

## Exploring the Biocontrol Potential of Endophytes against *Phytophthora megakarya* in Cocoa Cultivation

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**Abstract:** Cocoa (*Theobroma cacao* L., Malvaceae) is a perennial tree crop, which over the years been negatively affected by black pod disease which is caused by *Phytophthora megakarya*. This is a major pathogen responsible for black pod disease in cocoa, significantly reducing the yield and threatening cocoa production. Endophytes are asymptotically within plant tissues, with antagonistic properties. The potential of using endophytic fungi that were isolated from healthy cocoa tissues as biocontrol agents against *Phytophthora megakarya* was observed in this study. After isolating from an infected cocoa pod, the strains of *P. megakarya* were used to inoculate healthy cocoa tissues against endophytes using a dual culture test. The endophytes were evaluated for antagonistic activities against *Phytophthora megakarya* *in vitro*. The efficiency of these endophytes was assessed by identifying a few secondary metabolites. Results from this research showed that the endophytic isolates namely: *Trichoderma asperellum* (84.92%), and *Trichoderma harzianum* (78.53%) exhibited strong inhibitions against the *Phytophthora megakarya*, indicating their potentials as biocontrol agents against the pathogen, while *Colletotrichum gloesporioides* (16.80%), and *Botryoshaeria dothidea* (17.90%) recorded weak inhibitions against *Phytophthora megakarya* *in vitro*. Secondary metabolites elaborated by isolates were alkaloids (0.984%), apicidin (0.630%) harzianic (0.577%), helvolic acid (0.531%), ergotamine (0.491%) and flavonoids (0.009%) which were present in the isolates (*T. harzianum*, *T. asperellum*, *C. gloesporioides*, and *B. dothidea*). Findings from this study demonstrated that endophytes from cocoa tissues could significantly reduce *P. megakarya* infection rates on cocoa. Thus, the potentials inherent in the use of endophytes as sustainable eco-friendly biocontrol strategy in the controlling of black pod disease of cocoa.

Key word: Antagonistic, biocontrol, endophytes, metabolites, *Phytophthora megakarya*

### INTRODUCTION

The cocoa (*Theobroma cacao* L., Malvaceae) is a perennial crops and it's one of the most attractive crop for farmers that support livelihood due to the specific climate and inevitability imperative of profits over in the long term (Jean-Marie *et al.*, 2022). Cocoa is cultivated for its beans used as raw material for the manufacture of chocolate production. Chocolate is the leading product in the global confectionery trade, cosmetics, and starch (Afoakwa, 2014). Globally, in 2023 cocoa production was estimated at 4,953,000 tons, predominantly sourced from West Africa, South, and Asia (ICCO, 2023). The annual cocoa production in Nigeria is around 300,000 to 320,000 metric ton ranking as the world's fourth-largest producer and it's a major export cash crop in Nigeria. (Esan *et al.*, 2025).

However, farmer's lack of attention to plantation/farm maintenance has led to the emergence of pests and pathogens that

significantly reduce yields and worsen rural poverty. Despite the interest in cocoa cultivation, yields remain low due to constraints of diseases such as black pod caused by *Phytophthora megakarya* (Bailey *et al* 2016). This pathogen (black pod) has resulted in yield losses of 70% when environmental conditions are favourably for its growth and the yield loss could increase, to as high as 100% if left untamed especially in areas with high rainfall and humidity (Wardhany and Adzim, 2018). The decline in cocoa productivity can be attributed to low farm management practices, old trees, and the incidence of diseases (Wessel and Quist-Wessel, 2015). In Nigeria, the several effective of integrated pest and disease management (IPDM) practice are being recommended as a means of preventing *phytophthora megakarya* (black pod disease of cocoa).

Physical, biological agents, and chemical fungicides, (control by regulatory agencies, plant quarantine and certification agencies),

