

Inhibition of Fungal Pathogens of *Pleurotus tuber-regium* (Fries) Singer using *Trichoderma harzianum* and *Trichoderma Viride*

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Abstract: This study was carried out to compare the potentials of *Trichoderma Harzanium* and *Trichoderma viride* to inhibit the radial mycelial growth of three fungal pathogens (*Aspergillus niger*, *A. flavus* and *A. ochraceus*) of *Pleurotus tuber-regium*. Each *Trichoderma* strain was paired with each pathogen on 9 cm Petri plates of acidified potato dextrose agar using three pairing methods. These were: inoculation of pathogens before the antagonists (*Trichoderma* spp.), inoculation of pathogens simultaneously with the antagonist and inoculation of pathogens after the antagonist. Plates obtained from the three treatments were then incubated for different period of 48, 72, 96, 120, 144 and 168 hours. Varying estimated growth inhibitions of pathogens by *Trichoderma* strains were subjected to analysis of variance (ANOVA) to determine the significance of differences using SPSS version 17.0. The result of the study depicted that in all the three treatment combinations, the radial mycelial growth of all the *Aspergillus* pathogens were inhibited by the two species of *Trichoderma* antagonist. However, the degree of inhibition was treatment specific. For instance, the percentage inhibition was significantly higher ($P < 0.05$) in all the treatments where the antagonist was inoculated before the introduction of the pathogen while the least inhibition was observed when the pathogen was inoculated before the introduction of the antagonist. *A. niger* was not highly inhibited compared to the other pathogens. In addition, *T. harzianum*. Appeared to demonstrated greater antagonistic activity between 24 and 144 hours of incubation. However, at the 168th hour of incubation there appeared to be no significant difference between the antagonistic activities of the two *Trichoderma*spp against the investigated pathogens. Results of the present study suggest that *Trichoderma* spp. used in this study could be exploited as biopesticides in the control of *Aspergillus*. Pathogens of *P.tuber-regium*.

Keywords: *Trichoderma Harzanium*, *Trichoderma viride*, *Aspergillus* spp., pathogens, antagonists

1. Introduction

Pleurotus tuber-regium is an edible fungus in the class Basidiomycetes with a large group of about 16, 000 species. It is one of the popular indigenous edible fungus growing with distinct fleshy sporophores on wood (Alexopolous et al., 1996). *Pleurotus* spp. are delicacies in different parts of the world because of their excellent flavored taste (Jonathan et al., 2014). *Pleurotus tuber-regium* contains no starch, has low sugar content and high amount of fibre; hence it serves as the least fattening food (Adesina et al., 2011). Furthermore, its sclerotia serves as a valuable ingredient in the preparation of various native soups and sauces. *Pleurotus tuber-regium* are been used trado-medically for the treatment of a number of ailments such as headache, stomach pain, fever and cold. For instance, it was documented that its sclerotia combined with different herbs could be used to treat whooping cough, smallpox, asthma, and dysentery (Osemwegie et al., 2002). The bioactive metabolites and medicinal properties of *P. tuber-regium* have played an important role in the development of new biotech products and biopharmaceuticals. Most *Pleurotus* spp. contains polysaccharides like alpha and beta glucans that form components of modern drugs (Fasidi and Olorunmaje, 1994).

It has been reported that fungal pathogens can either partially or completely killed mushrooms by parasitizing their mycelia (Oyelakin et al., 2014). In this regard, it was documented that, *Pleurotus* spp. are susceptible to range of diseases caused by pathogenic bacteria and fungi and thus reducing its yield and quality (). Early detection of disease

can help a grower effectively control a disease outbreak. The disease type, distribution and level of damage are parameters included to help determine the disease patterns and sources of infection (Jonathan et al., 2012). Several approaches that have been adopted in control of fungal pathogens include Due to the global concerns against the use of environment harming pesticides, the development of environmental friendly strategies of suitable and available biological agents have been proposed (Olawuyi et al., 2014). In this connection, the effectiveness of *Trichoderma* spp. as biocontrol and ecofriendly agent had been confirmed in control of fungal pathogens of plants (Sobowale et al., 2007).

