Trop J Nat Prod Res, November 2020; 4(11):961-965 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effectiveness of *Trichoderma koningii* Extract on *Aspergillus* Species Isolated from Rotting Tomato (*Solanum lycopersicum* Mill.)

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ARTICLE INFO

Article history:
Received 05 August 2020
Revised 15 October 2020
Accepted 13 November 2020
Published online 30 November 2020

ABSTRACT

Pre and post-harvest losses caused by fungal pathogens have been a significant challenge to agriculture and the national economy. This study investigated the effect of *Trichoderma koniigii* extract on three *Aspergillus* spp (*A. niger, A. nidulans,* and *A. flavus*) isolated from rotting tomato. N-hexane and Ethyl acetate was used as extraction solvents while the growth inhibitory potential of the extract was tested at 1.0 g/mL, 2.5 g/mL, 5 g/mL, and 10 g/mL concentrations. The whole experiment was set-up in a completely randomized design (CRD). Data and observations were analyzed using ANOVA and LSD. The results revealed that the growth inhibitions differed significantly from one fungus to the other. *A. niger* and *A. flavus* showed significant growth inhibition at 5 g/mL, and 10 g/mL concentrations ($p \leq 0.05$, $R^2 = 0.76$), while *A. nidulans* did not show inhibition at all the different concentrations. From the results, ethyl acetate with a high significant value (P = 0.0406 at 50%) might be a better solvent than n-hexane. The study suggests that *T. koningii* extract possesses active biocontrol property against pre and post-harvest pathogens of crops.

***Keywords*:** *Trichoderma koningii*, Growth inhibition, Tomato, Biocontrol, Pathogens, *Aspergillus spp*.

Introduction

Plant diseases play a significant part in the destruction of natural resources in agriculture.[1,2] Soil-borne pathogens cause economic losses, fungi being the most aggressive. The spread of several plant-pathogenic fungi, such as *Fusarium*, *Alternaria*, *Colletotrichum*, *Rhizoctonia*, *Helminthosporium,* and *Aspergillus*, has appreciated in the last few years due to the introduction of new farm practices, which brings about harmful actions on economic crops.[3-5] Biological control of plant pathogens was employed as a potential control measure in the last few years, and a continuous search for these biological agents is increasing.[6,7] Bio-control provides an eco-friendly approach to plant disease management and can be incorporated into physical and cultural controls and the limited use of chemicals for effective integrated pest management (IPM) system.[8,9] The use of antagonistic microorganisms in controlling soil-borne plant pathogens is a possible non-chemical means of plant disease control.[5,10] *Trichoderma* spp. are commonly used to control plant pathogens and have long been recognized as effective antagonists against plant pathogenic fungi.[6,11] The activity of this species has been known since 1930. Today, modern technologies for incorporating them in biological control of various diseases have been developed,[11] and their isolates have been successfully used to control the impact of soil-borne pathogens in greenhouses and under opened-field conditions.[12] A study by Tran[13] showed that they parasitize not only fungal plant pathogens, but also produce toxic compounds (e.g., the antibiotics; gliotoxin, gliovirin, and peptaibols), and a battery of lytic enzymes, mainly chitinases, glucanases, and proteases, produced by *Trichoderma* spp, which destroys fungal pathogens.[14,15] The production of these antibiotics by *Trichoderma* strains also shows the enormous variety and use potential as an inexhaustible source of antibiotics, from the acetaldehydes gliotoxin and viridin[16,17] to alpha-pyrones[18] terpenes, polyketides, isocyanide derivatives, piperazines, and complex families of peptaibols.[15] These compounds produce synergistic effects, with efficacious inhibitory activity on many fungal plant pathogens.[19] Since there is no general solution to controlling plant pathogens, there is a need to develop a bio-control system by engaging in studies to screen for antibiotic production.[20] Therefore, this experiment was carried out to determine the growth inhibitory potential of *Trichoderma koningii* extract on *Aspergillus* spp, obtained from rotting tomato.

Materials and Methods

Sample collection

Rotting tomatoes of 200 g were collected from the Ojoo market, the Bodija market, the Eleyele market, and tomato farm in Ido local government, all in Oyo State, Nigeria. The samples were taken into the laboratory using sterile bags. A soil sample of 300 g from a tomato farm was also collected in a sterile polythene bag for the isolation of *Trichoderma koningii.*

Preparation of culture medium

The culture medium was prepared by dissolving 39 g of Potato Dextrose Agar in a conical flask containing 1000 mL of sterile distilled water. It was then sterilized at 121°C for 15 min in an autoclave and allowed to cool down to about 45°C. 500 uL of lactic acid was then added to inhibit bacterial growth.

