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The Efficacy of Paecilomyces SP and Penicilium Digitatum on Blac Pod Disease Pathogen on the Field

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Abstract: *Phytophthora palmivora* the blac pod disease pathogen is nown to cause the greatest damages to cocoa tree. All measures put in place to control the pathogen were not successful. This study therefore investigated the biological control of the pathogen by *Paecilomyces sp* and *Penicillium digitatum* isolated from rhizosphere and rhizoplane in farmers' field. The suspension (10^8propagules/ml) of each fungal isolates was prepared by blending the culture of the isolates on solid rice substrate with distilled water, 20% pectin and Ammonium diphoshate at PH 5.5. The experimental plots were set up in three cocoa farms untreated for many years. The efficacy of these potential antagonists was compared to fungicide (Metalaxyl-m and Cuprous oxide) while water was used as control. All data generated were analysed using Analysis of variance (ANOVA). The results revealed that the effects of the test potential antagonists on pod infection differed significantly at 5%. The mean percentage infection of pods was low in plots treated with the potential antagonists and chemical but high in control plots. This trend was observed throughout the periods of trial. The applied biological control agents effectively hampered the establishment of the pathogen on the pods, enhanced flowering and increased cocoa pod production in the field.

Key words: Pests · Diseases · Integrated control

INTRODUCTION

Cocoa (*Theobroma cacao* L.) family Sterculiaceae is useful in the production of cocoa butter, cocoa cae, cocoa oil, shoe polish, alali and native soap, livestoc feed ingredient and cocoa powder Aigbeaen [1]. In all countries where cocoa is grown, most damage is caused by *Phytophthora palmivora* the blac pod disease pathogen. Annual crop losses may range from 30-90% [2]. Curative measures for the disease have not been successful. When cocoa seedlings were sprayed with Bordeaux, control obtained is often inconsistent or unsatisfactory, it caused pod injury, loss of biodiversity, spoilage of land and non-target organism are affected [3, 4].

On this note, 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, [5] and [6] had earlier reported the *in vitro* potentials of some screened fungi isolated from cocoa farms at controlling the growth of *Phytophthora palmivora.* It was on this note that this research investigated the efficacy of *Paecilomyces sp* and *Penicillium digitatum* isolated from cocoa farm at preventing the infection of cocoa pods by the pathogen on the farm.

MATERIALS AND METHODS

Isolation of Potential Antagonists and Pathogen: *Paecilomyces sp* and *Penicillium digitatum* were isolated from cocoa rhizosphere and rhizoplane in farmers' fields at Aba- Ijesha in Ataunmosa L.G.A. Osun State Nigeria. The organisms were identified at the Microbiology Department Mycology unit University of Ilorin Nigeria. The pathogen (*Phytophthora palmivora)* was obtained from Cocoa Research Institute of Nigeria (CRIN), Ibadan.

Inoculum Production: Five mycelia plugs of 3-day- old culture of each potential antagonist were transferred to a sterilized potato broth medium (200 g/l) and the suspension was left on shaer for 5days at 28±2°C and

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95rpm. Thirty milliliters suspension of each was separately added to a polythene bag filled with 300g of sterilized rice,70ml distilled water and sealed. The bags were opened after 48hrs and the contents were spread on trays under aseptic condition and incubated further for 3days at 28±2°C for initiation of spore production. The suspension of each was prepared by blending the rice substrate with distilled water, 20% pectin and ammonium diphosphate (2:1 v/v) at PH5.5 for ten seconds in a blender and filter through cheesecloth. The final concentration of each of the suspensions was adjusted to 10^8 propagules/ml. The suspensions were separately stored in 2l container each and put in bigger container paced with ice bloc at 4°C for further use [7, 8, 9, 10].

Application of Treatments: The experimental plots were set up in year 2009 and 2010 growing seasons in three cocoa farms located in Aba- Jesha about 45m to Ile - Ife Osun State Nigeria which was left untreated for many years. The pathogen pressure in these farms was high. Four treatments were made in each of the farms: two potentials antagonists *Paecilomyces sp* and *Penicillium digitatum,* a chemical fungicide (Metalaxyl-m) 2.55g/lit. as recommended by the manufacturer and water was used as control. Each treatment plot composes of fifteen cocoa trees. Routine management practices were made in the field before and throughout the period. Two applications were made in this trial for over a period of eight wees starting from August, 2007. All treatments were applied on pods only in liquid suspension using a hand operated sprayer at 500 to 1000 ml per tree depending on pod load.

