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Research Article

In vitro assessment of botanical biopesticides against storage-associated fungal pathogens of maize (*Zea mays* L.)

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ABSTRACT: Maize (*Zea mays* L.) is essential to food security and the economy of Côte d'Ivoire, serving as a key staple crop for both local consumption and export. However, its production is threatened by various biotic constraints, among which fungal pathogens are prominent. In particular, molds of the genera *Aspergillus* and *Pythium* are responsible for the degradation of stored maize kernels. These fungi produce mycotoxins that are potentially dangerous to human and animal health, compromising crop quality and leading to considerable economic losses. The objective of this study was to investigate a biological control method against these fungi by evaluating the effectiveness of five biopesticides formulated with aromatic plant extracts: BIOSAKINE 50 EC, NORDINE 50 EC, NOSTAG 50 EC, RHOSO 50 EC, and WACHET 50 EC. *In vitro* tests were carried out to assess their impact on mycelial growth and fungal sporulation. Results revealed variable efficacy depending on the biopesticide and concentration used. The products WACHET 50 EC, NOSTAG 50 EC and RHOSO 50 EC showed a remarkable ability to inhibit mold growth, reaching total inhibition at 2000 ppm in some cases. In contrast, the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC were less efficient. The mycelial regrowth tests also demonstrated that certain biopesticides, like WACHET 50 EC at high concentrations, exhibited fungitoxic effects, while others displayed a fungistatic action. These findings indicate that biopesticides may serve as a feasible and eco-friendly substitute for chemical pesticides in managing maize pathogens. However, further studies on a larger scale and under real conditions are needed to confirm these results before widespread adoption by growers and traders.

KEYWORDS: Biological control, biopesticides, food safety, fungal pathogens, maize, mycotoxins

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INTRODUCTION

Maize (*Zea mays* L.), a representative grain of the Poaceae family, holds a crucial position in the food security and economic advancement of numerous developing nations (N'Da *et al.,* 2022). In West Africa, and particularly in Côte d'Ivoire, this crop occupies a strategic position. It is an essential source of calories and nutrients for rural and urban populations, while representing a livelihood for thousands of local farmers. According to Lemi and Debela (2023), corn is one of the main cereals produced and consumed in Africa, contributing to nearly 30% of the total caloric intake of households. In Côte d'Ivoire, maize production is mainly concentrated in the country's northern regions, favoured by a climate conducive to its development (N'Da *et al.,* 2022). However, despite its importance, the corn industry faces major constraints, notably post-harvest losses due to fungal contamination. Among the fungal pathogens of greatest concern are fungi of the *Aspergillus* and *Pythium* genera, which are responsible for the qualitative and quantitative degradation of corn kernels during storage.

These microorganisms often proliferate under inadequate storage conditions, such as excessive humidity or high temperatures (Mafe *et al.,* 2024). In addition, *Aspergillus* sp. are known to produce mycotoxins, such as aflatoxins, which are classified as carcinogenic to humans by the World Health Organisation (Navale *et al.,* 2021). These biological contaminants pose a twofold challenge: a serious health risk to consumers and significant economic losses for producers and retailers.

Conventional methods of combating these pathogens, based mainly on the use of synthetic chemicals, are now facing several limitations. In addition to their often prohibitive cost for smallholders, these products present ecotoxicological risks, encourage the emergence of resistance in pathogens, and can leave harmful residues in food (Ngegba *et al.,* 2022; Nadeem *et al.,* 2023). In this context, the use of plant-based biopesticides, particularly those derived from aromatic plant extracts, appears as a sustainable and environmentally friendly alternative. Plant extracts, rich in bioactive compounds such as phenols, terpenoids, and alkaloids, have demonstrated their antifungal (Tuo *et al.,* 2022; Abdi-Moghadam *et al.,* 2023; Bamba *et al.,* 2023), bactericidal (Dubale *et al.,* 2023), and insecticidal potential (Hussain *et al.,* 2023) in several studies. These biopesticides offer an innovative approach to mold management in corn grain storage, with minimal impact on the environment and human health. They not only preserve the quality of agricultural produce but also improve the sustainability of production systems. With this in mind, the present work aimed to explore the efficacy of biopesticides derived from aromatic plants in controlling contamination by Aspergillus and Pythium in maize stocks. This research, aligned with the Sustainable Development Objectives (SDOs), especially those related to food security (SDO 2) and environmental protection (SDG 15), aims to propose innovative biological solutions to meet the current challenges facing the maize sector in Côte d'Ivoire and beyond.

