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Research article

### Evaluation of the potential antagonist's potential use to regulate the growth and toxin formation of the fungus *Fusarium graminearum*

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#### Abstract

**Background and Aim.** Fungi of the genus *Fusarium* are the most frequently isolated pathogens of major agricultural crops. Due to their high pathogenicity and toxigenicity, they cause significant economic losses. Fungi of the genus *Fusarium* have the ability to synthesize numerous toxic metabolites, including deoxynivalenol, zearalenone et al. Toxins enter the human and animal food chains, causing serious human and animal diseases. The aim of this study was to evaluate the possibility of using a potential antagonist to regulate the growth and toxin formation of the fungus *Fusarium graminearum*.

**Materials and Methods.** *Fusarium graminearum*, a producer of fusariotoxin deoxynivalenol, and *Trichoderma Tr2* isolate, previously isolated from the soil of the Republic of Tatarstan were used in this study. The effect of in vitro interactions between *Trichoderma Tr2* and *F. graminearum* on the growth rate of fungal mycelium was evaluated. And the effect of the in vitro interaction between *Trichoderma Tr2* and *F. graminearum* on the amount of *F. graminearum* and deoxynivalenol production after 10 days of incubation at 25 °C on rice grain.

**Results.** *Trichoderma Tr2* significantly ( $p < 0.05$ ) reduced the growth of *F. graminearum* mycelium on potato agar by 74.3% compared to the control variant. Grain inoculation with *Trichoderma Tr2* isolate significantly ( $p < 0.05$ ) reduced the amount of *F. graminearum* on rice grains after 10 days of incubation, reducing the number of CFU fungi by 44% compared to control vials. A similar pattern was found in the analysis of mycotoxin. The detectable level of deoxynivalenol was also significantly reduced ( $p < 0.05$ ) when grain was treated with *Trichoderma Tr2* isolate, which reduced toxin accumulation by 68.7% compared to control vials, both the final concentration of deoxynivalenol and the concentration of deoxynivalenol per CFU by 44.1%.

**Conclusion.** The results of this study suggest that the soil fungus *Trichoderma Tr2* isolate may be of biotechnological interest. In future studies, it will be possible to determine effective levels of application in the field, test on other species and strains, and develop an optimal strategy for using the future product.

**Keywords:** antagonism; deoxynivalenol; mycotoxin; *Fusarium*; *Trichoderma*.

#### Introduction

Microscopic fungi and their toxic metabolites significantly reduce the quality of food and feed and pose a serious threat to food safety. Mycotoxins cause biochemical, physiological, and pathological changes in living organisms and have a toxic effect even at low concentrations [1, 2]. Fungi of the genus *Fusarium* are the most frequently isolated pathogens of important agricultural crops [3]. They are commonly found in soil, plant debris, and various organic substrates, where they live as saprophytes, feeding on dead organic matter. Due to their high pathogenicity and toxigenicity, they cause significant

economic damage. Fungi of the genus *Fusarium* have the ability to synthesize numerous toxic metabolites, including deoxynivalenol, zearalenone et others. Toxins enter the human and animal food chains, causing severe human and animal diseases [4, 5, 6].

New solutions aimed at reducing the entry of mycotoxins into the food chain are relevant. The results of studies show that most *Fusarium mycotoxins* are stable and persist at the stage of food processing [7, 8]. This highlights that limiting the presence of toxic fungi in agricultural products is extremely important, and in response to this challenge, there is a growing interest in biological methods for controlling fungal contamination and controlling toxin formation. The literature reports the use of the phenomenon of antagonism for various applications [9, 10, 11].

The aim of this study was to evaluate the possibility of using a potential antagonist to regulate the growth and toxin formation of the fungus *Fusarium graminearum*.

### Materials and Methods

This study used *Fusarium graminearum*, a producer of fusariotoxin deoxynivalenol, and *Trichoderma Tr2 isolate*, which previously demonstrated antagonistic properties to *F. graminearum* [12]. It was obtained from soil samples collected from various regions of the Republic of Tatarstan.

To prepare the mushroom inoculum, *Trichoderma Tr2* isolate was grown for 7 days at 25 °C in test tubes on Chapek-Dox agar. To obtain spore suspensions, the grown colonies were washed with 10.0 cm<sup>3</sup> of peptone water, filtered through sterile gauze to remove mycelium, and the spore concentration was adjusted to 10<sup>6</sup> CFU /cm<sup>3</sup> by serial dilution. *F. graminearum* was grown on potato agar at 25 °C for 7 days, and Petri dishes were used to remove agar plugs (5 mm) from the edge of growing colonies with a sterile sampler. To obtain spore suspensions, the grown colonies were also washed with 10.0 cm<sup>3</sup> of peptone water, filtered through sterile gauze to remove mycelium, and the spore concentration was adjusted to 10<sup>6</sup> CFU /cm<sup>3</sup> by serial dilution.

