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Characterization of Fungal Diseases in Palm Oil Nurseries and Implementation of Control Methods

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Abstract: Palm oil (*Elaeis guineensis* Jacquin) occupies an important place in the Ivorian economy. However, its cultivation is subject to numerous attacks by pests and fungal pathogens. The aim of the present work was to evaluate *in vivo* and *in vitro* the antifungal effect of three synthetic fungicides at different concentrations on the evolution of curvulariosis in palm oil nurseries in Côte d'Ivoire. To this end, a trial was carried out in the Boubo palm oil nursery to evaluate *in vivo* the effect of synthetic fungicides on the severity and incidence of curvulariosis. Samples of leaves showing characteristic symptoms of the disease were then taken from the palm oil nursery and sent to the laboratory for analysis. Two fungi were isolated from these symptoms. These were: *Fusarium* sp., and *Curvularia* sp. Among these fungi, *Curvularia* sp. was the most isolated, with an isolation frequency of 94.28%. The *in vitro* effect of the three synthetic fungicides on mycelial growth of the *Curvularia* sp. fungus showed that only the synthetic fungicide Flash (Benomyl) was effective, with a higher inhibition rate of 70.77% on mycelial growth of the pathogen at the 50 ppm concentration. The same was true for disease development in the nursery. These results indicate that Flash fungicide is best suited to the control of curvulariosis in palm oil nurseries.

Keywords: Côte d'Ivoire, Curvulariosis, Palm Oil, Synthetic Fungicides

1. Introduction

The palm oil (*Elaeis guineensis* Jacquin) is a monocotyledonous plant in the Palmaceae family. Of African origin, palm oil is the most widely used vegetable oil in the world. It is the world's leading vegetable oil, ahead of soy [1].

Introduced to Côte d'Ivoire as part of the cash crop diversification policy initiated since independence, the country currently boasts nearly 250,000 hectares of palm groves [2]. Annual production is estimated at 600,000 tonnes of palm oil. Côte d'Ivoire is West Africa's leading palm oil producer, and second in Africa behind Nigeria [2].

In Côte d'Ivoire, the palm oil industry directly or indirectly supports almost 2,000,000 people, or 10% of the total population [3]. The country exports almost half of its production to other African countries to guarantee its economic, social and environmental stability [3]. To achieve this position and guarantee the sustainability of the sector, the country has adopted agricultural policies to increase yields for small-scale growers in response to growing demand, and to improve the services offered by cooperatives, which are under pressure from increasing competition from intermediaries [3]. However, despite constant efforts on the part of the Ministry of Agriculture and professional agricultural organizations, it has to be said that the Ivorian palm oil sector is subject to numerous biotic constraints. Indeed, Ivorian palm oil is

attacked by pests but, also by fungal pathogens [4]. Phytopathogenic agents include *Curvularia* sp., the causal agent of palm oil curvulariosis [5]. According to this author, under favorable ecological conditions, this disease causes leaf necrosis in palm oil nurseries, leading to premature leaf senescence in young seedlings. The PALMCI Boubo palm oil agro-industrial unit is subject to severe attacks by this disease. In fact, a mortality rate of almost 14% was recorded during the nine months of nursery work in 2022. On the other hand, the preventive control methods adopted up to now have mainly concerned the choice of planting material and synthetic chemical products.

In the current context of agriculture, i.e. respect for the environment and reducing the impact of human activity on the planet and its biodiversity, it is important to develop sustainable control methods. In view of these objectives, the use of chemical molecules (fungicides, pesticides) and the implementation of cultivation practices with a high environmental impact must be reduced in palm oil plantations. Better knowledge of the ecosystem in which the crop is grown, the biology of the plant and its pathogens should enable the development of more reasoned and environmentally-friendly methods [6]. In view of the scale of mortality caused by this disease in palm oil nurseries, effective control strategies need to be proposed to ensure the sustainable survival of palm oil seedlings. The general objective of this work is to evaluate *in vitro* and *in vivo* the effect of three synthetic fungicides at different concentrations on the evolution of curvulariosis in palm oil nurseries in Côte d'Ivoire. More specifically, the aim was to:

1. Isolate and characterize the fungi responsible for the symptoms observed;
2. Evaluate *in vitro* the effect of synthetic fungicides on the mycelial growth of *Curvularia* sp.;
3. Evaluate *in vivo* the effect of synthetic fungicides on the development of curvulariosis symptoms.

2. Material and Methods

2.1. Study Site

This study was carried out in 2022 during the months of April to July in the department of Divo (Côte d'Ivoire), capital of the Lôh Djiboua region, specifically in the small locality of the Integrated Agricultural Unit of the PALMCI company (Boubo). The department is characterized by a tropical climate according to the Köppen-Geiger classification [7]. Average annual rainfall is 1,200 mm, with a wet season from march to november and a dry season from december to february [8]. The average annual temperature ranges from 27 to 28°C.

Isolation of fungal strains and *in vitro* testing were carried out in the phytopathology laboratory of the Plant Physiology and Pathology Teaching and Research Unit (PPP-TRU) at the University of Félix HOUPHOUËT-BOIGNY, Cocody, Côte d'Ivoire.

2.2. Material

2.2.1. Plant Material

The plant material consisted of leaves from young palm oil seedlings showing various symptoms characteristic of curvulariosis from the site (Figure 1). The C1001F variety was selected on the basis of its tolerance to fusariosis, production stability, low growth rate in height, uniformity, drought tolerance and average yield per hectare. It was acquired from the National Agricultural Research Center (CNRA) of the Mé in Côte d'Ivoire. Eight-month-old young palm seedlings were used for this study. The number of leaves on these plants varied between eight and twelve.

