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Fungitoxicity of *Trichoderma longibrachiatum* (Rifai) Metabolites against *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus tamarii*

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Abstract

An experiment was carried out to evaluate the fungitoxic effect of *Trichoderma longibrachiatum* (Rifai) *metabolite* on *F. oxysporum*, *A. niger* and *A. tamarii*. The fungi were collected from the International Institute of Tropical Agriculture (IITA) and Nigerian Institute of Science Laboratory Technology (NISLT). *T. longibrachiatum* was cultured on 1/4 strength Potato Dextrose Broth (PDB) following standard procedures. Its metabolite was extracted using 50 ml n-hexane with 50 ml Potato Dextrose Broth (PDB). The metabolite was purified by filter sterilization using a sterile 0.22 millipore filter disc after centrifuging at 900 rpm for 20 minutes. Petri plates of each fungus were later impregnated in triplicates with the *T. longibrachiatum* metabolites using four concentrations (10%, 25%, 50% and 100%), and three volumes (1 ml, 2 ml, and 3 ml). Petri plates of fungi without the metabolite and Petri Plates of fungi with n-hexane and PDB served as control. All Petri Plates were incubated at 28˚C for 7 days. Radial and diametric growth of each fungus on all Petri Plates were taken daily at 24 hours intervals. Data obtained were analysed using SAS (version 9.3). Growth inhibition of *F. oxysporum*, *A. niger* and *A. tamarii* was significantly higher than in control in that order ($P \leq 0.05$). Inhibition of the fungi by metabolites extracted with both PDB and n-hexane was significantly better than in control. Generally, inhibition by metabolite extracted with PDB was better than that extracted with n-hexane. Growth inhibition at all the concentrations of the metabolite was significantly better than in the control ($P \leq 0.05$). Generally, inhibition at 100%, 50% and

25% concentrations was better than that at 10% concentration of the metabolite. *Trichoderma longibrachiatum* metabolite can thus be said to possess promising fungitoxic potential even at concentrations as low as 25%. Both PDB and n-hexane are good extraction media for the metabolite.

Keywords

Antifungal Metabolites, *Trichoderma longibrachiatum*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus tamari*

1. Introduction

On a yearly basis, a huge percentage of economic plants are destroyed by plant pathogens. Pesticides and organic compounds are widely used to control plant pathogens in many countries. However, apart from the high cost, degradation of such compounds is very difficult and the concentration and/or accumulation of them in food chains continue to lead to higher toxicity levels in man and animals, as well as the environment [1] [2]. There is therefore an increasing focus on alternate methods for the control of plant diseases [3] [4]. *Fusarium oxysporum* is a pathogen documented to be the cause of several diseases in different crops [5] [6]. *Aspergillus niger* and *A. tamarii* are also known to be amongst the *Aspergillus* species causing common postharvest diseases in different plants [7]. Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins.

Trichoderma species are fungi that are present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. They are favoured by the presence of high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere competent, *i.e.* able to colonize and grow on roots as they develop. In addition to colonizing roots, *Trichoderma* species attack, parasitize and otherwise gain nutrition from other fungi. They are known worldwide as biological agents used in protecting crops against plant pathogens under greenhouse and field conditions. Their mode of action against plant pathogens is known to include antibiosis, mycoparasitism, competition for space and nutrients etc. [4] [8] [9] [10] [11]. They have been severally documented to suppress soil-borne pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Pythium* species [12] [13] [14] [15].

Trichoderma species are highly efficient producers of many extracellular enzymes. They are known to produce different volatile organic compounds [16] [17]. The metabolites secreted by them are as well known to be used as bio-fungicides to fight plant diseases caused by pathogenic fungi [18]-[23]. The experiment was set up to investigate the fungitoxicity of *Trichoderma longibrachiatum* metabolite against the growth of *Fusarium oxysporum*, *Aspergillus niger* and *Aspergil-*

lus tamarii.

2. Methodology

2.1. Collection of Fungal Isolates

Cultures of *Trichoderma longibrachiatum*, *Fusarium oxysporum*, *Aspergillus niger and Aspergillus tamarii* were collected from the International Institute of Tropical Agriculture (IITA), Ibadan.

2.2. Extraction of Metabolites from *T. longibrachiatum*

The metabolite of *T. longibrachiatum* was extracted using n-hexane and 1/4 strength Potato Dextrose Broth (PDB). A 5 mm cork borer was used to cut 10 mycelia discs from 7-day old cultures of *T. longibrachiatum*. A modified method of Spiegel and Chet [18] was used. *T. longibrachiatum* disc was transferred into each 250 ml flasks containing 100 ml of 1/4 strength of Potato Dextrose Broth (PDB), 50% n-hexane and 50% PDB. This was incubated and agitated on the horizontal shaker for 7 days at room temperature. After seven days of incubation and shaking, spores and mycelia of the fungi were removed from the broth culture of 1/4 strength Potato Dextrose Broth, 50% n-hexane and 50% PDB, by filtration using Whatman No. 1 filter paper. They were further purified by centrifuging at 9000 rpm for 20 mins and then filter sterilized using a sterile 0.22 millipore filter disc. The antimicrobial potential of the extracted metabolite was then tested against the three selected fungi at various concentrations of 10%, 25%, 50% and 100%.

