions, during the growth of the pathogen on its plant host, it produces conidiospores then in the end of season, it produces ascospores as well as chlamidiospores which remain in soil or plant residues (Rasiukeviciute and Kelpsiene 2018; Nazish and Jaitly, 2021).

Many attempts has been conducted to control F. graminearum, for instance, chemical fungicides, however, these practices are cost effective as well as fungicides can develop a resistance in the pathogen when it used frequently. Recently, biological control of plant pathogens is used widely as promising method when the biological agent is applied and prevented or reduced the infection by plant diseases. Trichoderma harzianum has been shown great reduction in the radial growth of many plant pathogens that cause various plant diseases, and it significantly reduced F. graminearum on wheat (Mahmoud 2016). Moreover, Schoneberg et al. (2015) reported that strains of Tricoderma were reduced the area of F. graminearum growth by 45 t0 93% as well as decreased the number of ascospores up to 100% on wheat straw. Thus, the objective of the current study is to evaluate the ability of the biocontrol agent T. harzianum to protect seed and seedlings of wheat from the infection by F. graminearum pathogen.

2. Materials and Methods

The culture medium used to isolate and grow fungi

Potato Dextrose Agar (P.D.A.): This medium was prepared by dissolving 39g of commercialized P.D.A powder in 1L of distilled water then it mixed very well until a clear solution is obtained. After that, the solution was autoclaved at 121^0C for 15-20 minutes and left to cool down at laboratory temperature. 250mg/L of chloramphenicol was added to the sterile and cooled P.D.A medium to rid of bacteria growth then the medium was poured into sterile Petri dishes to isolate and grow the fungi used in this study.

Isolation of pathogen and obtaining the biological control agent

Fusarium graminearum isolates used in this study were isolated from infected spikes of wheat collected during 2019 growing season. Wheat spikes was cut into 0.5 to 1cm pieces and washed well many times then put on filter paper to get rid of water and placed in Petri plates on PDA medium then incubated for five days at 25± 2^oC. Colonies of the fungus were purified and re-cultured on 9-cm sterile Petri plates the identified by its morphological characteristics under the microscope with the help of Fusarium key (Leslie and Summerell 2006). A very affective isolate of T. harzianum was obtained from plant pathology laboratory of Faculty of Agriculture, University of Kufa.

The effect of F. graminearum on the germination percentage of wheat seeds used

Germination percentage was tested by inoculated the centre of another Petri plates contain PDA with 0.5cm disk of F. graminearum hyphae and incubated for 48h. Wheat seeds were sterilized by 2% sodium hypochlorite solution for 3min then washed with sterilized distilled water for several times and planted on edges of the fungus colonies. Four replicates each with ten seeds were contaminated with F. graminearum and another four replicates each with ten seeds were sprayed with sterilized distilled water as a control. Then, all replicates were incubated at 25± 2^oC for 7 days. Afterward, the number of rotting seeds was calculated as follows:

$$\text{Germination percentage} = \frac{\text{The number of germinated seeds}}{\text{Total seeds number}} \times 100$$

Antagonistic ability of T. harzianum against F. graminearum on PDA medium

The double culture method was used to examine the antagonistic ability of T. harzianum against F. graminearum on PDA medium. 9cm Petri plate containing PDA was divided into two equal parts, the first part was inoculated with 0.5cm disc from 7days age of F. graminearum colony and the second part was inoculated with 0.5cm disc from 7days age of Trichoderma colony. The experiment was conducted with three replicates of F. graminearum and no addition of Trichoderma as control treatment. Plates were incubated at 25± 2°C for 7 days, after that, the percentage of inhibition was measured following the equation of Swami and Alane (2013).

$$\text{Inhibition \%} = \frac{\text{Average of colony diameter in control - Average of colony diameter in treatment}}{\text{Average of colony diameter in control}} \times 100$$

The effect of F. graminearum and T. harzianum on the germination of seeds and the growth of seedlings wheat in plastic pots

The inoculum of F. graminearum and T. harzianum was prepared by contaminating Panicum miliacem seeds. Soil samples from different sites growing by wheat were collected randomly from 0-30cm depth and sterilized using autoclave for 1 hour then left for 24 h and resterilised for the same period. 0.5g of F. graminearum and T. harzianum was added to 100gof sterile soil in 9×10 cm plastic pots and the control treatment was prepared similarly but with sterile Panicum miliacem seeds only. 10 wheat seeds (surface sterile) were grown in each pot and pots were distributed randomly and irrigated as needed. After 10 days, the percentage of seed germination and seedling damping off was calculated, and the vegetative, root length were calculated after 28 days of planting.

Effect of adding F. graminearum and T. harzianum to the soil and their interaction on the germination of seeds and the growth of seedlings wheat

Wheat seeds were sterilized by 2% sodium hypochlorite solution for 3min then washed with sterilized distilled water for several times and planted in 9×10 cm plastic pots containing sterile soil then 0.5g of F. graminearum and T. harzianum was added separately. 10 wheat seeds (surface sterile) were grown in each pot. After 10 days, the percentage of seed germination and seedling damping off was calculated, and the vegetative, root length were calculated from the following treatments:

1- Soil only.
2- Soil + F. graminearum.
3- Soil + T. harzianum.
4- Soil + F. graminearum + T. harzianum.

