



**CHEMICAL AND BIOLOGICAL CONTROL OF *Lasiodiplodia theobromae*, AGENT PATHOGEN ASSOCIATED WITH DECLINE MANGO IN BURKINA FASO**

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**ABSTRACT**

Mango (*Mangifera indica*) is one of the most important fruit trees in the tropics and subtropics of the world. Mango decline is currently one of the main diseases of mango in Burkina Faso. Preliminary studies revealed *Lasiodiplodia theobromae* associated the disease. This study was focus on research for solutions to mango decline in Burkina Faso. Six synthetic fungicides and three biopesticides were tested *in-vitro* on the mycelial growth of *Lasiodiplodia theobromae*. Fungicides, biopesticides, fertilizers and agricultural practices were used individually or in combination as treatments (T1 to T5) for the field trial in four localities. The results revealed antifungal effect of six fungicides and two biopesticides on mycelial growth on *L. theobromae*. Manga Plus<sup>®</sup>, Idefix<sup>®</sup> and Neco<sup>®</sup> induced 100% efficacy rate on the champignon at C1= 1000ppm. At the same dose, Banko<sup>®</sup>, Nativo<sup>®</sup>, Référence<sup>®</sup>, Azox<sup>®</sup>, and Proraly<sup>®</sup> had a mean inhibition rate of mycelial growth greater than 60%. T1 (Nativo<sup>®</sup> + Manga plus<sup>®</sup>+ NPK) and T2 (Nativo<sup>®</sup> +Manga Plus<sup>®</sup>) significantly reduced the incidence and severity of the disease. However, Plantsain<sup>®</sup> and Solsain<sup>®</sup> have been less effective against the disease. Therefore, as part of an integrated management of the disease in Burkina Faso, Neco<sup>®</sup> could be better predicted because of its biological nature respectful of the environment, the health of producers and consumers.

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**INTRODUCTION**

Mango (*Mangifera indica*) produces delicious fruits and is one of the most important fruit crops in the world's tropical and subtropical areas. In terms of fruit production, mango occupies the first place in Burkina Faso. Production and marketing of mangoes are now one of the promising sectors. In 2018, fresh mango production has increased from around 90 000 tons in 2017 to 200 000 tons (APROMAB, 2018). In terms of resources, marketing of fresh and dried mangoes generated more than 21 617,643.11 USD. Despite all these encouraging data, mango sector meets many phytosanitary difficulties that could compromise its productivity. Nowadays, mango decline, or sudden death syndrome or mango plant mortality (Khaskheli *et al.*, 2011), is considered one of the most serious threats in all mango producing regions (Kazmi *et al.*, 2005).

In 2015, mango decline outbreak was observed in Burkina Faso and continued to expand in different production areas (Dianda *et al.*, 2018). The disease is characterized by several types of symptoms (Ploetz *et al.*, 1996, Masood *et al.*, 2010, Ismail, 2011, Seyd *et al.*, 2014). It results in a wilting of leaves gradually evolving towards twigs and branches drying with or without defoliation. In addition, these symptoms are sometimes associated with gummosis by the presence of brownish exudates on the trunks and branches. Browning may

also appear on trunks and branches, and bark cracks. Root rot has often been reported associated with symptoms of mango decline. Generally, symptoms associated with this disease are expressed as twig tip dieback that advances into the old wood with branches that dry and die, and leaves scorch and fall, eventually causing death of plant (Saeed *et al.*, 2017). According to Ploetz, (2003), dieback is considered the most destructive disease causing significant losses of yields and low quality of mangoes. Studies have identified the Ascomycetous fungus *Lasiodiplodia theobromae* (Patouillard) Griffon & Maublanc (sin. *Botryodiplodia theobromae* Pat.) (Sutton, 1980) as the causative agent of the disease in various producing regions of the world including Brazil, Korea, India, Oman, Pakistan, United States of America, Egypt, Ghana, the United Arab Emirates and Peru (Sharma and Gupta, 1994; Ploetz *et al.*, 1996; Al Adawi *et al.*, 2003; Khanzada *et al.*, 2004; de Oliveira Costa *et al.*, 2010; Hong *et al.*, 2012, Ismail *et al.*, 2012; Ablormeti, 2016; Saeed *et al.*, 2017; Rodriguez *et al.*, 2017). Moreover, *L. theobromae* may attack almost all the organs of other trees such as cocoa, guayavier and cashew producing various types of symptoms. In addition, this fungus has been reported as responsible for leaf blight of Jack fruit (Haquet *et al.*, 2005) and for post-harvest diseases, including fruit rots for some fruit species such as mangoes (Nuret *et al.*, 2018).

Before considered as an opportunistic fungus, *L. theobromae* is now a serious threat for a number of crops in several production areas. The fungus may persist as an endophyte but turns virulent after plant weakening under abiotic stress. The unbalanced use of fertilizer, intercropping in the tree grooves, zero pruning and certain abiotic constraints such as drought, high temperatures, sunshine, water stress, salinity and nutritional deficiencies can contribute to the development and progression of the decline caused by *L. theobromae* (Naqvi *et al.*, 2015)

Different control strategies have been investigated to reduce the many losses caused by the mango decline. Genetic resistance to *L. theobromae* was searched among some mango varieties by direct stem inoculation of young trees. Indeed, Twenty varieties of mango viz., Langra, Chaunsa, Sindhri, Neelum, Jagidar, Anwar Ratol, Saleh Bhai, Saroli, Almas, Zafran, Gulab Khasa, Anmol, Sawarnarica, Bagan Pali, Dusheri, Bombay, Mehran, Rampuri, Lal Badshah and Tuta Pari screened against *Lasioidiplodia theobromae* in Pakistan. Of these, only Bagan Pali, Saroli and Saleh Bhai showed resistance against *L. theobromae*, whereas, Langra, Sindhri and Almas were highly susceptible (Khanzada *et al.*, 2015).