MATERIALS AND METHODS

Fungal material

The fungal material used consisted of an isolate of *Aspergillus* sp. and an isolate of *Pythium* sp. (Figure 1).

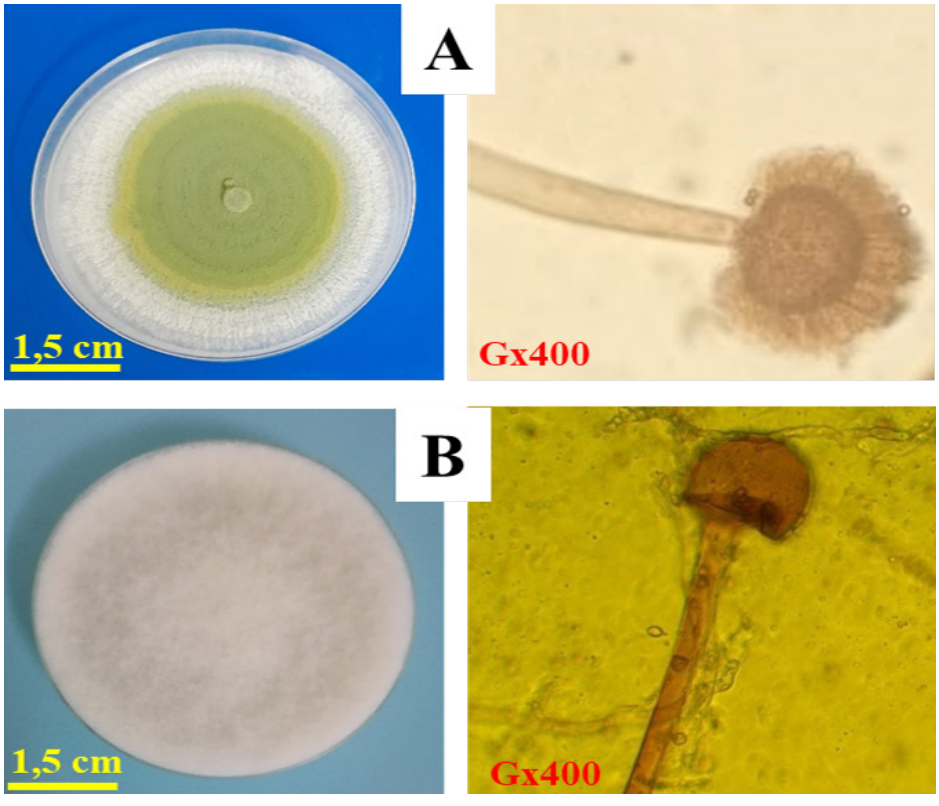


Figure 1. Fungal material used in this study. **(A).** Macroscopic and microscopic appearance of the fungal isolate of *Aspergillus* sp. **(B).** Macroscopic and microscopic appearance of the fungal isolate of *Pythium* sp.

These strains were extracted from maize grains gathered from market supplies in the autonomous district of Abidjan, Côte d'Ivoire. They are presently stored in the fungal culture repository of the Teaching and Research Department in Plant Physiology and Pathology at the University Félix HOUPHOUËT-BOIGNY, Côte d'Ivoire.

Biological control materials

The biological control materials used comprised five biopesticides: BIOSAKINE 50 EC, NORDINE 50 EC, NOSTAG 50 EC, RHOSO 50 EC and WACHET 50 EC. These biopesticides were developed from extracts of aromatic plants and supplied by the Industrial Research Unit (URI) on Biopesticides, located at the Bingerville Science and Innovation Park, a division of Félix HOUPHOUËT-BOIGNY University in Côte d'Ivoire.

Preparation of synthetic PDA culture medium

The growth medium employed in this research was the synthetic Potato Dextrose Agar (PDA) medium, widely recognised for its favourable properties in supporting the growth of various microorganisms, including fungi and yeasts. The preparation of this medium followed a precise protocol to ensure its sterility and effectiveness. The medium was developed by combining 20 g potato puree, 20 g D-glucose, and 20 g agar-agar in an Erlenmeyer flask. The total volume of the mixture was adjusted to 1 L with distilled water, followed by sterilisation in an autoclave at 121°C and 1 bar pressure for 30 min. Once sterilised, the medium was cooled to a temperature of approximately 45-50°C before being amended with biopesticides and poured into sterile Petri dishes with a diameter of 90 mm.

