International Scholars Journals

Advances in Food Science and Technology ISSN: 6732-4215 Vol. 8 (3), pp. 001-004, March, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Full Length Research Paper

Effect of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium

E.I. Eziashi[1*], N.U. Uma[2], A.A. Adekunle[2] and C.E. Airede[1]

[1]Nigerian Institute for Oil Palm Research (NIFOR), Plant Pathology Division, P.M.B. 1030 Benin City, Edo State. Nigeria.
[2]Department of Botany and Microbiology, University of Lagos, Akoka, Yaba, Lagos State. Nigeria.

Accepted 14 September, 2019

Metabolites released from *Trichoderma viride*, *T. polysporum*, *T. hamatum* and *T. aureoviride* were tested in culture medium against *Ceratocystis paradoxa*, which causes black seed rot in oil palm sprouted seeds. The *Trichoderma* metabolites had similar fungistatic effects on the growth of *C. paradoxa* except those from *T. aureoviride.* The inhibition varied depending on the *Trichoderma* species producing the metabolites; from 2.0% to 64% in volatile, 0.0% to 74% in non-volatile and 0.0% to 81% from direct- diffusible metabolites. *C. paradoxa* growth was significantly reduced in the presence of metabolites produced by *T. viride* and *T. polysporum* than the other species. *T. aureoviride* had the least growth inhibition, and medium containing direct-diffusible metabolite supported highest inhibition of *C. paradoxa.*

Key words: Metabolite, *Trichoderma, Ceratocystis paradoxa*, Fungistatic.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is said to be indigenous to West Africa (Zeven, 1965; Corner, 1966) but also can be found throughout a belt of land extending approximately between latitude 16^{o}N and 10^{o}S where annual rainfall ranges from 1524- 3048 mm and temperatures average 30^{o}C (Billows and Beekwith, 1913). The oil palm industry provides direct employment to about four million Nigerian people in about twenty oil palm growing states in Nigeria, and indirectly to other numerous people involved in processing and marketing (Ahmed, 2001).

Despite the enormous potential of the oil palm, there is problem with soil borne fungus *Ceratocystis paradoxa* causing black seed rot disease in oil palm sprouted seeds. The cause of the dry basal rot in adult palm, conducted by Robertson (1962), was also shown to be due to the Ascomycete, *C. paradoxa* of which the imperfect stage is known as *Thielaviopsis paradoxa*. It is a soil inhabitant, widely distributed throughout the tropics of Africa and Asia and causes disease of several other crops (Rajagopalan, 1965).

Biological control of plant pathogens by micro-organisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). *Trichoderma* species produce both volatile and non-volatile metabolites that adversely affect growth of different fungi (Bruce et al., 1984; Corley et al., 1994; Horvath et al., 1995; Moses et al., 1975). Dennis and Webster (1971b) found that some *Trichoderma* isolates produced volatile components, which were inhibitory to the growth of other fungi. Acetaldehyde was identified tentatively as one of the metabolites of *Trichoderma viride* inhibitory to other fungi. Dennis and Webster (1971a) also found that many isolates of *Trichoderma* species produced non- volatile antibiotics, which were active against a range of fungi. The relative abilities of the three biotypes of *T. harzianum* to colonize compost in competition with *Agaricus bisporus* and their influence on *A. bisporus* growth may be associated with secondary metabolite production (Seaby, 1987). Harman et al. (1980) and Nelson et al. (1988) r eported the use of

*Corresponding authors E-mail: eziashius@yahoo.com.

Table 1. Growth of *C. paradoxa* in the presence of volatiles released by *Trichoderma* species

Treatment	Radial growth (cm per day) of *C. paradoxa*			Inhibition rate of mycelial growth
	Days			
	1	2	3	(%)
T. viride	2.8	0.2	0.2	64*
T. polysporum	3.0	0.1	0.1	63*
T. hamatum	3.1	1.7	2.8	16*
T. aureoviride	3.9	1.9	2.9	2
Control	4.1	2.2	2.7	0.0

Mean values followed by * are significant ($p<0.05$) according to oneway analysis of variance as compared to the inoculated control. Data were expressed as % of control colonies without antagonist and values are average of 3 replicates.

