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On-Farm Evaluation of *Trichoderma asperellum* on the Suppression of *Phytophthora megakarya* Causing Pod Rot of *Theobromae cacao* in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOA designed the study. Authors SOA and ARA wrote the protocol and performed part of the applications. Author DOA harnessed the data, wrote the first draft of the manuscript, managed literature searches and the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The study examines the inclusion of biological control hitherto neglected, as part of the IPM strategies in a farmer participatory approach.
Study Design: The experimental plots were set up within a completely randomized block experimental design with 3 replicates.
Place and Duration of Study: The field trials were carried out in 2009 at Iloro-Idanre, Owode, Khalime and Bendeghe communities in the South -West and South - South of Nigeria.
Methodology: The bio-control agents used (*Trichoderma*) was isolated from the leaves of cacao tree and natural forest reserve soils in Ibadan, Nigeria, the culture was stored and maintained in sterile distilled water on small plugs of modified potato dextrose agar. **Five Treatments:** Funguran OH, *Trichoderma*, Funguran OH (1) + +*Trichoderma* (5) times, Funguran OH (2) +*Trichoderma* (4) and Sterile distilled water were applied. Disease incidence ratings were taken after the each application till the end of the trial. Data were taken on number of pod and cherelles production, while number of damaged and *Phytophthora* pod rot were taken as total pod loss on each tree in each plot.
Results: The pod productions in all the treatments differ significantly from the untreated

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control, likewise are the number of cherelles production in the treatments as well as the percent of *Phytophthora* pod rot incidence obtained from Iloro-Idanre. Both the number of pod production and cherelles production differ significantly in all the treatments and compare to the control. However, the pod production was highest (328) in trees treated with funguran application and the least percent (6.9%) of *Phytophthora* pod incidence was recorded in the same followed by that of *Trichoderma* and funguran combination and *Trichoderma* alone respectively in Khalime. The pod production in *Trichoderma* and untreated control do not differ significantly in Owode community though both differ significantly from other treatments. However, cherelles production of 111 cherelles was also highest in the untreated control but least in funguran treatment (37.0).

Conclusion: Combination of spraying application of *Trichoderma* with copper based fungicides will drastically reduce the rate of application thereby lower the cost of fungicides incurred on disease control. Highest percentage incidence of black pod disease was however observed in the unsprayed control.

Keywords: Trichoderma asperellum; phytophthora megakarya; biological control; black pod disease.

1. INTRODUCTION

Theobroma cacao L. is one of the most important cash crops grown by farmers in Central and West Africa representing more than 59.9% of the world production [1]. Cacao, the source of cocoa beans used to make chocolate, is a tropical, low - input, perennial tree crop grown by small scale farmers. Unfortunately, the crop suffers from a number of devastating diseases, among the most important is black pod disease caused by 24 various species of *Phytophthora* [2]. Small holder production in Central and 25 Northern South America has been devastated by frosty pod rot, and production in West Africa is threatened by the highly virulent black pod pathogen, *Phytophthora megakarya* [3-5]. Diseases of cacao can account for losses of more than 30% of the potential crop and this, along with old, less productive trees has caused a steady decline in global production. Black pod, caused by various *Phytophthora spp*. is the most widely spread and destructive disease of cacao causing losses that have been estimated in recent years at 30% of pod production and up to 95% in cacao farms in Cameroon and Gabon [6]. *Phytophthora* spp. pathogenic to cacao pods are arguably some of the most important cacao pathogen in Africa because of the annual crop losses inflicted and the costs associated with its management. Due to various reasons, black pod is difficult to control. Chemical control of black pod by spraying with copper fungicide is a well established control method but not completely effective in wetter areas. In addition, fungicidal control can be expensive and polluting. So, an urgent need exists for an effective biologically based integrated approach to the management of such plant diseases. In Central America and South East Asia, *P. palmivora*is the most prevalent spp. whereas in Africa the more aggressive *P. megakarya*is the most important. A renewed interest in biological control of plant diseases in agriculture and horticulture has evolved partly as a response to public concern about the use of hazardous agrochemicals [7]. In the last two decades, attempts have been made to use biocontrol agents against *Phytophthora* species. Effective biocontrol microorganisms are expected to contribute to reduction in the use of chemical fungicides, and increasing farmer's profit margins. The most important, well studied antagonists against several pathogens are fungi like *Amelomyces* spp., *Aspergillus* spp., *Chaetomium globosum*, *Conithyrium minitans*, *Fusarium* sp., *Gliocladium virens, Penicillium citrinum, Peniophora gigantean, Trichoderma* spp. and *Sporodesmium* sp. [8]. *Trichoderma* is currently the most extensively researched biocontrol fungus and has been

