

Evaluation of the Antifungal Efficacy of Biopesticides on the Growth of *Sclerotium rolfsii* Isolates from Vegetable Crops in Côte d'Ivoire

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Abstract *Sclerotium rolfsii* Sacc. is a polyphagous telluric fungus responsible for a large number of diseases affecting the yield of horticultural crops, particularly vegetables. Conventional methods of controlling this pest, which involve the use of synthetic fungicides, are toxic for the environment and consumer's health. The aim of this study was to evaluate in vitro the efficacy of five biopesticides (NECO 50 EC, ASTOUN 50 EC, FERCA 50 EC, BIOSAKINE 50 EC and NORDINE 50 EC) on the mycelial growth of five (5) *S. rolfsii* isolates from different localities in Côte d'Ivoire. For each biopesticide, seven (7) concentrations (100; 200; 400; 600; 800; 1000 and 2000 ppm) were tested. The experiment was repeated 3 times over time in the Laboratory of the Plant Physiology and Pathology Teaching and Research Unit of the UFR Biosciences. The effect of the biopesticides was compared with that of two reference fungicides, azoxythrobin and chlorothalonil combined with carbendazine, at concentrations of 0.1; 1; 5; 10; 25; 50 and 100 ppm. Each concentration was incorporated into Potatoes Dextrose Agar (PDA) culture medium after cooling to 45°C. The efficacy of these products was assessed by measuring the mycelial growth of the various isolates on the reverse side of the Petri dishes on two perpendicular axes every day, over a period corresponding to the complete growth of the unamended controls. The results of the study show that the fungicides applied have different levels of toxicity on *S. rolfsii* isolates. The biopesticides NECO 50 EC and ASTOUN 50 EC effectively inhibited mycelial growth in 60% of isolates at the 800 ppm concentration. Mycelial growth of all isolates (100%) was effectively inhibited at concentrations of 1000 and 2000 ppm three days after incubation. The use of these biopesticides would be an alternative to the use of synthetic fungicides.

Keywords: biological control, *Sclerotium rolfsii*, collar rot, market-garden, biopesticides

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1. Introduction

Plant wilt caused by crown rot remains a major concern for vegetable growers [1]. One of the agents involved in this physiological disorder is *Sclerotium rolfsii* Sacc. [2,3,4]. This telluric fungus persists in the soil for several years thanks to sclerotia, which are the fungus's storage organs (dormant survival structures). When conditions of humidity and heat are favorable, the sclerotia germinate and produce an abundant mycelial mass on plant roots and crowns. This contact stimulates the secretion of oxalic acid and endopolygalacturonase concomitant with rapid mycelial growth, and is the key condition for establishing effective infection [2].

In addition, the humidity and heat common to the tropical zones to which Côte d'Ivoire belongs, favours the development of this highly polyphagous fungus on the main vegetables exported or consumed locally, both in the field and in storage [5]. The rotting and wilting caused by this pathogen results in significant economic losses. In 1959, the U.S. Department of Agriculture estimated losses of 10 to 20 million dollars due to *S. rolfsii* attacks in North Carolina [6]. In India, losses of up to 25% of production were reported in 1988 [7]. Stem rot caused by *S. rolfsii* is a major threat to peanuts grown under irrigated conditions [7].

In Côte d'Ivoire, this fungus is present in the perimeters of market-garden crops in different agro-ecological zones [8], with a higher incidence in agro-ecological zone I, a zone of high market-garden production. As a result, *S.*

rolfsii makes it difficult to grow solanaceous crops in the zones to which it is attached.

S. rolfsii can be controlled by various methods, including synthetic fungicides, crop rotation and soil solarization [2,9]. However, the effectiveness of these methods is hampered by the pathogen's resistance. What's more, conservation structures are spread by production tools, animals and run-off water. These conditions make the application of chemical products less effective. Faced with these difficulties, growers' resort to increasing the recommended doses, with the consequent risk of environmental pollution and mostly cancerous diseases for users and consumers [10]. Faced with these consequences, we need to find alternative solutions that are more respectful of the environment and consumer's health.

