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Susceptibility of *Mycosphaerella fijiensis* to Mefentrifluconazole in Industrial Banana Plantations in Côte d'Ivoire : An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Banana Sigatoka, caused by Mycosphaerella fijiensis, severely impacts banana production in Côte d'Ivoire. This study evaluates the susceptibility of M. fijiensis isolates to mefentrifluconazole, a new

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triazole fungicide, compared to difenoconazole. *In vitro* tests on 17 isolates from industrial plantations revealed IC50 values ranging from 0.005 to 0.073 mg/L (average 0.037 mg/L), indicating high sensitivity. Growth inhibition rates were higher for mefentrifluconazole, demonstrating its superior efficacy. These results suggest mefentrifluconazole as a promising alternative for managing black spot and mitigating resistance development.

Keywords: Black spot; banana; mycosphaerella fijiensis; mefentrifluconazole; Côte d'Ivoire.

1. INTRODUCTION

Black Sigatoka, caused by *Mycosphaërella fijiensis* Morelet, is currently the main foliar disease affecting banana and plantain production (De Lapeyre, et al., 2010; Zandjanakou-Tachin, et al, 2013). The pathogen attacks banana leaves by damaging the leaf surface, thereby reducing the photosynthetic capacity of the leaves, which affects plant growth and development (Patrick, et al., 2023). The strategic approach to the introduction of appropriate agronomic techniques for improving the productivity of industrial banana plantations and better control of Mycosphaërella fijiensis populations necessarily involves rational management of chemical applications. In Côte d'Ivoire, repeated applications of systemic fungicides, particularly triazole fungicides have led to the development of loss of susceptibility within populations of this pathogen (N'guessan, 2008, Essis et al.,2008.) The emergence and development of resistance of *M. fijiensis*, to 14α-demethylase inhibitors (DMIs) has become a critical problem in industrial dessert banana plantations in Côte d'Ivoire (N'guessan, et al., 2016). Mefentrifluconazole, (2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1- (1H-1,2,4-triazol-1-yl) propan-2-ol, is an active substance in the new subclass of isopropanol-triazole agricultural fungicides.

The mode of action of this fungicide is the inhibition of C14-demethylase in the biosynthesis of sterols in membranes and belongs to FRAC code 3 (Ishii et al., 2021). It has strong selective fungicidal activity. A comprehensive toxicity testing programme according to OECD (Organisation for Economic Co-operation and Development) guidelines showed that mefentrifluconazole is non-genotoxic and non-carcinogenic (Tesh, et al., 2019). Mefentrifluconazole, the first isopropanol-azole fungicide belonging to a new DMI subclass (Ishii, et al., 2021), developed by BASF, could be an alternative for managing the triazole resistance of *M fijiensis* strains to from dessert banana production basins in Côte d'Ivoire (Gogbeu, et al., 2022).

It is in this context of improving control strategies for banana black spot that the present study is being carried out. The aim is to characterise the susceptibility of *M. fijiensis* to the active ingredients currently in use. Specifically, the aim is to determine the pathogen's level of susceptibility to mefentrifluconazole and difenoconazole. The pathogens came from 17 conventional plantations that had been treated more or less intensively, and were compared with pathogen populations from farmers' plantations that had never been treated.

2. MATERIALS AND METHODS

2.1 Materials

The plant material used in this study consisted of the leaves of banana cultivars (Grande Naine and Williams) whose fruit are dessert bananas intended for export. The banana plants were sampled in 17 industrial plantations. A final site in the city of Abidjan consisted of banana plants where no fungicide had been applied.

2.2 Fungal Material

In the laboratory, the study was carried out specifically on strains derived from monospore (conidial) cultures isolated from dessert and plantain banana leaves collected from different plantations. The leaves were at stages 3 and 4 of black Cercosporiosis.

2.3 Synthetic Fungicides

The fungicides used in our study are synthetic fungicides, intended mainly for the control of black cercosporiose. They were chosen on the basis of their current and forthcoming use in Côte d'Ivoire to control black cercosporiose.

These are mefentrifluconazole and difenoconazole, two active ingredients from the triazole family. They are renowned for their inhibitory action on the young stages of black spot. Their *in vitro* activity has been evaluated on the elongation of the germ tube of *M. fijiensis* conidia.

2.4 Methods

2.4.1 Taking leaf samples

Samples were taken from leaves that were still alive and bearing stage 3 and 4 lesions of black cercosporiosis that were well isolated from each other. Samples were taken from 30 banana plants in an area of 2 to 4 ha previously defined for the annual monitoring of the sensitivity of strains to the fungicides applied. From these banana trees, fragments of leaf blades measuring 15 cm x 25 cm were taken, with lesions as large as possible (at least 1 cm x 2 cm) to be cut individually and analysed separately in the laboratory.

