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## ESTIMATING THE ANTIFUNGAL ACTIVITY OF BIOPESTICIDE FORMULATIONS AGAINST *FUSARIUM* SP., RESPONSIBLE FOR THE POSTHARVEST LOSSES IN MAIZE

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### ABSTRACT

Maize (*Zea mays* L.) is the staple food of many people in developing countries. However, during postharvest storage of the grain, various biotic stresses have been reported. Among these are molds caused by a complex of fungi, including those of the *Fusarium* genus. To combat these postharvest molds, growers and traders frequently use synthetic pesticides during grain preservation. However, the abusive use of these synthetic pesticides poses a problem of environmental pollution and human health. Faced with this situation, sustainable management strategies are being sought as alternatives to chemical control of these molds. Therefore, this study was initiated with the aim of contributing to food safety through the biological control of *Fusarium* fungi, one of the agents responsible for postharvest molds. *In vitro* mycelial radial growth inhibition tests at five concentrations (200, 400, 800, 1000 and 2000 ppm) were performed with five biopesticides (BIOSAKINE 50 EC, NORDINE 50 EC, NOSTAG 50 EC, RHOSO 50 EC and WACHET 50 EC) formulated with aromatic plant extracts. Results showed that the biopesticide RHOSO 50 EC completely inhibited the radial mycelial growth of all *Fusarium* sp. strains at a concentration of 2000 ppm. The biopesticides RHOSO 50 EC, WACHET 50 EC, and NOSTAG 50 EC were effective in inhibiting the radial mycelial growth of *Fusarium* strains. The biopesticides NORDINE 50 EC and BIOSAKINE 50 EC were the least effective. The biopesticides RHOSO 50 EC, WACHET 50 EC, and NOSTAG 50 EC can be proposed as part of an integrated pest management program against *Fusarium* fungi, which are responsible for postharvest mildew in maize.

**Keywords:** Biopesticides, survey, fungal genera, Post-harvest, maize, molds, *Zea mays*.

### INTRODUCTION

Maize (*Zea mays* L.) is the second most widely grown cereal in the world, after rice (Awata *et al.*, 2019). Along with millet, sorghum, and rice, it forms the staple diet of many populations in developing countries, especially in West Africa (N'Da *et al.*, 2022). These cereals, which are rich in starch, proteins, and minerals, play a considerable

role in the diets of these populations because of their high energy value (Semassa *et al.*, 2016).

In Côte d'Ivoire, maize, with production estimated at over 1.3 million tons in 2022, is an important crop that plays an economic role (FAO, 2024). It is grown in all the country's agro-ecological zones, with a more pronounced concentration in the northern part of the country, which accounts for 60% of production (N'Da *et al.*, 2022). Maize cultivation is therefore an important source of income for the populations of the northern region. In Côte d'Ivoire, maize is consumed in various forms, such as braised or boiled fresh cobs, roasted dried kernels, and the flour used to make tô (N'Da *et al.*, 2022). However, poor postharvest storage of this cereal leads to the

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development of fungal molds, which reduces its marketability and nutritional qualities because of the mycotoxins produced. According to the World Health Organization (WHO), almost 25% of food stuffs are disposed of each year because of mycotoxin contamination, which is equivalent to 1 billion tons of food lost.

The presence of mycotoxins in maize kernels causes the production of toxic secondary metabolites, which represent a health risk to consumers (Dédi and Diomandé, 2017; García-Díaz *et al.*, 2020). Among the fungi that produce mycotoxins on maize kernels after harvest is the genus *Fusarium* (Bryla *et al.*, 2022). To deal with this fearsome fungal pathogen in many crops, growers use synthetic fungicides. However, intensive use of these synthetic fungicides can lead to contamination of the biosphere and food web, as well as the eradication of non-target species and the emergence of resistant fungal species. Consequently, in view of all these health problems and the economic losses caused by mycotoxins, particular attention needs to be paid to researching alternative methods to reduce the synthetic fungicides. Research into biological control methods using formulations based on plant extracts could undoubtedly make it possible to control these various fungi. Indeed, these formulations based on aromatic plant extracts have a broad spectrum of activities against insect pests of crops and pathogenic fungi of many crops. Recent studies by Tuo *et al.* (2022) have demonstrated the efficacy of certain biopesticides formulated with aromatic plant extracts in controlling banana black spot caused by *Mycosphaerella fijiensis*. In addition, certain compounds such as thymol,

eugenol, 1,8-cineole, citronellal, arcurcumene, citronellol, and carvacrol contained in biopesticides have antifungal properties (Kassi *et al.*, 2021; Doumbouya *et al.*, 2021; Tuo *et al.*, 2022). Moreover, formulations based on plant extracts are only slightly toxic to mammals (Isman, 2006). The present study was initiated with a view to contribute to food security through biological control of fungi of the genus *Fusarium*, one of the agents responsible for post-harvest molds.

#### MATERIALS AND METHODS

**Fungal material:** The fungal material used in the present study consisted of three *Fusarium* isolates (Figure 1) obtained from maize kernels taken from stocks stored in market warehouses in the autonomous district of Abidjan (Côte d'Ivoire). Isolation was carried out using two methods: the grain disinfection method and the grain non-disinfection method. Isolates are kept at the mycotheque of the Plant Physiology and Pathology Teaching and Research Unit of the Université Félix HOUPHOUËT-BOIGNY (Côte d'Ivoire).

**Biological control materials:** The biological control material used in this study consisted of five (5) biopesticides (Biosakine 50 EC, Nordine 50 EC, Nostag 50 EC, Rhoso 50 EC and WACHET 50 EC). These biopesticides were developed from leaf extracts of certain aromatic plants from the Ivorian flora. They were supplied by the Industrial Research Unit (URI) on Biopesticides located at the Bingerville Science and Innovation Center, University of Félix Houphouët Boigny (Côte d'Ivoire). The main characteristics of these biopesticides are listed in Table 1 (Tuo *et al.*, 2021).

