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## Effect of some biopesticides based on essential oil and plant extracts on postharvest mango Stem-end rot disease caused by *Lasiodiplodia theobromae*

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### ABSTRACT

*Lasiodiplodia* species causing mango Stem-end rot disease (SER) are mainly controlled using synthetic products, which can harm humans and environmental health. Therefore, developing an eco-friendly control method, such as using plant extract products, is imperative. In this study, we evaluated the inhibitory effect of 3 biopesticides based on essential oils (ASTOUN 50 EC, FERCA 50 EC, and NECO 50 EC) at 300, 500, 700, 1000 ppm and *Moringa oleifera* leaves extracts (Methanolic and aqueous) at 5, 10, 15 and 20 g/250 ml on mycelial growth of *Lasiodiplodia theobromae* *in vitro*. Subsequently, 135 fruits (cv. Kent) per treatment were inoculated ( $1 \times 10^5$  spores/ml) and treated with each biopesticide (700, 1000 and 2000 ppm) and *M. oleifera* leaves extracts (15 and 20 g/250 ml) *in vivo* to evaluate their efficacy on mango SER development. The results showed that the biopesticides ASTOUN (*Cymbopogon citratus*) and NECO (*Occimum gratissimum*) completely inhibited the mycelial growth of *L. theobromae* at 700 and 1000 ppm. Similarly, *M. oleifera* methanolic extract has the highest inhibitory rate (65.45 %) compared to aqueous extract (42.44%). Moreover, 1000 and 2000 ppm of biofungicides and 15 and 20 g/250 ml of *M. oleifera* methanolic extract significantly reduced mango SER development compared to the control. This study provides evidence that these plant extracts are effective alternatives to mango SER management that are consistent with sustainable agriculture principles, promote ecological balance, and reduce the environmental impact of conventional agriculture.

### 1. Introduction

Mango (*Mangifera indica* L.) is an economically important fruit worldwide, and its demand is constantly increasing [1]. Its production was estimated at more than 55 million tonnes, with Asia representing the most significant producer worldwide [2]. However, despite its importance, mango production faces several threats, notably mango fungal diseases [3]. *Lasiodiplodia* spp are causal agents of mango dieback and Stem end-rot disease, a severe disease for mango growers [4]. It can cause about 60 % of losses in mango production in affected plantations, which may increase to 100 % of fruit loss in absence of proper management strategies to control the diseases [5,6]. In response to this threat, chemical fungicides have been reported to control the fungal

pathogens [7,8]. However, this method has limitations: chemical fungicides can be harmful to humans and the environment [9]. Moreover, it has been proven that using chemical fungicides increased the expression of resistance genes in *Lasiodiplodia* spp. [10]. There have been strong advocacies on finding sustainable alternatives, including biological methods to control *Lasiodiplodia* spp pathogens. Medicinal plants and biocontrol agents are renowned for their fungicidal and bactericide effects with negligible side effects [11–13]. Their antimicrobial activity is due to their bioactive compounds, such as flavonoids, phenolic acids, alkaloids, isothiocyanates, tannins, saponins, aliphatic aldehydes, and terpenoids [14,15]. Numerous studies have demonstrated the potent antimicrobial effects of plant extract against phytopathogens [16–20]. However, there is limited data on the potential efficacy of plant extract

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on the development of *L. theobromae*, causing mango Stem-end rot (SER) disease. Filling this knowledge gap will help develop an eco-friendly control method for *L. theobromae*. Therefore, the present study provides answers to two important questions, including the effect of tree biofungicides based on essential oils such as NECO 50 EC (*Ocimum gratissimum*), ASTOUN 50 EC (*Cymbopogon citratus*), FERCA 50 EC (*Eucalyptus citryodera*) and plant extracts (aqueous and methanolic) of *Moringa oleifera* against *L. theobromae* *in vitro*, and their effects against postharvest *L. theobromae* in inoculated mangoes *in vivo*.