2.4.2 Preparation of culture media

The culture media used were agar media containing the various fungicides. They were prepared in two stages. First, pure agar was prepared. It was used at 2%, i.e. 2 g of agar per 100 ml of distilled water. This mixture was autoclaved at 121°C for 20 min at a pressure of 1 bar. Next, the triazole fungicides were prepared as a 1000 ppm stock solution and then distributed in the pure agar at different concentrations.

The concentration range was - 0, 0.03, 0.1, 0.3 and 1 ppm for the active ingredients mefentrifluconazole and difenoconazole. The amended media were dispensed into Petri dishes at a rate of 10 to 12 ml per dish. For each sample to be analysed, two Petri dishes were used for each concentration.

2.4.3 Isolation of strains of *Mycosphaerella fijiensis*

All strains were derived from monoconidial isolation. During isolation, the presence of conidia was checked under the light microscope after the trapping phase. To do this, a few lesions were cut out individually and placed in direct contact with the culture media contained in control Petri dishes (0 ppm), then immediately removed using the method of Koné (2008). After the verification phase, using the same procedure, the lesions from the selected fragments were cut out and placed in direct contact with culture media amended with different concentrations of fungicides. A Petri dish divided into thirty-two segments at the base using an indelible pen was used for each concentration. Conidia were isolated from each segment and the Petri dishes were incubated for 48 h at 25°C (Fig. 1).

2.4.4 Evaluation of the susceptibility of *Mycosphaerella fijiensis* to fungicides

In the case of triazoles, this evaluation consisted of measuring the length of the germ tube under the microscope of the strains using a micrometer. The percentage inhibition of germ tube growth in the presence of each fungicide was calculated compared to controls without fungicides using the following formula:

Lm (T0): Average length of germ tubes on control medium

Lm (Tx): Average length of germ tubes on medium amended with different concentrations of fungicides.

$$Inhibition\ Rate = \frac{Lm\ (T0) - Lm(Tx)}{Lm\ (T0)}\ X\ 100$$

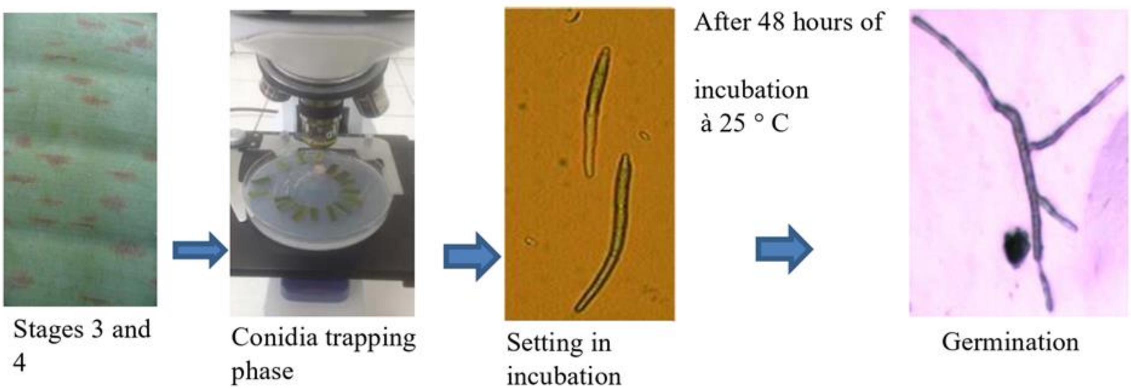


Fig. 1. Technique for culturing conidia

The distribution of growth inhibition classes at the discriminating dose of 0.1 ppm was estimated graphically in order to assess the 'shift' in the sensitivity of the strains in relation to the never-treated plantation (Baseline). The connection between the active ingredient logarithm concentration and the inhibition rate was used to estimate the concentrations which inhibit germ tube growth by 50% (IC 50) and 95% (IC 95), in relation with the control (0 ppm) (FRAC/DM/Working Group Banana 2021 recommendations).

3. RESULTS

3.1 Susceptibility of Strains to Mefentrifluconazole and Difenoconazole Molecules

3.1.1 Susceptibility of strains to mefentrifluconazole and difenoconazole molecules in never-treated plantations (wild)

In plantations that had never received a fungicide, inhibition rates of the germ filaments of isolates were greater than 70% whatever the fungicide and concentration may be, reflecting their effectiveness on the development of the fungus. At a concentration of 0.1 ppm, inhibition rates were 88.6% for mefentrifluconazole and 84.7% for difenoconazole (Fig. 2).

The IC50 and IC95 values were 4.30 x 10-7 mg/l and 8.56 x 10-7 mg/l respectively for mefentrifluconazole, and 3.46 x 10-4 mg/l and 0.651 mg/l for difenoconazole.