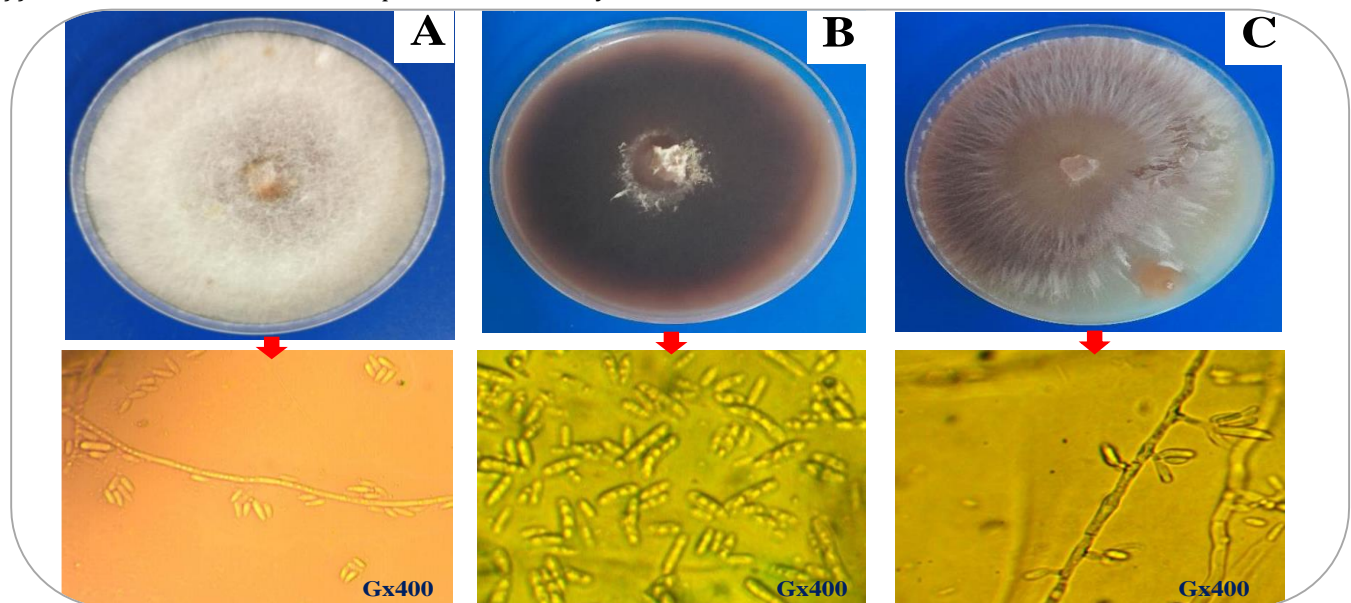


Figure 1. Macroscopic and microscopic characteristics of *Fusarium* fungal isolates

Table 1. Biopesticide characteristics

Commercial name	Matter Active	Formulation type	Recommended doses
BIOSAKINE 50 EC	Alpha-zingiberene + Arcurcumin	50 EC	5-10 ml/L (3-6 L/ha)
NORDINE 50 EC	Carvacrol and 1,8-Cineole	50 EC	5-10 ml/L (3-6 L/ha)
NOSTAG 50 EC	(Thymol + Eugenol) and (Geranial+Neral)	50 EC	5-10 ml/L (3-6 L/ha)
RHOSO 50 EC	(Thymol + Eugenol) and (Carvacrol+1,8 Cineole)	50 EC	5-10 ml/L (3-6 L/ha)
WACHET 50 EC	(Thymol+ Eugenol) and (Citronellal + Citronellol)	50 EC	5-10 ml/L (3-6 L/ha)

### ***In vitro* evaluation of the antifungal activity of biopesticides: Preparation of the culture medium:**

The culture medium used was potato dextrose agar (PDA). It was prepared by adding 20 g of potato puree, 20 g of D-glucose, and 20-g agar to an Erlenmeyer flask. The volume of this mixture was adjusted with distilled water to 1 L and then autoclaved at 121°C under a pressure of 1.5 bar for 30 min.

### **Inhibition of mycelial growth by biopesticides and mycelial pellet recovery: Mycelial growth inhibition test using biopesticides:**

To assess the *in vitro* effect of five biopesticides (BIOSAKINE 50 EC, NORDINE 50 EC, NOSTAG 50 EC, RHOSO 50 EC and WACHET 50 EC) on the mycelial growth of *Fusarium* isolates (*Fusarium oxysporum*, *Fusarium* sp.1 and *Fusarium* sp.2), preliminary tests were carried out to determine the minimum and maximum concentrations to be used. Concentrations of 200, 400, 800, 1000, and 2000 ppm were selected for this *in vitro* evaluation. Pre-prepared culture media were supercooled at 45°C in a laminar flow hood. These media were then amended by aseptically incorporating the various biopesticides at the indicated concentrations using a sterile micropipette, with the addition of a drop of Tween 20 (surfactant role). The whole mixture was homogenized under magnetic stirring to emulsify. Media amended with different concentrations of biopesticides were dispensed into 90-mm Petri dishes at a rate of 20 ml per dish. Two perpendicular lines were drawn on the reverse side of each Petri dish, with the point of intersection indicating the center of the dish (Doubouya *et al.*, 2021). Petri dishes containing frozen PDA-biopesticide culture medium were seeded in the center with a 5-mm diameter mycelial disk of each fungal genus isolate. Mycelial disks (5 mm diameter) were obtained from the growth front of 7-day-old fungal cultures. In control dishes, culture medium alone was introduced and the same fungal strains were deposited at

the intersection of the two axes (Doubouya *et al.*, 2021). Five Petri dishes were seeded per concentration and biopesticide and for the control, and the experiment was replicated three times over time. Petri dishes were sealed with kerosene and incubated at 25 ± 2°C for 12 h. Biopesticide efficacy was assessed by measuring the mycelial growth diameter of each fungal isolate every 24 h after inoculation until the control plates were filled (depending on the strain). The average of these measurements was used to calculate the inhibition rate (IR) of fungal growth compared with the control using the following formula from Bamba *et al.* (2023):

$$IR (\%) = \frac{D_0 - D_t}{D_0} \times 100$$

With:

D<sub>0</sub> = average diameter of the control

D<sub>t</sub> = Average diameter of the treated

The inhibitory concentrations CI<sub>50</sub> (inhibitory concentration at 50 p.c.) and CI<sub>90</sub> (inhibitory concentration at 90 p.c.) were determined graphically from the linear relationship between the decimal logarithm of the biopesticide concentration on the abscissa and the probit values derived from the mycelial growth inhibition percentages on the ordinate (Soro *et al.*, 2010).

