Journal of Animal & Plant Sciences, 35(1): 2025, Page: 293-306
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.1.0024

BIOLOGICAL CONTROL OF POST-HARVEST FUNGAL PESTS OF DESSERT BANANA (*Musa acuminata*) IN CÔTE D'IVOIRE

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ABSTRACT

Banana is the world's fourth most important crop of the food market next to rice, wheat and maize. In Côte d'Ivoire, banana is the third most important food crop. However, this crop is prone to several fungal diseases which hamper its post-harvest preservation. To control postharvest fungal diseases and with a view to finding an alternative to chemical control, the efficacy of the Bio-fungicides NECO 50 EC, ASTOUN 50 EC, PRORALY 50 EC, and FERCA 50 *in vitro* and *in vivo* were tested on postharvest fungi of dessert banana. Explants of the banana epidermis showing characteristic symptoms of phytopathogenic fungi and arranged in completely randomized design, were removed using a slide and cultured on PDA medium in Petri dishes. this study was repeated five times. Different concentrations of Bio-fungicides at 100, 200, 400, 500 and 1000 ppm incorporated into PDA culture media before fungus cultivation were used to assess bio-fungicide efficacy. Three fungal pathogens: *Colletotrichum* sp., *Fusarium* sp., and *Botryodiplodia* sp. were found. associated with symptomatic banana fruits. The highest growth inhibition of phytopathogenic fungi was obtained with the synthetic fungicide MIRAGE 450 EC (62.88%) at 0.5 ppm and the bio-fungicide ASTOUN 50 EC (60.78%) at 1000 ppm. With NECO 50 EC Bio-fungicide at 1000 ppm, an inhibition of 57.07% was achieved. In contrast, the lowest average inhibition of 37.42% was recorded with the bio-fungicide FERCA 50 EC . In view of their efficacy in *in vivo* and *in vitro* experiments, these Bio-fungicides could be used as an alternative to synthetic fungicides in the control of postharvest diseases of dessert banana.

Key words: Bio-fungicides, dessert banana, fungi, synthetic fungicides.

Published first online February 05, 2025 Published final February 18, 2025

INTRODUCTION

Dessert bananas (*Musa acuminata* L.) are a staple food and a source of income in over 120 developing countries for millions of people (Chabi *et al*. 2018). Global banana production is estimated at over 12 million tons of fruit per year (OECD/FAO, 2023). Côte d'Ivoire is the second largest producer after Nigeria in West Africa, with production of over 500 thousand tons. (Faostat, 2024), and remains the leading African supplier of the Grande Naine and William varieties to the European Union market (Maseko *et al*. 2024).

Despite its importance, this crop is prone to biotic constraints which threatens its production. Among these constraints, fungal diseases such as black Sigatoka and *cladosporiosis*, caused by *Mycosphaerella fijiensis* and *Cladosporium musae*, respectively, are the main causes of reduced yield. These diseases affect the host's foliage (Tuo *et al*. 2022). In addition, other fruit-specific fungal diseases in post-harvest conditions, mainly crown rot and anthracnose, cause significant damage and result in considerable economic losses ranging from 10% to 80% (Peralta-Ruiz *et al*. 2023). These diseases are caused by a complex of fungi such as *Colletotrichum* sp., *Botryodiplodia* sp., and *Fusarium* sp. (Zakaria, 2023; Peralta-Ruiz *et al*. 2023). Similarly, *Curvularia lunata* is responsible fruit rot of banana (Khan and Javaid, 2020). To combat these conditions during fruit packaging, production structures resort to synthetic fungicides, the systematic use of which can lead to resistance problems for certain fungal species. Moreover, although relatively effective, the use of these products could have harmful effects on the environment, human and animal health (Brühl *et al*. 2023). To remedy this situation, new alternative approaches to synthetic chemicals are being proposed (Javaid *et al*. 2023). Among these alternate strategies, use of natural antifungal compounds from plants (Ferdosi *et al*. 2022; Jabeen *et al*. 2022), and application of biocontrol agents such as species of

Trichoderma, Penicillium and *Aspegillus* (Javaid *et al*. 2021; Khan and Javaid, 2021, 2022), and plant growth promoting rhizobacteria (Javed *et al*. 2021; Sharf *et al*. 2021) are the most common . The present study was initiated with a view to reducing post-harvest production losses due to *Colletotrichum* sp., *Botryodiplodia* sp., and *Fusarium* sp. using biological products based on plant extracts.

MATERIALS AND METHODS

Study location: The study was conducted in June 2022 in the plant pathology laboratory of the Biosciences, Training and Research Unit of the Université Félix HOUPHOUËT-BOIGNY (Abidjan / Côte d'Ivoire).

Plant material: The banana sample consisted of healthy and necrotic dessert bananas of the Grande Naine variety of Cavendish subgroups. Four bio-fungicides, NECO 50 EC (thymol, eugenol), ASTOUN 50 EC (geranial, neral, myrcene), PRORALY 50 EC (thymol, eugenol, citronellol, citronellal), and FERCA 50 EC (citronellol, citronellal), were used in this study. These bio-fungicides, produced from aromatic plant extracts (Tuo *et al*, 2021), procured from the Industrial Research Unit (URI) of the Université Félix HOUPHOUËT-BOIGNY. MIRAGE 450 EC (prochloraz) and CARTE 10% (carvacrol thyme and terpinen-4-ol), synthetic and biological fungicides registered for use on bananas in Côte d'Ivoire, were also used as positive controls.

Preparation of the culture media: Potato dextrose agar (PDA) culture medium was used for fungal isolation. It consisted of potato puree (mineral source), glucose (carbon source), and agar-agar (gelling agent). To prepare 1l of PDA medium, 20 g of potato purée, 20 g of glucose, and 20 g of agar-agar were dissolved in 500 ml of distilled water (Westphal *et al*. 2021). The mixture was adjusted to 1l with distilled water in a jar., and the resulting solution was autoclaved at 121°C under a pressure of 1 bar for 30 min. The resulting PDA medium was cooled in Petri dishes at a rate of 18-20 ml per dish under a Steril-BIO BAN compact laminar flow hood.

Fungal isolation: Fungi were isolated on PDA medium. This was performed using fragments of the banana epidermis bearing various symptoms characteristic of fungal diseases (Dita *et al*. 2018). Using a sterile slide, explants were taken from the epidermis of diseased bananas at the symptom growth front. Explants were sterilized by soaking in 70% ethanol for 1 min and then in 3% sodium hypochlorite for 3 min. After soaking, the explants were rinsed three times with distilled water. The explants were then blotted on to sterile blotting paper and seeded into Petri dishes containing PDA culture medium using sterilized forceps. Cultures were incubated at room temperature in the laboratory at 27 ± 2°C for 72-96 h under a 12 h photoperiod (Gu *et al*. 2022). After incubation, fungi growing on the culture medium were removed from the mycelial growth front with a punch and placed in the center of a new Petri dish containing the new PDA medium. This operation was repeated until pure isolates were obtained. Homogeneous colonies were coded and used for morphological identification.