Distribution of strain sensitivity to 0.1 ppm in never-treated plantations

Analysis of the distribution of inhibition rates showed that isolates from untreated plantations were all susceptible to mefentrifluconazole and difenoconazole. This distribution was bimodal (80%-90% and 90%-100%) with 100% of isolates inhibited (Fig. 3).

3.1.2 Susceptibility of strains to mefentrifluconazole and difenoconazole in industrial production areas

Growth inhibition rates at 0.1 mg/l of germ tubes obtained with mefentrifluconazole ranged from 74.7% to 64.7% in isolates from industrial plantations (Fig. 4). These inhibition rate values showed a strong inhibitory action of mefentrifluconazole on mycelial growth. The highest rates were obtained from KOFFIBAM isolates (74.7%).

For isolates treated with difenoconazole at a concentration of 0.1 mg/l, germ tube inhibition rates ranged from 73.0% to 55.8%. Generally speaking, these rates were lower than those of isolates treated with mefentrifluconazole (Fig. 4). The lowest rates of growth inhibition by difenoconazole were obtained in isolates from TIABAM (55.8%), which seems to be a loss of sensitivity of the fungus in this plantation.

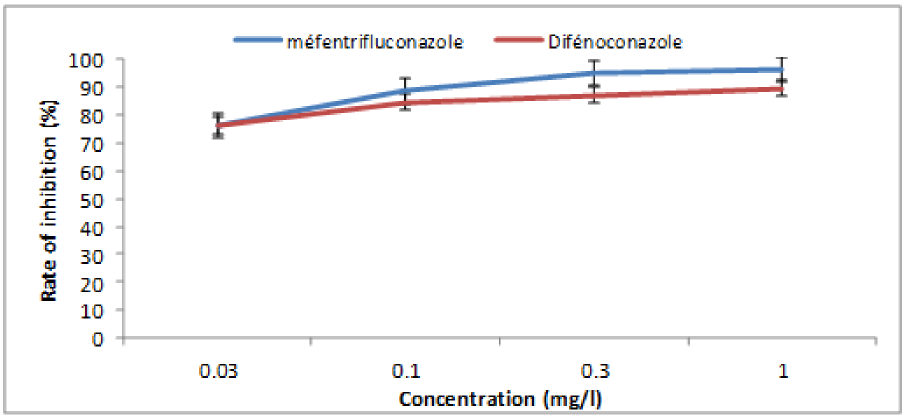


Fig. 2. Growth inhibition rates of *M. fijiensis* germ mycelia to mefentrifluconazole and difenoconazole in never-treated plantations

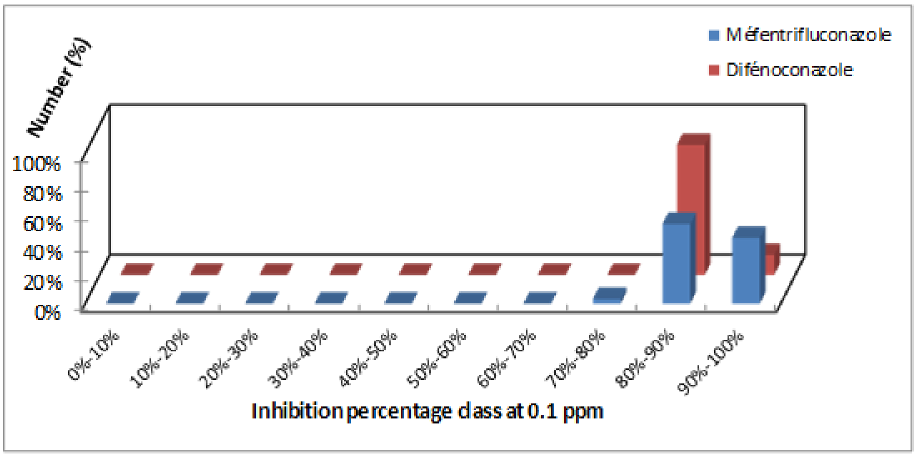


Fig. 3. Distribution of germ tube inhibition rate values at 0.1 mg/l for strains from plantations never treated with fungicides