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Citation: Oyesola OL, Sobowale AA, Obembe OO. Effectiveness of *Trichoderma koningii* Extract on *Aspergillus* Species Isolated from Rotting Tomato (*Solanum lycopersicum* Mill). Trop J Nat Prod Res. 2020; 4(11):961-965. doi.org/10.26538/tjnpr/v4i11.19

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Isolation of fungi from tomato fruits
Rotting portions of the tomato were picked and inoculated on Potato Dextrose Agar media and then incubated at room temperature for five days under standard growth condition. Pure cultures were subcultured, and stock cultures were put on slants in McCartney bottles.

Isolation of Trichoderma
T. koningii was isolated from soil by serial dilution up to 10^{-5} and inoculated into a sterile Petri dish using a pour-plate method, and incubated for five days under standard growth condition.

Identification of isolates
The isolated fungi were identified according to morphological and microscopical characters. All isolates were refreshed by growing at the optimum growth conditions at the beginning of the experiments.

Extraction of extract from T. koningii
The extract of *T. koningii* was extracted using two solvents; n-hexane and ethyl acetate. 5 mm cork borer was used to cut 30 mycelia discs from 7-day old cultures of *T. koningii*. The modified method of Jiaqi *et al.* [21] was used. The *T. koningii* discs were transferred into two different 500 mL flasks containing 300 mL of 1/2 strength of 50% n-hexane and 50% ethyl acetate. The flasks were incubated and agitated on a horizontal shaker for seven days at room temperature.

Extract purification
After seven days of incubation and shaking, spores and mycelia of the fungi were removed by filtration using Whatman No. 1 filter paper. They were further purified by centrifuging at 5000 rpm for 20 mins and then filter-sterilized using a sterile 0.22 millipore filter disc. The extracts' antimicrobial potential was then tested against the three selected fungi at 1.0 g/mL, 2.5 g/mL, 5 g/mL, and 10 g/mL concentrations.

Impregnation of test plates with extract and data collection
Potato Dextrose Agar (39 g) was dissolved in 1 L of distilled water in a conical flask and then sterilized at 121°C for 15 min. The sterilized Potato Dextrose Agar was later placed in a water bath, and the temperature was cooled to and maintained at 45°C. Sterile lactic acid was added to inhibit the growth of bacteria. 1 mL of the extract was mixed separately with the Potato Dextrose Agar in sterile plates by gently swirling plates to ensure homogeneity. Potato Dextrose Agar plates with fungus only and solvents (n-hexane and ethyl acetate) only were used as control 1 and 2, respectively. The mixture of the extract and Potato Dextrose Agar in the sterile Petri Plates were left to cool, and gel. 5 mm fungal discs of the tested fungi were collected and carefully inoculated onto separate agar plates. The plates were incubated at 28°C for seven days, and radial growth was measured daily. Each test was done in triplicate.

Statistical analysis
Data collected were subjected to analysis of variance (ANOVA) using the Generalized Linear Model option of SAS 9.3 version, and the Least Significance Difference (LSD) was used to compare the means ($p < 0.05$).

