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In vitro biological control of four fungi responsible for leaf diseases of yam (*Dioscorea spp*) in Côte d'Ivoire

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ABSTRACT

Yam (*Dioscorea spp*) is a major food crop in many parts of the world, particularly in West Africa. In Côte d'Ivoire, yam occupies the first place in terms of food crops. However, this crop is faced with enormous parasitic constraints including leaf diseases. Anthracnose is the most frequent fungal disease in Côte d'Ivoire and has a formidable impact on yam production as it causes yield losses that can reach more than 90%. This study was conducted to evaluate the effect of three biofungicides (NECO, ASTOUN, FERCA) and a synthetic fungicide (REFERENCE) on the mycelial growth of *Colletotrichum sp*, *Pestalotia sp*, *Botryodiplodia sp* and *Curvularia sp*, four fungi responsible for yam anthracnose. It revealed that the biofungicides NECO and ASTOUN were the most effective on the *in vitro* mycelial growth of *Colletotrichum sp* with respective inhibition rates of 92.67 and 97.06% at the 2000 ppm dose. The synthetic fungicide REFERENCE and the biofungicide ASTOUN were more fungitoxic on the fungus *Pestalotia sp* by reducing its growth to 100% at 100 ppm and 1000 ppm respectively. FERCA biofungicide was the least effective on mycelial growth of the pathogens. These fungicides may provide a basis for field control of anthracnose of yam in Côte d'Ivoire.

Keywords: Anthracnose, biofungicide, fungi, leaf diseases, yam.

INTRODUCTION

In West Africa, yam occupies an important place in economic and nutritional terms, but also in cultural ceremonies. In terms of volume, yam is the leading food crop in Côte d'Ivoire. It occupies 63.72% of the food crop area with an annual production of about 5.7 million tons (Kouakou et al., 2019). Among the food yam species of the *Dioscorea* genus cultivated in West Africa, the *Dioscorea cayenensis-rotundata* complex, because of its organoleptic qualities, is the most widespread and accounts for more than 90% of total production. Yam cultivation contributes to the food security of 300 million people in tropical countries. However, several constraints affect production and cause huge losses. These are constraints such as ecological factors, bacteria, insects and microscopic fungi. They constitute bottlenecks for yam cultivation, the main one being the attack of fungal agents. Indeed according to (Kouadjo et al., 2018), anthracnose is the most frequent fungal disease in Côte d'Ivoire and having a formidable impact on yam production as it

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causes yield losses that can reach more than 90%. Thus, the problem of foliar diseases of yam raises as a research question that of biological control of the responsible fungi. In Côte d'Ivoire, the flora abounds with plants with proven antifungal properties, notably *Eucalyptus citriodera* (Camara et al., 2010), *Ocimum gratissimum* and *Cymbopogon citratus* (Maïno, 2018), but their efficacy has been little or not at all evaluated in the fight against foliar fungi of yam. Yet, pesticides based on these plants could be alternatives to synthetic products used in the control of these pests. The present study proposes to evaluate the effects of three biopesticides compared to a synthetic product on the *in vitro* growth of four fungi isolated from yam leaves showing symptoms of anthracnose.

MATERIALS AND METHODS

Materials

Plant and fungal material

The plant material consisted of yam leaves of the *Dioscorea alata* (Florido, Bètè bètè), *Dioscorea Cayenensis- rotundata* (Kponan, Krenglè, Assawa Blanc, Assawa jaune, Fassadjô) complexes, and some traditional varieties. These leaves showed characteristic symptoms of fungal attack (Figure 1). They were collected in yam production localities in the central, northern and eastern regions of the country. From these leaves, fungi of the genus *Colletotrichum* sp, *Pestalotia* sp, *Curvularia sp* and *Botryodiplodia sp* were isolated (Figure 2).

Fungicides

Four fungicides were evaluated in this study: three biopesticides named NECO, ASTOUN and FERCA and REFERENCE, a synthetic fungicide. Tween 20 was used to make the different biopesticides miscible in the PDA culture medium.

Methods

In vitro evaluation of the effects of biopesticides on the mycelial growth of four fungi

Preparation of the culture medium

The culture medium used is the PDA medium (Potato Dextrose Agar). It's made of potato puree (mineral source), glucose (carbon source) and agar (for the solidification of the medium). The preparation of 1 L of PDA medium requires 20 g of each of the above products weighed with a precision balance (0.001g). The mixture is placed in an Erlenmeyer flask and made up to 1 L with distilled water. This medium was autoclaved at 121°C for 30 minutes under a pressure of 1 bar. The obtained medium was distributed in 9 cm diameter Petri dishes under a laminar air flow hood, in the presence of a flame**.**

Addition of biopesticides to the culture media

After cooling the culture media to 45-50°C, the biological fungicides FERCA, ASTOUN and NECO and the synthetic fungicide (REFERENCE) were added. These different fungicides were added to the PDA culture medium to obtain the concentrations of 500, 1000, 1500 and 2000 ppm for the biofungicides and 5, 25, 50 and 100 ppm for the synthetic fungicide. The synthetic fungicide was used in this experiment as a positive control. The negative control consisted solely of the PDA culture medium.

