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In Vitro Evaluation of Some Biopesticides Based on Plant Extracts and Trichoderma Spp Effects on Lasiodiplodia Theobromae Causing Mango Stem-End Rot Disease in Côte D'Ivoire

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In vitro evaluation of some biopesticides based on plant extract and *Trichoderma* spp effects on *Lasiodiplodia theobromae* causing mango Stem-end rot disease in Côte d'Ivoire

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Abstract

Plant pathogens are mainly controlled using synthetic products, which harm human and environmental health. In the current study, we aimed to assess the efficacy of some plant extracts and biocontrols agents inhibiting *Lasiodiplodia theobromae* development. We studied the inhibitory effect of 3 biopesticides based on essential oils (ASTOUN 50 EC, FERKA 50 EC, and NECO 50 EC), *Moringa oleifera* extracts (Methanolic and aqueous), and 4 isolates of *Trichoderma* spp on mycelial growth of *L. theobromae*. Results showed that biopesticides ASTOUN and NECO at 700 and 1000 ppm completely inhibited the mycelial growth of *L. theobromae*. Similarly, *M. oleifera* methanolic extract has the highest inhibitory rate (65.45 %) compared to aqueous extract (42.44%). Through dual assay, we found that *Trichoderma* spp isolates reduced *L. theobromae* development from 30 to 40 %. Therefore, this study shows how possible it is to use plant extracts and *Trichoderma* species to control mango Stem-end rot (SER). However, further studies should be undertaken *in vivo* to further confirmed.

Keywords: Biocontrol; Biopesticide; *Lasiodiplodia* sp; mango, Environnement, Human health

1 INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important fruits worldwide, and demand is increasing (Alkan & Kumar, 2018). Its production faces several threats, notably mango fungal diseases (Shahbaz *et al*., 2009). *Lasiodiplodia* spp are causal agents of mango dieback and Stem end-rot (SER), a severe disease for mango growers (Malik *et al*., 2005). It can therefore induce about 60 % of mango trees in affected sites and may rise to 100 % fruit loss if proper management strategies are not in place (Haggag, 2010; Johnson, 2008). In response to this threat, chemical fungicides have proven to be essential for controlling fungal pathogens (Da Silva *et al*., 2012; Karaoglanidis *et al*., 2003). However, this method has limitations: chemical fungicide harms humans and the environment (Bansal, 2020). Moreover, it has been proven that using chemical fungicides increased the expression of resistance genes of *Lasiodiplodia* spp. (Wang *et al*., 2021). Therefore, researchers are focused on finding alternative ways, like developing biological methods to control *Lasiodiplodia* spp pathogens. Thus, medicinal plants and biocontrol agents are renowned for having fungicidal and bactericide effects while having negligible side effects (Cox *et al.*, 2000; Katooli *et al*., 2011; Lewis *et al.*, 1996). Their antimicrobial activity is due to their bioactive compounds, such as flavonoids, phenolic acids, alkaloids, isothiocyanates, tannins, saponins, aliphatic aldehydes, and terpenoids (Dewick 1997). For example, the plant extract of Thyme oil, Moringa oleifera, Syzygium aromaticum and cinnamomum zeylanicum are among plants used to control *L. theobromae* the causal agent of SER (Alam *et al*., 2017a; Perumal *et al*., 2016). In addition, biocontrol agents' use were revealed to effectively control plant diseases. Therefore, *Basilus subtilus* was found causing cell degradation of SER-causing pathogens (Demoz, 2005; Diskin *et al*., 2009). Among biocontrol, *Trichoderma* species were sufficiently applied to control *L. thoebromae*, notably the *Trichoderma harzianum* and *T. viride* (Kota,2006; Sivakumar *et al*., 2000). The antimicrobial effects of plant extract and *Trichoderma* sp isolate against phytopathogens have been realised worldwide (Alam *et al*., 2017; Guimarães *et al*., 2019; Karan *et al*., 2020; Konsue *et al*., 2020; Saeed *et al*., 2017). However, there is limited data on the potential efficacy of plant extract and *Trichoderma* spp on the development of *Lasiodiplodia theobromae* causing mango SER. Filling this knowledge gap will help develop an eco-friendly control method for *L. theobromae*. Hence, the present study was carried out to study the antifungal activity of 3 biopesticides (based on essential oils), plant extracts of *Moringa Oleifera* and four isolates of *Trichoderma* spp on the mycelial growth of *L. theobromae* causing mango SER.

