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Research Article

Determination of *In Vitro* Biocontrol Efficacy of *Trichoderma Harzianum* Against Some Wheat Pathogen *Fusarium* Species

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ABSTRACT

Recently, phytopathogenic problems have been increasing day by day in cereal production and have adverse effects on its production. One of the most common methods applied to control phytopathogens is the use of synthetic chemicals. The impacts of pesticides on the environment and food safety have made their use controversial. Therefore, developing and applying methods that can replace agricultural chemicals is an issue that needs to be emphasized. In this sense, biocontrol can be an alternative approach against agrochemicals in terms of sustainable agriculture. In the present study, it was aimed to examine the biocontrol efficiency of *Trichoderma harzianum* against *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium avenaceum*, causing product losses in wheat by dual culture tests and double plate assays. Results showed that *T. harzianum* had remarkable inhibition of mycelial growth of all *Fusarium* species in dual culture assays. Moreover, no statistically crucial differences were found in the double plate tests.

Keywords: Biocontrol, *Trichoderma harzianum*, *Fusarium* spp., wheat.

1. INTRODUCTION

The genus *Fusarium* consists of dangerous phytopathogenic fungi that infect several host species including wheat, maize, barley and oats, as well as other crops.[1-5] Diseases such as root rot, leaf spot and crown blight caused by *Fusarium* species cause yield and quality losses in cereals, and contamination of products with toxic compounds such as mycotoxins, which pose a significant health risk.[6]

There are no reliable and efficient methods for controlling *Fusarium* spp.[7] Moreover, disease control is mainly carried out using chemical fungicides. These synthetic fungicides potentially cause environmental pollution, health risks to living organisms, and the emergence of resistance to pathogens.[8] Nowadays, biological control of plant diseases has gained importance as an environmentally friendly approach. For this reason, *Trichoderma* strains are remarkable for biological control of plant diseases due to their powerful bioprotective properties.[9] The multifunctional mechanisms of *Trichoderma* species have been reported as nutrient and space competition in the rhizosphere, induction of systemic acquired resistance, mycoparasitism, antibiosis and promotion of plant growth and development.[10-14] Among these, the mycoparasitism ability of *Trichoderma* genus is considered their primary action mechanism.[15] Furthermore, *T. harzianum* antagonizes phytopathogens by producing several bioactive secondary metabolites and lytic enzymes.[12,16,17] *In vitro* techniques such as direct and indirect confrontation tests are commonly regarded as the easiest and fastest assessment strategies. They are commonly used to select antagonistic *Trichoderma* species and eliminate those with no biocontrol potential.[18]

This *in vitro* study was performed to investigate the biocontrol efficiency of *T. harzianum* against

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phytopathogenic *F. culmorum*, *F. graminearum* and *F. avenaceum*, which are the main problems of cereal production areas and to integrate alternative approaches to chemicals into the agricultural system in the control of wheat diseases.

2. MATERIALS AND METHODS

2.1. Fungal isolates

In this study, three phytopathogenic *Fusarium* isolates (*F. graminearum* PH-1, *F. culmorum* UR99, *F. avenaceum* av51 strains) and *T. harzianum* K20 strain as biocontrol agents which had been previously characterised with molecular techniques were used in *in vitro* assays. All isolates were kindly provided from the Molecular Biology and Genetics Laboratory, of the Faculty of Fine Arts, Istanbul Yeni Yuzyıl University. The pathogens and antagonistic fungi were grown at 26 ± 1ºC for 7 days on potato dextrose agar (PDA). The fungal discs with a 5 mm diameter of 7-day-old fresh cultures were used in all experiments.

2.2. Dual culture assay

Dual culture tests were conducted according to the method proposed by Rahman[19]. Briefly, mycelium discs (5 mm) of *Fusarium* spp. and *T. harzianum* were taken from the margins of 7-day-old cultures and placed at a distance of 3 cm in a PDA medium. Plates prepared by inoculating only pathogens were considered as a control group. All groups were incubated at 26 ± 1 ºC for 7 days.

The efficacy of the antagonist was calculated as (%) inhibition by the following formula[20]:

$$\text{İnhibisyon (\%)} = \frac{\text{Cd} - \text{Td}}{\text{Cd} - 5\text{mm}} * 100$$

In the formula; Cd=radial growth of the pathogen in pure culture, Td= radial growth of the pathogen in dual culture or double plate.