Petri dishes containing the PDA medium, which the different products were added in increasing concentration were inoculated with a 0.5 cm diameter mycelial pellet. For each fungus, the mycelial pellets were taken from the growth front of a 7-day-old strain. To determine the radial growth of the mycelium, daily measurements were taken for 7 days from two perpendicular lines drawn on the reverse side of each Petri dish. Fungi that did not grow were transplanted onto new PDA media. The purpose of this action was to determine if the biofungicides were fungistatic or fungitoxic.

The rate of inhibition (Ti) of mycelial growth was determined daily from the formula of Hmouni et al. (1996) **:**

$$\text{Ti}\ (\%) = \frac{Do\ - Dc}{Do} \times 100$$

Ti : rate of inhibition

Do : the average mycelial growth diameter of the fungus in the control dishes ;

Dc : the average mycelial growth diameter of the fungus at the concentration of the fungicide (biological or chemical).

Statistical analysis

The data obtained were subjected to statistical analysis with Statistica 7.0 software. In case of a significant treatment effect, the means were compared according to the Newmann Keuls test at the 5% threshold. The statistical analysis performed was the chi-square analysis.

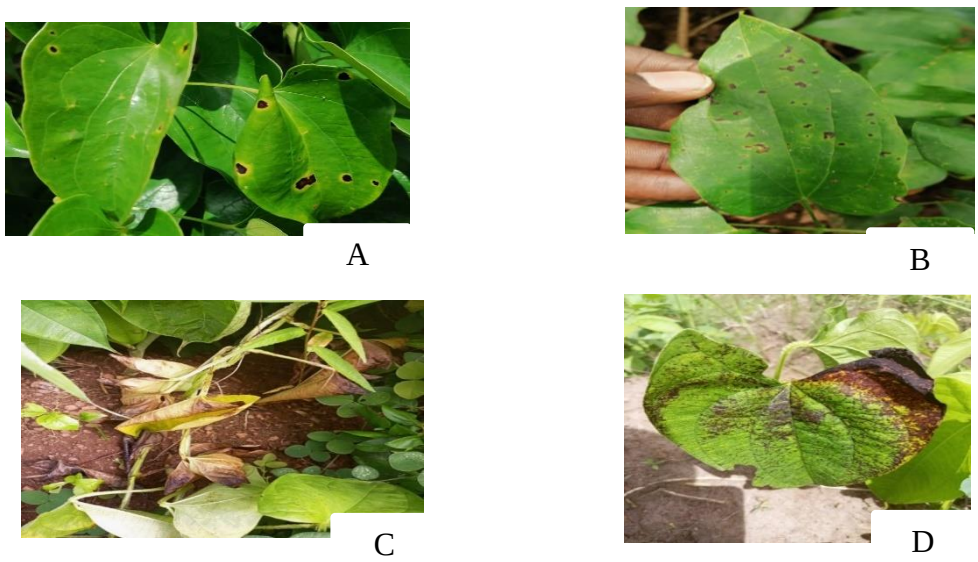

Figure 1 : Different symptoms of anthracnose observed on yam leaves.

A : black spots with yellow outlines (krenglè) ; B : black spots without contours (krenglè) ; C : death of the whole plant (bètè bètè) ; D : irregularly contoured burns (bètè bètè)

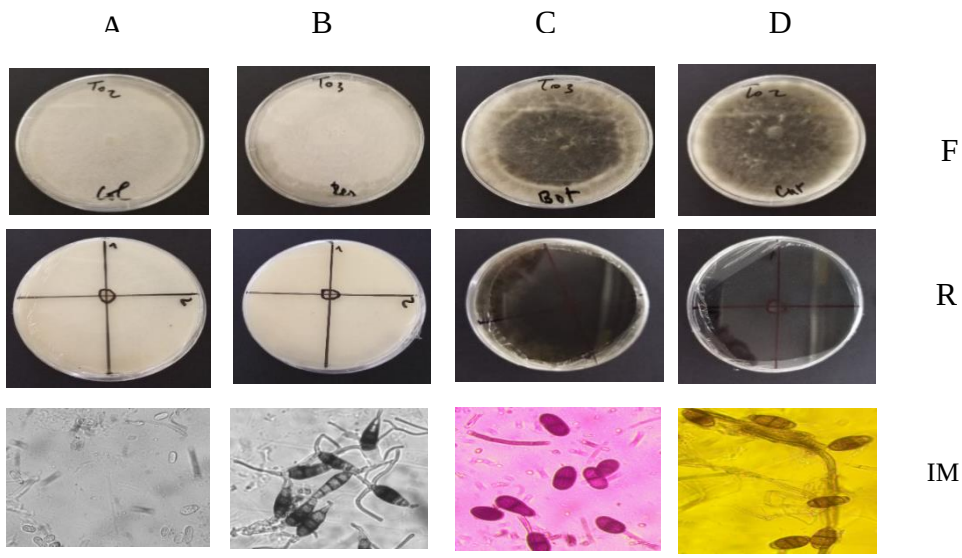

Figure 2 : Different fungi isolated from yam leaves

A : *Colletotrichum* sp ; B : *Pestalotia* sp ; C : *Curvularia* sp ; D : *Botryodiplodia* sp

F : front view ; R : back view; IM : conidies

RESULTS

Effects of fungicides on the *in vitro* growth of anthracnose fungi

Effect of NECO biopesticide on fungi

The rate of inhibition of mycelial growth of the fungus *Colletotrichum sp* was significant (greater than 90%) on days 1 and 2 for all doses compared to the control. For the 1500 and 2000 ppm concentrations, this rate remained above 50% throughout the experiment (Figure 3 A).

