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# Fungicides and Biological Products Activities towards Fungi Causing Diseases on Banana and Vegetable in Côte d'Ivoire

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## 1. Introduction

Banana and plantain are among important crop playing a major role as respectively exportation crop and foodstuff. Plantain are produced for local consumption and represent the fourth important crop of developing world (Arias *et al.* 2003). African bananas are either grown as a subsistence crop, often in backyard gardens or in intercropped agricultural systems. Besides providing food and nutritional security, bananas, because of their ability to be harvested all year round, produce a surplus and thus constitute an important financial resource for small-scale farmers. On a continental scale, the value of bananas exported from African countries was, in 2005, estimated at US\$ 78 millions (FAO, 2006). However, the majority of the crop (up to 85 %) is normally used for local consumption and marketing (Ploetz and Mourichon, 1999). Banana is an important food for the populations in Côte d'Ivoire. Exports of bananas are established in around 360 000 tons (FAO, 2007). The production areas are almost 6 000 ha (Kobenán *et al.*, 2006). As for plantain, its production is estimated at 1 510 778 tons and it is classified 3<sup>rd</sup> foodstuff behind yam and cassava (FAO, 2007). This production makes Côte d'Ivoire the 3<sup>rd</sup> producer country of plantain in West Africa after Nigeria and Ghana (FAO, 2007), and the principal supplier of the countries in West Africa.

Tomato represents the second vegetable behind the traditional cultivar of eggplant (N'drowa) in Côte d'Ivoire (Ndabalishye, 1995). Tomato is the first condiment in the world according to its alimentary importance and its therapeutics virtues (FAO, 2005; Rao and Agarwal, 2000). The world production of tomato raises 120 millions tons / year according to FAO (2005).

Fungi belonging to *Mycosphaerella* genus cause the most important leaf diseases of banana and plantain (Stover and Simmonds, 1987; Carlier *et al.*, 1996; Koné, 2008) worldwide *Mycosphaerella fijiensis* [anamorph: *Pseudocercospora fijiensis* (Stewart *et al.*, 1999)] causes black

leaf streak disease (black Sigatoka) and *M. musicola* (anamorph : *Pseudocercospora musae*) is the causal agent of yellow Sigatoka (Stover and Simmonds, 1987). *Mycosphaerella musae* causes a leaf speckle disease which is considered of minor importance except in Australia (Stover, 1972). A more recently identified *Mycosphaerella* species, *M. eumusae* (anamorph: *Pseudocercospora eumusae*) is the causal agent of septoria leaf spot of banana (Carlier *et al.*, 2000). Black leaf streak disease caused by *Mycosphaerella fijiensis* is present in all areas where bananas or plantains are grown. The infection process of these *Mycosphaerella* species is similar, except that symptoms develop faster and are more severe on banana infected with *M. fijiensis* and *M. eumusae* than with *M. musicola* (Balint-Kurti *et al.*, 2001). Since it was observed in Fiji in the early 1960s (Rhodes, 1964; Mourichon and Fullerton, 1990), *M. fijiensis* has spread rapidly to new banana- and plantain-growing areas, being the most pathogenic and of greatest concern to both commercial banana growers and in countries where banana and plantain are staple crops. Although yellow Sigatoka has been reported to be a significant problem at higher altitudes and cooler temperatures (Mouliom-Pefoura *et al.*, 1996), most of the banana and plantain production areas in West Africa are located in lower altitude zones characterized by high temperature regimes. Struggle methods for those diseases are pesticides. In Côte d'Ivoire, Banana leaves are also colonized by *Cladosporium musae* causing Cladosporium leaf speckle and disease development is more frequent on Pisang mas or Figue Sucrée (*Musa AA*), (Koné *et al.*, 2006; Koné *et al.*, 2007a). *Deightoniella torulosa* considered as a minor disease is more frequently described on plantain (Koné *et al.*, 2007b). The fungus can alone induce symptoms particularly on plantain (Koné *et al.*, 2007b) and was also described in Savannah (South Georgia) in USA (Kone *et al.*, 2008a, b).

Crop protection by using pesticides is one of the most important ways to increase yield in reducing pathogenic fungi impact.

Foliar disease management strategies are focused on agronomic, use of resistant cultivar and chemicals in simple or integrated approaches. The protectant fungicide Mancozeb is primarily used, while systemic fungicides such as propiconazole (Tilt, Bumper and Aurora), tebuconazole (Folicur) and benomyl (Benlate) may be applied during the wet season.

In banana fields fungicides are used alone or in mixture for foliar disease control. In Côte d'Ivoire several companies are involved in fungicides commercialization (Table 1). Nowadays, *Mycosphaerella* pathogens are controlled by developing an integrated approach to control the pathogens. Disease control strategies are focused on *Mycosphaerella fijiensis*, the most foliar pathogenic fungi in Côte d'Ivoire banana field (Koné *et al.*, 2008c). Several applications of fungicides occur according to the geographic regions base on monitoring program consisting of a field survey to detect resistance. Fungicides application is related to disease evolution.

Fungicides belonging to triazoles (propiconazole, Bumper), benzimidazoles (Peltis), Strobilurin (Trifloxystrobin), Spiroketalamins (Spiroxamine) are the essential products used for the control of banana foliar diseases. There is a concern to control Cladosporium leaf speckle and leaf black spot caused by *Deightoniella torulosa* because any fungicide was recommended and evaluated against *Cladosporium musae* and *Deightoniella torulosa* except the works of Kone *et al.* (2008c) and Camara *et al.* (2007 and 2010). To recommend fungicides, investigations were performed *in vitro* using synthesis and biological fungicides (Camara *et al.*, 2007, Koné *et al.*, 2008c; Camara *et al.*, 2010).

Tomato production is strongly influenced by some viruses, bacteria and soil born fungi in Côte d'Ivoire. The severity of soil born fungi parasites become more important in view of

Groupe	Commercial name	Active compound	quantity	Recommended dose
<b>Systemic, IBS</b>				
<b>Triazoles</b>	Tilt 250 EC/ Bumper 25 EC Référence 250 EC	Propiconazole	250 g/ l	0.4 l/ ha
	Folicur 250 EW-Junior 250 EW	Tébuconazole	250 g/ l	0.4 l/ ha
	Opal 7.5 EC	Epoxiconazole	75 g/ l	1 l/ ha
	Bayfidan 250 OL	Triadimenol	250 g/ l	0.4 l/ ha
	Sico 250 EC Difecor 250 EC	Difénoconazole	250 g/ l	0.3 – 0.4 l/ ha
	Trical 250 EC	Triadimefon	250 g/ l	0.4 l/ ha
	Eminent	Tétraconazole	250 g/ l	0.4 l/ ha
	Punch 40 EC	Flusilazole	400 g/ l	0.25 l/ ha
<b>Benzimidazoles</b>	Peltis 40/ Callis 400 OL			
	Fungis 400 SC	Méthyl- thiophanate	400g/ l	0.7 - 1 l/ ha
	Benlate OD / Flash OD	Bénomyl	500 g/ kg	0.3 - 0.5 kg/ ha
<b>Strobilurines</b>	Bankit	Azoxystrobine	250 g/ l and 0.4 l/ ha	1 l/ ha
	Téga 075 EC	Trifloxystrobine	75 g/ l	1 l/ ha
<b>Spiroketalamines</b>	Impulse 800 EC	Spiroxamine	800 g/ l	0.4 l/ ha
<b>Penetrants, IBS</b>				
<b>Morpholines</b>	Calixine 75 EC	Tridémorphe	750 g/ l	0.6 l/ ha
	Volley 88 OL	Fenpropimorphe		0.5 l/ ha
<b>Protectants</b>				
<b>Anilino- pyrimidines</b>	Siganex 600 SC	Pyriméthanil	600 g/ l	0.5 l/ ha
	Banko 720 SC	Chlorothalonil*	720 g/ l	1 - 2 l/ ha
<b>Dithiocarbamates</b>	Antracol 70 WG	Probineb	70 g/ l	2 - 3 Kg/ ha
	Ivory 80 WP/ Dithane F 448 SC	Mancozèbe*		2 kg/ ha

EC : Emulsionnable EW : Emulsion OL : liquid dissolve in water SC : Concentrated suspension  
OD : Powder disperse in water WG: Autodispersible compounds

\*: Fungicides use for both vegetables and banana diseases.

Table 1. Fungicides recommended to control black leaf streak disease of banana in Côte d'Ivoire

damages in field (Soro *et al.*, 2008). Nevertheless it is difficult to practice gardening market in the tropical countries because there are a lot of pathogens including *Pythium*, *Fusarium* and *Macrophomina* (Soro *et al.*, 2008).

In San José, there was much discussion about the potential of new fungicides, rational ways of using them, the advantages of forecasting systems, and the management of resistance to

fungicides (Jacome *et al.*, 2003). It was emphasized that effective and rational control required a greater knowledge of different aspects of the pathogen epidemiology. The use of fungicides remains the strategy by which other strategies are compared. In the past, the selection pressure by different active ingredients has given rise to the disastrous situation where fungicide-resistant pathotypes are continuously selected. This strategy is no longer acceptable in a society increasingly concerned about the environment (Romero, 2007). The pesticides become more important to control the diseases and pests. There is increasing public concern over the level of pesticide residues in food. The bad utilization of pesticides causes the apparition of some resistant viruses, bacteria and fungi, why it is necessary today to found alternative products to control pests (Rose *et al.*, 2003; Punja, 2003). This concern has encouraged us to look for other solutions instead of synthetic pesticides in the second hand. Recently there has been considerable interest in GRAS (generally regarded as safe) compounds. Naturally occurring biologically active compounds from plants are examples of GRAS compounds. These plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds. This means that essential oils could be used as alternative anti-fungal and anti-bacterial treatments for fresh produce. The potential for these types of plant extracts is considerable. It is a resource that has not been fully explored. In the majority of the tropical countries, forest destruction and agriculture cause a strong reduction of forest cover. The majority of the market gardening is practiced on fallow; what makes the pressure parasitic very strong in tomato culture in Côte d'Ivoire. The use of the natural extracts in the control of the parasites of the cultures will make it possible on the one hand to reduce the degradation of these ecosystems and on the other hand, to improve the vegetable productions (Bakayoko, 2005; Ouattara, 2006; Makumbelo *et al.*, 2008). In order to contribute to the reduction of the degradation of the wooded areas, some woody species were compared to the fungicides assessed *in vitro* and *in vivo* for their antifungal activities against three telluric fungi strains (*Pythium*, *Fusarium* and *Macrophomina*) in tomato culture. Some authors showed that the powder extracts and essential oils are capable to repress the parasites of the cultures (Smolinska and Horbowicz, 1999; Smolinska, 2000; Soro *et al.*, 2008; Soro *et al.*, 2010).

This book chapter is first a contribution about synthetic fungicides and biological fungicides evaluated against foliar fungi of banana and recommended for the control of vegetables diseases by using natural products.

## 2. Material and methods

### 2.1 Material

#### 2.1.1 Pathogenic fungi

##### Banana

Pathogenic fungi of banana including *Mycosphaerella fijiensis*, *Deightoniella torulosa* and *Cladosporium musae* isolated from plantain have been used to assess fungicide and biological products activities.

##### Vegetables

Four fungi including *Pythium aphanidermatum*, *Macrophomina phaseoli*, *Fusarium oxysporum* f. sp. *radicis lycopersici* and *Sclerotium rolfsii* were used. *Pythium aphanidermatum* was characterized by a whitish thalle constituted of non partitioned mycelial filaments (Fig. 1 A and E). *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl) has a purplish red thalle on the PDA

culture medium (Fig. 1 B). To the microscopic level, it presents the macro or microconidies (Fig. 1 F) and of the chlamydospores. *Macrophomina phaseoli* is a fungus that presents a blackish gray thalle on the PDA culture medium (Fig. 1 C). To the microscope, the fungus doesn't present any spores but of the microsclerotes (Fig. 1 G). *Sclerotium rolfsii* present the filaments of flaky aspect of white coloration on the PDA culture medium (Fig. 1 D). These filaments condense to give white mycelia with brown or black coloration sclerotes (Fig. 1 H).

