International Scholars Journals

African Journal of Environmental and Waste Management ISSN 2375-1266 Vol. 8 (5), pp. 001-004, May, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Potentials of two *Trichoderma* species as antagonistic agents against *Colletotrichum destructivum* of cowpea

O. A. Akinbode[1]* and T. Ikotun[2]

[1]Institute of Agricultural Research and Training, I.A.R and T, Ibadan, Nigeria.

[2]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria.

Accepted 11 November, 2020

Antagonistic potentials of two *Trichoderma* species that is *Trichoderma harzianum* and *Trichoderma pseudokoningii* against the pathogen, *Colletotrichum destructivum* of cowpea was examined *in vitro*. Each of the *Trichoderma* species was paired with the pathogen in 9 cm Petri dishes with Acidified Potato Dextrose Agar (APDA) as the growth medium. Pathogen and antagonists were paired using three methods of pairing. Mycelia radial growth of both the pathogen and the antagonists were taken. The two *Trichoderma* species significantly inhibited radial growth of the pathogen. Introduction of the antagonists before the pathogen gave the best growth inhibition of *C. destructivum*. The inhibitory effect of the two *Trichoderma* species significantly differed from each other at P 0.05. *T. pseudokoningii* had better inhibition of the mycelia growth of *C. destructivum* than *T. harzianum. T. pseudokoningii* and *T. harzianum* have good antagonistic potentials against *C. destructivum.*

Key words: *Colletotrichum destructivum, Trichoderma pseudokoningii, Trichoderma harzianum,* antagonists, inhibition, pathogen.

INTRODUCTION

The importance of cowpea, *Vigna unguiculata* L. walp is notable as one of the legume crops by many farmers in Africa. It is cultivated for its many uses such as green manure, its seeds, as vegetable crop, and also as a cash crop and cover crop (Prasanna, 1985; Kormawa et al., 2002) . It grows along with other crops on the fields in many agro-ecological zones of Nigeria (Emechebe and Shoyinka, 1985).

In recent times, the production of cowpea has been on decline due to many factors, most especially, biological factors which include diseases and pest attack. Anthracnose disease of cowpea is one of the most destructive diseases of cowpea that cause a great reduction in cowpea yield (Allen et al., 1998). This disease is caused by *Colletotrichum destructivum* O'Gara (Allen et al*.,* 1998) . It persists in almost all forms; seed-borne, soil borne and survives for long in diseased tissues (Amusa et al., 1994, Bailey et al*.*, 1990).

Various control measures against pathogen have been taken these include the use of fungicides and integrated control methods (Emechebe and Shoyinka, 1985; IITA, 1995). However, the efficacy of fungicides has been reduced by the development of resistance by the pathogen when exposed continuously to the chemical and deterioration of environmental quality (Poincelot, 1986). Chemical control method is expensive for the small holders as it costs a lot of money to hire labour and purchase machine and chemicals which makes it unsustainable economically, hence, the search for alternative to chemicals.

Biological control of plant disease, on the other hand lacks most of the above limitations but it is safe and sustainable (Cook and Baker, 1982, Janisiewiez and Korsten, 2002; Spadaro and Gullino, 2005; Sobowale et al., 2008). Some strains of *Trichoderma* species had been identified as potential biological control agents of plant pathogenic fungi on many crops including maize (Sobowale et al., 2008), cocoa (Adedeji et al*.*, 2008) and banana fruits (Adebesin et al*.*, 2005). *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers.ex.Gray, *Trichoderma polysporium* (Link ex Pers.), *Trichoderma longibrachiatum* Rifai, *Trichoderma koningii* Oudem are

*Corresponding author. E-mail: foyedeji@yahoo.com.

important control agents of plant pathogens. They were found to be effective in controlling a large number of plant pathogenic fungi. These bioagents were used for the control of foliar, soil- borne and post harvest diseases in various crops in the field, greenhouses and in storage (Papavizas, 1985; Adejumo et al., 1999; Sobowale et al., 2005, 2008) the exploration of biological control strategy against *C. destructivum* of cowpea therefore becomes a viable option in view of its reported effectiveness in the control of other plant diseases. This study is therefore aimed at evaluating two *Trichoderma* strains for their antagonistic activities against *C. destructivum in vitro*, and to also examine effect of pairing method on growth suppression of the pathogen by the *Trichoderma* species for further development to biological control package.

MATERIALS AND METHODS

Isolation and identification of pathogen and antagonists

The pathogen, *C. destructivum* was isolated from naturally infected stems of cowpea (*V. unguiculata);* which were cut and taken to the laboratory for isolation. Several smaller tissues from the infected parts were cut and surface sterilized by soaking for five minutes in 10% sodium hypochlorite (NaOCl) and then rinsed in five changes of sterile distilled water.