### **Mycelial pellet recovery test for *Fusarium* isolates:**

During inhibition tests, when no mycelial growth was observed for a given concentration and biopesticide, the Petri dishes were opened and the mycelial disk was transplanted into a new Petri dish containing PDA culture medium without biopesticide. All plates were stored under the same conditions for 7 days. The lethal inhibitory concentration (LIC) was determined from the lowest concentration for which no mycelial growth or mycelial pellet recovery was observed on the PDA culture medium (Soro *et al.*, 2010). Thus, when recovery was observed at a

given concentration of a biopesticide, the biopesticide was declared fungistatic for the *Fusarium* isolate. In contrast, when there was no recovery, the biopesticide was declared fungitoxic for the fungal genus at that concentration (Soro *et al.*, 2010).

**Assessment of biopesticide activity on isolate spore production:** In each of the Petri dishes used to measure mycelial growth, 3 explants 5 mm in diameter were removed and placed in a test tube containing 3 ml of sterile distilled water. The tubes were shaken in 3-s sequences for 15 s to separate spores from conidiospores. The resulting suspensions were filtered through muslin to remove mycelial fragments. The number of spores were counted using a Malassez slide at a rate of ten counts per suspension to obtain a total of at least 100 spores. The number of spores per unit area (mm<sup>2</sup>) was counted, followed by the number of particles in 1 ml. The percentage of sporulation inhibition (SI) was then determined using the following formula (Doubouya *et al.*, 2010):

$$SI (\%) = \frac{NS0 - NSt}{NS0} \times 100$$

NS0 = Average number of spores in the control  
 NSt: Average number of spores in treated tubes

**STATISTICAL ANALYSIS**

Data related to *in vitro* tests with biopesticides were first subjected to arc-sine transformation to normalize them. Subsequently, a two-way analysis of variance (ANOVA 2)

was used to study the interaction between biopesticides and their concentrations to determine their efficacy. ANOVA 2 was also used to study the interaction between fungal isolates and biopesticide concentrations. Data on the growth rate and spore production of different fungal isolates were subjected to single-criteria analysis of variance (ANOVA 1). Separation of post ANOVA means (1 and 2) was performed using the Student Newman–Keuls test at the 5% probability threshold (Casanoves *et al.*, 2012).

**RESULTS AND DISCUSSION**

**Effects of biopesticides on mycelial growth of *Fusarium oxysporum*:** Figure 2 shows the rates of mycelial growth inhibition of the *Fusarium oxysporum* strain as a function of biopesticides and concentrations. The biopesticides NOSTAG 50 EC, RHOSO 50 EC, and WACHET 50 EC recorded the best inhibition rates at all concentrations. These three (3) biopesticides completely inhibited the mycelial growth of the *Fusarium oxysporum* strain at 2000 ppm and gave inhibition rates of over 50% at 800 and 1000 ppm. Therefore, they were highly effective against the *Fusarium oxysporum* isolate. In contrast, the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC gave inhibition rates below 30% at all concentrations. The biopesticides BIOSAKINE 50 EC and NORDINE 50 EC therefore have very low efficacy against the *Fusarium oxysporum* strain.

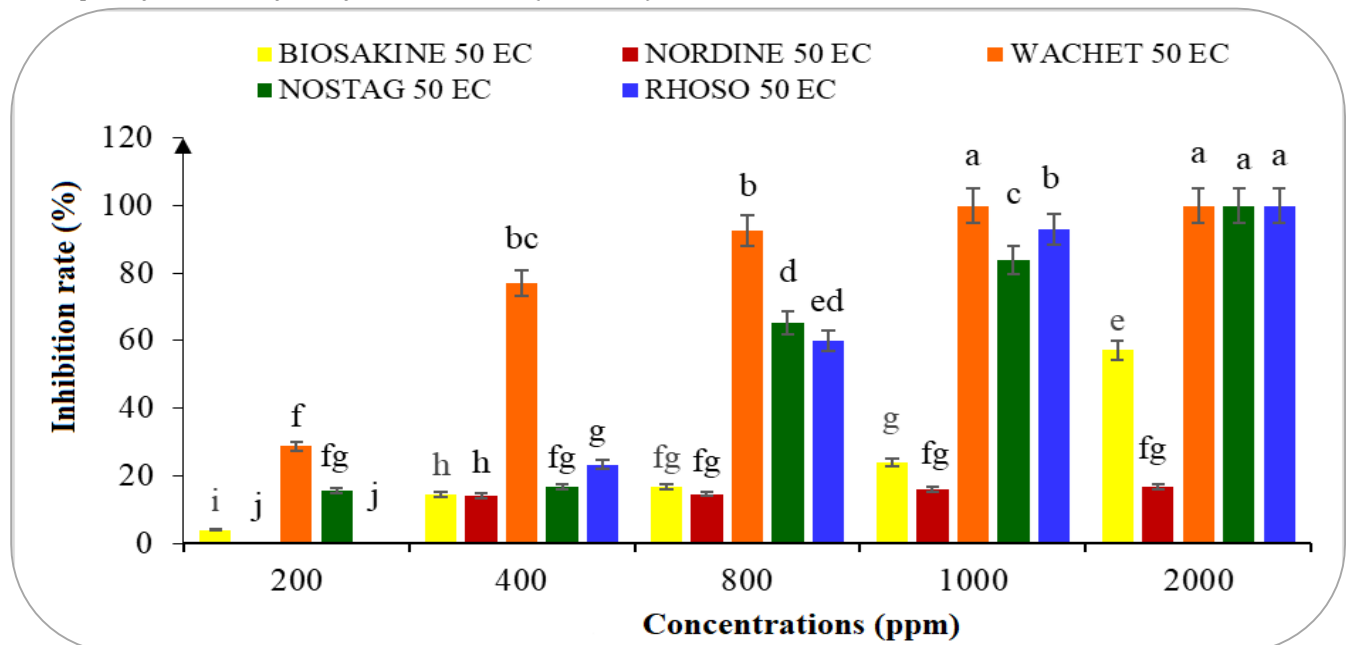


Figure 2: *Fusarium oxysporum* strain mycelial growth inhibition rate as a function of biopesticides and concentrations. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