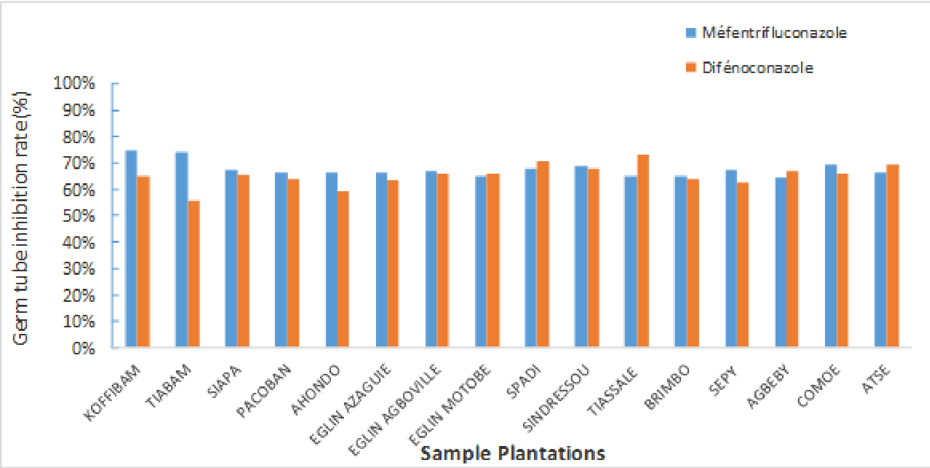


Fig. 4. Growth inhibition rate of M. fijiensis to mefentrifluconazole and difenoconazole molecules isolated from commercial plantations

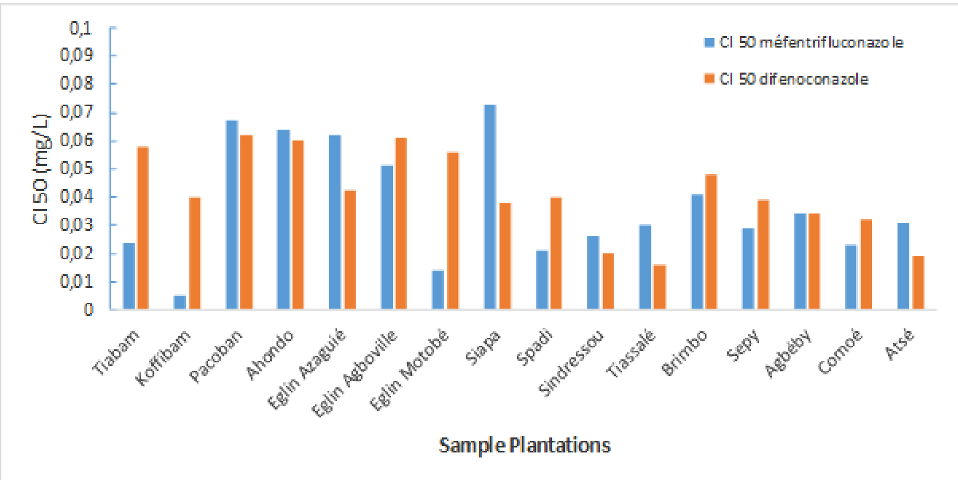


Fig. 5. IC50 of *Mycosphaerella fijiensis* isolates from industrial plantations

Regardless of location, IC50 values obtained from isolates from commercial plantations treated with mefentrifluconazole were low. The mean IC50 was 0.037 mg/l. As for the IC 95, the average obtained was 1.04 mg/l. The IC50 obtained showed that the isolates were all sensitive to mefentrifluconazole (Fig. 5).

The mean IC50 evaluated was 0.045 mg/l in isolates treated with difenoconazole. This value is lower than that of isolates treated with mefentrifluconazole, but is still low. This suggests that isolates from commercial plantations are sensitive to difenoconazole. The mean IC 95 was 1.10 mg/l.

Analysis of the IC50 and IC95 data generally showed that the IC50 values recorded were below 0.1 mg/l. Mefentrifluconazole showed very satisfactory efficacy in the laboratory on the germination of mycelial filaments. The efficacy of difenoconazole was also satisfactory on analysis of the laboratory results.

3.1.3 Distribution of inhibition rate values for M. fijiensis isolates at 0.1 mg/L from industrial plantations

At TIABAM, the distribution of inhibition rate values at 0.1 mg/l showed a better fungistatic action of mefentrifluconazole compared with difenoconazole. 50% of the inhibited strains were in the modal class of 70-80%, while for difenoconazole, the amplitude of the numbers evaluated was less than 40%. It was in the 60-70% class (Fig. 6A).

In Koffibam, the range of distribution was between 50-90% with 41% of inhibited strains in the 80-90% class. The spread of the distribution of isolates to difenoconazole indicated a weak inhibitory action compared to mefentrifluconazole. In fact, the majority of strains were distributed in the 30-90% class (Fig. 6B).

A better fungistatic action of mefentrifluconazole compared with difenoconazole was observed at PACOBAN. 53% of the strains inhibited by mefentrifluconazole were located in the 60-70% modal class, whereas for difenoconazole, the range of numbers assessed was 41% (Fig. 6C).

