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THE EFFICACY OF THREE *ASPERGILLUS* ISOLATES FOR THE BIOLOGICAL CONTROL OF COCOA BLACK POD DISEASE PATHOGEN (*Phytophthora palmivora*) ON THE FIELD

ADEBOLA, M.O*.[1] AND AMADI, J.E.[2]

Department of Biol. Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State.

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Abstract

Theobroma cacao (cocoa tree), is a small (48 m tall) evergreen tree in the family Sterculiaceae (synonym Malvaceae). Its seeds are used to make cocoa powder and chocolate. Phytophthora palmivora, the cocoa black pod disease pathogen causes the greatest damage to Cocoa tree. Previous efforts made to combat this menace of cocoa such as agronomic and horticultural practices and the use of chemical were inadequate. This study investigated the efficacy of three Aspergillus species (Aspergillus fumigatus, Aspergillus repens and Aspergillus niger) isolated from the cocoa rhizosphere and rhizoplane as biological control agents of Phytophthora palmivora. The suspension of each of the three fungi was prepared by blending solid rice substrate with distilled water, 20% pectin and ammonium diphosphate (2:1 v/v) at pH 5.5 for ten seconds in a blender and filtered through cheesecloth. The final concentration of each of the suspensions was adjusted to 10^8 propagules/ml. The experimental plots were set up in three cocoa farms untreated for many years at Aba Ijesa, 45Km from Ile Ife, Osun State of Nigeria. The efficacy of the three fungal isolates was compared to the fungicide (Metalaxyl-m) while water was used as control. The effects of the test antagonists on pod infection differed significantly ($P< 0.05$). The percentage infection of pods by the pathogen was very low and found not to be significantly different ($P< 0.05$) in plots treated with chemical (11%) and A. repens (11%). The three antagonists effectively reduced the pressure of disease over time in this experiment. Percentage infection rose steadily in the control till the end of the experiment. The application of the three biocontrol agents effectively hampered the establishment of the pathogen on the pods, enhanced flowering, and increased cocoa pod production in the field.

Key words: Efficacy, *Aspergillus,* Black pod disease, Pathogen, Cocoa

INTRODUCTION

Phytophthora palmivora, the cocoa black pod disease pathogen had been reported to cause the greatest damage to Cocoa tree (*Theobroma cacao* L.), family Malvaceae. Bowers *et al.* (2001) reported that crop loss to this pathogen may range from 30- 90% amounting to about 450,000 tonnes annually. Previous efforts made to combat this menace were agronomic and horticultural practices, the use of chemical fertilizers and pesticides. These different methods used, had contributed significantly to improvement in crop productivity and quality. However, the excessive use of chemicals had been reported to cause pod injury, which led to superficial blackening of the pod surface due to death of epidermal cells and often caused some burning of young, tender leaves and stunted growth. The aftermath of this is loss of biodiversity, spoilage of land and water and the development of resistance by the pathogen (Newhall, 1971; WHO, 1987; Purdy and Schmidt, 1996; Fontem *et al.*, 2005; He *et al.*, 2005). Therefore, all efforts were directed towards preventing the incidence of the disease. But in spite of these, control obtained is often inconsistent or unsatisfactory and the reduction in crop loss has not been achieved by farmers. According to Agbeniyi and Adenikinju (2000), spraying of cocoa should not be directed only to the green pods but also to cocoa stem where mosses grow and harbour the inoculum.

* Author for Correspondence
2 Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria

But Samuel and Hebbar (2003) reported that this is very expensive and uneconomical. Also, because of the aftermath effects of chemical, there are strict regulations on the use of chemicals and the political pressure to remove the most hazardous chemicals from the market (Kamil and Yahaya, 1999; Adebola and Amadi, 2010). Therefore, the use of biological control could be be a better alternative or complimentary method for the management of this disease.