shown on a number of occasions to provide a protective effect against some fungi [9] and research has been done on biological control potential of *Trichoderma* spp. against several pathogens attacking vegetables, fruits, field and industrial crops. Past research indicated that *Trichoderma* can parasitize fungal pathogens and produce antibiotics [10]. Cocoa production can be viewed as an 18th century agriculture system that provides a product for a 21st century industry. Until disease-tolerant cultivars are readily available and adequate extension services are provided, a low-input IPM strategy disseminated through farmer field school training is seen as a short-to-medium-term solution to the current challenges in pest management. In Nigeria, [11-13] have demonstrated the efficiency of *Trichoderma* strains as effective biocontrol agents against *Phytophthora* pod - rot of cocoa and also demonstrated the comparative efficacy and economic viability of integration of bio-agents and fungicides [as an Integrated Pest Management (IPM) strategy] in bringing about effective control of *Phytophthora* pod-rot, increasing yield as well as increasing revenue accruable in on-station trials. On-farm research and application of biological control fungus *Trichoderma asperellum* to enhance disease management, along with rational use of fungicides will be discussed in this paper. The study examines the inclusion of biological control hitherto neglected, as part of the IPM strategies in a farmer participatory project.

2. MATERIALS AND METHODS

2.1 Bio-Control Agents

The bio-control agents used was isolated from the leaves of cacao tree and natural forest reserve soils in Ibadan, Nigeria and stored in sterile distilled water on small plugs of modified potato dextrose agar. Isolate of *Trichoderma* were cultured and maintained on potato dextrose agar. Eight day old cultures of *Trichoderma* were flooded with sterile distilled water harvested by scrapping the plates gently. The obtained suspensions 50ml were then adjusted to 108 conidial/ml and mixed to get a 300ml solution of a 1.5% sterile cassava flour liquid suspension. The biocontrol isolate suspension was stored in a refrigerator and transported to the field.

2.2 Field Trial

The field trials were carried out in 2009 at Iloro-Idanre, Owode, Khalime and endeghe communities in the South -West and South - South of Nigeria. The experimental plots were set up within a completely randomized block experimental design with 3 replicates, in cacao fields left untreated with chemical fungicides for 5 years due to abandonment but was newly re-opened, on which the pathogen pressure was well established. Five treatments (F, Tr, Tr + F, Tr + F1 and C) where F is Funguran OH; Tr = *Trichoderma*; Tr+F = Funguran OH sprayed only once + *Trichoderma*5 times; Tr+F1 = Funguran OH sprayed twice +*Trichoderma* 4 times; C = Sprayed with sterile water. Six applications of each treatment were made during this field trial. All treatments were applied in liquid suspension using a hand -operated sprayer and 150ml/tree was applied in one pass over each plot [14]. Disease incidence ratings were taken for each tree in each plot every week after the first application till the end of the trial. Data were taken on number of pod and cherelles production, while number of damaged and *Phytophthora* pod rot were taken as total pod loss on each tree in each plot. All data collected were subjected to analysis of variance (ANOVA) while the means were separated with Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

The output of the different treatments application of *Trichoderma* and chemical fungicide (funguran OH) in Iloro-Idanre are as shown in Table 1.

Table 1. Pod production and incidence of *Phytophthora* Pod rot in Iloro-Idanre

Treatment	Pod production	Cherelles production	*Phytophthora* Pod rot	
			no. of Pod	% pod rot
F	215.0b	100.0b	12	5.6
Tr	224.0a	172.0a	11	4.9
Tr+F	122.0e	51.0e	20	16.4
Tr+F1	160.0c	72.0d	22	13.8
C	153.0d	91.0c	28	18.3

Each value is the mean of 3 replicates. Means followed by the same letter in the same column are not significantly different according to Least Significant Difference (LSD) (5%)

The highest pod and cherelle production of 224 and 172 respectively were observed in *Trichoderma* treatment while the least (122 and 51 respectively) was observed in a combination of *Trichoderma* and funguran treatment. However the lowest percent of *Phytophthora* pod rot incidence (4.9%) was observed in *Trichoderma* treatment whereas it was higher(16.4%) in the combination of *Trichoderma* and funguran, both of which differ significantly (P=0.05) compared to 18.3% in the untreated control (Table 1). The pod productions in all the treatments differ significantly from the untreated control, likewise are the number of cherelles production in the treatments as well as the percent of *Phytophthora* pod rot incidence obtained from Iloro-Idanre (Table1). The effects of the different treatments on the control of *P. megakarya* was the primary target of this research work, which could be seen from the result in the Tables above, of this but significant effect of *Trichoderma* could be seen in Iloro-Idanre as it influenced the pod production of the cacao tree. This study shows that the treatments *Trichoderma* and funguran when applied alone improved the number of pod production in Iloro-Idanre and Khalime which differ significantly from the untreated control. *Trichoderma* treatment applied in Iloro-Idanre has the highest number of pod and cherelles production with the least occurrence of *Phytophthora* spp. after applications. This shows *Trichoderma* to be effective as a bio-control agent of *P. megakarya*, which is in agreement with earlier work of [15]. In Table 2, both the number of pod production and cherelles production differ significantly in all the treatments and compare to the control. However, the pod production was highest (328) in trees treated with funguran application and the least percent (6.9%) of *Phytophthora* pod incidence was recorded in the same followed by that of *Trichoderma* and funguran combination and *Trichoderma* alone respectively in Khalime. The untreated control has 473 numbers of cherelles produced and the highest percent (14.8%) of *Phytophthora* incidence in this community.