All doses were effective in reducing the growth of the fungus *Pestalotia sp* with an inhibition rate ranging from 55.81 to 96.30% during the first two days of the experiment. On the other days, the 2000 ppm dose was more effective with reduction rates of 75.66, 71.38 and 69.24% on days 3, 4 and 5 respectively (Figure 3 B).

The effect of NECO biopesticide was significant at all doses on the mycelial growth of *Curvularia sp* during the first three days of the experiment with oxillative rates between 35.80 and 93.64%. The reduction rate remained below 40% during the last two days with a minimum rate of 4.48% recorded with the 500 ppm dose on day 5 (Figure 3 C).

The reduction rates of mycelial growth of the fungus *Botryodiplodia sp* were day and dose dependent. Indeed, the reduction rates were significant for all doses during the first 3 days with values ranging from 53.60 to 100% (Figure 3 D). The 1500 and 2000 ppm doses were very effective on the first day by completely inhibiting the mycelial growth of the fungus. For the other four days, the reduction rate remained above 60% for these two doses.

Effect of ASTOUN biopesticide on fungi

All concentrations tested had a remarkable effect on the mycelial growth of the fungus *Colletotrichum sp*. The efficacy of this fungicide in reducing mycelial growth was not only concentration dependent but also time dependent. The inhibition rate varied from 11 to 100%. Mycelial growth of the fungus was completely inhibited on the first day at the 1000 ppm dose and reduced by more than 80% during all 5 days for the 2000 ppm concentration. (Figure 4A).

The susceptibility of the fungus *Pestalotia sp* differed according to the concentration of the biofungicide. Indeed, the concentration of 500 ppm was weakly fungitoxic. Its inhibition rate was less than 50% on days 3, 4 and 5. The product was highly fungitoxic at 1000 and 2000 ppm. These doses allowed a reduction rate ranging from 37.22 to 100% during the 5 days of the evaluation. As for the 1500 ppm dose, it caused a reduction rate of 77.61, 64.76, 48.41, 36.54 and 25.44% on days 1,2,3,4 and 5 respectively. Severe toxicity was observed at 1500 ppm, the growth of the fungus was totally inhibited during the 5 days (Figure 4B).

The effect of this biofungicide on the pathogen *Curvularia sp* was almost the same at 500, 1000 and 1500 ppm with inhibition rates that varied from 97.66 to 6% from day 1 to day 5. However, the 2000 ppm dose reduced mycelial growth of the fungus by 93.98% on day 1 before completely inhibiting it on the remaining four days (Figure 4C).

The effect of ASTOUN biopesticide on mycelial growth of *Botryodiplodia sp* was notable for all doses as the inhibition rate ranged from 100 to 14.78% from day 1 to day 4 (Figure 4D). The lowest rates were 3.4 and 6.9% obtained with the 500 and 1500 ppm concentrations on day 5, respectively. Only the 2000 ppm concentration completely inhibited mycelial growth of the fungus throughout the experiment.

Effect of FERCA biopesticide on Fungi

FERCA biofungicide showed almost similar effect on mycelial growth of the pathogen *Colletotrichum sp* at all concentrations. For the lowest concentration used (500 ppm) inhibition rates ranged from 66.11% to 18.27% from day 1 to day 5. No concentration of this product was able to completely inhibit mycelial growth of the pathogen. With the 1000, 1500 and 2000 ppm concentrations the inhibition rates improved significantly on the first three days ranging from 80.95 to 28.48% growth reduction (Figure 5A).

All doses used had average effects on mycelial growth of *Pestalotia sp*. There was a similar evolution of inhibition rates for all

concentrations with values ranging from 83 to 14% from day 1 to day 4. The fifth day is characterized by a loss of fungitoxicity of the product which is reflected by the significant drop in inhibition rates for the four doses. None of the concentrations was able to completely inhibit the growth of the fungus during all 5 days (Figure 5B).

The effect of FERCA Biofungicide on reducing mycelial growth of *Curvularia sp* diminished over the days for all concentrations. There was a similar variation in reduction rates for all doses used from day 1 to day 4.

Effect of REFERENCE synthetic fungicide on fungi

The synthetic fungicide had approximately equal effects on mycelial growth of the pathogen *Colletotrichum sp* at all concentrations used. The product was clearly effective during the first three days of the experiment during which the reduction rates varied between 21 and 96%. The product lost its fungitoxicity on day 5 at the 50 ppm concentration (Figure 6A).

The synthetic fungicide REFERENCE was particularly effective against *Pestalotia sp* during the first two days of the trial, completely inhibiting mycelial growth of the pathogen at all concentrations. The lowest reduction rate was 52.11% on day 5 at the 50 ppm concentration. The 25 ppm dose was more effective on the growth of this fungus. Indeed, with this concentration, the growth of the fungus starts only at day 5. The reduction in mycelial growth is therefore concentration dependent (Figure 6B).