Macroscopic and microscopic characterization of the fungi: Pure colonies aged between 6 and 14 days were identified using the determination key (Bosch-Roig *et al*. 2021**)** based on the morphological characteristics of the various isolates (colour, shape and appearance). The macroscopic and microscopic characteristics of the fungi were determined using the naked eye and an Amscope photonic microscope Model T490A equipped with a camera, respectively. For each fungal isolate, a 6-mm diameter mycelial disk was grown on PDA medium in the center of a Petri dish. Petri dishes were incubated at 27 ± 2°C under a 12 h photoperiod until the mycelial filaments reached the periphery of the dish. Thallus growth was observed daily. For microscopic characterization, the mycelium of each strain was removed superficially from the PDA medium after 14 days of culture, using sterilized forceps. Samples were mounted between a slide and a coverslip and observed at different magnifications under a light microscope (Huang *et al*. 2021). The mycelial structure and spore shape were observed and recorded.

Study of the *in vitro* antifungal effects of bio-fungicides on fungi: For the study of antifungal activity, concentrations of 100, 200, 400, 500, and 1000 ppm of each of the bio-fungicides tested and concentrations of 0.1, 5, 10, 25, and 50 ppm for synthetic fungicides were added to the PDA medium. The amended media were homogenized and dispensed into 9-cm-diameter Petri dishes. Then, a mycelial disk of approximately 6 mm diameter, taken with a sterilized punch from a 7-day-old culture, was placed in the center of a Petri dish containing PDA medium amended with Bio-fungicides and synthetic fungicides. Fungi were cultured at room temperature in a laminar flow hood. The plates were incubated in a tray at room temperature (27 ± 2°C) with a 12h photoperiod. Each treatment was replicated five times in separate Petri dishes, and the experiment was repeated five times. The efficacy of the bio-fungicides was assessed by measuring the rate of inhibition of mycelial growth (Shin *et al*. 2017):

$$= \frac{Do-Dc}{Do} \times 100$$

T_i (%)

T_i: mycelial growth inhibition rate; Do: average mycelial growth diameter of the fungus in control boxes; Dc: average mycelial growth diameter of the fungus at fungicide concentration C (biological or chemical).

Evaluation of the *in vivo* antifungal effects of bio-fungicides on dessert bananas: This study assessed the *in vivo* efficacy of Bio-fungicides in preventing or delaying the onset of fungal disease symptoms on " hands " from banana bunches. . Batches of samples were collected and treated with the different fungicides previously used. The efficacy of the different bio-fungicides was assessed using a completely randomized design, at different doses of 0.1; 0.15; 0.2; 0.3 and 0.4%, and compared with those of synthetic fungicides at doses of 0.25% for MIRAGE 450 EC and 0.5% for CARTE 10%. The Fungicides were applied by spraying and brushing the apex and crown of bananas using a 2-liter volume garden pump sprayer (Ambang *et al*. 2023). Untreated fruits constituted the control. After treatment, the fruits were stored in a laboratory cold room at 13°C for 21 days to assess the green life of the bananas, thus simulating export storage conditions. During this study, the severity index, incidence of post-harvest diseases and mass loss of treated fruits were evaluated. The treated bananas were then placed in bins for each treatment modality and left in ambient air for 72 h. Observations were then made every 3 days for 12 days.

Statistical analysis: The collected data were analyzed using STATISTICA version 7.1 software. ANOVA was used to assess the effect of Bio-pesticides on the phytopathological parameters of bananas. In the event of rejection of the null hypothesis (H_0), the Newman-Keuls test at the 5% threshold was used to classify the means into homogeneous groups.

RESULTS

Characteristics of pathogenic fungi associated with symptoms: Various fungal microorganisms were isolated from symptomatic banana tissues. Among them, three genera associated with symptoms were identified: *Colletotrichum* sp., *Fusarium* sp., and *Botryodiplodia* sp. (Fig. 1). The *Colletotrichum* sp. presented a cottony, light-gray appearance on the surface of the Petri dish and a gray colour on the reverse side. The *Fusarium* sp. strain showed a raspy, pinkish-white mycelial appearance on the surface and underside of the Petri dish. Finally, for the *Botryodiplodia* sp , the mycelial appearance was cottony, with a dark gray surface and black underside (Table 1). Microscopic observation of the strains revealed three forms of conidia, notably oval, unicellular conidia for *Colletotrichum* sp., oval and thick-walled septate conidia for *Botryodiplodia* sp., and arched conidia for *Fusarium* sp. (Fig. 1).