2.3. Impregnating Test Plates with *Trichoderma* Metabolites and Data Collection

Acidified Potato Dextrose Agar (APDA) was prepared and dispensed into several Petri plates. Thereafter, 1 ml, 2 ml, and 3 ml of the metabolite were mixed separately with the Potato Dextrose Agar in the sterile plates by gently swirling plates to ensure homogeneity. The APDA plates with no metabolite served as a control. The plates were left to cool and gel, after which 5 mm fungal disc of each of the three pathogens was inoculated separately on the Agar plates under sterile conditions. The plates were incubated at 28˚C for 7 days and radial and diametric growth were measured daily. All experiments were done in triplicates.

2.4. Data Collection and Analysis

Percentage inhibition of pathogen was calculated by the following formula:

$$L = \left(R_1 - R_2\right)/R_1 \times 100,$$

where:

L = Inhibition of mycelia growth;

R_1 = Growth measurement of the pathogen in control;

R_2 = Growth of the pathogen in the presence of *Trichoderma longibrachiatum*

metabolite extract on daily basis [24].

Results obtained were subjected to analysis of variance (ANOVA) at the National Centre for Genetic Resources and Biotechnology (NACGRAB) using the Generalized Linear Model option of SAS (version 9.3).

3. Results

Table 1 shows the collapsed analysis of variance (ANOVA) table for inhibitory effect of *T. longibrachiatum* metabolite on the isolated pathogens. For *Fusarium oxysporum*, F-values for model, metabolite, concentration, days of incubation and volume of metabolite ($P > 0.0001$) were all highly significant. F-values for the interaction between concentration and day ($P > 0.0001$), metabolite and concentration ($P > 0.0001$), concentration and volume ($P > 0.0001$), metabolite and day ($P > 0.0001$), volume and day ($P > 0.0001$) as well as metabolite and volume ($P > 0.0219$) were all highly significant. F-values for interaction among metabolite, concentration and day ($P > 0.0001$), concentration, volume and day ($P > 0.0001$), metabolite, concentration and volume ($P > 0.0001$), metabolite, volume and day ($P > 0.0025$) were all also highly significant. For *Aspergillus niger*, F-values for model, metabolite, concentration, days of incubation and volume

Table 1. ANOVA table for effectiveness of *T. longibrachiatum* metabolite against growth of the isolated fungi *Fusarium oxysporum*.

	Fusarium oxysporum	*Aspergillus niger*	*Aspergillus tamarii*
Source	**P > F**	**P > F**	**P > F**
Model	0.0001**	0.0001**	0.0001**
Concentration	0.0001**	0.0001**	0.0001**
Day	0.0001**	0.0001**	0.2145
Metabolite	0.0001**	0.0001**	0.8814
Volume	0.0001**	0.0001**	0.0236*
Concentration * Day	0.0001**	0.0001**	0.1938
Metabolite * Concentration	0.0001**	0.0001**	0.9317
Concentration * Volume	0.0001**	0.0001**	0.0711
Metabolite * Day	0.0001**	0.0001**	0.5326
Volume * Day	0.0001**	0.0001**	0.7003
Metabolite * Volume	0.0219*	0.0001**	0.7801
Metabolite * Conc. * Day	0.0001**	0.0001**	0.4991
Conc. * Volume * Day	0.0001**	0.0001**	0.9990
Metabolite * Conc. * Vol.	0.0001**	0.0001**	0.9286
Metabolite * Volume * Day	0.0025**	0.0002**	0.0001**
Error			

* = Significant, ** = Highly Significant.

of metabolite ($P > 0.0001$) were all highly significant. F-values for interaction between concentration and day, metabolite and concentration, concentration and volume, metabolite and day, volume and day, as well as metabolite and volume ($P > 0.0001$) were highly significant. F-values for interaction among metabolite, concentration and day, concentration, volume and day, metabolite, concentration and volume ($P > 0.0001$) as well as metabolite, volume and day ($P > 0.0002$) were all highly significant. For *Aspergillus tamarii*, F-values for model and metabolite ($P > 0.0001$) as well as volume of metabolite ($P > 0.0236$) were all highly significant. F-value for interaction among metabolite, volume and day ($P > 0.0001$) was also highly significant.