**Effects of biopesticides on mycelial growth of *Fusarium sp. 1* isolates:** The biopesticides WACHET 50 EC and RHOSO 50 EC completely inhibited (100%) the mycelial growth of *Fusarium sp.1* at concentrations of 1000 and 2000 ppm (Figure 3). The biopesticide NOSTAG 50 EC completely inhibited (100%) mycelial growth at 2000 ppm (Figure 3). The biopesticide WACHET 50 EC gave inhibition rates of over 50% at 400 and 800 ppm. The biopesticides BIOSAKINE 50 EC and NORDINE 50 EC showed a very weak inhibitory effect on *Fusarium sp.1* mycelial growth at all concentrations. Inhibition rates for both biopesticides ranged from 0 % to 21.90% for BIOSAKINE 50 EC and from

0 % to 22.01% for NORDINE 50 EC (Figure 3).

**Effects of biopesticides on mycelial growth of the *Fusarium sp. 2* isolate:** Figure 4 shows the rate of inhibition of mycelial growth in the *Fusarium sp.2* isolate. In this figure, the biopesticides RHOSO 50 EC and WACHET 50 EC completely inhibited (100%) the mycelial growth of the *Fusarium sp.2* isolate at a concentration of 2000 ppm. The biopesticide WACHET 50 EC showed an inhibition rate of 67.42% at 400 ppm. The biopesticides NORDINE 50 EC and BIOSAKINE 50 EC showed very low inhibition rates of mycelial growth of the *Fusarium sp.2* isolate.

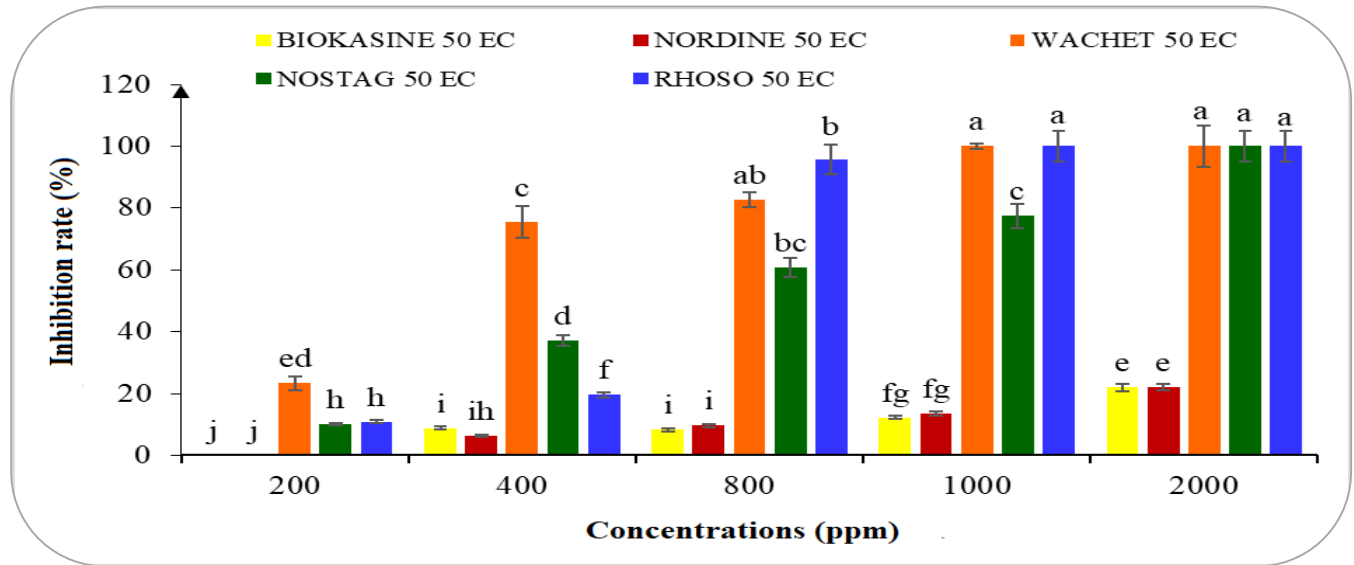


Figure 3. Rate of inhibition of mycelial growth of *Fusarium sp.1* isolate as a function of biopesticides and concentrations. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

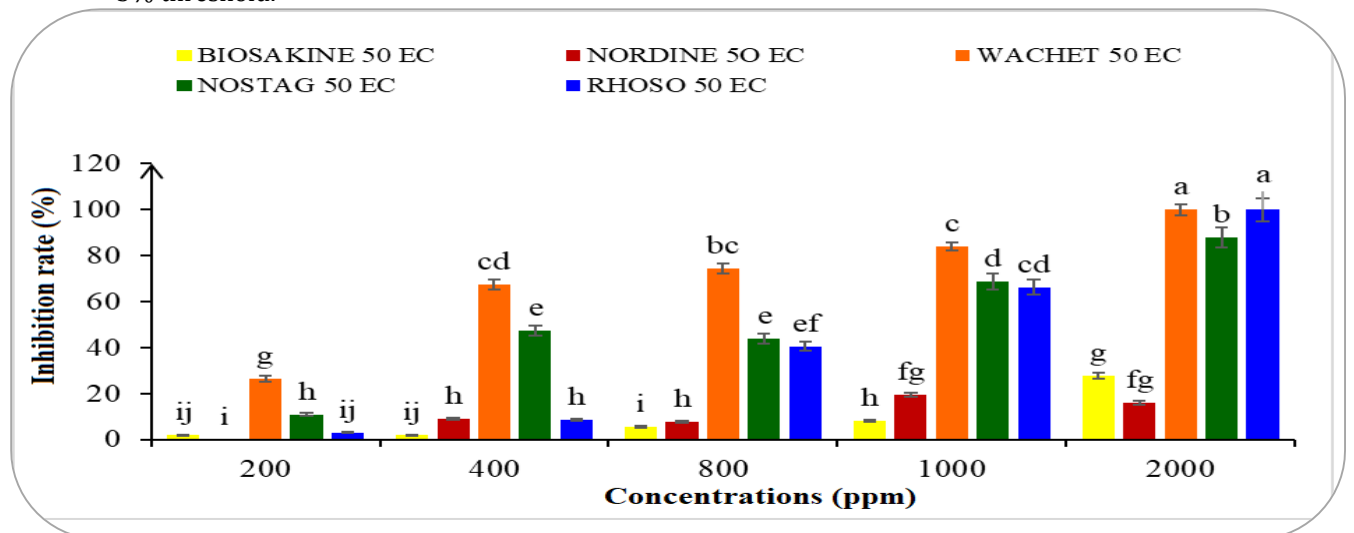


Figure 4. Rate of inhibition of mycelial growth of *Fusarium sp.2* isolate as a function of biopesticides and concentration. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