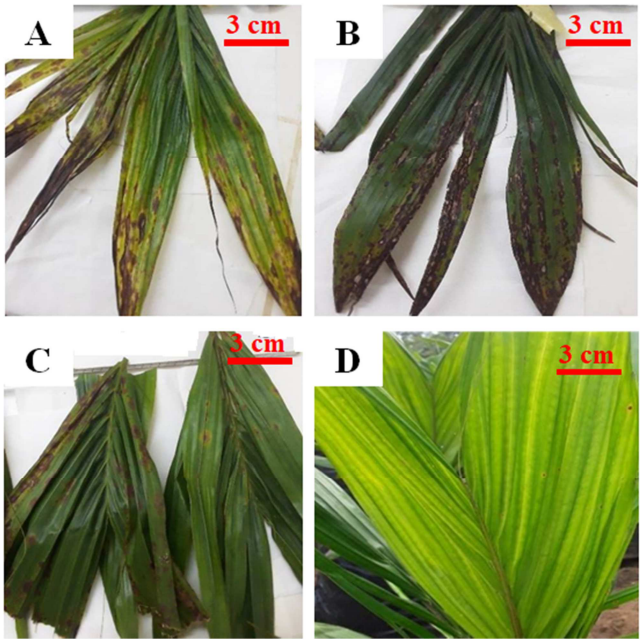


Figure 1. *Leaf samples from young palm oil seedlings.*

A, B, C: Leaf samples showing characteristic symptoms of curvulariosis
D: Samples of apparently healthy leaves

2.2.2. Chemical Materials

The chemical material used for control in this study was essentially composed of three synthetic fungicides. These were the synthetic fungicides Confirm, Banko Plus and Flash, whose physico-chemical characteristics are given in Table 1. These preventive and curative fungicides were supplied by PALMCI.

Table 1. *Characteristics of synthetic fungicides used for pest control.*

Trading name	Active ingredients	Concentrations	Types of formulation	Mode of action
Banko Plus 650 SC	Chlorothalonil + Carbendazine	550 g/l + 100 g/l	Concentrated suspension	Contact
Confirm 250 SC	Azoxystrobin	250 g/l	Concentrated suspension	Systemic
Flash 500 WP	Benomyl	500 g/kg WP	Wettable powder	Systemic

2.3. Methods

2.3.1. Characterization of Pathogens Responsible for Observed Symptoms

(i). Pathogen Isolation and Purification

The culture medium used for pathogen isolation and purification was Potato Dextrose Agar (PDA). Pathogens associated with the symptoms observed on the leaves of young palms oil were isolated using the modified method of [9]. Explants were extracted from leaf samples at the symptom growth front. Explants were successively disinfected with a solution of 70% ethanol for 1 minute and 3% sodium hypochlorite for 3 minutes, followed by three successive rinses with sterile distilled water. Using sterile forceps, in a fume hood and close to the flame, explants were seeded onto PDA culture medium in 9 cm diameter Petri dishes, at a rate of 4 explants per dish. The plates were sealed with stretch film and incubated at room temperature (25 ± 2°C). After three days' incubation, the various pathogen colonies visible around the fragments were picked and transferred to new Petri dishes containing PDA culture medium. This process was repeated several times until pure strains were obtained [10].

(ii). Identification of Isolates

a. Macroscopic Identification of Isolates

Fungal strains were identified using the Botton *et al.* [11] identification key. Cultural characteristics were described in terms of coloration, appearance and growth pattern of mycelial colonies of fungal strains.

b. Microscopic Identification of Isolates

Fungal strains were identified using the Botton *et al.* [11] identification microscopic description was carried out on 15-day-old strains. Colony explants were mounted between slide and coverslip and observed under the microscope. Organs such as mycelium and spores were described [11].

(iii). Microscopic Identification of Isolates

After isolate identification, the isolation rate of each fungal pathogen isolated was determined using the following formula:

$$\text{Isolation rate } (\%) = \frac{\text{Number of strains of afungus}}{\text{Total number of strain isolated}} x\ 100$$

2.3.2. In vitro Evaluation of the Antifungal Activity of Synthetic Fungicides on the Growth of Curvularia **sp.** *Mycelium*

(i). Dosing and Strain Inoculation

Of the three synthetic fungicides used, two - Banko Plus and Confirm - were in liquid form, while Flash fungicide was in powder form. For each fungicide, a 100 ml stock solution at 1000 ppm was prepared by solubilizing in sterile distilled water. Potato Dextrose Agar (PDA) culture media were autoclaved at 121°C, under a pressure of 1 bar, for 30 minutes. After the media had been supercooled at 45°C, fungicides were added to the PDA media to obtain concentrations of 0.1, 1, 10, 20 and 50 ppm. The fungicide-supplemented PDA media were homogenized and dispensed into 9 cm-diameter Petri dishes at a rate of 20 ml per dish. Mycelial disks 6 mm in diameter were cut from 15-day-old strains and placed in the center of Petri dishes containing the PDA-fungicide mixture. Five Petri dishes were used for each concentration and fungicide, and a further five Petri dishes were used as fungicide-free controls. Cultures were incubated at room temperature (25 ± 2°C), with a 12-hour photoperiod.