Fungicide treatments were also investigated against *L. theobromae*. *In-vitro* mycelial growth of (KUCC0027) was significantly inhibited by Carbendazim and thiophanate-methyl used at 1 ppm (Khanzada *et al.*, 2005). After the 3<sup>rd</sup> spray the trees sprayed with Carbendazim or Thiophanate-methyl showed promising results.

They produced large number of new shoots and complete disappearance of typical symptoms of the disease (Khanzada *et al.*, 2005). Similar studies in Ghana detected carbendazim, funguran, mancozeb, prochloraz and zamir effective in inhibiting *L. theobromae* mycelial growth *in-vitro* (Ablormeti, 2016). Application of these different fungicides at intervals of two weeks in orchards showed that carbendazim was the most effective in reducing disease and regenerating trees (Ablormeti, 2016). However, excessive use of fungicides may result in chemical residues in fruit, environmental pollution and adverse effects on human health (Okigbo & Osuinde, 2003). The current trend is towards the application of biological control as an alternative to chemical treatments. *In-vitro* tests with products of biological origin, including plant extracts, essential oils, or antagonistic *Trichoderma* isolates etc. showed promising efficacy in inhibiting mycelial growth of *L. theobromae*. Indeed, aqueous extracts of garlic (*Allium sativum*) had inhibitory effect against *L. theobromae* growth and conidia production *in-vitro* (Nur *et al.*, 2018; Raheet *et al.*, 2018). In addition, *Trichoderma* spp inhibited *L. theobromae* and *L. pseudotheobromae* growth (Raheet *et al.*, 2018) suggesting that garlic and *Trichoderma* spp could be tested for ecological disease management. Given the importance of mango decline in Burkina Faso and the lack of effective adaptation and ecological control measures, this study aims to identify strategies for chemical or biological control *in vitro* and *in vivo* against mango decline.

## MATERIALS AND METHODS

### Fungal strains

Three *L. theobromae* strains (N6B, K12B and C4B) were used for *in-vitro* tests of mycelial growth inhibition (Table 1). Strains were isolated from infected mangoes organs, purified on potato-dextrose agar (PDA) media up to single-spore cultures (Dianda *et al.*, 2018) and then characterized on the basis of morphological and molecular tests. Molecular tests were done by Polymerase Chain Reaction (PCR) assays with specific primers Lt347-F(AACGTACCTCTGTTGCTTTGGC) and Lt347-R(TACTACGCTTGAGGGCTGAACA) (10 µM) defined by (Xu *et al.*, (2015). Pathogenicity of these strains was confirmed by the detached organ method described in (Ismail, 2011; Munirah *et al.*, 2017), by inoculating leaves and mangoes and also nursery plants of the Amelie variety (Dianda *et al.* no published).

**Table 1** Origins of the *Lasioidiplodia theobromae* strains isolated from mango trees in Burkina Faso used in this study.

N°	Strain	Organ isolated	Locality	Geographic coordinates	Burkina Faso province
1	N6B	Twig	Tombolo 3	N11°05.308'W001.07.882'	Nahouri
2	C4B	Root	Toumousseni	N10.82142°W004.63026°	Comoé
3	K12B	Twig	Koloko	N11°05.287' W005°20.216'	Kéné Dougou

### Fungicid and biopesticides tested

Two categories of products were selected for this study, including synthetic fungicides available in local markets and biopesticides. Two doses were tested. They are C1= 1000ppm and C2=500ppm. The detail of products including their brand name, active ingredient, concentration/formulation and manufacture are given in Table 2.

## METHODOLOGY

### *In-vitro* antifungal effects of fungicides and biopesticides on *L. theobromae* growth

The "poisoned food" technique described by Kiran *et al.* (2010) has been adopted to evaluate the *in-vitro* efficacy of fungicides and biopesticides: The require quantity or volume of each product was aseptically added to 1 l of PDA culture medium kept in a water bath at 55 °C. The PDA-fungicidal mixture was gently agitated and poured in Petri dishes to solidify, after which a 5-mm mycelia disc from a 7 day-old culture of *L. theobromae* was placed in the center of each Petri dish. Petri dishes containing PDA without fungicide were inoculated to serve as control. Each of the treatments was replicated five times corresponding five petri dishes. Dishes were incubated at ambient temperature (28 ± 2 °C) and the observation started 24 hours after incubation and continued until the complete growth of the mycelium in the control containing the three strains. All manipulations were performed under aseptic conditions.

### Efficacy of the treatments in the field trials

**Choice of sites and trees:** Four localities (Peni, Dafinso, Koloko and Pô) selected are among the major mango production zones. In addition, high incidences and severities were recorded in these localities. Mangoes trees of Amelie

which branches were been partially drying especially scores 3 and 4, according to the severity scale of (Ramos *et al.*, 1997) were selected for the field trials. Amelie is one of commercial varieties in Burkina Faso and presented the highest prevalence of the disease (Dianda *et al.*, 2018).

**Choice of products and agricultural practices :** Nativo (Systemic fungicide) and Manga Plus (Contact fungicide) are approved by the Sahelian Pesticides Committee (CSP). These synthetics fungicides and Plantsain (Arômes of *Trichoderma harzianum*), Solsain (conidia of *Trichoderma harzianum*) are accessible by farmers in Burkina Faso. These products were used to control *L.theobromae* in the field. Cuvettes of 2m radius and 30cm deep were made around the trunks of the trees for the correction of the problems of water retention and nutrients. Pruning of dead branches was been to reduce the inoculum rate on already attacked branches and contribute a good vegetative growth.

**Choice of treatments for the experiment**

All four treatments and control were used for experiment. The table III described products combinaison in each treatment during the field trial in the four localities

year (Supplemental data). Thus, all diseased branches of mango trees used in this experiment were pruned and cuvettes were made around tree which fertilizers were applicated. The effect of the treatments were evaluated by assessing the disease severity and incidence before and after foliar spray during a one-year period with 4 evaluations.