Biopesticides based on plant extracts are therefore a promising alternative to conventional control methods, as they are less polluting, as are certain biopesticides based on natural antagonists such as *Trichoderma harzianum* present in the soil.

There is also growing interest in the use of plant extract-based biopesticides as natural alternatives to synthetic fungicides. The active ingredients of these biopesticides are various volatile compounds extracted from plants [11,12]. These active ingredients possess a range of biological properties, including antifungal, antibacterial and insecticidal activity [13,14,12]. In recent years, research into the use of biopesticides to combat crop diseases has increased in Côte d'Ivoire [15,16,17].

The aim of this study is to increase knowledge of biological substances that could enable effective management of *S. rolfsii*. More specifically, we tested *in vitro* the efficacy of five plant extract-based biopesticides

on the mycelial growth of different *S. rolfsii* isolates from various agro-ecological zones in Côte d'Ivoire.

2. Materials and Methods

2.1. Materials

2.1.1. Fungal material

The fungal material used in this study consisted of 5 isolates of *Sclerotium rolfsii* from different agro-ecological zones and from various crops showing symptoms of crown necrosis (Table 1). These isolates were selected on the basis of their high incidence in the five agro-ecological zones of Côte d'Ivoire.

Table 1. Origin and years of isolation of *Sclerotium rolfsii* isolates

Codes	Year of isolation	Towns	Agroecological zones	crops
Song 73	2021	Songon	ZAE I	Tomato
Yamb 22	2021	Yamoussoukro	ZAE IV	Parsley
Sega 4	2022	Séguéla	ZAE V	Eggplant
Dalc 14	2022	Daloa	ZAE II	Eggplant
Korb 2	2021	Korhogo	ZAE VI	Groundnut

2.1.2. Chemical and biological control materials

The fungicides used in this study consist of five biopesticides (NECO 50 EC, ASTOUN 50 EC, FERCA 50 EC, NORDINE 50 EC and BIOSAKINE 50 EC) and two synthetic fungicides (Banko Plus and Callicuivre). The characteristics of these products are given in Table 2.

Table 2. Characteristics of the biopesticides evaluated

Commercial name	Active ingredients	Chemical family	Formulation type	Recommended doses
NECO 50 EC	Thymol and Eugenol	Biopesticide	50 EC	5-10 ml/L (3-6 L/ha)
ASTOUN 50 EC	Geranial and Neral	Biopesticide	50 EC	5-10 ml/L (3-6 L/ha)
FERCA 50 EC	Citronellal et citronellol	Biopesticide	50 EC	5-10 ml/L (3-6 L/ha)
BIOSAKINE 50 EC	Alpha-zingiberene and arcurcumene	Biopesticide	50 EC	5-10 ml/L (3-6 L/ha)
NORDINE 50 EC	Carvacrol and 1.8-cineole	Biopesticide	50 EC	5-10 ml/L (3-6 L/ha)
BANKO PLUS 660 SC	Chlorothalonil (550 g/l) and carbendazim (110 g/l)	Synthesis fungicide	660 SC	50-75 ml/15 L (2 L/ha)
CALLICUIVRE 50 WP	Azoxystrobin 75 g/l	Synthesis fungicide	50 WP	5 kg/ha

2.2. Methods

2.2.1. Macroscopic and Microscopic characterization of *Sclerotium rolfsii* Isolates

2.2.1.1. Preparation of Culture medium

Potato Dextrose Agar (PDA) culture medium was used to characterize isolates of *S. rolfsii* fungal strains. This medium was prepared by adding 20 g mashed potatoes, 20 g D-glucose and 20 g Agar-agar to an Erlenmeyer flask. The volume of the mixture was adjusted with distilled water to 1 L, then autoclaved at 121°C under a pressure of

1 bar for 30 min. The medium was left to cooling at 45°C, then amended with antibiotics against bacteria. The mixture was homogenized under magnetic stirring, then dispensed into 90 mm-diameter Petri dishes at a rate of 18 ml per dish, under a Steril bioban 48 laminar flow hood.