(ii). Measurement of Radial Mycelial Growth

Radial mycelial growth of fungal colonies was observed every 24 hours. The average diameter of colonies was measured until the surface of the culture medium in the control Petri dish was completely covered by the fungus. Radial mycelial growth was measured along two perpendicular lines drawn on the underside of each Petri dish, intersecting at a point in the middle of the mycelial disc. These measurements were taken over 13 days. Fungicide efficacy was assessed on the basis of the rate of inhibition (Ic) of mycelial growth. For each concentration, the average inhibition rate of mycelial growth was calculated using the following formula from Hmouni *et al.* [12]:

$$\text{Ir } (\%) = \frac{\text{D0}-\text{Dc}}{\text{D0}} \text{x } 100$$

Ir (%): Inhibition rate as a percentage of mycelial diameter;

D0: Average mycelial diameter (cm) of control colonies;

Dc: Average mycelial diameter (cm) of colonies treated with concentration (c) of synthetic fungicides.

(iii). Inhibitory Concentrations at 50 and 90% (IC_{50} and IC_{90})

CI_{50} and CI_{90} inhibitory concentrations were determined using ed50v10 software. These concentrations are those of synthetic fungicides that reduced fungal growth by 50% and 90% respectively [11].

2.3.3. In vivo Evaluation of Fungicide Efficacy

(i). Experimental Set-up

The experimental set-up used in this study was a Fisher complete randomized block with ten (10) treatments and three (3) replicates (Figure 2). The treatments were as follows: untreated control plants, plants treated with the synthetic fungicides Confirm 250 SC, Banko Plus 650 SC and Flash 500 WP at three different concentrations (Table 2). Treatments were distinguished from each other as follows: the individual plots in each block were marked with adhesive strips of different numbers. The blocks were laid out parallel and 2 m apart (Figures 2 and 3). The elementary plots each consisted of 20 palm oil plants spaced 0.6 m by 0.5 m (density of 600 plants). Each block comprised 10 elementary plots separated by 1.6 m and consisting of 4 rows 2.4 m long. Each elementary plot was 1.5 m wide, giving a total surface area of 530 m^2.

(ii). Trial Monitoring and Parameters Measured

Assessments were made every 14 days for 6 weeks, and involved 12 of the 20 plants in each elementary plot. During this study, three doses of each fungicide were used. These were half, double and the dose registered by the phytosanitary firm (Table 2). The ability of fungicides to protect plants or reduce pathogen infections was evaluated. This was done through the incidence and severity index of the disease.

Table 2. *Doses of synthetic fungicides used for applications.*

Trading name	Active ingredient	Registered dose (ppm)	Half the registered dose (ppm)	Double the registered dose (ppm)
Banko Plus 650 SC	Chlorothalonil 550 g/L + Carbendazine 100 g/L	5330	2660	10660
Confirm 250 SC	Azoxystrobin 250 g/l	2660	1330	5330
Flash 500 WP	Benomyl 500 g/kg WP	2660	1330	5330

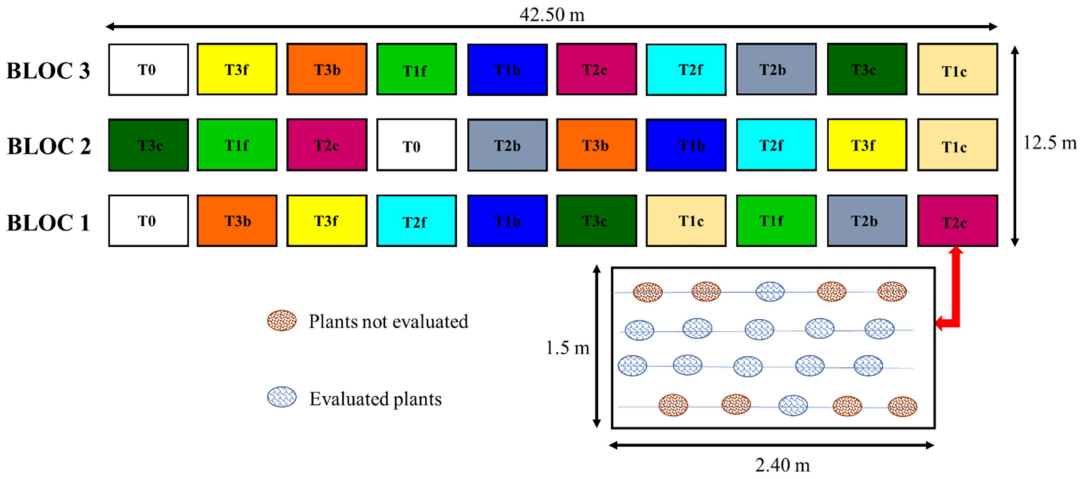


Figure 2. *Schematic of experimental set-up.*

- T0: Untreated control
- T1b: Treatment with Banko Plus 2660 ppm
- T1c: Treatment with Confirm 1330 ppm
- T1f: Treatment with Flash 1330 ppm
- T2b: Treatment with Banko Plus 5330 ppm
- T2c: Treatment with Confirm 2660 ppm
- T2f: Treatment with Flash 2660 ppm
- T3b: Treatment with Banko Plus 10660 ppm
- T3c: Treatment with Confirm 5330 ppm
- T3f: Treatment with Flash 5330 ppm

Figure 3. *The experimental oil palm nursery plot at the PALMCI Boubo site.*

a. Assessment of Disease Incidence on Palm Oil Leaves

The rate of disease infection was assessed through the ratio between the number of leaves showing disease symptoms and the total number of leaves treated. It was calculated using the following formula [14]:

$$\text{Infection rate (\%)} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \text{x100}$$

b. Assessment of Disease Severity on Palm Oil Seedlings

Disease severity on treated plants was assessed using the modified 0-6 rating scale [15] shown in Table 3.

The *Curvularia* sp. severity index was determined for each plant using the following equation established by [16].

$$\text{SI (\%)} = \frac{\Sigma \text{ (Xi x ni)}}{\text{Z x N}} \text{ x 100}$$

SI: Severity index,
Xi: infection score or index,
ni: number of times the Xi note is reached,
N: total number of plants observed per plot,
Z: highest score on the scale.