Statistical analysis

Complete Randomized Design (C.R.D) was used to arrange laboratory experiments, and Randomized Complete Block Design (R.C.B.D) was used for the arrangement of field experiment. Means were compared using the least significant difference (L.S.D.) at 5% level of significance (P>0.05). All data of experiments were analyzed using Genstat program (version 12 Vsn Intersectional, Hemel Hempstead, UK).

3. Results and Discussion

Results of current study showed that F. graminearum was reduced the percentage of germination of wheat seeds to 0.00 compare to control treatment which reached 86.66, and the pathogenic fungus caused 100% of seeds rot in comparison with 13.33% in control treatment(Table 1). The reason for that may be attributed to the parasitism of the pathogenic fungus and its enzymes on wheat seeds or secreting toxic substances such as Zearalenone, Trichothecin which kills seed embryos immediately after germination. Results also indicated that T. harzianum was increased the germination percentage up to 90% compare to control.

Table 1: The effect of F. graminearum on the germination percentage of wheat seeds in Petri plates.

Treatments	**Percentage %**	
	Seeds germination	**Rotting seeds**
Control	86.66	13.33
F. graminearum	0.00	100
T. harzianum	90.00	10
L.S.D 0.05	6.66	6.63

Results showed high antagonistic ability of T. harzianum against F. graminearum and inhibited the growth of the pathogenic fungus by 58.33%. Several mechanisms used by the bio control agent T. harzianum to control pathogenic fungi including producing antibiotics, enzymes and toxins that can inhibit the growth of pathogens, parasitism, competition on nutrition and space, also some fungi such as Pleurotus ostreatus need specific media to grow and reproduce (Hussein et al. 2019). Trichoderma isolates can inhibit the growth many plant pathogens effectively as many studies has been shown great control of these isolates to F. oxysporum f.sp. lycopersici, F. solani and Rhizoctonia solani (Suleiman et al. 2019).

The effect of F. graminearum and T. harzianum on the germination of seeds and the growth of seedlings wheat in plastic pots

Results showed that there was significant reduction in seed germination when the pathogenic fungus F. graminearum was reduced the germination percentage to 46.33% compare to control treatment which reached 83.33%. While, F. graminearum was recorded the highest percentage of rotting seeds amounted 53.33% and the reason for that is the toxins that produced by the fungus and lead to reduce the germination of seeds (Kaur et al. 2020; Alaa et al., 2021). Results also indicated that F. graminearum was significantly reduced the length of total vegetative and root of wheat seedlings as it reached 8.80cm and 3.16cm respectively in comparison with 15.70 and 4.86cm in control treatment. Whereas, the biological control agent T. harzianum was enhanced the growth of seedlings and increased the length of total vegetative and root of wheat as it recorded 19.86 and 10.90cm respectively compare to control treatment. The biological control agent can promote the growth of plants using various direct mechanisms such as increase the availability of insoluble nutrients and minerals solubilization (Hajieghrari and Mohammadi 2016; Yadav et al. 2016).

Table 2: The effect of F. graminearum and T. harzianum on the germination of seeds and the growth of seedlings wheat in plastic pots.

Treatments	% percentage		Length (cm)	
	Seed germination	Rotting seed	Total vegetative	Total root
Control	83.33	16.66	15.70	4.86
F. graminearum	46.66	53.33	8.8	3.166
T. harzianum	86.66	16.66	19.86	10.90
L.S.D 0.05	16.31	16.30	3.39	2.56

Effect of adding F. graminearum and T. harzianum to the soil and their interaction on the germination of seeds and the growth of seedlings wheat

Table 3 results showed that F. graminearum was negatively affected seed germination percentage as it reached 36.66% compare to 90% in control. While, the percentage of rotting seeds was reached 63.33% in the treatment of the pathogenic fungus in comparison with 10% in control treatment. F. graminearum was also impacted greatly the total vegetative and root of wheat seedlings. The adding of T. harzianum to the soil in the plastic pots was increased seed germination percentage and reduced the percentage of rotting seeds. T. harzianum was promoted the length of both, total vegetative and root of wheat seedlings as it reached 21.16 and 9.36cm respectively compare to 15.93 and 6.62cm in control treatment.

Table 3: Effect of adding F. graminearum and T. harzianum to the soil and their interaction on the germination of seeds and the growth of seedlings wheat.

Treatments	% percentage		Length (cm)	
	Seed germination	Rotting seed	Total vegetative	Total root
Soil only	90.00	10.00	15.93	6.62
Soil + F. graminearum	36.66	63.33	10.43	4.83
Soil + T. harzianum	93.33	6.66	21.16	9.36
Soil + F. graminearum + T. harzianum	73.33	26.66	14.50	8.00
L.S.D 0.05	13.31	13.30	2.67	2.02

4. Conclusion

Current study indicated that F. graminearum was very pathogenic to the seed of wheat as it reduced the germination percentage and increased rotting seeds. The biocontrol agent T. harzianum was showed a high antagonistic ability against F. graminearum and inhibited the growth of this pathogenic fungus. T. harzianum was protected the seed and seedlings of wheat from the infection by F. graminearum, in addition to promote the total vegetative and root growth.

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