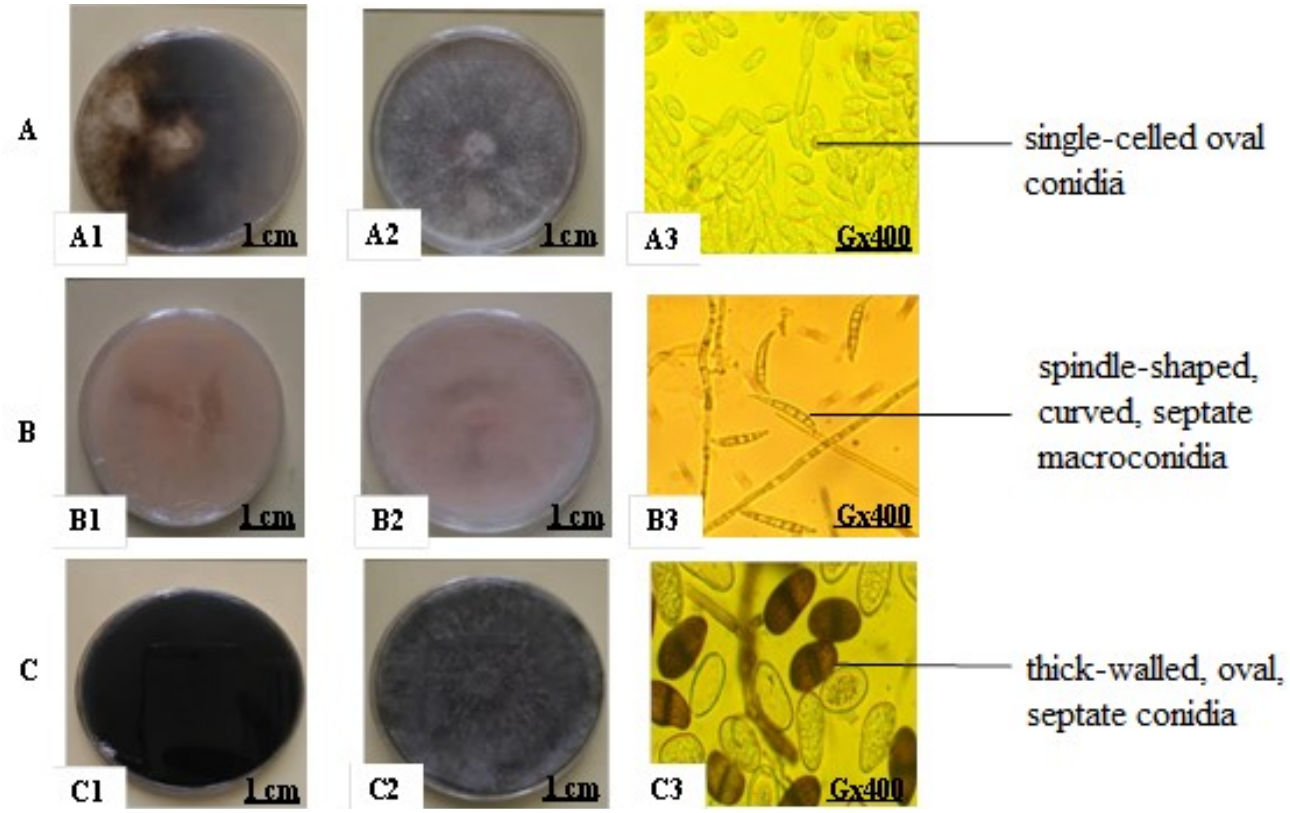

Fig. 1. Macroscopic and microscopic aspects of different fungal strains isolated from banana tissue.
A: *Colletotrichum* sp.; B : *Fusarium* sp. ; C : *Botryodiplodia* sp. Appearance of mycelium on the surface (A2, B2) and underside (A1, B1) of the Petri dish and shape of conidia (A1, B3, C3)

Table 1. Macroscopic and microscopic description of isolated fungal strains

Fungal strains	Mycelial appearance	Mycelial color	
		Surface Petri dish	Reverse Petri dish
Colletotrichum sp.	Cottony	Light-gray	Gray
Fusarium sp.	Ras-fibrous	White-pink	White-pink
Botryodiplodia sp.	Cottony	Gray-dark	Black

Effects of fungicides on the mycelial growth of *Colletotrichum* sp., *Fusarium* sp., and *Botryodiplodia* sp. isolates as a function of concentration and incubation time.

Inhibition rates varied with fungicide concentration and fungal culture incubation time. Fungi showed varying degrees of sensitivity to the applied concentrations of NECO, FERCA, PRORALY, and ASTOUN Bio-fungicides as a function of time.

Effects of fungicides on the mycelial growth of *Colletotrichum* sp.: The highest inhibition rate was obtained using the bio-fungicide NECO 50 EC at a concentration of 1000 ppm. At the 1000 ppm concentration, the 100% inhibition rate was maintained until the fourth day after incubation and then dropped to 92.28% by the seventh day. This inhibition rate is higher than that of the positive controls, which at 50 ppm recorded values of 62.51% and 17.35% for MIRAGE 450 EC and 10% CARTE, respectively. On the other hand, the lowest rate of 12.52% was recorded with the bio-fungicide FERCA 50 EC. Next, the bio-fungicide ASTOUN 50 EC showed the second best inhibition rate, ranging from 100% to 58.06% between day one and day seven for the 1000 ppm concentration (Fig. 2).

Effects of fungicides on the mycelial growth of *Fusarium* sp.: The rate of inhibition of mycelial growth was higher at concentrations of 1000, 500, and 400 ppm for the Bio-fungicide ASTOUN 50 EC than for the other fungicides. ASTOUN 50 EC showed a 100% inhibition rate over a 4-day period. The bio-fungicide NECO 50 EC showed a 100% inhibition rate over a 3-day period at concentrations of 1000 and 500 ppm. In contrast, a progressive decrease in the inhibition rate was observed from day four to seven for ASTOUN 50 EC. It varied from 100% to 66.79% for 1000 ppm, from 100% to 60.44% for 500 ppm, and from 100% to 34.48% for 400 ppm. On the other hand, for NECO 50 EC Bio-fungicide, a decrease in the inhibition rate was observed from day three to seven, with variations ranging from 100% to 62.03% for 1000 ppm and then from 100% to 57.26% for 500 ppm. For the 400 ppm concentration, the inhibition rate varied from 100% on day two to 53.90% on day seven (Fig. 3).

Effects of fungicides on the mycelial growth of *Botryodiplodia* sp.: Of all the bio-fungicides tested, only NECO 50 EC and PRORALY 50 EC showed the highest inhibition rates of 93.46% and 84.81% respectively, up to day 4 after incubation for the 1000 ppm treatments. These rates were higher than the mycelial growth inhibition rates induced by the fungicides MIRAGE 450 EC and CARTE 10%, which were 53.73 and 36.86% respectively, at the 50 ppm concentration (Fig. 4).

Mycelial growth inhibition rates as a function of the fungicides applied: The efficacy of the fungicides tested in inhibiting the mycelial growth of the different fungal strains varied significantly between the different fungicides tested (Fig. 5). The highest inhibition rates were recorded with the synthetic fungicide MIRAGE 450 EC (62.88%) and the bio-fungicide ASTOUN 50 EC (60.78%), followed by the Bio-fungicide NECO (57.07%). In contrast, the lowest average inhibition rate, 37.42%, was obtained with the Bio-fungicide FERCA (Fig. 5).

Inhibition rates of fungicides on the mycelial growth of different fungal isolates: Inhibition rates also varied according to the strain and fungicide. Fig. 6 shows that for the *Colletotrichum* sp strain, the highest inhibition rate (69.97%) was obtained using the Bio-fungicide ASTOUN 50 EC. Conversely, the lowest inhibition rates (58.7 and 59.28%) were recorded with MIRAGE 450 EC and CARTE 10% respectively. For the *Fusarium* sp. strain, no significant difference was observed between the Bio-fungicides NECO 50 EC and MIRAGE 450 EC. On the other hand, the highest inhibition rate (79.23%) was recorded with the synthetic fungicide MIRAGE 450 EC, whereas the Bio-fungicides NECO 50 EC and ASTOUN 50 EC recorded rates of 75.43% and 68.03%, respectively. Finally, with the *Botryodiplodia* sp. strain, the highest rates of mycelial growth inhibition were observed with the Bio-fungicides PRORALY 50 EC (53.75%) and NECO 50 EC (52.31%).

Effect of fungicide concentrations on fungal disease incidence in dessert bananas: Evaluation of disease incidence showed variation according to the fungicide and concentration applied (Tables 2, 3, 4, 5 and 6). The incidence of banana crown rot varied from 40% to 100%. The lowest incidence (40%) was recorded with the bio-fungicide NECO 50 EC (Table 2) at concentration C5 (0.4%), followed by NECO 50 EC and ASTOUN 50 EC, at concentrations of C4 (0.3%) and C5 (0.4%) respectively, with an incidence of 50% (Table 3). On the other hand, the incidence of bananas treated with

synthetic fungicides was 60% and 63% for MIRAGE 450 EC and CARTE 10%, respectively, whereas that of untreated bananas was 100% (Table 6).