Table 3. *Modified curvulariosis severity scale [15].*

Notes	Percentages	Characteristics
0	0	no symptoms
1	1%	of the leaf blade with symptoms,
2	1 to 5%	of the leaf blade with symptoms,
3	6 to 15%	of the leaf blade with symptoms,
4	16 to 33%	of the leaf blade with symptoms,
5	34 to 50%	of the leaf blade with symptoms,
6	51 to 100%	of the leaf blade with symptoms.

2.4. Statistical Analysis

Statistica version 7.1 was used to process the data obtained, involving analysis of variance (ANOVA I and II) between the different means. When a significant difference was observed, the Newman-Keuls statistical test, at the 5% threshold, was used to separate the means.

3. Results

3.1. Characteristics of Isolated Pathogens

3.1.1. Identification of the Different Fungal Genera Isolated

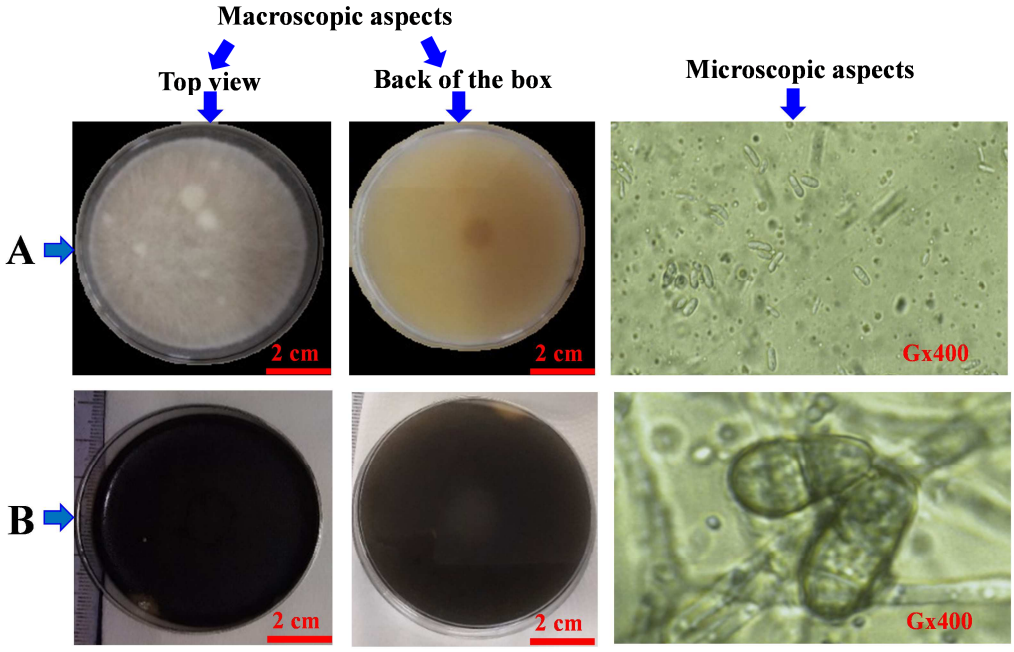
From the characteristic symptoms of curvulariosis observed on the leaves of young palm oil seedlings, 52 isolates were purified. Analysis of the macroscopic and microscopic examination of the purified colonies led to the identification of two fungal genera, *Fusarium* and *Curvularia* (Figure 4). The *Curvularia* fungal genus yielded an isolation rate of 94.28%, compared with 5.71% for the *Fusarium* genus.

3.1.2. Description of Fusarium Isolates

Pure 15-day-old isolates of the *Fusarium* sp. strain showed an abundant aerial thallus, initially white to pink in color, with a yellow underside (Figure 4). The spores varied in size, some oval with curved ends, others rounded (Figure 4).

3.1.3. Description of Curvularia Isolates

In Petri dishes, 15-day-old isolates of the *Curvularia* strain showed a short, black thallus on both sides (Figure 4). Mycelial growth was initially concentrated in the middle of the dish, and then spread to the tip. Microscopic observations of the isolate revealed a class of spores with different conidial sizes and number of septa. Conidia are solitary, simple, curved, fusiform, ovoid, with 3 transverse septa, pale to dark brown, smooth (Figure 4).



A: *Fusarium* sp.; B: *Curvularia* sp.

Figure 4. *Macroscopic and microscopic aspects of the two fungal genera isolated from palm oil leaves.*

3.2. Effect of Fungicides on Mycelial Growth of Curvularia sp.

Figures 5 to 9 show the daily evolution of the *in vitro* inhibition rate of *Curvularia* sp. mycelial growth as a function of different concentrations of synthetic fungicides.

3.2.1. Effect of Synthetic Fungicides at a Concentration of 0.1 ppm

Figure 5 shows that at a concentration of 0.1 ppm, no product totally inhibited mycelial growth of the *Curvularia* sp. strain. However, the synthetic fungicides Confirm and Flash had considerable inhibition rates between day 1 and day 6, with 31.03 and 44.25% respectively. As for the synthetic fungicide Banko Plus, the rate of inhibition of mycelial growth was low over the thirteen days of evaluation, ranging from 13.21 to 1.35%.

3.2.2. Effect of Synthetic Fungicides at 1 ppm Concentration

Figure 6 shows the effect of the synthetic fungicides Banko Plus, Confirm and Flash on mycelial growth of *Curvularia* sp. at a concentration of 1 ppm. From day one to day four, the fungicides Flash and Confirm inhibited mycelial growth the most, ranging from 47.12 to 19.64 and 59.60 to 36.63% respectively. From day four onwards, the rates of inhibition of fungal mycelial growth by the synthetic fungicides Banko Plus and Confirm remained similar until the last day of the experiment. The best product in this concentration was the synthetic fungicide Flash, which progressively inhibited strain growth until day eleven (Figure 6).

