

Original Research Article

Effects of some fungicides and nematicides used in banana plantation on pathological characteristics of *Metarhizium* sp., biological agent control of banana weevil *Cosmopolites sordidus* Gemar (Coleoptera: Curculionidae)

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ABSTRACT

Keywords

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The use of biological agent in environment where chemical is regular entail risks of failure that might be elucidated by suitable tests. Effects of 3 fungicides (Methyl-thiophanate, Propiconazole and Trifloxystrobin) and 2 nematicides (Oxamyl and Ethoprophos) on some pathogenicity characters of autochthonous isolate of *Metarhizium* sp. were evaluated. The germination inhibition rate (Tig) increase with the concentration of fungicides, ranging from 18.11% to 46.88% for the Methyl-thiophanate, 2.08% to 39.94% for the Trifloxystrobin and 29.51% to 42.54% for Propiconazole. Mycelial growth and sporulation were most affected by fungicides. The inhibition rate reached 56.52%, 71.80% and 81.96% for the mycelial growth and 24.71%, 99.07% and 90.27% for sporulation respectively on media containing Methyl-thiophanate, Trifloxystrobin and Propiconazole. Germination rate of these spores decreased with higher concentrations also affecting significantly their infectivity. Maximal mortality rate of adults weevils exposed to conidia produced on media containing high concentrations of Methyl-thiophanate and Propiconazole was 33% while 11% was obtained for Trifloxystrobin. However, TL50 only obtained with lowest doses were 14 to 21 days compared to 12 days obtained with the control. Unlike fungicides, most doses of Oxamyl and Ethoprophos tested stimulate conidial germination, mycelial growth and the sporulation of the *Metarhizium* isolate.

Introduction

The banana weevil [*Cosmopolites sordidus* Gemar (Coleoptera: Curculionidae)], is the major insect constraints of bananas and plantains. Yield losses due to its damage are over 40% (Gold et al., 2001). Chemical

control is the dominant fighting strategy because of its efficiency. But this method is increasingly controversial because of concerns for environmental pollution, toxicity, adverse effects on human health

and biodiversity and development of insecticide resistance (Faurie et al., 2009). Alternative control measures such as the use of biological control has been proposed by several authors (Schiffers, 2011). This biological control using antagonistic organisms such as *Beauveria* and *Metarhizium* fungi was performed successfully by Gold et al (2003). In Côte d'Ivoire, field survey using the application of the fungus in traps and plywood plates showed a performance of autochthonous isolates of *Metarhizium* sp on adults weevil causing a mortality rate reaching 100% (Aby et al., 2010; 2011; Aby 2013). Although most studies on biological control of *C. sordidus* have been performed with strains within the both genera *Beauveria* and *Metarhizium*, few authors have been interested on their compatibility with chemicals used in the field as fertilizer or against others diseases and pests. Thus, this study aimed to determine in laboratory the effect of some fungicides and nematicides on pathological characteristics of local isolate of *Metarhizium* sp. to help identify the conditions for an efficient implementation of this biological agent in banana plantations and propose an integrated strategy against the most harmful insect of banana *Cosmopolites sordidus*.

Material and Methods

Biological agent

The biological agent used was entomopathogenic fungus, a local isolate of *Metarhizium* sp (lab name: BME2) isolated on a mycosed weevil collected through pseudo stern trap in the banana plantation of Batia, Tiassalé region of Agnéby Tiassa, 150 km north of Abidjan.

Insect target

Adult of banana weevils used for tests were collected through disk on stump traps in

SAKJ plantation located in Ayamé, the southern region Comoé, 230 km from Abidjan. The Insects were reared in CNRA's laboratory in plastic boxes 20 x 15 x 15 cm, and regularly fed with pieces of corm.

Pesticides

Three fungicides and two nematicides were used at different concentrations (Table I). The concentrations 1 µg/l, 0.1 µg/l and 5ppm were laboratory concentrations of Propiconazol, Methyl-thiophanate and Trifloxystrobin corresponding to concentrations of these pesticides used in bananas fields against *Mycphaerella fijiensis*, the causal agent of black Sigatoka.

Oxamyl and Ethoprophos were the nematicides. Laboratory concentrations corresponding to those used in fields are 4 µg/l.

Media preparing

Stock solutions were first prepared by dissolving the pesticide in sterile distilled water. Then, 0.1 ml of methyl thiophanate, 0.16 ml of Propiconazole and Trifloxystrobin were separately dissolved in 40 ml of sterile distilled water to obtain 1000 µg/l for each fungicide. For Oxamyl and Ethoprophos 1.2 ml and 1.6 ml were respectively dissolved in 40 ml of sterile distilled water.

PDA medium was used for fungus culture, prepared in Erlenmeyer flasks and autoclaved for 30 min at 121°C and 1 bar. Pesticides (Table I) are incorporated in the culture medium. The mixture is homogenized with a stirrer and then poured into Petri dishes. Controls were PDA and agar media without pesticides.

Effect of pesticides on conidia germination

Metarhizium sp. isolate BME2 was grown on PDA medium for 21 days in darkness and freshly collected conidia were used for the experiment. Conidial suspensions were prepared by scraping conidia from Petri into sterile distilled water. The conidial suspensions were filtered through several layers of cheesecloth to remove mycelial mats. Five drops of conidial suspensions were spray on colonn of agar medium containing the pesticide. Three replicates were performed for the different concentrations of pesticide. The Petri dishes were placed in darkness for 48 hours at room temperature.