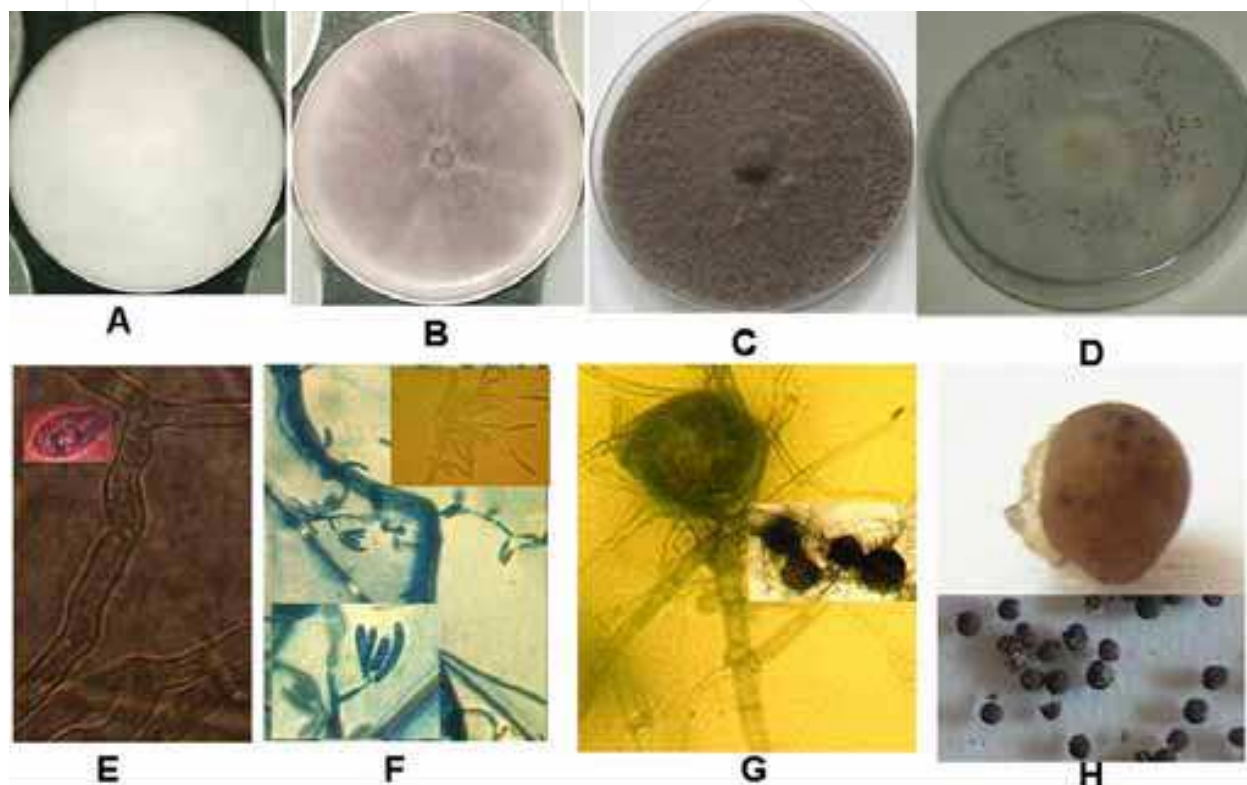


Fig. 1: Fungi isolated from tomato roots and stem at Songon in South Côte d'Ivoire  
A: Thalle of *Pythium aphanidermatum*; B: *Fusarium oxysporum radialis lycopersici*; C: *Macrophomina phaseoli*; D: *Sclerotium rolfsii*; E: Mycelial filament of *Pythium* sp.; F: Conidies of *Fusarium oxysporum* f. sp. *radialis lycopersici*; G: Sclerotes of *Macrophomina phaseoli*; H: Sclerotes of *Sclerotium rolfsii* brown or black.

## 2.1.2 Fungicides products

### 2.1.2.1 Banana

Fungicides used *in vitro* and in field traitements in Côte d'Ivoire used to control *Mycosphaerella* disease were listed in table 1. The essential oils of *Monodora myristica*, *Eucalyptus torelliana*, *Eucalyptus platyphylla*, *Melaleuca quinquenervia*, *Eucalyptus citriodora* and *Ocimum gratissimum* were assessed *in vitro* against *Deightoniella torulosa* and *Mycosphaerella fijiensis*.

### 2.1.2.2 Vegetables

#### Antifungal chemicals

Two fungicides of synthesis have been used during all these experiments; "Banko-Plus" of which the formulation of basis is the chlorothalonil (550 g/ L) more of the carbendazime (100

g/ L) in the dose of 1.5 L/ ha and Ivory 80WP whose chemical formula is the Mancozeb (80%) with the "Callicuire" composed of 56% of cuprous oxide used to the dose of 2 kg/ ha.

#### Antifungal natural products

Ten plants were tested (Table 2). These ten (10) natural extracts were ranged in 3 basic groups: spices plants including *Peper guinense* L., *Xylopi aethiopia* L., *Zingiber officinalis* L.; aromatic plants such as *Ocimum basilicum* L., *Ocimum gratissimum*, *Melaleuca quinquenervia* L. and non spicy and non aromatic plants are *Cola nitida* L., *Cola acuminata* L., *Combretum racemosum* L., *Ricinus communis* L. These plants were used in oil, powder and aqueous extracts formulation.

Scientific name	Family	Part of the used plant	Place of the harvest	Excerpt produces
<i>Xylopi aethiopia</i> (Dunal) A. Rich.	Annonaceae	fruits	Bingerville	powder / essential oil
<i>Peper guinense</i> Schum et Tonn.	Piperaceae	fruits	Bingerville	essential oil
<i>Ocimum basilicum</i> L.	Labiaceae	leaves	Agnibilékro	essential oil
<i>Ocimum gratissimum</i> L.	Labiaceae	leaves	Dabou	essential oil
<i>Melaleuca quinquenervia</i> L.	Myrtaceae	leaves	Abidjan	essential oil
<i>Zingiber officinalis</i> L. (Roscoe)	Zingiberaceae	rhizomes	Divo	powder / essential oil
<i>Combretum racemosum</i> P. Beauv	Combretaceae	leaves	Dabou	powder
<i>Cola nitida</i> (Vent.) Schott & Endl	Sterculiaceae	walnut	Anyama	powder
<i>Cola acuminata</i> (Vent.) Schott & Endl	Sterculiaceae	walnut	Anyama	powder
<i>Ricinus communis</i> L.	Euphorbiaceae	seeds	Abidjan	siccative oil

Table 2. Plant species used for the antifungal tests

## 2.2 Methods

### 2.2.1 Banana

#### 2.2.1.1 Field traitement and observation

Fungicides applications were performed by using air plan. Observations consisted of disease evolution based on symptoms presence on the leaves.

#### 2.2.1.2 *In vitro* activity of fungicides against *Mycosphaerella fijiensis*, *Cladosporium musae* and *Deightoniella torulosa*

Tests were performed first against *Mycosphaerella fijiensis*. Propiconazole was used at recommended concentration (0.4 L/ ha) followed by testing concentrations 1.5 or 2 times

inferior to the recommended one. According to the different concentration, tests were performed with 4 fungicides. Because of total inhibition due to the synthetic fungicides in relation with active ingredient of each product, stock solutions were prepared at 100 ppm., 10 ppm., 1 ppm. After autoclaving media, stock solutions were used to amend media to the following final concentrations: 0.01 ppm., 0.05 ppm., 0.1 ppm., 0.5 ppm. and 1 ppm. Seventeen millilitres of amended media were poured in 9 cm diameter plates. Susceptibility of each fungus was evaluated by measuring mycelium growth of each colony. A mycelium disk of 6-mm-diameter was removed at the edge of the colony and placed at the center of plate containing amended medium. Plates with non amended medium were used as control. Five plates were used for each fungicide. Inhibition rate was estimated. Experimentations were repeated 3 times.

#### 2.2.1.2.1 *In vitro* activity of essential oils against *Mycosphaerella fijiensis* and *Deightoniella torulosa*

In the case of foliar diseases of banana, PDA culture medium was autoclaved and cooled in a water bath to 40 °C. The essential oils were mixed with sterile molten PDA to obtain final concentrations of 1000 ppm, 3000 ppm, 5000 ppm, 7000 ppm and 10000 ppm. The PDA was poured into 9 cm plates (15 ml/ plate).

#### 2.2.1.2.2 Measurement of mycelia growth and germination of the spores

Susceptibility of each fungus was evaluated by measuring mycelium growth of each colony. A mycelium disk of 6 mm diameter was removed at the edge of the colony and placed at the center of plate containing amended medium. Plates with non amended medium were used as control. Five replicate plates were used for each fungicide, natural extracts or essential oils. The plates were incubated at 28 °C and photoperiod 12/ 12. The colony radius was measured every 24 h for *Deightoniella torulosa* and 72 h for *Mycosphaerella fijiensis*, excluding the plug. An average was taken of two measurements made on each plate. The assessment of the inhibition rate was estimated. Experiments were repeated 3 times. The rate of inhibition was calculated using the following formula (Hmouni *et al.*, 1996):

$$\text{Inhibition rate} = (T_0 - E / T_0) \times 100 \quad (1)$$

$T_0$  = mean value of control treatments radial growth

$E$  = mean value of assay treatments radial growth

The minimal inhibition concentration (MIC) is calculated from the smallest concentration that inhibits the mycelial growth of every fungus. The sufficient concentrations to kill the resumption of the mycelial growth have been used to calculate the lethal doses of  $CI_{50}$  and  $CI_{90}$  with the help of the linear equations of inhibition of the mycelial growth according to the reviewed formula of (Paranagama *et al.*, 2003). Experiments were performed three times. Activity of fungicide has been evaluated on spore's germination using spores suspension calibrated to  $10^5$  ou  $10^6$  conidia/ ml. Different dilutions were realized until the final dilution estimated to  $10^2$  conidia/ ml. Eight hundred  $\mu\text{L}$  (800  $\mu\text{L}$ ) of this final dilution were spread out on plates containing amended or non-amended medium and incubated at  $28 \pm 2$  °C. Germination was evaluated in counting 20 spores among those germinating conidia were counted. The rate of germination has been evaluated by the percentage of germinating conidia. For mycelia or conidia germination, the concentration that inhibits 50% was calculated by linear regression.

For *Deightoniella torulosa*, after 21 days of culture growing on various media, each plates according to the concentration of the fungicides is scraped using a curved Pasteur pipette, in



the presence of 10 ml of distilled water. A drop is then taken and assembled between blade and plate evaluated qualitatively to under the optical microscope the presence of spore.

## 2.2.2 Vegetables

### 2.2.2.1 *In vitro* evaluation on mycelial radial growth

Concerning pathogenic fungi of vegetables, five concentrations (1, 2, 4, 6, 8 g/ L or 250, 500, 1000, 2000, 4000 ppm) have been kept either for the excerpts of powder or for the essential oils according to the behavior of the different fungi strains (Neri *et al.*, 2006). A mycelial disk of 5 mm was removed at the edge of the colony and placed in a plate of 9-cm-diameter containing 20 ml of PDA. The set of these plates kept at  $27\pm 2$  °C, under photoperiod 12 h during 7 days (Paster *et al.*, 1993). At the end of the 7<sup>th</sup> day, the plug was transfert on a new medium and the product is fungicide if there is a not repulse mycelial. In the contrary case, product is said fongistatic. This experiment has been repeated 3 times (Neri *et al.*, 2006; Hmouni *et al.*, 1996). Measurement of the mycelia growth is done as describe in the case of banana.

### 2.2.2.2 *In vivo* evaluation of natural products

The seeds of the three varieties of tomato have been treated to the ethanol 70% during 3 minutes then they have been rinsed with the sterile distilled water 3 times during 3 min for every rinsing. Under the hood in presence of a flame, 25 seeds have been deposited on the paper blotter in plates of 11 cm of diameter. The seedling is kept at the steamroom in  $27\pm 2$  °C to the obscurity and watered once per day until the apparition of the leaves (Hibar *et al.*, 2005). To this stage, the seedlings are planted out in containing ferries of the sterile soil of Songon-Dabou and conserved under shelter at the ambient temperature until the transplantation.