For isolates from AHONDO, the distribution of individuals showed that 50% of those treated with 0.1 mg/L mefentrifluconazole were in the 70-80% modal class. However, 6% of strains were in the 40-50% class (Fig. 6D).

At Eglin Azaguié, the sensitivity of isolates to difenoconazole began to drift. In fact, 7% of individuals were inhibited at less than 50% of their germ filament. For strains treated with mefentrifluconazole, although the range of numbers was 40.6% at the 70-80% class, a risk of drift in susceptibility to mefentrifluconazole is likely. 3% of the numbers showed less than 50% inhibition of germinative mycelia (Fig. 6E).

At Eglin Motobé, although all the mycelial filaments were inhibited by more than 50%, the distribution of inhibition classes compared with that of the baseline showed a ‘shift’, which could reflect a future drift of mefentrifluconazole in this plantation. Analysis of the distribution of the inhibition rate of strains treated with difenoconazole shows that the modal class is also 60-70%, with a population of 80%. The number of conidia whose germ tubes were less than 50% inhibited was 3% (Fig. 6F).

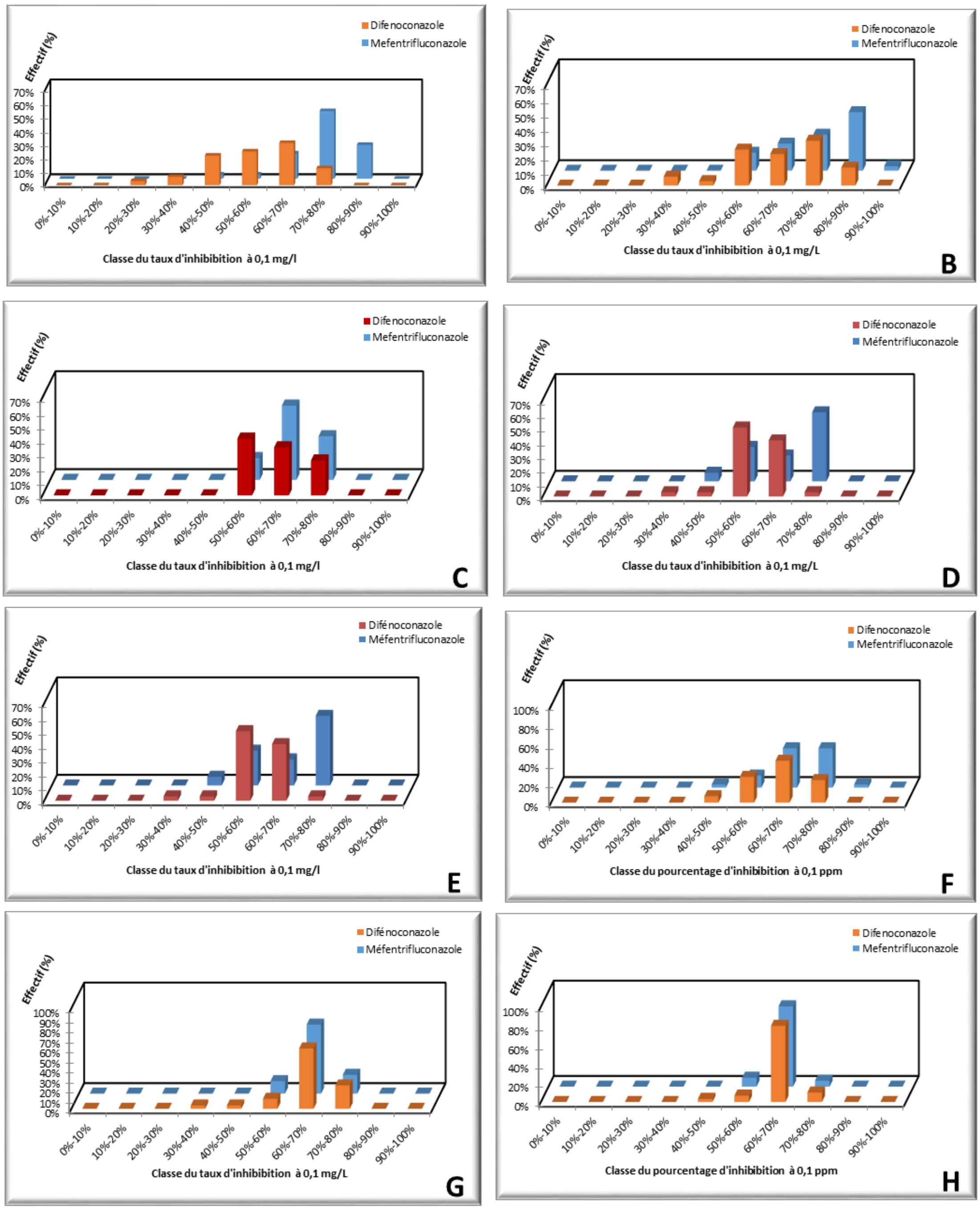
At Agboville, mycelial filaments were inhibited to over 50%, showing satisfactory action of mefentrifluconazole in this plantation. Inhibition of isolates treated with difenoconazole also remained acceptable at a dose of 0.1 mg/L, although 6% of isolates showed germ tubes inhibited to less than 50% (Fig. 6G).