Trichoderma is a unique genus that is made up of fungi most commonly used as biocontrol fungi against many pathogens *in vitro* and *in vivo* (Paavanen-Huhtala et al. 2000). The different mechanisms by which members of this genus bring about their biocontrol activity include mycoparasitism, competition, and antibiosis amongst others (Campbell 1988; Wells 1988; Sharma and Sankaran 1988). Amongst several other reports, *T. viride*, isolated from roots of maize plants was reported to suppress radial colony extension of *F. verticillioides in vitro* (Yates et al. 2000). *T. harzianum* amongst others are also reported to be effective in controlling pigeon pea wilt of *Fusarium oxysporum* f. sp. *Udum* (Somasekhara et al. 1996) and maize stem rot of *Fusarium verticillioides*. (Sobowale et al., 2007, 2009). Recently, the fumonis in content of maize seeds was reduced subsequent to dual inoculation of maize stem with *Fusarium verticillioides* and certain *Trichoderma* species (Sobowale, 2019). Similarly, selected isolates of *Trichoderma gamsii* was shown to induce different pathways of systemic

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resistance in maize upon *Fusarium verticillioides* challenge (Galletti et al. 2020) while *T. pseudoharzanum*T17 isolated from a Chinese forestry model base demonstrated a significant antifungal properties toward *Fusarium oxysporum* (Zhou et al. 2020). In the present study, the potentials of *Trichoderma viride* and *Trichoderma Harzanium* to inhibit three fungal pathogens (*Aspergillus niger*, *A. flavus* and *A. ochraceous*) of edible *Pleurotus tuber-regium*. was investigated.

2. Material and Methods

Isolation and Identification of Fungal Pathogens from Infected mushrooms (*P. tuber-regium*)

Ten samples of naturally infected mushrooms (*P. tuber-regium*) were collected from a local farm in Igboora, Ibarapa Local Government, Oyo State, Nigeria (). Fragments from the rotting parts of the fruiting bodies (stipe and cap) were prepared and surface sterilized in 1% sodium hypochlorite (for 5 minutes) and later rinsed in five separate beakers with sterile distilled water. Sterile forcep was then used to pick up and place the rotted fragments onto sterile filter papers which were wrapped for 5 minutes. Thereafter, the dried fragments were plated in Petri plates with acidified potato dextrose agar (APDA). The plates were incubated at 28–30°C for 10 days. Resulting mixed cultures of fungal colonies were subcultured appropriately to obtain pure cultures (Sobowale et al., 2009). Identification of the purified cultures was done using Keys described by compendium of soil fungi (Malloch, 1997). The frequency of occurrence of each of the pathogens was estimated. Pathogenicity test of the three isolates was done in order to confirm their pathogenicity status.

Pairing of *T. harzanium* and *T. viride* with Isolated Fungal Pathogens

Pure cultures of the antagonist agents used (*T. harzanium* and *T. viride*) were collected from the Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. They were subcultured on acidified Potato Dextrose Agar. *T. harzanium* and *T. viride* were screened against the three isolated pathogens (*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus ochraceous*) in an in vitro pure culture of PDA plates according to the three treatment described above (Innocenti et al., 2003). Each *Trichoderma* strain was paired with each pathogen on 9 cm Petri plates of acidified potato dextrose agar using three pairing methods. These were: inoculation of pathogens before the antagonists (*Trichoderma spp.*), inoculation of pathogens simultaneously with the antagonist and inoculation of pathogens after the antagonist. Plates obtained from the three treatments were then incubated for different period of 48, 72, 96, 120, 144 and 168 hours.

The interaction of antagonists with pathogens along with the control plates were observed and recorded. The radial growth of mycelia of the pathogens was calculated in relation to the growth of the control as follows;

$$\% \text{ Inhibition of Mycelial growth} = \frac{P_c - P}{P_c} \times 100$$

Where P_c = mycelial radical growth of the pathogens in control

P = mycelial radical growth of pathogens in antagonists.

Varying estimated growth inhibitions of pathogens by *Trichoderma* strains were subjected to analysis of variance (ANOVA) to determine the significance of differences. A two-tailed P value of less than 0.05 was considered to be statistically significant. Values that were significantly different were separated using the Duncan Multiple Range test using SPSS for Windows Version 17.0 statistical package.