The fungal inoculum has been deposited directly all around of the hypogeeal part of the stem of the tomato plant. Five plants out of the ten that account every variety have been inoculated with 10 ml of mycelial solution of each of the three fungi. The seedlings of the same age and the same plant species are planted out in ferries control containing the same soil sterilized two times at 121 °C during 30 min to the pressure of 1.5 bars.

Control with the pesticides were performed using 4 weeks old seedlings.

The synthesis fungicides, Mancozeb and Banko-Plus (the control positive) are used to the concentrations of formulation for the set of the three fungi strains.

In using powder of plant, the non spicy plant extracts (*Cola nitida* and *Combretum racemosum*) was brought to 25 g/ dm<sup>3</sup> whereas those of the plant to spices (*X. aethiopica* and *Z. officinalis*) were brought of 10 g/ dm<sup>3</sup>.

The essential oils (*Xylopi aethiopica*, *Zingiber officinalis* and *Ocimum gratissimum*) are brought all to the concentration of 20 µl/ dm<sup>3</sup>. The seedlings are treated by 20 µl/ dm<sup>3</sup> of the essence homogenized in 100 ml of "Tween 20" and the whole is mixed in 900 ml of distilled water. The treatment consists in watering the root system of the plant with 100 ml of this solution. The death rate has been valued 30 days after the inoculation. The rate of the dead plants served to value the infectious potential of the different fungi. This experience was repeated 3 times.

*2.2.2.2.1 Index of withering (IW) and index of mortality (IM) of the tomato plants in greenhouse were recorded to assess product efficiency.*

The assessment of the symptoms is achieved 30 days after the transplantation of the seedlings (Woo *et al.*, 1996) while being based on a scale of notation of the symptoms

proposed by Vakalounakis & Fragkiadakis (1999) and that understands four active securities of zero to three:

0 : healthy plant,

1 : light yellowing, light rot of the pivot and the secondary roots and rot of the collar,

2: yellowing important of the leaves with or without withering, stunted of the plants, stern rot of the pivot and the secondary roots, rot important of the collar and brown discoloration of the vessels of the stem,

3: dead of the plant.

These notations acted as basis to calculate the index of mortality that corresponds to the average of the securities assigned to the ten plants (number of repetition by elementary treatment). Besides, the percentage of the plants that has a notation of the symptoms superior or equal to 2 is taken like criteria to value the severity of the attacks of *Fusarium*, *Pythium* and *Macrophomina*. The severity of the mycopathogen is valued like follows:

- fungus strain is said very virulent when the index of mortality is between 50 and 100%, the cultivar is said very sensitive;
- fungus strain is said virulent when the index of mortality is consisted between 20 and 50%, the cultivar is said sensitive;
- fungus strain is said fairly virulent when the index of mortality is between 10 and 20%, the cultivar is said moderately resistant;
- fungus strain is said little virulent when the index of mortality is between 5 and 10%, the cultivar is said resistant;
- fungus strain is said very little virulent when the index of mortality is between 1 and 5%, the cultivar is said very resistant;
- fungus strain is said non virulent when the index of mortality is equal to 0 and the cultivar is said immune according to the formula modified of Bambang (1987). The experiment has been repeated three times.

#### 2.2.2.2.2 Dry biomass

The dry biomass foliar and root is valued 60 days after the seedling to the level of the biologic struggle test. The plant matter is put to dry at the steamroom in 105 °C until obtaining of a constant weight on 3 days (Woo *et al.*, 1996).

### 2.3 Stastitcal analysis

Data were first tested for normality and then subjected to analysis of variance (ANOVA). Significant differences between values were determined using Newman-keuls test ( $p < 0.05$ ), following ANOVA. Statistical analysis was performed using SAS V7 and graphs were produced using Microsoft Office Excel.

## 3. Results

### 3.1 Control of Banana foliar diseases

#### 3.1.1 Field disease control strategies against banana leaf spot diseases in Côte d'Ivoire

Disease control by fungicide application varies according to geographic location because of the monitoring program. The number of application of fungicides is also different according the wet or dry season. The number of fungicide application is higher in the wet season. During black sigatoka disease evolution, a number of measures are implemented to ensure

the pathogen. Agronomic practices include plant destruction by burning the leaves in order to reduce the sources of inoculum.

In commercial plantation, foliar disease management is undertaken through combine methods including cultural and chemical practices. Due to the higher cost of chemical and the lack of sufficient knowledge, small holder farmers do not use them. An important diversity of systemic fungicides is available according to their efficacy and the distribution. In Côte d'Ivoire, fungicides used against Sigatoka disease, particularly black leaf streak disease are more utilised. All the fungicides available were only evaluated and homologation is focused on *Mycosphaerella fijiensis*. Resistance to protectant fungicide is lower due to the multisite action of these products. Protectant fungicides are well efficient on pathogen present on the leaves at the moment of their application. The lack of systemic activity make them more efficient during a time exceeding a week. Mancozeb and chlorothalonil belonging to the protectant fungicides groups are particularly used alone or in mixture in the strategy to avoid resistance strains appearance.

Systemic fungicides contain diverse active compounds that can remain 14 days in humid period and 28 days during dry period after application. Fungicides belonging to benzimidazoles are different.

Benzimidazoles containing benomyl (Benlate) and methyl-thiophanate (Peltis, Callis) are the first systemic fungicides used in 70's for the control of black leaf streak disease caused by *Mycosphaerella fijiensis*. The fungicides are unisite inhibitors and resistant strains have been detected in Côte d'Ivoire.

Morpholines represent the second group of systemic fungicides commercialized in 80's and used in Côte d'Ivoire is represented by Tridemorph (Calixine) as active compounds.

Fungicides used are ergosterol biosynthesis inhibitors. Several products possessing different compounds have the same inhibition site that consists to inhibit cytochrom P-450. The inhibition leads to eliminate methyl groupment in position 14.

Products containing propiconazole (triazole fungicide) are systemic and have a good activity. Propiconazole shows more efficacy in June where inoculum development beginning in May could be a factor of severity because of humidity and night temperature elevation.

Fungicides containing Tebuconazole are systemic and can easily spread out on the leaf surface.

Fungicides belonging to strobilurines have two active compounds including azoxystrobin (Bankit) and Trifloxystrobin (Tega). Trifloxystrobin is conidia germination inhibitor. Disease evolution is stopped and the product reduces inoculum level.

Fungicides of Spiroketamine family are considered as a new group recommended to the control of sigatoka disease. Spiroxamine is one of the fungicides having preventive and curative action.

### **3.1.2 In vitro essay**

#### **3.1.2.1 Fungicide effect**

##### *3.1.2.1.1 Inhibition of mycelial growth*

Mycelium growth is inhibited at the recommended concentration that is 0.4 L/ha and also at fungicide amount reaching 1.5 to 2 times inferior to recommended concentration and 1.5 times superior to recommended amount (Fig. 2 A, B and C). The inhibition rate is 100 % for all the fungi whatever the concentration. After 3 weeks, all the plugs that have been inhibited on

media amended with different concentrations of propiconazole did not grow after transplantation on a new medium without fungicide. The inhibition rate of mycelium becomes higher when the product concentration is higher. Media amended with tebuconazole, trifloxystrobin and propiconazole gave higher inhibition rate compare to media amended with spiroxamine (Fig. 2 A and B). A total inhibition of *Mycosphaerella fijiensis* mycelium reaching 100% at 0.5 and 1 ppm that do not cause inhibition in *Cladosporium musae* were observed (Fig. 2 A and B). The inhibition of mycelium growth reaching 50 % is inferior to 0.6 ppm and is 0.14, 0.4 and 0.52 ppm respectively for tebuconazole, trifloxystrobin and propiconazole (Table 3). Spiroxamine gave less inhibition on *Cladosporium musae* and *Mycosphaerella fijiensis* compare to other molecules. Reduction of *Cladosporium musae* mycelium growth can reach 70 to 80% on media amended with trifloxystrobin and propiconazole (Fig. 2 B). Inhibition rate of *Deightonella torulosa* mycelium can reach 15 to 70% and 0.5, 0.63 and 0.72 are the inhibiting concentration that can affect 50% of the mycelial growth.

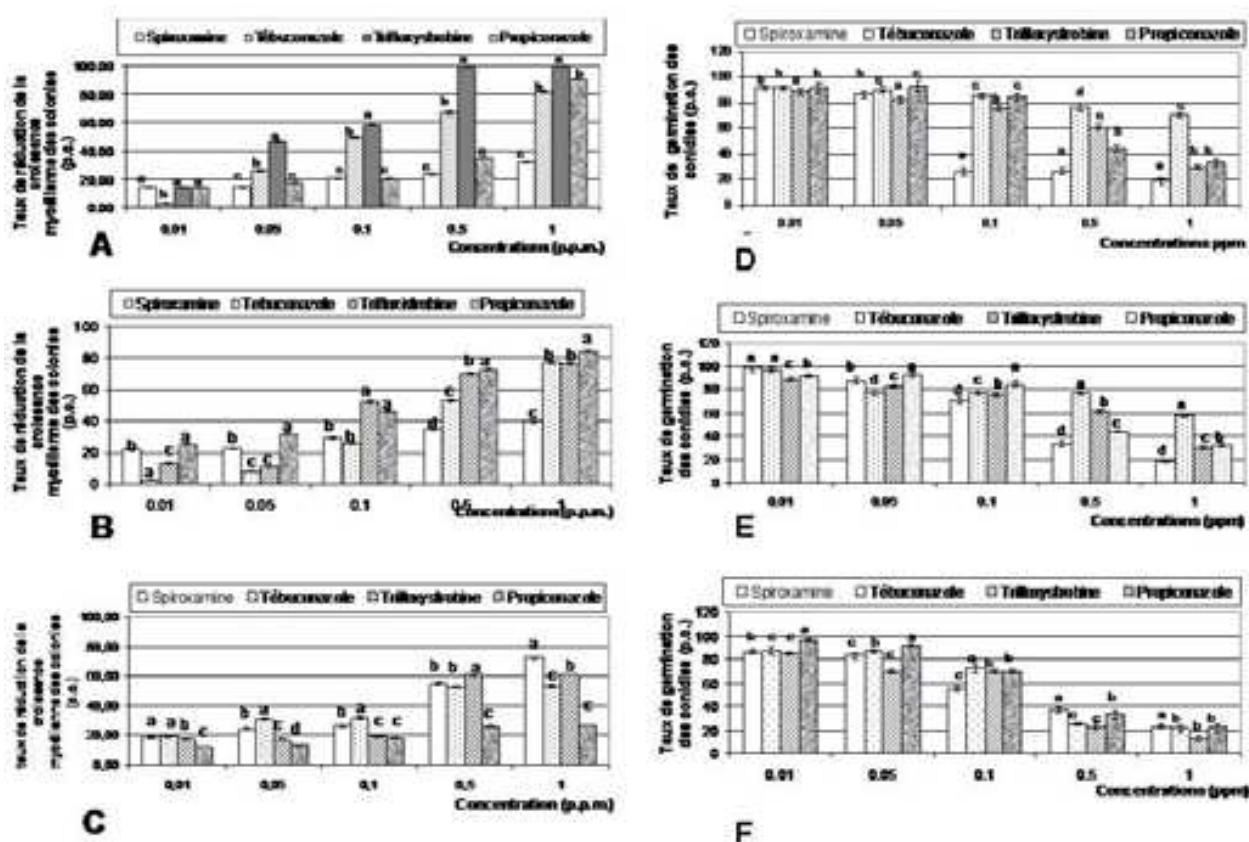


Fig. 2. *In vitro* effect of different molecule on foliar fungi growth and conidia germination A and D : *Mycosphaerella fijiensis* ; B and E : *Cladosporium musae* ; C and F : *Deightonella torulosa*