The effects of REFERENCE on mycelial growth of *Curvularia sp* were time dependent. All doses had a significant effect on the pathogen on the first two days before dropping off on the last three days to a minimum value of 2.82 on day 5 at the 100 ppm concentration. None of these four doses was able to completely inhibit the mycelial growth

Fungitoxic and fungistatic activities of each fungicide on the different fungi

- NECO Biopesticide

The 2000 ppm concentration of NECO biofungicide had a fungitoxic effect on the mycelial growth of the fungus *Pestalotia sp* (Table 1).

However, by day 5 no concentration had fungitoxic effects greater than 10 except for the 2000 ppm dose which showed a rate of 11.40% (Figure 5C).

FERCA was effective on the fungus *Botryodiplodia sp* at 1000, 1500 and 2000 ppm with a rate of mycelial growth reduction exceeding 30% during the first 4 days of the experiment. Only the 2000 ppm dose was able to completely inhibit the growth of *Botryodiplodia sp* fungus throughout the evaluation (Figure 5D).

- ASTOUN Biopesticide

The concentrations of 1000, 1500 and 2000 ppm of ASTOUN biofungicide were fungitoxic for all the fungi while for the of the fungus over the entire duration of the experiment (Figure 6C).

Regarding the growth of the *Botryodiplodia sp* fungus, there was a significant difference between the 5, 25 ppm and 50, 100 ppm doses. In fact, at the doses of 5 and 25 ppm the growth of the pathogen starts respectively at day 4 and 5 of the experiment while it was totally inhibited at the doses of 50 and 100 ppm. The reduction in mycelial growth is therefore concentration-dependent (Figure 6D).

Cumulative effects of fungicide concentrations on mycelial growth of individual fungi

When the effects of each fungicide on all fungi were considered, there was a significant difference between products ($F = 340.98$; $p = 0.0000$). The biopesticides NECO and ASTOUN were the most fungitoxic with mycelial growth inhibition rates above 50% for all four fungi (Figure 7). These two biopesticides were followed by the synthetic fungicide REFERENCE, which inhibited mycelial growth of the fungus *Pestalotia sp*. by 88.14%. The least effective fungicide was the biopesticide FERCA, which had mycelial growth reduction rates ranging from 13 to 36% for all four fungi. Inhibition rates increased with concentration for NECO and REFERENCE.

concentration of 500, 1500 and 2000 ppm the product had a fungistatic effect on the mycelial growth of the fungus *Colletotrichum sp* (Table 1)

- ***FERCA biopesticide***

None of the doses of the biopesticide FERCA was able to completely inhibit the mycelial growth of the different fungi (Table 1**).**

- ***Synthetic Pesticide (REFERENCE)***

The dose of 2000 ppm totally inhibited the mycelial growth of the pathogen *Pestalotia sp*, this concentration was fungitoxic for this fungus (Table 1).