Results and Discussion

Results obtained from this study showed that *Trichoderma koningii* extract possesses significant antifungal potential against fungi associated with rotting tomato, some of which could be pathogenic. Jiaqi *et al.* reported that *Trichoderma* species could control their target fungi using different control mechanisms.[21] The growth inhibition of the selected fungi by *T. koningii* extract at all concentration levels is shown in Tables 1, 5, 6, and 7. The F-value for the model ($P > 0.0001$), selected fungi ($P > 0.0001$), incubation days ($P > 0.0001$) were highly significant. However, F-value for solvent ($P > 0.0759$) was not significant (Table 6). The highly significant F-values ($P > 0.0001$) for the models for the selected fungi' growth inhibitions at all concentrations of the extract showed models' appropriateness. The highly significant F-values for selected fungi ($P > 0.0001$) at all concentrations of *T. koningii* extract showed that the growth inhibitions of these fungi at all extract concentrations differed significantly from one fungus to the other. This means a particular concentration of the metabolite will have significantly different inhibitory activity on different fungi. The non-significant F-values for extraction solvents at all concentrations (Tables 1, 5, and 7) except at 5 g/mL mean that both solvents are generally suitable for the extraction of *T. koningii* metabolite. However, the significant F-values for extraction solvents (Table 6) at 5 g/mL (Table 8) suggest that one of the solvents might still be better than the other in *T. koningii* metabolite extraction. It means ethyl acetate might be a better extraction solvent for the metabolite, even though both are good for its extraction.
Table 3 shows the comparison of growth inhibitions of the selected fungi by the extract at different incubation days. There was a significant difference ($p \leq 0.05$) in the selected fungi' growth inhibitions among the incubation days. The F-values for incubation days, which were all highly significant at all concentrations ($P > 0.0001$), imply that the growth inhibitions of the selected fungi differed significantly from one day of incubation to the other (Tables 1, 5, 6, and 7). This is supported by the results obtained in comparing growth inhibitions at different incubation days (Table 3). The results also mean that the higher the incubation days, the higher the growth inhibitions ($p \leq 0.05$). It means that the period of contact between the extract and fungus determines the extract's significant inhibitory activity. This suggests that the longer the duration of contact between the metabolite and the fungi, the higher the chances of inhibition of the fungi. This agrees with the report of Sobowale *et al.*, which concluded that the longer the duration (incubation days), the better the antagonistic potential of the *Trichoderma* against fungal pathogens.[22] The fungi' growth inhibition, which was not significantly different among incubation days 5, 6, and 7, means that there was little or no additional mycelia by the fungi at the latter days of incubation. Table 2 compares the means of growth inhibitions of the selected fungi by *T. koningii* extract at the different concentration levels. There were significant differences in the growth inhibitions of *A. nidulans*, *A, niger*, and *A. flavus* by *T. koningii* extract ($p \leq 0.05$). The difference in the growth inhibition of *A. nidulans*, which was significantly higher than that of other selected fungi at all concentrations, showed that *A. nidulans* was the most inhibited fungus by the metabolite of *T. koningii*. This means, of the three selected fungi, *A. nidulans* was the most sensitive fungus to the inhibitory effect of *T. koningii* metabolite.

Table 1: ANOVA table for growth inhibition of selected fungi isolated from rotting tomato by 1.0 g/mL *T. koningii* extract

Source of Variation	DF	SS	MS	F value	Pr > F
Model	11	1082.33	98.39	54.98	0.0001
Selected fungi	2	559.56	279.78	156.33	0.0001
Solvents	1	5.74	5.74	3.21	0.0759
Incubation Days	6	513.88	85.65	47.86	0.0001
Replicates	2	3.14	1.57	0.88	0.4183
Error	114	204.03	1.79		
Corrected Total	136	1286.35			

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Means comparison of growth inhibition of selected fungi from rotting tomato by *T. koningii* extract

Selected Fungi	1.0 g/mL	2.5 g/mL	5 g/mL	10 g/mL
Aspergillus nidulans	1.4167[c]	1.1869[c]	1.0502[c]	0.8357[b]
Aspergillus niger	4.8143[b]	4.3169[b]	3.6798[b]	3.614
Aspergillus flavus	6.4810[a]	6.3048[a]	5.2821[a]	
$LSD_{0.05}$	0.58	0.58	0.62	0.52
R^2	0.84	0.83	0.76	0.76

($p \leq 0.05$)

The significant difference in the growth inhibitions of *A. niger* and *A. flavus* at all concentrations except 10 g/mL concentration showed that *A. flavus* was the least inhibited by the metabolite. This agrees with Gayoung and Sung-Eun's reports that some antifungal agents inhibit the growths of Aspergillus species.[23] The significant difference in growth inhibitions of *A. niger* compared with its controls at all concentrations of *T. koningii* metabolite means that the metabolite could inhibit the growth of *A. niger* significantly. It showed that the metabolite still has significant inhibitory potential against this fungus ($p \leq 0.05$) even at low concentration. The growth inhibition of *A. nidulans* and *A. flavus*, which was significant compared to control only at 5 g/mL and 10 g/mL concentrations (Tables 4), means that *T. koningii* metabolite will most likely inhibit the growth of these fungi at higher concentrations significantly. It showed that *T. koningii* metabolite's inhibitory effect on *A. flavus* and *A. nidulans* might be associated significantly with its concentration.