One hundred conidia were observed per Petri dish. A conidium has germinated when the length of the germinated tuber is greater than the half length of the conidium. All tests were performed twice. The Inhibition of the Germination Rate (TIG) as a

percentage was obtained by the following formula:

$$Tg = \frac{\text{Germinated conidia}}{\text{conidia}} \times 100$$

$$Tig = \frac{\text{Tg of the control} - \text{Tg of the treatment}}{\text{treatment}} \times 100$$

Effect of pesticides on mycelial growth (Cm)

The effects of the pesticides were studied on PDA medium. PDA medium without pesticide was used as a control. For each concentration, a washer 5 mm diameter of fungus culture 15 days aged was placed at the centre of the Petri dish containing the medium and sealed with parafilm. Mycelial sides growth was measured from the middle of the washer every 2 days. Three replicates were conducted for each concentration. The Petri dishes were placed in darkness at room temperature (28 ± 2°C) in an incubation room.

$$Cm = \frac{\sum_{j=2}^n (m_j - m_{j-2})}{\text{Replication}}$$

m_j is colony diameter/day, m_{j-2} is colony diameter day-2 and n is observation days

The inhibition rate of mycelial growth (TiCm) is expressed:

$$Ticm = \frac{\text{Cm of control} - \text{Cm of treatment}}{\text{Cm of control}} \times 100$$

Effect of pesticides on sporulation

After 21 days culture duration for the isolate on PDA medium with a pesticide, the colony formed was scraped with a sterile spatula and stirred at 150 rot / min. for 30 min. in 100 ml of sterile distilled water. The

solution obtained was filtered through a two-layered cheese cloth. The spores' concentration was determined using a Hemocytometer. The sporulation inhibition (TISP) was expressed:

$$Tisp = \frac{\text{Sporulation of control} - \text{Sporulation of treatment}}{\text{treatment}} \times 100$$

Germination of conidia produced on media containing fungicides

After 21 days culture on PDA containing the pesticide, the rates of the germinated spores produced were determined as described above.

Pathogenicity of conidia produced on media containing fungicides

The pathogenicity of conidia produced on media containing fungicides was evaluated by inoculation of adults weevils. For each treatment, five weevils were immersed for 30s in conidia suspension containing 2 drop of Tween 80. The weevils are then placed in plastic containers (12 x 8 x 7 cm) and fed with pieces of pseudo stem. Mortality was monitored on daily basis during 33 days and dead weevils were progressively removed. Only mycosed weevils were considered to determine the pathogenic factors LT50 and Maximal Mortality rate. Three replicates were performed for each concentration of pesticides.

Statistical analysis

Inhibition rates calculated for each parameters studied were analysed using one-ways analysis of variance (ANOVA) in STAT XL version 7.5.3 software. The Duncan test at 5% threshold was used to compare means and classifies averages groups. The LT50 were determined through the trend curves presented by the mortality rate registered with time after inoculation.

Results and Discussion

Effect of fungicides on conidial germination

On the medium without fungicide (the control), the conidial germination rate was 77.59 %.

Effect of Methyl-thiophanate on conidial germination

The Tig was inversely proportional with the concentration of the fungicide.

Thus the dose 3 µg/l and 5 µg/l respectively induced the Tig of 43.93 and of 46.88% and were significantly higher ($F = 8.88$; $P = 0.001 < 0.05$) than those of lower concentrations 1 µg/l and 0.5 µg/l (Table II).

Effect of Trifloxystrobin on conidial germination

The Tig due to Trifloxystrobin was inversely proportional with doses of the fungicide. Approximately 1 µg/l of trifloxystrobin caused the highest Tig 39.94% which was significantly different ($F = 7.852$; $P = 0.001 < 0.05$) from those induced by lower concentration.

It was following by doses 0.1 µg/l and 0.5 µg/l causing respectively 17.53% et 21.88% and finally doses 0.01 µg/l and 0.03 µg/l which induced the lowest inhibitions rate 2.08% and 7.75% respectively (Table II).

Effect of propiconazole on conidial germination

The highest average inhibition rate with propiconazole was induced by high concentrations of the fungicide. However, no significant difference ($F = 0.940$; $P = 0.426 > 0.05$) were observed between the inhibition rate induced by different concentrations of the fungicide (Table II).

Effect of fungicides on mycelial growth

On the control medium, mycelial growth averaged 2.15 mm /day.

Effect of Methyl-thiophanate on mycelial growth

Inhibition of mycelial growth rate increased proportionally with the concentrations of the fungicide in the medium. Analysis of variance was separated by concentrations in three different groups ($F = 65.73$ $P < 0.0001 < 0.05$). The first group consisting of the dose of $5 \mu\text{g/l}$ caused a relatively high inhibition reaching 56.52 %. It was followed by $3 \mu\text{g/l}$ dose causing 43.47% inhibition. The last group includes $1 \mu\text{g/l}$ and $0.5 \mu\text{g/l}$ with the lowest inhibition rate 17.87 and 13.76% respectively, but statistically similar (Table III).

Effect of Trifloxystrobin on mycelial growth

The inhibition rate of mycelial growth due to Trifloxystrobine varied with the concentration. Significant differences highlighted 3 groups ($F = 145.76$; $P < 0.0001 < 0.05$). The first group consisting of doses of 0.5 and $1 \mu\text{g/l}$ resulting in the highest inhibition rate 69.35 and 71.80% respectively. Concentration of $0.1 \mu\text{g/l}$ induced an intermediate inhibition rate 39.42%. and doses of $0.01 \mu\text{g/l}$ and $0.03 \mu\text{g/l}$ resulted inhibition rates 6.83 and 13.04% respectively (Table III).

Effect of propiconazole on mycelial growth

The highest concentration $0.1 \mu\text{g/l}$ induced the highest inhibition rate reaching 81.96%, significantly ($F = 186.626$; $P < 0.0001 < 0.05$) higher than the inhibition rates 68.11% and 23.55 % induced by $0.03 \mu\text{g/l}$ and $0.01 \mu\text{g/l}$ respectively (Table III).

Effect of fungicides on sporulation

The concentration of conidia obtained on the control medium was $44.8 \cdot 10^5$ conidia /ml.