**Experiment in the differents orchards**

The experiment was designed using the Randomized Complete Block Design (RCBD) with six replications by traitement. The two field trials were installed respectively in May 2017 and 2018. For each of the two experiments in the mango tree orchard attacked the monitoring was conducted annually.

**Mode and frequency of treatment applications**

The products and practices agricultural were applied at the level of the aerial and underground part of the trees concerned. A volume (V = 300l) of the mixture (water + product) was retained for a treatment of one hectare of 100 mango trees. This volume was determined after a field test following the method of (PIP, 2013).

**Table 2** Fungicides and biopesticides used in the study

Brand name	Manufacturer's address	Active ingredient	Doses and quantity			Chemical family	Category
			Field trial	1000ppm	500ppm		
Banko Plus <sup>(C)</sup>		Carbendazime (100 g.l <sup>-1</sup> )	2l.ha <sup>-1</sup>	10ml	Nt	phtalic derivatives + Benzimidazoles, Strobilurines	
Azox <sup>(C)</sup>		Azoxtrobine (250 g.l <sup>-1</sup> )	1 l.ha <sup>-1</sup>	4ml	Nt		
NATIVO <sup>(C)</sup>	01 BP 1930 Bobo-Dioulasso	(Tebucon-azole 200 g.l <sup>-1</sup> )	1 l.ha <sup>-1</sup>	5ml	2,5ml	Triazole	Fungicides
300 SC	Tel: (226) 20 97 20 18 / 20 97 20 36						
Référence <sup>(C)</sup>	Fax: (226) 20 97.13.75	Propiconazol (250 g.l <sup>-1</sup> )	0.5 l.ha <sup>-1</sup>	4ml	2ml	Triazole	
IDEFIX <sup>(C)</sup>	Email: saphyto@fasonet.bf	Hydroxyde de cuivre (656g/kg)	2Kg.ha <sup>-1</sup>	1,5g	0,76g	Fongicide minéral	
Manga Plus <sup>(C)</sup>		Mancozeb (800 g/kg)	2Kg.ha <sup>-1</sup>	1,3g	0,65g	Dithiocarbamate	
Plantsain <sup>(C)</sup>	Email:bioprotect.b@gmail.com Website:www.bioprotect-b.com Tel.: 00226 70 22 48 41	Arômes de <i>Trichoderma harzianum</i> (50ml/l)	5 l.ha <sup>-1</sup>	20ml	Nt	Microbial	
Neco <sup>(C)</sup>	Phytopathology and Agrophysiology laboratory of the	<i>Ocimum gratissimum</i> essential oil (50ml/l)	1.2 l.ha <sup>-1</sup>	20ml	10ml	Vegetable	Biopesticides
Proraly <sup>(C)</sup>	University of Félix Houphouët-Boigny, in Côte d'Ivoire Contacts: 09 03 03 77 / 01 08 92 75	Tymol Eugenol Citronollol (50ml/l)	1.2 l.ha <sup>-1</sup>	20ml	Nt	Vegetable	

Legend : C1 and C2: Two dose tested, Nt: Not tested; (C) : commercial

**Table 3** Characteristics of the traetments, periods and the localities used in the fields trial

Treatments	First year of the field trial at 2017 to 2018.		Second year of the field trial at 2018 to 2019.	
	In Peni	Products and agricultural practices	In Dafinso, Koloko and Pô	Products and agricultural practices
T1	Nativo + Manga Plus + NPK		T1	Nativo + Manga Plus + NPK
T2	Nativo + Manga Plus		T2	Nativo + Manga Plus
T3	Nativo		T3	Plantsain +organic manure
T4	Manga Plus		T4	Plantsain + Solsain
T5	Control		T5	Control

In a preliminary experiment, we showed that pruning of branches affected and cuvettes around the tree helped recovering vegetative growth for more than 30% of the branches after three months and up to 48% after one year. However, we also showed that shaping the soil into a slight bowl around the tree to retain water near the tree helped did not reduce wilting symptoms of diseased branches after one

The plants were copiously sprayed three times with the fungicide and biopesticides after every 14 days interval (Khazanda *et al.*, 2005).Fertilizers were applied only once during the first spray.

**Parameters evaluated**

**Determination of the inhibitory effect of fungicides and biopesticides on mycelial radial growth of *L.theobromae***

The rate of inhibition of mycelial growth, the fungicidal and fungistatic effects were the main parameters evaluated. The radial growth of the mycelia was measured using a graduated ruler from daily after incubation on two perpendicular lines drawn on the reverse side of each Petri dish.

The percentage of mycelial growth inhibition was calculated using the formula of Sahi *et al.* (2012).  $I = \left( \frac{X - Y}{X} \right) * 100$

X : Average mycelia growth of control; Y : Average mycelia growth of treated plates. At the end of the 6<sup>th</sup> days after incubation (DAI), the mycelial explants whose radial growth

was null in the Petri dishes containing the poisoned media were transferred aseptically into a freshly prepared, non-poisoned PDA medium. The cultures were incubated under the same conditions previously described and examined daily under a microscope for ten days to detect a possible start of mycelial growth.

If the fungus restarted its growth, the product used was said fungistatic. On the other hand, if the fungus did not develop after 10 days of observations, the product used was classified as fungicide.

**Field evaluation of the treatments for control of mango tree decline disease**

The effects of the treatments on mango plants were evaluated by assessing the disease severity and incidence before and after spray. Moreover rate of vegetative growth of branches per mango tree was evaluated. Disease incidence was calculated as number of infested plants showing symptoms out

$$Ti = \frac{ni \times 100}{N}$$

: number of infested plants, N : number of mango plants observed.

The mean severity (S) per treatment was calculated according to the formula.  $S = \sum(xini)/n$  with xi : represents the severity scores of the disease according to the scale of (Ramos *et al.*, 1997). The rate of vegetative growth of branches per mango tree (Tr) was evaluated according to the formula:  $Tr = (nr * 100)/N$  with nr : number of branches presented vegetative growth; N : total of branches concerned. After the average rate of vegetative growth was calculated per treatment.