Many beneficial fungi and bacteria that occur naturally and associated with cocoa were reported to show potential as antagonists of major cocoa pathogens (Kamil and Yahya, 1999; Bong *et al*., 2000; Samuel and Habber, 2003). Adebola and Amadi (2010a & b) reported the effectiveness of *Aspergillus fumigatus, A. repens A. niger, Paecilomyces* sp and *Rhizopus stolonifer* isolated from the rhizosphere of black pod-infected cocoa trees to inhibit growth of the pathogen *in vitro*. The culture filtrates of these organisms were also reported to hinder the growth of the pathogen in liquid medium. On this note, this study investigated the efficacy of these three fungi (*Aspergillus fumigatus*, *Aspergillus repens* and *Aspergillus niger*) isolated from the cocoa rhizosphere and rhizoplane as biological control agents of *P. palmivora* on the field.

MATERIALS AND METHODS

Inoculum production

The inoculum was produced on rice according to Hebbar and Lumsden (1999). Five mycelial plugs of 3-day- old culture of each potential antagonist were transferred to a potato broth medium (200g/l) autoclaved twice for 60 mins at 1atm. Each of the suspensions was left on a shaker for 5days at 28 ± 2°C and 95rpm.

Ten polythene bags were separately filled with 300 g of rice and 70 ml distilled water each. They were autoclaved for three consecutive days and cooled to room temperature. Thirty millilitres of each of the liquid cultures of potential antagonist was poured into the bags containing the rice. The bags were opened after 48 hrs and the contents were spread on trays and incubated for further 3 days at 28 ± 2°C for initiation of spore production. Finally, they were dried for 6 days. The suspension of each was prepared by blending solid rice substrate with distilled water, 20% pectin and ammonium diphosphate (2:1 v/v) at pH 5.5 for ten seconds in a blender and filtered through cheesecloth. The final concentration of each of the suspensions was adjusted to 10^8 propagules/ml. (Bong *et al*., 2000; Krauss and Soberanis, 2000; Tondje *et al*.,2006). The suspension was stored in containers which were placed in a container packed with ice-blocks at 4°C for further use (Tondje *et al*., 2006).

Application of treatments

The experimental plots were set up in three cocoa farms untreated for many years. The pathogen pressure in these farms was high. Five treatments were made in each of the farms: Three potential antagonists (*Aspergillus fumigatus*, *Aspergillus repens* and *Aspergillus niger*), a chemical fungicide (Metalaxyl-m, 2.55g/lit. as recommended by the manufacturer) and water was used as control. Each treatment plot comprises of three cocoa trees. Routine management practices were made in the field before and throughout the period. Two applications were made fortnightly in this trial for over a period of eight weeks starting from August to September each year. All treatments were applied on pods only in liquid suspension using a hand operated sprayer at 500 to 1,000 ml per tree depending on pod load.

Data collection

The number of diseased pods was recorded for each tree in each plot fortnightly after the first application till the end of experimentation. Different measures were used in assessing disease severity: total number of pods, total number of healthy pods and total number of diseased pods on each tree in each plot. All the ripe

and diseased pods were removed from each tree after having been recorded. To compare the efficacy of the various treatments on the control of black pod, percentage infection was calculated (total number of infected pods/ total number of pods x 100) and the data were subjected to ANOVA and DMRT (Gomez et al., 1984).

RESULTS

The results of the field trial revealed that the effects of all the treatments applied were significantly different ($P \leq 0.05$) on the mean pod production within and between the experimental farms after the second week of the first application (Table 1). The trees treated with *Aspergillus repens* produced the highest number of healthy pods (30) followed by the chemical (Metalaxyl- m), *Aspergillus niger*, *Aspergillus fumigatus* and the control. Untreated trees (the control) produced the highest mean number of infected pods (6). The mean number of pods produced by trees treated with *A. repens* and chemical were not significantly different ($P>0.05$). The trees treated with *A. repens* produced the highest total mean number of pods (32.6 pods) followed by trees treated with the chemical, *A. niger* and least with *A. fumigatus* (27pods). However, the mean percentage infection of pods with the *P. palmivora* was found to be least in trees treated with *A. repens* (7%) and highest in untreated trees (14%).