2.3. Double plate assay

The inhibition effects of volatile organic compounds produced by *T. harzianum* were determined on the mycelial growth of three pathogens by double plate tests according to Dennis and Webster.[21] Seven-day-old cultures of both the biocontrol agent and the pathogens were used in all tests.

Mycelial discs of pathogens and *T. harzianum* were placed on PDA, separately. The margins of the plates were sealed with parafilm and incubated as mentioned earlier. The control groups comprised the pathogen mycelial discs and a blank agar disk. The inhibition percentage was calculated using the formula previously mentioned in Dual culture assays section.

2.4. Statistical analyses

All assays were performed with triplicate and repeated two times. All data were subjected to variance analysis and differences among means were determined by Tukey's multiple range test ($p<0.05$) using Minitab 17 statistical software. All data were presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Dual culture assay

The inhibition effect of *T. harzianum* K20 against *F. culmorum*, *F. graminearum* and *F. avenaceum* was evaluated in dual culture on the 7th day of incubation. *T. harzianum* K20 significantly inhibited radial growth of all *Fusarium* species. The highest percent inhibition was observed on *F. graminearum* (Table 1, Figure 1).

Trichoderma species, which are widely used in biological control, are known to have different action mechanisms against pathogenic fungi.[22,23] However, different species of the genus *Trichoderma* or different isolates of the present species have been reported to show differences in their biocontrol ability.[24] As previously reported in many studies, dual culture tests are commonly utilised to determine antagonistic activity.[25] In a study conducted by Ann and co-workers[26], it was reported that *F. verticillioides* mycelial growth was obstructed by 32% by *Trichoderma* sp. in dual culture assays. Dal Bello[27]examined the biocontrol efficiency of three different *T. harzianum* isolates against *F. graminearum*, which causes blight of seedlings in wheat plants. The result showed that the biocontrol activity among the isolates was different from each other. Moreover, *T. harzianum* isolates had inhibition by 58.7-74% on the *F. graminearum* radial growth. Similarly, *T. harzianum* significantly inhibited the growth of *F. culmorum* in dual culture assays.[28]

Table 1. Percent inhibition of *Fusarium* species by *T. harzianum* in the dual culture.

	Inhibition (%)		
T. harzianum	***F. graminearum***	***F. culmorum***	***F. avenaceum***
	79,07 ± 0,3[a]	66,86 ± 1,15[b]	43,75 ± 1,81[c]

*Means shown with different letters in the lines are statistically different according to the Tukey ($p<0.05$).

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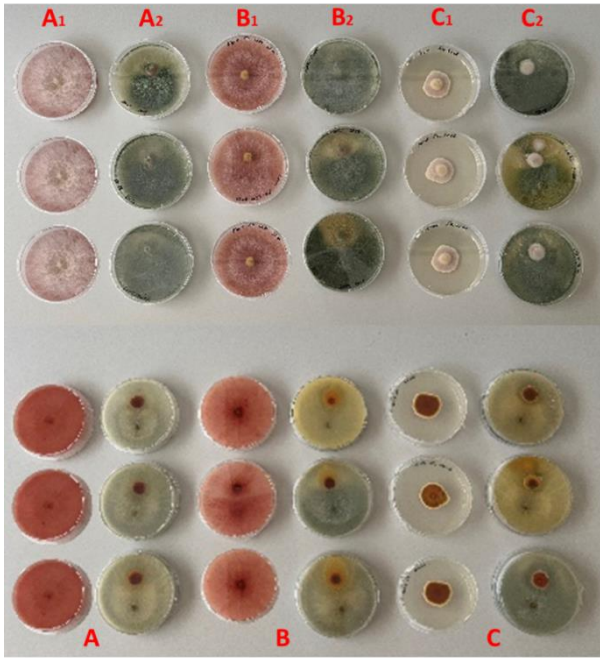

Figure 1. Inhibition of mycelial growth of *Fusarium* spp. by *T. harzianum* in dual culture; A) ***F. graminearum;*** (A_1) Control, (A_2) *F. graminearum* + *T. harzianum.* B) ***F. culmorum***; (B_1) Control, (B_2), *F. culmorum* + *T. harzianum.* C) ***F. avenaceum***; (C_1) Control, (C_2), *F. avenaceum* + *T. harzianum* (the upper part of the figure illustrates the front side of Petri dishes, bottom part illustrates the back side)

Table 2. Percent inhibition of *Fusarium* species by *T. harzianum* in the double plate assays.