### 3.1.2.1.2 Activity on conidia germination

In *Mycosphaerella fijiensis* (Fig. 2 D), conidia germination is different according to product concentration. Germination rate can reach 80% at 0.01 and 0.05 ppm. Tebuconazole gave less activity compare to other fungicides. Germination of *Cladosporium musae* conidia depend on the product (Fig. 2 E). Germination is lesser in tebuconazole. Germination rate of *Deightonella torulosa* (Fig. 2 F) conidia can reach 80% at 0.01 and 0.05 ppm. Concentrations of

product required to inhibit 50% of conidia germination are inferior to those inhibiting 50% of mycelium growth.

actives compound	Ci <sub>50</sub> of mycelium growth (ppm)					
	<i>Mycosphaerella fijiensis</i>		<i>Cladosporium musae</i>		<i>Deightoniella torulosa</i>	
	Ci50	R <sup>2</sup>	Ci50	R <sup>2</sup>	Ci50	R <sup>2</sup>
Spiroxamine	2.06	0.91	1.50	0.83	0.54	0.97
Tébuconazole	0.40	0.75	2.32	0.93	0.72	0.77
Trifloxystrobine	0.14	0.71	0.39	0.71	0.63	0.82
Propiconazole	0.52	0.94	0.29	0.89	2.44	0.79

Table 3. Ci50 Values according to the fungicide

### 3.1.2.1.3 *In vitro* activity of essential oils on *Mycosphaerella fijiensis* and *Deightoniella torulosa*

#### 3.1.2.1.3.1 *Mycosphaerella fijiensis*

Essential oils of *Monodora myristica*, *Eucalyptus torelliana*, *Melaleuca quinquenervia*, *Eucalyptus citriodora* and *Ocimum gratissimum*, were compared for their *in vitro* antifungal activity. The inhibitory effects on the mycelial growth of *Mycosphaerella fijiensis* are respectively shown in the fig. 3. *Monodora myristica* essential oil showed a good aptitude to reduce the mycelial growth of *Mycosphaerella fijiensis*. The growths of mycelium were reduced of 50% and 70% after 6 days, respectively for 1000 and 3000 ppm. The amounts of 7000 ppm inhibited strongly the growth of the fungus (Fig. 4 A) with a rate of 100% during the 18 first days and 97% three days later. At 10000 ppm, there was a total inhibition (100%) during all the experiment. The CI<sub>50</sub> parameter which corresponds to the amount inhibiting for half the mycelial growth, is 744.046 ppm (Table 4). The essential oil of *Eucalyptus torelliana* showed high fungitoxic activity on the mycelial growth with the concentrations of 5000, 7000 and 10000 ppm. These amounts allowed a reduction ratio of growth superior to 70%. A moderate toxicity was observed with 3000 ppm which reduced the growth of half 17 days after. At 1000 ppm this oil is slightly fungitoxic. With this last concentration, the growth was reduced of 30% six days after (Fig. 4 B). The CI<sub>50</sub> was 3158.900 ppm (Table 4). The essential oil of *Melaleuca quinquenervia* showed a good aptitude for the reduction of the mycelial growth of *M. fijiensis* for the whole of the concentrations used (Fig. 4 C). The rates of inhibition vary between 60 and 100% according to time and of the concentrations. The strongest concentrations (5000, 7000 and 10000 ppm) completely inhibited the mycelial growth during the first 15 days after the start of the experiment. From this date, the mycelial growth slightly restarts. This one was reduced by 60% the 6<sup>th</sup> day for the concentration of 1000 ppm. CI<sub>50</sub> is equal to 738.58 ppm (Table 4). Essential oils of *Eucalyptus citriodora* and *Ocimum gratissimum* inhibited all the mycelial growth of *M. fijiensis* at all the concentrations (Fig. 4 D and E).

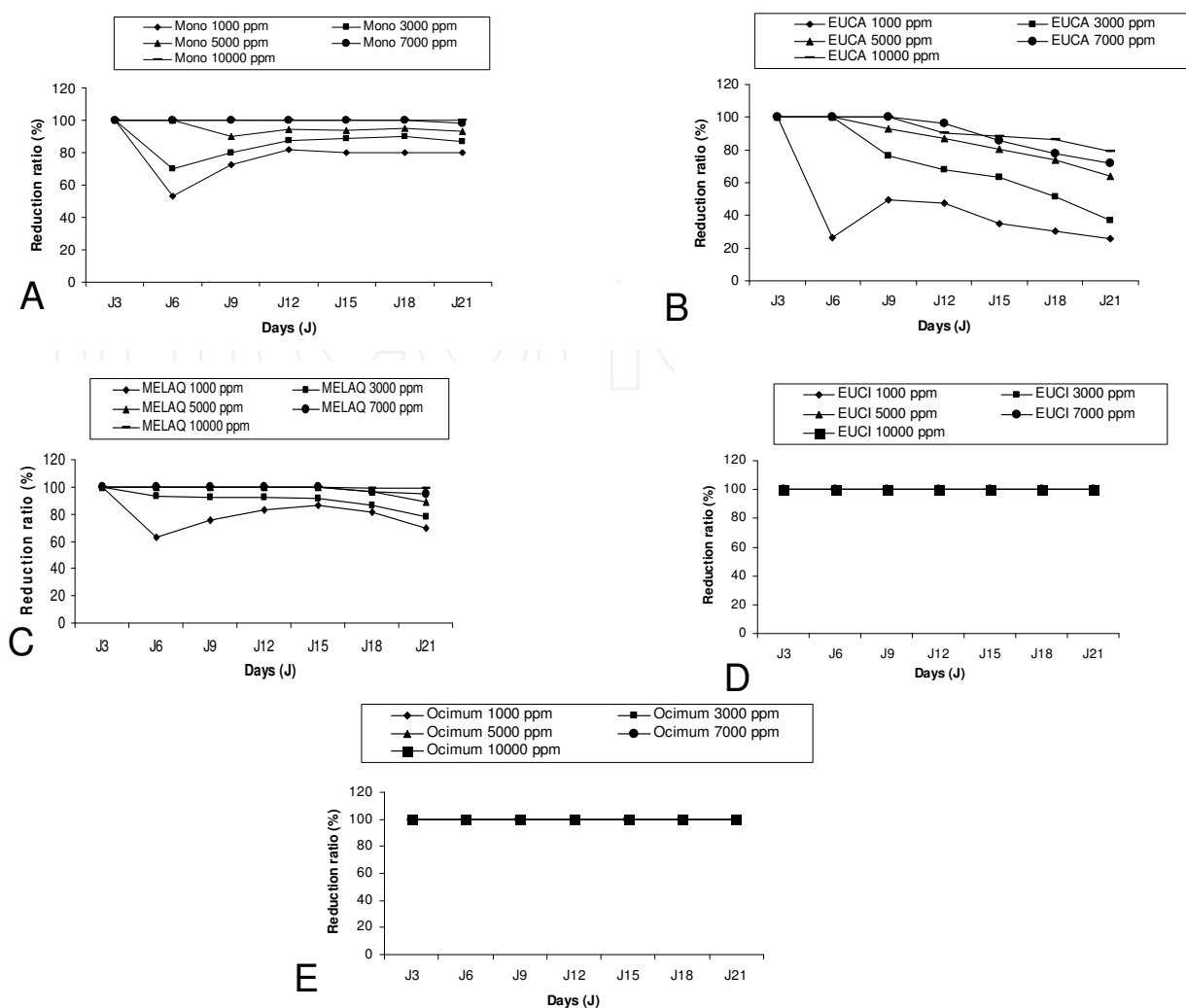


Fig. 3. Reduction of the mycelial growth of *Mycosphaerella fijiensis* at different concentrations of *Monodora myristica* (A), *Eucalyptus toreilliana* (B), *Melaleuca quinquenervia* (C), *Eucalyptus citriodora* (D) and *Ocimum gratissimum* (E) essential oils

### 3.1.2.1.3.2 *Deightoniella torulosa*

Antifungal activity of essential oils is different from one and another. The essential oil of *Monodora myristica* was shown fairly fungitoxic with the concentrations of 5000, 7000 and 10000 ppm (Fig. 4 A). For each one of the last amounts, 50% of reductions of the mycelial growth are respectively obtained the 11<sup>th</sup>, 13<sup>th</sup> and 15<sup>th</sup> day. This essential oil is slightly fungitoxic with the concentrations of 1000 and 3000 ppm. With these concentrations, 50% of reductions of the mycelial growth are reached respectively to the 2<sup>nd</sup> and 8<sup>th</sup> day. The  $CI_{50}$  at the 14<sup>th</sup> day is of 23 860 ppm and  $CI_{90}$  at the same time is of 40450 ppm (Table 5). With the essential oil of *Ocimum gratissimum*, the mycelial growth was inhibited to 100% with the concentrations higher or equal to 3000 ppm (Fig. 4 B). The fungus grows on the culture medium with essential oil at the concentration of 1000 ppm, and filled all surface of the plate at the 17<sup>th</sup> day of the experiment. A reduction of 50% of the mycelial growth was reached to the 12<sup>th</sup> day.  $CI_{50}$  at the 14<sup>th</sup> day is of 2020 ppm and  $CI_{90}$  at the 14<sup>th</sup> day is of 14940 ppm. No mycelial growth is observed with the essential oil amounts higher or equal to 3000 ppm. The mycelial fragments taken in limp of plates to this last concentration and transferred on a

new culture medium PDA start again their growth. The rate of resumption of mycelial growth was 66.7% after 24 h, it reached 100% 48 hours after their transfer. The essential oil amounts higher than 3000 ppm do not allow a resumption of growth of the mycelium once this one transferred on new culture medium PDA.

The essential oil of *Melaleuca quinquenervia* showed a good aptitude for the reduction of the mycelial growth of *Deightoniella torulosa* for the whole of the concentrations used (Fig. 4 C). The rates of inhibition vary from 0 to 100% according to time and from the concentrations. The mycelial growths were reduced by 50% at the 11<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> days respectively for the concentrations of 1000, 3000 and 5000 ppm. The amounts of 7000 ppm and 10000 ppm have a marked effect on the reduction of the fungus growth with a rate of inhibition higher than 50%. The 14<sup>th</sup> day of the culture in the presence of essential oil of *Melaleuca quinquenervia* have  $CI_{50}$  of 7997 ppm and  $CI_{90}$  is equal to 19383 ppm (Table 5). The essential oil of *Eucalyptus platyphylla* was shown highly fungitoxic on the mycelial growth at the concentrations of 7000 and 10000 ppm. These amounts made it possible to obtain a reduction ratio of growth superior to 50%. An average toxicity, observed with 5000 ppm, reduced the growth of half, at the 16<sup>th</sup> day. At 1 000 and 3000 ppm this oil is slightly fungitoxic. With these last concentrations, the growth was reduced 50% respectively at the 3<sup>rd</sup> day and the 10<sup>th</sup> day (Fig. 4 D). The 14<sup>th</sup> day, the  $CI_{50}$  and  $CI_{90}$  are respectively 12256 and 20 080 ppm. *Deightoniella torulosa* is thus more sensitive to the essential oils of *Melaleuca quinquenervia* than with that of *Eucalyptus platyphylla*.