Table 3: Comparison of growth inhibition of selected fungi from rotting tomato by *T. koningii* extract among different incubation days

Days	1.0 g/mL	2.5 g/mL	5.0 g/mL	10 g/mL
1	0.7222[e]	0.7250[e]	0.6833[d]	0.7306[f]
2	2.0806[d]	2.0417[d]	1.4306[d]	1.0833[f]
3	3.7028[c]	3.3750[c]	2.7194[c]	2.0444[e]
4	4.7972[b]	4.4694[b]	3.6917[b]	2.8972[d]
5	5.6750[a]	5.2583[ab]	4.5167[a]	3.5972[c]
6	6.2444[a]	5.7556[a]	4.9972[a]	4.2833[b]
7	6.4389[a]	5.9278[a]	5.3228[a]	4.9500[a]
$LSD_{0.05}$	0.58	0.58	0.62	0.53
R^2	0.84	0.83	0.76	0.76

($p \leq 0.05$)

Table 4: Comparison of growth inhibition of selected fungi from rotting tomato by 1.0 g/mL *T. koningii* extract with the controls

Selected Fungi	1.0 g/mL	2.5 g/mL	5.0 g/mL	10 g/mL	Control 1 (Fungus only)	Control 2 (Fungus+ Solvent)
A. nidulans	1.4167a	1.1869[a]	1.0502[a]	0.8357[a]	1.3250a	1.1929a
A. niger	4.8143a	4.3169[a]	3.6798[a]	3.6143[a]	5.5929b	5.8286b
A. flavus	6.4810a	6.3048[a]	5.2821[a]	3.9440[a]	6.0964a	6.1500a
$LSD_{0.05}$	0.58	0.58	0.62	0.53		
R^2	0.84	0.83	0.76	0.76		

($p \leq 0.05$)

Table 5: ANOVA table for growth inhibition of selected fungi from rotting tomato by 2.5 g/mL *T. koningii* extract

Source of variation	DF	SS	Ms	F value	P ≤ 0.05
Model	11	986.49	89.68	50.17	0.0001
Selected Fungi	2	559.17	279.58	156.42	0.0001
Solvents	1	2.04	2.04	1.14	0.2871
Incubation Days	6	423.45	70.57	39.48	0.0001
Replicates	2	1.84	0.91	0.51	0.5991
Error	114	203.77	1.79		
Corrected Total	136	1190.26			

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: ANOVA table for growth inhibitions of selected fungi from rotting tomato by 5.0 g/mL *T. koningii* extract

Source of variation	DF	SS	MS	F value	P ≤ 0.05
Model	11	739.24	67.20	33.18	0.0001
Selected fungi	2	383.47	191.74	94.67	0.0001
Solvents	1	8.69	8.69	4.29	0.0406
Incubation days	6	346.95	57.82	28.55	0.0001
Replicates	2	0.12	0.06	0.03	0.9700
Error	114	230.89	2.03		
Corrected Total	136	970.89			

Table 7: ANOVA table for growth inhibitions of selected fungi from rotting tomato by 10 g/ml *T. koningii* extract

Source of variation	DF	SS	MS	F value	P ≤ 0.05
Model	11	527.35	47.94	32.52	0.0001
Selected Fungi	2	244.87	122.44	83.05	0.0001
Solvents	1	0.69	0.69	0.47	0.4941
Incubation Days	6	274.83	45.80	31.07	0.0001
Replicates	2	6.95	3.48	2.36	0.0992
Error	114	168.07	1.47		
Corrected Total	136	695.42			

Table 8: Comparison of the extraction solvents for *T. koningii* extract

Concentrations	Solvents	Means
1.0 g/mL	N-Hexane	4.0238[a]
	Ethyl Acetate	4.4509[a]
$LSD_{0.05}$	0.47	
R^2	0.84	
2.5 g/mL	N-Hexane	3.8087[a]
	Ethyl Acetate	4.0635[a]
$LSD_{0.05}$	0.47	
R^2	0.83	
5.0 g/mL	N-Hexane	3.6000[a]
	Ethyl Acetate	3.0748[b]
$LSD_{0.05}$	0.50	
R^2	0.76	
10 g/mL	N-Hexane	2.7238[a]
	Ethyl Acetate	2.8722[a]
$LSD_{0.05}$	0.43	
R^2	0.76	

($p \leq 0.05$)

Conclusion

The study shows *T. koningii* metabolite's potential to inhibit the growth of *A. nidulans*, *A. niger* significantly, and *A. flavus* isolated from rotting tomato (*Solanum lycopersicum*) fruits. *T. koningii* metabolite is effective against *A. nidulans* even at low concentrations. However, it is effective against the growth of *A. niger* and *A. flavus* at higher concentrations. Growth inhibitions of the fungi are higher at higher incubation days. The results also suggest that antibiosis could be one of the mechanisms of action by *T. koningii*. The effectiveness of *T. koningii* metabolite could also depend significantly on its concentration. N-hexane and ethyl acetate are suitable extraction solvents for the metabolite of *T. koningii*, though ethyl acetate might be better.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to this article's content.

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