Effect of Methyl-thiophanate on sporulation

Inhibition rates of the sporulation caused by fungicide doses were relatively low ($\square 50\%$) compared to those induced by Trifloxystrobin and propiconazole. The highest concentration $5 \mu\text{l}$ caused the highest inhibition rate 48.57% significantly different ($F = 5.67$ $P = 0.04 < 0.05$) from the lower doses $0.5 \mu\text{g/l}$; $1 \mu\text{l}$ and $3 \mu\text{l}$ which induced 20.21% respectively; 22.25% and 24.71% inhibition rate looking statistically similar (Table IV)

Effect of Trifloxystrobin on sporulation

The sporulation of the fungus was almost completely inhibited by the three doses $0.1 \mu\text{g/l}$, $0.5 \mu\text{g/l}$ and $1 \mu\text{g/l}$ causing inhibition rate 89.30%, 98.93% and 99.07% respectively and significantly different ($F = 66.89$ $P < 0.0001 < 0.05$) with those due to $0.01 \mu\text{g/l}$ and $0.03 \mu\text{g/l}$ (Table IV)

Effect of propiconazole on sporulation

Inhibition rates caused by the highest doses ($0.1 \mu\text{g/l}$ and $0.03 \mu\text{g/l}$) were 90.27% and 84.26 % respectively and statistically different ($F = 4.07$; $P = 0.03 < 0.05$) from the inhibition rate due to the lower concentration $0.01 \mu\text{g/l}$ (Table IV).

Germination of conidia produced on media containing fungicides

The conidial germination rate of the control was 82.75% higher than those of conidia produced on media containing fungicides.

Germination of conidia produced on medium containing Methyl-thiophanate

Conidia produced on the medium containing $5 \mu\text{g/l}$ of the fungicide had the lowest germination rate 49.7% showed significant

difference ($F = 11.33$; $P = 0.0001 < 0.05$) with those of conidia produced on media containing lower doses 0.5.

1 and 3 $\mu\text{g/l}$ which were 66.98%, 66.53% and 61.81%, respectively (Table V). The germination inhibition rates explained by these lower concentrations did not differ significantly.

Germination of conidia produced on medium containing Trifloxystrobin

From 0.5 $\mu\text{g/l}$ of Trifloxystrobin in the culture medium, the germination rate of conidia produced becomes less than 50%. And these levels induced by high doses were statistically lower ($F = 16.832$; $P < 0.0001 < 0.05$) than those of conidia produced on media containing the lowest concentrations (0.01 $\mu\text{g/l}$; 0.03 $\mu\text{g/l}$ and 0.1 $\mu\text{g/l}$) of the fungicide.

The lowest concentration 0.01 $\mu\text{g/l}$ induces conidial germination rate of 78.40% statistically equal to that of untreated conidia (Table V).

Germination of conidia produced on medium containing Propiconazole

The highest germination rate was obtained with the conidia produced on the media containing the smaller concentrations of the fungicide. The conidia produced on medium containing 0.01 $\mu\text{g/l}$ was 69.19% significantly lower ($F = 21.894$; $P < 0.0001 < 0.05$) than germination rate of the conidia from the control medium.

Conidia produced on media containing the highest concentrations 0.03 $\mu\text{g/l}$ and 0.1 $\mu\text{g/l}$ recorded the lowest germination rates reaching 50.59% and 43.25% respectively (Table V).

Infectivity of conidia produced on media containing the fungicides

Infectivity of conidia produced on medium containing Methyl-thiophanate

Mortality rates of banana weevils' adult due to conidia produced on media containing Methyl-thiophanate were all lower than those due to the control conidia. These mortality rates decrease with increasing concentration in the medium. Thus, 75, 67%, 50, 33% and 33% of mortality rates were obtained from 0 ppm (control), 0.5 $\mu\text{g/l}$, 1 $\mu\text{g/l}$, 3 $\mu\text{g/l}$, and 5 $\mu\text{g/l}$ respectively (Figure 1). TL50 were also not achieved (maximum mortality rate \square 50%) on conidia produced on media containing high concentration 3 and 5 $\mu\text{g/l}$, and those of conidia produced on media containing lower concentrations 0.5 $\mu\text{g/l}$ and 1 $\mu\text{g/l}$ which were 14 days and 21 days longer respectively and are well above TL50 (12 days) due to untreated conidia (Figure 1 b and c).

Infectivity of conidia produced on medium containing Propiconazole

Mortality rates induced by the conidia from media containing Propiconazole are also decreasing with increasing concentrations. The concentrations 0, 0.01, 0.03 and 0.1 $\mu\text{g/l}$ gave 75, 50, 33 and 33% of maximal mortality (Figure 1). Only the lowest rate allowed having a LT50 10 day which was lower than the control.

Infectivity of conidia produced on medium containing Trifloxystrobin

The dose of Trifloxystrobin in the culture medium had influenced the pathogenic parameters of the fungus.

Mortality rates decreased from the control to the highest concentrations 75 (control), 67 (0.03 µg/l), 50 (0.1 µg/l), 50 (0.5 µg/l) and 11% (1 µg/l). The TL50 14 days and 21 days were obtained with the lowest doses, 0.03 and 0.1 µg/l, but still greater than the control's (12 days) (Figure 2).

Effect of nematicide on conidial germination

Effect of Oxamyl on conidial germination

Conidial germination rate of the fungus on the control medium was 77.59%. The concentrations 3, 5 µg/l, 10 µg/l and 15 µg/l caused inhibition rate reaching -19.79%, -16.97%, -14.42% and -11.16%, respectively (Table VI). Negative values of these inhibition rates indicate a stimulation of conidial germination due the nematicide. The stimulation was higher on media containing the lower concentration of the nematicide although there was no significant difference ($F = 0.64$; $P = 0.604 > 0.05$).

Effect of Ethoprophos on conidial germination

Concerning Ethoprophos, inhibition rates caused by doses 3 µg/l, 5 µg/l, 10 µg/l and 15 µg/l were -20.50%, -13.84%, -13.54% and -13.49%, respectively (Table VI). Negative values indicate a stimulation of the conidial germination by the nematicide. As shown by the use of Oxamyl, the stimulation was higher on the media amended with smaller quantities of the nematicide although significant differences in the inhibition rates were observed between all the concentrations effects ($F = 0.249$; $P = 0.859 > 0.05$).

