African Scientist Vol. 11, No. 4 December 31, 2010
Printed in Nigeria

1595-6881/2010 $12.00 + 0.00

http://www.klobex.org/afs

AFS 2010127/11403

Antagonistic activities of *Paecilomyces* and *Rhizopus* species against the cocoa black pod pathogen (*Phytophthora palmivora*)

M. O. Adebola and J. E. Amadi*

Department of Plant Biology, University of Ilorin, PMB 1515, Ilorin, Nigeria

(Received August 17, 2010; Accepted September 27, 2010)

ABSTRACT: Two test fungal antagonists (*Paecilomyces sp.* and *Rhizopus stolonifer)* were isolated from cocoa rhizosphere and rhizoplane while the black pod pathogen (*Phytophthora palmivora)* was isolated from freshly infected cocoa pods. *In vitro* screening using the dual culture technique was conducted to assess their potential as biological control agents against the pathogen. The results revealed that in dual cultures, the test antagonists effectively checked the growth of *P. palmivora*. The antagonists grew faster than the pathogen and produced zones of inhibition. In liquid medium tests, the culture filtrates of the test fungi also inhibited the growth of *P. palmivora*. *Rhizopus stolonifer* was slightly more efficient (45%) than *Paecilomyces sp.*(43%) in inhibiting the growth of *P. palmivora*.

Keywords: *Paecilomyces*, *Rhizopus*, *Phytophthora*, antagonist, inhibition.

Introduction

Black pod disease of cocoa caused by *Phytophthora palmivora* is an important yield-suppressing disease of cocoa especially in Africa. In Nigeria, in spite of regular attempts every season using copper and Metalaxyl-based fungicides coupled with appropriate farm sanitation to achieve a lasting control of this disease, there are still regular outbreaks (Ndoumbe-Nkang *et al.*, 2004). Apart from being expensive and its adverse effects on the environment, heavy reliance on these chemicals can be associated with non-targeted effects (WHO, 1987), loss of biodiversity, pollution of land and water and may lead to development of resistance by the pathogen (He *et al.*, 2005; Fontem *et al.*, 2005; Tondje *et al.*, 2006). Therefore, alternative or complimentary methods are needed for management of black pod disease. One such option is a biological control method which is relatively cheaper, less laborious, and environmentally friendly. This method is more durable in its effect and has the advantage of not requiring repeated periodic applications as is the case with chemical fungicides (Okigbo, 2000). The objective of this study therefore, is to investigate the potentials of *Paecilomyces* and *Rhizopus* species isolated from the cocoa rhizosphere and rhizoplane as biological control agents of *P. palmivora.*

*To whom correspondence should be addressed.
E-mail: jamadi2009@yahoo.com

Materials and Methods

Isolation of potential antagonists and pathogen

Two native potential antagonistic fungi were isolated from cocoa rhizosphere and rhizoplane in farmers' fields at Aba-Ijesha in Atakunmosa LGA of Osun State, Nigeria. Healthy cocoa leaves, stems and roots were cut into small pieces, surface-sterilized for 3 minutes with sodium hypochlorite and rinsed twice with sterilized distilled water. They were then placed on water agar and incubated at room temperature (28 ± 2^{o}C) for 7 days, to allow for the growth of all associated organisms (Tondje *et al.*, 2006). The pathogen (*Phytophthora palmivora)* was isolated from freshly infected cocoa pods obtained from Cocoa Research Institute of Nigeria (CRIN), Ibadan. Infected pods were surface-sterilized with 70% ethanol and the epidermis was stripped off with a sterile scalpel. The pod tissue was plated directly on sterile water agar plates and incubated at 28 ± 2^{o}C for 72 hours after which subcultures were made on PDA (Odigie and Ikotun, 1982). Stock cultures of isolates were maintained at 4^{o}C for subsequent studies.

Screening fungal isolates for antagonism

Dual culture technique

Inhibition of *P. palmivora* by the two test fungi was evaluated on PDA using a dual culture technique. Three 5-mm diameter mycelial plugs of each of the potential antagonists were inoculated at the periphery of three sterile culture plates and incubated for two days at 28 ± 2^{o}C (Evans, 2002; Holmes *et al.*,2006). The three plates were each doubly inoculated with a 5-mm diameter mycelial plug of *P. palmivora* 5cm away from the test antagonist inoculum. The plates were re-incubated for another one week at 28 ± 2^{o}C. The control plates were doubly inoculated with sterile agar plugs. The growth of *P. palmivora* and the test antagonists was recorded. Percentage growth inhibition in the pathogen was assessed using this formula:

$$\frac{(R1-R2) \times 100}{R1}$$

(where R1= radial growth of pathogen in control and R2= the radial growth of pathogen in the test plate). The diameter of the zone of inhibition (ZI) was also calculated as being the distance between the pathogen and the test antagonist in each plate (Royse and Ries, 1973, Whipps, 1987; Reddy and Hynes, 1993).