	Inhibition (%)		
	F. graminearum	***F. culmorum***	***F. avenaceum***
T. harzianum	41,46 ± 3,14[a]	34,03±5,05[a]	29,37± 1,71[a]

*Means shown with the same letters in the lines are not statistically different according to the Tukey ($p<0.05$).

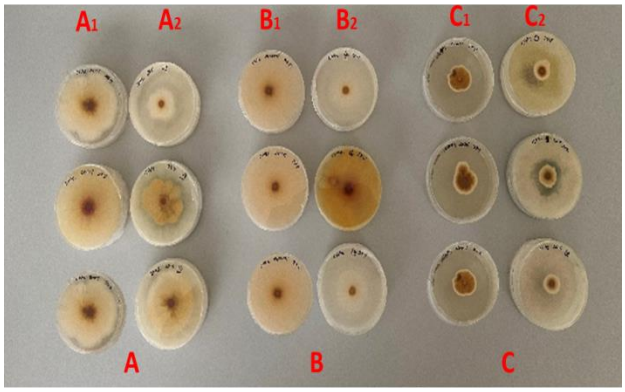

Figure 2. Inhibition of mycelial growth of *Fusarium* spp. by volatile compounds secreted by *T. harzianum*; A) ***F. graminearum***; (A_1) Control, (A_2), *F. graminearum* + *T. harzianum.* B) ***F. culmorum***; (B_1) Control, (B_2), *F. culmorum* + *T. harzianum.* C) ***F. avenaceum***; (C_1) Control, (C_2), *F. avenaceum* + *T. harzianum.*

Trichoderma species, which are widely used in biological control, are known to have different action mechanisms against pathogenic fungi.[22,23] However, different species of the genus *Trichoderma* or different isolates of the present species have been reported to show differences in their biocontrol ability.[24] As previously reported in many studies, dual culture tests are commonly utilised to determine antagonistic activity.[25] In a study conducted by Ann and co-workers[26], it was reported that *F. verticillioides* mycelial growth was obstructed by 32% by *Trichoderma* sp. in dual culture assays. Dal Bello[27]examined the biocontrol efficiency of three different *T. harzianum* isolates against *F. graminearum*, which causes blight of seedlings in wheat plants. The result showed that the biocontrol activity among the isolates was different from each other. Moreover, *T. harzianum* isolates had inhibition by 58.7-74% on the *F. graminearum* radial growth. Similarly, *T. harzianum* significantly inhibited the growth of *F. culmorum* in dual culture assays.[28]

3.2. Double plate assay

The inhibition effects of volatile compounds secreted by *T. harzianum* on the mycelial growth of three pathogens were evaluated on the seventh day of incubation. The inhibition percent by antagonist varied between 29,37 and 41,46. No statistically significant difference was found in the double plate assays (Table 2, Figure 2).

It is obviously known that *Trichoderma* produces many volatile organic compounds and these compounds have crucial roles in inhibiting the phytopathogens.[29,30] The biocontrol potential of *T. harzianum* has been reported against various plant pathogenic fungi in previous studies.[16,17] In the current work, the radial growth of three *Fusarium* species in double plate assays was inhibited by volatile compounds secreted by *T. harzianum*. However, no statistically significant difference was found among them. Similar results were also reported for *Trichoderma* sp.-*F. graminearum* pairs.[31] The most significant advantage of antibiosis via volatile organic compounds is the control of pathogens without physical contact.[32] When the results of the *in vitro* tests are examined, it can be easily understood that the percent inhibition rates obtained in the dual culture assays are significantly higher than the double plate. The importance of volatile organic compounds secreted by *Trichoderma* has also been reported in many earlier studies.[33,34] Our findings are consistent with previous studies.

4.CONCLUSIONS

The current study investigated the *in vitro* biocontrol efficacy of *T. harzianum* K20 on *F. graminearum*, *F. culmorum* and *F. avenaceum*. The findings showed that *T. harzianum* K20 could be an effective antagonistic agent against mentioned pathogens, especially *F. graminearum.* Further research which would be

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performed *in vivo* tests could be useful in determining the role of *T. harzianum* in plant defense against cereal diseases caused by *Fusarium* species.

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Conflict of interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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