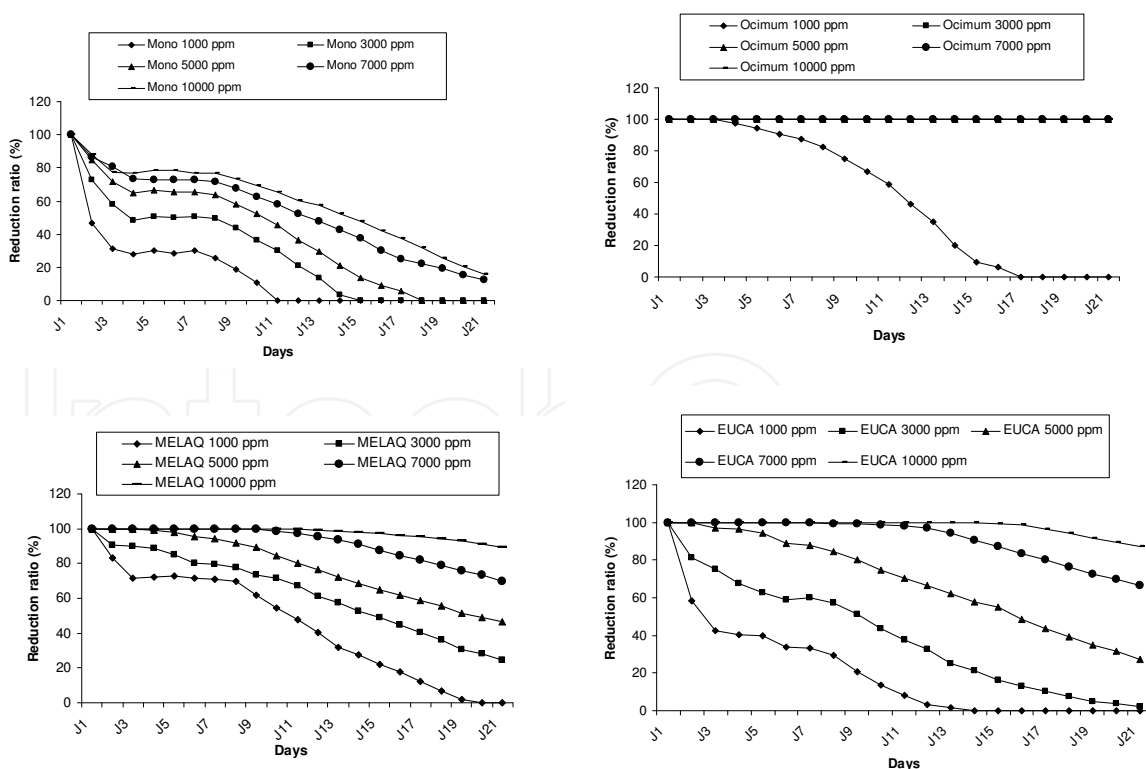


Fig. 4. Reduction of mycelial growth of *Deightoniella torulosa* according to time and the concentrations of *Monodora myristica* (A), *Ocimum gratissimum* (B) *Melaleuca quinquenervia* (C) and *Eucalyptus platyphylla* (D) essential oils

Antifongic parameters	Essential oils			
	<i>Melaleuca</i>	<i>Eucalyptus</i>	<i>Ocimum</i>	<i>Monodora</i>
CI <sub>50</sub> (ppm)	7 997 a	12 256 b	2 020 a	23 860 b
CI <sub>90</sub> (ppm)	19 383 a	20 080 b	14 940 a	40 450 b

NB: On the same line, the concentrations followed by the same letters has, a and b are not significantly different with  $p < 0.05$  according to the test of Newman-Keuls.

Table 5. Concentrations of essential oils (in ppm) reducing 50% (CI<sub>50</sub>) and 90% (CI<sub>90</sub>) the mycelial growth at the 14<sup>th</sup> day of incubation of *Deightoniella torulosa* on the cultures medium.

### 3.1.2.1.3 Conidia production

The essential oil of *Ocimum gratissimum* at all the concentrations prevents the production of the spores of *Deightoniella torulosa*. The essential oil of *Monodora myristica* is ineffective on the production of spores at all the concentrations. As for those resulting from *Melaleuca quinquenervia* and *Eucalyptus platyphylla* essential oils, they prevented the sporulation of the fungus at the concentrations of 7000 and 10000 ppm (Table 6).

Essential oils	Concentrations (ppm)				
	1000	3000	5000	7000	10000
<i>Ocimum gratissimum</i>	-	-	-	-	-
<i>Monodora myristica</i>	+	+	+	+	+
<i>Melaleuca quinquenervia</i>	+	+	+	-	-
<i>Eucalyptus platyphylla</i>	+	+	+	-	-

+ means that there is presence of spores  
- means that there is absence of spores

Table 6. Production of spores after 21 days on medium culture PDA containing essential oils at various concentrations

## 3.2 Control of vegetable diseases

### 3.2.1 *In vitro* effects of fungicides, natural extracts and essential oils on soil born pathogens

#### 3.2.1.1 Mancozeb

The rate of inhibition of mancozeb illustrated by table 6 shows a low sensitivity of *Fusarium* compared to *P. aphanidermatum* and *M. phaseoli* to the concentrations lower than 3 g/ L. The threshold of significance is largely exceeded to 1 g/ L for *Pythium* and *Macrophomina* whereas it is reached only with 1.2 g/ L at *Fusarium*. All the 3 fungi strains are completely



inhibited for the concentrations higher than 2 g/ L and no other resumption of mycelial pastille was observed. The rate of germination of the spores of *Fusarium* is null for all the inhibiting concentrations at 100%. The mancozeb is thus well a fungicide of synthesis for these three mycopathogens.

### 3.2.1.2 Banko-Plus

The rate of inhibition of Banko-Plus illustrated by table 6 shows a strong sensitivity of *Fusarium* compared to *P. aphanidermatum* and *M. phaseoli* to the concentrations lower than 1000 ppm. The threshold of significance is largely exceeded with 2000 ppm for the three fungi. The rate of inhibition to 100% is reached with 4000 ppm for the whole of the fungi. All the concentrations higher than 4000 ppm completely inhibited the resumption and the growth of the mycelial pastilles. The rate of germination of the spores of *Fusarium* is null for all the inhibiting concentrations at 100%. Banko-Plus is thus well a fungicide of synthesis for these three mycopathogens.

### 3.2.1.3 Aqueous extract of *Cola nitida* (Vent.) Schott & Endl (Sterculiaceae), *Cola acuminata* (Vent.) Schott & Endl (Sterculiaceae) and *Combretum racemosum* P. Beauv (Combretaceae)

The aqueous extract of *C. nitida* inhibits in various manners the mycelial growth of the three fungi. The minimum threshold of significance (25%) is reached for *Pythium* and *Fusarium* with 10 g/ L. The inhibiting minimal concentration (MIC) with 50% is reached to 20 g/ L and no fungus was inhibited at 90% by this aqueous extract. The aqueous extract of *C. nitida* is more effective on *Fusarium* than the two others fungi and this difference is significant at 15 and 25 g/ L. *Cola nitida* spring like fongistatique and not a fungicide for the whole of these three fungi (Table 6).

The aqueous extract of *Cola acuminata* is significantly effective on *Macrophomina* with the concentration of 15 g/ L. As for *Fusarium* and *P. aphanidermatum* they did not raise any inhibiting concentration with the threshold of significance (25%). No concentration could inhibit the mycelial growth of only one fungus with 50%. For all the concentrations lower or equal to 15 g/ L, the aqueous extract of *C. acuminata* is rather activator of the mycelial growth of *Pythium* and *Fusarium*.

The simultaneous repiquate of the mycelial pastilles of the three mycopathogenes in the Petri dishes with various concentrations of *Combretum racemosum* shows a stronger inhibition at *Fusarium* compared to *P. aphanidermatum* and *M. phaseoli* (Table 6). The aqueous extract of *C. racemosum* is significantly effective on *Fusarium* with the concentration of 0.7 g/ L. As for *M. phaseoli* and *P. aphanidermatum* they have minimal concentrations of 0.9 g/ L and 1.9 g/ L respectively. The rate of inhibition at 100% is reached to 6 g/ L for the strains of *Fusarium* and *P. aphanidermatum*; on the other hand *M. phaseoli* reaches his rate of maximum inhibition to 8 g/ L. Beyond 6 g/ L all the 3 fungi strains are completely inhibited and no other resumption of mycopathogen is noted (Table 6).

The minimal inhibiting concentrations (MIC) and the CI<sub>50</sub> and CI<sub>90</sub> vary between 0.03 g/ L to 0.52 g/ L for the MIC and between 0.25 to 1.26 g/ L for the CI<sub>50</sub> and 2.25 to 3.54 for the CI<sub>90</sub>. The fungi strains of *Pythium*, *Fusarium* and *Macrophomina* have a very significant sensitivity with respect to the aqueous extract of *Combretum racemosum*.

The aqueous extract of *Combretum racemosum* can thus be used as well like a fungicide just like antifongistatique for these three mycopathogens.

### 3.2.1.4 Powders fruits of *Xylopiæ aethiopia* (Dunal) A. Rich. (Annonaceae) and the rhizomes of *Zingiber officinalis* L. (Roscoe) (Zingiberaceae)

The inhibiting capacity of the powder of the fruits of *Xylopiæ aethiopia* varies from 30 to 60% for the smallest concentration of 1 g/ L (Table 6). The inhibition of the mycelial growth increases with the concentration of the extract for all fungi. *Pythium aphanidermatum* has a very strong sensitivity to the powder of the fruits of *X aethiopia*. Indeed, it is the only strain which was inhibited with more than 90% (25 g/ L).

No lethal inhibiting concentration was given with the powder of the fruits of *Xylopiæ* against the whole of these mycopathogens. The inhibiting minimal concentrations (MIC) and the inhibiting concentrations with 50% and 90% were given to 1 g/ L; 4.6 g/ L and 12.4 g/ L respectively. The fungi strains of *Pythium*, *Fusarium* and *Macrophomina* thus have a significant sensitivity with respect to the powder of the fruits of *X aethiopia*.

The minimal inhibiting concentrations (MIC) of *Z. officinalis* with the threshold of significance (25%) vary from 5 to 10 g/ L for *Pythium*, *Fusarium* and *Macrophomina*

Products	Mycopathogens	Mycelia growth		
		MIC (g/ L)	CI <sub>50</sub> (g/ L)	CI <sub>90</sub> (g/ L)
Mancozeb	<i>P. aphanidermatum</i>	< 0.5 mg/ L	1 mg/ L	0.70
	Forl	0.69	1.53	3.34
	<i>M. phaseoli</i>	< 0,5 mg/ L	< 0.5 mg/ L	< 1 mg/ L
C. <i>racemosum</i>	<i>P. aphanidermatum</i>	0.52	1.26	3.04
	Forl	0.03	0.25	2.25
	<i>M. phaseoli</i>	0.17	0.78	3.54
Z. <i>officinalis</i>	<i>P. aphanidermatum</i>	1.24	3.63	10.61
	Forl	1.49	4.61	14.28
	<i>M. phaseoli</i>	1.42	4.55	14.49
C. <i>nitida</i> <i>rouge</i>	<i>P. aphanidermatum</i>	0.57	5.08	> 25
	Forl	0.59	3.04	15.80
	<i>M. phaseoli</i>	1.04	5.84	> 25
X. <i>aethiopia</i>	<i>P. aphanidermatum</i>	0.94	2.09	4.66
	Forl	1.25	3.82	11.67
	<i>M. phaseoli</i>	1.20	3.10	7.98

Table 6. Minimal inhibiting concentrations (MIC), CI<sub>50</sub>, CI<sub>90</sub> (g/ L) of the natural extracts and mancozeb on *Pythium aphanidermatum*, *Fusarium. oxysporum* f. sp. *radicis-lycopersici* and *Macrophomina phaseoli*

respectively. The rough extract of *Z. officinalis* could not inhibit only one fungus at 90%. On the other hand all the fungi are inhibited with more than 50% with the concentration of 20 g/ L. The fungi strains *Pythium*, *Fusarium* and *Macrophomina* thus have a significant sensitivity with respect to the rough extract of *Z. officinalis* at the strong concentrations (Table 6).

### 3.2.1.5 Siccative oil of the fruits of *Ricinus communis* L. (Euphorbiaceae)

The siccative oil of *R. communis* is not active to 5 ml/ L on the stocks of *P. aphanidermatum*, *Fusarium* and *M. phaseoli*. The significant minimal inhibiting concentration for the fungi is at 15 ml/ L. All the fungi were resistant to the siccative oil of *R. communis* for all the concentrations. Minimal inhibiting concentration (MIC) raised is obtained to 10 ml/ L. The inhibiting concentrations with 50% are obtained with the strongest concentration (25 ml/ L) and no concentration is raised to 90%. The sensitivity of these fungi to the siccative oil of *R. communis* is very low. It is nonfungicidal oil for these fungi (Table 6).