## 2. Material and methods

### 2.1. Identification of *L. theobromae*

The isolate FRF01 of the causal agent, *L. theobromae* used in this current study, was isolated from mango Stem-end rot (SER) disease symptoms in Côte d'Ivoire. Then, it was identified based on morphological characteristics and confirmed by the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) and translation elongation factor 1- $\alpha$  (tef1- $\alpha$ ). Furthermore, pathogenicity tests were confirmed on mango fruits.

### 2.2. Inhibiting test of *L. theobromae* with biopesticides based on essential oil

In this current study, three biopesticides (ASTOUN 50 EC, NECO 50 EC, FERCA 50 EC) formulated by the Industrial Research Unit on biopesticides (URI) of Félix Houphouët-Boigny University (UFHB) were used. The biopesticides have been formulated with several plant essential oils, such as *Cymbopogon citratus*, *Ocimum gratissimum* and *Eucalyptus citryodera*, respectively, for ASTOUN, NECO and FERCA. The biopesticides were added to 20 ml Potato Dextrose Agar (PDA) medium to obtain final concentrations of 300 (C1), 500 (C2), 700 (C3) and 1000 (C4) ppm. The amended fungicide media were homogenized under agitation and emulsified in 2 % 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. [21].

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

*M. oleifera* leaves were used for extractions. The leaves were harvested in the Scientific pole of UFHB and dried in the URI laboratory at room temperature. The method applied by Mamka & Gwa [21] was adapted for methanolic and aqueous extracts. Extractions 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. The solution was left for 24 h and subsequently 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. Measurement of mycelial radial growth

The inhibition rate (I) of mycelial growth of *L. theobromae* by biopesticides and *Moringa oleifera* extract was calculated using the formula reported in Ref. [23].

$$I (\%) = [(Do - Dc) / Do] \times 100 \quad (1)$$

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

### 2.5. Pathogen preparation

The strain FRF01 (accession number: OQ269654) of *L. theobromae* isolated from mango SER symptoms and tested for their virulence by inoculation in artificially wounded mango fruit. Spore suspensions were prepared by growing the pathogens on a 2 % water Agar (WA) containing sterilized pine (*Pinus nigra* Arnold) needles and incubated at 25 °C with a 8 h photoperiod for four weeks, to induce the formation of fruiting bodies and sporulation. Spores were collected and resuspended in sterile distilled water. After filtering through eight layers of sterile cheesecloth, the spores were counted and brought to a concentration of  $1 \times 10^5$  spores/ml [24]. The resultant suspensions were shaken in a vortex mixer for 30 s before inoculation.

### 2.6. Efficacy of the biopesticides and *M. oleifera* plant extract on mango stem-end rot disease

Following the *in vitro* assay, we opted for concentrations of 700, 1000, and 2000 ppm for the biopesticides ASTOUN, NECO, and FERCA, alongside 15 g/250 ml and 20 g/250 ml concentrations of *Moringa oleifera* plant extract (aqueous and methanolic forms) in order to perform the treatments *in vivo*. Additionally, 2 % Tween 20 surfactant was incorporated. Resultant emulsions were vigorously shaken to ensure a uniform and consistent mixture.

Mango fruit (cv. Kent) harvested in orchards in northern Côte d'Ivoire were divided into groups of 45 fruits per treatment. All the fruits, free from evident wounds and rot, were disinfected in 1 % sodium hypochlorite solution. They were rinsed in tap water, dried at room temperature, and perforated with a sterile tip at the peduncle region (3 mm deep and 3 mm wide, three wounds per fruit). A volume of pathogen suspension (10  $\mu$ l;  $1 \times 10^5$  spores/ml) was added to each wound. The fruits were kept at room temperature for 12 h to help the establishment of the pathogen. Then, 10  $\mu$ l of biopesticides or plant extract emulsion was dropped into each inoculated wound. An inoculated control (without biofungicides or plant extract application) fruits was also prepared [24]. All treatments were stored in cold chambers at  $26 \pm 2$  °C for 12 days. The diameter of the rot around each wound was measured after 12 days of storage. Each trial was performed three times. The severity of the disease (lesion diameter) in the inoculated fruits was measured and expressed in centimetres. The disease incidence was calculated according to Xing et al. [25] using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected wounds}}{\text{Total no of inoculated wounds}} \times 100 \quad (2)$$

### 2.7. Data analysis

Data were subjected to Analysis of Variance (ANOVA) at a 5 % significance level, and means were compared among treatments for each measured parameter using Tukey's honest significance test. Data were analysed using R software version 4.2.2.