Test for inhibition of mycelial growth using Biopesticides

To assess the impact of five biopesticides (BIOSAKINE 50 EC, NORDINE 50 EC, NOSTAG 50 EC, RHOSO 50 EC, and WACHET 50 EC) on fungal mycelial development (*Aspergillus* sp. and *Pythium* sp.), *in vitro* tests were conducted using concentrations of 200, 400, 800, 1000, and 2000 ppm. The biopesticides were carefully incorporated into the PDA medium at temperatures between 45°C and 50°C, with the addition of a drop of Tween 20 as a surfactant. The mixtures were blended and poured into Petri dishes at a volume of 18-20 ml per dish. Each dish was inoculated with a 5-mm mycelial disc from 7-day-old fungal cultures placed at the centre. The control dishes contained only PDA medium. Five repetitions were conducted for each concentration and biopesticide, with the experiment being repeated three times. The dishes were incubated at 25 ± 2°C under a 12-h light/dark cycle and monitored every 24 h to assess the growth diameter. The Inhibition Rate (IR) was calculated relative to the controls using the method of Tuo *et al.* (2024) :

$$IR(\%) = \frac{D0-Dt}{D0} \times 100$$

With :

D0 = average diameter of the control

Dt = Average diameter of the treated

The sensitivity of the isolates and the toxicity of the fungicides (Table 1) were evaluated according to the scale of Kumar *et al.* (2007). The lethal inhibitory concentration (LIC) is the minimum concentration that fully halts mycelial growth after 7 days of incubation on PDA (Cruz *et al.,* 2022; Nabila *et al.,* 2025).

Mycelial plug regrowth test for different fungal genera

In the inhibition tests, when no mycelial growth occurred at a specific concentration, the mycelial disk was relocated to PDA medium without the biopesticide

Table 1. Fungicide efficacy level according to the scale of Kumar *et al.* (2007)

Scale	Class of Mycelial Inhibition Rate	Level of sensitivity/resistance of the fungus	Effectiveness level of the fungicide
1	I > 90 %	Highly sensitive	Very good efficiency
2	75 % < I ≤ 90%	Sensitive	Good efficiency
3	60 % < I ≤ 75 %	Moderately resistant	Average efficiency
4	40 % < I ≤ 60 %	Resistant	Low efficiency
5	I < 40 %	Highly resistant	Very low efficiency

and incubated for 7 days under identical conditions. The lethal inhibitory concentration (LIC) is the minimum concentration that stops any growth or mycelial recovery. If recovery is noted, the biopesticide is deemed fungistatic; if not, it is considered fungitoxic (Nabila *et al.,* 2025).

Assessment of biopesticide effectiveness on spore generation of isolates

In every Petri dish used to assess mycelial development, three 5-mm diameter explants were positioned in a tube containing 3 ml of sterile distilled water. After stirring (3 seconds x 5 cycles) to dislodge the spores, the suspension was strained through muslin to eliminate mycelial particles. Spore counting was performed using a Malassez slide (10 counts/suspension, total ≥ 100 spores), allowing the calculation of the number of spores per mm² and per ml. The inhibition percentage of sporulation (IS) was subsequently measured following the method outlined by Doumbouya *et al.* (2010):