**Cumulative effects of biopesticides concentrations on the growth of different strains: Cumulative effects of biopesticides concentrations on mycelial growth of *Fusarium oxysporum* isolates:** The most effective biopesticide against the *Fusarium oxysporum* strain was WACHET 50 EC, which inhibited mycelial growth by 79.71%. This was followed by the

biopesticides RHOSO 50 EC and NOSTAG 50 EC, which inhibited the mycelial growth of the *Fusarium oxysporum* isolate by 55% and 54%, respectively. The biopesticides BIOSAKINE 50 EC and NORDINE 50 EC were the least effective, inhibiting the mycelial growth of the *Fusarium oxysporum* isolate by 23.03 and 11.71%, respectively (Figure 5).

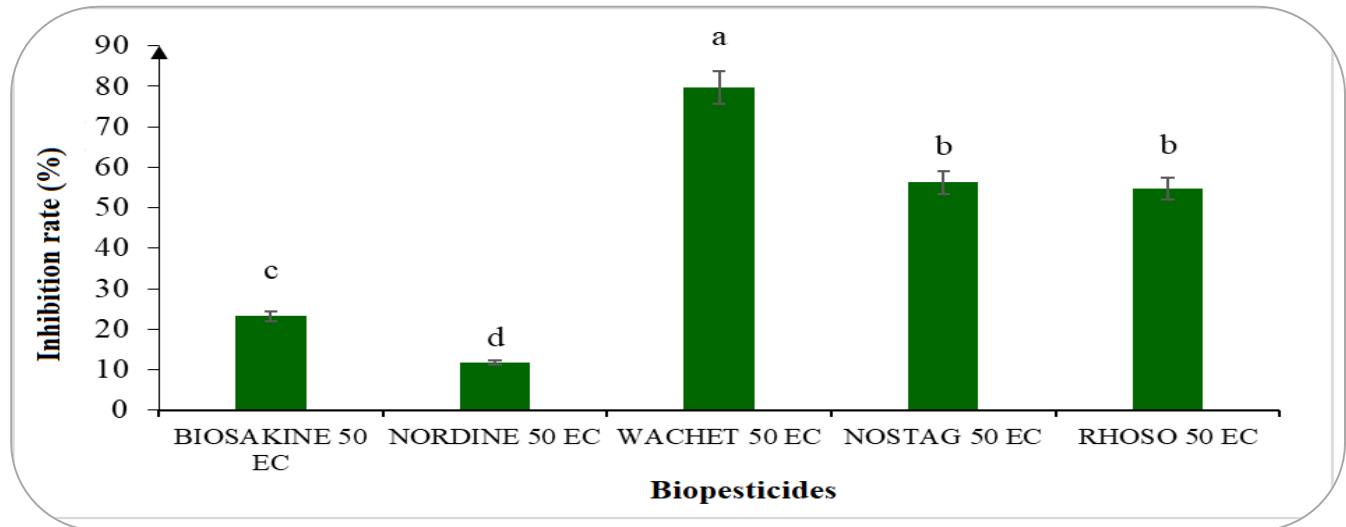


Figure 5. Rate of inhibition of mycelial growth of *Fusarium oxysporum* isolate as a function of biopesticides. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

**Cumulative effects of biopesticides concentrations on mycelial growth of the *Fusarium sp.1* isolate:** *Fusarium sp.1* was highly sensitive to the biopesticide WACHET 50 EC. The latter inhibited the mycelial growth of the *Fusarium sp.1* isolate by 76.30% (Figure 6). The

biopesticides RHOSO 50 EC and NOSTAG 50 EC, with inhibition rates of 65.21 and 57.14, respectively, showed good efficacy in controlling the *Fusarium sp.1* isolate. The biopesticides BIOSAKINE 50 EC and NORDINE were less effective in controlling *Fusarium sp.1* isolates (Figure 6).

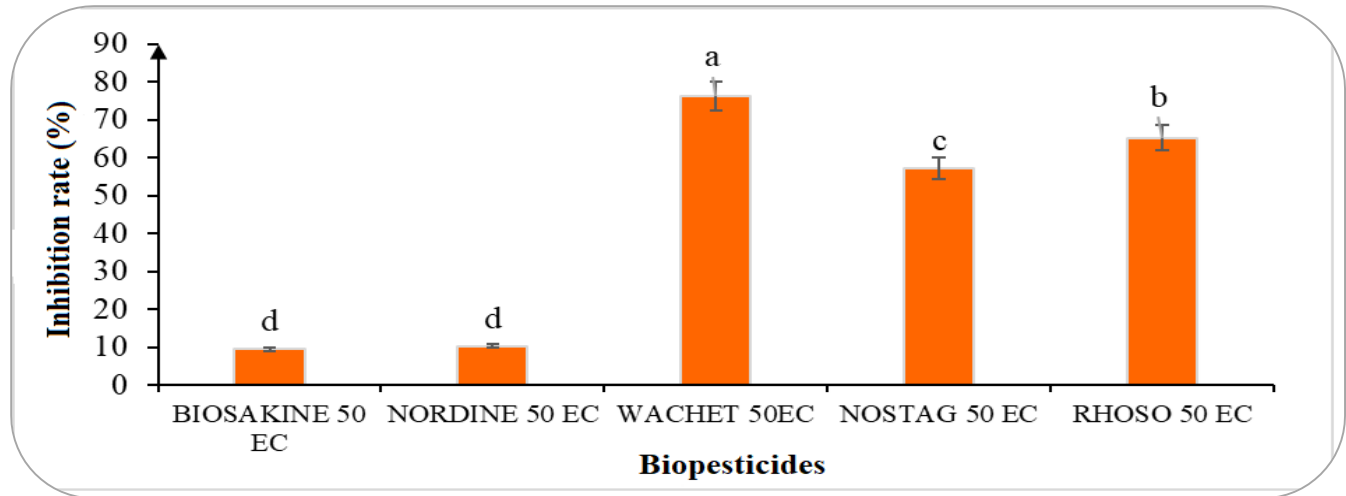


Figure 6. Rate of inhibition of mycelial growth of *Fusarium sp.1* isolate as a function of biopesticides. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