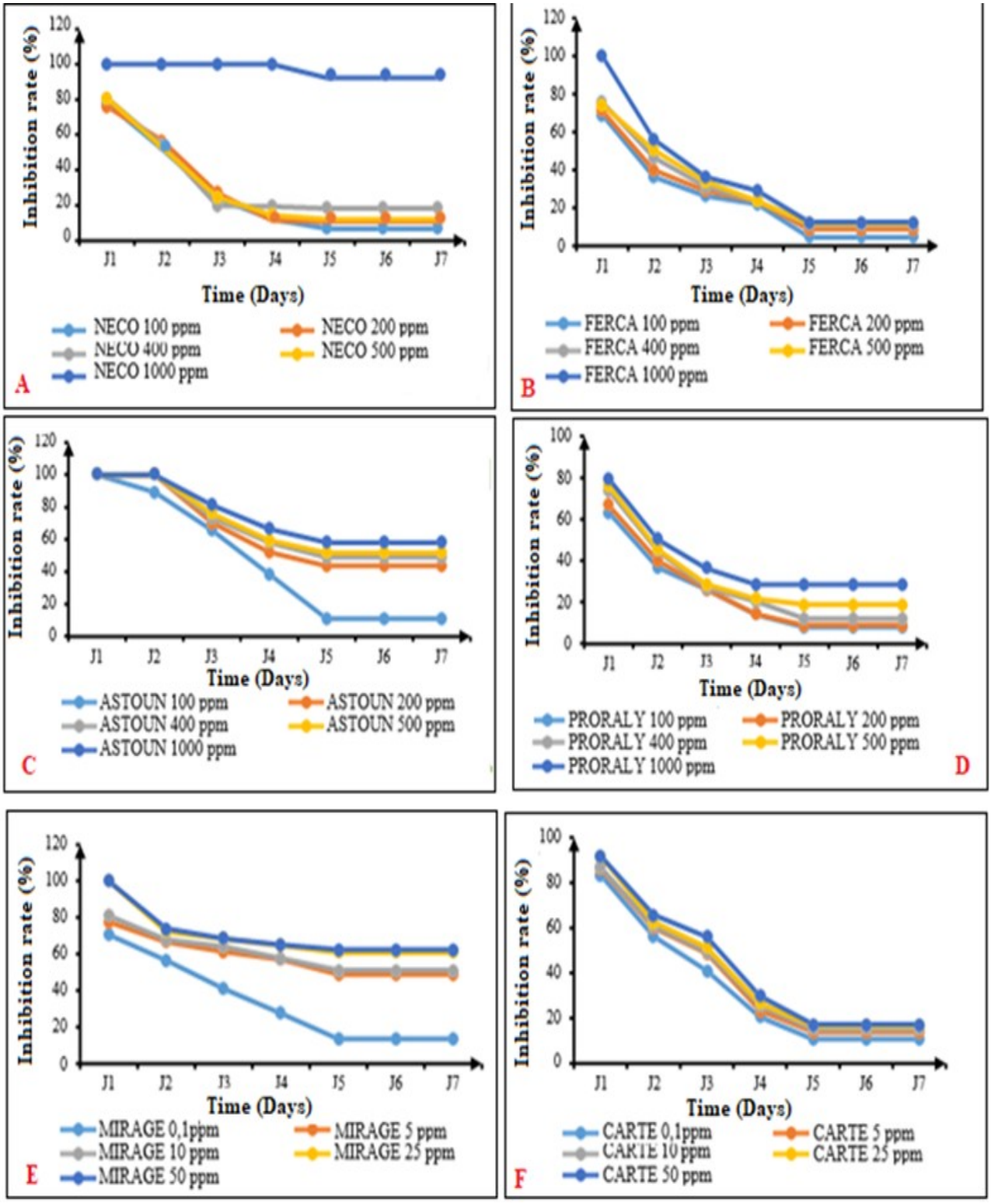

Fig. 2. Reduction in mycelial growth of *Colletotrichum* sp. as a function of time and fungicide concentration

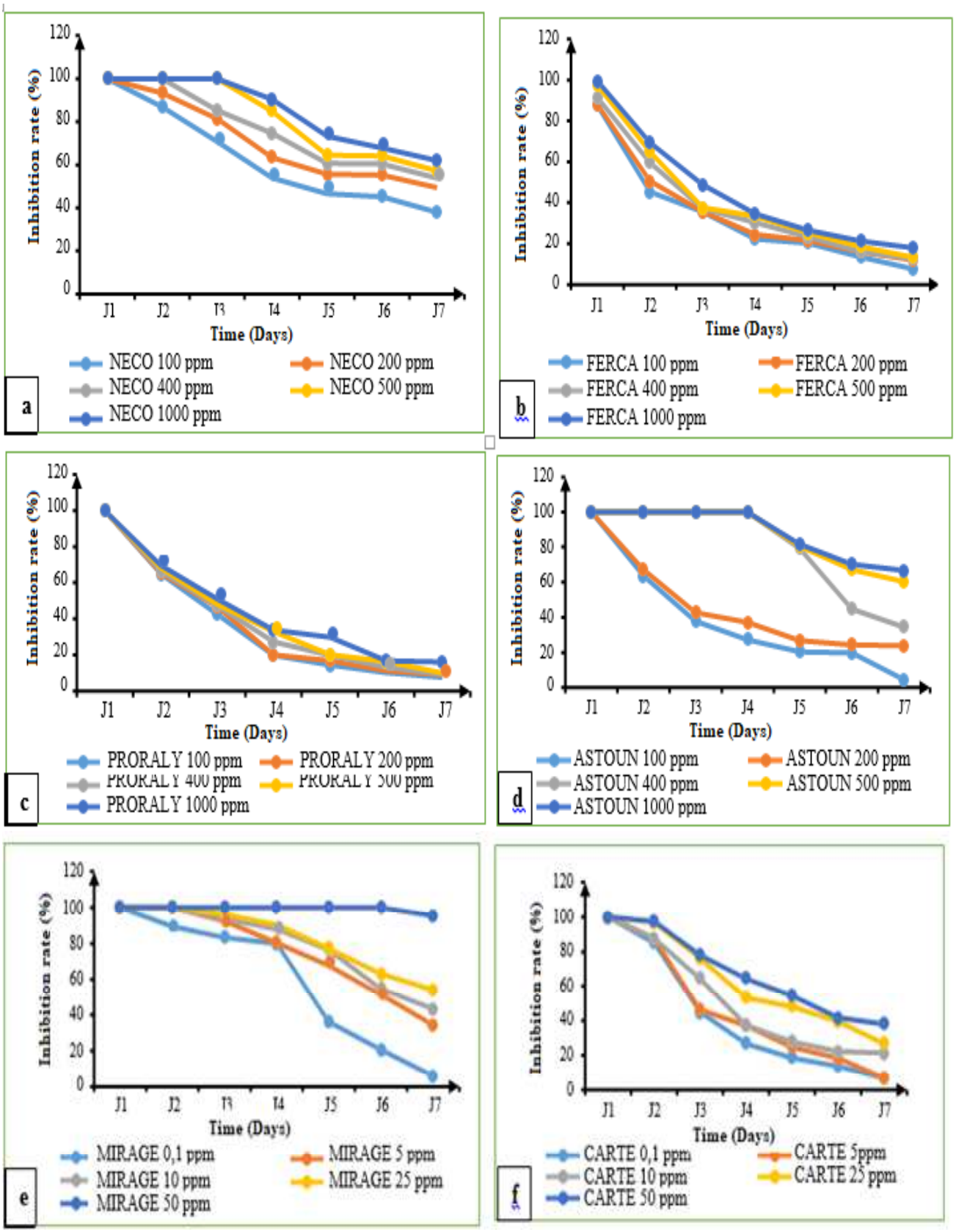

Fig. 3. Reduction in mycelial growth of *Fusarium* sp. as a function of time and fungicide concentration