pruning, resistant cultivars, and phyto-sanitation are some of the methods (Tinzaara *et al.*, 2024). However, Chemical method is widely used and more than 10 fungicides are homologated each year against cocoa pathogen (black pod) disease. However, 77% of farmers do not respect the official spray recommendations for chemicals (Chaudhary *et al.*, 2022). On the other hand, the management of cocoa diseases is at present, heavily reliant on chemicals. The application of fungicides to control cocoa black pod disease has only caused negative effects on the environmental, pollution through the accumulation of chemical residues, soil and groundwater contamination, disrupting ecosystems and killing useful organisms, but also the appearance of more resistant strains of these phytopathogenic agents (Bateman and Croizer *et al.*, 2012) and which have resulted in various human diseases such as skin irritation, respiratory problems, or long-term health problems. Chemical also affect the non-target organisms in the ecosystem thereby killing the beneficial microorganisms. However, endophytes are microorganisms that reside within plant tissues without causing harm; they have emerged as promising candidates for biological control and can enhance plant health through multiple mechanisms (Chaudhary *et al.*, 2022). Moreover, some plant endophytes have fungistatic or fungicidal properties that could be exploited as alternatives to chemicals against cocoa black pod caused *phytophthora megakarya*. One of the promising new agents in the biological control of plant diseases is fungal endophytes. Fungal endophytes are referring to as fungi, residing in plant tissue (Chitnis *et al.*, 2020). These endophytic fungi protect their hosts from harmful organisms and unfavorable environments by producing bioactive secondary metabolites directly and indirectly (Fadiji and Babalola, 2020). Therefore, this study aimed at identifying and exploring the potentials of endophytic fungi isolated from healthy cocoa tissues against *Phytophthora megakarya* that causes

black pod disease of cocoa. This is with a view to determining their suitability as sustainable eco-friendly strategy for the management of the disease.

## MATERIALS AND METHODS

**Collection of Samples:** Samples of cocoa pods with symptom of black pod were collected randomly with a sterile Ziploc bag from selected cocoa farm plots within Adebayo area, Idi-Ayunre, Ibadan. Oyo State Nigeria. The cocoa pods were surface sterilize with water to remove the debris and sterilized with 2% sodium hypochlorite solution. The samples were rinsed thoroughly with sterile distilled water to remove residual of hypochlorite and dissected using sterile scalpel, inoculated into sterile cooled medium potato dextrose agar (PDA) and incubated. Discrete colonies on the plates were sub-cultured to obtain a pure culture of *Phytophthora megakarya*.

**Non-Infected Samples:** Non-infected fully mature cocoa pods with no signs of diseases were brought to the laboratory in a sterile Ziploc bag using sterile appropriate tools to prevent contamination, rinsed with sterile running water to remove debris and disinfected with 70% ethanol for 1-2 minutes. The cocoa pods was then transfer to a solution of 2.5% sodium hypochlorite (Bleach) for 2-5 minutes and later rinsed with 70% of ethanol to remove residual of hypochlorite. The samples were finally rinsed thoroughly 3-5 times with sterile distilled water to remove the traces of the sterilizing agents. The cocoa pods tissues were cut into small segments with the aid of sterile scalpel under aseptic conditions, and inoculated into sterile and cooled prepared potato dextrose agar (PDA) plates, in an inoculating chamber. The inoculated plates were incubated at 27°C for fungal growth. The isolated endophytes were sub-cultured into freshly prepared PDA plates to obtained pure cultures. Each of the isolated endophytes was characterized and identified under a compound light microscope (10X) using routine procedures (Khalil *et al* 2021).

**Antagonistic Activity of the Endophytes**

**Isolates:** The antagonistic action of the endophytes was observed based on the interaction between endophytes and *Phytophthora megakarya*, which were assessed after incubation in the dual cultures assay (Pakora et al 2018). The endophytes isolated were confirmed for their antagonism against *Phytophthora megakarya* by using dual culture technique.

The mycelia of the endophytes and *P. megakarya* were placed on the opposite edges of freshly prepared potato dextrose agar plates (dual cultures). The control plates contained only *P. megakarya*. Both the dual culture and control plates were incubated simultaneously at 18°C to 27°C for 3-7 days in the cooled incubator. The mycelia growth/extension diameters of *Phytophthora megakarya* and each of the endophytes were observed measured and recorded. The average percentage mycelia inhibitions against the pathogen (*Phytophthora megakarya*) by the endophytes were also computed.

**Statistical Analysis:** The data obtained from the study were statistical analyzed and compared using Fisher's LSD Test with the aid of MINITAB 17 statistical package.