Data Collection: Disease incidence was recorded for each tree in each plot after two wees of the first application and later every other wee 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 ripped and diseased pods were removed from each tree after having being recorded. To compare the efficacy of the various treatments on the control of blac pod, percentage infection was calculated and the data were subjected to ANOVA and Duncan multiple range tests was used to separate the means [10].

RESULTS

The results of this field trial revealed that at the end of second wee after the first application the total mean number of pods produced by trees in plots (Table 1) treated with *Paecilomyces sp*, chemical *and* Penicillium digitatum were not significantly different ($P< 0.05$). The plots treated with *Paecilomyces sp* produced the highest number of pods (34pods) followed by *Penicillium digitatum,* chemical (Metalaxyl-m) and contro (33, 31 and 28pods respectively). However, the mean percentage infection of pods with *P. palmivora* was found to be least in plots treated with *T harzianum* (7%) and highest in untreated plots (14%). These trends were observed throughout the wees of trial. At wee four, the percentage infection of pods by the pathogen was relatively high but not significantly different ($P< 0.05$) among all the treatments. At wee six, the percentage infection of pods dropped except in control. Both the percentage pod infection and mean pod production in plot treated with chemical and *Paecilomyces sp* were not significantly different ($p<0.05$). At wee eight, the percentage pod infection in plots treated with chemical and *Paecilomyces sp* were low and not significantly different ($p<0.05$). The highest mean total number of pods produced was recorded in the plots treated with chemical (30 pods).

The effect of all the treatments on total number of pods produced and percentage infection of *P. palmivora* was found to be significantly different ($P < 0.05$) among the wees of trial. The highest number of pods recorded at wee two (Table 2) was significantly different ($P < 0.05$) from other wees. The percentage infection was not significantly different ($P < 0.05$) at wees four, six and eight. The results obtained from all the farms used for the trial were not significantly different ($P <0.05$) (Table 3).

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 blac pod disease pathogen. Fortnight phytosanitation was observed and the exercise was carried out at early hour of afternoon when temperature range was still about 35°C. The disease incidence rose from 14.1% at the wee two after the first application of the treatment to 38% at wee eight. The initial lower incidence might be due to removal of diseased pods and all possible sources of pathogen inoculums. Throughout the period of trial the chemical ept the incidence of the disease at bay with the highest incidence of 7.57% at wee two and lowest (0.8%) at wee eight. However, *Paecilomyces sp* had much lower incidence of the disease at wee two than the chemical. This was in agreement with earlier report that fungi antagonists produced nonanoic acid which inhibit

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

	Wee two				Wee four				Wee six				Wee eight			
Treatment	TP	NHP	NIP	PI	TP	NHP	NIP	PI	TP	NHP	NIP	PI	TP	NHP	NIP	PI
Water	28a	24a	4a	14b	23a	18a	5a	21b	18a	14a	4a	22c	12a	7a	5a	42c
P. digitatum	33b	30b	2a	8a	27b	24b	3a	11a	30c	27b	3a	10b	27b	25b	2a	7b
Paecilomyces sp	34b	32b	2a	7a	29b	27b	3a	10a	27b	25b	2a	7a	25b	24b	1a	4a
Chemical	31b	28b	2a	8a	28b	25b	3a	11a	28b	27b	2a	7a	30c	29c	1a	3a

*Mean per plot in a column followed by different letters differ significantly at $P < 0.05$

TP=Total number of pod NHP= Number of health pods NIP= Number of infected pods PI= Percentage infection

Table 2: Pod production during the period of trial

WEE	*Total no of pods	*No of Healthy pods	*No of infected pods	* % infection
2	305c	27c	3a	10a
4	28b	24b	4a	14b
6	26b	23b	3a	12b
8	22a	19a	3a	13b

*Mean per farm in a column followed by different letters differ significantly at $P < 0.05$

Table 3: 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

*Mean per farm in a column followed by different letters differ significantly at $P< 0.05$

spore germination and mycelia growth of two cocoa pathogen *Crinipellis perniciosa and* Moniliophthora roreri [11, 10]. At wee eight which was the end of field trial, plots treated with *Paecilomyces sp* gave the best control of the pathogen followed by *Penicillium digitatum.* These antagonists might able to colonized 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 [12]. 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 Adebola and Amadi, [5]. The percentage infection was observed to be high at wee 4 probably due to constant and increasing rains. Generally, these potential antagonists were effective as biocontrol agents against *P. palmivora* the cocoa blac pod pathogen.

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