## 3. Results

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

The results of the effect and inhibition rate of biopesticides ASTOUN 50 EC, NECO 50 EC and FERCA 50 EC on the mycelial growth of *L. theobromae* are presented in Figs. 1 and 2, respectively. The results showed that all biopesticides inhibited *L. theobromae* growth at different concentrations. ASTOUN and NECO at the four tested concentrations were the most efficient in inhibiting *L. theobromae* growth compared to FERCA ( $P < 0.05$ ). A complete (100 %) inhibition rate was achieved at 700 ppm and 1000 ppm for ASTOUN, and 1000 ppm for NECO. All FERCA concentrations showed a similar effect in inhibiting the mycelial

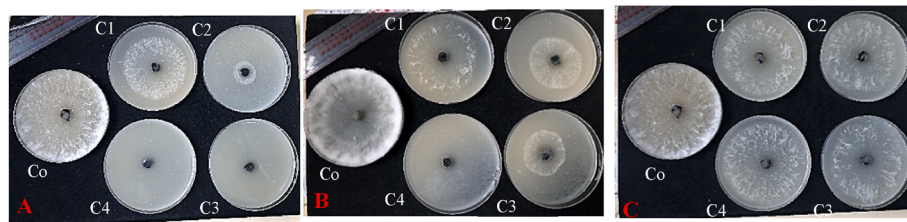


Fig. 1. Effect of biopesticides on the mycelial growth of *Lasiodiplodia theobromae* after 3 days incubation on PDA medium. (A) ASTOUN 50 EC; (B) NECO 50 EC; (C) FERCA 50 EC. Co= Control (0 ppm); C1 = 300 ppm; C2 = 500 ppm; C3 = 700 ppm; C4 = 1000 ppm.

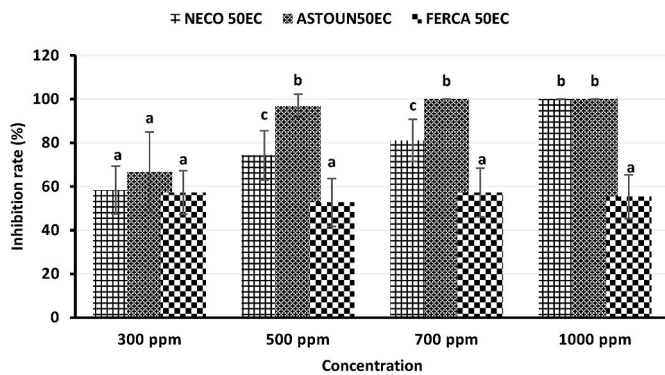


Fig. 2. Inhibition rate of the biopesticides ASTOUN, NECO and FERCA on the mycelial growth of *L.theobromae*. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.

growth of *L. theobromae* with 52.7, 55.33, 57.24 and 57.27 % at 300, 500, 700 and 1000 ppm, respectively.

Fig. 3 shows the results of *L. theobromae* mycelial growth according to incubation period and biopesticides concentrations. Therefore, the inhibitory activity of biopesticides was significantly impacted by the duration of the incubation period ( $P < 0.05$ ). Although ASTOUN at concentrations of 700 and 1000 ppm and NECO at 1000 ppm were effective in completely inhibiting the mycelial growth of *L. theobromae* during the incubation period, their inhibitory effect gradually diminished, allowing mycelial growth to resume from day 1 to day 3 at 300 and 500 ppm (Fig. 3).