**RESULTS**

The mycelia extension diameters (cm) of the endophytes improved and increased as the days of incubation increased (Table 1). The lowest mycelia growth/extension was observed on *Botryosphaeria dothidea* (2.42 cm), followed by *Colletotrichum gloesporioides* (2.85 cm) at day 3, while *Trichoderma asperellum* (4.12 cm), followed by *Trichoderma harzianum* (4.38cm) which had significantly highest mycelia growth/extensions ( $P \leq 0.05$ ). The same tendency was observed on Days 5 and 7, with the significantly highest mycelia extension ( $P \leq 0.05$ ) recorded for *Trichoderma harzianum* (7.26 cm) and *Trichoderma asperellum* (7.67 cm). Diagonally in the Table (Days 3-7) *Colletotrichum gloesporioides* inoculated

against *P. megakarya*, gave the lowest mycelia diameters (2.85-6.88 cm), followed by *Botryosphaeria dothidea* (2.42-6.45 cm), *Trichoderma harzianum* (3.76-8.58 cm) and *Trichoderma asperellum* (4.12-8.58 cm) were however, higher than those of the other endophytes (Table 1).

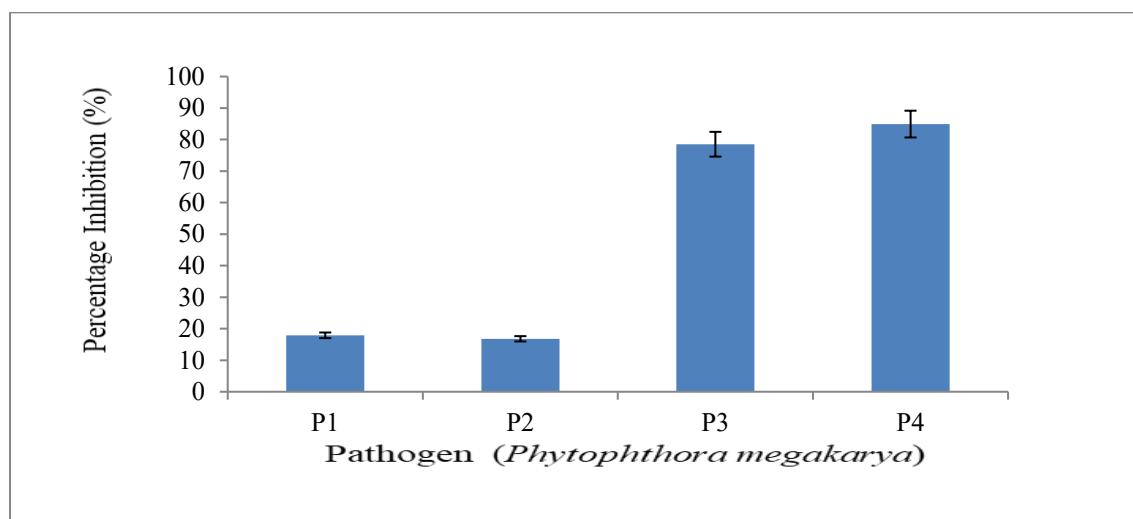
The average percentage growth inhibition of *P. megakarya* against inoculated endophytes in the dual culture plates (antagonistic test) is displayed (Figure 1 and 2). The percentage of *P. megakarya* that were cultured against *Trichoderma asperellum* (84.92%) and *Trichoderma harzianum* (78.53%) showed the significantly highest percentage of inhibition ( $P \leq 0.05$ ), suggesting that the pathogen was effectively controlled. When compared Plate 1 and 2 to Plate 3 and 4; the percentages of inhibition recorded for the other *P. megakarya* isolates were very much lower (*C. gloesporioides* 16.80% – *B. dothidea* 17.90%), suggesting relatively mild inhibitory qualities (Figure 1).

As shown in Table 3, the secondary metabolites produced by endophytic fungal were harzianic acid, flavonoids, alkaloids, helvolic acid, apicidin and ergotamine. The highest metabolites produced were observed in alkaloids (0.98%) produce by *Colletotrichum gloesporioides* and harzianic acid (0.97%) in *Trichoderma asperellum* respectively similar trend occur in apicidin *C. gloesporioides* (0.63%) and *T. asperellum* (0.53%) which are far higher than all the metabolite produce by the other endophytic fungal Isolates and this make the two a potential biocontrol agent. The presence of alkaloids (0.89%) in *T. harzianum*, apicidin 0.46%, helvolic acid 0.41% and harzianic acid 0.38% also make *T. harzianum* and *B. dothidea* 0.87% a potential biocontrol of *Phytophthora megakarya*. The lowest metabolites produced by *Botryosphaeria dothidea* were harzianic acid (0.002), (0.0042), while flavonoids produced the lowest in all the endophytes, which show weak inhibitory properties.