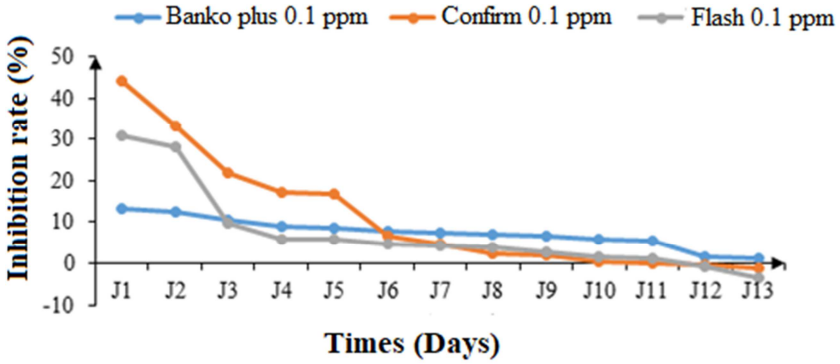


Figure 5. *Daily variation in the rate of inhibition of mycelial growth of Curvularia* sp. *at a concentration of 0.1 ppm.*

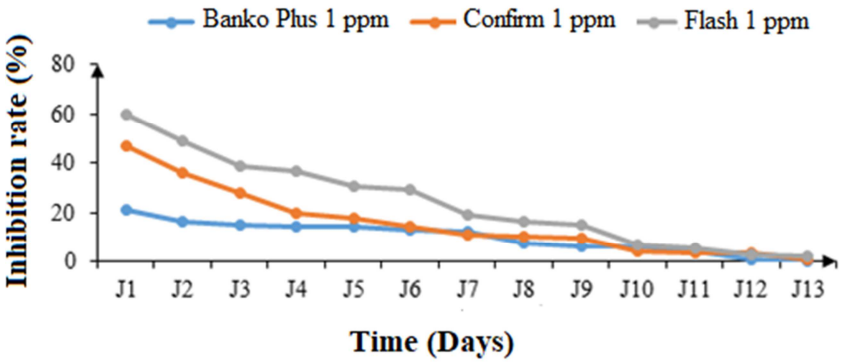


Figure 6. *Daily variation in the rate of inhibition of mycelial growth of Curvularia* sp. *at 1 ppm.*

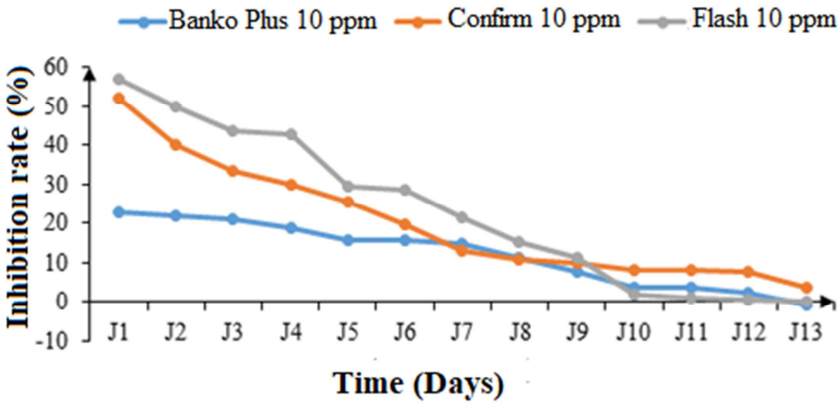


Figure 7. *Daily variation in the rate of inhibition of mycelial growth of Curvularia* sp. *at 10 ppm.*

3.2.3. Effect of Synthetic Fungicides at 10 ppm Concentration

Figure 7 shows the daily evolution of the rate of inhibition of mycelial growth of *Curvularia* sp. at a concentration of 10 ppm. The synthetic fungicide Flash, with inhibition rates ranging from 57.02 to 11.09%, was the most effective during the first 9 days. The synthetic fungicides Confirm and Banko Plus showed respective inhibition rates ranging from 52.29 to 3.88 and 22.77 to -0.85%. These rates recorded during the evaluation of *Curvularia* sp. mycelial growth show that none of the three products totally reduced the growth of the *Curvularia* sp. strain at the 10 ppm.

3.2.4. Effect of Synthetic Fungicides at 20 ppm Concentration

Observation of the results in Figure 8 revealed that at the 20 ppm concentration, the synthetic fungicide Flash had the highest inhibition rate during the first 6 days. These rates ranged from 57.62 to 29.82%. The synthetic fungicides Banko Plus and Confirm had the lowest inhibition rates, 52.87-6.28 and 52.87-2.43% respectively. No fungicide inhibited pathogen mycelial growth by more than 50% at this concentration at the end of the evaluation.

3.2.5. Effect of Synthetic Fungicides at 50 ppm Concentration

The results of the inhibition rate of mycelial growth of *Curvularia* sp. strain as a function of products are shown in figure 9. Their analysis showed that at the 50 ppm concentration, the synthetic fungicide Flash significantly reduced mycelial growth of the strain over the thirteen days of evaluation, ranging from 82.73 to 49.42%. The synthetic fungicides Banko Plus and Confirm had a similar effect on strain growth at this dose throughout the evaluation. These two fungicides had inhibition rates ranging from 53.44 to 6.79% for Banko Plus and from 54.59 to 6.01% for Confirm. Unlike these two fungicides, which saw their inhibition rate of mycelial growth drop to less than 10% on the last day, Flash fungicide maintained its inhibition rate on the last two days of the experiment at 49.42%.