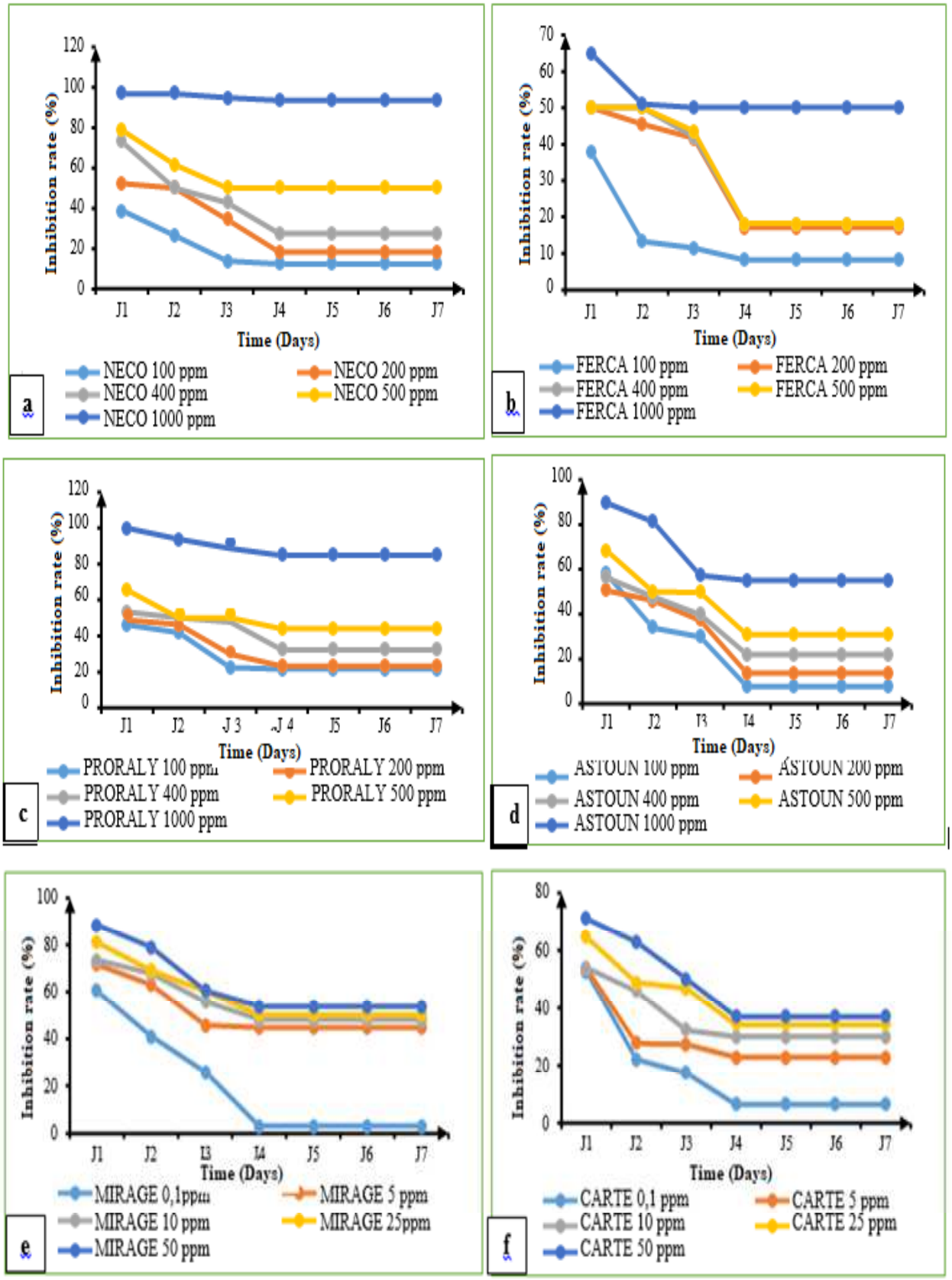

Fig. 4. Reduction in mycelial growth of *Botryodiplodia* sp. as a function of time and fungicide concentration

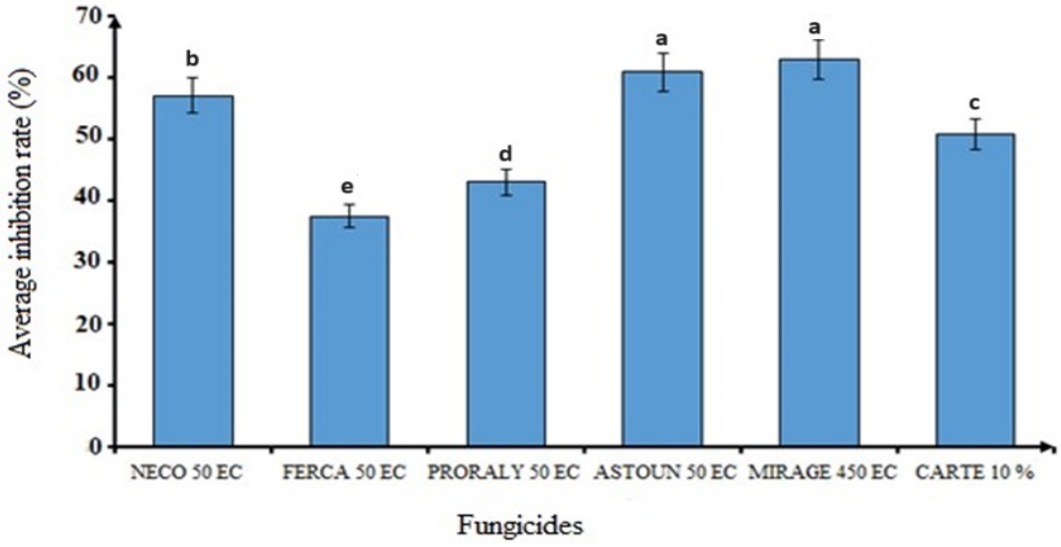

Fig. 5. Average inhibition rates of fungicides tested on mycelial growth of fungal isolates