These were picked onto sterile filter papers with sterile forceps and wrapped in sterile paper towel for 5 min to dry. They were later plated on 15 ml Acidified Potato Dextrose Agar (APDA) in 9 cm diameter sterile Petri plates, and incubated for 10 days at 28 ± 2°C. Method employed in isolating *Trichoderma* species were the soil plate method (Warcup, 1950) and soil dilution plate method (Tuite, 1969). Pure cultures of the *C. destructivum* was obtained by subculturing into separate Petri plates and incubated again till pure culture was gotten. And these were maintained on APDA agar slant in sterile McCartney bottles and kept at 4°C till use. The two *Trichoderma* species were compared with already identified species from International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria.

Dixenic culturing of *T. pseudokoningii* and *T. harzianum* with the pathogen

The experiment was laid out in a Complete Randomized Design (CRD) with three treatments and three replicates. The treatments were the two bioagents versus the pathogen and the 3rd is the control. Each bioagent was paired against *C. destructivum* on APDA contained in 90 mm diameter Petri dishes following a method described by Sobowale (2002): (i) Five millimetre plug of each of the bioagents was placed at 20 mm away from the edge of the Petri dish, and after 24 h *C. destructivum* was placed 20 mm away for the other edge of the same Petri dish. (ii) *C. destructivum* was placed 20 mm away from the edge of the plate and at the same time 5 mm plug of the antagonists separately were placed 20 mm away from the edge of the plate. (iii) Five millimetre plug of the pathogen, *C. destructivum* was placed at 20 mm away from the edge of the Petri dish, and after 24 h the bioagents, individually were placed at the other edge of the plate.

The dixenic cultures were incubated at 28 - 30°C for 10 days, while observation on mycelia growth of both bioagents and pathogen were done every 24 h after 48 h of inoculation, taking mycelia growth at right angle to one another and found the average measurements. The measurements were subjected to analysis of Variance (ANOVA) using the General Linear Model option to check the performance of the two antagonists: *Trichoderma* against *C. destructivum* and the effective pairing method for inhibition of mycelia of the pathogen.

RESULTS

T. pseudokoningii paired with *C. destructivum*

When *T. pseudokoningii* was inoculated earlier than *C. destructivum,* the *Trichoderma* grew very fast covering the entire 90 mm Petri plate less than seven days of pairing, actually, the plate was full at the 5th day of incubation. The pathogen *C. destructivum* grew to 0.7 cm (7 mm) diameter on the average in the Petri plates. The pathogen mycelia were overgrown completely by the antagonist by the 7th day. With the introduction of the pathogen before the antagonist, that is when *C. destructivum* was introduced before the antagonist, *T. pseudokonningii*, the later grew still much faster than the pathogen in all Petri plates. By the third day of pairing, radial growth of *C. destructivum* was 2.10 cm diameter which was overgrown by *T. pseudokoningii*. By the 7th day of pairing, *T. pseudokoningii* had filled the plate, sporulating on the pathogen mycelia. Simultaneous inoculation of *C. destructivum* and *T. pseudokoningii, T. pseudokoningii* grew fast, stopped the pathogen growth at an average of 1.7 cm diameter by the third day of incubation. The antagonist sporulated on *C. destructivum* mycelia.

T. harzianum paired with *C. destructivum*

T. harzianum inoculated earlier than *C. destructivum,* the antagonist, *T. harzianum* very grew heavily. But, inhibited the growth of the pathogen to its point of inoculation with average mycelia growth as 1.13 cm. By the 5th day, the antagonist suppressed the mycelia growth of the pathogen. Earlier introduction of *C. destructivum* than *T. harzianum* into the plate. The *T. harzianum* grew fast, making connection with the pathogen to 2.43 cm. Simultaneous introduction of both pathogen and antagonist, *T. harzianum* grew fast, almost touching the *C. destructivum*. Fifth day of inoculation there was no clear zone of inhibition. By 7th day of pairing, *T. harzianum* had completely grown its mycelia mass as well as *C. destructivum.* In all the three pairing methods, contact between the two organisms was made within 24 h of pairing and in all the two *Trichoderma* species inhibited *C. destructivum*'s growth.

T. pseudokoningii and *T. harzianum* in the 3 pairing methods

Fast and heavy sporulation of *T. pseudokoningii* and

Table 1. Mycelia growth inhibition of *C. destructivum* by *T. pseudokiningii* and *T. harzianum* at 7days of incubation of the three pairing methods.

Antagonists	Mycelia growth of *C. destructivum*
Control	9.48a
T. pseudokoningii	1.91c
T. harzianum	2.57b
R^2	0.75

*Average with different letters are significantly different from each other.