$$IS(\%) = \frac{NSO - NSt}{NSO} \times 100$$

NS0 = Mean spore count in the control group

NSt= Mean spore count in the treated tubes

Statistical data analysis

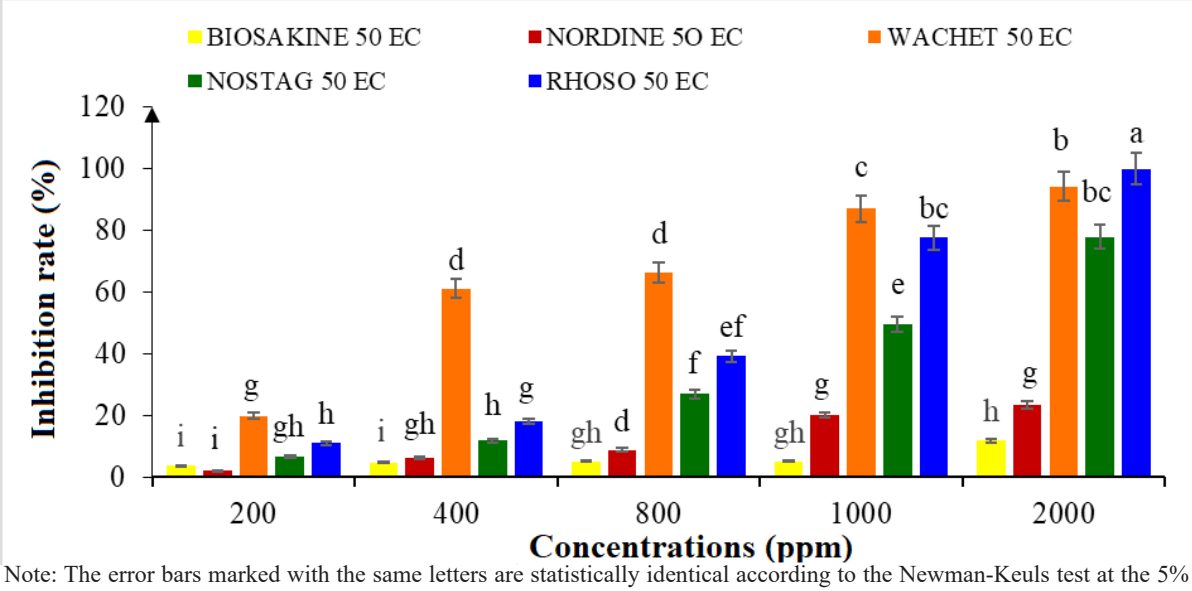
The results from *in vitro* tests with biopesticides were adjusted using an arcsine transformation. A two-way Analysis of Variance (ANOVA 2) was performed to assess the interaction between biopesticides, concentrations, and fungal strains to evaluate their efficacy. The data on the growth and spore production of the strains were examined using a one-way analysis of variance (ANOVA 1). Post-ANOVA mean comparisons were conducted using the Student-Newman-Keuls test at a significance level of 5%.

RESULTS

Impact of biopesticides on the mycelial development of fungal strains

Impact of biopesticides on the fungal development of *Aspergillus* sp.

Figure 2 demonstrates the impact of various biopesticide concentrations on the mycelial development of *Aspergillus* sp. All the tested biopesticides demonstrated the ability to reduce this growth, with the sensitivity of the fungal pathogen varying depending on the type of biopesticide and its concentration. The highest inhibition rates were observed with the biopesticides WACHET 50 EC, NOSTAG 50 EC, and RHOSO 50 EC, regardless of the concentration used. In contrast, the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC exhibited the lowest inhibition



Note: The error bars marked with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

Figure 2. Inhibition rate of mycelial growth of *Aspergillus* sp. as a function of biopesticides and concentration.

rates across all concentrations. The inhibition rates ranged from 5.09% to 11.76% for BIOSAKINE 50 EC and from 2% to 23.41% for NORDINE 50 EC. Notably, the biopesticide WACHET 50 EC achieved inhibition rates above 50% at concentrations of 400, 800, 1000, and 2000 ppm. Ultimately, full suppression of mycelial development (100%) was attained with the biopesticide RHOSO 50 EC at a concentration of 2000 ppm.

Impact of Biopesticides on the Fungal Development of *Pythium* sp.