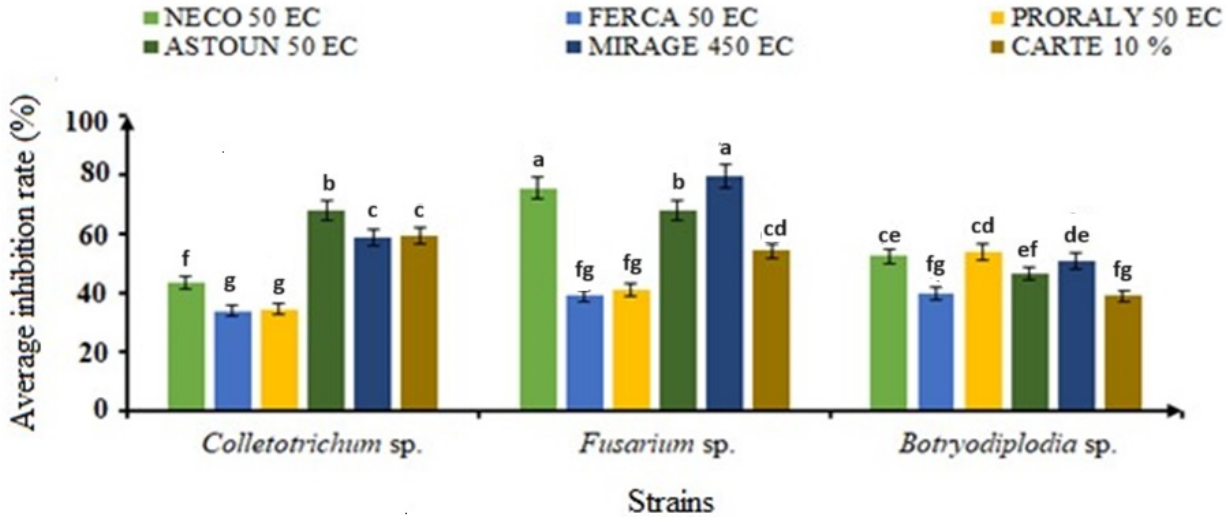

Fig. 6. Average inhibition rate per strain according to Bio-fungicides

The lowest incidence (33.33%) of anthracnose was recorded using the bio-fungicide NECO 50 EC at concentration C5. On the other hand, at the C4 concentration (0.3%), the incidence was identical to that of MIRAGE 450 EC for all treatments, with an average of 40%, except for 10% CARTE (Table 6). The incidence of apex rot on bananas ranged from 0% to 100%. Bananas treated with the various bio-fungicides showed no symptoms at concentrations C4 and C5, and C3 only for Bio-fungicide NECO. However, for the fungicides CARTE 10% and MIRAGE 450 EC, the incidence was 6.66%, compared with 100% for the untreated control.

Effect of bio-fungicide concentrations on disease severity index after application to bananas: Disease severity indices varied with the fungicide and concentration. For crown rot (Table 7), the highest severity indices of 7.7 % and 6.96% were recorded for untreated bananas and those treated with FERCA 50 EC bio-fungicide at concentration C1, respectively. The severity index varied slightly from 2.6 % to 3.6% for all fungicides except for FERCA 50 EC bio-fungicide at concentration C3. For anthracnose (Table 8), the highest index (12.33%) was recorded for untreated bananas, followed by C1 (0.1%) for all bio-fungicides tested. The lowest severity indices, ranging from 4.16 to 4.83, were observed with bio-fungicides at C4 and C5 concentrations. Concerning apex rot (Table 9), the disease severity index was nil (0%) for bananas treated with bio-fungicides at C4 and C5 doses, in contrast to the synthetic fungicides (MIRAGE 450 EC and CARTE 10%), where the severity index was 0.66%. For the untreated control, the severity index was 2.56% (Table 9).

Table 2. Disease incidence as a function of NECO 50 EC concentrations

		Incidence (%)		
Fungicide	**Concentration**	**Crown rot**	**Anthracnose**	**Apex rot**
NECO 50 EC	C1	100 ± 0,00[a]	100 ± 0.00[a]	100 ± 0.00[a]
	C2	100 ± 0.00[a]	100 ± 0.00[a]	66.6 ± 33.39[b]
	C3	100 ± 0.00[a]	100 ± 0.00[a]	0 ± 0.0[c]
	C4	50 ± 17.77[b]	40 ± 12.89[b]	0 ± 0.0[c]
	C5	40 ± 12.89[c]	33.33 ± 12.33[c]	0 ± 0.0[c]
	Average	78	74.66	33.32
	Coefficient of variation (%)	7.86	6.75	20.01
	Probability	0.0001	0.0001	0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman-Keuls test. C1 = 0.1%; C2 = 0.15%; C3 = 0.2%; C4 = 0.3%; C5 = 0.4%.

Table 3. Incidence of disease as a function of ASTOUN 50 EC concentrations.

		Incidence (%)		
Fungicide	**Concentration**	**Crown rot**	**Anthracnose**	**Apex rot**
ASTOUN 50 EC	C1	100 ± 0.00[a]	100 ± 0.00[a]	78.6 ± 11.3[a]
	C2	100 ± 0.00[a]	100 ± 0.00[a]	66.6 ± 33.39[a]
	C3	100 ± 0.00[a]	100 ± 0.00[a]	33.33 ± 22.2[b]
	C4	60 ± 28.88[b]	40 ± 12.89[b]	0 ± 0.0[c]
	C5	50 ± 17.77[c]	36.66 ± 11.33[c]	0 ± 0.0[c]
	Average	82	75.33	35.7
	Coefficient of variation (%)	11.37	6.43	37.45
	Probability	0.0001	0.0001	0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman-Keuls test. C1 = 0.1%; C2 = 0.15%; C3 = 0.2%; C4 = 0.3%; C5 = 0.4%.

Table 4. Disease incidence as a function of PRORALY 50 EC concentrations

		Incidence (%)		
	Concentration	**Crown rot**	**Anthracnose**	**Apex rot**
PRORALY 50 EC	C1	100 ± 0.00[a]	100 ± 0.00[a]	66.66 ± 33.39[a]
	C2	100 ± 0.00[a]	100 ± 0.00[a]	66.66 ± 33.39[a]
	C3	100 ± 0.00[a]	100 ± 0.00[a]	33.33 ± 11.33[b]
	C4	70 ± 34.42[b]	40 ± 12.89[b]	0 ± 0.0[c]
	C5	66 ± 33.33[b]	36.66 ± 11.33[b]	0 ± 0.0[c]
	Average	87.2	75.33	33.33
	Coefficient of variation (%)	15.53	6.43	46.86
	Probability	< 0.0001	< 0.0001	< 0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman-Keuls test. C1 = 0.1%; C2 = 0.15%; C3 = 0.2%; C4 = 0.3%; C5 = 0.4%.

Table 5. Incidence of disease as a function of FERCA 50 EC concentrations

		Incidence (%)		
	Concentration	**Crown rot**	**Anthracnose**	**Apex rot**
FERCA 50 EC	C1	100 ± 0.00[a]	100 ± 0.00[a]	66.66 ± 33.39[a]
	C2	100 ± 0.00[a]	100 ± 0.00[a]	66.66 ±33.39[a]
	C3	100 ± 0.00[a]	100 ± 0.00[a]	33.33 ± 11.33[b]
	C4	78 ± 22.22[b]	40 ± 12.89[b]	0 ± 0.0[c]
	C5	67 ± 35.25[c]	36.66 ± 11.33[b]	0 ± 0.0[c]
	Average	89.00	75.33	33.33
	Coefficient of variation (%)	13.42	6.43	46.86
	Probability	< 0.0001	< 0.0001	< 0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman-Keuls test. C1 = 0.1%; C2 = 0.15%; C3 = 0.2%; C4 = 0.3%; C5 = 0.4%.

Table 6. Incidence of disease as a function of concentrations of MIRAGE 450 EC and CARTE 10 %.