Table 2. Effect of 3 pairing methods of *T. pseudokoningii* and *T. harzianum* on *C. destructivum.*

Methods	*Average mycelia growth (cm) of *C. destructivum*
24 h before *C. destructivum*	0.96a
Simultaneous inoculation	2.80b
24 h after *C. destructivum*	2.96b

*Average with different letters are significantly different from each other.

T. harzianum was observed on the pathogen, *C. destructivum* mycelia, such that the pathogen was completely overgrown by the antagonists with respect to time. The *trichoderma* species had completely parasitized the mycelia mass of *C. destructivum*. Growth inhibition of the pathogen, *C. destructivum* by the antagonists showed significant difference (P 0.05) from that of the control (Table 1). The mean growth inhibition of *C. destructivum* by *T. pseudokoningii* was also significantly different from that of *T. harzianum* (R^2 = 0.75). Inoculation of antagonist before pathogen was significantly different (P = 0.05) from the other two pairing methods which were not significantly different from each other (Table 2).

Pairing of *T. pseudokoningii* and *T. harzianum* in separate pairing methods

The growth inhibition of pathogen by the two *Trichoderma* species were significantly different (P = 0.05) from control when inoculated 24 h before the pathogen, *C. destructivum*. The average growth inhibition of *C. destructivum* by *T. pseudokoningii* differed significantly (R^2 = 0.71) from that of *T. harzianum* (Table 3). Early introduction of the pathogen before the antagonists, showed significant difference (P = 0.05, R^2 = 0.40) of the inhibition when compared with the control (Table 3). Average growth inhibitions of C. destructivum by *Trichoderma* species were not significantly different from each other. Simultaneous inoculation of the pathogen and antagonists showed significant difference (P=0.05, R2 = 0.53) of the growth inhibition of the *C. destructivum* by the two *Trichoderma* species compared with the control.

DISCUSSION

Results obtained from the *in-vitro* study showed that *T. pseudokoningii* could be used effectively against the seedborne pathogen, *C. destructivum*. When the pathogen was paired with *T. pseudokoningii*, a remarkable and almost total control of the pathogen was achieved; it was able to stop further growth of the pathogen by the third day after pairing. The fast mycelia growth of *T. pseudokoningi* and *T. harzianum* irrespective of pairing method with *C. destructivum*, showed the inhibitory ability of the two *Trichoderma* species. The ability of the two *Trichoderma* species to grow aggressively to colonize and occupy the Petri plates, with little or no space for the pathogen buttress the fact by Sharma and Sankara (1988), Prescott et al. (2002) that a good antagonist should be to able to compete well for space and nutrients. *T. pseudokoningii* exhibited mycoparasitism on *C. destructivum* due to its ability to compete well for space and nutrient. The zone of inhibition by *T. harzianum* on *C. destructivum* irrespective of the pairing methods shows antibiosis mode of inhibition.

The antagonistic potentials of the two *Trichoderma* species against *C. destructivum* were shown in all the three pairing methods. This suggests the ability of each of the *Trichoderma* species to inhibit the mycelia growth of *C. destructivum* irrespective of time of application. This tells us that if *C. destructivum* occurs on cowpea field before the two *Trichoderma* species were introduced; either of the two can still suppress it to some extent. Strains of *T. pseudokoningii* were successful in inhibiting growth of *F. verticillioides in vitro* in a similar manner, Sobowale et al. (2005), were also able to check occurrence of the pathogen significantly within maize (*Zea mays*) stem in the field, irrespective of pairing methods (Sobowale et al., 2007). The significance of mycelia suppression of *C. destructivum* by *T. pseudokoningii* over that of *T. harzianum* suggests that *T. pseudokoningii* better competitive ability than *T. harzianum* against *C. destructivum. T. pseudokoningii* appeared to be more promising than *T. harzianum* in checking the occurrence of the pathogen on cowpea fields.

Inoculating antagonist before pathogen had a significant advantage and preference over the other two pairing methods in aiding effective growth inhibition of the pathogen. This means that inoculating antagonists before pathogen is better than the other two pairing methods. In inhibition of *C. destructivum* growth, it is better for the antagonists to be on the field before the pathogen comes in.

The highly significant interaction between the

Table 3. Comparative effect of mycelia growth inhibition on the introduction of *T. pseudokoningii* and *T. harzianum* against *C. destructivum* using 3 pairing methods at 7th day of incubation.

Antagonists	Before pathogen	Before antagonist	Pathogen and antagonist same time
Control	8.67a	8.67a	8.67a
T. pseudokoningii	2.57b	3.68b	3.40b
T. harzianum	1.61c	3.10c	3.10b
R^2	0.71	0.40	0.53

*Average with different letters in the same column are significantly different from each other.

Trichoderma species and pairing methods showed mycelia suppression of C*. destructivum* by either *T. pseudokoningii* or *T. harzianum,* as the pairing method had significant influence on the pathogen. Overall, for a more competitive exclusion of *C. destructivum* from cowpea plant, it is better for *Trichoderma* species to be on the field before the occurrence of the pathogen.