3. Results and Discussion

The most frequently occurring fungal pathogens isolated from sporophores of the naturally infected *P. tuber-regium* in this study were *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus ochraceous*. The percentage frequency of occurrence of these pathogens was, and respectively. All the three pathogens were isolated from the stipe and cap of the mushroom. Okhuoya et al. (1996) had reported that *Sclerotium rolfsii*, a destructive soil pathogen common in tropical soils, was found to cause stipe rot of the fruit bodies of *Pleurotus tuber-regium*. In another development, Oranusi et al. (2014) evaluated the bacterial and fungal flora associated with the sclerotia of *Pleurotus tuber-regium*. These authors reported the presence of species of *Aspergillus*, *Bacillus*, *Pseudomonas*, *Klebsiella*, and *Staphylococci*. However, the pathogenicity status of these isolated microorganisms was not established by their report. It appears that this is the first study that established the pathogenicity status of *A. niger*, *A. flavus* and *A. ochraceous* to *Pleurotus tuber-regium*. However, the phytopathogenicity of *Aspergillus* fungi is not in doubt as Raji and Raveendran (2013) documented that *Aspergillus niger* is a saprophyte in soil which causes black mould of onion, garlic and shallot; stem rot of *Dracaena*; root stalk rot of *Sansevieria*; and boll rot of cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune as well as Crown rot of groundnut.

The *in vitro* inhibition (%) of radial mycelial growth of *Pleurotus tuber-regium* fungal pathogens by *Trichoderma Harzanium* and *Trichoderma viride* after different treatment combinations is shown in Table 1. In all the three treatment combinations, the radial mycelial growth of all the *Aspergillus* pathogens were inhibited by the two species of *Trichoderma* antagonist. However, the result indicated that the degree of inhibition was treatment specific. For instance, the percentage inhibition was significantly higher ($P < 0.05$) in all the treatments where the antagonist was inoculated before the inoculation of the pathogen while the least inhibition was observed when the pathogen was inoculated before the introduction of the antagonist. In this particular treatment, *A. niger* was not highly inhibited compared to the other pathogens. In addition, *T. harzanium*. appeared to demonstrated greater antagonistic activity between 24 and 144 hours of incubation. However, at the 168th hour of incubation there appeared to be no significant difference between the antagonistic activity of the two *Trichoderma spp* against the investigated pathogens.

The findings from this study showed that *Trichoderma spp.* inhibited the growth of *Aspergillus spp.* which agrees with the report of Gomathinayagam, (2012), who observed that

T. harzanium was an active hyper parasite which prevented the mycelia growth of *Bipolarisoryza* in rice disease. Similarly, Salami (1999) reported that *T. viride* was a potential biocontrol agent of pepper plants against soil-borne pathogens (*Phytophthora infestans*) and enhanced the growth of pepper seedlings. In a related development, Okhuoya et al. (1996) had reported that *Sclerotium rolfsii*, a destructive soil pathogen common in tropical soils, was found to cause stipe rot of the fruit bodies of *Pleurotus tuber-regium*. The rot occurs only when the pathogen was inoculated into the soil before seeding and before primordial emergence. The pathogens caused 100% inhibition of

primordia and sporophore formation when the soil was inoculated before seeding and highly reduced yield when the soil was inoculated with the pathogen just before primordial emergence. However, no fruit body rot occurred on the mushroom when the soil was inoculated with the pathogen after primordial emergence from the soil. The significance of the pairing methods showed that the inoculation of antagonist before pathogens aided the inhibition of mycelia growth of *Aspergillus* spp. which is significantly ($P < 0.05$) better than other two pairing methods as similarly reported by (Zegeye et al., 2011).

Table 1: In vitro Inhibition (%) of Radial Mycelial Growth of *Pleurotus tuber-regium* fungal pathogens by *Trichoderma Harzanium* and *Trichoderma viride* after different treatment combinations.

