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Original Article

Screening Five Fungal Isolates as Potential Antagonists to *Phytophthora palmivora* on Cocoa Pod Pericarp

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ABSTRACT

Investigations were carried out on the antagonism of *Penicillium digitatum, Aspergillus repens*, *Paecilomyces* sp*., Botryodiplodia theobromae* and *Alternaria tenuis* to *Phytophthora palmivora* (cocoa black pod pathogen) on cocoa pod pericarp blocks (10x1x1cm). The result revealed the antagonistic activity of the tested organism when each was placed together with pathogen on either side of the cocoa pod pericarp of length 10cm long. *Penicillium digitatum* and *Paecilomyces* sp were able to check the invasion of the pathogen by colonizing the greater part of the pericarp (70% each), followed by *Aspergillus repens* (62%)and *Alternaria tenuis* (34%) . The test antagonists were unable to colonize the samples previously invaded by the pathogen and therefore unable to eliminate the pathogen that was already established. When the test antagonists were placed on sterile intact cocoa pod, only *B. theobromae* produced spectacular rot and loss in weight. However, when they were inoculated on sterilized cocoa pericarp of length 10cm long, the entire block was colonised with rots and loss in weight. The decay capacity was observed to be significant ($P < 0.05$) with loss ranging from 28% in *Paecilomyces* sp to 57% in *B. theobromae*. Lesions were produced by all when the mixture of the pathogen with each of the antagonists was tested on cocoa pod surface; the radii of lesions obtained were significantly different ($P < 0.05$). *Alternaria tenuis* produced the largest lesion (37.9%) closely followed by *B. theobromae (*26.0%) while *Penicillium digitatum* produced the least lesions (9.8%).

Key words: Cocoa, Black pod, Antagonists, Pathogen, Confrontation

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INTRODUCTION

Cocoa tree (*Theobroma cacao* L.), Family Sterculiaceae is a native to the rainforest of Tropical America (the central and Western Amazon region of South America). The tree is subject to numerous constraints due to plant and animal parasites or viruses. Among the diseases caused by these parasites are: black pod disease (*Phytophthora palmivora;* cocoa swollen shoot (viral); *Monilia* pod rot (*Moniliophthora roreri*); witches broom (*Crinipellis* perniciosa); *Botryodiploidia* pod rot (*Botryodiploidia theobromae*); collar crack (*Armellaria mellea*); cushion gall

(*Calonetria rigidiuscula*) (Evans *et al*., 1998;). *Phytophthora palmivora* cocoa black pod disease pathogen caused an estimated loss in production in Asia, Africa and Brazil of 450,000 tonnes annually, worth an estimated value of $423million. Annual crop losses may range from 30 - 90% (Bowers *et al*., 2001). The impact of the disease varies from country to country (Bong *et al*., 2000). Black pod rot occurs in almost all cocoa producing countries, with worldwide loss estimated at 10%. Direct crop losses of up to 90% occur in wetter areas such as Nigeria (Gregory and Maddison, 1981). Curative measures for the disease have not been successful. Newhall (1971) reported that when cocoa seedlings were sprayed with Bordeaux it often caused some burning of young, tender leaves and caused stunted growth. According to Purdy and Schmidt (1996), high volume spraying of chemical caused pod injury, which leads to superficial blackening of the pod surface due to death of epidermal cells. Apart from being very expensive for many African farmers, heavy reliance on such chemicals have been associated with non-target effects: loss of biodiversity, spoilage of land and water and may also lead to the development of resistance by the pathogen (Fontem *et al*., 2005; He *et al*., 2005; WHO, 1987). Burying pod husks has little effect on the viability of the pathogen and only increases their population in the soil. Frequent harvesting and frequent removal of infected pods will generally reduce black pod but are not practiced as much as they should (Tondje *et al*., 1993; Nduombe –Nkeng *et al*., 2004). The use of resistant varieties of cocoa is apt to breaking down under adverse weather condition or from the appearance of new strain of the pathogen. Breeding resistant cocoa variety is more laborious, and most varieties now grown are susceptible to *Phytophthora.* It will therefore be difficult to obtain completely resistant cultivars. Moreover, according to Tondje *et al*., (2006) no cocoa variety is completely resistant to black pod disease caused by *Phytophthora.* Following from this, alternative or complimentary methods are needed for management of this disease. One such option could be a biological control approach using antagonistic microorganisms (Adebola and Amadi, 2010). This paper reports on the results on evaluation of the potentials of *Penicillium digitatum, Aspergillus repens*, *Paecilomyces sp., B. theobromae* and *Alternaria tenuis* for the control of cocoa black pod disease cause by *Phyphthora palmivora.*