To assess the effect of *Trichoderma Tr2* isolate on the growth rate of *F. graminearum mycelium*, a modified method was used [13]. Aliquots (1.0 cm<sup>3</sup>) of mushroom suspensions were mixed with 19.0 ml of molten potato agar, homogenized, and poured onto Petri dishes (final concentration 10<sup>6</sup> cells/cm<sup>3</sup>). After solidification, agar plugs with *F. graminearum mycelium* were placed in the center of the cup. Control cups consisted of cups with sterile peptone broth (1.0 cm<sup>3</sup>) mixed with potato agar (19 cm<sup>3</sup>) and inoculated in the center with *F. graminearum* agar blocks. Experimental and control plates were incubated for 7 days at 25 °C in three repetitions. The diameter of the fungal colony (mm) was estimated daily by measuring two radii located at right angles to each other using a caliper. The relative decrease in mycelium growth was calculated by the formula:

Inhibition (%) = [(CD-TD)/CD] × 100 where CD is the control diameter and TD is the antagonism diameter.

Biological control was performed on rice as follows: 25 g of autoclaved rice grains were added to glass vials. 1 cm<sup>3</sup> of *F. graminearum* spore suspensions (10<sup>6</sup> CFU / cm<sup>3</sup>) and 1cm<sup>3</sup> of *Trichoderma Tr2* isolate suspensions (10<sup>6</sup> CFU /cm<sup>3</sup>) were sprayed, and incubated in three repetitions at 25 °C for a period of 10 days. Control vials were inoculated with 1 cm<sup>3</sup> of fungal suspension (10<sup>6</sup> CFU /cm<sup>3</sup>) and 1cm<sup>3</sup> of peptone broth instead of *Trichoderma Tr2* isolate suspension. At the end of incubation, mycotoxin deoxynivalenol was quantified according to the method described in [14].

The obtained experimental data were processed by the generally accepted method of variational statistics using the student confidence criterion using special programs.

### Results and Discussion

Table 1 presents the results of the in vitro interaction between *Trichoderma Tr2* and *F. graminearum* in terms of mycelial growth rate.

Table 1. Effect of in vitro interaction between *Trichoderma Tr2* and *F. graminearum* on mycelial growth rate.

	Colony diameter, cm	Mycelium growth rate, cm / • day	Mycelium growth reduction, %
Control <i>F. graminearum</i>	7.4 ± 0.32	0.93 ± 0.08	00.00
Experiment <i>F. graminearum</i> + <i>Trichoderma Tr2</i>	1,9,9 ± 0,26*	0,1717 ± 0,04*	74,3,3 ± 8,6,6

Note: \*  $p \leq 0.05$

The results presented in Table 1 demonstrate a pronounced antagonistic interaction between *Trichoderma Tr2* isolate and *Fusarium graminearum* under in vitro conditions. In the control variant, where *F. graminearum* was cultivated alone, the colony diameter reached  $7.4 \pm 0.32$  cm, with a mycelial growth rate of  $0.93 \pm 0.08$  cm/day, indicating the typical growth dynamics of this species on potato agar. In contrast, the presence of the antagonist significantly suppressed the development of the pathogen. When *Trichoderma Tr2* isolate was introduced into the growth medium, the colony diameter of *F. graminearum* decreased to  $1.9 \pm 0.26$  cm, while the mycelial growth rate declined to  $0.17 \pm 0.04$  cm/day. These changes correspond to a 74.3% reduction in mycelial growth, which was statistically significant ( $p \leq 0.05$ ). Such a strong inhibitory effect suggests that the *Trichoderma Tr2* isolate possesses pronounced antagonistic properties toward *F. graminearum*. The mechanism of inhibition may be associated with several well-known biological control strategies characteristic of *Trichoderma* species, including competition for nutrients and space, secretion of antifungal metabolites, production of cell wall-degrading enzymes, and direct mycoparasitism. Numerous studies have demonstrated that species of the genus *Trichoderma* are capable of producing hydrolytic enzymes such as chitinases,  $\beta$ -1,3-glucanases, and proteases, which contribute to the degradation of fungal cell walls and suppression of pathogenic fungi. Therefore, the obtained results confirm that the *Trichoderma Tr2* isolate exhibits significant antagonistic activity against *F. graminearum*, which makes it a promising candidate for further studies on biological control of *Fusarium* infections.

The effect of *Trichoderma Tr2* on the quantity of *F. graminearum* and deoxynivalenol production is presented in Table 2.

Table 2. Effect of in vitro interaction between *Trichoderma Tr2* and *F. graminearum* on fungal growth and deoxynivalenol production after 10 days of incubation at 25 °C

	CFU <i>F.</i> <i>graminearum</i> 10 <sup>6</sup> / cm <sup>3</sup>	Deoxynivalenol content, mcg • * g of rice	Decrease in deoxynivalenol production, % deoxynivalenol	Content, mcg / • CFU	Decrease in deoxynivalenol production, %
Control	7.5 ± 2.3	243.4 ± 26.7	00.0	32.4	00.0
Experiment <i>F. graminearum</i> + <i>Trichoderma</i> <i>Tr2</i>	4,2 ± 1,1*	76,2 ± 15,9*	68,7	18,1	44,1,1

Note: \*  $p \leq 0.05$

The results shown in Table 2 indicate that the antagonistic interaction between *Trichoderma Tr2* and *F. graminearum* affects not only the growth of the pathogen but also its ability to produce the mycotoxin deoxynivalenol (DON).