3.2.6. Effect of the Three Synthetic Fungicides on Mycelial Growth of Curvularia sp.

Figure 10 shows the rate of inhibition of *in vitro* mycelial growth of *Curvularia* sp. as a function of concentration. This figure shows that the best product for this experiment was the synthetic fungicide Flash at 50 ppm concentration, with a rate of 70.77%. The synthetic fungicide Banko Plus, with an inhibition rate of 7.46%, was the least effective on the mycelial growth of *Curvularia* sp. As for the synthetic fungicide Flash, its inhibition rate evolved over the course of the experiment, with concentrations of 7.49; 23.29; 23.94; 27.95; 70.77% at 0.1; 1; 10; 20 and 50 ppm respectively.

3.2.7. Inhibitory Concentration CI_{50} and CI_{90} of the Three Synthesis Products

Table 4 shows the inhibitory concentrations reducing *Curvularia* sp. mycelial growth by 50% (IC_{50}) and 90% (IC_{90}) according to the products. These results show that Flash fungicide had the lowest inhibitory concentrations (IC_{50} = 33.66 ppm and IC_{90} = 69.73 ppm). In fact, the synthetic fungicide Flash was more fungitoxic on the mycelial growth of *Curvularia* sp. isolates. The effect of the other two fungicides on the mycelial growth of the fungus was weak, as they had high IC_{50} inhibitory concentrations (167.01 ppm for Confirm and 107.53 ppm for Banko Plus) compared with the fungicide Flash.

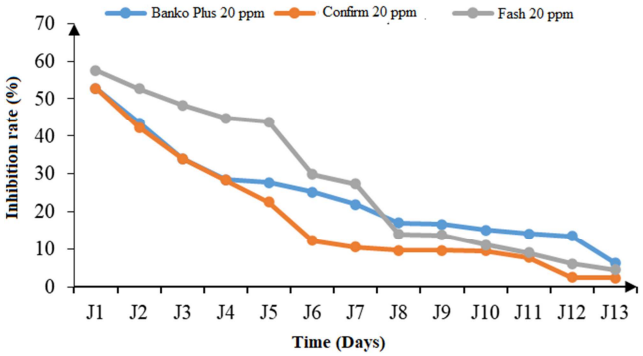


Figure 8. *Daily variation in the rate of inhibition of mycelial growth of Curvularia* sp. *as a function of 20 ppm concentration.*

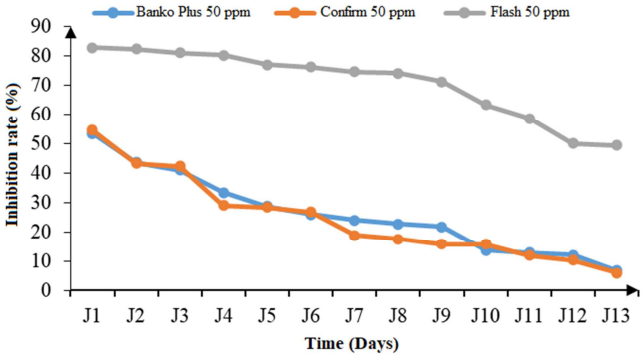


Figure 9. *Daily variation in the rate of inhibition of mycelial growth of Curvularia* sp. *as a function of 50 ppm concentration.*

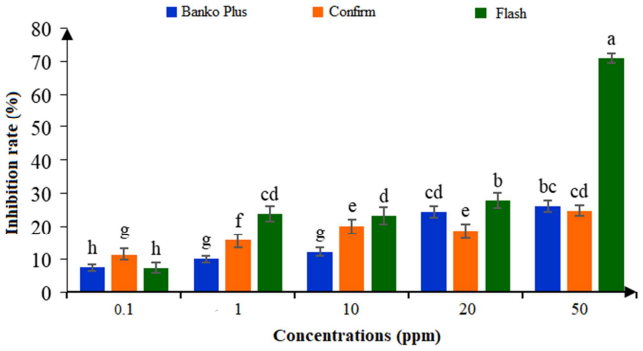


Figure 10. *Inhibition rate of in vitro mycelial growth of Curvularia* sp. *as a function of concentration thirteen days after cultivation.*

Bars topped by the same letters are statistically identical according to the Newman-Keuls test at Threshold α = 0.05.

Table 4. *IC_{50} and IC_{90} inhibitory concentrations of three synthetic fungicides on mycelial growth of Curvularia* sp.

Inhibitor concentrations (ppm)	Synthetic fungicides		
	Banco Plus	**Confirm**	**Flash**
IC_{50}	107.53	167.01	33.66
IC_{90}	215.19	356.62	69.73

IC_{50}: Inhibitory concentration that inhibits mycelial growth of the fungus at 50 (%),
IC_{90}: Inhibitory concentration that inhibits mycelial growth of the fungus at 90 (%).