**Table 1: Mycelia Growth Diameter of Endophytes (cm) against *P. megakarya***

| Fungal Pathogen | Days of Incubation  |                    |                     |
|-----------------|---------------------|--------------------|---------------------|
|                 | 3                   | 5                  | 7                   |
| FP <sub>1</sub> | 2.42 <sup>f-h</sup> | 6.47 <sup>b</sup>  | 6.39 <sup>bc</sup>  |
| FP <sub>2</sub> | 2.85 <sup>d-f</sup> | 5.48 <sup>c</sup>  | 5.84 <sup>b-d</sup> |
| FP <sub>3</sub> | 3.76 <sup>bc</sup>  | 7.26 <sup>ab</sup> | 8.50 <sup>a</sup>   |
| FP <sub>4</sub> | 4.12 <sup>a</sup>   | 7.67 <sup>a</sup>  | 8.50 <sup>a</sup>   |
| Control         | 4.38 <sup>a</sup>   | 5.85 <sup>bc</sup> | 6.74 <sup>bc</sup>  |

Keys: FP<sub>1</sub>= *Botryosphaeria dothidea* against *P. megakarya*, FP<sub>2</sub>= *Colletotrichum gloesporioides* against *P. megakarya*, FP<sub>3</sub>= *Trichoderma harzianum* against *P. megakarya*, FP<sub>4</sub>= *Trichoderma asperellum* against *P. megakarya*. Values with same letters along same columns are not significantly different at  $P \leq 0.05$  using Fishers LSD test

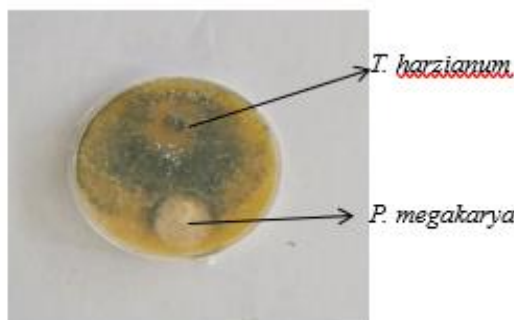
**Figure 1: Average Percentage Inhibition of *P. megakarya* by Endophytic Fungi**

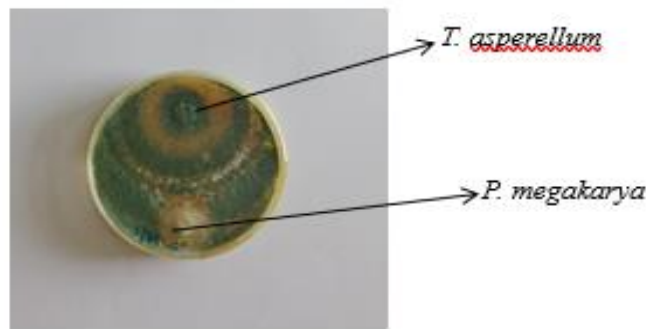
Keys: P1: *Botryosphaeria dothidea*, P2: *Colletotrichum gloesporioides*, P3: *Trichoderma harzianum*, P4: *Trichoderma asperellum*

**Table 2: Metabolites Produced by Endophytic Fungal Isolates**

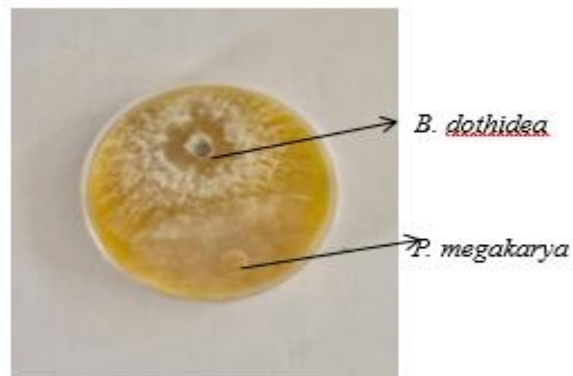
| Samples | Harzianic Acid     | Flavonoids         | Alkaloids          | Helvolic Acid      | Apicidin           | Ergotamine         |
|---------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| E1      | 0.002 <sup>d</sup> | 0.008 <sup>b</sup> | 0.871 <sup>d</sup> | 0.358 <sup>d</sup> | 0.042 <sup>d</sup> | 0.328 <sup>b</sup> |
| E2      | 0.577 <sup>a</sup> | 0.009 <sup>a</sup> | 0.984 <sup>a</sup> | 0.531 <sup>a</sup> | 0.630 <sup>a</sup> | 0.491 <sup>a</sup> |
| E3      | 0.384 <sup>c</sup> | 0.003 <sup>d</sup> | 0.894 <sup>c</sup> | 0.414 <sup>b</sup> | 0.459 <sup>c</sup> | 0.156 <sup>d</sup> |
| E4      | 0.488 <sup>b</sup> | 0.004 <sup>c</sup> | 0.970 <sup>b</sup> | 0.380 <sup>c</sup> | 0.526 <sup>b</sup> | 0.191 <sup>c</sup> |