The periods of evaluations of the effectiveness of the treatments were variable according to the parameter concerned.

**Statistical analysis**

The Excel 2013 spreadsheet was used for data entry, data organization and graphing. The average inhibition rates of the different fungicides, the average of disease severity and the mean rate of vegetative growth in the different treatments were subjected to variance analysis (ANOVA) using the XLSTAT 2014 software. The separation of averages was done by the Newman Keuls and Duncan test at the 5% level.

**RESULTS AND DISCUSSION**

**Inhibitory effect of products on mycelial radial growth of *L. theobromae* at C1= 1000ppm**

The nine products showed variable inhibitory levels on *L. theobromae* mycelial growth. The inhibition rate of each product on each of the three strains was almost similar. Table IV shows the average inhibition rate of mycelial growth of the three strains (C4B, N6B and K12B) by the products during 6 days of incubation. There was complete inhibition (100%) of mycelial radial growth of *L. theobromae* during this period by three of the products namely Neco, Idefix and Manga Plus. At 6 Days After Incubation (DAI), they are followed by Nativo (89,19%) and Reference (86,35%). Proraly, Banko and Azox registered intermediate efficacy with inhibition rates between

62,3% and 80,13%. Plantsain compared to the control, has no effect on inhibition of mycelial growth of *L. theobromae*.

**Table 4** Percentage inhibition of mycelial radial growth of *L. theobromae* by Potato Dextrose Agar (PDA) amended with nine products at C1= 1000ppm.

Treatments	2DAI	3DAI	4DAI	5DAI	6DAI
Témoin	0,00a	0,00a	0,00a	0,00a	0,00a
Banko	100,00c	100,00c	100,00c	76,48bc	75,74bc
Proraly	100,00c	100,00c	73,64b	70,37b	66,05b
Azox	100,00c	100,00c	100,00c	80,93bcd	80,12cd
Nativo	100,00c	100,00c	100,00c	89,84de	89,20de
Reference	100,00c	100,00c	100,00c	86,85cd	86,36cd
Idefix	100,00c	100,00c	100,00c	100,00e	100,00e
Manga Plus	100,00c	100,00c	100,00c	100,00e	100,00e
Plantsain	50,54 b	17,41b	0,00 a	0,00a	0,00a
Neco	100,00c	100,00c	100,00c	100,00e	100,00e

Means followed by same letters in a column are not significantly different according the test of Newman Keuls at (5%).

**Inhibitory effect of products on mycelial radial growth of *L. theobromae* at C2= 500ppm**

Neco, Manga Plus, Idefix, Nativo, Reference and Azox recorded the highest inhibitory rates of mycelial growth of the three strains of *L.theobromae* at C1= 1000ppm. These six fungicides tested at C2=500ppm also showed varying inhibitory rates on mycelial growth of the three *L. theobromae* strains. They were less effective during the incubation period compared to C1 = 1000ppm. Nevertheless, all six fungicides resulted in a 100% inhibition rate until the 3 (DAI). This rate is constant for Neco, Manga Plus and Idefix until the 4DAI. At 6 DAI all six fungicides had an inhibition rate greater than 60%.

**Table 5** Percentage inhibition of mycelial radial growth of *L. theobromae* by Potato Dextrose Agar (PDA) amended with six products at C2= 500 ppm.

Treatements	2DAI	3DAI	4DAI	5DAI	6DAI
Témoin	0,00	0,00	0,00a	0,00a	0,00a
Nativo	100,00	100,00	69,38b	69,38b	67,04b
Référence	100,00	100,00	64,69b	64,69b	62,41b
Idefix	100,00	100,00	100,00c	100,00c	80,56c
Manga Plus	100,00	100,00	100,00c	100,00c	78,58c
Neco	100,00	100,00	100,00c	71,73b	68,58b

Means followed by same letters in a column are not significantly different according the test of Newman Keuls at (5%).

**Fungicidal and fungistatic effect of the products tested**

The nine products tested were classified into two groups depending on mycelial growth and color of the culture medium during the incubation period.

Neco, Idefix and Manga caused total inhibition of mycelium growth of the three *L. theobromae* strains when tested at C1=1000 ppm. Thus, these three products had a fungicidal effect on the three fungal strains. There was no color change in the culture media containing these products. In contrast, a restart of mycelial growth after the incubation period was noted for Nativo, Propiconazol, Banko, Azox, Proraly and Plantsain at the two doses tested. Culture media treated with these fungicides turned red during the incubation period.

Fungicidal or fungistatic effect of products tested depended sometimes of the dose used.

**Efficacy of different in-vivo treatments in mango orchards.**

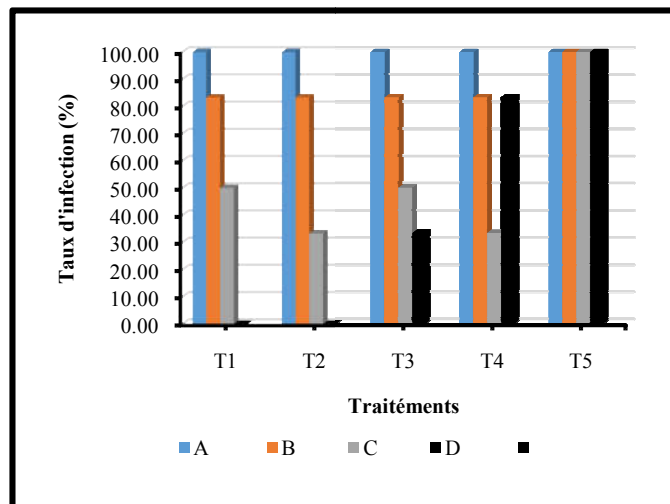
**First year of the field trial at 2017 to 2018 in Peni**

**Vegetative growth :** All four treatments including the control enregistered vegetative growth on branches pruned. The number of branches having vegetative growth increased progressively in the treatments during the period of the experiment.