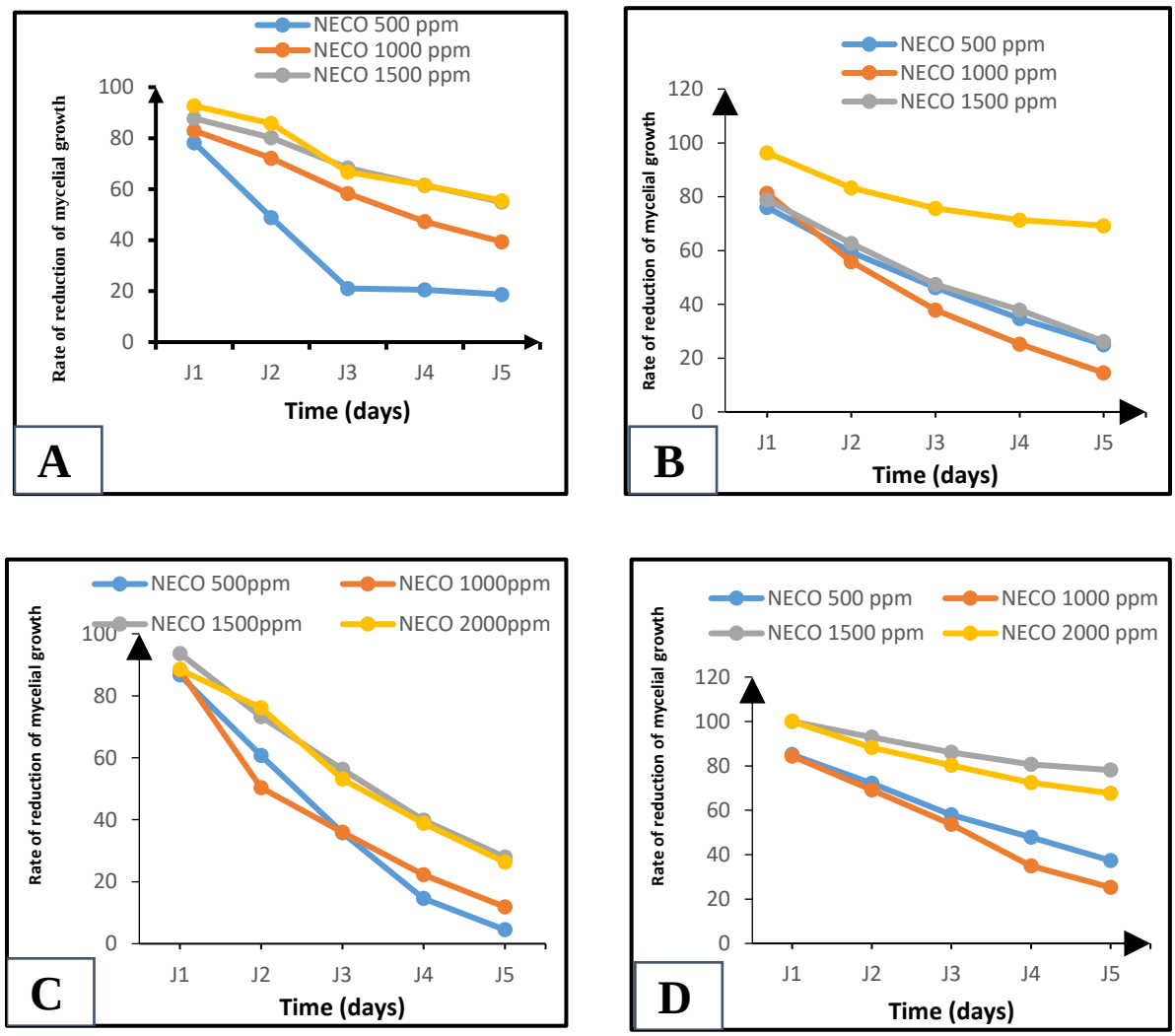

Figure 3: Rate of reduction of mycelial growth of four yam pathogenic fungi as a function of time and NECO biofungicide concentration.

A : *In vitro* effect of NECO on the fungus *Colletotrichum sp*

B : *In vitro* effect of NECO on the fungus *Pestalotia sp*

C : *In vitro* effect of NECO on the fungus *Curvularia sp*

D : *In vitro* effect of NECO on the fungus *Botryodiplodia sp*

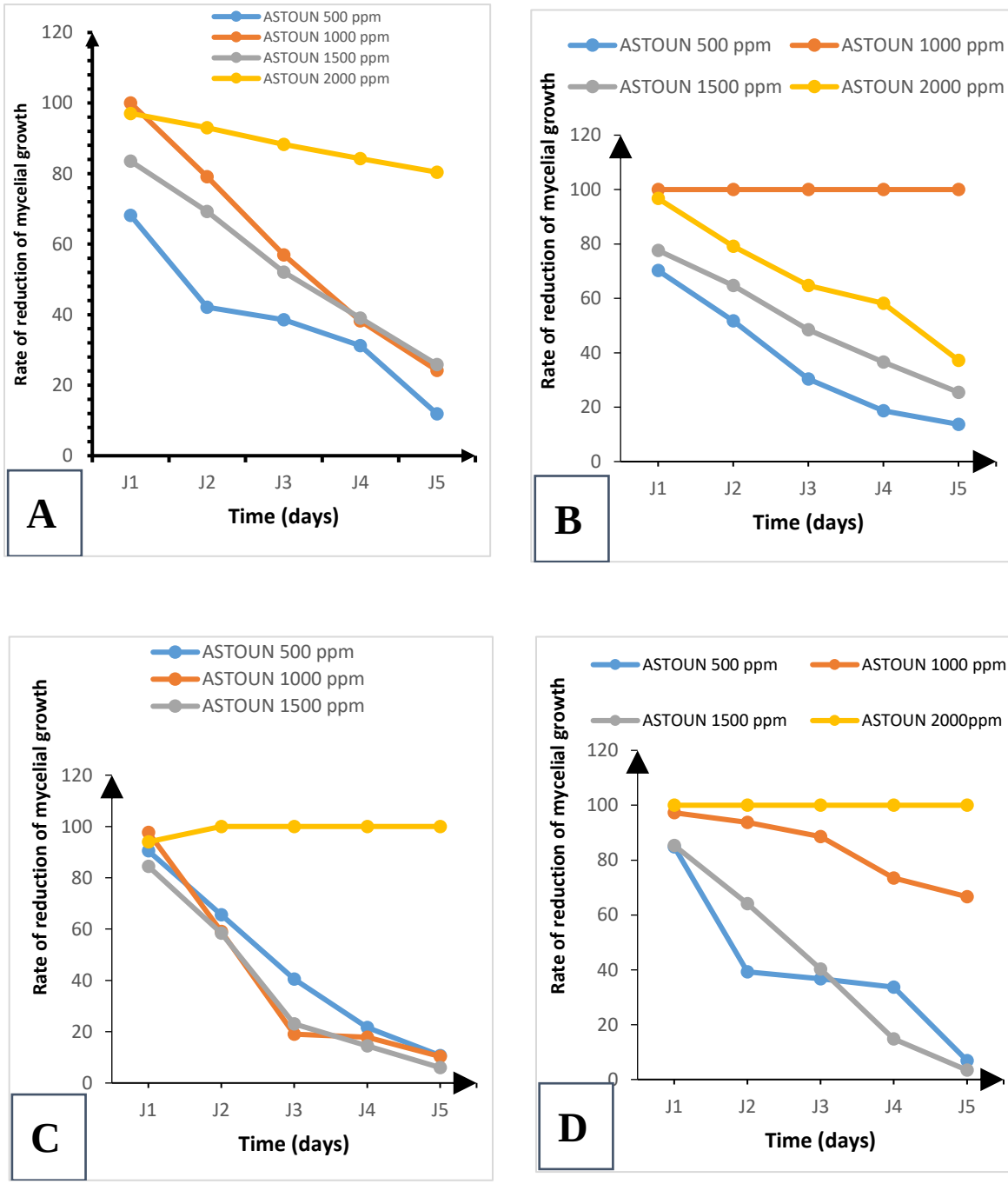

Figure 4 : Rate of reduction of mycelial growth of four yam pathogenic fungi as a function of time and ASTOUN biofungicide concentration.