In the control samples inoculated with *F. graminearum* only, the fungal population reached  $7.5 \pm 2.3 \times 10^6$  CFU/cm<sup>3</sup>, while the concentration of deoxynivalenol accumulated in rice grain reached  $243.4 \pm 26.7$   $\mu$ g/g. These values confirm the high toxigenic potential of the pathogen under favorable growth conditions. However, when the *Trichoderma Tr2* isolate was introduced together with the pathogen, a

significant reduction in fungal growth and toxin formation was observed. The number of viable fungal cells decreased to  $4.2 \pm 1.1 \times 10^6$  CFU/cm<sup>3</sup>, representing a 44% reduction compared with the control. At the same time, the accumulation of deoxynivalenol decreased markedly to  $76.2 \pm 15.9$  µg/g, corresponding to a 68.7% reduction in toxin production. Moreover, the toxin production calculated per fungal unit (µg DON/CFU) also decreased by 44.1%, indicating that the antagonist not only limits the growth of *F. graminearum* but also suppresses its toxigenic activity. This observation suggests that the *Trichoderma Tr2* isolate may interfere with the metabolic pathways responsible for mycotoxin biosynthesis or create environmental conditions unfavorable for toxin production. These findings highlight the dual protective effect of the *Trichoderma Tr2* isolate: inhibition of pathogen proliferation and suppression of mycotoxin accumulation. Such characteristics are particularly important for the development of biological strategies aimed at improving the safety of agricultural products and reducing the risk of mycotoxin contamination in the food and feed chains.

### Conclusion

The present study evaluated the antagonistic potential of the soil fungus *Trichoderma Tr2* isolate against the phytopathogenic fungus *Fusarium graminearum*, a well-known producer of the mycotoxin deoxynivalenol. The results obtained under laboratory conditions clearly demonstrate that the *Trichoderma Tr2* isolate exhibits strong antifungal activity and significantly suppresses both the growth of the pathogen and its ability to produce mycotoxins.

The in vitro experiments showed that the presence of *Trichoderma Tr2* reduced the growth rate of *F. graminearum* mycelium by more than 70%, indicating a pronounced antagonistic interaction between these microorganisms. In addition, the biological control experiment performed on rice grain confirmed that the antagonist effectively limited fungal development and significantly decreased the accumulation of deoxynivalenol. The observed reduction in toxin production suggests that the *Trichoderma Tr2* isolate may influence the metabolic activity of the pathogen, which is particularly important from the perspective of food and feed safety.

Species of the genus *Trichoderma* are widely recognized as effective biological control agents due to their ability to colonize soil, compete with phytopathogenic fungi, and produce various antifungal metabolites and hydrolytic enzymes. Chemical and pharmacological studies of secondary metabolites of various *Trichoderma* species have shown that these fungi produce a number of compounds potentially applicable in medicine, biotechnology, and agriculture [15]. These properties allow them to suppress plant pathogens through several mechanisms, including competition for ecological niches, secretion of inhibitory compounds, and direct mycoparasitism. Therefore, the antagonistic activity demonstrated by the *Trichoderma Tr2* isolate in this study is consistent with the well-known biological characteristics of this genus.

From a practical standpoint, the results obtained indicate that the *Trichoderma Tr2* isolate may represent a promising biological agent for the control of *Fusarium* infections and the prevention of mycotoxin contamination in agricultural products. The use of biological control agents such as *Trichoderma* may provide an environmentally friendly alternative to chemical fungicides, contributing to sustainable agricultural practices and improved food safety.

However, further research is required to fully assess the practical applicability of this isolate. Future studies should focus on determining optimal application rates, evaluating the effectiveness of the *Trichoderma Tr2* isolate under field conditions, investigating its activity against different *Fusarium* species and strains, and assessing its stability and safety in agricultural ecosystems. In addition, molecular and biochemical studies could help clarify the mechanisms underlying the antagonistic activity observed in this study.

In conclusion, the results obtained demonstrate that the soil fungus *Trichoderma Tr2* isolate possesses significant antagonistic activity against *Fusarium graminearum* and is capable of reducing both fungal growth and deoxynivalenol production. These findings suggest that this isolate may have considerable biotechnological potential and could serve as a promising candidate for the development of biological preparations aimed at controlling *Fusarium* contamination in agricultural systems.

**Authors' Contributions**

SS and AG: developed the research concept; ASH and EM: research; SS and EM: comprehensive literature search, analyzed the collected data and prepared a draft of the manuscript; RV and YK: completed the final editing and proofreading of the manuscript; All authors read, reviewed, and approved the final version of the manuscript.

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