Keys: E1= *Botryosphaeria dothidea* E2= *Colletotrichum gloesporioides* E3= *Trichoderma harzianum* E4= *Trichoderma asperellum*. Values with same letters along same columns are not significantly different at  $P \leq 0.05$  using Fishers LSD test

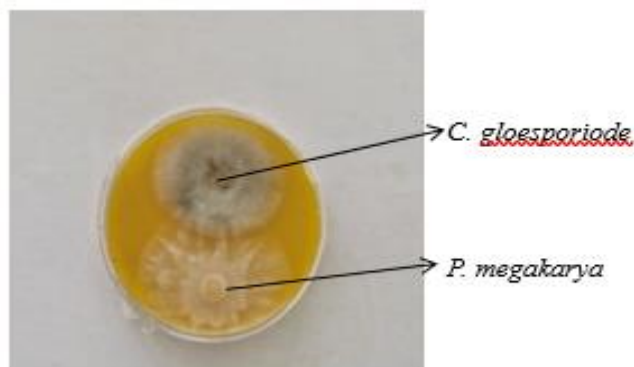
**Plate 1: Antagonistic Effect of *Trichoderma harzianum* on *Phytophthora megakarya***



**Plate 2: Antagonistic Effect of *Trichoderma asperellum* on *Phytophthora megakarya***



**Plate 3: Antagonistic Effect of *Botryosphaeria dothidea* on *Phytophthora megakarya***



**Plate 4: Antagonistic Effect of *Colletotrichum gloesporioides* on *Phytophthora megakarya***



**Plate 5: Pure Culture *Phytophthora megakarya* on Carrot Agar from Infected Cocoa Pods**

## DISCUSSION

Endophytes, which live within plant tissues of cocoa pods without causing harm, have

shown great prospective as biocontrol agents in this study. The isolated endophytic fungi from healthy cocoa pods were classified

according to Yao *et al.* (2023). The isolates found were two isolates of *Trichoderma* namely *Trichoderma asperellum* and *Trichoderma harzianum* with *Botryosphaeria dothidea*, and *Colletotrichum gloesporioides*, the occurrence confirms the finding of Fadiji and Babalola *et al.* (2020). This trend was also earlier observed by Agbeniyi and Adedeji (2003). Due to its antibiosis, parasitism, and competition, of plant resistance mechanisms, *Trichoderma harzianum* and *Trichoderma asperellum* are well-known biocontrol agents that are effective against *Phytophthora megakarya*, a plant pathogen of cocoa. This agreed with the findings of Woo *et al.* (2015). *Trichoderma* species are extensively known for their biocontrol prospective due to their aptitude to parasitize pathogens, enhance plant development, and produce various antimicrobial compounds. According to Fadiji *et al.* (2020). *Trichoderma harzianum* and *Trichoderma asperellum* are particularly effective against *Phytophthora megakarya* due to their production of secondary metabolites like alkaloids, apicidin and harzianic acid, which help in inhibits the growth of pathogens. The *Trichoderma harzianum* is effective in reducing the incidence of *Phytophthora megakarya* by

employ mechanisms such as parasitism, where they attach to and degrade the cell walls of *Phytophthora megakarya*, and competition for space and nutrients and colonizing the pathogen of the plants, thereby preventing the pathogen from establishing itself. In addition, *Trichoderma* species are known to produce enzymes like chitinases and glucanases that degrade the cell walls of pathogens, further aiding in the biocontrol of *Phytophthora megakarya*. This agreed with the reports of Chitnis *et al.* (2020), as an effective biocontrol agent.

## CONCLUSION

*Trichoderma harzianum* and *Trichoderma asperellum*, unlike *Botryosphaeria dothidea*, and *Colletotrichum gloesporioides*, endophytes used in this study exhibited clear parasitism, and antimicrobial efficiency against *Phytophthora megakarya*, and as such, appropriate for further improvement as biocontrol agents to control pod rot disease of cocoa. The findings recommended the extraction of the metabolites for incorporation into substances for the treatment of plant infections and diseases that result into black pod of cocoa. This is with the view targeted at reducing economic losses cause by the disease.

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