Table 2. Growth of *C. paradoxa* in the presence of non-volatiles released by *Trichoderma* species.

Treatment	Radial growth (cm per day) of *C. paradoxa*			Inhibition rate of mycelial growth
	Days			
	1	2	3	(%)
T. viride	1.3	0.4	0.6	74*
T. polysporum	1.3	0.7	0.6	71*
T. hamatum	1.6	0.7	0.9	64*
T. aureoviride	4.1	1.7	3.2	0.0
Control	4.2	2.4	2.4	0.0

Mean values followed by * are significant ($p<0.05$) according to oneway analysis of variance as compared to the inoculated control. Data were expressed as % of control colonies without antagonist and values are average of 3 replicates.

Trichoderma hamatum for the control of *Pythium* seed rot and *Rhizoctonia* root rot in pea.

Although, benomyl is recommended for the control of *C. paradoxa* (Omamor, 1985), information on the use of bioagent for control is lacking. This paper present different effect of metabolites produced by *T. viride*, *T. polysporum*, *T. hamatum*, and *T. aureoviride* against *C. paradoxa*, which causes black seed rot in oil palm sprouted seeds.

MATERIALS AND METHODS

The effect of volatile metabolites from *Trichoderma viride*, *T. polysporum, T. hamatum*, and *T. auroeviride* against *C. paradoxa* were tested in the assemblage described by Dennis and Webster (1971b). Two bottons of Petri dishes containing PDA were individually inoculated with a disc of pathogen and antagonist, and bottons were adjusted and attached by tape. *Trichoderma* species were inoculated 48 h earlier, since growth of *C. paradoxa* was more rapid. The control sets did not contain the antagonist.

The effect of non-volatile metabolites from *Trichoderma* species against *C. paradoxa* was by the method described by Lundberg and Unestan (1980) and Dennis and Webster (1971a). Initially, mycelial agar plugs (4 mm diameter) removed (with a no 2 cork borer) from the edge of a young culture *Trichoderma* species were transferred to the center of Petri dishes (90 mm diameter) containing PDA and a sterilized cellophane disc (Courtauld Films: 50mm thick) adjusted on the medium surface, where the antagonist was grown for 3 days. Then the cellophane containing the *Trichoderma* growth was removed and on the same medium a disc of the pathogen was placed. The control treatments had *C. paradoxa* growing similarly on PDA medium where previously there was a cellophane disc without antagonist.

The effect of direct-diffusible metabolites from *T. viride*, *T. polysporum*, T*. hamatum*, and *T. aureoviride* against *C. paradoxa* were determined by transferring seven days old mycelial plug (4 mm diameter) removed from growing edge each of *Trichoderma* species to the center of Petri dishes containing PDA, previously left overnight to allow excess water to evaporate. The uninoculated Petri dishes were placed over the inoculated Petri dishes of *Trichoderma* species and attached firmly by tape. The Petri dishes were untapped after 3 days. Seven old mycelial disc (4 mm diameter) of *C. paradoxa* was inoculated on previously uninoculated Petri dishes. The control sets did not contain the antagonist, only had the uninoculated Petri dishes placed over the same uninoculated Petri dishes and attached also firmly by tape.

The studies of volatile, non-volatile and direct-diffusible metabolites were conducted in three replicates repeated twice. The cultures were incubated at 27-28^{o}C. Growth rates were recorded daily by measuring colony diameter according to Lilly and Barnett (1951). The inhibition percentage was obtained using the formula $1\% = [(C^2-C^1)/ C^2)] \times 100$ (Edington et al., 1971).