2.2.1.2. Macroscopic and Microscopic Aspects of *Sclerotium rolfsii*

For the macroscopic and microscopic study, Petri dishes containing the previously prepared and solidified PDA culture medium were seeded in the center with a 6 mm diameter mycelial disk taken from the growth front of 5-day-old *S. rolfsii* mother fungal isolates. The seeded

Petri dishes were incubated at ambient laboratory temperature ($25 \pm 2^\circ\text{C}$) with a 12 h photoperiod until the mycelial filaments of the different strains reached the periphery of the Petri dish. Subsequently, macroscopic double-sided observation of colony growth was carried out according to the method of Botton *et al.* [18]. This method consisted in determining the color and texture of the fungal isolates. After macroscopic observation, a sample of each *S. rolfsii* isolate was taken superficially on PDA medium, using the platinum loop, and placed on a glass slide to which two drops of methylene cotton blue were added. The slide carrying the sample was then gently heated by the Bunsen burner to facilitate the reaction. Finally, the slide was covered with a coverslip. Several successive microscopic observations were made at different magnifications using an Amscope optical microscope equipped with an immersion camera [18]. Thus, the identification of the *S. rolfsii* isolates used was confirmed on the basis of the presence of connection clamps as mentioned by Botton *et al.* [18]. The sclerotia produced by each *S. rolfsii* isolate were counted 28 days after incubation.

2.2.2. Antifungal Evaluation of Products *in Vitro*

The antifungal activity of five biopesticides (NECO 50 EC, ASTOUN 50 EC, FERCA 50 EC, BIOSAKINE 50 EC and NORDINE 50 EC) compared with that of two synthetic fungicides (Banko Plus and Callicuivre) was carried out on PDA culture medium. Biopesticides were tested at concentrations of 100, 200, 400, 600, 800, 1000 and 2000 ppm, while synthetic fungicides were evaluated at concentrations of 0.1; 1; 5; 10; 25; 50 and 100 ppm. Each synthetic fungicide (Banko Plus and Callicuivre) was diluted with sterile distilled water to obtain a stock solution with a concentration of 1000 ppm, from which the different concentrations were obtained by dilution [13].

A drop of Tween 20 (surfactant) was added to the amended biopesticide medium under magnetic stirring to emulsify it. The effect of Tween 20 was tested beforehand to ensure that it was harmless to *S. rolfsii* isolates. Media thus amended with different concentrations of fungicides were dispensed into 90 mm Petri dishes at a rate of 18 ml per dish. Two perpendicular lines were drawn on the underside of each Petri dish prior to dispensing the culture medium under sterile conditions, their point of intersection indicating the center of the dish [16]. Petri dishes containing frozen PDA-fungicide culture media were inoculated in the center with a 5 mm diameter mycelial disk of *S. rolfsii* culture. The 5 mm diameter mycelial discs were taken from the growth front of 7-day-old fungal cultures. In control dishes, culture medium alone was introduced and the same fungal strains were deposited at the intersection of the two axes [13]. Five (5) Petri dishes were replicated per concentration and fungicide as well as for the control, and the experiment was replicated three times over time. Petri dishes were sealed with paraffin and incubated at $25 \pm 2^\circ\text{C}$ under a 12 h photoperiod.

Fungicide efficacy was assessed by measuring the diameter of mycelial growth of each *S. rolfsii* isolate every 24 h after inoculation. The average of these measurements was used to calculate the inhibition rate (IR) of fungal

growth compared with the control, using the modified formula of Nyaka *et al.* [19]:

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

With:

D_0 = average diameter of the control

D_t = average diameter of treated

Isolate susceptibility and fungicide toxicity were assessed according to the scale of Kumar *et al.* [20] modified by Yao *et al.* [21] recorded in Table 3. The lethal inhibitory concentration (LIC) was determined from the lowest concentration for which no mycelial growth or mycelial pellet recovery was observed on PDA culture medium at the end of seven (7) days of incubation [22].

Table 3. Level of fungicide efficacy according to the scale of Kumar *et al.* [20] modified by Yao *et al.* [21].