Table 2 gives the comparison of effectiveness of the metabolite of *T. longibrachiatum* at different concentrations against the growth of isolated pathogens. For *F. oxysporum*, growth inhibition of the pathogen at all the concentrations of the metabolite differed significantly from the control. Growth inhibition at 100% concentration was significantly higher than that at other concentrations. Growth inhibition at 25% concentration was also significantly higher than that at 50% concentration, which in turn, was significantly higher than that at 10% concentration ($P = 0.05$, $R^2 = 0.99$). For *A. niger*, growth inhibition of the fungus at all the concentrations were significantly higher than control. However, growth inhibition at 10% concentration was significantly lower than that in other concentrations ($P = 0.05$, $R^2 = 0.99$), which were not significantly different from themselves. For A. *tamarii*, growth inhibition of the fungus at all the concentrations was significantly higher than control. Growth inhibition at 25% concentration was significantly higher than that in other concentrations. Growth inhibition at 10% concentration was also significantly higher than that in 50% and 100% concentrations, while that at 50% concentration was as well significantly higher than that at 100% concentration ($P = 0.05$, $R^2 = 0.99$). Table 3 compares the growth inhibition of the isolated fungi (*Fusarium oxysporum*, *Aspergillus niger and A. tamarii*,) by *Trichoderma longibrachiatum* metabolite among days of incubation. Growth inhibition at all days of incubation was significantly higher than control ($P = 0.05$, $R^2 = 0.99$).

Table 2. Effectiveness of *Trichoderma longibrachiatum* metabolite at different concentrations against growth of *Fusarium oxysporum*, *Aspergillus niger and A. tamarii*.

Concentration (%)	*Fusarium oxysporum*	*Aspergillus niger*	*Aspergillus tamarii*
	Means (cm)	Means (cm)	Means (cm)
Control	2.833^{a}	3.467^{a}	6.490^{a}
10	2.570^{b}	3.033^{b}	5.717^{b}
25	2.121^{c}	2.715^{c}	5.596^{c}
50	2.245^{d}	2.844^{c}	5.942^{d}
100	2.020^{e}	2.769^{e}	6.490^{e}
$LSD_{0.05}$	0.178	0.264	0.435
R^2	0.992	0.989	0.991

Table 4 compares effectiveness of *T. longibrachiatum* metabolite at different volumes on growth of the isolated fungi (*F. oxysporum*, *Aspergillus niger and Aspergillus tamarii.*). Growth inhibition of the three pathogens at the three different volumes was significantly higher than control. For *F. oxysporum*, growth inhibition at 3 ml of the metabolite was significantly higher than that at 2 ml, which in turn was as well significantly higher than that at 1ml of the metabolite. For *Aspergillus niger*, growth inhibition at 2 ml of the metabolite was significantly higher than that at 3 ml, which in turn was as well significantly higher than that at 1 ml of the metabolite ($P = 0.05$, $R^2 = 0.99$). For *Aspergillus tamarii*, growth inhibition at 3 ml of the metabolite was also significantly higher than that at 2 ml, which in turn was significantly higher than that at 1 ml of the metabolite ($P = 0.05$, $R^2 = 0.99$). Table 5 compares the growth inhibition of the three pathogens by metabolites of *T. longibrachiatum* extracted with different

Table 3. Comparison of growth inhibition of the isolated fungi by *Trichoderma longibrachiatum* metabolite among days of incubation.

Days of Incubation	*Fusarium oxysporum*	*Aspergillus niger*	*Aspergillus tamarii*
	Means (cm)	Means (cm)	Means (cm)
Control	2.833[a]	3.467[a]	6.490[a]
7	2.759[b]	3.200[b]	5.761[b]
6	2.711[c]	3.122[c]	5.755[c]
5	2.526[d]	3.021[d]	5.541[d]
4	2.172[e]	2.860[e]	4.524[e]
3	1.690[f]	2.291[f]	3.193[f]
2	1.234[g]	1.623[g]	1.087[g]
1	0.523[h]	0.805[h]	6.490[h]
$LSD_{0.05}$	0.178	0.264	0.435
R^2	0.992	0.989	0.991

Means with different letters in a column are significantly different ($P \leq 0.05$).

Table 4. Impact of volume of *T. longibrachiatum* metabolite on its effectiveness in inhibiting growth of the isolated fungi.

Volumes Used (ml)	*Fusarium oxysporum*	*Aspergillus niger*	*Aspergillus tamarii*
	Means (cm)	Means (cm)	Means (cm)
Control	2.833[a]	3.467[a]	6.490[a]
1 ml	2.292[b]	2.838[b]	5.768[b]
2 ml	2.257[c]	2.739[c]	5.726[c]
3 ml	2.168[d]	2.944[d]	5.687[d]
$LSD_{0.05}$	0.178	0.264	0.435
R^2	0.992	0.989	0.991

Means with different letters in a column are significantly different ($P \leq 0.05$).

Table 5. Effectiveness of *Trichoderma longibrachiatum* metabolite extracted with different media against growth of the isolated fungi.