		Incidence (%)		
	Concentration	**Crown rot**	**Anthracnose**	**Apex rot**
MIRAGE 450 EC	C"	60 ± 28.88^{b}	40 ± 12.89^{b}	66.66 ± 2.39^{b}
CARTE 10 %	C'	63 ± 32.62^{b}	36.66 ± 11.33^{b}	66.66 ± 2.39^{b}
Untreated control	C0	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
	Average	74.33	58.88	37.77
	Coefficient of variation (%)	27.57	13.7	4.2
	Probability	< 0.0001	<0.0001	<0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman-Keuls test. C0 = 0%; C'= 0.15% ; C" = 0.5%.

Table 7. Crown rot severity index as a function of bio-fungicides concentrations

Fungicides	**Severity index (%)**				
	C1	**C2**	**C3**	**C4**	**C5**
ASTOUN 50 EC	5.6 ± 1.22^{b}	3.6 ± 1.2^{bc}	3.16 ± 1.21^{bc}	3.16 ± 1.21^{b}	2.66 ± 1.17^{b}
NECO 50 EC	4.3± 1.23^{b}	3.6 ± 1.21^{bc}	3. 3 ± 1.2^{b}	3 ± 1.17^{b}	2.66± 1.17^{b}
PRORALY 50 EC	4.83 ± 1.22^{b}	3.33 ± 1.21^{bc}	3.16 ± 1.21^{bc}	3 ± 1.19^{b}	2.66 ± 1.18^{b}
FERCA 50 EC	6.96 ± 2.4^{a}	4 ± 1.3^{b}	3.3 ±1.21^{b}	3 ± 1.19^{b}	2.83 ±1.19^{b}
MIRAGE 450 EC	2.6± 1.17^{c}	2.6± 1.17^{c}	2.6 ± 1.17^{c}	2.6 ± 1.17^{c}	2.6 ± 1.17^{b}
CARTE 10%	2.33 ± 1.02^{c}	2.33 ± 1.02^{c}	2.33 ± 1.02^{c}	2.33 ± 1.02^{c}	2.33 ±1.02^{c}
Untreated control	7 ± 2.4^{a}	7 ± 2.4^{a}	7 ± 2.4^{a}	7 ± 2.4^{a}	7 ± 2.4^{a}
Average	4.8	3.78	3.59	3.44	3.24
Coefficient of variation (%)	31.66	35.71	37.32	38.66	40.74
Probability	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman Keul test; C1 = 0.1%; C2 = 0.15%; C3 = 0.2%; C4 = 0.3%; C5 = 0.4%.

Table 8. Anthracnose severity index as a function of Bio-fungicide concentrations

Fungicides	**Severity index (%)**				
	C1	**C2**	**C3**	**C4**	**C5**
ASTOUN 50 EC	9.16 ± 2.1^{b}	7.16 ± 1.6^{c}	7.33 ±1.4^{b}	5.66 ± 1.3^{cd}	4.33 ± 1.2^{d}
NECO 50 EC	9 ± 1.8^{b}	7 ± 1.4^{c}	6.66 ± 1.5^{c}	4.83 ± 1.3^{d}	4.16 ± 1.1^{d}
PRORALY 50 EC	8.5 ± 1.8^{bc}	8 ± 1.4^{bc}	7.33 ± 1.4^{b}	6 ± 1.3^{c}	5 ± 1.1^{c}
FERCA 50 EC	10.83 ± 2.1^{b}	9.16 ± 1.5^{b}	7.33 ± 1.4^{b}	6 ± 1.3^{c}	5.66 ± 1.2^{c}
MIRAGE 450 EC	7.33 ± 1.2^{c}	7.33 ± 1.2^{c}	7.33 ± 1.2^{b}	7.33 ± 1.2^{b}	7.33 ± 1.2^{b}
CARTE 10 %	7.66 ± 1.1^{c}	7.66 ± 1.1^{c}	7.66 ± 1.1^{b}	7.66 ± 1.1^{b}	7.66 ± 1.1^{b}
Untreated control	12.33 ± 2.4^{a}	12.33 ± 2.4^{a}	12.33 ± 2.4^{a}	12.33 ± 2.4^{a}	12.33 ± 2.4^{a}
Average	9.52	8.49	7.99	7.11	6.63
Coefficient of variation (%)	17.64	18.61	18.52	18	19;90
Probability	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean values followed by the same letter in are not significantly different at the 5% threshold with the Student-Newman Keul test; C1 = 0.1%; C2 = 0.15%; C3 = 0.2%; C4 = 0.3%; C5 = 0.4%.

Table 9. Apex rot severity index as a function of Bio-fungicides concentrations

Fungicides	**Severity index (%)**				
	C1	**C2**	**C3**	**C4**	**C5**
ASTOUN 50 EC	1.6 ± 0.24^{b}	1 ± 0.021^{ab}	0.3 ± 0.007^{c}	0 ± 0.00^{d}	0 ± 0.00^{d}
NECO 50 EC	1.33 ± 0.54^{b}	0.33 ± 0.007^{c}	0.15 ± 0.003^{c}	0 ± 0.00^{d}	0 ± 0.00^{d}
PRORALY 50 EC	1.66 ± 0.024^{b}	1.44 ± 0.004^{b}	0.33 ± 0.007^{c}	0 ± 0.00^{d}	0 ± 0.00^{d}
FERCA 50 EC	1.56 ± 0.46^{b}	1.41 ± 0.4^{b}	0.66 ± 0.009^{c}	0 ± 0.00^{d}	0 ± 0.00^{d}
MIRAGE 450 EC	0.86 ± 0.027^{c}	0.86 ± 0.027^{bc}	0.86 ± 0.027^{bc}	0.86 ± 0.027^{c}	0.86 ± 0.027^{c}
CARTE 10 %	1.41 ± 0.04^{b}	1.41 ± 0.04^{b}	1.41 ± 0.04^{b}	1.41 ± 0.04^{b}	1.41 ± 0.04^{b}
Untreated control	2.56 ± 1.08^{a}	2.56 ± 1.08^{a}	2.56 ± 1.08^{a}	2.56 ± 1.08^{a}	2.56 ± 1.08^{a}
Average	1.6	1.28	0.89	0.69	0.69
Coefficient of variation (%)	21.25	17.18	17.97	23.18	23.18
Probability	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman Keul test; C1: 0.1%; C2:0.1%; C3:0.2%; C4:0.3%; C5: 0.4%.

Weight loss of bananas after fungicide application: The average rate of weight loss varied with the treatment and concentration. This variation ranged from 14.94% to 20.17%. The lowest rate (14.94%) was recorded with ASTOUN 50 EC at concentration C1. On the other hand, the highest rate (20.17%) was observed with the bio fungicide NECO 50 EC at concentration C5 (Table 10).