At the end of week 4 after the first application (Table 2), the trees treated with *A. repens* and the chemical produced the highest mean number of healthy pods (25pods each) while the untreated trees produced the least mean number of healthy pods (18 pods). The untreated plots produced the highest mean number of infected pods (5 pods) followed by *A. fumigatus*. The trees treated with *A. repens* and the chemical produced the least number of infected pods (3 pods each). In all the treatments, the total mean number of pods produced was not significantly different ($P > 0.05$) except in the control. Trees treated with *Aspergillus niger* produced the highest mean number of pods (28 pods) while the control produced the least (23 pods). However, the highest percentage number of infected pods was recorded in untreated plots (21%). The percentage infection of pods by the pathogen was very low and found not to be significantly different ($P>0.05$) in plots treated with the chemical (11%) and *A. repens* (11%).

At week 6, the plots treated with chemical (Metalaxyl - m) produced the highest mean number of healthy pods (27 pods) while the untreated plots produced the least (14 pods)(Table3). Plots treated with chemical and *Aspergillus niger* produced the least number of infected pods (2 pods each). The highest mean number of pods produced was obtained in the plots treated with *A. fumigatus* (29 pods) while the control plots produced the least pods (18 pods). The highest mean percentage infection of pods with *P. palmivora* was recorded in untreated plots (22%). The plots treated with chemical were least infected and was significantly different ($P<0.05$) from all other plots. However the percentage infection of the plots treated with *Aspergillus niger* was very low.

At week 8 (Table 4), the plots treated with chemical produced the highest mean number of healthy pods (29 pods) followed by *A. repens* with 22 pods while the least mean number of healthy pods (7 pods) was recorded in control plots. The control plots produced the highest mean number of infected pods (5 pods). Plots treated with chemical were the least infected. The highest mean number of pods produced was recorded in the plots treated with chemical (30 pods) but these were not significantly different ($P < 0.05$) from plots treated with *A. repens* (25 pods). The percentage infection by *the P. palmivora* in the plots treated with chemical were very low (3%) and significantly different ($P<0.05$) from the remaining plots. The percentage infection produced in plots treated with *A. repens, A. niger and A. fumigatus*. (8%, 10% and 11%, respectively) were relatively low when compared with control plots (42%).

The effect of all the treatments on healthy pods, infected pods, total number of pods and percentage infection of *P. palmivora* on pods production was found to be significantly different ($P < 0.05$) among the weeks of trial. The highest mean number of healthy pods and mean total number of pods were recorded at

week 2 (Table 5) which was significantly different ($P < 0.05$) from other weeks. The highest number of infected pods was recorded at week 2 while it was least at week 8. However, percentage infection was not significantly different ($P < 0.05$) at weeks 4 and 6. The results obtained from all the farms used for the trial were not significantly different ($P < 0.05$) from one another (Table 6).

In all the treatments(except the control), trends observed showed that at the second week after the first application the disease pressure was generally high but later decreased at weeks 4, 6 and 8. The percentage infection rose steadily from week 2 to the end of the trial period in week 8. However, the percentage infection was highest in control plots with 14% at the end of second week and rose to 42% at week 8.

In chemical and *A. repens*-treated plots the average percentage pod infection was low throughout the trial periods. The percentage pod infection produced by chemical at week 2 was 8% and declined to 3% at week 8. In A. *fumigatus*, and *Aspergillus niger* treated plots, the average percentage pod infection rose from week 2 (11% and 10%, respectively), to 15% and 14%, respectively at the end of week 4. However, decrease was observed as from week 6 (14% and 13%) to the end of trial period in week 8 with 11% and 10%, respectively which was lower than what was obtained at week 2.

Table 1: Effects of treatments on cocoa pod production 2 weeks after spraying.

Treatment	Total no of pods	No of healthy pods	No of infected pods	% infection
Water	28ab	24a	4a	14d
A. fumigatus	27a	24a	3a	11c
A. niger	29bc	26b	3a	10*bc*
A. repens	32bc	30c	2a	7a
Chemical	31bc	28b	2a	8a

Table 2: Effects of treatments on cocoa pod production 4 weeks after spraying.