A : *In vitro* effect of ASTOUN on the fungus *Colletotrichum sp*

B : *In vitro* effect of ASTOUN on the fungus *Pestalotia sp*

C : *In vitro* effect of ASTOUN on the fungus *Curvularia sp*

D : *In vitro* effect of ASTOUN on the fungus *Botryodiplodia sp*

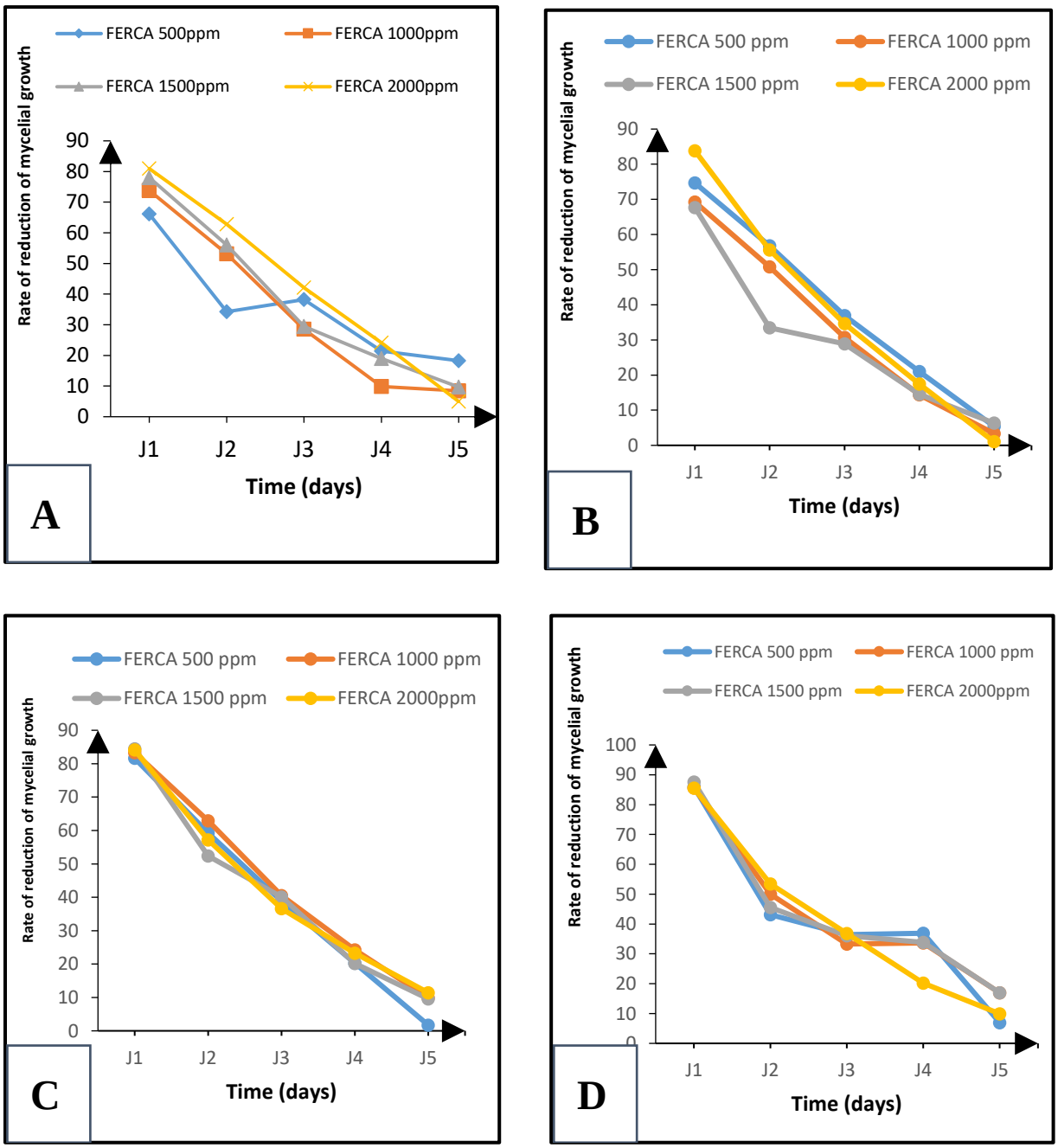

Figure 5 : Rate of reduction of mycelial growth of four yam pathogenic fungi as a function of time and FERCA biofungicide concentration.
A : *In vitro* effect of FERCA on the fungus *Colletotrichum sp*
B : *In vitro* effect of FERCA on the fungus *Pestalotia sp*
C : *In vitro* effect of FERCA on the fungus *Curvularia sp*
D : *In vitro* effect of FERCA on the fungus *Botryodiplodia sp*