Treatments	Time of Incubation (Hour)											
	48		72		96		120		144		168	
	TH	TV	TH	TV	TH	TV	TH	TV	TH	TV	TH	TV
Ao ₄ A	27.77 ^c	70.83bc	50.53bc	37.03cd	51.30bc	41.20de	55.57bc	33.73d	55.57bc	50.00a	54.60e	54.60cd
An ₄ A	23.33 ^e	25.20e	22.20de	34.83cd	36.30cd	35.33e	37.33de	41.67d	47.70cd	42.57a	44.63f	42.93d
Af ₄ A	79.17 ^{bc}	49.53cd	42.23cd	45.83cd	30.57d	53.20cd	31.80e	53.93c	35.70cd	29.43b	62.83f	56.70c
Ao + A	33.33 ^e	50.83cd	52.77bc	54.63bc	54.37bc	60.33cd	59.53bc	60.53bc	59.53bc	24.27bc	66.70d	66.70bc
An + A	50.00 ^d	27.90de	36.27cd	31.10d	46.67cd	32.67e	50.00cd	42.07d	60.00bc	49.77a	47.53f	61.70bc
Af + A	65.00 ^{cd}	91.43ab	40.73cd	75.00ab	50.57bc	80.80ab	53.87bcd	80.60a	56.20bc	12.77de	82.37c	61.27bc
A ₄ Ao	92.23 ^{ab}	95.83a	95.57a	87.40a	94.90a	89.10a	94.67a	90.03a	94.67a	6.17a	91.70a	91.70a
A ₄ An	75.00 ^c	64.00c	66.70b	66.70ab	90.37a	66.00bc	88.67a	70.00b	90.30a	18.47cd	77.10a	72.53b
A ₄ Af	97.50 ^a	71.40bc	96.33a	85.67a	66.13b	87.83a	67.70b	86.07a	69.07b	11.13de	87.63ab	94.93b
Control (Ao)	3.00 ^f	4.00e	6.00e	4.50e	6.50e	5.50f	7.50f	6.00e	7.50e	7.00e	3.00g	8.00e
Control (Af)	4.00 ^f	3.50e	4.50e	4.50e	6.00e	5.20f	6.50f	5.50e	7.00e	6.00e	7.00g	8.00e
Control (An)	4.00 ^f	3.50e	4.50e	4.50e	4.50e	5.00f	5.00f	6.00e	6.50e	6.50e	8.00g	8.00e

A- Values are means of three replicates. Within a column, values with the same superscript are not significantly different at $P > 0.05$ Antagonist Organism TH-*Trichoderma Harzanium* TV-*Trichoderma viride*

- Ao₄A – *Aspergillus ochraceous* inoculated before Antagonist
- Ao +A - Simultaneous inoculation of *A. ochraceous* with antagonist
- An₄A - *Aspergillus niger* inoculated before Antagonist
- Af + A - Simultaneous inoculation of *A. flavus* with antagonist
- Af₄A - *Aspergillus flavus* inoculated before Antagonist
- An + A - Simultaneous inoculation of *A. niger* with antagonist
- A₄Ao - Antagonist inoculated before *A. ochraceous*
- A₄An - Antagonist inoculated before Antagonist *A. niger*
- A₄Af - Antagonist inoculated before *A. flavus*