The observation of highest pod production in funguran application in Khalime community, the highest cherelles production and the least percent of *Phytophthora* in the untreated control require further experimental studies. The untreated control in Bendeghe community (Table 3) has the highest number of pod production (129), while the number of cherelles produced is significantly high (151) following the highest of 154 in funguran application. The least percent incidence (9.8%) of *Phytophthora* pod rot was found in a combination of *Trichoderma* and funguran followed by that of funguran (12.9%) treatment. The untreated control in this community has the highest number of pod production of 129 pods but also recorded the highest percent (17.8%) of *Phytophthora* pod incidence (Table 3). It could be

observed in this study that the efficiency of *Trichoderma* and funguran OH treatments when applied alone and in combinations differ significantly from one community to the other when compared to the untreated control. This however could be linked to the climatic and ecological diversity of the communities as the applications were done at the same period. The pod production in *Trichoderma* and untreated control do not differ significantly in Owode community (Table 4), though both differ significantly from other treatments. However, cherelles production of 111 cherelles was also highest in the untreated control but least in funguran treatment (37.0). Whereas, the percents incidence of *Phytophthora* (23.8%) was least in the funguran treatment which differ significantly from the untreated control which has (72.1%) the highest *Phytophthora* incidence (Table 4).

Table 2. Pod production and incidence of *Phytophthora* Pod rot in Khalime

Treatment	Pod Production	Cherelles Production	*Phytophthora* Pod rot No. of Pod	% Pod rot
F	328.0a	391.0c	22	6.7
Tr	297.0b	394.0b	35	11.7
Tr+F	192.0d	333.0d	20	10.4
Tr+F1	176.0e	324.0e	24	13.6
C	256.0c	473.0a	38	14.8

Each value is the mean of 3 replicates. Means followed by the same letter in the same column are not significantly different according to Least Significant Difference LSD (5%)

Table 3. Pod production and incidence of *Phytophthora* Pod rot in Bendeghe

Treatment	Pod production	Cherelles production	*Phytophthora* Pod rot no. of Pod	% Pod rot
F	77.0d	154.0a	10	12.9
Tr	72.0e	124.0d	12	16.7
Tr+F	102.0b	52.0e	10	9.8
Tr+F1	98.0c	128.0c	14	14.3
C	129.0a	151.0b	23	17.8

Each value is the mean of 3 replicates. Means followed by the same letter in the same column are not significantly different according to Least Significant Difference LSD (5%)

Table 4. Pod production and incidence of *Phytophthora* Pod rot in Owode

Treatment	Pod production	Cherelles production	*Phytophthora* Pod rot no. of Pod	% Pod rot
F	63.0b	37.0d	15	23.8
Tr	68.0a	99.0b	26	38.2
Tr+F	43.0d	41.0c	13	30.2
Tr+F1	55.0c	44.0c	20	36.4
C	68.0a	111.0a	49	72.1

Each value is the mean of 3 replicates. Means followed by the same letter in the same column are not significantly different according to Least Significant Difference LSD (5%)

The results also show diversity in the pod production and reduction of *Phytophthora* pod rot under different treatments. Except in Iloro-Idanre, the treatment with the highest number of pod production do not have the least incidence of *Phytophthora* pod rot. Thus as reported

by [2], there is a need to investigate the integrated pest management (IPM) strategies including biocontrol, rational use of chemicals and technology transfer. For a holistic control measure the integration of botanicals into the IPM for black pod disease need to be investigated.

4. CONCLUSION

Combination of spraying application of *Trichoderma* with copper based fungicides will drastically reduce the rate of application thereby lower the cost of fungicides incurred.

Highest percentage incidence of black pod disease was however observed in the unsprayed control. This integrated disease management is recommended to farmers as it reduce chemical residue on soil, in cocoa beans and also reduce cost of disease management by farmers translating to reduction in cost of cocoa production.

COMPETING INTERESTS

Authors declare that there are no competing interests.

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