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CONFRONTATION BETWEEN FOUR FUNGAL ISOLATES AND *Phytophthora palmivora* ON COCOA POD

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ABSTRACT

Cocoa pod pericarp blocks (10x1x1cm) were challenge-inoculated with any one of four test antagonists (Trichoderma harzianum, Aspergillus fumigatus, A. niger and Rhizopus sp.) and Phytophthora palmivora, the causal organism of cocoa black pod disease. Results revealed that all the test antagonists were effective in checking the growth of the pathogen. All the test antagonists were able to colonize a good proportion of the cocoa pericarp which had previously been colonized by P. palmivora. However, none of the test antagonists was able to eliminate the pathogen completely from the inoculated pericarp. The test antagonists colonized the entire surface of the cocoa pod pericarp when inoculated before the pathogen and did not allow the pathogen to establish. Inoculation of intact mature green cocoa pods with aqueous suspension of the propagules of each of the test fungal antagonists did not produce any lesion. However, when a mixture of the pathogen with each of the test antagonists was used, lesions were produced.

INTRODUCTION

It has been reported that at least five major cacao diseases destroy approximately 45% of the total crop annually in twelve major producing counties (1 and 2). In ranking the diseases according to their economic impact among other considerations, it was shown that 52% of the loss was caused by the cocoa black pod pathogen, *Phyphthora palmivora* (3 and 4). In Asia, Africa and Brazil the estimated loss is 450,000 tonnes annually, worth an estimated value of $42.3 million (5). At present, the strategies available to control this disease are cultural practice, phytosanitation, fungicidal sprays (copper and matalaxyl) and breeding for resistance. However, these strategies are expensive, labour intensive and moreover, there is no black pod disease- resistant variety yet. Heavy use of chemicals can lead to fungicide resistance in pathogens in addition to the issue of non-target effects on beneficial microorganisms, humans as well as on the environment (6). Many researchers have shown that certain microorganisms are useful as biocontrol agents for the control of plant pathogens. For instance, *Trichoderma* species have been used to suppress *Pythium* species.

The aim of this work was to evaluate the potentials of four fungal organisms namely *Trichoderma harzianum, Aspergillus fumigatus, A. niger* and *Rhizopus stolonifer* for use for the bicontrol of the cocoa black pod organism, *P. palmivora.*

MATERIALS AND METHODS

The test isolates

Four fungal isolates, *Trichoderma harzianum, Aspergillus fumigatus, A. niger* and *Rhizopus stolonifer* were isolated from cocoa phylloplane and rhizosphere in farmers' fields while the pathogen was isolated from freshly infected pods obtained from Cocoa Research Institute of Nigeria (CRIN), Ibadan and stored in sterile distilled water.

Challenge inoculations (Confrontation)

The effects of challenge inoculations of cocoa pods and pericarps were tested in this study at three different levels. The organisms used for the challenge inoculations were *P. palmivora* and *T. harzianum*.

(i) Sterile cocoa pod pericarp blocks (10x1x1cm) were used. The blocks were weighed and each was placed on sterile glass rods in Petri dish containing PDA at the centre and a small quantity of sterile water round the agar. The pericarp blocks were inoculated at one end with *P. palmivora* and at the other end with *T. harzianum* using 5mm-diameter agar discs of appropriate inoculum. The control was inoculated at both ends with 5mm agar discs of the same fungus. This experiment was replicated three times and incubated for 4 weeks at 28 ± 2°C. Colonization was determined by re-isolating the inoculated fungi from the interior of the cocoa pericarp. Re-isolated organisms were compared with the inoculum and identifications made (7).

(ii) Cocoa pod wood previously invaded by *P. palmivora* was challenged with *T. harzianum*. The pod wood was inoculated at both ends with *P. palmivora* and incubated for 4weeks. The inoculated pieces of wood were challenge-inoculated by transferring them into culture plates of *T. harzianum*. After another 4weeks of incubation the fungi were re-isolated from the interior of the wood block and identified. Three replicates were also made.

(iii) The reaction of cocoa pod to antagonists and pathogen (cotton placement method).