2 Materials and methods

2. 1 *L. theobromae* isolate

L. theobromae isolate FRF01 was isolated from a mango fruit with SER symptoms in Côte d'Ivoire. The fungus was identified based on macro- and micro-morphological characteristics and confirmed by the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) and translation elongation factor 1-α, (*tef1-α*) sequencing (accession numbers: OQ067836 and OQ269654 for ITS and EF1 respectively). BLASTn analysis of the sequences revealed 100 % identity with *Lasiodiplodia theobromae* strain CBS111530 (NCBI Accession: EF622054). In addition, *L. theobromae* isolate FRF01 pathogenicity was confirmed on mango fruits.

2.2 Inhibition tests of *L. theobromae* growth with plant essential oils

In this current study, three biopesticides (ASTOUN 50EC, NECO 50EC, FERCA 50EC) formulated by the Industrial Research Unity on biopesticides (URI) of Félix Houphoët-Boigny University (UFHB) were used. They have been formulated with several plant essential oils, such as *Cymbopogon citratus*, *Ocimum gratissimum* and *Eucalyptus citryodera*, respectively, for ASTOUN, NECO and FERCA. Biopesticides were added to 20 ml Potato Dextrose Agar (PDA) medium to obtain final concentrations of 300, 500, 700 and 1000 ppm. The amended fungicide

media were homogenised under agitation and emulsified in Tween 20 to improve their solubility in water. Solutions were then distributed in 90 mm diameter Petri dishes at the rate of five Petri dishes per concentration. Finally, a control without biopesticide was prepared under the same conditions.

2.3 *Moringa oleifera* extraction method and inhibiting test with *L. theobromae*

M. oleifera leaves were used for extractions. Therefore, leaves were harvested and collected in the scientific pole of UFHB and dried in URI laboratory. The method applied by Mamkaa & Gwa, 2018 was adapted for methanolic and aqueous extracts. Extracts were conducted by adding 5, 10, and 15 g of powder plant extract to 250 ml of sterile distilled water or methanol in a 1000 ml Pyrex flask. Then left for 24 hours before being filtered through four layers of sterile filter cloth. Then 5 ml of plant extracts of each concentration level were mixed in sterile Petri dishes containing 20 ml of Potato Dextrose Agar (PDA) solution and allowed to solidify before pathogen inoculation.

2.4 Dual confrontation assay

Four isolates of *Trichoderma* spp were used in dual confrontation tests. They were isolated in our laboratory from healthy mango organs in growing mango production areas in Côte d'Ivoire. The Mycelial disks (5 mm in diameter) of each isolate of *Trichoderma* sp and *L. theobromae* obtained from the margins of 7-day-old PDA cultures were placed on opposite sides (50 mm distance) of a 90 mm PDA Petri dishes (Stracquadanio *et al*., 2020). Petri Dish were then incubated for 72 h. The radial growth of *L. theobromae* was then measured in the presence (treatment) or absence (control) of *Trichoderma* species. Each assay used five biological replicates repeated three times for each isolate of *Trichoderma* sp.

2.5 Measurement of mycelial radial growth

The inhibition rate (I) of mycelial growth of *L. theobromae* by biopesticides and *Moringa oleifera* extract were calculated according to the following formula (Attrassi e*t a*l., 2019).

$$I(\%) = \frac{Do-Dc}{Do} \times 100$$

Where Do= average mycelial growth diameter in control Petri dish, Dc= average mycelial growth diameter in Petri dish at the concentration (c) (Treatment).

For the dual confrontation assay, the percentage of radial growth inhibition (%RGI) was calculated by using the formula of (Vincent, 1947):

$$RGI(\%) = \frac{C-T}{C} \times 100$$

Where C = growth in control (absence of *Trichoderma spp*) and T = growth in treatment (Presence of *Trichoderma* spp)

2.6 Data analysis

Data collected were subjected to Analysis of Variance (ANOVA), and means were compared among treatments for each measured parameter using Fisher's Least Significant Difference (L.S.D).