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

The results of the inhibition rate of *M. oleifera* extract on the mycelial growth of *L.theobromae* according to extract type are presented in Figs. 4

and 5. The evaluation of the inhibitory activities of aqueous and methanolic extracts of *M. oleifera* showed that *M. oleifera* extracts had significant inhibitory activity against *L. theobromae* compared to the control group ( $P < 0.05$ ). The methanolic extract had the highest inhibition percentage (65.46) compared to the aqueous extract (42.44) (Fig. 4). The inhibition rate recorded varied from 6.84 to 52.26 % for aqueous extract and 36.51–76.36 % for methanolic extract (Fig. 4). Concentrations 15 g/250 ml and 20 g/250 ml of the methanolic extract had the highest inhibition effect on *L. theobromae* development with an inhibition rate above 76 % compared to 42 and 52 % for aqueous extract for the same concentrations ( $P < 0.05$ ) (Fig. 5).

### 3.3. Effect of biofungicides based on essential oil on the development of mango stem-end rot

Figs. 6 and 7 show the incidence and severity of mango SER

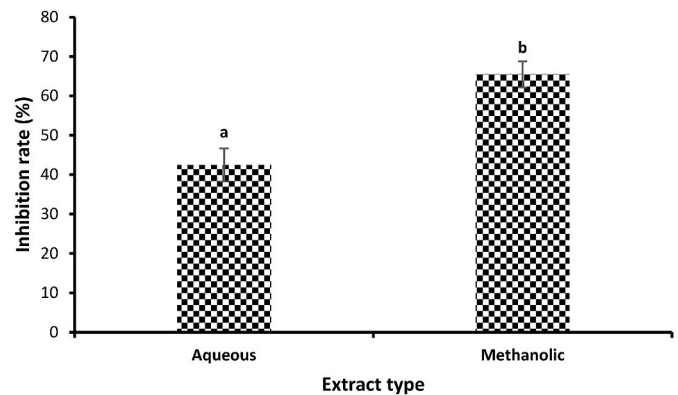


Fig. 4. Inhibition rate of *M. oleifera* extract on the mycelial growth of *L. theobromae* according to extract type. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.