**Cumulative effects of biopesticides concentrations on mycelial growth of the *Fusarium sp.2* isolate:** The highest inhibition rate (70.30%) of mycelial growth of the *Fusarium*

*sp.2* isolate was obtained using the biopesticide WACHET 50 EC. The biopesticides NOSTAG 50 EC and RHOSO 50 EC, with inhibition rates of 51.20 % and 43.70%, respectively,

showed average efficacy on the mycelial growth of *Fusarium* sp.2 isolates. As for the biopesticides BIOSAKINE 50 EC and

NOSTAG 50 EC, their efficacy was very low in controlling the *Fusarium* sp.2 isolate (Figure 7).

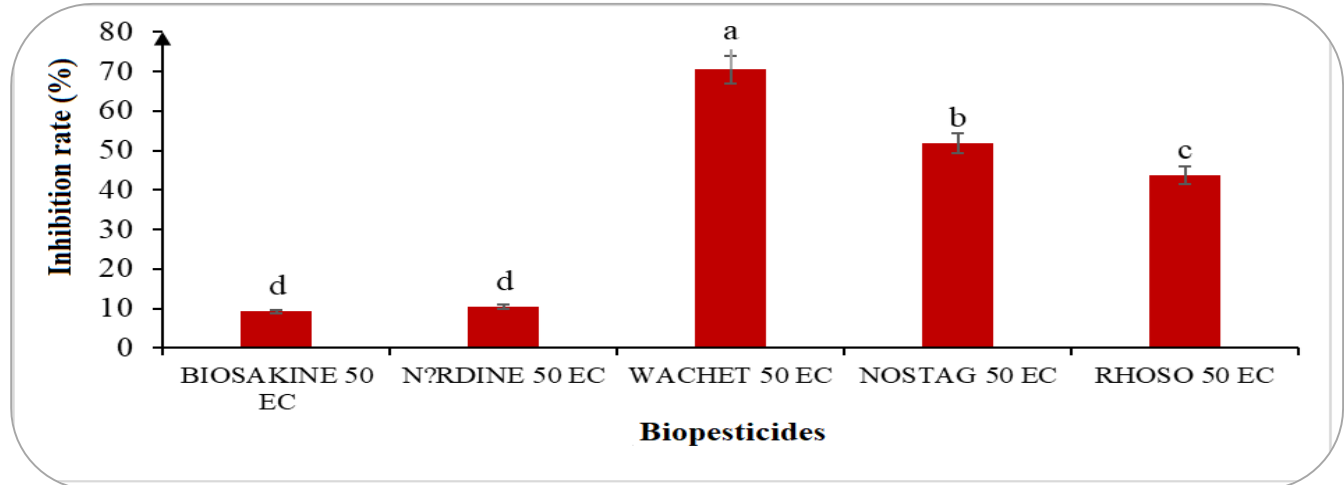


Figure 7. Rate of inhibition of mycelial growth of *Fusarium* sp.2 isolate as a function of biopesticides. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

**Cumulative effects of biopesticides on mycelial growth of all *Fusarium* isolates:** Figure 8 provides information on the average rate of mycelial growth inhibition of different *Fusarium* isolates by biopesticides. Analysis of this figure shows that the biopesticides WACHET 50 EC and NOSTAG 50 EC were

the most effective, inhibiting the mycelial growth of all isolates by 73.08% and 61.08%, respectively. The biopesticides NORDINE 50 EC and BIOSAKINE 50 EC were the least effective, inhibiting 29.41% and 19.26% of mycelial growth, respectively, in all isolates (Figure 8).

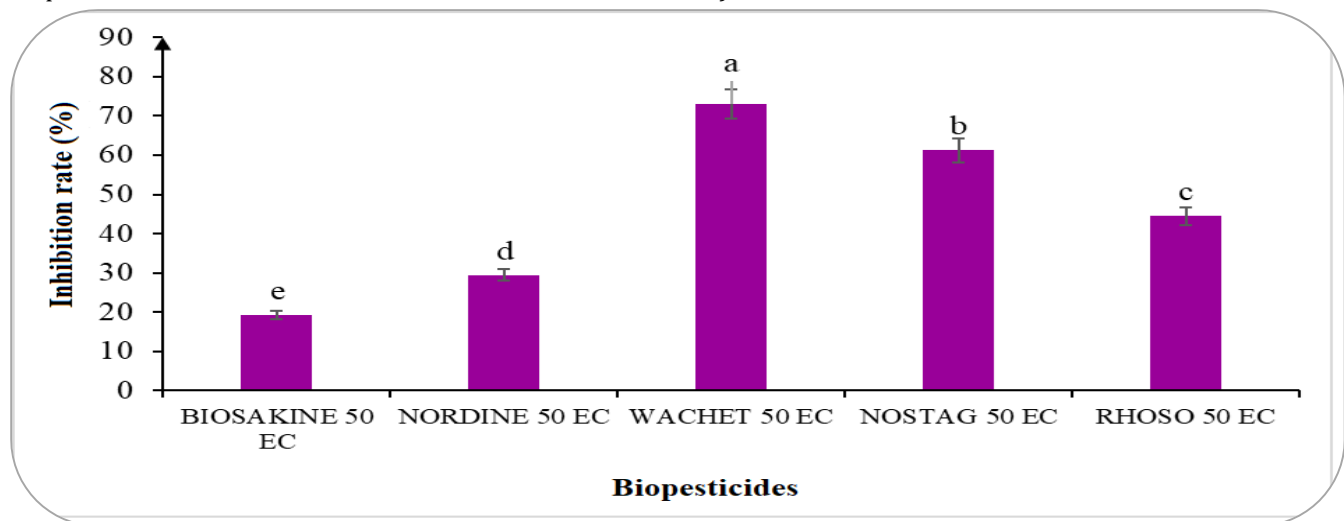


Figure 8. Average rate of mycelial growth inhibition of *Fusarium* isolates as a function of biopesticides. Error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