However, the treatments showed a variability in the average rate of vegetative growth. About, T1 (recorded the highest average rate (84,96%) and the lowest value (48,8%) was observed in the control (T5). Other treatments recorded average rates of vegetative growth intermediate (Fig1).

**The effect of treatments on the incidence of the disease**

All four treatments and control had variable incidence rates of the disease. T5 (control) did not result in a reduction in the incidence of the disease during the period of the experimental. It recorded an incidence rate of (100%) after one year of the experimental test. However, T1 and T2 led to progressive reductions in the rate of incidence of disease. After one year of the experimental trial, no characteristic symptoms of the disease were observed in these two treatments. T3 and T4 reduced also the rate of symptomatic mango trees respectively, by 50% and 33.33%. After one year, T3 recorded a rate of 33.33%. However, the incidence rate of T4 increased to (80%). This increase is due to the appearance of symptoms on the new shoots of mango trees (Fig 2).



**Figure 2** Effect of six treatments on disease incidence rate compared to control (%)

A : Before the 1st spray ; B : Before the 2nde spray C : Before the 3rd spray D : After one year of implementation of the experimental trial.

**The health status of mango trees in the different treatments**

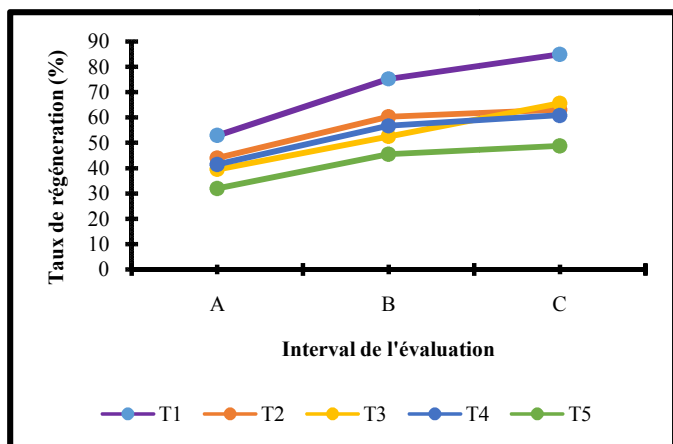
At the end of the experimental test, observations of the different parts of the canopy were made. Depending the health of the leaves of mangoes tree, treatments were divided two categories.

At the end of the experimental trial, T1 and T2 mango trees were not affected by the disease. However, mango trees of T4 and T5 presented the characteristic symptoms of the disease. Indeed, T5 recorded a rate mortality of (50%) of mangoes tree. The Figure3 presents the sanitary evolution of two mango trees respectively from T1 Fig 3(A & B) and T5 Fig 3(C& D).

**The effect of the treatments on the severity of the disease**

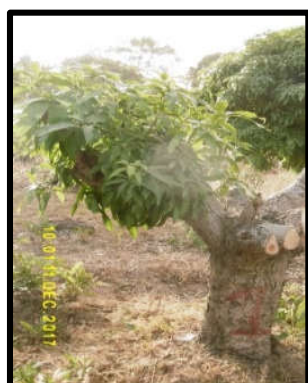
All of the treatments including the control had an initial mean severity score of (S=3,5) before the implementation of the experiment trial. The vegetative growth on branche pruned after three months of the installation of the experimental test, resulted in a considerable reduction of the average severity (S = 2) in the different treatments. At the end of the experimental trial, T1 and T2 had a mean severity score of (S = 1) corresponding to the absence of symptoms on mango trees according to the scale used. The average value of 1,33 collected at T3 level also reflects an acceptable control in reducing the severity of the disease.

The mean severity (S=3,5) of the control remained unchanged and even increased (S=3,83) at the end of the experimental test. It is followed by T4, which recorded at the same period average severities of (S=2,83) (Fig4).



**Figure 1** Average rate of branches with vegetative growth per the four treatments and the control

A: Three month after the implementation of the experimental trial ;B: Six month after the implementation of the experimental trial; C: One year after the experiment.



A



B

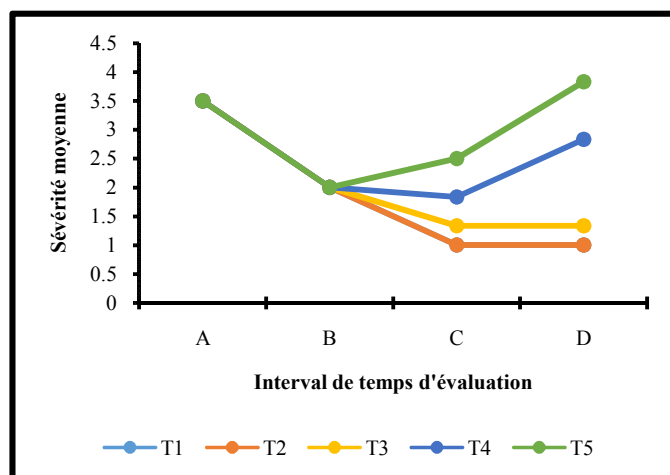


C



D

**Figure 3** Health status of two mango trees during two different periods the experimental test (A&B: Mango tree of T1 without symptoms after respectively 7 and 11 month; C: Mango tree of T5 without symptoms after 7monthD: died of mango tree T5 after 11month.



**Figure 1** The average severity of six treatments during four periods in experimental trials in comparison with the control.

A : Before the implementation of the experimental trial, B : After three months of the experimental test ; C : After ten month of the experimental test ; D : After one year of the experimental test.

According to the results of this first experimental test in Peni, the combinations T1 (Nativo + Manga Plus + NPK ) and (Nativo +Manga Plus) could be effective in strategy to control the disease.

**Second year of the field trial at 2018 to 2019.**

**Vegetative growth :** it was also observed on mango trees of all the treatments from all three localities. After one year of implementation of the experimental trial treatments T1 (Nativo + Manga Plus + NPK) and T3 (Plantsain + Organic Fumure) recorded the highest rates of vegetative growth of mango trees in all the localities. Analysis of the variance of the average rates of vegetative growth at the threshold of (5%) shows no significant difference between these two treatments in each locality concerned. T2 (Nativo + Manga Plus) and T4 (Plantsain + Solsain) were statistically equivalent but different to (T1 and T3). T5 (Control) recorded the lowest vegetative growth rates (24,88 to 34,44%). For each of the four treatments used in the three localities, the lowest values come from the field trial of Pô Fig 5.