All isolates were more than 50% inhibited in the SIAPA plantation; the effect of mefentrifluconazole was satisfactory. Class distribution indicated that 13% of the numbers treated with difenoconazole were inhibited to less than 50%. A drift in sensitivity to difenoconazole was observed (Fig. 6H).

At SPADI, 50% of the strains inhibited by mefentrifluconazole were found to be in the 70-80% modal class (Fig. 6I). For difenoconazole, the range of numbers assessed was 40% in the 70-80% class. 23% of those treated with difenoconazole had mycelial filaments inhibited by more than 80%. The distribution of values showed a better fungistatic action of mefentrifluconazole and difenoconazole at 0.1 mg/L.

At Sindressou, the distribution of strains by inhibition class showed that 47% of isolates treated at 0.1 mg/L with mefentrifluconazole fell into 2 modal classes: 70-80% and 60-70% (Fig. 6J). All filaments were more than 50% inhibited, showing satisfactory action of mefentrifluconazole in this plantation. The strains treated with difenoconazole showed a modal class in the 60-70% range, with 67% effective (Fig. 6J).

In Tiassalé, the distribution of inhibition classes for strains treated with mefentrifluconazole showed a modal class of 60-70%, with a population of 63% (Fig. 6K). Although all strains were more than 50% inhibited, there was a 'shift' compared with the never-treated wild-type strains. The difenoconazole-treated strains showed a modal class in the 70-80% range, with a population of 73%. The distribution of isolates in the growth inhibition class at Brimbo showed that 75% of individuals treated with mefentrifluconazole were in the 60-70% modal class (Figure 6 K). The distribution of inhibition rate values for isolates treated with difenoconazole showed that the modal class was also 60-70%, with a population of 57%. Although all mycelial filaments were more than 50% inhibited, the distribution of inhibition classes compared with the baseline also showed a shift. This could indicate a future shift in the sensitivity of strains to these 2 molecules in this