**Effects of biopesticides on sporulation of different *Fusarium* isolates:** The results shown in Table 2 relate to the effects of biopesticides on the sporulation of different *Fusarium* isolates. Analysis of this table shows that biopesticides influenced the sporulation of all *Fusarium* isolates, with variances from isolate to isolate, biopesticide to biopesticide, and concentration to

concentration. Thus, the biopesticide BIOSAKINE 50 EC was ineffective against the sporulation of all *Fusarium* isolates, with inhibition rates ranging from 0.40 % to 19.10% at all concentrations. In contrast, the biopesticides WACHET 50 EC and RHOSO 50 EC completely inhibited (100%) sporulation of all *Fusarium* isolates at 2000 ppm.

Table 2. Average sporulation inhibition rate of *Fusarium* isolates as a function of biopesticides and concentrations

Biopesticides	Concentrations (ppm)	Fungus strains		
		<i>Fusarium oxysporum</i>	<i>Fusarium</i> sp.1	<i>Fusarium</i> sp.2
BIOKASINE 50 EC	200	0.40	03.40	3.63
	400	8.84	10.40	2.71
	800	6.57	11.21	8.93
	1000	7.83	15.79	7.90
	2000	10.97	19.10	11.45
NORDINE 50 EC	200	5.46	5.58	0.00
	400	10.33	11.28	6.70
	800	13.66	13.53	6.39
	1000	15.55	20.75	8.70
	2000	16.33	19.83	13.31
WACHET 50 EC	200	56.05	44.94	46.86
	400	73.99	40.57	73.98
	800	94.10	82.29	76.44
	1000	100.00	100.00	78.31
	2000	100.00	100.00	100.00
NOSTAG 50 EC	200	20.49	0.00	50.53
	400	25.43	3.20	55.42
	800	29.60	39.65	57.41
	1000	30.66	33.86	55.43
	2000	100.00	74.79	63.31
RHOSO 50 EC	200	20.27	42.53	0.00
	400	27.00	75.40	13.29
	800	26.34	100.00	14.69
	1000	85.90	100.00	78.33
	2000	100.00	100.00	100.00

**Effects of biopesticides on mycelial regrowth of different**

***Fusarium* isolates:** The results reported in Table 3 provide information on the effects of biopesticides on mycelial regrowth of the various strains. Analysis of this table shows that all the *Fusarium* isolates used, whose mycelial growth had been completely (100%) inhibited by the biopesticide RHOSO 50 EC at a concentration of 2000 ppm, all resumed mycelial growth after being transplanted onto new Petri dishes containing PDA culture medium without the product. Therefore, the biopesticide RHOSO 50 EC was fungistatic at a

concentration of 2000 ppm for the *Fusarium* isolates. Mycelial growth of *Fusarium oxysporum*, *Fusarium* sp.1, and *Fusarium* sp.2 isolates was completely inhibited (100%) by the biopesticide WACHET 50 EC at a concentration of 2000 ppm. However, once transplanted onto new Petri dishes containing PDA culture medium without the product, only *Fusarium oxysporum* and *Fusarium* sp.2 isolates resumed growth. Therefore, the biopesticide WACHET 50 EC was fungistatic for *Fusarium oxysporum* and *Fusarium* sp.2 strains, and fungitoxic for the *Fusarium* sp.1 strain (Table 3).

Table 3. Effects of biopesticides on mycelial regrowth of different strains

Biopesticides	Total inhibitor concentrations (ppm)	Fungus strains		
		<i>Fusarium oxysporum</i>	<i>Fusarium</i> sp.1	<i>Fusarium</i> sp.2
NOSTAG 50 EC	800	0	0	0
	1000	0	0	0
	2000	+	+	0
NORDINE 50 EC	2000	0	0	0
RHOSO 50 EC	1000	0	+	0
	2000	+	+	+
WACHET 50 EC	800	0	0	0
	1000	+	+	0
	2000	+	-	+

0: there was no total inhibition of mycelial growth at this concentration,  
 +: mycelial growth resumed,  
 - no resumption of mycelial growth in the strain.



### Influence of *Fusarium* isolates on IC<sub>50</sub> and IC<sub>90</sub> inhibitory concentrations of different biopesticides:

The results in Table 4 show that IC<sub>50</sub> and IC<sub>90</sub> inhibitory concentrations varied from one biopesticide to another and from one *Fusarium* isolate to another. The biopesticide IC<sub>50</sub>s ranged from 86.83 to 7870.03 ppm for all *Fusarium* isolates. In contrast, biopesticide IC<sub>90</sub>s ranged from 1217.21 to 15173.35 ppm for all *Fusarium* isolates. In general, the lowest IC<sub>50</sub> values were obtained using the biopesticide WACHET 50 EC in all *Fusarium* isolates. In contrast, the lowest C<sub>90</sub> values were recorded

with the biopesticides WACHET 50 EC and NOSTAG 50 EC in the *Fusarium oxysporum* isolate. However, the highest IC<sub>50</sub> value (7870.03 ppm) was obtained using the biopesticide NORDINE 50 EC in the *Fusarium oxysporum* isolate. The lowest IC<sub>50</sub> value (86.83 ppm) was obtained using the biopesticide WACHET 50 EC in the *Fusarium* sp.1 isolate. In contrast, the lowest IC<sub>90</sub> values (1217.21 ppm) were observed with the biopesticide WACHET 50 EC in the *Fusarium oxysporum* isolate. (Table 4) and the highest (15173.35 ppm) with the biopesticide NORDINE 50 EC in the *Fusarium oxysporum* isolate (Table 4).