3 Results and Discussion

3.1 Efficacy of biopesticides ASTOUN, NECO, and FERCA on mycelial growth of *L. theobromae*

Biopesticides could be an excellent alternative to synthetic chemical fungicides used in agriculture. In this section, we evaluate the effect of biopesticides ASTOUN 50 EC (*Symbopogon citratus* , NECO 50EC (*Occimum gratissimum*) and FERCA 50EC(*Eucalyptus citryodera*) on the mycelial growth of *L.theobromae*. The results revealed that ASTOUN and NECO at the four tested concentrations were the most efficient in inhibiting *L.theobromae* growth compared to FERCA ($P<0.05$) (Figure 1,2). They reached 100 % of the inhibition rate at 700 ppm and 100 ppm for ASTOUN, and 1000 ppm for NECO. All FERCA concentrations show a similar effect in inhibiting the mycelial growth of *L. theobromae* with 52.7, 55.33, 57.24 and 57.27 % at 300, 500, 700 and 100 ppm, respectively (Figure 2). The difference in inhibition activity between these biopesticides would probably be due to each product's chemical composition. The degree of sensitivity of *L. theobromae* to different concentrations of this biological fungicide could also play a role in these findings (El *et al*., 2014). Indeed, ASTOUN contains thymol and citronellol as active components, while NECO contains thymol and eugenol molecular (Kassi *et al*., 2014), which have been reported to have fungicidal and bactericidal activity (Cox *et al*., 2000). Our results agree with several studies that have proven the fungicide effect of ASTOUN, NECO and FERCA on plant pathogenic fungi (N'goran *et al.*, 2022; Silue *et al*., 2018; Kassi *et al*., 2014;). The biopesticide FERCA could not inhibit the development of *L. theobromae* at all concentrations. This is probably due to its composition, which may differ from ASTOUN and NECO. This is corroborated by the result found by N'goran *et al*. (2022). They found that FERCA biopesticide was the least effective, inhibiting mycelial growth of *Phytophthora katsurae*, the causal agent of the premature nut fall and the heart rot of the coconut tree, compared to ASTOUN and NECO.

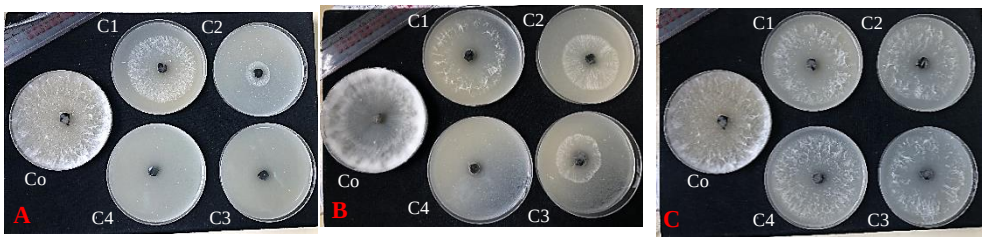

Figure 1: Effect of biopesticides on the mycelial growth of *Lasiodiplodia theobromae* after 3 days incubation on PDA medium. (A) ASTOUN 50 EC; (B) NECO 50 EC; (C) FERCA 50 EC. Co= Control (0 ppm); C1= 300 ppm; C2= 500 ppm; C3= 700 ppm; C4= 100 ppm