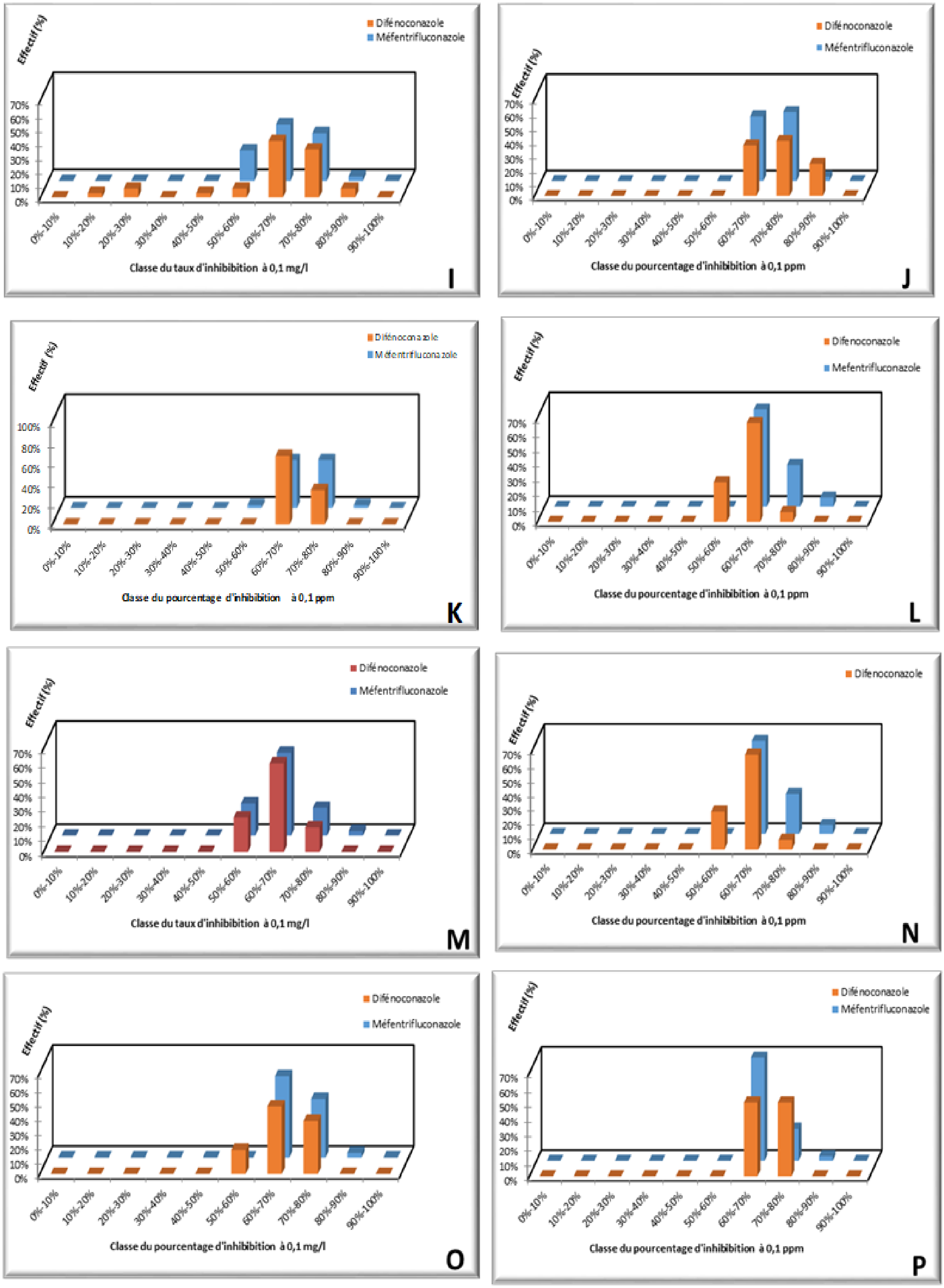


Fig. 6. Distribution of inhibition rate values at 0.1 mg/L of *M. fijiensis* isolates from :
A- TIABAM ; B- KOFFIBAM ; C- PACOBAN ; D- AHONDO ; E- Eglin Azaguié ; F-Eglin Agboville G- Eglin Motobé ; H- SIAPA ; I-SPADI ; J- SINDRESSOU ; K- TIASSALE ; L- BRIMBO ; M- SEPY ; N-AGBEBY ; O-COMOE ; P-ATSE

plantation (Fig. 6L). At SEPY, the distribution of strains showed three classes ranging from 60% to 90%, with a modal class of 60-70% (Fig. 6M). The modal class of 66% shows that the effect of mefentrifluconazole was satisfactory. The strains treated with difenoconazole showed a distribution ranging from 50% to 80%, with 67% of the strains in the 60-70% modal class. A total of 27% of strains were located in the 50-60% range (Fig. 6M). Monitoring of the effect of difenoconazole in this plantation is necessary.

At AGBEBY, the strains treated with mefentrifluconazole showed a distribution ranging from 50% to 80%, with 56% in the 60-70% modal class.

This distribution was similar to that obtained with difenoconazole-treated strains, but with an amplitude of 60% at the modal class of 60-70% (Fig. 6N).

The numbers of inhibited strains obtained in the 50-60% range for mefentrifluconazole and difenoconazole were 22% and 23% respectively. Sensitivity to these 2 active ingredients needs to be monitored.

At Comoé, the distribution of individuals treated with mefentrifluconazole fell into 3 classes ranging from 60% to 90%. The number of individuals in the 60-70% modal class was 56%. There were 41% of strains classified in the 70-80% interval and 3% in the 80-90% class (Fig. 6O). In this plantation, mefentrifluconazole had a satisfactory effect on germ tube growth. The strains treated with difenoconazole showed a distribution ranging from 50% to 80%, with 17% in the 50-60% class. Although all the strains were more than 50% inhibited, vigilance regarding sensitivity to difenoconazole should be necessary for this plantation.

At Atsé, the distribution of strains by growth inhibition class showed that 75.0% of strains treated with 0.1 mg/l mefentrifluconazole were in the 60-70% modal class (Fig. 6P). 22% of inhibited strains were in the 70-80% range and 3% in the 80-90% range. The inhibitory action of mefentrifluconazole on mycelial growth was satisfactory. The distribution of strains to difenoconazole was bimodal, with 50% of the numbers in the 60-70% class and the other 50% in the 70-80% class.

4. DISCUSSION

The effect of mefentrifluconazole was assessed as part of the search for improved control strategies for black cercosporiosis and for new active ingredients to manage the loss of sensitivity of Mycosphaerella fijiensis to certain triazoles in conventional banana production. Under *in vitro* conditions, after its incorporation into the culture medium, a reduction in conidial germ tube elongation was observed at 0.1 mg/L. Experiments showed that there were differences in the susceptibility of *Mycosphaerella fijiensis* to mefentrifluconazole and difenoconazole. Germ tube growth inhibition of conidia treated with mefentrifluconazole was higher than those treated with difenoconazole. Recent studies carried out to assess the susceptibility and risk of resistance of *Corynespora cassiicola* to isopyrazam and mefentrifluconazole showed that mefentrifluconazole showed the strongest inhibition of mycelial growth (Ma, et al., 2020).