RESULTS AND DISCUSSION

Volatile, non- volatile and direct-diffusible metabolite tests showed that all the *Trichoderma* species except *T. aureoviride* inhibited the growth of *C. paradoxa*. The inhibition varied from 2.0% to 64% in volatile (Table 1),

Table 3. Growth of *C. paradoxa* on medium containing direct-diffusible metabolite produced by *Trichoderma* species.

Treatment	Radial growth (cm per day) of *C. paradoxa*			Inhibition rate of mycelial growth
	Days			
	1	2	3	(%)
T. viride	1.2	0.2	0.3	81*
T. polysporum	1.2	0.2	0.5	79*
T. hamatum	1.7	0.4	0.6	70*
T. aureoviride	4.0	1.8	3.2	0.0
Control	4.1	2.2	2.7	0.0

Mean values followed by * are significant ($p<0.05$) according to oneway analysis of variance as compared to the inoculated control. Data were expressed as % of control colonies without antagonist and values are average of 3 replicates.

0.0% to 74% in non-volatile (Table 2) and 0.0% to 81% in direct-diffusible metabolite (Table 3). The results of volatile and non-volatile indicated that mycelial growth of *C. paradoxa* was significantly ($p<0.05$) inhibited by *T. viride* than the other species tested. The results of direct-diffusible metabolites showed that mycelial growth of *C. paradoxa* was significantly ($p< 0.05$) inhibited by *T. polysporum* up to 81% (table 3). *T. aureoviride* recorded the least inhibition in all the tests from 0.0% to 2.0%. In all the tests conducted, *T. polysporum, T. viride*, and *T. hamatum* significantly ($p<0.05$) reduced mycelial growth of *C. paradoxa* in medium containing direct-diffusible metabolite when compared with media containing volatile and non volatile metabolites, and the control.

Biological activity of antagonist fungi and bacteria may partially be associated with production of antibiotic (Etebarian et al., 2000; Faull et al., 1994; Pusey and Wilson, 1984). The production of antibiotics; Trichodermin (Godtfredsen and Vangedal, 1964), ergokonin (Kumeda et al., 1994), viridin (Grove et al., 1996; Grove et al., 1965) and viridin fungin A, B and C (Harris et al., 1993) by *Trichoderma viride* have been involved in biological control. However, it has not been possible to extract these substances from *Trichoderma* species tested in this study. The metabolites produced by *T. viride* in these experiments are likely similar to metabolites produced by *T. viride* by the other investigators mentioned above. This investigation suggests that metabolites released by these *Trichoderma* species are toxic and fungistatic to *C. paradoxa*. Medium containing direct-diffusible metabolites should be re-employed for further studies since it gave the best results compared to volatile and non- volatile tests. *Trichoderma* species involved in this study should be investigated for their ability to control *C. paradoxa* in commercial situation.

ACKNOWLEDGEMENTS

E.I. Eziashi wish to thank the Director, Board and Management members of Nigerian Institute for Oil Palm Research for the award of scholarship and provision of oil palm sprouted seeds.

REFERENCES

Ahmed SA (2001). Agriculture and Food Security in Nigeria. Paper prepared for a forum with Mr. President on Agriculture and Food Security. Council chambers, Presidential Villa, Abuja. January 2001.

Baker R, Paulitz TC (1996). Theoretical basis for microbial interactions leading to biological control of soilborne plant pathogens In: Principles and Practice of Managing Soilborne Plant Pathogens. Hall, R. (ed.). The American Phytopathol. Soc. St. Paul, MN. pp. 50-79.

Billows HC, Beekwith H (1913). Palm Oil and Kernels, The consols of the West Coast. Charles Birchell, Liverpool.

Bruce A, Austin WJ, King B (1984). Control of growth of Lentimus lepideus by volatiles from *Trichoderma*. Trans. Br. Mycol. Soc. 82: 423-428.