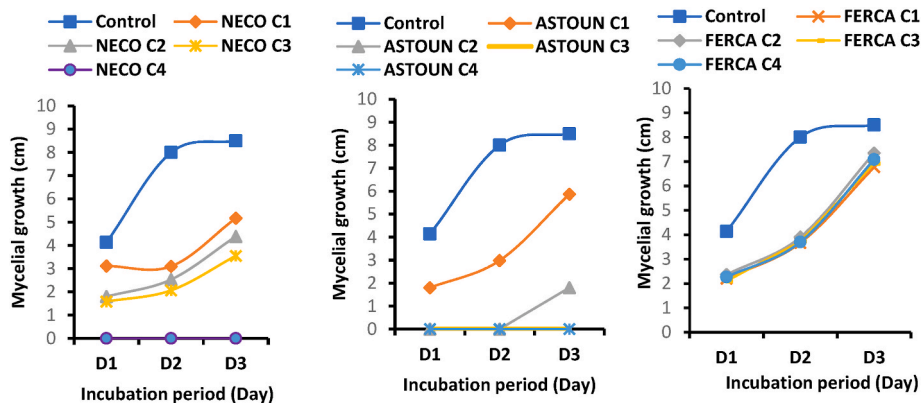
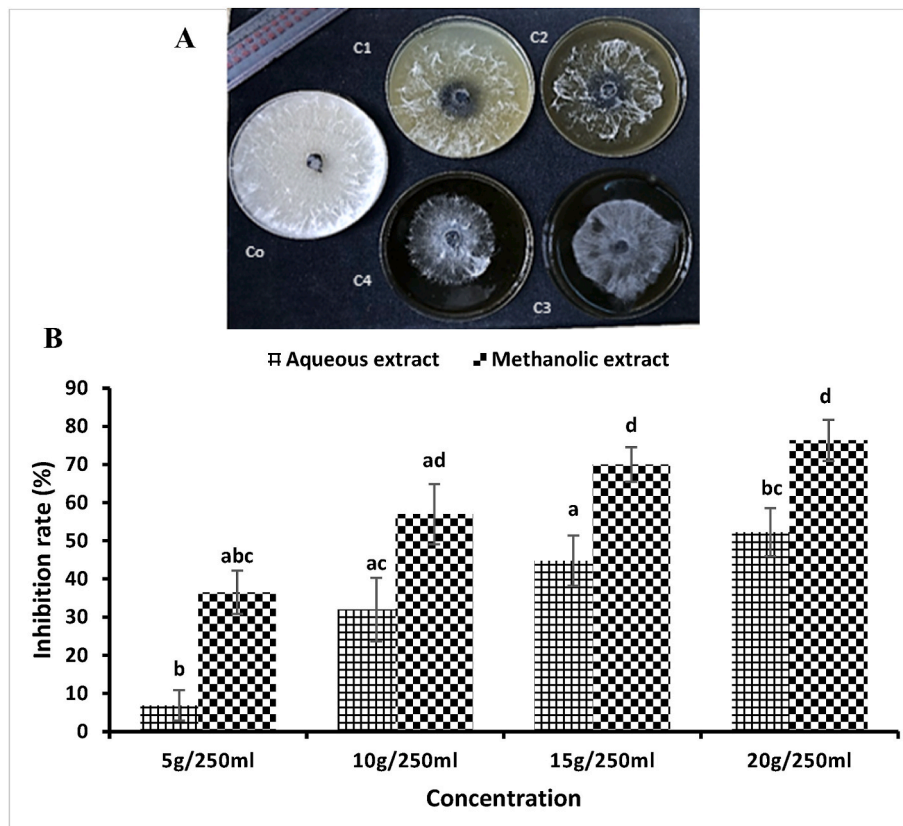
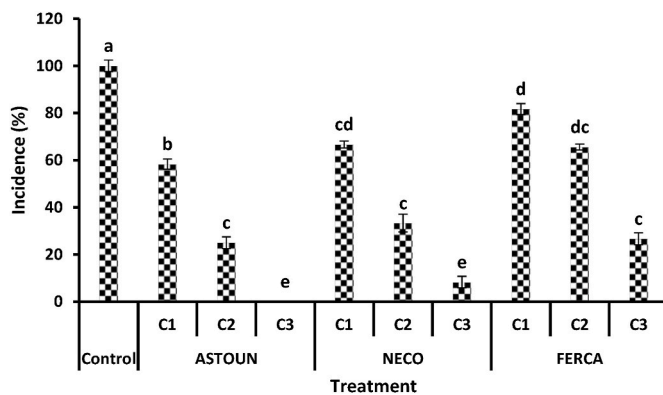


Fig. 3. *L. theobromae* mycelial growth according to incubation period and biopesticides concentrations. C1 = 300 ppm; C2 = 500 ppm; C3 = 700 ppm; C4 = 1000 ppm.



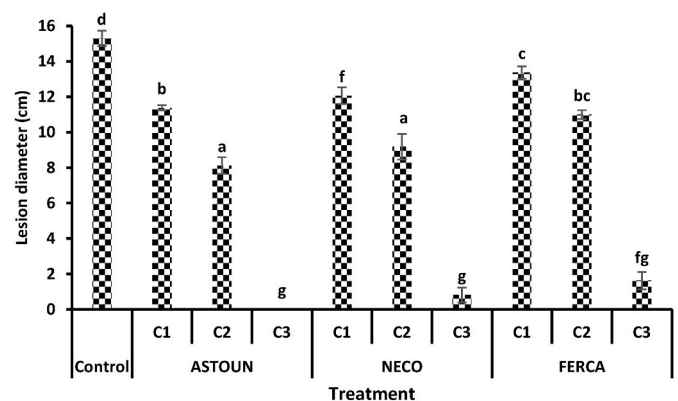
**Fig. 5.** Inhibitory effect of *M. oleifera* extract on *L. theobromae* mycelial growth. (A) representative photo of the mycelial growth after 3 days of incubation on PDA medium. (B) inhibition according to extract type and concentration. Co = 0 g/250 ml (control); C1 = 5 g/250 ml; C2 = 10 g/250 ml; C3 = 15 g/250 ml; C4 = 20 g/250 ml. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.