Comparative analysis of IC50 values revealed that levels of susceptibility of *M. fijiensis* to mefentrifluconazole could vary from one locality to another. Indeed, the sensitivity of the 17 isolates to mefentrifluconazole varied considerably, with minimum and maximum EC50 values of 0.005 mg/L and 0.073 mg/L respectively. The work of (Peng, et al., 2024) on cucumber cultivation in China, on the inhibitory activity of mefentrifluconazole in 101 isolates of *C. cassiicola* also showed the variability of susceptibility from one locality to another.

The growth inhibition class distribution of the isolates according to the active ingredients showed that the variability of their susceptibility is related to the number of applications made in their original plantations. A slight shift in *M. fijiensis* populations was observed in the commercial plantations sampled, in contrast to isolates from untreated plantations, which showed a unimodal distribution. According to Parisi, et al (1991), the selection pressure exerted by fungicide applications causes a gradual reduction in the susceptibility of the pathogen's populations. This assertion is confirmed in our study, as difenoconazole, which made up 14% of the triazole molecules sprayed in Ivorian commercial plantations (N'guessan et al., 2016), is currently the most widely used triazole. However, an analysis of the different nodal classes in the distribution of inhibition rates suggests that *M. fijiensis* isolates remain sensitive to difenoconazole and mefentrifluconazole in the laboratory.

Recent studies on the inhibitory activity of mefentrifluconazole against susceptible and resistant isolates of older DMIs using the *M. fructicola* complex, *Colletotrichum* spp. and *Alternata sp*. from peach, *C. beticola* from sugar

beet and *P. xanthii* from cucumber, showed cross-resistance with these fungicides *in vitro* and in plantations (Ishii, et al*.,* 2021). These authors concluded that mefentrifluconazole had an intrinsic efficacy comparable to that of other DMI fungicides. These results corroborate our own, which reveal the efficacy of mefentrifluconazole on Mycosphaerella fijiensis isolates from dessert banana production basins in the Côte d'Ivoire.
As an isopropanol triazole fungicide, mefentrifluconazole inhibits the biosynthesis of ergosterol, a vital component of the fungal cell membrane. By interfering with ergosterol production, mefentrifluconazole disrupts the structure and function of the plasma membrane in fungal cells (Liu, et al., 2022). This disruption affects the fluidity, integrity and permeability of the membrane, ultimately leading to inhibition of fungal growth or death.

Polygenic determinism involving genes that contribute to susceptibility or resistance of *M fijiensis* to mefentrifluconazole was not demonstrated in our study, however analysis of the CYP51 gene in *M fijiensis* indicated that six mutations were associated with different degrees of *in vitro* susceptibility to propiconazole (Cañas-Gutiérrez, et al., 2009). Other recent studies on C. cassiicola in the control of cucumber target spot suggested that the risk of developing resistance to mefentrifluconazole was moderate, but that overexpression of CcCYP51A and CcCYP51B could be associated with resistance to mefentrifluconazole in C. cassiicola (Peng, et al., 2024).

Mutations in the structural gene for 14-alpha demethylase (ERG11/CYP51), which lead to a reduction in the affinity of the target protein for DMI fungicides, have also been observed in powdery mildew (Delye, et al., 1998, Delye, et al., 1997) and M. graminicola (Leroux, et al*.,* 2007).

Mefentrifluconazole has been shown to be highly effective in controlling major fungal diseases of pome and stone fruits, grapevine, potato, soybean (Heinecke et al. 2019) and other crops and has recently been registered in Côte d'Ivoire for the control of banana black spot.

5. CONCLUSION

This study made it possible to characterise the level of sensitivity of *Mycosphaerella fijiensis* to mefentrifluconazole and difenoconazole. Mefentrifluconazole was more effective than difenoconazole at inhibiting ergosterol biosynthesis. In the laboratory, mefentrifluconazole was found to be more effective than difenoconazole in inhibiting ergosterol biosynthesis on the industrial plantations sampled in Côte d'Ivoire.

Mefentrifluconazole is a promising fungicide for controlling banana black spot in Côte d'Ivoire.

Difenoconazole is still remarkably effective in the laboratory on certain industrial plantations in Côte d'Ivoire.

We speculate that mefentrifluconazole may have a different mode of binding to CYP51 in M. fijiensis. Further research is needed to elucidate the polygenic determinism of the fungus to mefentrifluconazole. We also recommend that field tests to measure in situ bioefficacy be carried out in order to confirm these results in the field.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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