Effect of nematicides on mycelial growth

On the control medium, mycelial growth was 2.02 mm /days.

Effect of Oxamyl on mycelial growth

The high concentration 15 µg/l exhibited inhibition rate on mycelial growth reaching 33.92% and makes significant difference ($F = 20.39$; $P < 0.0001 < 0.05$) with stimulating effect due to the lowest doses -6.34%, -2.98% and -0.44% induced by the doses of 3 µg/l, 5 µg/l and 10 µg/l respectively (Table VII).

Effect of Ethoprophos on mycelial growth

Similarly, the impact of Ethoprophos on mycelial growth was inhibited in the culture media amended with the highest concentration 15 µg/l. But the stimulation effects were induced by low doses 3, 5 and 10 µg/l. Indeed, two significant different groups ($F = 19.70$; $P < 0.0001$) were observed. The first group was formed by the concentration 15 ppm with 20% inhibition rate and second group was composed by 3 µg/l, 5 µg/l and 10 µg/l which caused stimulation effects (Table VII).

Effect of the nematicides on sporulation

On the control medium, the number of conidia was $37, 2.10^5$ spores /ml.

Effect of Oxamyl on sporulation

The incidence of oxamyl on sporulation varied with concentration of nematicide in the medium.

Inhibitions of the sporulation have been caused by the doses 10 µg/l and 15 µg/l resulting in 58.39% and 11.25% respectively (Table VIII) while stimulating effects (negative values of the inhibition rate) were significantly ($F = 23.884$ $P < 0.0001 < 0.05$) induced by the lower concentrations 3 µg/l (-56.8%) and 5 µg/l (-18.6%).

Effect of Ethoprophos on sporulation

The concentrations of Ethoprophos tested exhibited different effects on the sporulation. The highest doses 10 µg/l and 15 µg/l inhibited sporulation of the fungus whereas the lower doses, 3 µg/l and 5 µg/l boosted the sporulation (Table VIII).

Three groups were significantly ($F = 28.822$; $P < 0.0001 < 0.05$) distinguished. The first was the highest concentrations creating inhibition 63.22% and 63.95%, respectively, the 2th was 3 µg/l resulting in a little stimulating effect (-2.92%) and the 3rd group was 5 µg/l producing a stimulation effect reaching 78.69% (Table VIII).

This study aims to evaluate the ability of *Metarhizium* sp. to germinate, grow, sporulate and infect the banana weevil *Cosmopolites sordidus*, in an environment where fungicides and nematicides are applied frequently to protect banana.

Effect of the fungicides on conidial germination

Conidial germination of *Metarhizium* sp BME2 isolate used in this study is inhibited by the three different fungicides Methyl-thiophanate (Benzimidazole), Trifloxystrobin (Strobilurin) and propiconazole (Triazole) tested at various concentrations as the case of most fungicides *Phomopsis amaranthicola*, bioherbicide against *Amaranthus* spp (Wyss et al., 2004). Following the work of Grant et al (1990) which showed the temporal complete inhibition of conidial germination of *Colletotrichum gloeosporioides*, a bioherbicide used against *Malva pusilla*, by the majority of the tested fungicides. However, the inhibition rates obtained in the case study were relatively low, below 50% as well as those obtained by Aby (2013).

And the inhibition rate ranging from 18.11% to 46.88% would seem to be contrary to the action of methyl-thiophante, which is heterocyclic Carbamate nitrogen belonging to the subfamily of benzimidazole. The benzimidazole is an anti-mitotic microtubule affecting cell divisions (Couteux and Lejeune, 2005), thus the formation and growth of the germ tube (Davidse et al., 1978).

Kobenan et al (2011; 2012) have shown that *Mycosphaerella fijiensis* causing black Sigatoka disease, is very sensitive to Methyl-thiophanate at 5 µg/l. But our results are consistent with those of Atrassi et al (2007) who reported a weak inhibition of conidia germination in several fungi such as *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Rhizopus stolonifer* and *Alternaria alternata* under the action of benomyl and thiabendazole (benzimidazoles).

Inhibitions of conidial germination due to Trifloxystrobin (Strobilurin) were the lowest ranging from 2 to 39.94% and contrasting with the results obtained by Kobenan et al (2011 and 2012) on the germination of *Mycosphaerella fijiensis*. The analogues of strobilurin have also strongly inhibited the conidial germination of various fungi (Stierl et al., 2002) including *Alternaria alternata* (Moshe, 2005).

The tests with propiconazole gave inhibition rate of conidial germination ranging from 29.51 to 42.54%, lower than 50% compared to those obtained with benzimidazole and strobilurin tested by Aby (2013).

The results, compared to the classification by Wiss et al (2004), indicate an incompatibility between the fungicides tested and the fungus *Metarhizium* sp. BME2 isolate used.

Effect of fungicides on mycelial growth.

Mycelial growth was more affected than the conidial germination and the highest inhibition rates were also recorded by the laboratory concentrations corresponding to those applied in the field: 5 µg/l for Methyl-thiophanate, 1 µg/l for Trifloxystrobin and 0.1 µg/l for propiconazole. The inhibition rate of mycelial growth were 56.52, 71.80 and 81.96% respectively similar to those of Aby (2013). Especially the inhibition rate induced by trifloxystrobin and propiconazole confirm their modes of action. The Trifloxystrobin is a heterocyclic carbamate nitrogen belonging to the Strobilurins subfamily and recognized as an inhibitor of mitochondrial complex III of the respiratory chain and thus, the production of cellular energy (Couteux and Lejeune, 2005) for 'germ tube elongation and the mycelium growth. The Propiconazole is a Carbamate heterocyclic nitrogen belonging to the Triazoles subfamily. It is an inhibitor of sterol biosynthesis (IBS) that prevents the conversion of lanosterol to ergosterol, a final product of the formation of the fungal cell wall (Koller and Scheinpflug, 1987). This strobilurin and triazole tested were hardly compatible to mycelial growth in the scale classification done by Wiss et al (2004).