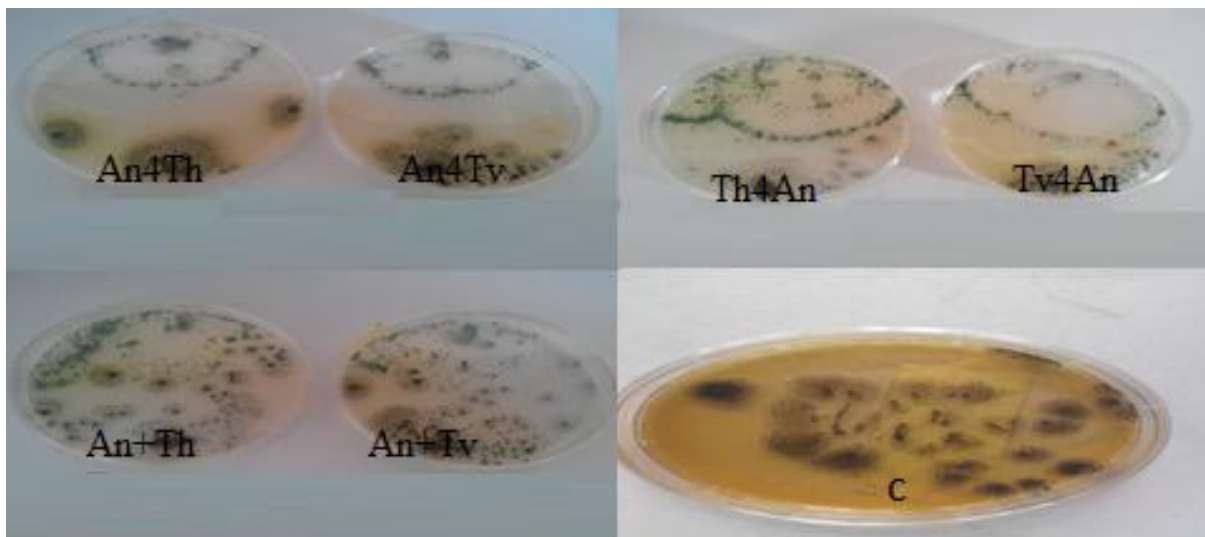


Plate 1: In vitro inhibition of *Aspergillus niger* by *Trichoderma Harzanium* and *Trichoderma viride* on Potato Dextrose Agar (PDA).

An+Th = *Aspergillus niger* simultaneous incubation with *Trichoderma Harzanium*.

An+Tv = *Aspergillus niger* simultaneous incubation with *Trichoderma viride*.

An4Th = *Aspergillus niger* inoculated before *Trichoderma Harzanium*.

An4TV = *Aspergillus niger* inoculated before *Trichoderma viride*.

Th4An = *Trichoderma Harzanium* inoculated before *Aspergillus niger*.

Tv4An = *Trichoderma viride* inoculated before *Aspergillus niger*.

C= Control plate of *A. niger* unpaired with *Trichoderma* spp.

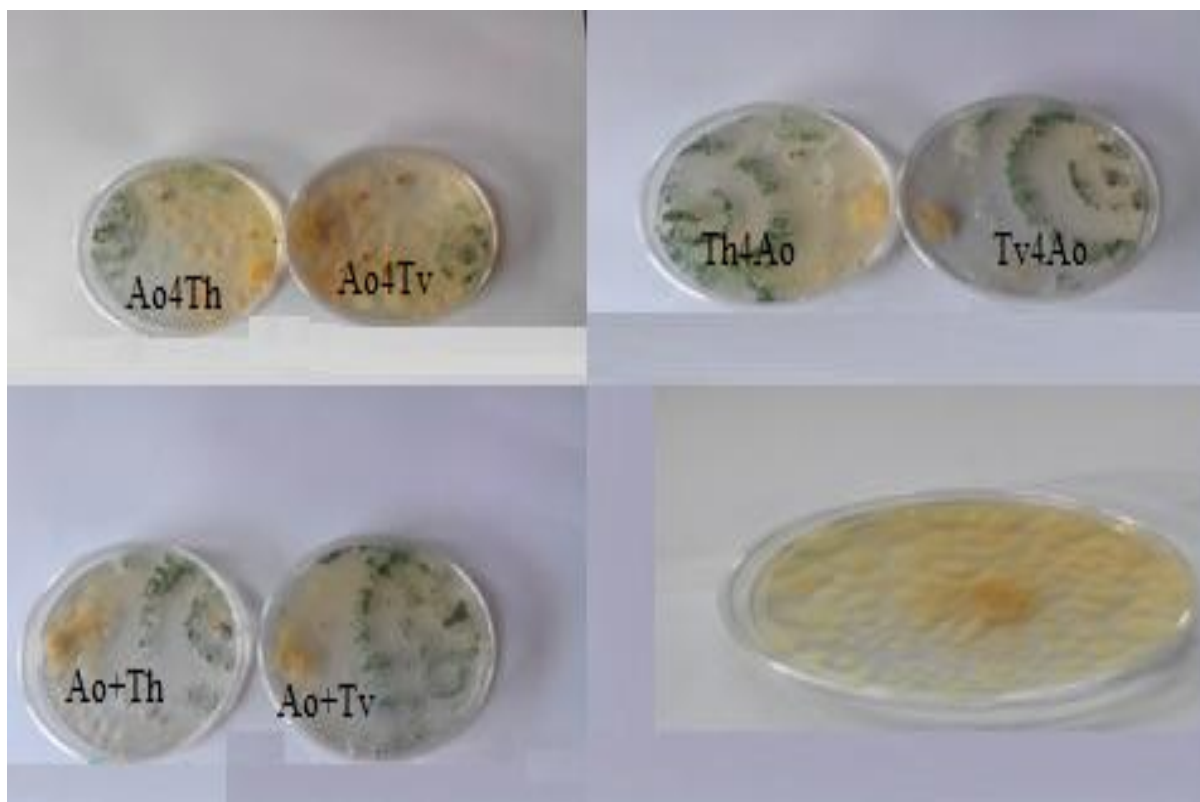


Plate 2: *In vitro* inhibition of *Aspergillus ochraceus* by *Trichoderma Harzanium* and *T. viride* on Potato Dextrose Agar (PDA)

Ao+Th = *Aspergillus ochraceus* simultaneous incubation with *Trichoderma Harzanium*.