**Fig. 6.** Incidence of mango SER according to the biofungicides based on the essential oil. C1 = 700 ppm; C2 = 1000 ppm; C3 = 2000 ppm. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.

according to the biofungicides (ASTOUN, NECO and FERCA) based on the essential oil. The incidence and severity varied according to the specific biopesticide employed and its concentration. Among the concentrations examined, the biofungicide ASTOUN derived from *Cymbopogon citratus* showed the least incidence of mango stem-end rot: 58.22 % for C1, 25 % for C2, and complete prevention (0 %) for C3. In addition, NECO (*Occimum gratissimum*) exhibited incidence rates of 66.66 % (C1), 33.33 % (C2), and 8.33 % (C3), while FERCA (*Eucalyptus citryodera*) had respective values of 81.66 % (C1), 65.57 % (C2), and 26.66 % (C3) (Fig. 6). This pattern was verified through severity assessments, as illustrated in Fig. 7.

Hence, across all concentrations, the biofungicides significantly



**Fig. 7.** Severity of mango SER according to the biofungicides based on essentials at different concentrations. C1 = 700 ppm; C2 = 1000 nppm; C3 = 2000 ppm. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.

reduced the lesions' diameter compared to the control group ( $P < 0.05$ ). At concentration C1, ASTOUN exhibited the smallest lesion diameter (11.37 cm), followed by NECO (12.06 cm) and FERCA (13.37 cm). However, at concentrations C2 and C3, the effect of ASTOUN (C2 = 8.12 cm, C3 = 0.00 cm) and NECO (C2 = 9.18 cm, C3 = 0.81 cm) was not statistically significant compared to the control group ( $P > 0.05$ ). Nonetheless, ASTOUN and NECO resulted in a greater reduction in lesion diameter than FERCA (C2 = 11 cm, C3 = 1.62 cm).

### 3.4. Effect of *Moringa oleifera* aqueous and methanolic extracts on the development of mango stem-end rot

The results illustrating the effect of *M. oleifera*'s methanolic and aqueous extracts are presented in Figs. 8 and 9, showing the incidence and severity of mango SER according to the biofungicides based on *M. oleifera* plant extract. The incidence rates were estimated as 13.33 % (20 g/250 ml) and 66.66 % (15 g/250 ml) for the methanolic extract and 66.66 (20 g/250 ml) and 80 % (15 g/250 ml) for the aqueous extract. In contrast, the control group exhibited a 100 % incidence rate (Fig. 8). In terms of severity, the methanolic extract resulted in the smallest lesion diameter at 20 g/250 ml (1.12 cm) and 20 g/250 ml (4.48 cm) compared to aqueous extract ( $P < 0.05$ ). On the other hand, the aqueous extract's effect at 15 g/250 ml (10.31 cm) and 20 g/250 ml (9.84 cm) was less pronounced and did not display significant differences compared to methanolic extract ( $P > 0.05$ ) (Fig. 9).

## 4. Discussion

### 4.1. Efficacy of biopesticides ASTOUN, NECO and FERCA on mycelial growth of *L. theobromae* and the development of mango stem-end rot

Biopesticides could be an excellent alternative to synthetic chemical fungicides used in agriculture. In this study, we evaluated the effect of biopesticides ASTOUN 50 EC (*Cymbopogon citratus*), NECO 50 EC (*Ocimum gratissimum*), FERCA 50 EC (*Eucalyptus citryodera*) on the mycelial growth of *L. theobromae* and the development of mango stem-end rot diseases *in vivo*. All treated mango fruits showed significant inhibition of mango stem-end rot compared to control fruits ( $P < 0.05$ ). The incidence and severity of disease decreased by increasing the concentration of biofungicides (essential oils) and or plant extracts. This could be due to the antifungal properties and or the antioxidant enzyme activities. These results are consistent with the studies of [26], which emphasized the action of thyme oil against postharvest decay in apricot and plum and those of [27] on essential oil vapour treatment on the postharvest disease control and different defence responses in two mango cultivars. Perumal et al. [27] observed that the fruits exposed to different postharvest treatments (with essential oils) exhibited a slight increase in antioxidant enzyme activities. Hence, the biofungicide ASTOUN and NECO were more effective across all concentrations on mango stem-end rot disease development than FERCA. The difference in inhibition activity among these biopesticides could be due to their specific chemical composition. In addition, the degree of sensitivity of *L. theobromae* at different concentrations of the biological fungicide can also be a contributing factor [28]. The essential oils of *Cymbopogon citratus* (ASTOUN 50 EC) contain thymol and citronellol as active