Extraction solvent	*Fusarium oxysporum*	*Aspergillus niger*	*Aspergillus tamarii*
	Means (cm)	Means (cm)	Means (cm)
Control	2.833[a]	3.467[a]	6.490[a]
N-hexane	2.410[b]	2.968[c]	5.742[b]
PDB	2.068[c]	2.713[b]	5.711[c]
$LSD_{0.05}$	0.178	0.264	0.435
R^2	0.992	0.989	0.991

Means with different letters in a column are significantly different ($P \leq 0.05$).

solvents. Growth inhibition of the three pathogens by metabolites extracted with both solvents (n-hexane and PDB) was significantly higher than control. For both *F. oxysporum* and *Aspergillus niger*, growth inhibition by metabolite extracted with PDB was significantly higher than that extracted with n-hexane. However, growth inhibition of *Aspergillus tamarii* by metabolite extracted with n-hexane was also significantly higher than that by metabolite extracted with PDB ($P = 0.05$, $R^2 = 0.99$).

4. Discussion

The significant F values for metabolite, days of incubation, concentration and volume of *Trichoderma* metabolite ($P > 0.0001$) showed the effectiveness of the *Trichoderma* metabolite in inhibiting growth of the fungal pathogens compared to control. It showed that days of incubation as well as concentration and volume of the metabolite also played critical role in the effectiveness of the *Trichoderma* metabolite in its inhibitory potential. This is also corroborated by the highly significant F value ($P > 0.0001$) for model, which showed the appropriateness of the fitted model (*i.e.* "goodness of fit"), where day of incubation, metabolite, concentration and volume of metabolite were all independent variables and growth inhibition of the fungal pathogens being the dependent variable. The significant F values for the interaction between metabolite and concentration ($P > 0.0001$), interaction between metabolite and volume ($P > 0.0219$) and also interaction between metabolite and days of incubation ($P > 0.0001$) all show that the effectiveness of the *T. longibrachiatum* metabolite in growth inhibition of *Fusarium oxysporum* and *Aspergillus niger* differed significantly from one concentration to the other; and from one volume of metabolite to the other, and also from one day of incubation to the other ($P = 0.05$, $R^2 = 0.99$). This, however, is not true of *Aspergillus tamarii* where interaction among most of the variables were not significant. This suggests that whereas the effectiveness of *T. longibrachiatum* metabolite against growth of *Fusarium oxysporum* and *Aspergillus niger* depends amongst others, on the interaction among the variables, the contrary is true for *A. tamarii*. It suggests that the effectiveness of *T. longibra-*

chiatum metabolite against *A. tamarii* does not depend on any interaction among the variables.

The results obtained with metabolite concentration strongly suggest that 100% concentration of the *Trichoderma* metabolite could be said to generally exhibit the most effective growth inhibition. Inhibition of the pathogens by the metabolite at 3 ml can also be said to be the most effective compared to the other volumes ($P = 0.05$, $R^2 = 0.99$). The results obtained here support the suggestions of Luckner [25] and Vicaino *et al.* [26]. Rifai [12] also wrote extensively on the biocontrol potential of *Trichoderma* species. The results obtained with metabolites extracted with both solvents compared to control ($P = 0.05$, $R^2 = 0.99$) showed the appropriateness of the two solvents (n-hexane and PDB) as extraction media for the *Trichoderma* metabolite. Both of them could thus be said to be good extraction solvents for *T. longibrachiatum* metabolite. The better result obtained with metabolite extracted with n-hexane might not be unconnected with its organic nature [18] [27]. Gams and Bissette [28] reported the degree to which *Trichoderma species* suppressed the growth of peanut moulds such as *Fusarium* species, *A. niger*, *A. flavus*, *A. parasiticus* and *A. ochraceous* might involve the production of volatiles and/or production of extracellular enzymes. Volatiles from *Trichoderma species* have been reported to be able to arrest the hyphal growth of different fungal pathogens on agar plates. Most strains of *Trichoderma* are reported to possess the potential to produce volatiles and non-volatile toxic metabolites such as harzianic acid, alamethicins and tricholin that hinder growth of other microorganisms [29]. Some aggressive *Trichoderma* spp. used as biocontrol agents are also known to be capable of producing either antibiotics or extracellular enzymes or both.

5. Conclusion

Conclusively, the metabolite of *Trichoderma longibrachiatum* could be said to possess promising antagonistic potential against *Fusarium oxysporum*, *Aspergillus niger* and *A. tamarii* in that order. Generally, the effectiveness of the metabolite is better at 100% and 50% concentrations and at a volume of 3 ml. Both PDB and n-hexane are good extraction solvents for *Trichoderma longibrachiatum* metabolite. Further studies are however necessary to ascertain its effectiveness *in vivo* over a long duration.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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