**The effect of treatments on the incidence of the disease**

After one year of the experimental trial, characteristic symptoms of the disease were observed in all three localities (Dafinso, Koloko and Pô). Indeed, branches drying and mortalities of mango trees were recorded at the T3, T4 and (T5) control. T3 and T4 enregistred a rate mortality of 33,33% and T5 (50%) in the field trial of Pô. Alone, T5 enregistred mortality of mango tree in Dafinso and Koloko with a rate (16,66%) in these two localities. Leafs necrosis showing the initial symptoms of the disease were observed a few mango trees of T1 and T2 in Pô. In the experimental trials of Koloko and Dafinso, no symptoms of the disease were recorded on the mango trees of these two treatments. The Figure 6 shows the incidences of the disease recorded in four treatments and the control. The Treatments (T4 and T5) and control (T3) showed the highest incidence rates of the disease. However, disease

incidence were zero at the treatments (T1 and T2) in the localities of Dafinso and Koloko Fig 6.

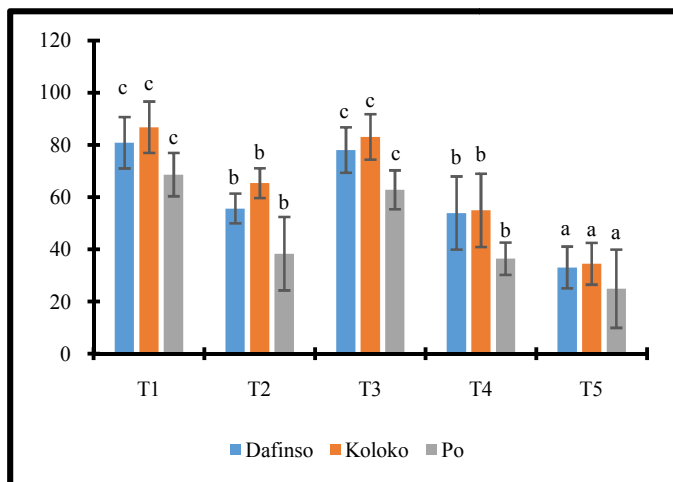


Figure 2 Average rate of vegetative growth after one year per treatment in the three localities. Means followed by same letters in a column are not significantly different according to the test of Duncan at (5%).

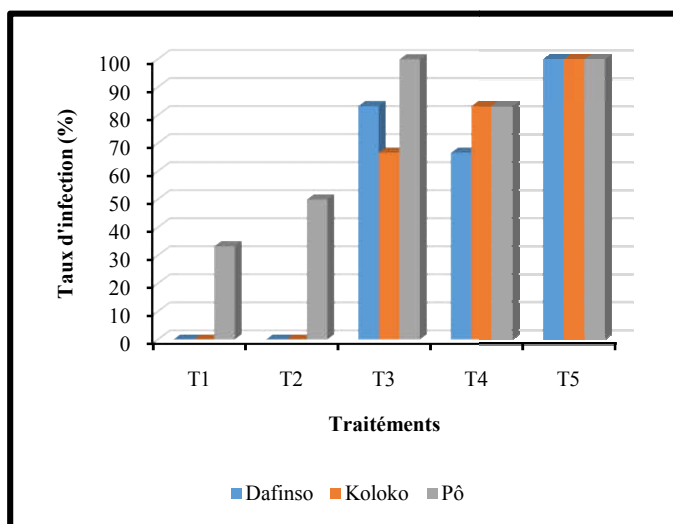


Figure 3 Effect of four treatments on disease incidence rate compared to control (%) in Dafinso, Koloko and Pô. After one year of implementation of the experimental trial.

### The effect of the treatments on the severity of the disease

A reduction in mean severity was recorded at each treatment except for the control. T1 and T2 resulted in a considerable reduction in the mean severity of the disease ( $S = 1$ ) in the fields trials of Dafinso and Koloko. For each of the four treatments including the control, the highest average severity of the disease was observed in the field trial of Pô. Indeed, the mean severities of T1 and T2 were respectively ( $S = 1,5$ ) and ( $S = 2,16$ ) in this locality. T3 and T4 were less effective in reducing the severity of the disease. Their average severity was respectively ( $S = 3,66$  and  $S = 3,5$ ) Fig7.

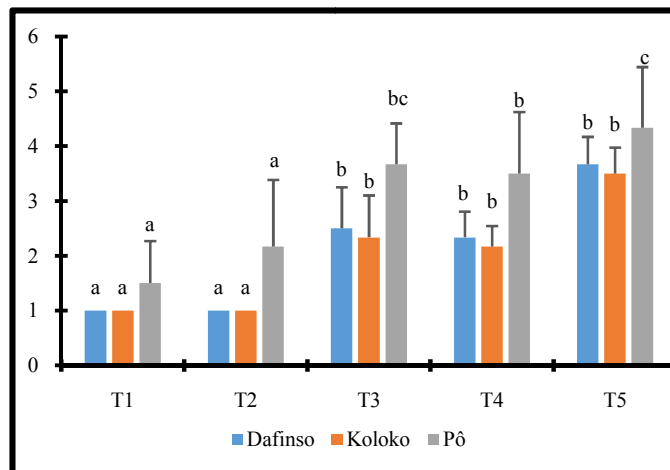


Figure 7 The effect of four treatments compared to the control in reducing the average severity of mango decline in three localities in Burkina Faso. The values followed by the same letter are not significantly different at the 5% threshold according to the Duncan test in the three localities.

## DISCUSSION

### In-vitro antifungal activity of fungicides and biopesticides on mycelial of *L.theobromae*

The results obtained showed an inhibitory effect of all the nine products used on the mycelial growth of *L. theobromae*. However, there was a variability in the inhibition rates between the different treatments depending on the duration of incubation and the two doses used.