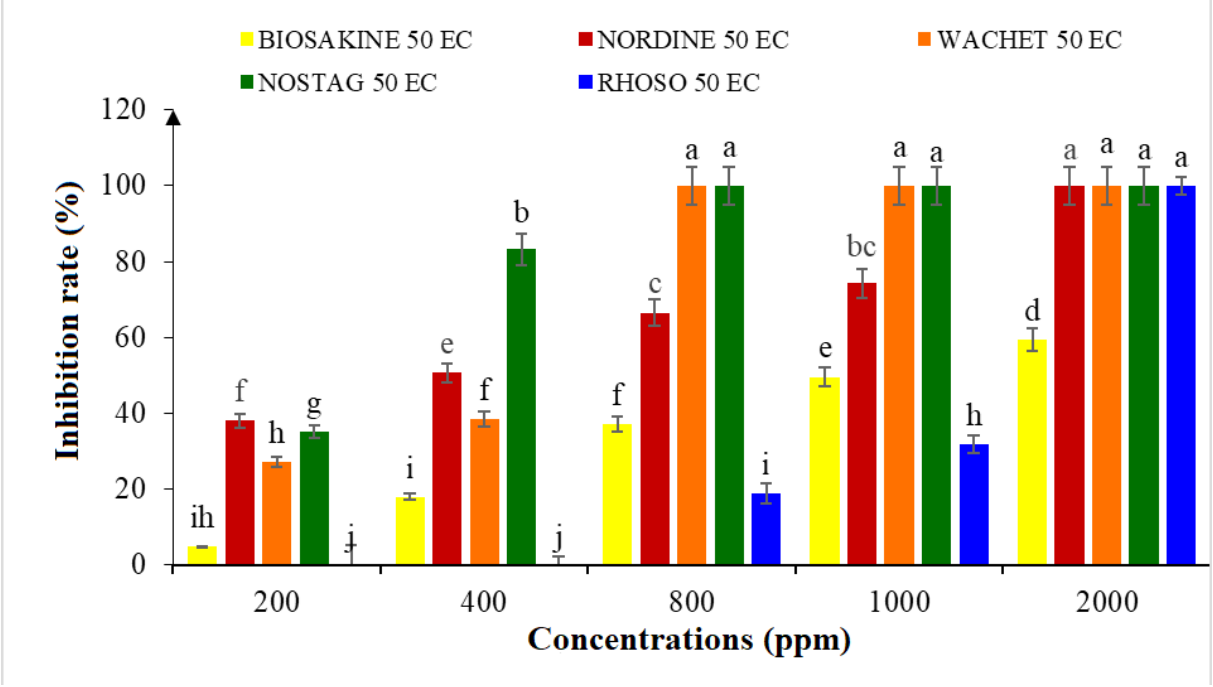
The levels of 800, 1000, and 2000 ppm of the biopesticide WACHET 50 EC, along with the 2000 ppm concentration of the biopesticides NORDINE 50 EC and RHOSO 50 EC, fully (100%) prevented the mycelial development of *Pythium* sp. Furthermore, the concentrations of 800 and 1000 ppm of the biopesticide NORDINE 50 EC, along with the 400 ppm concentration of the biopesticide RHOSO 50 EC, exhibited strong efficacy, with inhibition rates well over 50%. In contrast, the biopesticide BIOSAKINE 50 EC demonstrated minimal suppression of mycelial growth of *Pythium* sp. at all concentrations, except for the 2000 ppm concentration, which achieved an inhibition rate of 59.48% (Figure 3).

Impact of biopesticides on the mycelial regeneration of various strains

Table 2 presents the effects of biopesticides on the mycelial recovery of the strains. The results show that the biopesticide RHOSO 50 EC at 2000 ppm, which completely inhibits mycelial growth, allows for its recovery once the strains are transferred to a medium without the product, classifying it as fungistatic. The biopesticide NORDINE 50 EC at 2000 ppm also inhibited the growth of *Pythium* sp., but growth resumed after replanting. The biopesticides NOSTAG 50 EC and WACHET 50 EC at 2000 ppm inhibited the growth of *Pythium* sp., and recovery only occurred at lower concentrations (800 and 1000 ppm). Therefore, the biopesticide WACHET 50 EC is fungitoxic to *Pythium* sp. at 2000 ppm, as no recovery occurred.

Influence of strains on inhibitory concentrations CI50 and CI90 of different biopesticides

The inhibitory concentrations CI50 and CI90 vary between biopesticides and strains. CI50 values range from 86.83 to 8565.39 ppm, while CI90 values range from 1117.71 to 16286.53 ppm (Table 3). The biopesticide WACHET 50 EC generally gives the lowest CI50 and CI90 values. However, the highest CI50 (8565.39 ppm) is



Note: Error bars with the same letters are statistically identical according to the Newman-Keuls test at the 5% threshold.

Figure 3. Mycelial growth inhibition rates of *Pythium* sp. depending on biopesticides and concentration.