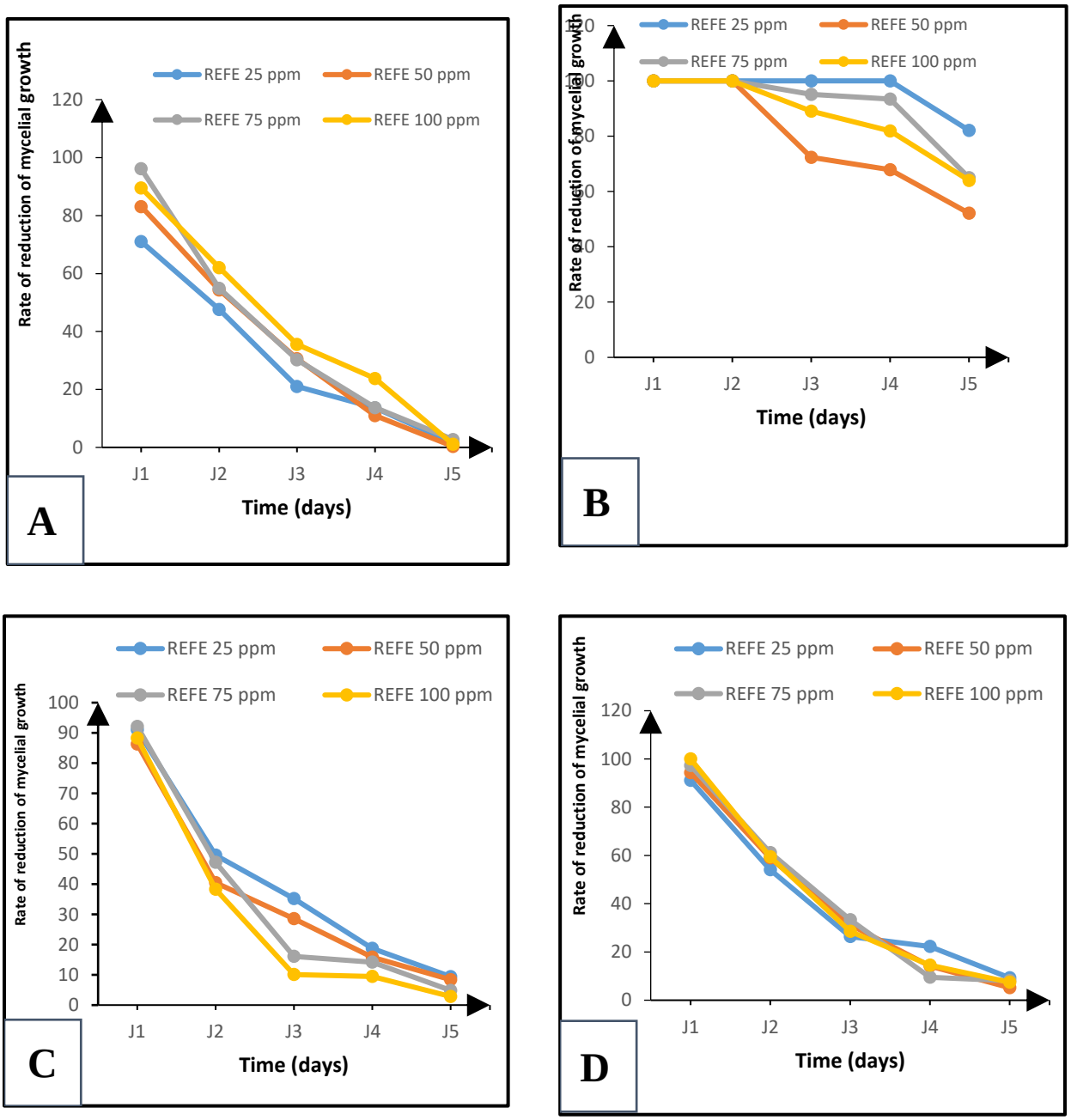

Figure 6 : Rate of reduction of mycelial growth of four yam pathogenic fungi as a function of time and biofungicide concentration REFERENCE.
A : *In vitro* effect of the synthetic fungicide REFERENCE on the fungus *Colletotrichum sp*
B : *In vitro* effect of the synthetic fungicide REFERENCE on the fungus *Pestalotia sp*
C : *In vitro* effect of synthetic fungicide REFERENCE on the fungus *Curvularia sp*
D : *In vitro* effect of synthetic fungicide REFERENCE on the fungus *Botryodiplodia sp*