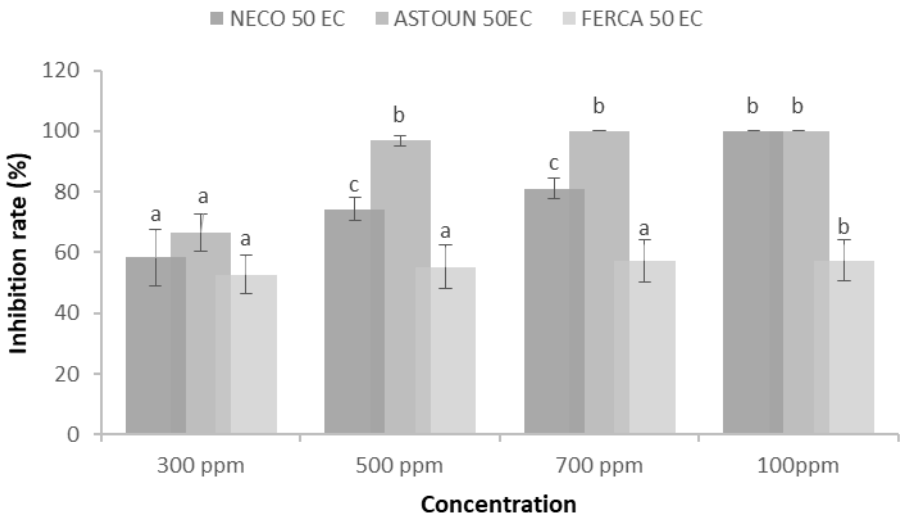

Figure 2 : Inhibition rate of the biopesticides ASTOUN, NECO and FERCA on the mycelial growth of *L.theobromae*. Diferent letters indicate a signifcant variance of $P<0.05$

The incubation period significantly affected the inhibitory activity of *L. theobromae* mycelial growth for all biopesticides (P<0.05). Although, in general, a part of ASTOUN and NECO at 700 and 1000 ppm maintained the inhibition rate at 100 % during the incubation period, the inhibition effect decreased, allowing mycelial growth from day 1 to day 3 for all biopesticides (Figure 3). On the other hand, at 300 ppm, the mycelial growth of *L. theobromae* constantly increased from day 1 to day 3 (Figure 3). We hypothesised that this is because of having volubility properties; aromatic components could evaporate from the Petri dish (Edris & Farrag, 2003). Furthermore, the possibility of active molecular degradation and the solubility of the active substances in water may contribute to the efficacy loss of biopesticide with time (Oluma & Elaigwe, 2006).