REFERENCES

Adebesin AA, Odebode AC, Ayodele MA (2005). fungal species associated with market fruits of Musa species in South Western Nigeria. Bull. Sci. Assoc. Nig., 26: 299-305.

Adejumo TO, Ikotun T, Florini DA (1999). Biological control of Protomycopsis phaseoli, the causal agent of leaf smut of Cowpea. J. Phytopathol., 147: 371-375.

Allen DJ, Thottappilly G, Emechebe AM, Singh BB (1998). Diseases of cowpea. In: the pathology of food and pasture legumes. Allen DJ, Lenne JM(ed). Cab international in association with ICRISAT, p. 750.

Amusa NA, Ikotun T, Osikanlu YOK (1994). Screening Cowpea and Soybean cultivars for resistance to anthracnose and brown blotch diseases using phytotoxic metabolites. Afr. Crop. Sci. J., 2: 221-224.

Bailey JA, Nash C, Connell RJD, Skipp RA (1990). Infection process and host specificity of a Colletotrichum specie causing anthracnose of Cowpea, *Vigna unguiculata*. Mycol. Res., 94(6): 810-815.

Cook RJ, Baker KF (1982). Biological control of plant pathogens. W.H Freeman San Francisco, p. 433.

Emechebe AM, Shoyinka SA (1985). Fungal and bacterial diseases of Cowpea in Africa. In: Cowpea Research Production and Utilization. Singh SR, Rachie KO (eds). John Wiley and Sons limited, New York.

International Institute of Tropical Agriculture, IITA (1985). Soybean production manual. International Institute of Tropical Agriculture, Ibadan, Nigeria, 10: 200.

Janisiewiez WJ, Korsten L (2002). Biological control of postharvest diseases of fruits. Ann. Rev. Phytopathol., 40: 411-441.

Kormawa PM, Chianu JN, Manyong VM (2002). Cowpea demand and supply patterns in West Africa: the case of Nigeria. In: Challenges and Opportunities for enhancing sustainable Cowpea production. Fatokun CA,Tarawali SA, Singh BB, Kormawa PM,Tamo M (eds).IITA,Ibadan, Nigeria, pp. 375-386.

Odebode AC, Sobowale A (2001). Antagonistic activities of fungal flora isolated from pepper phylloplane on postharvest pathogen of pepper (capsicum annum). Acta Phytopathologica et entomologica Hungarica, 36(3-4): 289-292.

Papavizas GC (1985). Trichoderma and Gliocladium: Biology, ecology and potential for biocontrol. Ann. Rev. Phytopathol., 23: 23-54.

Poincelot RP (1986). Towards a more sustainable Agriculture.Lib.07 Congress Cataloguing – in – Pub. Dat., pp. 53-150.

Prasanna KPR (1985). Seed health testing of Cowpea with special reference to anthracnose caused by Colletotrichum lindemuthianum. Seed Sci. Technol., 13: 821-827.

Prescott LM, Harley J, Klein D (2002). Biotechnological Applications In microbiology. The McGraw –Hill companies, Inc. fifth edition, pp. 1020-1022.

Sharma JK, Sankara KV (1988). Biocontrol of Plant Diseases. Boca Raton (FL): CRC Press. Biocontrol of rust and leaf spot diseases. KFRI Scientific Paper, 133(2): 1-23.

Sobowale AA (2002). Bioligical control of Fusarium moniliforme (Sheld.) on maize stems by some fungal isolates from maize phyllosphere and rhizosphere. Ph.D. Thesis.

Sobowale AA, Cardwell KF, Odebode AC, Bandyopadhayay R, Jonathan SG (2005). Growth inhibition of Fusarium verticillioides (Sacc.) Nirenberg by isolates of Trichoderma pseudokoningii strains from maize plant parts ant its rhizosphere. J. Plant Prot. Res., 45(4): 249-265.

Sobowale AA, Cardwell KF, odebode AC, Bandyopadhayay R, Jonathan SG (2007). Persistence of Trichoderma species within maize stem against Fusarium verticillioides. Arch. Phytopathol. Plant Prot., 40(3): 215-231.

Sobowale AA, Cardwell KF, Odebode AC, Bandyopadhayay R, Jonathan SG (2008). Antagonistic potential of Trichoderma longibrachiatum and T.hamatum resident on maize (Zea mays) plant against Fusarium Verticillioides (Nirenberg) isolated from rotting maize stem. Arch. Phytopathol. Plant Prot., pp. 1-10.

Spadaro D, Gullino ML (2005). Improving the efficacy of biocontrol agents against soil borne pathogens. Crop Prot., 24: 601-613.

Warcup JH (1950). The soil-plate method for isolation of fungi from soil. Nature, 166: 117-118.