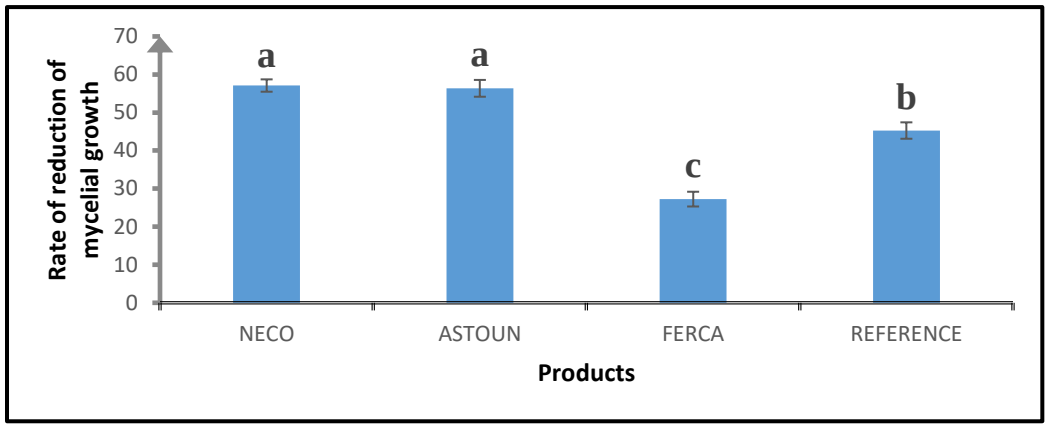

Figure 7: Average rate of inhibition of mycelial growth of fungi according to the products.

Table 1: Fungitoxic activities biopesticides and synthetic fungicide on different fungi.

		Resumption of mycelial growth			
Products	Concentrations	*Colletotrichum sp*	*Pestalotia sp*	*Curvularia sp*	*Botryodiplodia sp*
NECO	2000 ppm		-		
ASTOUN	500 ppm	+			
ASTOUN	1000 ppm		-		
ASTOUN	1500 ppm	-	-		
ASTOUN	2000 ppm	-	-	-	-
ASTOUN					
REFERENCE	100 ppm		-		

- : Means no recovery in growth + : Means resumption of growth

DISCUSSION

This work demonstrated the comparative effect of biological fungicides (NECO, ASTOUN, FERCA) and the synthetic fungicide REFERENCE on the *in vitro* growth of the four fungi. All fungicides used significantly reduced *in vitro* mycelial growth of all fungi. The biopesticides NECO and ASTOUN were the most effective products on the four pathogens followed by the synthetic fungicide. The fungitoxicity of these two biofungicides lies in their composition. Indeed, NECO and ASTOUN are based on essential oils of natural plants. The composition in aromatic compounds as well as the structure of NECO would be at the origin of this effectiveness. Indeed, as shown by Camara et al. (2007) the product would act directly on the pathogen and on the propagation organs. This antifungal effect of NECO would be due to the action of phenolic compounds such as thymol which is the majority compound of the essential oil of *Ocimum gratissimum* from which NECO was formulated (Kassi et al., 2014). Thymol is known to be toxic and reportedly targets the cytoplasmic membrane and wall of microorganisms (Chami, 2005). Also, camphor and 1.8- cineole two of the constituents of NECO would inhibit germination of propagating or infecting organs and growth of pathogens Camara et al. (2007). These results are similar to those obtained by Silué et al. (2018) who showed the efficacy of the biological fungicide NECO in reducing mycelial growth of *Colletotrichum sp* at concentrations of 300, 400, 500 ppm. The work of Yéo (2017) also showed that NECO completely reduced the growth of *Sclerotium rolfsii* at the concentrations of 3000, 5000,

7000 and 10000 ppm. At 2000 ppm ASTOUN product completely inhibited the mycelial growth of *Curvularia sp* and *Botryodiplodia sp*, at 1500 ppm the growth of *Pestalotia sp* fungus was completely inhibited throughout the experiment. These results are corroborated by those of Kaboré et al. (2007) and Tiendrébéogo et al. (2017) who also found that the essential oils of different plants which are the essential compounds of ASTOUN namely *Cymbopogon citratus*, could inhibit 100% of the mycelial growth of some fungi. Other authors such as Issoufou et al. (2016), Tiendrébéogo et al. (2017) and Sirima et al. (2020) have proven the efficacy of essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* on the growth of other fungi and insect pests.

The biological fungicide FERCA had a less marked effect on the inhibition of mycelial growth of the different fungi. This may be due to the composition of this product which is made on the basis of essential oil of natural plant whose major compounds are limonene (26.42%), 1.8 -cicerole (20.04%), gamma-terpinene (18.91%) but also to the concentration because at the concentration of 2000 ppm the growth of *Botryodiplodia sp* was totally inhibited throughout the experiment. These results corroborate those of N'goran et al. (2022) who showed that FERCA is the least effective in reducing only half the mycelial growth of *Phytophtora katsurae.*

Conclusion

This study allowed us to show the comparative effect of biofungicides on the four fungi associated with the symptoms of yam anthracnose in Côte d'Ivoire. All the products used acted according to the concentrations used and the time. Among the fungicides used,
ECO and ASTOUN were more effective in inhibiting the mycelial growth of champions *in vitro*. REFERENCE synthetic fungicide was more effective than FERCA biofungicide. ASTOUN and NECO actually contain properties giving them antifungal properties. Thus NECO and ASTOUN could be used as a means of biological control in the *in vivo* fight against leaf fungi of yam in culture.

COMPETING INTERESTS

The authors declare that they have no competing interests in the publication of this work.

AUTHORS' CONTRIBUTIONS

SG guided the methodological approach and the results obtained. He participated in the analysis of the data and the drafting of the proposed manuscript. BC defined the theme and objectives of this study. FJMKK contributed to the definition of the methodological approach and the obtained results. AK participated in the collecte the field. DK corrected the form and content of the proposed manuscript. All authors read and approved this manuscript.

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