Table 10. Average rate of weight loss as a function of fungicide concentration

Treatments	Concentrations	Mass loss (%)
ASTOUN 50 EC	C1	14.94 ± 0.34^{c}
	C2	17.1 ± 0.42^{bc}
	C3	18.38 ± 0.37^{b}
	C4	18.39 ± 0.36^{b}
	C5	18.47 ± 0.51^{b}
NECO E0 EC	C1	18.11 ± 0.42^{b}
	C2	18.77 ± 0.37^{b}
	C3	18.8 ± 0.46^{b}
	C4	18.17 ± 0.44^{b}
	C5	20.17 ± 0.39^{a}
PRORALY 50 EC	C1	17.88 ± 0.29^{bc}
	C2	18.27 ± 0.33^{bc}
	C3	17.98 ± 0.40^{bc}
	C4	17.96 ± 0.43^{bc}
	C5	18.71 ± 0.39^{b}
FERCA 50 EC	C1	18.19 ± 0.54^{bc}
	C2	18.52 ± 0.45^{b}
	C3	18.94 ± 0.28^{b}
	C4	18.9 ± 0.29^{b}
	C5	19.83 ± 0.37^{a}
MIRAGE 450 EC	C0	18.82 ± 0.46^{b}
CARTE 10 %	C'	18.49 ± 0.34^{b}
Untreated control	C''	17.81 ± 0.38^{bc}
	Average	18.33
	Coefficient of variation (%)	2.12
	Probability	< 0.0001

Mean values followed by the same letter in the same column are not significantly different at the 5% threshold with the Student-Newman-Keuls test. C1= 0.1% ; C2 = 0.15% ; C3 = 0.2% ; C4 = 0.3% ; C5 = 0.4% ; C0 = 0.0% ; C'= 0.15% ; C'' = 0.5%.

DISCUSSION

In this study, a variety of fungi was isolated from bananas showing disease symptoms. These results indicate that export-ready dessert bananas are subject to latent infections and therefore face enormous parasitic constraints. Our findings are in concurrence with **(**Alvindia, 2013**)** who also isolated , many fungal genera from organic banana have been isolated in the Philippines . Similarly, several morphotypes of *Fusarium* sp., *Botryodiplodia* sp., and *Colletotrichum* sp. pathogens implicated in post-harvest banana diseases have been reported **(**Zakaria, 2023**)**. The symptoms observed in this study were characterized by crown and distal tip rot and epicarp necrosis. This diversity of symptoms could be attributed to the morphology of the banana, which comprises three different parts (crown, epicarp and distal tip). Each of these parts is susceptible to infection by one or more pathogens. Similar observations have shown that bananas can be infected on both the epicarp and crown (Triest and Marijkx, 2016; Ewané *et al*. 2013). In fact, infection of the crown could be due to incisions made in this area during separation, which involves detaching banana hands from the flower stalk using secateurs. This is a likely entry point for pathogens. Studies have also shown that crown rot is the most important symptom of post-harvest banana disease **(**Baria *et al*. 2021**)**. Other reports have also confirmed that crown rot is the most important symptom in bananas intended for export (Khan *et al*. 2014**)**.

In vitro evaluation of the antifungal activity of the Bio-fungicides NECO 50 EC, FERCA 50 EC, ASTOUN 50 EC, and PRORALY 50 EC and the positive control fungicides MIRAGE 450 EC and CARTE 10% (synthetic and organic products, respectively) on three fungal isolates (*Colletotrichum* sp., *Fusarium* sp. and *Botryodiplodia* sp.) showed variable efficacy between the different fungicides. The inhibition of mycelial growth is

linked to the nature of the product and the concentration applied. The best rates of inhibition of mycelial growth of these fungi recorded with the Bio-fungicides NECO 50 EC, ASTOUN 50 EC, and PRORALY 50 EC at a dose of 1000 ppm, suggest that these Bio-fungicides have good antifungal properties. The Bio-fungicide NECO was more effective against *Colletotrichum* sp. and *Botryodiplodia* sp., with inhibition rates of 92.29% and 93.46%, respectively.

The Bio-fungicide ASTOUN 50 EC is effective against Fusarium sp. Both Bio-fungicides (NECO 50 EC and ASTOUN 50 EC) act on the essential functions of fungi, inhibiting their metabolism. These Bio-fungicides inhibit mycelium proliferation, conidial germination, and toxin production. Previous studies demonstrated the efficacy of these Bio-fungicides in vitro through their ability to strongly inhibit the radial growth of *Colletotrichum gloeosporioides* at 1000 ppm (N'Goran *et al*. 2023). In addition, the *in vitro* antifungal efficacy of the fungicide NECO 50 EC at concentrations 1000 and 2000 ppm was evaluated three days after incubation, with a 100% inhibition rate of *Sclerotium rolfsii* growth (Bamba *et al*. 2023). The results were similar at the concentrations (1000 and 500 ppm) used in this study. On the other hand, the Bio-fungicide FERCA 50 EC proved to be the least repressive on the different strains during *in vitro* tests, with an average inhibition rate of 37.42%, thus presenting dissimilarity (Macías *et al*. 2023). This low activity could be due to an antagonistic effect between the different major components (Citronellal and Citronellol) involved in the formulation of the product, reflecting the low sensitivity of these fungi to this Bio-fungicide. However, contrary results showed that the use of the active ingredient of FERCA 50 EC demonstrated strong antifungal activity against *Candida albicans* (Trindade *et al*. 2022). On the basis of our results, we suggest that NECO 50 EC and ASTOUN 50 EC can be used as replacements for MIRAGE 450 EC or CARTE 10% for better post-harvest preservation of banana at a dose of 1000 ppm. The *in vivo* evaluation showed that all Bio-fungicides tested had some effects on disease progression with incidence rates ranging from 0% to 67% depending on concentrations. This study showed severity indices of 8% at C4 (0.3%) and C5 (0.4%) concentrations in all Bio-fungicides, these observed severity indices were lower than those of the controls. The efficacy of Bio-fungicides may be due to the aromatic compound composition, direct action of active ingredients on the pathogen and on the propagation organs (Šunjka *et al*. 2022). Previous studies have demonstrated that banana plants in plots treated with NECO 50 EC Bio-fungicide were less prone to infection with *Mycosphaerella fijiensis*, the causative agent of black Sigatoka, than those in control plots (Kassi *et al*. 2014). The average rate of mass loss of bananas ranged from 14.94% to 20.17%. However, the highest weight loss (20.17%) observed with bananas treated with NECO 50 EC Bio-fungicide in contrast to controls suggests that the Bio-fungicide is responsible for the rapid degradation of some banana phytocompounds.

Conclusion: Evaluation of Bio-fungicides for post-harvest control of fungal pests in dessert bananas showed that the effectiveness of Bio-fungicides depends on their composition. The bio-fungicides ASTOUN 50 EC and NECO 50 EC showed similar efficacy to MIRAGE 450 EC and CARTE 10%, which are synthetic and biological fungicides commonly used to control post-harvest fungal diseases of dessert bananas. The Bio-fungicides ASTOUN 50 EC and NECO 50 EC could therefore provide an alternative to chemical control of post-harvest diseases in dessert bananas.

Author Contributions: "Conceptualization: A.L.N.D.G.E., T.S., and G.N.B.; methodology, K.K.J.V. and K.D.; software, G.N.B., and T.S.; validation, A.L.N.D.G.E. and K.D.; formal analysis, writing original draft preparation: G.N.B, D.J.P and T.S.; writing review and editing: G.N.B, D.J.P. and T.S., visualization: A.L.N.D.G.E.; supervision: K.D."

Conflicts of Interest: "The authors declare no conflict of interest."

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