Table 4. IC<sub>50</sub> and IC<sub>90</sub> inhibitory concentrations of biopesticides as a function of fungal strains

Biopesticides	Inhibitor concentrations (ppm)	Fungus strains		
		<i>Fusarium oxysporum</i>	<i>Fusarium</i> sp.1	<i>Fusarium</i> sp.2
BIOSAKINE 50 EC	IC <sub>50</sub>	1917.56	4327.16	3617.45
	IC <sub>90</sub>	3466.56	7724.36	6302.84
NOSTAG 50 EC	IC <sub>50</sub>	752.58	727.17	831.51
	IC <sub>90</sub>	1555.92	1585.24	1909.58
NORDINE 50 EC	IC <sub>50</sub>	7870.03	4342.72	5818.35
	IC <sub>90</sub>	15173.35	7831.04	10798.36
RHOSO 50 EC	IC <sub>50</sub>	793.63	579.29	992.14
	IC <sub>90</sub>	1516.74	1369.67	1704.74
WACHET 50 EC	IC <sub>50</sub>	94.28	86.83	266.57
	IC <sub>90</sub>	1217.21	1585.24	1909.58

### DISCUSSION

The present study evaluated the *in vitro* antifungal effect of five biopesticides (BIOSAKINE EC, NOSTAG 50 EC, NORDINE 50 EC, RHOSO 50 EC and WACHET 50 EC) on the mycelial growth of three (3) isolates of *Fusarium* sp. (*Fusarium oxysporum*, *Fusarium* sp.1 and *Fusarium* sp.2). The results revealed different levels of antifungal efficacy of the biopesticides, depending on the concentration. The 3 species of *Fusarium* sp. appear to be sensitive to different biopesticide active ingredients. Induced inhibition rates differ from one biopesticide to another and from one concentration to another. Analysis of the effect of varying biopesticide concentrations on the radial mycelial growth of all *Fusarium* sp. isolates showed that the antifungal activity displayed was greater at higher concentrations. This shows that a dose-dependent effect was demonstrated for each biopesticide on the different *Fusarium* sp. isolates used. This result is in line with the work of Kossonou *et al.* (2019), who demonstrated a dose-dependent effect in *in vitro* tests of the antifungal activity of extracts from five local plants on *Colletotrichum higginsianum*, *Fusarium oxysporum*, and *Rhizopus stolonifer*, pathogens of papaya (*Carica papaya* L.) and tomato (*Solanum lycopersicum* L.). Therefore, the efficacy of biopesticides is linked to the quantity of active

ingredients in the environment. Variability in the sensitivity of *Fusarium* sp. isolates exposed to the active ingredients of different biopesticides appears to be related to the chemical composition of the biopesticides. Indeed, according to Silué *et al.* (2018) and Tuo *et al.* (2022), the antifungal activity of a biopesticide depends as much on its composition in aromatic compounds as on its structure. All the biopesticides used were developed from aromatic plant extracts that contain volatile compounds and have antifungal properties (Tuo *et al.*, 2021). Thus, the main compounds in biopesticides are apha-zingiberene and arcurcumene for BIOSAKINE 50 EC ; carvacol and 1,8-cineole for NORDINE 50 EC; Thymol, Eugenol, Geranial, and Neral for NOSTAG 50 EC; Thymol, Eugenol, Carvacol, and 1,8-Cineole for RHOSO 50 EC; and Thymol, Eugenol, Citronellal, and Citronellol for WACHET 50 EC. Most of these volatile compounds are also the main constituents of certain essential oils of aromatic plants with antifungal properties. Indeed, it has been established that certain constituents of essential oils can positively influence their overall activity (Doubouya *et al.*, 2021). This finding is confirmed by the work of Soro *et al.* (2010) on the telluric mycopathogen, *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato cultivation, who demonstrated the efficacy of the essential oil of

*Ocimum gratissimum*. According to Soro *et al.* (2010), the radial mycelial growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* *in vitro* was significantly inhibited by *Ocimum gratissimum* essential oil at a concentration of 250 ppm. According to these authors, thymol, the most represented chemical compound in this essential oil, was responsible for this efficacy. Similarly, Toé *et al.* (2022) demonstrated the fungicidal efficacy of three essential oil-based biopesticides in controlling the main fungi associated with cowpea seeds in Burkina Faso. According to these authors, the effectiveness of the treatments on the fungi was attributed to the presence of essential oil chemical molecules still active in the formulations.

The biopesticides RHOSO 50 EC and WACHET 50 EC showed good efficacy in controlling the *Fusarium* sp. isolates used. Their effectiveness may be explained by the fact that these biopesticides are combinations of several active ingredients. In addition to their own compounds, these two biopesticides share thymol and eugenol. According to Djeugap *et al.* (2011), the active ingredients in plant extracts can act on pathogens individually or synergistically, inhibiting their development. Considering this information, it could be said that in the case of the biopesticides RHOSO 50 EC and WACHET 50 EC, the active molecules comprising these biopesticides acted synergistically. The results of this *in vitro* study provide great hope to the scientific community, as well as to maize growers and traders in Côte d'Ivoire, with respect to the use of biopesticides. These products could be tested *in vitro* on other fungal pathogens responsible for mold during maize storage, and *in vivo* on maize samples before storage. These biopesticides could provide an alternative in the search for sustainable mold control management strategies for maize stocks.

#### CONCLUSION

The biopesticide RHOSO 50 EC completely inhibited the radial mycelial growth of all *Fusarium* sp. strains at a concentration of 2000 ppm. The biopesticides NOSTAG 50 EC, RHOSO 50 EC, and WACHET 50 EC showed good efficacy against mycelial growth and sporulation in all strains. However, the biopesticides NORDINE 50 EC and BIOSAKINE 50 EC were less effective in controlling these corn mold pathogens. The use of the biopesticides NOSTAG 50 EC, RHOSO 50 EC, and WACHET 50 EC in the control of these pathogens may be a promising solution. However, this study needs to be further investigated before it can be disseminated to maize traders and growers.

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**Contribution of Authors:**

Tuo Seydou	:	Conceived the theme of the study, formulated the biopesticides, helped conduct the various field experiments, performed statistical analyses, and wrote the first draft of the manuscript.
Aboure A. Hermine	:	Collected field data and isolated pathogens associated with maize molds in the laboratory and reviewed the manuscript.
Ouattara Brahim	:	Contributed to the experiments and reviewed the manuscript.
Bamba Barakissa	:	Contributed to the experiments and reviewed the manuscript.
Camara Brahim	:	Supervised laboratory research and reviewed the manuscript
Koné Daouda	:	Supervised the study and reviewed the manuscript