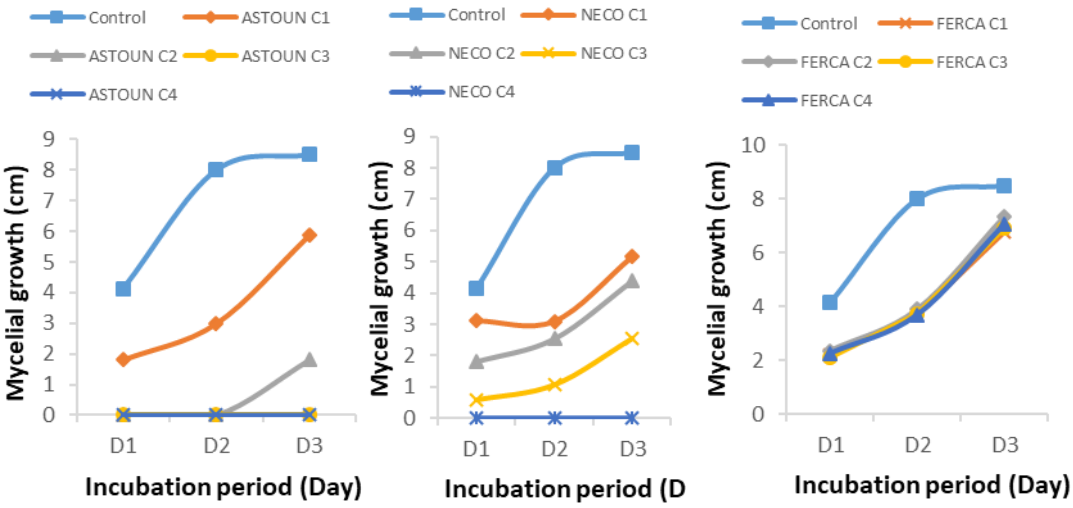

Figure 3 : *L. theobromae* mycelial growth according to incubation period

3.2 Effect of *Moringa oleifera* aqueous and methanolic extracts on mycelial growth of *L. theobromae*

Among alternative methods to chemical pesticides, the medicinal plant *Moringa oleifera* has been studied worldwide. In this current study, we assessed the inhibitory activity of aqueous and methanolic extracts of *M. oleifera* at four concentrations (5g/250 ml, 10g/250 ml, 15g/250 ml, 20g/250 ml) on the mycelial growth of *L.theobromae*. Results showed that *M. oleifera* extracts had significant inhibitory activity against *L.theobromae* (P<0.05). The methanolic extract had the highest inhibition percentage (65.46) compared to the aqueous extract (42.44). Thus, the inhibition rate recorded varied from 6.84 to 52.26 % for aqueous extract and 36.51 to 76.36 % for methanolic extract (Figure 4). Concentrations 15g/250 ml and 20g/250 ml of the methanolic extract have the most inhibited *L.theobromae* development with an inhibition rate above 76 % compared to 42 and 52 % for aqueous extract for the same concentrations (Figure 5). We assumed that methanolic extract had the highest inhibition percentage may be because of the extraction method, such that the use of solvents of an alcoholic nature allows us to obtain extracts with much more concentrated bioactive molecules compared to aqueous extract. Several others have confirmed this finding (Fontana *et al.*, 2021; Mamkaa & Gwa, 2018). Indeed, *M. oleifera* contains saponins, alkaloids, phenols, tannins, flavonoids, steroids, phlorotannins and terpenes, which are responsible for their antifungal activity (Nweke, 2015). The general increase in percentage growth inhibition with an increase in extract concentration agrees with the finding of Mamkaa and Gwa (2018).

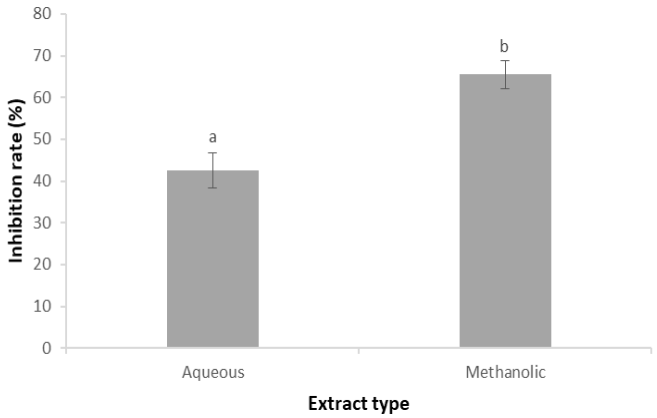

Figure 4: Inhibition rate of *M.oleifera* extract on the mycelial growth of *L.theobromae* according to extract type. Diferent letters indicate a signifcant variance of P<0.05