Treatment	Total no of pods	No of healthy pods	No of infected pods	% infection
Water	23a	18a	5b	21e
A. fumigatus	27bc	23b	4ab	15b
A. niger	29c	25b	4ab	14b
A. repens	28bc	25b	3a	11a
Chemical	28bc	25b	3a	11a

Means followed by different letters within the same column differ significantly at $P < 0.05$

Table 3: Effects of treatments on cocoa pod production 2 weeks after spraying.

Treatment	Total no of pods	No of healthy pods	No of infected pods	% infection
Water	18a	14a	4a b	22e
A. fumigatus	29c	25b	4ab	14c
A. niger	28c	26b	3ab	7a
A. repens	28c	25b	2a	11b
Chemical	28c	27b	2a	7a

Table 4: Effects of treatments on cocoa pod production 2 weeks after spraying.

Treatment	Total no of pods	No of healthy pods	No of infected pods	% infection
Water	12a	7a	5b	14d
A. fumigatus	18b	16b	2a	11c
A. niger	21c	19b	2a	10c
A. repens	25cd	23c	2a	8b
Chemical	30cd	29d	1a	3a

Means followed by different letters within the same column differ significantly at $P < 0.05$

Table 5: Effects of treatments on cocoa pod production 2 weeks after spraying.

Week	Total no of pods	No of healthy pods	No of infected pods	% infection
2	30c	27b	3a	10a
4	28b	24b	4a	14b
6	26b	23b	3a	12b
8	22a	19a	3a	13b

Table6: Effect of farms used on all the treatments

Farm	Total no of pods	No of healthy pods	No of infected pods	% infection
A	26a	23a	3a	12a
B	27a	24a	3a	12a
C	27a	24a	3a	12a

Means followed by different letters within the same column differ significantly at $P < 0.05$

DISCUSSION

The field trial was conducted in August 2007 in cocoa farms untreated for many years with high pathogen pressure. This period was usually the epidemiological period of the black pod disease pathogen. Fortnightly, phytosanitation was observed and the exercise was carried out at early hours of afternoon when temperature range was still about 30°C - 35°C. The disease incidence was much higher in the control plots throughout the weeks of experiment from 14.1% at week 2 after the first application of the treatment to 38% at week 8. However, the initial lower incidence might be due to removal of diseased pods and all possible sources of pathogen inoculums. Throughout the period of the trial, the chemical kept the incidence of the disease at bay with the highest incidence of 7.57% at week 2 and lowest (0.8%) at week 8. This trend has also been reported by Tondje *et al. (1993). A. repens* had a much lower incidence of the disease at week 2 than the chemical. The percentage infections were found to increase at week 4 but dropped at week 6 and week 8. All the treatments except untreated plots increased the percentage of healthy pods produced significantly. This might be attributed to the good cultural practice throughout the period of trial.

At weeks 6 and 8, the highest percentage infection of pods was recorded in the control. At week 8 which was the end of the field trial, plots treated with *Aspergillus repens* gave the best control of the pathogen. The antagonist might be able to colonize the meristematic tissue of actively growing cocoa pod thereby excluding the entry of the pathogen by acting as barriers to colonisation, using their innate antagonistic abilities (mycoparasitism or antibiosis) to maintain their positions. Alternatively, they might reduce inoculum pressure by parasitizing and colonizing the pseudostroma and spores of the pathogen as it develops on the cocoa pod (Holmes *et al*., 2006). The results obtained from the three farms used for the experiment were not significantly different. This probably confirmed the homogeneity of these farms. The addition of nutritional supplement (pectin and sucrose) might have possibly improved the ability of the tested antagonists to colonize the pods and promoted sporulation in the field as earlier reported by Hjorth *et al*. (2003); Adebola and Amadi (2010b). The percentage infection was observed to be high as from week 4 probably due to constant and increasing rains. Generally, these potential antagonists were effective as biocontrol agents against *P. palmivora*, the cocoa black pod pathogen.

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