The inhibition rate (13.73 to 56.52%) caused by Methyl-thiophanate were relatively low than the others mentioned above, indicating its relative compatibility with the fungus. Similar results were obtained with benomyl and Thiabendazole, two benzimidazoles on mycelial growth of *Aspergillus Niger* and *Aspergillus fumigatus* (Atrassi et al., 2007)

Effect of fungicides on sporulation

Sporulation was more affected than conidial germination. Inhibition rate reaching 90.27% and 99.07% were caused by the

concentration of propiconazole and Trifloxytrobine respectively used in the field confirming their mode of action. Trifloxystrobin strongly inhibit sporulation by preventing the production of essential cellular energy in the mitosis which is the process of this imperfect fungus to produce conidia. According to the compatibility classes of Wiss et al. (2004), the dose of Propiconazole and Trifloxystrobin tested in the laboratory corresponding to those used in the fields would be incompatible with the sporulation of *Metarhizium* sp. The inhibition rate (from 64.10 to 98.93%) confirms propiconazole and Trifloxystrobin. For Benzimidazole (Methyl-thiophanate) the dose applied in the field induced relatively low inhibition rate (below 50%). This fungicide would be more compatible with sporulation than the other fungicides tested. Tolerance effect could be explained with that low level of inhibition as it is found with systemic single-site fungicides (Bacon, 2002). That resistance can be naturally present within the fungus population or rarely result from a genetic mutation (Gilbert, 1999; Bacon, 2002; Sholberg et al., 2005).

Germination and infectivity of conidia produced on media containing fungicides

The Conidia produced on media containing fungicides showed germination rates from 40 to 79% but lower than those obtained with conidia produced on media without fungicide (82.75%). The use of fungicides reduced the conidia produced germination. This decrease in the germination could be explained by the presence of fungicide residues within the conidia produced. The low germination rate exhibited by the conidia produced on media containing fungicides was corroborated with the pathogenic parameters. The TL50 average was 15 days compared to the 12 days due to

the conidia produced on the control medium. Mortality rates induced by the conidia produced on media containing fungicides varied from 16 to 67%, and were lower than 75% average induced by the conidia produced on the control medium. The fungicides would affect germ tube

elongation, appressoria formation which is essential to establish infection on insect's bodies. Aby (2013) demonstrated the effect of these fungicides on the growth of germ tubes of *Metarhizium* isolates revealed by deformation or atrophy that may limit the infection

Table.1 Concentrations and active ingredients of pesticide used

Pesticides	Active ingredients	Concentrations used (µg/l)					
		0	0,01	0,03	0,1	0,5	1
Fongicides	Trifloxystrobine (Strobilurines)	0	0,01	0,03	0,1	0,5	1
	Propiconazole (Triazoles)	0	0,01	0,03	0,1		
	Méthyl-thiophanate (Benzimidazoles)	0	0,5	1	3	5	
Nématicides	Oxamyl (Carbamates)	0	3	5	10	15	
	Ethoprophos (Organophosphorés)	0	3	5	10	15	

Table.2 Inhibition rate of conidial germination of *Metarhizium* sp isolate BME 2 grown on agar medium containing the fungicides Methyl-thiophanate, Propiconazole and Trifloxystrobin

Doses (µg/l)	Fongicides		
	Méthyl-thiophanate	Trifloxystrobine	Propiconazole
0,01	n.e	2,08 ± 4,06 c	29,51 ± 6,79 a
0,03	n.e	7,75 ± 6,42 c	34,38 ± 9,60 a
0,1	n.e	17,53 ± 6,42 bc	42,54 ± 9,60 a
0,5	18,11 ± 5,32 b	21,88 ± 6,02 b	n.e
1	19,44 ± 7,53 b	39,94 ± 6,42 a	n.e
3	43,93 ± 7,53 a	n.e	n.e
5	46,88 ± 7,21 a	n.e	n.e

n.e: not done

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Table.3 Inhibition rate of mycelial growth of *Metarhizium* sp BME2 isolate grown on PDA containing fungicides Methyl-thiophanate, Propiconazole and Trifloxystrobin

Doses (ppm)	Fongicides		
	Méthyl-thiophanate	Tryfloxytrobine	Propiconazole
0,01	n.e	6,83 ± 4,03 c	23,55 ± 2,62 c
0,03	n.e	13,04 ± 3,08 c	68,11 ± 3,45 b
0,1	n.e	39,42 ± 3,77 b	81,96 ± 3,10 a
0,5	13,76 ± 2,73 c	69,35 ± 4,03 a	n.e
1	17,87 ± 4,17 c	71,80 ± 3,71 a	n.e
3	43,47 ± 3,86 b	n.e	n.e
5	56,52 ± 3,47 a	n.e	n.e

n.e: not done,

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Table.4 inhibition rate of the sporulation isolate of *Metarhizium* sp isolate BME 2 grown on PDA containing the fongicide Methyl-thiophanate, Propiconazole and Trifloxystrobin

Doses (µg/l)	Fongicides		
	Méthyl-thiophanate	Tryfloxytrobine	Propiconazole
0,01	n.e	11,21 ± 6,94 c	64,10 ± 6,79b
0,03	n.e	17,57 ± 9,82b	84,26 ± 9,60a
0,1	n.e	89,30 ± 8,35a	90,27 ± 9,60 a
0,5	20,21 ± 9,32b	98,93 ± 8,96 a	n.e
1	22,25 ± 7,12b	99,07 ± 8,70 a	n.e
3	24,71 ± 8,88 b	n.e	n.e
5	48,57 ± 8,88 a	n.e	n.e

n.e: not done,

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Table.5 Conidial germination rate of *Metarhizium* sp isolate BME2 produced on PDA medium containing the fungicides Methyl-thiophanate, propiconazole and Trifloxystrobin

Doses (µg/l)	Fongicides		
	Méthyl-thiophanate	Tryfloxytrobine	Propiconazole
0	82,75 ± 3,52 a	82,75 ± 2,79 a	82,75 ± 3,82 a
0,01	n.e	78,40 ± 4,26a	69,19 ± 5,41b
0,03	n.e	65,00 ± 4,26b	50,59 ± 5,41c
0,1	n.e	56,33 ± 4,26b	43,25 ± 5,41c
0,5	66,98 ± 4,98b	45,00 ± 4,83c	n.e
1	66,53 ± 5,38b	40,66 ± 4,26c	n.e
3	61,81 ± 4,98 b	n.e	n.e
5	49,72 ± 4,98c	n.e	n.e

n.e: not performed. The dose of 0 ppm is the witness.