The good control of eight products (Neco, Idefix, Manga Plus, Banko, Nativo, Azox, Reference and Proral) would be inherent to the properties of their active ingredients. In this regard, studies have already revealed the efficacy of certain products on a large number of pathogenic fungi including Deuteromycetes and Ascomycetes. Indeed, Ablormeti, (2016) revealed a total effectiveness of three doses of Mancozeb on mycelial growth of *L. theobromae* responsible for mango decline in Ghana.

Tedihou *et al.* (2018) reported the combination of Banko Plus as a systemic fungicide and Mancozeb as a contact fungicide should help to control mango and citrus dieback in Togo. Both fungicides have been shown to be effective against *L. theobromae*, the probable pathogen of the disease in this country.

In Pakistan, no mycelial growth of *L. theobromae* associated with mango stem end rot was observed following the *in-vitro* antifungal test with Carbendazim, Azoxtrobin, (Tebuconazole + Trifloxystobin) and Propiconazol (Seyd *et al.*, 2014).

In the same country, Baloch *et al.* (2017) revealed that Nativo was more effective on *Lasiodiplodia theobromae*, a fungus responsible of the guava decline.

In addition, Neco has a total effectiveness on the mycelial growth of *L. theobromae* from the beginning till 6 DAI in the same way as Idefix and Manga Plus. This *O.gratissimum* essential oil even offered superior efficacy compared to other synthetic products (Banko, Azox, Reference and Nativo). Our results corroborate those Doumbouya *et al.* (2012) who showed that *O.gratissimum* oil has a marked efficacy on

*Pythium* sp. and much greater on the different life stages of *F. oxysporum* compared to callucivire and Banko plus. In addition, this essential oil completely inhibited *Colletotrichum capsici* and *Sclerotium rolfsii* growth at concentrations of 50 and 500 ppm, respectively (Khanna *et al.*, 1991). According to Kassi *et al.* (2014), Neco foliar spray can be an effective means of controlling *Mycosphaerella fijiensis* and can be combined in an integrated control system against banana black leaf streak disease. Nativo, Reference, Banko and Azox are the systemic fungicides used in this study. The active ingredients in these products are divided into three chemical families (Table 2). In general, this group of fungicides inhibits the processes of cellular biosynthesis and is important in curative control against fungi that grow very fast like *L. theobromae* (Corbaz, 1993). The fungicidal activity of triazoles is based on their ability to inhibit the biosynthesis of ergosterol, an essential membrane sterol of fungal strains (Roberts et Hutson., 1999; Onyewu *et al.*, 2003). This inhibition leads to an accumulation of non-demethylated sterols, modifying the shape and the physical properties of the fungal membrane (decrease of the fluidity) and causing in particular a change in the permeability and the malfunctioning of the membrane proteins. Benzimidazoles inhibit parasite mitosis by binding to a microtubule protein preventing their correct union in the spindle (Davidse and Flach, 1978). Strobilurins inhibit electron transfer in mitochondria. This disrupts the energy metabolism of the target fungi and prevents their growth.

They are therefore inhibitors of mitochondrial respiration and therefore of the energy production of cells (Giraud, 2018).

Manga and Idefix are the two contact fungicides tested in this study. Still called preventives, protective or residual fungicides, they attack the pathogen as unspecific, broad-spectrum poisons. They inhibit systems respiratory and enzymatic cellular. Indeed, they act on the spores of the fungi and prevent them to emit filaments penetrating the tissues of the host. Thus, these two fungicides could be used in preventive treatment against *L. theobromae* as well as the other species of fungus associated with mango decline in Burkina Faso (Corbaz, 1993; Dianda *et al.*, 2018). In addition, actives ingredients of Manga and Idefix belongs respectively to dithiocarbamates and mineral fungicides. The first chemical family (dithiocarbamates) acts on many fungi and Oomycetes by blocking three main metabolic processes: fatty acid and nucleic acid biosynthesis and mitochondrial respiration. The copper contained in Idefix can act preventively by inhibiting spore germination and hyphal growth. Nevertheless, it can sometimes acts in a curative way and limits the development of fungi. Neco and Proraly are the essential oils tested. Phenolic compounds of essential oils modify fungal cell permeability by interacting with membrane proteins.

This causes deformation of the cell structure and disrupts their functionality, entraining to loss of macromolecules leading to inhibition of fungal growth (Pramila *et al.*, 2012). Thus, the efficacy of Neco on the three *L. theobromae* strains used in this study is due to the action of thymol phenolic compound, which is the majority of the essential oil of *Ocimum gratissimum* (Kassi *et al.*, 2014). Thymol is known to be toxic

and would target the cytoplasmic membrane and the wall of microorganisms (Uribe *et al.*, 1985).

The Camphor and 1,8-cineole are two constituents of Neco. They can also inhibit the germination of spores and the growth of pathogens (Ishikawa *et al.*, 1986).

This study revealed the total efficacy of Neco on the mycelial growth of *L. theobromae*. This product could effectively replace the other six synthetic products (Banko plus, Azox, Reference, Nativo, Idefix and Manga) used in this study.

#### **Efficacy of treatments in the fields trial**

The results of the first experimental trial in 2017-2018 revealed a gradual reduction of incidence and severity of the disease in T1, T2, T3 and T4. This reduction was even complete after one year of the experimental trial at the treatment level (T1 and T2). These results are almost similar to those obtained at the level of the second experimental trial in 2018-2019 in the localities of Dafinso, Koloko and Pô for treatments (T1 and T2). The fungicides (Nativo and Manga Plus) associated with these treatments would be responsible for the decrease of the disease. These two fungicides were effective in inhibiting the growth mycelial of *L. theobromae in-vitro*. Our results are similar with those of Khanzada *et al.* 2005 which revealed a greater effectiveness of carbendazim followed by thiophanate-ethyl and Alliete to control mango decline. These authors noted that incidence and severity of the disease in mangoes treated with these fungicides had gradually decreased with the number of sprays. After the third spraying, trees treated with carbendazim or thiophanate-methyl gave promising results. In addition, Mahmood *et al.* (2002) noted that the first foliar spray of Topsin-M (thiophanate-methyl) @ 1 gL<sup>-1</sup> reduced *L. theobromae* infestation to 10% and the second spray of the same fungicide inhibited completely the fungus.