Ao+Tv = *Aspergillus ochraceus* simultaneous incubation with *Trichoderma viride*.

Ao4Th = *Aspergillus ochraceus* inoculated before *Trichoderma Harzanium*.

Ao4Tv = *Aspergillus ochraceus* inoculated before *Trichoderma viride*.

Th4Ao = *Trichoderma Harzanium* inoculated before *Aspergillus ochraceus*.

Tv4Ao = *Trichoderma viride* inoculated before *Aspergillus ochraceus*.

P = Control plate of *A. ochraceus* unpaired with *Trichoderma* spp.

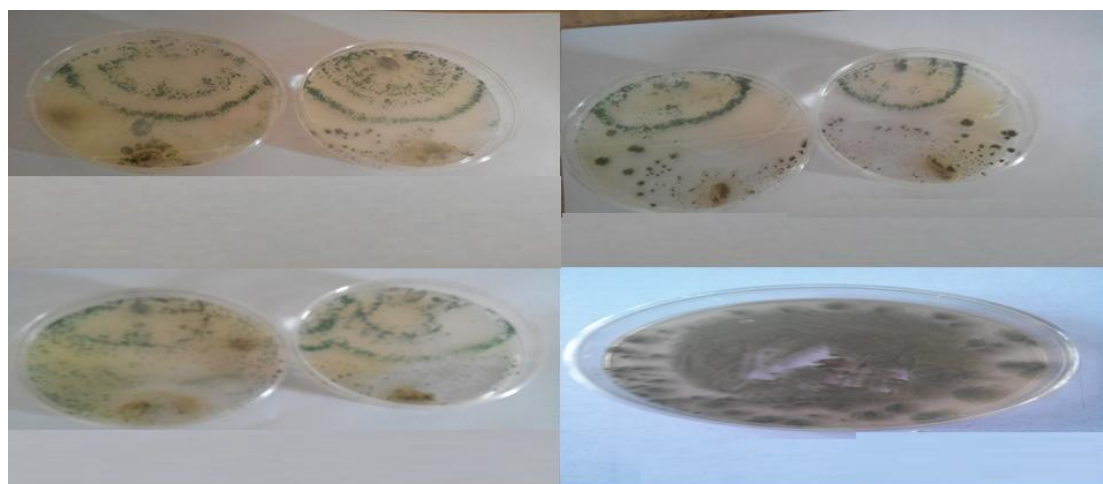


Plate 3: *In vitro* inhibition of *Aspergillus flavus* by *Trichoderma Harzanium* and *Trichoderma viride* on Potato Dextrose Agar (PDA).

Af+Th = Simultaneous *Aspergillus flavus* inoculation of *Trichoderma Harzanium*.

Af+Tv = *Aspergillus flavus* simultaneous inoculation with *Trichoderma viride*.

Af4Th = *Aspergillus flavus* inoculated before *Trichoderma Harzanium*.

Af4TV = *Aspergillus flavus* inoculated before *Trichoderma viride*.

Th4Af = *Trichoderma Harzanium* inoculated before *Aspergillus flavus*.

Tv4Af = *Trichoderma viride* inoculated before *Aspergillus flavus*.

C = Control plate of *A. flavus* unpaired with *Trichoderma* spp.

4. Conclusion

Results of the preset study indicate that time the application of antagonists (*Trichoderma* spp.) before the inoculation of the pathogens will result in higher degree of inhibition of the pathogen and that *A. niger* is least inhibited out of the three investigated pathogens. Furthermore, the antagonistic activity of *T. harzanium* and *T. viride* against the examined pathogens was not significantly different at the end of 168th hour of incubation. Results of the present study suggest that *Trichoderma* spp. used in this study could be exploited as biopesticides in the control of *Aspergillus* pathogens of *P.tuber-regium*.

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