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Table.6 Inhibition rate of conidial germination of *Metarhizium* sp. isolate BME2 grown on Agar medium containing the nematicides Oxamyl and Ethoprophos

Doses ($\mu\text{g/l}$)	Nématicides	
	Oxamyl	Ethoprophos
3	-19,79 \pm 4,59a	-20,50 \pm 9,02 a
5	-16,97 \pm 6,50a	-13,84 \pm 5,71a
10	-14,42 \pm 6,50a	-13,54 \pm 9,02a
15	-11,16 \pm 6,50a	-13,49 \pm 9,02 a

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Table.7 Inhibition rate of mycelial growth of *Metarhizium* sp. isolate BME2 grown on PDA medium containing the nematicides Oxamyl and Ethoprophos

Doses ($\mu\text{g/l}$)	Nématicides	
	Oxamyl	Ethoprophos
3	-6,34 \pm 3,98 b	-16,81 \pm 5,26 b
5	-2,98 \pm 5,09 b	-13,27 \pm 6,15 b
10	-0,44 \pm 4,44 b	-11,75 \pm 4,83 b
15	33,92 \pm 5,54 a	20,00 \pm 3,55 a

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Table.8 Inhibition rate of sporulation of *Metarhizium* sp. isolate BME2 grown on agar containing the nematicides Oxamyl and Ethoprophos

Doses ($\mu\text{g/l}$)	Nématicides	
	Oxamyl	Ethoprophos
3	-56,87 \pm 9,62 d	-2,92 \pm 12,02b
5	-18,65 \pm 13,61 c	-78,69 \pm 18,03c
10	11,25 \pm 12,83b	63,22 \pm 17,00a
15	58,39 \pm 14,16 a	63,95 \pm 16,25a

The inhibition rate topped by the same letter are not significantly different (Duncan 5%)

Figure.1 Mortality rates of *C. sordidus* inoculated with conidial suspensions produced on PDA media containing the fungicide Methyl-thiophanate and Propiconazole