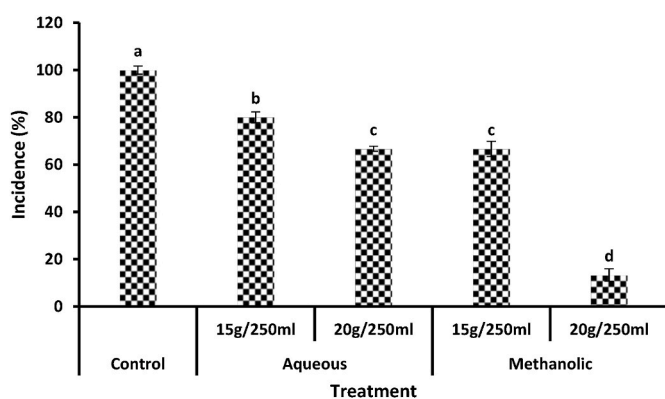


Fig. 8. Incidence of mango SER according to the biofungicides based on *M. oleifera* plant extract type at different concentrations. Control = 0 g/250 ml. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.

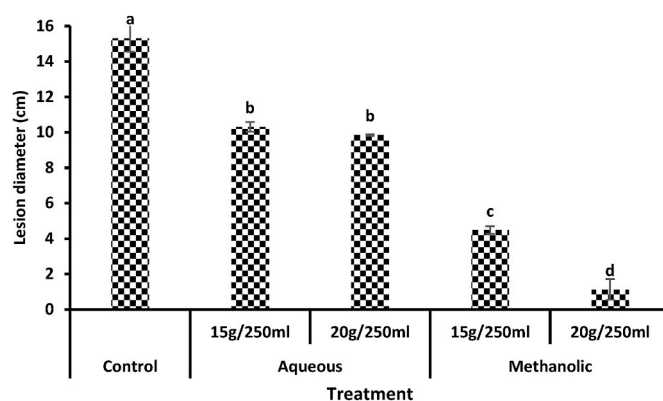


Fig. 9. Severity of mango SER according to the biofungicides based on *M. oleifera* plant extract at different concentrations. Control = 0 g/250 ml. Different letters indicate a significant variance ( $P < 0.05$ ) analysed by Tukey's range test.

components, while *Ocimum gratissimum* (NECO 50 EC) contains thymol and eugenol molecular which have been reported to have fungicidal and bactericidal activity [27,28]. Our results agree with several studies that have elucidated the fungicide effect of ASTOUN, NECO and FERCA on plant pathogenic fungi [29–32]. The biopesticide FERCA could not completely inhibit the development of *L. theobromae* at all concentrations. This is probably due to its composition, which differs from ASTOUN and NECO. This is corroborated with the result of N'goran et al. [31], who reported that at FERCA biopesticide was the least effective in 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.

### 4.2. Effect of incubation period on the inhibitory activity of biopesticides *in vitro*

The inhibitory activity of biofungicides was significantly impacted by the duration of the incubation period *in vitro*. Therefore, their inhibitory effect gradually diminished, allowing mycelial growth to resume from day 1 to day 3. We hypothesized that the aromatic components in the biopesticides could evaporate from the Petri dish due to their volatile properties, as suggested by Edris and Farrag [33]. 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 [33,34].