MATERIALS AND METHODS

Fungal Isolates

Five fungi *(Penicillium digitatum, Aspergillus repens*, *Paecilomyces sp., B. theobromae* and *Alternaria tenuis)* were isolated from cocoa phylloplane and rhizosphere in farmers' fields while *Phytophthora palmivora* was isolated from freshly infected pods obtained from Cocoa Research Institute of Nigeria (CRIN) Ibadan and stored in sterile distilled water.

Confrontation on Cocoa Pod Pericarp

(i) Eighteen blocks of Cocoa pod pericarp (10x1x1cm) were weighed, sterilized 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 an antagonist using colonized 5mm-diameter agar discs. The control was inoculated at both ends with 5mm- diameter agar disc. Three replicates of each were made and incubated for 4 weeks at 28 $\pm$ 2^{o}C. Colonization was determined by re-isolating the inoculated fungi from the interior of the cocoa pericarp. Re-isolated organisms were compared with

inocula and identification made (Sudirman *et al*., 1992; Adebola and Amadi, 2011).

(ii) Cocoa pod wood previously invaded by *P. palmivora* was challenged-inoculated with the antagonists at both ends with *P. palmivora* and incubated for 4weeks. The inoculated pieces of cocoa pod wood were challenged – inoculated by transferring into the culture plates of the antagonist fungi. After another 4weeks of incubation at 28 $\pm$ 2^{o}C, the fungi were re-isolated from the interior of the wood block and identified. Three replicates were made (Sudirman, *et al*., 1992).

(iii) The reaction of cocoa pods to antagonists and pathogen (cotton placement method): Ten intact surface sterilized green matured Cocoa pods were inoculated with 5ml of aqueous suspension at 5 x 10^5 of each of the fungi, using pad of absorbent cotton wool held in place by cellotape 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 *P. palmivora* with 5ml of aqueous suspension of each of the potential antagonists (5 x 10^5). The rate of spread of lesion caused by the antagonists or mixture of antagonist and pathogen were measured and recorded for 20 days (Toxopeus and Gorenz, 1969; Adebola and Amadi, 2011).

Statistical analysis

The results were analysed using Analysis of Variance (ANOVA)

RESULTS

The results from this study revealed that all the antagonists and the pathogen were able to colonize and cause weight loss on cocoa pod (Table 1). The highest weight loss was caused by *P. palmivora* (75%) followed by *B. theobromae* 57%. *Paecilomyces* sp. caused the least weight loss of 28% while it was 30% in *Penicillium digitatum* and 31% in *A. repens*. The result showed that the weight loss was not significantly different ($P>0.05$) among *Paecilomyces* sp., *P. digitatum* and *A . repens*. However, when the *P palmivora* was challenged on the pod pericarp(Table 2), *Paecilomyces* sp. and *P. digitatum* effectively challenged the pathogen by invading up to 70% each of the pod, thus, reduced the growth of the pathogen to 30% in each case. Followed closely was *A. repens* that invaded 62% of the pod pericarp. The result of confrontation between *B. theobromae* and *P. palmivora* showed that the competition was so keen. *B. theobromae* colonized 56% while the pathogen colonized 44%. The pathogen out competed *Alternaria tenuis* invaded 66% of the pod pericarp leaving only 34% for *A. tenuis*. The result revealed that none of the antagonists was able to colonized the entire cocoa pod pericarp used in this experiment. Only *Paecilomyces* sp., *P. digitatum* and *A. repens* were re-isolated from coca pod pericarp previously colonized by *P palmivora* after 4 weeks of incubation. *B. theobromae* and *A. tenuis* were not re-isolated. This result also showed that none of the antagonists was able to completely out competed the pathogen

from the cocoa pod samples previous colonized by *P palmivora*.