Effect of culture filtrates of test antagonists on *P. palmivora*

One hundred milliliters of potato dextrose broth (PDB) in 250ml Erlenmeyer flasks were separately inoculated with 4 disks of 5-mm diameter mycelial plugs of 7-day-old cultures of the test antagonists. The flasks were incubated at 28 ± 2^{o}C for 7, 14, 21 and 28 days. Filtrates were harvested at the various intervals and used for assay. Fifty milliliters (50ml) of sterile PDB were separately amended with 10ml of each of the different culture filtrates and then inoculated with 5-mm diameter mycelial plug of a 7-day-old culture of the pathogen. The control flasks were amended with 10ml of sterile distilled water. Each test was replicated three times. The flasks were incubated at 28 ± 2^{o}C for 7 days after which the mycelia in each flask were harvested, dried to constant weight in an oven and weight recorded. The percentage growth inhibition was calculated (Reddy and Hynes, 1993).

Statistical Analysis

All the data were analysed statistically using ANOVA and Duncan's multiple range rest (Steel and Torrie, 1980). Possible relationships between treatments with antagonists and control were established. All the analyses were done at P=0.05.

Results

Isolated Organisms

Two potential antagonistic fungi were isolated from cocoa phylloplane and rhizosphere. These isolates were identified as *Paecilomyces sp.* and *R. stolonifer*. The pathogen (*Phytophthora palmivora)* was isolated from freshly infected cocoa pods obtained from CRIN, Ibadan.

Antagonism in culture

The result of this study shows that the two fungi tested exhibited antagonistic activities against the pathogen, *P. palmivora*. There was significant difference ($P < 0.05$) in radial growth of pathogen in dual culture with test fungi and when it was grown singly in a culture. *Paecilomyces sp.* and *R. stolonifer* grew faster with time in dual cultures than the pathogen (Fig 1). The two fungi tested significantly ($P > 0.05$) inhibited the radial growth of the pathogen. *Paecilomyces sp.*was more efficient than *R. stolonifer* in inhibiting the pathogen (Table 1). There was no significant difference ($P > 0.05$) in the diameter of the zones of inhibition produced by the two test antagonists but *R. stolonifer* produced wider zone of inhibition. The zone of inhibition decreased over incubation period.

Effect of culture filtrate on antagonism

It was observed that the culture filtrates of the test antagonists generally inhibited the growth of the pathogen significantly ($p<0.05$). Percentage inhibition increased with the age of the culture (Table 2). The inhibitory effects of the culture filtrates of *Paecilomyces sp. and R. stolonifer* were not significantly different at $P < 0.05$.

Table 1: Radial growth (mm), and growth inhibition of Pathogen in dual culture.

Organisms	Radial growth (mm)	*Percentage Inhibition	Inhibition Zone
Paecilomyces sp.	9a	78a	13a
Rhizopus stolonifer	10a	76a	16a
Phytophthora palmivora	40b	-	-

*Results are means of three replicates
Means followed by different letters differ significantly at P = 0.05 (DMRT)

Table 2: Effect of age of culture filtrate on inhibition of pathogen growth

Age (Days)	Percentage inhibition*
7	35.5a
14	38.7ab
21	42.0b
28	49.5c

*Values are means of the two filtrates .
Means followed by different letters differ significantly at P= 0.05 (DMRT)

Discussion

Paecilomyces sp. and R. stolonifer were isolated in this study and were able to effectively challenge *P. palmivora*, the black pod pathogen *in vitro*. A lot of beneficial fungi and bacteria have been reported to occur naturally and some of them associated with cocoa have shown potential as antagonists of major cocoa pathogens (Kamil and Yahya, 1999; Bong *et. al*., 2000)). Samuel and Hebbar, (2003) had suggested that effort and expense in finding novel biological control species and strains can be saved by directing the search either by exploration for new strains in native area of the host or its pathogen or by studying the species that are known to be phylogenetically related to species that is effective in biological control. Since it has been observed that there are no biocontrol agents that have enough competitive ability to displace an already established pathogen, the dual inoculation and the introduction of potential antagonists two days before the pathogen was developed (Campbell, 1988). According to Robert, (1990) and Janisienwicz, (1988) the time lag between the arrival of the antagonist and later the pathogen on the phylloplane, allows adequate increase in cell concentration and subsequent colonization by antagonist before the arrival of the pathogen.