## 3.2.2 Essential oils

### 3.2.2.1 *Xylopiya aethiopica*

The table 6 illustrates the rate of inhibition of the mycelial growth of *Pythium*, *Fusarium* and *Macrophomina* by the essential oil of *X. aethiopica*. The inhibition of the mycelial growth increases with the concentration. The essential oil presents a total inhibition of *Fusarium* and *Pythium*. On the other hand *Macrophomina* could not be inhibited completely by the essential oil of *X. aethiopica*. Indeed, the mycelial pastilles of *Fusarium* and *Pythium* could not take again their growth in the box of Petri to the concentration of 4000 ppm and even after its transfer on a neutral culture medium. The lethal inhibiting concentration thus determines the fungicidal capacity of the essential oil of *Xylopiya* against the mycopathogens *Fusarium* and *Pythium*.

The weakest inhibiting concentrations with 50% and 90% were given with 94 ppm and 2761 ppm. The fungi strains *Fusarium*, *M. phaseoli* and *Pythium* thus have a very significant sensitivity with respect to essential oil of *X. aethiopica* (Table 7).

### 3.2.2.2 *Peper guinense*

The inhibition of the mycelial growth only increases with the concentration of the oil for the fungus *Pythium aphanidermatum*. The essential oil presents a total inhibition of *Pythium* at 2000 ppm. On the other hand *Fusarium* and *Macrophomina* could not be inhibited completely by the essential oil of *P. guinense*. Indeed, no mycelial resumption of *Pythium* was raised in the boxes of Petri to the concentrations of 2000 and 4000 ppm and even after its transfer on a neutral culture medium. The essential oil of *P. guinense* is thus fungicidal for the mycopathogen *P. aphanidermatum*.

The minimal inhibiting concentrations (MIC) vary from 100 ppm to 200 ppm. The inhibiting concentrations with 50% and the weakest 90% are obtained with *Pythium*. Those (CI<sub>50</sub> and CI<sub>90</sub>) obtained with *Fusarium* and *Macrophomina* are given at 250 ppm. All the fungi strains were inhibited with more than 75% to 2000 ppm. The sensitivity of these fungi is thus very significant with respect to the essential oil of *Peper guinense* (Table 7).

### 3.2.2.3 *Zingiber officinalis*

No fungus was completely inhibited. However, *Pythium* was very significantly inhibited (75%) by the essential oil of *Z. officinalis*. The essential oil of *Z. officinalis* is thus not

fungicidal for the mycopathogens *P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli*.

The table 7 presents the minimal inhibiting concentrations (MIC), the CI<sub>50</sub> and CI<sub>90</sub> which were given with 270 ppm, 350 and with 1050 ppm respectively for *Pythium*. *Pythium aphanidermatum* is the only strain which raised a CI<sub>50</sub> with 500 ppm. All the fungi strains were inhibited to 50% only that with 4000 ppm. The sensitivity of *Pythium* is very significant with respect to the essential oil of *Z. officinalis*; on the other hand *Fusarium* and *Macrophomina* are significantly less sensitive to this oil. Indeed, no inhibiting concentration with 75% was raised for two fungi with essential oil and no fungus strain presented a CI<sub>90</sub>.

#### **3.2.2.4 *Melaleuca quinquenervia***

All the three fungi were inhibited completely by the essential oil of *M. quinquenervia*. On the other hand, it is only at *Pythium* that no resumption of the mycelial growth was observed.

The minimal inhibiting concentrations (MIC) vary from 100 ppm with 200 ppm. The inhibiting concentrations with 50% and the weakest 90% are obtained with *Pythium*. Those (CI<sub>50</sub> and CI<sub>90</sub>) obtained with *Fusarium* and *Macrophomina* are given with 600 ppm and 4000 ppm respectively. All the fungi strains were inhibited with more than 75% to 2000 ppm. The sensitivity of these fungi is very significant with respect to the essential oil of *Melaleuca quinquenervia* (Table 7).

#### **3.2.2.5 *Ocimum basilicum***

All the strains were inhibited completely by the essential oil of *Ocimum basilicum*. However, all the mycelial pastilles took again their growth. The essential oil of *O. basilicum* is thus fungistatic for these three fungi. The essential oil of *Ocimum basilicum* is thus not fungicidal for the mycopathogens *P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli*.

The minimal inhibiting concentrations (MIC) are all lower than 200 ppm. The inhibiting concentrations with 50% and the weakest 90% are obtained with *Pythium*. The CI<sub>50</sub> and CI<sub>90</sub> obtained with *Fusarium* and *Macrophomina* are given below 500 ppm and only *Pythium* could reach the CI<sub>90</sub> (<4000 ppm). All the fungi strains were inhibited with more than 75% to 2000 ppm. The sensitivity of these fungi is thus very significant with respect to the essential oil of *Ocimum basilicum* (Table 7).

#### **3.2.2.6 *Ocimum gratissimum***

No significant difference was raised between the five concentrations for all the three fungi strains. The very significant threshold of inhibition (75%) is reached starting from the smallest concentration 250 ppm.

In addition, no resumption of the mycelial growth was observed at only one stock. The minimal inhibiting concentrations (MIC) are all lower than 100 ppm. The inhibiting concentrations with 50% and 90% are largely exceeded with the weakest concentrations (250 ppm). The threshold of inhibition very highly significant (75%) is reached by all the fungi strains with 250 ppm. The sensitivity of these fungi is thus very significant with respect to the essential oil of *Ocimum gratissimum*.

In addition, No resumption of the mycelial growth was observed at only one stock. The essential oil of *O. gratissimum* is fungicidal for *Pythium aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli* (Table 7).

		Mycelia growth		
	Mycopathogens	MIC (ml/ L)	CI <sub>50</sub> (ml/ L)	CI <sub>90</sub> (ml/ L)
<i>X. aethiopica</i>	<i>P. aphanidermatum</i>	2 µl	0.06	1.58
	Forl	0.05	0.46	3.77
<i>P. guinense</i>	<i>M. phaseoli</i>	7 µl	0.05	3.93
	<i>P. aphanidermatum</i>	0.86	1.84	3.91
	Forl	0.87	2.29	5.99
	<i>M. phaseoli</i>	0.90	2.33	6.01
<i>Z. officinalis</i>	<i>P. aphanidermatum</i>	0.56	1.58	4.43
	Forl	3.04	7.07	16.44
	<i>M. phaseoli</i>	1.55	9.04	52.92
<i>M. quinquenervia</i>	<i>P. aphanidermatum</i>	5 µl	0.13	3.08
	Forl	0.06	0.60	6.37
	<i>M. phaseoli</i>	0.26	1.04	4.12
<i>O. basilicum</i>	<i>P. aphanidermatum</i>	0.02	0.29	5.39
	Forl	0.11	1.03	9.63
	<i>M. phaseoli</i>	8 µl	0.27	9.77
<i>O. gratissimum</i>	<i>P. aphanidermatum</i>	5 µl	0.11	2.25
	Forl	< 1 µl	< 1 µl	0.01
	<i>M. phaseoli</i>	< 1 µl	< 1 µl	< 1 µl
Banko Plus	<i>P. aphanidermatum</i>	0.01	0.15	1.98
	Forl	0.24	0.87	3.19
	<i>M. phaseoli</i>	0.03 µl	6 µl	1.20

Table 7. Minimal inhibiting concentrations (MIC), CI<sub>50</sub>, CI<sub>90</sub> (g/ L) of the essential oils and Banko-Plus on *Pythium aphanidermatum*, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Macrophomina phaseoli*

### 3.2.3 *In vivo* effects of fungicides, natural extracts and essential oils on soil born pathogens

#### 3.2.3.1 Effect of Mancozeb on diseases

The transplantation of the tomato seedlings in the substrate treated by the mancozeb and inoculated by each of the three mycopathogens (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis lycopersici* and *M. phaseoli*) generated a weak attack of the latter (Table 8). Indeed the index of disease is practically null for the three isolates at the three cultivars except *Pythium* on the level of the cultivars Caraïbo (0.1) and Tropimech (0.1); nevertheless the percentage of the seedlings having a higher notation of the symptoms or equalizes to two is null. The fungicide of synthesis significantly reduced the index of the diseases (rot pythienne, fusariose and black rot) on the tomato seedlings.

The effect of mancozeb on the dry biomass of the seedlings of tomato and/ or inoculated and/ or treated is consigned in table 4. The biomass produced during the treatment of the fusariose by the mancozeb is a function of the variety of tomato. Mongal presents the strongest foliar biomass for all the treatments. All the inoculated seedlings and treated have a biomass higher than that of inoculated and the seedlings inoculated with Forl ( $6.53 \pm 0.31$  g) and treated produced the strongest biomass. The seedlings inoculated for all the three varieties produced a biomass significantly reduced compared to the healthy control or positive control.

The mancozeb reduced the impact of fungi on the dry biomass root on the level of the treated inoculated seedlings. Indeed, all the treated inoculated seedlings gave a biomass root higher than that of the seedlings inoculated at all the cultivars. The dry biomass root produced at Tropimech is significantly lower than that of Mongal and Caraïbo.

### 3.2.3.2 Effect of Banko-Plus on diseases

The transplantation of the tomato seedlings in the substrate treated by Banko-Plus and inoculated by each of the three mycopathogenes (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis lycopersici* and *M. phaseoli*) generated a weak attack of the latter (Table 8). The index of the diseases varies according to the fungi and the cultivars. It is null at Mongal and is higher at Tropimech (0.2) with Pythium and Forl (0.6). The percentage of the seedlings having a higher notation of the symptoms or equalizes to two is null for all the cultivars. The fungicide of synthesis significantly reduced the incidence of the fusariose and entailed a null mortality of the tomato seedlings at all the cultivars.

The biomass produced during the treatment of the fusariose by Banko-Plus is a function of the variety of tomato. Mongal presents the strongest foliar biomass for all the treatments. All the inoculated seedlings and treated have a biomass higher than that of inoculated and the seedlings inoculated with Forl ( $5.59 \pm 0.18$  g) and treated produced the strongest biomass. The seedlings inoculated for all the three varieties produced a biomass significantly reduced compared to the healthy or positive control.

Banko-Plus reduced the impact of fungi on the root dry biomass on the level of the treated inoculated seedlings. Indeed, all the treated inoculated seedlings gave a root biomass higher than that of the seedlings inoculated at all the cultivars. The root dry biomass produced at Tropimech is significantly lower than that of Mongal and Caraïbo.

### 3.2.3.3 Effect of the aqueous extract of the leaves of *Combretum racemosum* on diseases

The table 8 shows the tomato seedlings mended in the substrate treated by the aqueous extract of *Combretum racemosum* and inoculated by each of the three mycopathogenes (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli*). No mortality was raised at the positive control. The inoculated seedlings treated with the aqueous extract of *C. racemosum* strongly reduced the attacks of the fungi. The percentage of the seedlings which had a notation of symptoms higher or equal to two is null at Mongal inoculated with Forl and *Macrophomina*. The aqueous extract of *C. racemosum* significantly reduced the index of the diseases (rot pythienne, fusariose and black rot) on the tomato seedlings.

The tests carried out *in vivo* with the extract of the powder of *Combretum racemosum* made it possible to establish a positive relation between the product and the vegetative growth of the tomato seedlings. The foliar and root dry biomass are evaluated over 30 days after inoculation of the seedlings. The aqueous extract of *C. racemosum* significantly ( $p < 0.05$ ) increased the foliar and root biomass of the tomato seedlings inoculated by *P. aphanidermatum*, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) and *M. phaseoli* compared

to the healthy control. Indeed, the seedlings inoculated by the three fungi produced a biomass varying from 2.08 g to 3.54 g of foliar matter and 1.02 g to 1.67 g of root matter respectively. No significant difference on the level of the foliar biomass is raised between the treated and inoculated seedlings and the positive control at all the cultivars. On the other hand, the root biomass of the seedlings treated and inoculated has a varietal sensitivity according to fungi. The weakest biomass are obtained with Tropimech treated and inoculated with Forl ( $1.65 \pm 0.10$  g) and *M. phaseoli* ( $1.95 \pm 0.14$  g).

The aqueous extract of *C. racemosum* thus reduced the impact of fungi on the tomato seedlings; what supported an increase in the foliar and root biomass.