Nevertheless, there was a difference between the two fungicides (Nativo and Manga Plus) in controlling mango decline in Burkina Faso. Indeed, we observed characteristic symptoms of disease on new shoots of mango treated only with Manga Plus in the field trial in Peni. These results indicate a weak control of this contact fungicide which active ingredient is a Mancozeb in the management of mango decline. In contrast, the effectiveness of Nativo to control the disease could be due to its systemic properties allowing it to be maintained in the vascular system of the tree. These results corroborate those of (Ablormeti, 2016) who has shown at the end of orchard tests that carbendazim (systemic fungicide) was more effective in reducing the incidence and the severity of mango decline compared to funguran (contact fungicide) in Ghana. Nevertheless, this author believes that repeated applications of contact fungicides at a reduced time interval may help control the disease.

**Efficacy of products of biological origins :** T3 (Plantsain + Organic Fume) and T4 (Plantsain + Solsain) used in the second experimental trial (2018-2019) resulted in a very lowest reduction of incidence and severity of the disease. Dead mango trees were even noted on these treatments in the locality of Pô. Thus, Plantsain and Solsain used in this study as biofungicides would be less effective against this fungus. These results confirm the low rate of inhibition of mycelial



growth of *L.theobromae* that we obtained during the *in-vitro* antifungal efficacy test with Plantsain.

Nevertheless, these two biopesticides were made respectively using the aroma and spores of *Trichoderma harzianum* (www.bioprotect-b.com). *Trichoderma* spp are antagonists of a range of fungi (*Botrytis cinerae*, *Fusarium oxysporium*, *L. theobromae*, etc.) by producing volatile and non-volatile antibiotics that can control them (Dennis and Webster, 1971a et b; Mukhopadhyay et Kaur, 1990; Pan et Bhagat, 2008). In fact, *Trichoderma harzianum* was able to cause an inhibition rate (74,47%) on the mycelial growth of *L. theobromae*, the pathogen responsible of stem end rot in mangoes in India (Rahee *et al.*, 2018). *T. harzianum* isolates tested *in-vitro* resulted in inhibition of mycelial growth of *Aspergillus niger*, *F. oxysporum*, and *Fusarium solani* as the main fungal pathogens found in onions in Burkina Faso (Dabiré *et al.*, 2016). The low efficacy of Plantsain and Solsain found in this study could be related to the conidia of *T. harzianum* incorporated in these two biopesticides. According to Corbaz (2013), the results of biological control in the strict sense depend very much on the strains used as antagonists. About, within a population of a single species, there are considerable differences in the production of antibiotics, the ability to parasitize the pathogen, or even the behavior according to the temperatures.

Also, he reported that difficulties in the conservation of these living microorganisms may occur during storage and influence their ability to germinate, develop and take action as soon as they are transferred to the environment where they can act. About, inhibitory effect of *Trichoderma* spp is related to its ability to produce antibiotics such as trichodermin, trichodermol, harzianum A, harzianolide (Dennis and Webster, 1971a, Kucuk and Kivanc, 2004). Moreover, they are capable of producing cell wall degrading enzymes such as chitinases and glucanases that break down polysaccharides, chitins and the destructing cell wall of beta-glucanase (Elad, 2000). Moreover, the weak control on *L. theobromae* by plantsain and Solsain could also be explained by the mode of application used. Indeed, Dabiré *et al.* (2016) found that the antagonistic power of five isolates of *T. harzianum* on three pathogenic fungus species isolate from onions was higher in direct confrontation (antagonistic coefficient of 0,6 at 0,93) compared to the distance confrontation (antagonism coefficient ranging from 0,01 to 0,21). Thus, they consider that the modes of action of the antagonists and the possible forms of use are important parameters in the context of biological control.

**Agricultural practices in mango decline management :** T5 recorded a of vegetative growth in the two fields trials. This result confirm these of our preliminary study, about advantage of pruning of portions affected. In addition, to the improvement in vegetative growth T1 (Nativo + Manga Plus + NPK) and T2 (Nativo + Manga Plus) led to a considerable reduction in the incidence and severity of the disease. In summary, the fungicide treatments associated with these agricultural practices would contribute to a good management mango decline in Burkina Faso. Indeed, Parkash and Raoof, (1989) reported that the size of the affected parts, followed by the spraying of the Bordeaux mixture in the pruned parts, made it possible to effectively control the disease.

### Low efficacy of treatments in the field trial in the Pô locality

All the treatments showed a low level of control of mango decline in Pô compared to the two other localities (Dafinso and Koloko). These results could be explained by the fungus strains present in this locality that would be resistant to the fungicides applied. Moreover, the mortality rate (50%) confirms the high severity of the disease, which could have a negative impact on the effectiveness of the treatments provided in this locality.

## CONCLUSION

*In-vitro* antifungal tests revealed the inhibitory effect of the ten products tested. Eight products made up of six synthetic fungicides (Banko, Azox, Reference, Nativo, Idefix and Manga Plus) and two essential oils (Neco and Proraly) showed a significant level of control in their rate of inhibition on mycelial growth of *L. theobromae*. From the results of this study, fungicides (Nativo and Manga Plus) and agricultural practices (pruning of portion affected and Cuvettes around mangoes trees) could be used in the management of mango decline in Burkina Faso. Moreover, Neco could be associated with the means of combating of mango decline, as part of an integrated management strategy that respects the environment, the health of the producer and the farmer customer. However, field trial have to carry out with this product or others biological d'origins. These experiment would allow to confirm efficacy of Neco or to choice of other.

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