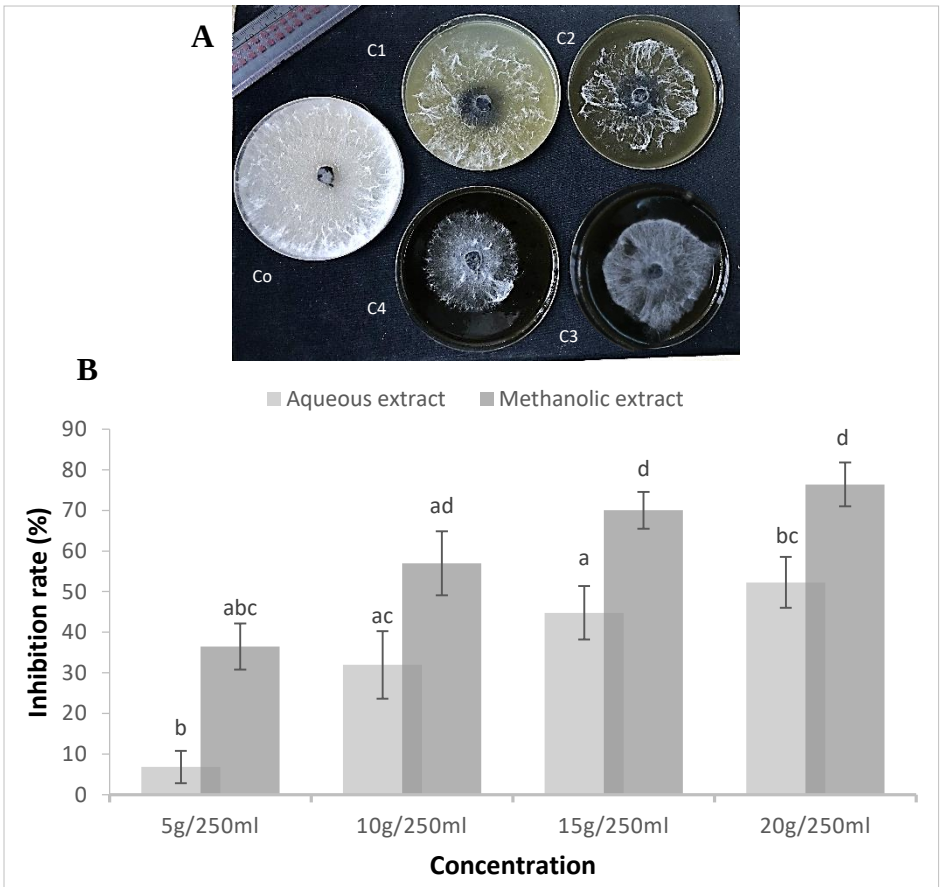

Figure 5: Inhibitoty effect of *M. oleifera* extract on *L. theobromae*. (A) representative photo on the mycelial growth after 3 days incubation on PDA medium. (B) inhibition according to extract type and concentration. Co=Control (0g/250 ml), C1= 5g/250 ml, C2= 10g/250 ml, C3= 15 g/250 ml, C4= 20g/250 ml. Diferent letters indicate a significant variance of P<0.05.

3.3 Efficacy of *Trichoderma* spp against *L.theobromae*

Researchers have proven that some endophyte microorganisms benefit the ecosystem, as they can eco-friendly control pathogens. Thus, the species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda *et al.*, 2011). Therefore, they have been controlling *Sclerotinia* spp. (Rabeendran *et al*., 2006), *Rhizoctonia solani*, *Pythium ultimum* (Lewis *et al*., 1996), *Fusarium oxysporum* (Mao *et al*., 2020), *Lasiodiplodia theobromae* (Li *et al*., 2022; Govender *et al*., 2005). In our study, the results of the dual confrontation assay revealed that *Trichoderma* spp isolates had a significant inhibitory effect against *L. theobromae* with a rate inhibition between 30 and 37 % ($P<0.05$). However, the isolates had similar efficacy inhibitory activity on *L. theobromae* ($P\geq0.05$) (Table 1). This could be explained by the nonvolatile metabolites and/or volatiles produced by *Trichoderma* spp contribution to its inhibitory activity against *L.theobromae*. Furthermore, volatiles produced by *Trichoderma* species has been reported to include molecular terpenes (Lee *et al*., 2016). Also, the inhibitory effect of *Trichoderma* spp could be due to antibiotics production among Trichodermin, Trichodermol, Harzianum A, and Harzianolide (Dennis & Webster, 1971) as well as some cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharide, chitins and beta-glucanase destroying cell wall (Elad, 2000). These results agree with many studies showing that the volatiles produced by *Trichoderma* species, notably *T. hamatum* had a significant inhibitory effect on the growth of *L. theobromae* (Li et *al*., 2022).

Table 1: Efficacy of the 4 *Trichoderma* spp on *L. theobromae*

Trichoderma spp	Radial growth of L.theobromae (cm)	% of inhibition
1BTrSyA7	4.61±0.18a	30.85±5.2a
AdjBrA7	3.98±0.36a	36.47±11.87a
MoRSy-2	4.43±0.19a	33.09±6.61a
MoRSyA8-1	4.26±0.2a	35.37±6.92a
AdjBrA6	4.53±0.77a	36.06±9.34a
Control	6.93±0.7b	

4 CONCLUSION

In order to deal with the harmful effect of chemicals on human health and the environment, researchers are finding alternative methods. Our study demonstrated that plant extract and biocontrol agent *Trichoderma* spp can contribute to eco-friendly control of *Lasiodiplodia theobromae* causing mango Stem-end rot. In addition, the biopesticides NECO, and ASTOUN were the most effective, with 100 % inhibition of *L. theobroma* of mycelial growth. However, further studies should be implemented *in vivo* to confirm the finding.

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