### 4.3. Effect of *Moringa oleifera* aqueous and methanolic extracts on mycelial growth of *L. theobromae* and the development of mango stem-end rot

The medicinal plant *M. oleifera* has been studied worldwide. In this current study, we evaluated the efficacy of aqueous and methanolic extract on the mycelial growth of *L. theobromae* and the development of mango stem-end rot diseases *in vivo*. Therefore, the methanolic extract had the highest inhibition percentage compared to the aqueous extract *in vitro* and *in vivo*. The difference in extraction methods can explain these findings. Indeed, the use of solvents of an alcoholic nature allows to obtain extracts with much more concentrated bioactive molecules than aqueous extract. These findings are consistent with several studies in the literature [21,35]. Numerous studies have reported plant extracts possessing antifungal potential against a range of fungal pathogens. For instance, *Moringa oleifera* and *Syzygium aromaticum* were reported to show significant antifungal activity against the mycelial growth of fungal pathogens causing SER [16]. *M. oleifera* contains saponins, alkaloids, phenols, tannins, flavonoids, steroids, phlorotannins and terpenes, which are responsible for their antifungal activity [36,37]. These

results are consistent with the findings of Mamkaa and Gwa [22], where there was a general increase in percentage growth inhibition as the extract concentration increased.

#### 4.4. Implications and potential applications

The findings of this study hold considerable practical significance, particularly within the domains of agriculture, horticulture, and the mango industry. The application of biopesticides derived from essential oils (ASTOUN 50 EC, FERCA 50 EC, and NECO 50 EC) and from *M. oleifera* leaves extracts presents an alternative for managing mango SER disease in mango cultivation with profound economic implications for farmers. Moreover, deploying these extracts as natural surrogates for chemical pesticides underscores their potential to advance sustainable agricultural practices. This paradigm shift will reduce dependence on chemical pesticides, reducing harmful impacts on the environment and reducing chemical residues in mango fruits. Additionally, these biopesticides' innate natural and non-toxic attributes may bolster consumer confidence, engendering heightened demand for mangoes and stimulating economic growth within the industry. Furthermore, mangoes treated with these natural alternatives may align with export markets' stringent pesticide residue regulations, opening new avenues for export and bolstering revenue streams for mango-producing regions. Disseminating knowledge about the efficacy of these extract plants to mango farmers and agricultural extension services can catalyze their widespread adoption across diverse mango-producing regions.

Continued exploration of this promising avenue by scientists and industry may lead to further research results and practical applications. Indeed, replacing chemical pesticides with natural extracts represents a promising avenue that promises to reduce exposure to chemical pollution, soil contamination, and damage from non-target organisms in the environment. Additionally, a shift towards reduced use of chemical pesticides in mango cultivation could lead.

#### 5. Limitations of the study

This study did not evaluate various environmental factors that can influence the efficacy of biopesticides, such as temperature, humidity, and weather conditions. In addition, the study did not test other biopesticide application methods, such as spraying and dipping, which can impact their effectiveness. Moreover, while the study showed that biopesticides are effective, it did not address their cost-effectiveness compared to conventional chemical treatments. Addressing these limitations in further research can help improve the understanding of the effectiveness of biopesticides in controlling mango stem-end rot disease. Despite its limitations, this study offers exciting findings which could promote sustainable agriculture practices by reducing the environmental impact of pesticide use and supporting organic or integrated pest management (IPM) approaches.

#### 6. Conclusion

Researchers are working to provide more sustainable alternative methods to limit the harmful effects of chemical pesticides on human health and the environment. Our study demonstrated that plant extract can contribute to eco-friendly control of *Lasioidiplodia theobromae* causing mango stem-end rot. The biopesticides (NECO, ASTOUN and FERCA) and *Moringa oleifera* leaves extracts effectively inhibited the mycelial growth of *L. theobromae* and mango stem-end rot disease development. Therefore, they can be considered a better alternative treatment for mango postharvest stem end rot disease control. Further studies should be implemented in the future in naturally infected fruits to offer an effective SER control measure to the biological mango fruit industry.

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#### Authors' contributions

The authors YSY and DK designed the work. YSY and SO collected plant materials interpreted the data, and drafted the article. The work was substantively revised, and the final version was approved by the authors DDD, BC, JR and DF.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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