#### 3.2.3.4 Effect of the powder of *Xylopiæ aethiopicæ* on diseases

The table 8 shows the tomato seedlings mended in the substrate treated by the powder of the fruits of *Xylopiæ aethiopicæ* and inoculated by each of the three mycopathogens (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli*). No mortality was raised at the positive control. The inoculated seedlings treated with the rough extract of *Xylopiæ aethiopicæ* strongly reduced the attacks of fungi on the tomato seedlings. The percentage of the seedlings which had a notation of symptoms higher or equal to two is null with Forl and *Macrophomina*. *Pythium aphanidermatum* showed the highest death rate at Caraïbo (5.6%) and Tropimech (4.2%). The rough extract of *X. aethiopicæ* significantly reduced the index of the diseases (rot pythienne, fusariose and black rot) on the tomato seedlings. An effect of phytotoxicity was raised with the powder of *X. aethiopicæ* without that not causing mortality at the tomato seedlings.

The biomass produced with the application of the powder of *X. aethiopicæ* on the tomato seedlings varies according to varieties'. The powder significantly increased the foliar and root biomass inoculated seedlings and treated or seedlings treated (pilot positive) compared to the healthy control and with inoculated. The three varieties react positively with the powder of *Xylopiæ*. Indeed, the three tomato cultivars carried out profits of 33.6%; 30.6%; 43.1% and 47.1%; 51.8%; 35.5% in foliar and root biomass at Mongal, Caraïbo and Tropimech respectively. The repressive effect of the powder of *Xylopiæ aethiopicæ* on the fungi is the result of this tendency in rise of the biomass of the tomato seedlings.

#### 3.2.3.5 Effect of the powder of *Ziniber officinalis* on diseases

No mortality was raised at the positive control; on the other hand an effect of toxicity was observed at all the cultivars Mongal (0.2) Caraïbo (0.3) and Tropimech (0.3). All seedlings inoculated by each of the three fungi (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici*) and treated with the rough extract of *Z. officinalis*, concerned mortalities varying from 30% to 60%. The rough extract of *Z. officinalis* did not have any effect on the incidence of the diseases of the tomato seedlings. The phytotoxicity of the extracts of *Z. officinalis* caused the chlorosis of the sheets of the treated tomato seedlings.

The biomass is reduced with the application of the powder of *Zingiber officinalis* on the tomato seedlings at all the cultivars. This fall of the foliar biomass is significant with the inoculum of *P. aphanidermatum* at Mongal ( $1.77 \pm 0.21$  g), Caraïbo ( $1.81 \pm 0.41$  g) and Tropimech ( $1.51 \pm 0.14$  g) compared to the respective healthy witnesses ( $7.25 \pm 0.45$  g;  $6.88 \pm 0.23$  g;  $6.16 \pm 0.28$  g). The powder of *Z. officinalis* had a negative effect on the foliar biomass of the tomato seedlings.

The dry root biomass also significantly lowered at all the cultivars treated with the powder of *Z. officinalis* compared to the healthy control. No significant difference is raised between the inoculated controls and inoculated treated at all the cultivars. The healthy control

presents also a dry root biomass significantly higher than that of the positive control except at Tropimech. Indeed, the same dry root biomass produced at the healthy control (3.33±0.22 g) does not present any significant difference with that of the positive witnesses (2.97±0.04 g). The rough extract of the rhizomes of *Z officinalis* significantly reduced the biomass of the tomato seedlings.

### 3.2.3.6 Effect of the aqueous extract of the nuts of *Cola nitida* on diseases

The index of the disease is null at Mongal and it did not exceed 0.01 at Caraïbo and Tropimech for the three fungi. The percentage of the seedlings having a notation of the symptoms higher or equal to two is null also at Mongal and varies from 2.08% to 3.13% at the two other varieties. The biofungicide extracts aqueous from *C. nitida* significantly reduced the index of the diseases on the tomato seedlings. Nevertheless, there was an effect of toxicity raised with the aqueous extract of *Cola*.

The aqueous extract of *Cola nitida* significantly increased the foliar and root biomass of tomato seedlings inoculated with *P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli* compared to those inoculated and untreated. Indeed, the seedlings inoculated with the fungi produced a foliar biomass varying from 2.08 to 3.37 g. of foliar dry matter and of 1.02 to 1.67 g of matter dry racinaire respectively. On the other hand, those inoculated with mushrooms and treated with the aqueous extract of *C. nitida* produced a biomass varying between 3.04 and 4.28 g from foliar dry matter and 2.24 and 2.97 g of matter dry racinaire respectively. The aqueous extract of *C. nitida* thus reduced the impact of the fungi on the tomato seedlings; what supported an increase in the foliar biomass and racinaire.

Treatments	No product			E <sub>1</sub> = <i>Combretum racemosum</i>			E <sub>2</sub> = <i>Xylocopa aethiopica</i>			E <sub>3</sub> = <i>Zingiber officinalis</i>			E <sub>4</sub> = <i>Cola nitida</i>			E <sub>5</sub> = Mancozeb		
	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T
Inoculated by <i>Pythium aphanidermatum</i>	1.6	1.5	2.5															
Inoculated by <i>Pythium aphanidermatum</i> treated by E <sub>a</sub>	60	60	90	0.7	1.1	0.9	0.4	1.9	1.7	1.0	1.3	2.4	0	0.6	0.1	0	0.1	0.2
Inoculated by <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	1.0	1.3	2.4															
Inoculated by <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i> treated by E <sub>a</sub>	40	50	90	0.2	0.4	0.9	0.3	0.6	0.6	0.5	1.4	1.3	0	0.5	0.3	0	0.1	0.6
Inoculated by <i>Macrophomina phaseoli</i>	0.8	1.0	1.9															
Inoculated by <i>Macrophomina phaseoli</i> treated by E <sub>a</sub>	30	40	70	0.4	0.6	0.3	0.3	0.5	0.4	0.9	1.3	1.0	0	0.1	0.1	0	0.3	0
Treated by E <sub>a</sub>				0	0.1	0.1	0.1	0.2	0.3	0.2	0.3	0.3	0	0.1	0.1	0	0	0
Healthy control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

M=Mongal; C=Caraïbo; T=Tropimech

Table 8. Effects of natural extracts and mancozeb on the incidence of the diseases of tomato varieties at 30 days after transplantation



### 3.2.3.7 Effect of the essential oil of the fruits of *Xylopiya aethiopica* diseases

The table 8 shows the tomato seedlings mended in the substrate treated by the essential oil of the fruits of *Xylopiya aethiopica* and inoculated by each of the three mycopathogenes (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli*). No mortality was raised at the positive control just like at the healthy control. The essential oil of *Xylopiya aethiopica* strongly reduced the attacks of fungi on the inoculated tomato seedlings and treated. With share *P. aphanidermatum* which caused the death of 30% (Mongal) 40% (Caraïbo) and 50% (Tropimech) of the seedlings, the percentage of the seedlings which had a notation of symptoms higher or equal to two is null at *Fusarium* and *Macrophomina*. The essential oil of *X. aethiopica* significantly reduced the incidence of the diseases (rot pythienne, fusariose and black rot) of the tomato seedlings however it presented an effect of toxicity.

The biomass produced during the treatment of the fusariose by the essential oil of *X. aethiopica* is a function of the variety of tomato. Mongal presents the strongest foliar biomass for all the treatments, 28.80 g (Pilot healthy); 9.70 g (Inoculated); 17.10 g (Pilot positive); 15.90 g (Inoculated and treated with essential oil). The seedlings inoculated for all the three varieties produced a biomass significantly reduced compared to the healthy or positive control and the treated inoculated seedlings. The Tropimech variety inoculated treated with essential oil produced the double of the root biomass (2.10 g) of the untreated inoculated seedlings (1.10 g).

### 3.2.3.8 Effect of the essential oil of the rhizomes of *Zingiber officinalis* on diseases

The table 8 shows the tomato seedlings mended in the substrate treated by the essential oil of the rhizomes of *Zingiber officinalis* and inoculated by each of the three mycopathogenes (*P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* and *M. phaseoli*). No mortality was raised at the positive control just like at the healthy control. The essential oil of *Z. officinalis* did not have any effect on the attacks of fungi on the level of the inoculated tomato seedlings and treated. *P. aphanidermatum* raised the index of mortality highest with 70% at Mongal; 80% at Caraïbo and Tropimech. No fungus was repressed by the essential oil of *Z. officinalis*.

The foliar and root dry biomass are evaluated over 30 days after inoculation of the seedlings.

The essential oil of *Z. officinalis* did not have any significant effect on the foliar and root biomass of the tomato seedlings treated and inoculated with *P. aphanidermatum*, *F. oxysporum* f. sp. *radicis-lycopersici* or *M. phaseoli* compared to the control. Indeed, the seedlings inoculated with mycopathogenes produced a foliar biomass varying from 2.08 to 3.37 g of foliar dry matter and of 1.02 to 1.67 g of root dry matter respectively. On the other hand, those treated and inoculated with fungi produced a biomass varying between 3.04 and 3.82 g from foliar dry matter and 0.68 and 1.45 g of root dry matter respectively. The essential oil of the rhizomes of *Z. officinalis* did not have any effect on the attacks of fungi on the tomato seedlings; what supported a reduction of the foliar and root biomass.

### 3.2.3.9 Effect of the essential oil of the sheets of *Ocimum gratissimum* on diseases

The effect of the oil essential of *Ocimum gratissimum* on the incidence of the diseases at 30 days after transplantation is consigned in table 8. The tomato seedlings mended in the substrate treated by the essential oil of *O. gratissimum* just like do not present any mortality at the positive control at the healthy control. The essential oil of *O. gratissimum* strongly repressed the attacks of fungi on the inoculated tomato seedlings and treated. With share *P. aphanidermatum* which caused the death of 10% of the seedlings at Mongal, the percentage of the seedlings which had a notation of symptoms higher or equal to two is null with *Macrophomina* and

*Fusarium*. The essential oil of *O. gratissimum* significantly repressed the incidence of the diseases (rot pythienne, fusariose and black rot) on the tomato different cultivars.

The biomass produced with the application of the essential oil of the sheets of *Ocimum gratissimum* on the tomato seedlings varies according to cultivars. Essential oil significantly increased the foliar and root biomass inoculated seedlings and treated or seedlings treated (pilot positive) compared to the healthy control and with inoculated. The three varieties react positively to the essential oil of the sheets of *O. gratissimum*.

The three tomato cultivars produced foliar biomass at the positive control, significantly no different from those of the healthy control except at Mongal (7.25±0.49 g and 6.37±0.35 g respectively). All the inoculated seedlings treated by the essential oil of *O. gratissimum* presented a better vegetative growth compared to the inoculated control. The difference between the treated inoculated seedlings and the healthy control is significant at all the cultivars.

The essential oil of *O. gratissimum* significantly increased the root dry biomass of the inoculated tomato seedlings treated of all the cultivars. No significant difference is noted between the healthy control Mongal (4.30±0.29 g) and same the cultivar inoculated by Forl (3.83±0.38 g). The essential oil of *O. gratissimum* significantly increased the biomass of the tomato seedlings at all the treated inoculated cultivars.