Table 2. Effects of biopesticides on the mycelial recovery of different strains

Biopesticides	Total inhibitory concentrations (ppm)	Fungal strains	
		***Aspergillus* sp.**	***Pythium* sp.**
NOSTAG 50 EC	800	0	+
	1000	0	+
	2000	0	-
NORDINE 50 EC	2000	0	+
RHOSO 50 EC	2000	+	+
WACHET 50 EC	800	0	+
	1000	0	+
	2000	0	-

Table 3. Inhibitory concentrations CI50 and CI90 of biopesticides based on fungal strains

Biopesticides	Inhibitory concentrations	Fungal strains	
		***Aspergillus* sp.**	***Pythium* sp.**
BIOSAKINE 50 EC	CI_{50} (ppm)	8565.39	1431.34
	CI_{90} (ppm)	16286.53	2796.34
NOSTAG 50 EC	CI_{50} (ppm)	1256.27	395.16
	CI_{90} (ppm)	2231.18	1117.71
NORDINE 50 EC	CI_{50} (ppm)	5360.23	400.03
	CI_{90} (ppm)	10094.92	1604.89
RHOSO 50 EC	CI_{50} (ppm)	896.43	1245.08
	CI_{90} (ppm)	1667.73	1879.07
WACHET 50 EC	CI_{50} (ppm)	371.86	302.46
	CI_{90} (ppm)	1667.78	1117.71

observed with BIOSAKINE 50 EC for the *Aspergillus* sp. strain, and the lowest (302.46 ppm) with WACHET 50 EC for *Pythium* sp. Regarding CI90, the lowest (1117.71 ppm) values were obtained with NOSTAG 50 EC and WACHET 50 EC for *Pythium* sp., and the highest (16286.53 ppm) was obtained with BIOSAKINE 50 EC for *Aspergillus* sp.

Impact of biopesticides on the spore production of various strains

The information shown in Table 4 pertains to the impact of biopesticides on the spore production of different strains collected from preserved maize seeds. Analysis of this table reveals that the biopesticides had an impact on the sporulation of all strains, with variations observed between strains, between biopesticides, and depending on the concentrations used. Specifically, the biopesticide BIOSAKINE 50 EC demonstrated no efficacy in reducing the sporulation of all strains, with inhibition percentages varying from 0 to 19.10%, regardless of the application rate. On the other hand, the biopesticide RHOSO 50 EC resulted in complete inhibition (100%) of the sporulation of all strains at the concentration of 2000 ppm. Moreover, the sporulation of the *Pythium* sp. strain was entirely suppressed (100%) at concentrations of 800, 1000, and 2000 ppm using the biopesticides WACHET 50 EC and NOSTAG 50 EC.

Table 4. Average inhibition rate of sporulation according to biopesticides and concentrations

Biopesticides	Concentrations (ppm)	Fungal strains	
		***Aspergillus* sp.**	***Pythium* sp.**
BIOKASINE 50 EC	200	2.10	0.00
	400	7.20	4.99
	800	5.03	5.73
	1000	8.20	7.08
	2000	10.30	6.70
NORDINE 50 EC	200	0.00	24.23
	400	0.00	22.02
	800	9.45	21.35
	1000	9.34	19.40
	2000	14.34	100.00
WACHET 50 EC	200	50.20	50.85
	400	55.30	49.35
	800	79.82	100.00
	1000	85.98	100.00
	2000	88.87	100.00
NOSTAG 50 EC	200	0.00	34.70
	400	6.30	35.70
	800	23.81	100.00
	1000	29.95	100.00
	2000	75.75	100.00
RHOSO 50 EC	200	3.30	28.39
	400	8.55	47.66
	800	14.75	51.35
	1000	78.60	65.83
	2000	100.00	100.00

DISCUSSION

The results confirm the effectiveness of biopesticides based on aromatic plant extracts in controlling fungal contamination of stored maize, particularly against the genera *Aspergillus* and *Pythium*. The impact of biopesticides on mycelial growth and sporulation of fungal isolates varied depending on the type of biopesticide and the concentration applied. The biopesticides WACHET 50 EC, NOSTAG 50 EC, and RHOSO 50 EC demonstrated significant suppression of mycelial growth in the isolates, achieving complete inhibition (100%) at elevated concentrations, particularly for RHOSO 50 EC at 2000 ppm. These findings corroborate those of Tuo *et al.* (2022), Bamba *et al.* (2023), and Yeo *et al.* (2023), who demonstrated the antifungal potential of aromatic plant extracts against various pathogens. In comparison, the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC demonstrated reduced effectiveness, indicating that their chemical formulation might be less potent against these particular pathogens. Regarding mycelial recovery, notably RHOSO 50 EC and