Lesions were not produced on intact cocoa pod inoculated with each antagonist and placed on a rectangular rod stand covered by polythene except the pods that were inoculated with *B. theobromae* and the pathogen that produced lesion after eight days of incubation (Table 3). However, lesions were observed when the mixture of the pathogen and each of the antagonists was inoculated on intact cocoa pod. The highest lesion was produce by the mixture of pathogen with A. tenuis (37.9mm radius) followed by *B. theobromae* with26.0mm. The lesions produced by mixture of pathogen with *P. digitatum(*9.8mm), *A. repens*(10.7mm) and *Paecilomyces* sp.(13.9mm)were small and not significantly different (P<0.05)

Table 1. Colonization of potential antagonist and the pathogen on cocoa pod pericarp blocks of 10cm long

Fungi	*% Colonization of samples	* % Weight loss
P. palmivora	100	75f
A. repens	100	31bc
Paecilomyces sp	100	28a
Alternaria tenuis	100	35d
B. theobromae	100	57e
P. digitatum	100	30ab

*Mean of three replicates after 4weeks of incubation . Means followed by different Letters differ significantly at P< 0.05

Table2: Confrontation between pathogen and the potential antagonists on cocoa pod pericarp

Antagonists with pathogen	* %Colonization of samples by	
	Pathogen	Antagonists
A.repens	38c	62c
Paecilomyces sp	30d	70d
Alternaria tenuis	66a	34a
B. theobromae	44b	56b
P. digitatum	30d	70d

*Mean of three replicates after 4weeks of incubation

Means in a column followed by different letters differ significantly at P< 0.05

Table 3 Reaction of cocoa pod to the mixture of potential antagonist and pathogen.

Pathogen with antagonists	*Radius of lesion (mm)
A.repens	10.7a
Paecilomyces sp	13.9a
Alternaria tenuis	37.9c
B. theobromae	26.0b
P. digitatum	9.8a

*Mean of three replicates after 20days of incubation

Means followed by different letters differ significantly at P < 0.05

DISCUSSSION

The antagonistic activity of the tested antagonists was in evidence when each was placed together with pathogen on either side of the cocoa pod pericarp of length 10cm long. *Penicillium digitatum* and *Paecilomyces sp* were able to check the invasion of the pathogen by colonizing the greater part of the pericarp. Probably they may be very effective at preventing the growth of the pathogen on the pods when used as biocontrol. *A. repens* gave moderate invasion, supporting the earlier report of Bailey and Garac-Eppinniza (1978) that *Aspergillus* species are competitors and hyperparasites that inhibit sporulation and prevent mycelium infection of cocoa pods by *P. palmivora*. However, *Alternaria tenuis* gave the least invasion of the cocoa pod pericarp as it was observed that the pathogen invaded the greater part of the pericarp suggesting that it might be less effective at controlling the pathogen on the pod when used as bicontrol agent.

When the mixtures of both the pathogen and the antagonists were introduced on the cocoa pod surface, the pathogen with *Alternaria tenuis* and with *B. theobromae* produced the largest lesions suggesting that they are weak competitors and hyperparasites in this study. Odigie and Ikotun, (1982) also reported an increase in the rate at which the pods were consumed when *P. palmivora* was introduced into pods pre-treated with *B. theobromae* compared to when *P. palmivora* was inoculated alone

meaning that the fungus might not be suitable as a bicontrol of cocoa pathogen. Lesions produced by *Penicillium digitatum, A. repens* and *Paecilomyces* sp were relatively very small and increase with days of incubation suggesting that they are good competitors and hyperparasites of *P. palmivora*. This result was in support of the earlier findings of Adebola and Amadi,(2011)that such antagonists might be able to challenge the establishment of the pathogen if they arrived earlier or about the same time with the pathogen on the surface of the pod.

The result showed that when each of the tested antagonists was placed on sterile intact cocoa pod, no rot or loss in weight was noticed as against when they were inoculated on sterilized wounded cocoa pod, where rots and loss in weight were observed. This possibly suggests a balanced interaction between the antagonists and the cocoa pod (Holmes *et al*., 2006) and that some of these antagonists might not be able to infect an intact cocoa pod except when wounded thus they might be suitable as biocontrol of cocoa pod pathogen on the field.

CONCLUSION

The result of this study revealed that though some of these tested antagonists could protect the pod from pathogen invasion; none of them could colonise the samples previously invaded by the pathogen. That is they could be used to prevent the invasion of *P. palmivora* on cocoa pods if they arrived earlier or about the sametime on the surface of the pod. But could not be used to eliminate the pathogens already on cocoa pods. *Penicillium digitatum, A. repens* and *Paecilomyces* sp are potential antagonists of *P. palmivora* cocoa black pod disease pathogen and a field trial is needed.

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