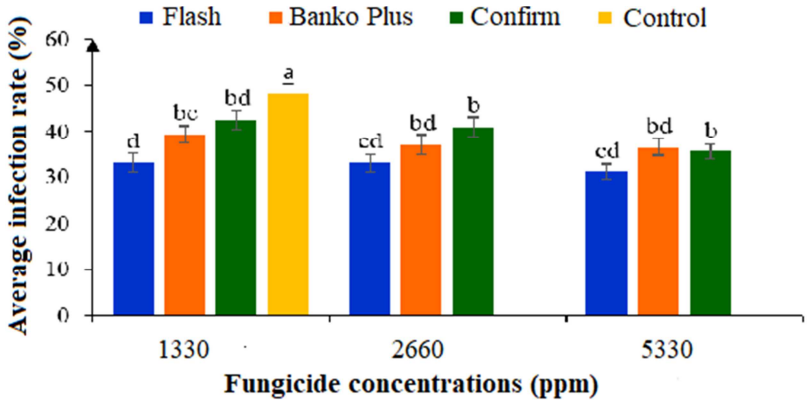
3.3. In vivo Effect of Synthetic Fungicides on the Development of Curvulariosis in Palm Oil Nurseries

3.3.1. Effect of Fungicides on the Infection Rate of Seedlings

Seedlings treated with synthetic fungicides showed variable infection rates depending on the product and dose used (Figure 11). Analysis of variance shows a significant difference at the 5% threshold between the fungicides tested. Analysis of Figure 11 shows that Flash was the most effective product in reducing disease incidence on young oil palm seedlings. In fact, Flash fungicide showed the lowest infection rates that reduced disease manifestation (33.23; 33.10 and 31.11%) at concentrations of 1330, 2660 and 5330 ppm respectively. The fungicides Banko Plus and Confirm were the least effective in reducing disease, with the highest infection rates of 42.59 and 39.40% at 1330 and 2660 ppm respectively, compared with the control.

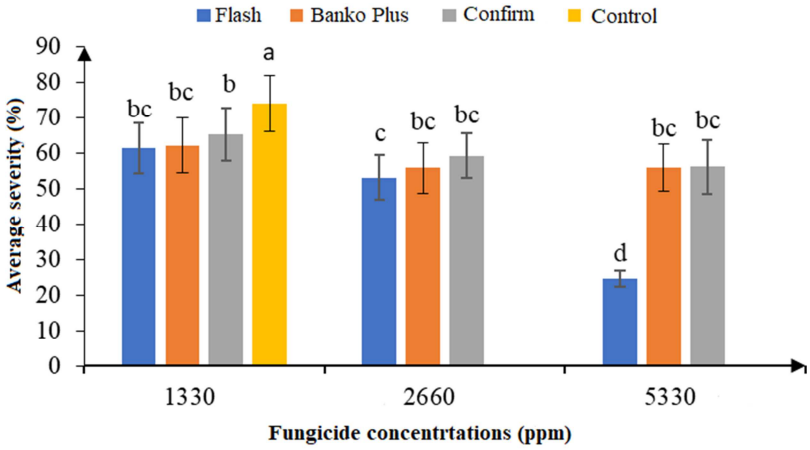
3.3.2. Effects of Fungicides on Disease Severity in Young Plants

The effect of different concentrations of the three synthetic fungicides on the severity of curvulariosis is shown in figure 12. The analysis shows that at a concentration of 5330 ppm, Flash fungicide was the best, with an average severity rate of 24.65% compared with the control (73.97%). On the other hand, the fungicides Banco plus and Confirm recorded the highest severities at 1330 ppm (62.07 and 65.15% respectively). At concentrations of 2660 and 5330 ppm, the severity rate for these two products remained almost similar throughout the evaluation.



Error bars surmounted by the same letters are statistically identical according to the Newman-Keuls test at the threshold of α = 0.05.

Figure 11. *Average infection rate of fungicide-treated palm plants as a function of concentration.*



Error bars surmounted by the same letters are statistically identical according to the Newman-Keuls test at the threshold of α = 0.05.

Figure 12. *Average severity of fungicide-treated palm plants as a function of concentration.*

4. Discussion

Two genera of fungi were isolated and identified in the course of this study from palm oil leaves showing characteristic symptoms of curvulariosis: *Fusarium* sp. and *Curvularia* sp. These were *Fusarium* sp. and *Curvularia* sp. The isolation rate for *Fusarium* sp. was 5.71% and for *Curvularia* sp. 94.28%. These rates show that the pathogen most responsible for palm oil leaf necrosis in the study area is *Curvularia* sp. [6] reported the presence of this fungus in palm oil nurseries in Southeast Asia. The presence of *Fusarium* sp. is not surprising, as several researchers such as Diabaté *et al.* [17] and Gogbé *et al.* [18] have shown its presence in palm oil plantations. Indeed, *Fusarium* sp. is a telluric fungal agent that causes fusarium disease in palm oil.