Treatments	No product			O <sub>1</sub> = <i>Xylopi</i> <i>aethiopica</i>			O <sub>2</sub> = <i>Zingiber</i> <i>officinialis</i>			O <sub>3</sub> = <i>Ocimum</i> <i>gratissimum</i>			O <sub>4</sub> = Banko- Plus		
	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T
Inoculated by <i>Pythium aphanidermatum</i>	1.6	1.5	2.5												
	60	60	90												
Inoculated by <i>Pythium aphanidermatum</i> treated by O <sub>n</sub>				1.1	1.6	1.5	1.8	1.6	1.3	0.4	0	0	0	0.1	0.2
				30	40	50	70	80	80	10	0	0	0	0	0
Inoculated by <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	1.0	1.3	2.4												
	40	50	90												
Inoculated by <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i> treated by O <sub>n</sub>				0.5	0.9	1.1	1.0	1.3	1.9	0	0	0	0	0.1	0.6
				0	0	0	40	40	80	0	0	0	0	0	0
Inoculated by <i>Macrophomina phaseoli</i>	0.8	1.0	1.9												
	30	40	70												
Inoculated by <i>Macrophomina phaseoli</i> treated by O <sub>n</sub>				0.7	0.9	0.8	1.2	1.8	1.2	0	0.3	0.2	0	0.3	0
				0	0	0	30	60	50	0	0	0	0	0	0
Treated by O <sub>n</sub>				0.2	0.2	0.3	0.6	0.6	0.6	0.1	0.1	0.1	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
Healthy control	0	0	0												
	0	0	0												

Index of wilting  
Index of mortality (%)

M=Mongal; C=Caraïbo; T=Tropimech

Table 9. Effects of essential oils and Banko-Plus on the incidence of the diseases of tomato varieties at 30 days after transplantation

### 3.2.4 Activity of essential oil compared to fungicides on *Sclerotium rolfsii*

Inhibition of mycelium growth is different according to the product and their concentration (Fig. 5). Between essential oil, all concentrations (250 to 6000 ppm) of *Chenopodium ambrosioides* and *Zingiber officinalis* inhibited mycelial growth. The most effective

concentration of *Melaleuca quinquenervia* and *Monodora myristica* are respectively 4000 and 6000 ppm. Mancozeb is the most effective fungicide inhibiting mycelium growth followed by Banko plus and Callicuivre. Callicuivre effect is different to others fungicides. There is no inhibition but the mycelium turned in green color from 1000 ppm more marked with the concentration. *Zingiber officinalis* and *Chenopodium ambrosioides* gave similar effect than

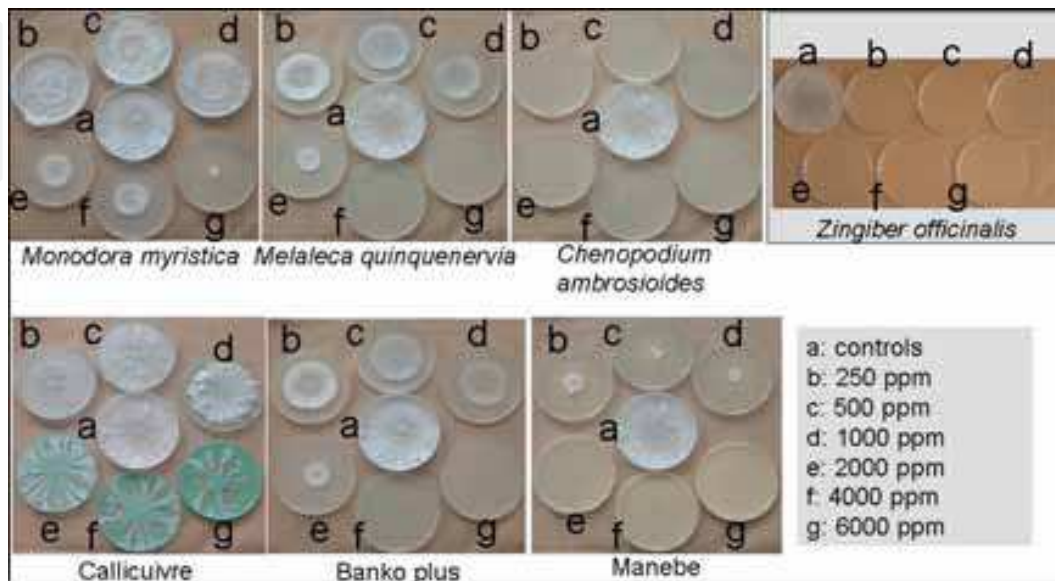


Fig. 4. *In vitro* activity of fungicides on mycelium growth of *Sclerotium rolfsii*

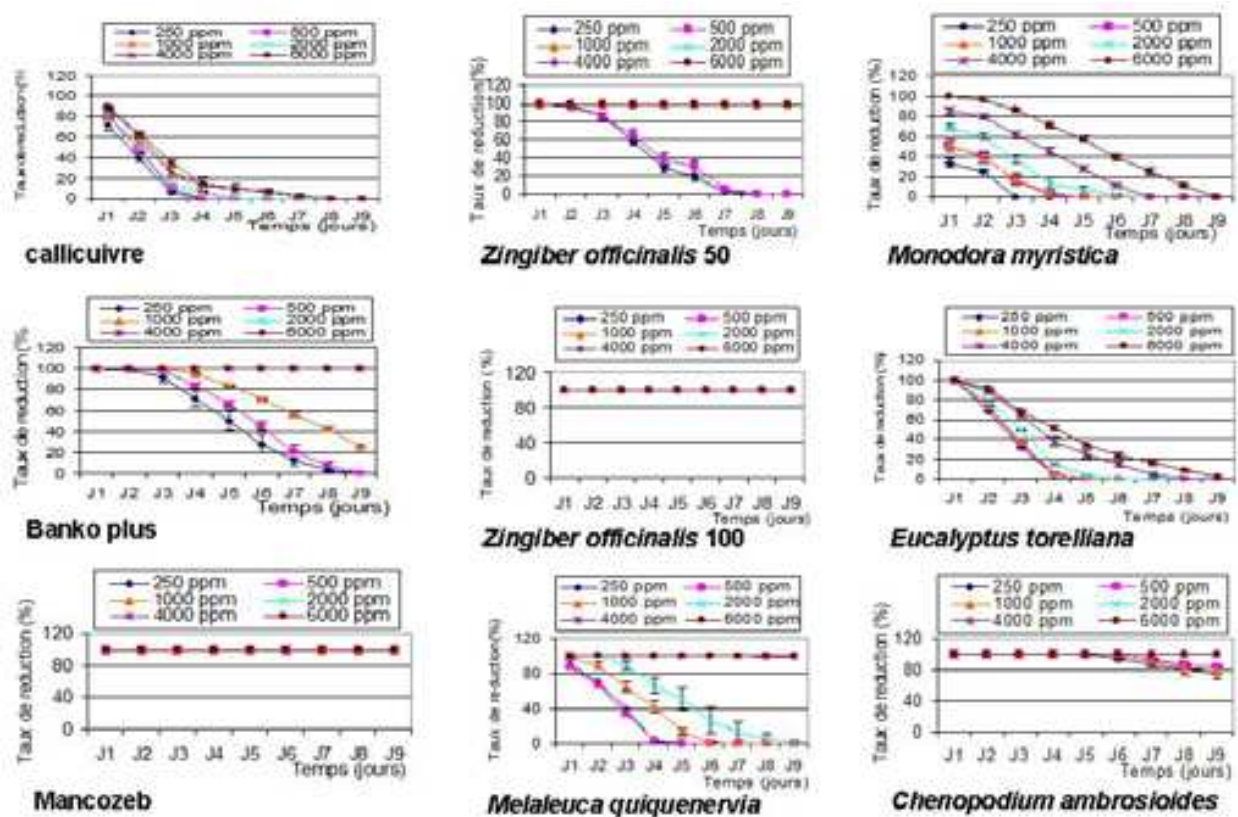


Fig. 5. Inhibition rate of essential oil and fungicides on mycelium growth of *Sclerotium rolfsii*

mancozeb. Essential oil of *Zingiber officinalis* and *Chenopodium ambrosioides* gave similar effect than Mancozeb (Fig. 6). Essential oils of *Melaleuca quinquenervia* gave similar effect than banko plus while essential oil of *Monodora myristica* reacted as Callicuivre (Fig. 5 and 6).

#### 4. Conclusion

Control of *Mycosphaerella* sp. pathogens and others pathogenic fungi of banana and vegetable need to use an integrated approach that will incorporate both cultural practices and the application of fungicides. In Côte d'Ivoire fungicide application in commercially growing banana varies according to geographic location. Around 20 applications of fungicides were performed during the year. Systemic fungicides including Tilt, Bumper (propiconazole), folicur (tebuconazole) were applied during wet periods while protectant fungicides such mancozeb is used during dried periods. Because of appearance of resistant strain, development of strategy base on the use of fungicide in mixture (systemic and biological) is an approach to encourage. It is required to use less fungicide to get banana free fungicide and to control *Mycosphaerella* leaf spots in decreasing pesticide input.

The various extracts tested *in vitro* gave results significantly different according to the fungi Forl, *P. aphanidermatum* and *M. phaseoli*. On the other hand no significant difference was raised between fungicides of synthesis and the aqueous extracts of *Combretum racemosum*. The tests of inhibition carried out with the aqueous extract of *C. racemosum* revealed antifongistatic and fungicidal activities significantly higher than the extracts of *Xylopiya aethiopica* on these three mycopathogenes. The strongest positive correlation observed between the amounts of the powder of *C. racemosum* and the inhibition of the three fungi was raised with *Pythium* and *Fusarium*. Compared to fungicides of synthesis, the aqueous extract of *C. racemosum* is fungicidal for three fungi.

The study carried out in greenhouse made it possible to highlight the infectious potential of the soils and to come out the need from it for adopting a method of control in order to reduce mortalities. Three of the identified telluric fungi parasites belong to the kinds *Pythium*, *Fusarium*, *Sclerotium*. A new fungus ever announced in Côte d'Ivoire on market gardenings was also identified; it is about *Macrophomina phaseoli*. The study of the infectious potential showed that the presence of the telluric fungi parasites tiny room the growth of the seedlings and induces a mortality whose rate varies according to varieties'. This reduction of growth is stronger at Tropimech with respect to 4 mycopathogenes on these soils. On the other hand the test of inoculation with *Pythium* alone showed that Caraïbo is the variety most sensitive to this fungus. The seedbeds of 20 and 25 days arise like the best ages for the transplantation of the seedlings of these three varieties of tomato. Indeed, the seedlings of 20 and 25 days of seedbed resist better the attacks of the mycopathogene *Pythium* sp.

This study showed the inhibiting effect of *Combretum racemosum* on Forl, *P. aphanidermatum* and *M. phaseoli*, all telluric mycopathogenes of the tomato cultures. They are agents responsible for the rot of the roots, tomato collet and stem. The tests of inhibition carried out with the natural extract gross of the powder of *C. racemosum* revealed an antifongistatic and fungicidal activity significant on these three mycopathogenes. The strong correlation observed between the concentration of the powder of *C. racemosum* and the inhibition of the three fungi will be used as a basis to determine the lethal amounts usable *in vivo* in order to extend its use to the plantations of tomato cultures. The natural extract of the powder of *C. racemosum* can be used as a biopesticide against these fungi for the alternative control in tomato plantation once that its antifongic activity will have been proven in cultures *in vivo* on tomato. The interest of this study locates at two levels; on the one hand,

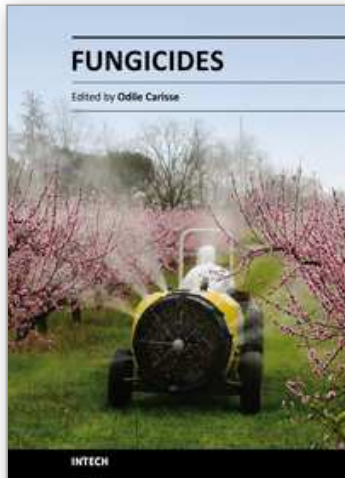
the advantage that *Combretum racemosum* meets easily in the forest belts of the Côte d'Ivoire and on the other hand it is a nontoxic plant for human consumption. A method of use on a large scale of the powder of this plant would be thus a good prospect to be considered for the market gardenings in Côte d'Ivoire.

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## **Fungicides**

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Plant and plant products are affected by a large number of plant pathogens among which fungal pathogens. These diseases play a major role in the current deficit of food supply worldwide. Various control strategies were developed to reduce the negative effects of diseases on food, fiber, and forest crops products. For the past fifty years fungicides have played a major role in the increased productivity of several crops in most parts of the world. Although fungicide treatments are a key component of disease management, the emergence of resistance, their introduction into the environment and their toxic effect on human, animal, non-target microorganisms and beneficial organisms has become an important factor in limiting the durability of fungicide effectiveness and usefulness. This book contains 25 chapters on various aspects of fungicide science from efficacy to resistance, toxicology and development of new fungicides that provides a comprehensive and authoritative account for the role of fungicides in modern agriculture.

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