Ten intact surface-sterilized green matured cocoa pods were inoculated with 5ml of aqueous suspension (5×10^5) of each of the fungi using pad of absorbent cotton wool. The cotton wool was held in place with a cello tape to retard drying out. The pods were hanged on rectangular rod stands, with moistened cotton wool placed on the floor of the stands to create a humid environment. The setup was covered with transparent polythene to maintain humidity. Inoculation of the pods was repeated by mixing 5ml of aqueous suspension of the pathogen with 5ml of aqueous

suspension of each of the potential antagonists (5×10^5). The rate of spread of lesions caused by the antagonists or mixture of antagonist and pathogen were measured and recorded for 20days.The result was analyzed using ANOVA.

RESULTS

Successful colonization of intact cocoa pod pericarp by both the pathogen and potential antagonists was observed in the present study. All the organisms tested were able to colonize the cocoa pod samples. However, the colonization was observed to be more rapid when the pathogen and the potential antagonists were paired on either side of an intact cocoa pod pericarp. *T. harzianum* invaded up to 78% of the inoculated sample followed by *A. niger* with up to 74% (Table I). All the four test antagonists (*A. fumigatus, T. harzianum, Rhizopus stolonifer and A. niger)* were able to colonize cocoa samples previously invaded by *P. palmivora*. Re-isolation after four weeks of incubation produced the test antagonists. However, it was observed under the conditions of this study that none of the test antagonists was able to completely eliminate the pathogen.

When each of the antagonists was tested on cocoa pod placed on a rectangular rod stand it was observed that no lesion was produced except on the pod covered with the pathogen. However, when a mixture of the pathogen and each of the antagonists was tested on the pods, lesions were observed. The radii of lesions obtained were significantly different ($P < 0.05$) (Table II).

Table I. Percentage colonization of cocoa pericarp by pathogen and the potential antagonists.

Pericarp

Fungi with pathogen	* % Colonization of samples by		
	Pathogen	Antagonists	%loss in weight
A. fumigatus	28a	72c	66.7a
T. harzianum	22b	78a	66.7a
R. stolonifer	28a	72c	66.7a
A. niger	26b	74b	66.3a

*Mean of three replicates after three weeks of incubation
Means in a column followed by different letters differ significantly at P= 0.05 (DMRT)

Table II. Reaction of cocoa pod to the mixture of potential antagonist and pathogen.

Pathogen with antagonist	*Radius of lesion
A. fumigates	12.4a
T. harzianum	3.8b
Rhizopus stolonifer	11.3a
A. niger	3.6b

*Mean of three replicates after 20days of incubation
Means in a column followed by different letters differ significantly at P = 0.05 (DMR)

DISCUSSIONS

The antagonistic activity of the isolates was in evidence when each was placed together with pathogen on either side of the cocoa pod pericarp. *T. harzianum, A. niger, A. fumigatus* and *R. stolonifer* were able to check the invasion of the pathogen. They probably produced substances that may be very effective at preventing the growth of the pathogen on the pods. It has earlier been reported

that the biocontrol activity of fungal endophytes can be due to a number of mechanisms including direct interaction with the pathogen by parasitism, antibiosis or competitive exclusion. Equally, it can be as a result of its interaction with the host plant defense mechanism (8). Tondje *et al.* (9) reported that nonanoic acid was isolated from an isolate of *T. harzianum* from an infected cocoa pod and was shown to inhibit spore germination and mycelial growth of two cocoa pathogens, *Crinipellis perniciosa* and *Moniliophthora roreri.* The result of this study revealed that though some of these potential antagonists could prevent the pod from pathogen invasion, none of them could colonize the samples previously invaded by the pathogen. This suggests that these test antagonists can be used to prevent cocoa invasion but may not be useful for total elimination of pathogens that have already established on cocoa pods.

When the test antagonists were tested on cocoa pod, it was observed that no lesions were produced. This close association with the host without the expression of stress or disease symptoms may suggest a balanced interaction between the two organisms. This shows that earlier arrival of the biocontrol agent before the pathogen is a major factor (8). The mechanism of action is probably that the antagonist may act as a physical barrier by colonizing the meristematic tissues of the host thereby excluding the entry of the pathogen. Alternatively, the antagonist may reduce inoculum pressure by parasitizing and colonizing the pseudostroma and spores of the pathogen as they develop. However, when a mixture of the pathogen and the antagonists was introduced on cocoa pod surface, the pathogen in combination with *A. fumigatus and Rhizopus stolonifer,* separately produced lesions. This confirmed the belief that these antagonists may be acting by challenging the establishment of the pathogen if they arrived earlier or about the same time with the pathogen on the surface of the host.

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