In this study, three synthetic fungicides were tested *in vitro* at five different concentrations (0.1; 1; 10; 25 and 50 ppm) against the pathogen *Curvularia* sp. All the synthetic fungicides used had a significant effect on the *in vitro* mycelial growth of *Curvularia* sp. Of these synthetic fungicides, Benomyl proved effective at a dose of 50 ppm on the *in vitro* mycelial growth of the fungus, with an inhibition rate of 70.77%. The fungitoxicity of this product's active ingredient lies in its systemic nature [19], which enables effective curative applications at the onset of infection. Furthermore, work carried out by Ella Ondo [20] and Bondoux [21] showed that Benomyl (the active ingredient in the synthetic fungicide Flash) is highly effective against all fungal contaminants in preserved apples and pears. At high doses, this fungicide inhibited *in vivo* sporulation of *Penicillium* spp. on rotted fruit at concentrations ranging from 4000 to 6000 ppm [22]. In addition, work by Rachida *et al.* [23] showed that Benomyl and methyl-thiophanate act almost in the same way on *Curvularia* spore germination. These results are in line with those of Olufolaji [24], who reported that Benomyl strongly inhibited *Curvularia cymbopogonis* spore germination. Other authors, such as El-Eraky *et al.* [25] and Sisterna and Ronco, [26] showed the effects of these fungicides on *Curvularia lunata*, *Alternaria alternata*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Aspergillus niger* and *Thielaviopsis paradoxa*. Dickinson and Wallace [27] reported that synthetic fungicides with a broad spectrum of activity were very active on different stages of *Curvularia cymbopogonis* development. However, Flash fungicide proved less active at lower concentrations on *Curvularia* sp. mycelial growth: concentrations of 0.1, 1 and 10 ppm had no significant effect on fungal growth, with relatively low inhibition rates ranging from 7.29 to 23.94%. Research by Rachida *et al*. [23] also showed the same resistance of *Alternaria tenuissima* and *Fusarium oxysporum* to this synthetic fungicide. Similar results were obtained by Mc Phee [28]. He showed that at a concentration of 100 ppm, mycelial growth and spore germination of *Alternaria alternata* were very high in the presence of this product. The ineffectiveness of benzimidazoles has been reported by numerous authors, including Burton and Dewey [29], who reported that Benomyl (Flash) at 1800 ppm failed to control rots caused by this pathogen. Similarly, work carried out in the same vein revealed that on fruit-growing stations in Morocco, *Penicillium expansum* developed positive cross-resistance to these products, whose efficacy had previously been proven [30]. This is in line with our results. Fungicides belonging to the benzimidazoles (Benomyl and Thiabendazole) act systemically and have a single site of action. They are antimitotic agents that specifically interfere with nuclear division and other processes linked to microtubule activity [31, 32]. Microtubules are major components of the cytoskeleton and the achromatic spindle, and any substance that interferes with the formation or function of these microtubules blocks cell divisions and the elongation of mycelial hyphae [33, 34]. Benzimidazole fungicides bind to the tubulin of many Ascomycota and Basidiomycota, but their interaction is weak with that of Oomycota [34].

On the other hand, Banko Plus and Confirm expressed low rates of inhibition of mycelial growth of *Curvularia* sp. at all doses. Indeed, all concentrations used *in vitro* of these synthetic fungicides did not significantly reduce mycelial growth of the fungus. Pathogen resistance to these fungicides has been reported by several authors, including Errampalli *et al.* [35] and Francès *et al.* [36] in *Penicillium expansum* and *Botrytis cinerea*. Studies by Tonon et al. [37] and Yao *et al.* [38] revealed the 100% inhibitory effect of the synthetic fungicide Banko Plus 650 SC on mycelial growth and sporulation of *Colletorichum gloeosporioides* and *Corynespora cassiicola*.

There was a significant difference between the IC_{50} and IC_{90} values of the three synthetic fungicides on the growth of *Curvularia* sp. The synthetic fungicide Flash was the most effective, with the lowest IC_{50} and IC_{90} values (33.66 and 69.73 ppm respectively). Brenneman and Murphy [39] and Decal *et al*. [40] used the mean IC_{50} of isolates of *Sclerotium rolfsii*, *Rhizoctonia solani* and *Monilia laxa* to show the difference in sensitivity of these species to the fungicides tested. In fact, the synthetic fungicide Flash was the most active on the mycelial growth of *Curvularia* sp. This inhibitory activity was recorded at concentrations as low as those used for the other fungicides tested.

The synthetic fungicides Confirm and Banko Plus are molecules commonly used in the chemical control of cryptogamic diseases, due to their fungitoxic effects on fungi. They are systemic fungicides of the benzimidazole family, acting as inhibitors of germination tube development and mycelial growth [41]. Their action is specific to the parasite and, consequently, they act on a very limited number of targets (oligosites), and are subsequently likely to give rise to resistant strains [42]. Benzimidazoles inhibit parasite mitosis by binding to a microtubule protein, preventing their proper assembly in the spindle [31].

In vivo treatment of palm oil plants with the synthetic fungicide Flash revealed that this chemical at a concentration of 5330 ppm was most effective in reducing the disease severity index, with infection rates ranging from 31.11 to 24.65%. The synthetic fungicides Banko Plus and Confirm were less effective both *in vivo* and *in vitro*. Infection rates varied according to the concentration of fungicides used. In curative treatments, synthetic fungicides were effective on

both sides in reducing the severity of the disease (Curvulariosis). Concentrations of 1330, 2660 and 5330 ppm of Banko Plus and Confirm achieved high infection rates and weakly reduced the expression of Curvulariosis. Adéyè A. T. [43] showed that regular application of the same product (Banko Plus) against onion anthracnose reduced the incidence of this disease by 60% and yield loss by 14%. Indeed, the effectiveness of a fungicide lies in its ability to inhibit fungal development at relatively low doses; and the effective dose is that at which inhibition is complete [44]. This is entirely rational, given that the massive use of synthetic fungicides would not only lead to the induction of resistant strains of fungi, but would also irreversibly cause environmental pollution with its many disastrous consequences.

5. Conclusions

In the search for a control solution for foliar fungal diseases of oil palm caused by *Curvularia* sp., this work was carried out in the nursery, in the field and in the laboratory. The results revealed the efficacy of one synthetic fungicide out of three tested. The synthetic fungicide Flash was the most effective in controlling *Curvularia* sp., inhibiting 82.73% of the fungus's mycelial growth *in vitro* at a dose of 50 ppm. The synthetic fungicides Confirm and Banko Plus were less effective on mycelial growth of the strain. *In vivo* test results showed that, at a concentration of 5330 ppm, the synthetic fungicide Flash was also the best, maintaining the disease severity rate at 24.65% compared with the control (73.97%). In addition, the activity of these synthetic products is fungistatic. To extend the range of products available to protect against curvulariosis, similar trials should be carried out with other synthetic fungicides. Biopesticides can also be tested on the pathogen to diversify the range of products for effective control of this disease.

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Conflicts of Interest

The authors declare no conflicts of interest.

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