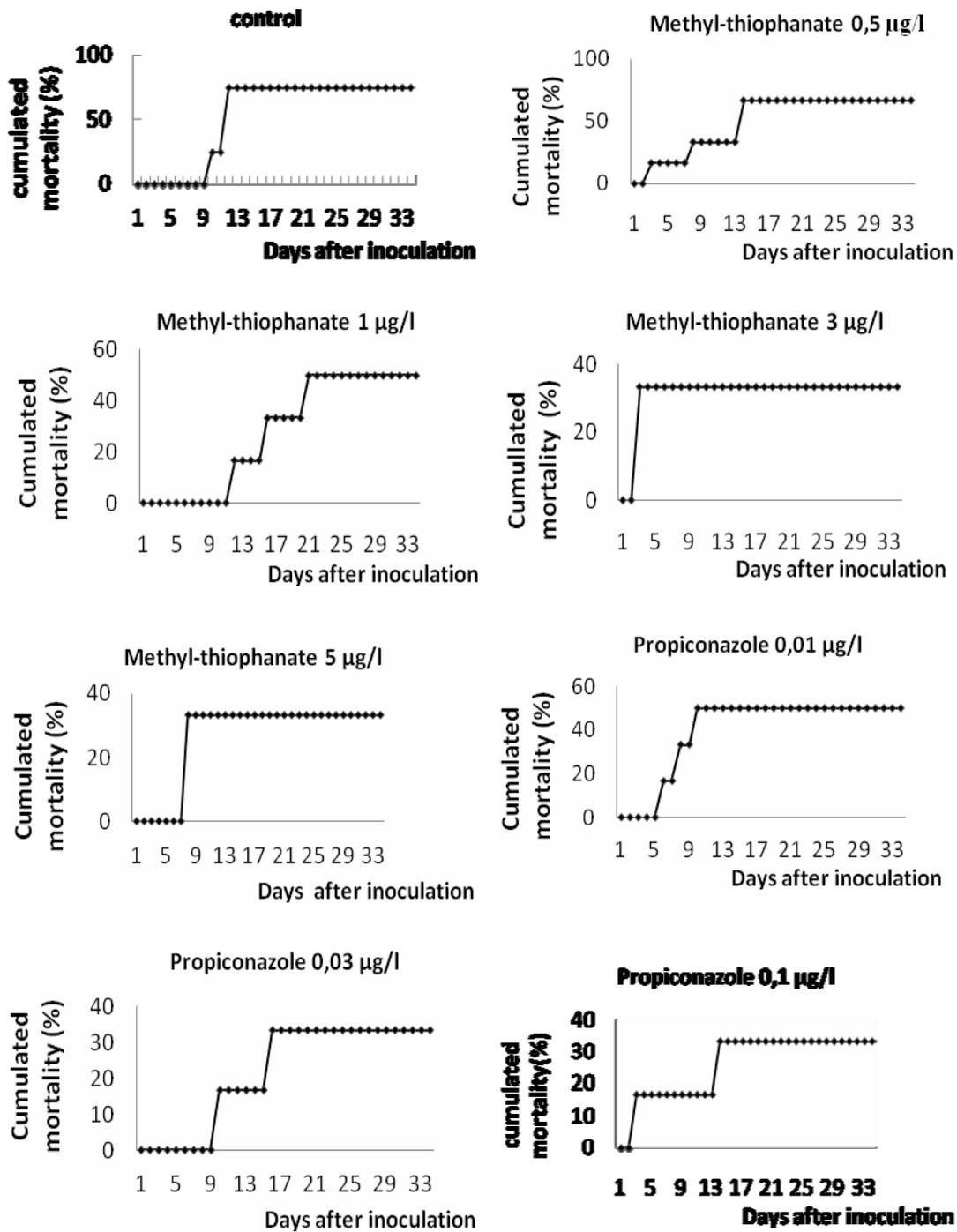
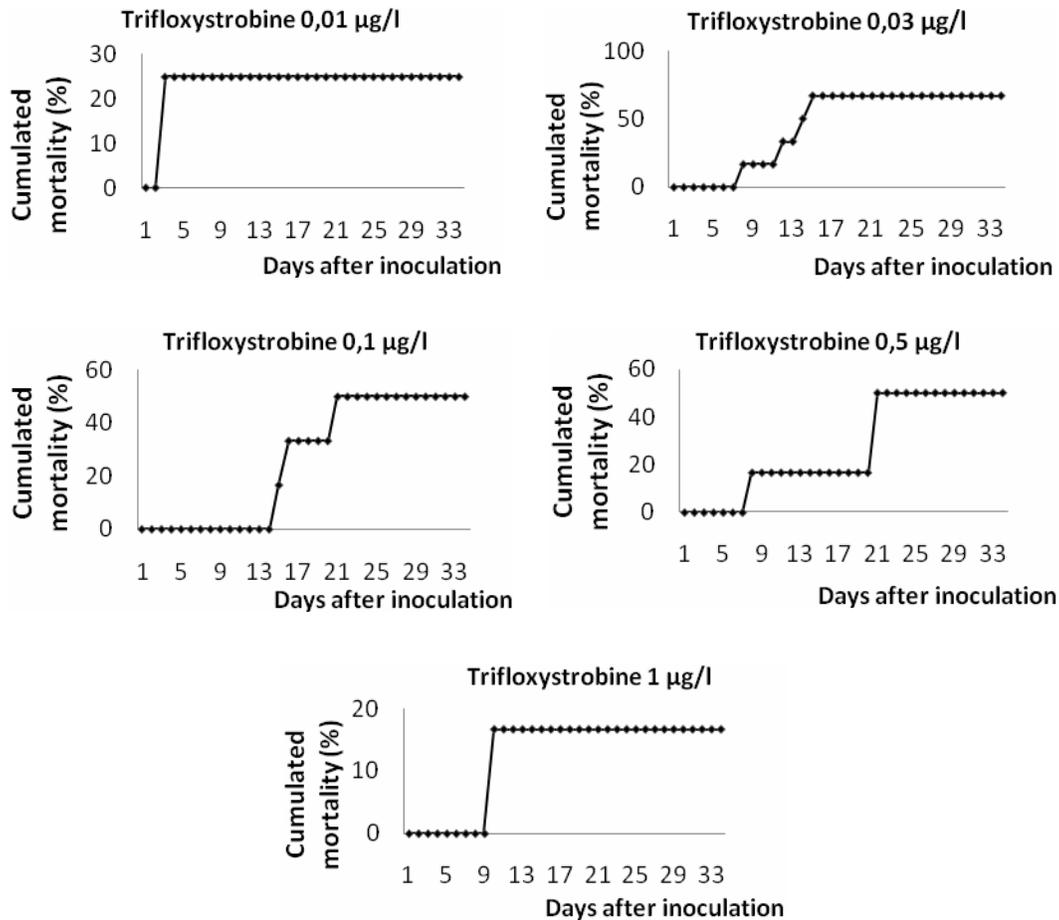


Figure.2 Mortality rates of *C. sordidus* inoculated with conidial suspensions produced on PDA media containing the fungicide Trifloxystrobin



Effect nematicides on conidial germination, mycelial growth and sporulation

Both nematicides Oxamyl and Ethoprophos stimulated germination of conidia. The results were similar to those of Aby (2013) and also compatible with the conidial germination *Metarhizium* sp. The application of these nematicides in banana fields could increase germination and pathogenicity of *Metarhizium* sp, which is a soil born fungus.

Low inhibitions and mycelial growth stimulation were recorded and indicate the compatibility between these nematicides and

the biocontrol agent. Aby (2013) also demonstrated they were no significant difference between mycelial growth on medium containing 5 µg/l of oxamyl and on the control medium. High doses of 10 µg/l and 15 µg/l Oxamyl and Ethoprophos inhibited sporulation of the fungus but for low doses (5 µg/l and 3 µg/l) only stimulation were observed. Stimulation due to nematicide was also observed by Culbreath *et al.* (1986). On *Paecilomyces lilacinus*, biological agent against the nematode *Meloidogyne* sp., Aby (2013) also demonstrated sporulation inducing at 5 µg/l of oxamyl and its compatibility to the fungus.

Conidial germination, mycelial growth and sporulation of *Metarhizium* sp were inhibited by the Methyl-thiophante, the Trifloxystrobin and the Propinaconazole at their laboratory concentration corresponding to the concentrations applied on banana fields against banana black Sigatoka. The use of *Metarhizium* sp as biological agent against *Cosmopolites sordidus* should be considered, although foliage of banana mat and systemic effect of these fungicides were barriers for fungicides to contact the inoculum. However, conidial germination, mycelial growth and sporulation could be obtained with these fungicides. And conidia produced in the presence of fungicide were also mortality cause of the banana weevil, *Cosmopolites sordidus* although their efficiencies were affected. Furthermore, most of the nematicides concentrations tested were compatible with the fungus and boosted sporulation and could be used for mass production of the inoculum.