Growth experiment results showed that the two test antagonists grew faster than the pathogen in dual culture. Fast growth is important in any organism if it is to be useful in biocontrol studies. This is because competition for available nutrients is likely to be a major factor in biocontrol mechanism of action. Dandurand and Knudsen (1993) reported that the effectiveness of biocontrol agents might depend partially on their ability to proliferate during a short period of favourable environmental conditions before they encounter any plant pathogen. *Paecilomyces sp.* and *R. stolonifer* showed strong antagonism and produced zones of inhibition at the point of contact with the pathogen. This might be due to the production of antifungal metabolites by the test fungi (Shanker *et al.*, 1993). The filtrates of the test organisms inhibited mycelial growth of the cocoa pathogen. The effect of the culture filtrates on the pathogen might be due to toxin production into the culture medium. Over time, more of the toxin would accumulate accounting for the observed increased toxicity. But complete inhibition of pathogen growth was not observed in this study. Corinne *et al.,(*2003) had also reported that biocontrol agents do not completely inhibit the pathogens they antagonize. Odigie and Ikotun, (1982) had earlier reported increased antifungal activity of *G. roseum* filtrate on *P. palmivora* with time. Going by the results of this study, *Paecilomyces sp. and R. stolonifer* may probably function as good biocontrol agents against *P. palmivora*, the cocoa black pod organism.

References

Bong, C.L.; Shari Fuddin, S. and Almad Kamil, M.J. (2000). Research on cocoa diseases and their management. Workshop on latest development and issues in cocoa cultivation, 22 July 2000, Tawau, Sabah, Malaysia.

Campbell, R.B. (1988). Biological control of microbial plant pathogens. Rot of *Citrus* fruit by *Debaryomyces hansenii. Plant Dis. 74:*134-137.

Dandurand, L.M. and Knudsen, G.R. (1993). Influence of *Pseudomonas fluorescens* on hyphal growth and Biocontrol activity of *Trichoderma harzianum*. In the spermosphere and rhizosphere of Pea. *The American Phyto. Soc. 83* (3): 265.

Evans, H.C.(2002). Globalization and the threat from invasive alien species. In paper present at the Henry A. Wallace. Inter-American scientific conference, February 25 – 27, Costa Rica.

Fontem, D.A.; Olanya, O.M.; Tsopmbeng, G.R. and Owona, M.A.P. (2005). Pathogenicity and metalaxyl sensitivity of *Phytophthora infestans* isolates obtained from garden huckleberry, potato and tomato in Cameroon. *Crop Prot. 24:* 449-456.

He, Z.L.; Yang, X.E.and Stoffella, J.F.(2005). Trace elements in agro ecosystems and impacts on the environment. *J. Trace Elements Med.. Biol. 19:* 125-140.

Janisienwicz, J.W.J. (1988). Biocontrol of post-harvest disease of Apples with antagonistic mixtures. *Phytopathol. 78 :* 194-198.

Kamil, M.J. and Yahya M.N. (1999). Screening epiphytic bacteria present on cocoa pods for antagonistic activities against *Phytophthora palmivora*, causal pathogen of black pod disease, In: *Sustainable Crop Protection Practices in the Next Millennium.*

Ndoumbe-Nkang, M.C.; Clias, E.; Nyemb, S.; Nyasse, D.; Bieysse, A.; Flori, I., and Sache, C. (2004). Impact of prunning diseased pods on cocoa black pod caused by *Phytophthora megakarya* and on cocoa production in Cameroon. *Crop Prot. 23:*415- 424.

Odigie, E.E. and Ikotun, T. (1982). *In vitro* and *in vivo* inhibition of growth of *Phytophthora palmivora* by antagonistic microorganisms. *Fitopatologia Brasileira. 7:* 157-167.

Okigbo, R. N. and Ikediugwu, F.E.O. (2000). Studies on biological control of postharvest rot of yams (*Dioscorea* spp.) with *Trichoderma viride. J. Phytopathol. 148*(6): 351- 355.

Reddy, M.C. and Hynes, R.K. (1993). Relationship between *in vitro* growth inhibition of pathogens and suppression of pre-emergence damping off and post emergence root rot of white bean seedlings in the green house by bacteria. *Can J. Microbiol. 40:*113-199.

Robert, R.C. (1990). Post-harvest biological control of Apple by *Crotococus laurentii*. *Phytopathol. 80:* 526-530.

Royse, D.J. and Ries, S.M. (1973). The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cinata*. *Phytopathol. 63:603-607.*

Samuel, J.G. and Hebbar, P. (2003*). Trichoderma*: its potential for control of diseases of cocoa. Fourteenth International Cocoa Research Conference pp. 669-675.

Shankar, M.; Kurtboke, D.I. and Sivasithamparam, K. (1993). Nutritional and environmental factors affecting growth and antifungal activity of a sterile red fungus against *Gaeumanomyces graminis var. tritici*. *Can. J. Microbiol. 33:*515-519.

Tondje, P.R. Berry, Hebber, K.P., Samuels, G., Bowers, J.H., Weise, S., Nyemb, E. Begonde, D., Foko, J., and Fontem, D. (2006). Bioassay of *Geniculosporium* species for *Phytophthora megakarya* biological control on cocoa pod husk pieces. *African Journal of Biotechnology 8:* 648-652.

Whipps, J.M. (1987). Effect of media on growth and interactions between a range of soil-borne glass house pathogens and antagonistic fungi. *New Phytol. 107*. 127-142.

WHO, (1987). Report of an informed consultation on the detection, isolation, identification and ecology of biocontrol agents of disease vectors. Geneva 41pp.