Corley DG, Miller-Wideman M, Durley RC (1994). Isolation and Structure of *harzianum* : a new richothecene from *Trichoderma harzianum.* J. Natl. Prod. 57: 422-425.

Corner BJH (1966). The natural history of the palms. Wiedenfeld and Nicholson. p. 202.

Dennis C, Webster J (1971a). Antagonistic properties of species groups of *Trichoderma* I. Production of non-volatile antibiotics. Trans. Brit. Mycol. Soc. 57: 25-39.

Dennis C, Webster J (1971b). Antagonistic properties of species groups of *Trichoderma* II. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57: 41-48.

Edington LV, Khew KL, Barron GI (1971). Fungitoxic spectrum of benzimidazole compounds. Phytopathology 61: 42-44.

Etebarian HR, Scott ES, Wicks TJ (2000). *Trichoderma harzianum* T39 and *T. virens* DAR 74290 as potential biological control agents for phytophathora erythroseptica. Eur. J. Plant Pathol. 106: 329-337.

Faull JL, Craene-Cook KA (1994). Production of an isonitrin antibiotic by an U-V induced mutant of *Trichoderma harzianum*. Phytochemistry 36: 1273-1276.

Grove JF, Mcloskey JP, Moffatt JS (1996). Viridin, Oart V. structure. J. Chem. Soc. C: 743-747.

Grove JF, Moffatt JS, Vischer EB (1965). Viridin. Part 1. Isolation and characterization. J. Chem. Soc. C: 3803-3811

Harris GH, Jones ETT, Meinz MS, Nallin-Omstead M, Bills GL, Zink D, Wilson KE (1993). Isolation and Structure elucidatrion of viridio fungins A, B and C. Tetrahedron Lett. 34: 5235-5238.

Harman GE, Chet I, Baker R (1980). *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp. Or *Rhizoctonia solani*. Phytopathology 70: 1167-1172.

Horvath EM, Burgel JL, Messner K (1995). The production of soluble antifungal metabolites by the biocontrol fungus *Trichoderma harzianum* in connection with the formation of conidiospores. Mat. Org. 29: 1-4.

Kumeda Y, Asao T, Lida A, Wada S, Futami S, Fuijita T (1994). Effects of ergokonin produced by *Trichoderma viride* on the growth and morphological development of fungi. Bokin Bobai 22: 663-670.
Lilly VG, Barnett NL (1951). Physiol. of Fungi. McGraw Hill, New York. p. 463.
Lundberg A, Unestan T (1980). Antagonism against fomes annosus. Comparison between different test methods *"in vitro*" and "*in vivo*". Mycopathologia. 70:107-115.
Moses MO, Jackson RM, Rodgers D (1975). The characterization of 6-pent-1-enyl. Pyrone from *Trichoderma viride*. Phytochemistry 14: 2706-2708.
Nelson EB, Harman GE, Nash GT (1988). Enhancement of *Trichoderma*-induced biological control of *Pythium* seed rot and pre-emergence damping0off of peas. Soil Biol. Biochem. 20: 145-150.
Omamor IB (1985). Black rot of sprouted seeds of the oil palm. Trans. Brit. Mycol. Soc. 84(1): 159-161.
Pusey PL, Wilson CL (1984). Post-harvest biological control of stone fruit brown rot by *Bacillus subtilis*. Plant Dis. 70: 587-590.
Rajagopalan K (1965). NIFOR. First annual report. 1964-5. pp. 88-89.
Robertson JS (1962). Dry Basal Rot, a new disease of oil palms caused by *Ceratocystis paradoxa*. (Dade) Moreau. Trans. Br. Mycol. Soc. 45: 475-478.
Seaby D (1987). Infection of mushroom compost by *Trichoderma* species. Mushroom J. 179: 355-361.
Zeven AO (1965). The origin of the oil palm (*Elaeis guineensis Jacq.*) a summary. J. Nig. Inst. Oil Palm Res. 4: 218-225.