References

- Aby N. 2013. Lutte biologique contre le charançon noir du bananier, *Cosmopolites sordidus* Germar (Coleoptere : Curculionidae) en Côte d'Ivoire : Caractéristiques entomopathologiques d'isolats locaux de *Metarhizium* sp testés sur les populations au laboratoire et en bananeraie. Thèse de Doctorat présentée à l'UFR de Biosciences de l'Université de Cocody, Côte d'Ivoire. 181p.
- Aby N. , Kobénan K., Traoré S. and Koné D. 2011. *Metarhizium anisopliae* as potential biological agent of banana weevil *Cosmopolites sordidus* in Côte d'Ivoire. International Journal of Biological and Chemical Sciences IJBCS, Vol. Dec. 2011.
- Aby N., Kobenan K., Kéhé M., Gnonhour P., Koné D. and Zouzou M. 2010. *Metarhizium anisopliae*: parasite du charançon noir du bananier *Cosmopolites sordidus* dans les bananeraies Ivoiriennes. Journal of Animal & Plant Sciences,. Vol. 7, Issue 1: 729- 741.
- Atrassi K., Benkirane R., Atrassi B. and Badoc A. 2007. Efficacité de deux fongicides benzimidazoles et anilopirimidine sur la pourriture des pommes en conservation. Bull. soc.pharm.Bordeaux. 195-210
- Bacon R. 2002. Gestion de la résistance aux fongicides. Agriculture et Agroalimentaire Canada.<http://www.agrireseau.qc.ca/petitsfruits/Documents/resistance%20aux%20fongicides.PDF>. Consulté le 12-09-2013.
- Chubilleau C., Pubert M., Comte J. and Giraud J. 2011. Pesticides et santé. Etude écologique du lien entre territoires et mortalité en Poitou-Charentes. Rapport 136. 222 p. <http://www.ors-poitou-charentes.org/pdf/kvUUK4Pesticides11.pdf>. Consulté le 31-08-2014.
- Couteux A. and Lejeune V. 2005. Index phytosanitaires ACTA, 41ème édition, 820p.
- Culbreath, A.K., Rodriguez-Kaban R. and Morgan-Jones G. 1986. Chitin and Paecilomyces lilacinus for control of Meloidogyne arenaria. Nematropica, 16(2):153-166.
- Davidse L.C. and Flach W. 1978. Interaction of thiabendazole with fungal ubulin. - Biochemistry Biophysique Acta, 543(1), 82-90.
- Faurie B., Cluzet S., Corio-costet M.F. and Merillon J.M. 2009. Methyl jasmonate/etephoncotreatments synergically induces stilbene production in vitisvinifera cell suspension but fails

- to trigger resistance to *Erysiphe necator*. *Journal International Sciences Vigne vin* 43 (2): 99-110.
- Gilbert G. 1999. La résistance des champignons aux fongicides. Semaine Hortic. http://www.agrireseau.qc.ca/legumes_champ/documents/R%C3%A9sistance.pdf. Consulté le 12-09-2013.
- Gold C.S., Jorge P.E., Karamura E.B. and Rukazambuga N.D. 2003. Biology and integrated pest management for the banana weevil *Cosmopolites sordidus* (Germar). *Integrated Pesticides Management Review*, 6: 79–155.
- Gold C.S., Pena J.E. and Karamura E.B. 2001. Biology and integrated pest management for the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Integrated Pest Management Reviews*, 6: 79-155.
- Grant NT, Prusinkiewicz E, Makowski RMD, Holmstrom-Ruddick B and Mortensen K 1990 Effect of selected pesticides on survival of *Colletotrichum gloeosporioides* f. sp. *malvae*, a bioherbicide for round-leaved mallow (*Malva pusilla*). *Weed Technology* 4, 701–715.
- Kobenan K., Traoré S. and Essi B. 2012. Niveau de sensibilité du champignon responsable de la cercosporiose noire aux fongicides systémiques appliqués sur les bananiers en plantations industrielles en Côte d'Ivoire. Rapport CNRA. 31 p.
- Kobenan K., Traoré S. and Essi B. 2011. Niveau de sensibilité du champignon responsable de la cercosporiose noire aux fongicides systémiques appliqués sur les bananiers en plantations industrielles en Côte d'Ivoire. Rapport CNRA. 27 p.
- Koller W. and Scheinpflug H. 1987. Fungal resistance to sterol biosynthesis inhibitors: a new challenge. *Plant Dis.*, 71: 1066-1074.
- Kouser S. and Qaim M. 2011. Impact of Bt cotton on pesticide poisoning in smallholder agriculture: A panel data analysis. *Ecological Economics*, 70: 2105–2113.
- Moshe R. 2005. Inhibition of germination and growth of *Alternaria alternata* and mouldy-core development in Red Delicious apple fruit by Bromuconazole and Syngnum. *Crop Protection*, 25: 253-258.
- Schiffers B. 2011. Lutte biologique et production intégrée, manuel 10. PIP éditions, Bruxelles, Belgique, 128p. <http://www.pip.coleacp.org./files/documents/COLEACP>. Manuel consulté le 21-02-2014.
- Sholberg P.L., Harlton C., Haag P., Levesque C.A., O'Gorman D. and Seifert K. 2005. Benzimidazole and diphenylamine sensitivity and identity of *Penicillium* spp. that cause postharvest blue mold of apples using β -tubulin gene sequences. *Postharv. Biol. Technol.*, 36(1), 41-49.
- Stierl R. A., Hanke W., Lorenz G., Koelhle H. and Ammermann E. 2002. Nicobifen (BAS 510 F) biological characteristics and redistribution. *Phytopathology* 92, S79 (Abst.).
- Wyss G.S., Charudattan R., Roskopf E.N. and Littell R. 2004. Effect of selected pesticides and adjuvants on germination and vegetative growth of *Phomopsis amaranthicola*, a biocontrol agent for *Amaranthus* spp. *European Weed Research Society, Weed Research* 44: 469-482.