NORDINE 50 EC at 2000 ppm were classified as fungistatic, as the isolates resumed growth after being transferred to a medium free of biopesticides. Conversely, WACHET 50 EC at 2000 ppm was identified as fungitoxic for *Pythium* sp., meaning its antifungal action is permanent at this concentration (Nabila *et al.,* 2025). This distinction between fungistatic and fungitoxic activity is crucial for guiding biological control strategies. The effect of biopesticides on isolate sporulation also showed significant differences. The biopesticide RHOSO 50 EC at 2000 ppm completely inhibited the sporulation of both isolates, which is a major advantage in limiting pathogen spread. Additionally, WACHET 50 EC and NOSTAG 50 EC prevented the sporulation of *Pythium* sp. at high concentrations, further reinforcing their potential as biocontrol agents. The analysis of inhibitory concentrations CI50 and CI90 reveals that WACHET 50 EC has the lowest values, confirming its effectiveness at reduced doses. In contrast, BIOSAKINE 50 EC has the highest CI50 and CI90 values, suggesting lower efficacy or the need for a higher concentration to achieve a significant effect (Tuo *et al.,* 2024). Looking ahead, the use of biopesticides based on aromatic plants represents a promising alternative to synthetic fungicides, reducing the risk of pathogen resistance and minimising negative impacts on human health and the environment (Ayilara *et al.,* 2023; Aremu *et al.,* 2024). However, further studies are necessary to refine optimal dosages, evaluate their storage stability, and investigate their compatibility with other maize preservation methods. Ultimately, this research is in line with the Sustainable Development Goals (SDGs), especially SDG 2 concerning food security and SDG 15 focused on the conservation of land ecosystems. By promoting sustainable biological solutions, it contributes to the development of more resilient and environmentally friendly agricultural systems. The implementation of these solutions requires collaboration among researchers, farmers, and policymakers to integrate these biopesticides into sustainable agricultural practices. Moreover, in-depth toxicological and ecotoxicological analyses should be conducted to assess the long-term impact of these biopesticides on the surrounding fauna and flora. It would also be necessary to examine their effect on the nutritional and organoleptic quality of treated maize to ensure complete safety for consumers. The expansion of biopesticides based on aromatic plants could be further strengthened through outreach and training programs for producers, encouraging large-scale adoption, and contributing to more sustainable agriculture that respects ecological balance.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates the effectiveness of several biopesticides based on aromatic plant extracts against molds responsible for maize degradation during storage. The *in vitro* results show that some biopesticides, notably WACHET 50 EC, RHOSO 50 EC, and NOSTAG 50 EC, have a strong capacity to inhibit mycelial growth and spore formation of pathogenic fungi such as *Aspergillus* and *Pythium*, even achieving total inhibition at high concentrations. These biopesticides have shown fungitoxic potential, offering an effective and environmentally friendly alternative to chemical pesticides, which are often costly and ecotoxic. However, further research under real conditions is needed to confirm the effectiveness of these biopesticides in managing maize mold and their large-scale adoption by producers. This study thus paves the way for sustainable management of fungal pathogens in maize production systems, addressing food security and environmental protection challenges.

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NOVELTY STATEMENT

This study pioneers maize protection in Côte d'Ivoire by assessing five biopesticides derived from aromatic plant extracts against Aspergillus and Pythium. Unlike chemical-focused research, it underscores the potent fungitoxic and fungistatic properties of natural alternatives, with select formulations achieving total fungal inhibition at specific doses. These findings advance eco-friendly, sustainable strategies for post-harvest maize preservation.

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AUTHOR CONTRIBUTION

TS and YG developed the study, planned the experiments, carried them out, and drafted the manuscript. SKG and BB gathered and structured the data, examined the results, and contributed to the manuscript writing. CB and KD oversaw the project, helped in manuscript preparation, and reviewed the paper. All authors have read and agreed to the published version of the manuscript.