Scale	Mycelial inhibition rate	Class Fungal susceptibility/resistance	Fungicide efficacy level
1	$I > 90\%$	Highly sensitive	Very good efficiency
2	$75\% < I \leq 90\%$	Sensitive	Good efficiency
3	$60\% < I \leq 75\%$	Moderately resistant	Medium efficiency
4	$40\% < I \leq 60\%$	Resistant	Low efficiency
5	$I < 40\%$	Highly resistant	Very low efficiency

2.3. Statistical Analysis

The data collected were analyzed using Statistica version 12.5 software. Inhibition rate data were arc-sine transformed for normalization. Analysis of variance with two classification criteria (ANOVA 2) was used to study the interaction between products and concentrations in order to determine their efficacy. ANOVA 2 was also used to study the interaction between isolates and concentrations of each product. Data on isolate growth rate and ability to produce sclerotia were subjected to a one-way analysis of variance (ANOVA 1). The DUNCAN post ANOVA test was performed to determine the different homogeneity classes at the 5% threshold in the event of a significant difference between the averages compared.

3. Results

3.1. Characteristics of the *Sclerotium rolfsii* Isolates Used

The *S. rolfsii* isolates showed different appearances. Isolates Song 73 and Dalc 14 from the towns of Songon and Daloa respectively showed a downy appearance with fibers that lined the surface of the medium (Table 4). However, isolates Yamb 22, Sega 4 and Korb 2 from the towns of Yamoussoukro, Séguéla and Korhogo respectively showed a downy mycelial colony with fine, spiky fibers that were thick around the edges (Table 4). All isolates were white except for the Korhogo isolate (Korb 2), which was beige. All isolates were fully grown

within 3 days of plating. From day 1 to day 2, the growth rate increased for all isolates. Then, on day 3, it slowed down. However, analysis of variance with one classification criterion showed no difference ($F = 0$; $p = 1$) between the growth rates of each isolate per day. The average was 2.83 cm/day for all isolates (Table 4).

A variable quantity of sclerotia was produced by the different *S. rolfsii* isolates. The number of sclerotia varied from 3 to 349 after 28 days of culture. Analysis of variance showed a statistical difference at the 5%

threshold according to Duncan's test between these quantities for each isolate. The Songon isolate (Song 73) produced the highest quantity of sclerotia, with a value of 349, followed by the Yamoussoukro (Yamb 22) and Séguéla (Sega 4) isolates, which recorded quantities of 82 and 63.33 sclerotia respectively. As for isolates from Daloa (Dalc 14) and Korhogo (Korb 2), they produced the lowest quantities of sclerotia, at 17.33 and 3 sclerotia respectively (Table 4).

Table 4. Macroscopic characteristics of *Sclerotium rolfsii* isolates

Isolate codes	Origin	Face staining	Reverse staining	Aspect	Number of sclerotia	Day 1	Day 2	Day 3	Daily growth rate (cm ⁻¹)
Song 73	ZAE I	White	White	Fluffy with fine, bristly fibers	349.00 a	2.05 c	3.77 b	2.68 ab	2.83 ± 0.25 a
Yamb 22	ZAE II	White	White	Fluffy with fine, bristly fibers, thick on edges	82.00 b	2.35 b	3.37 c	2.78 a	2.83 ± 0.18 a
Sega 4	ZAE III	White	White	Fluffy with fine, bristly fibers, thick on edges	63.33 c	2.30 bc	3.87 ab	2.33 b	2.83 ± 0.27 a
Dalc 14	ZAE IV	White	White	Fluffy with fine, bristly fibers	17.33 d	2.48 ab	4.22 a	1.80 c	2.83 ± 0.36 a
Korb 2	ZAE V	Beige	Beige	Fluffy with fine, bristly fibers, thick on edges	3.00 e	2.65 a	3.24 c	2.61 ab	2.83 ± 0.10 a
F					3066,65	6,44	11,90	10,61	0.000
P					<0,0001	0,008	0,001	0,001	1.000

ZAE : Agroecological zones, F : Ratio of variance, P : Probability

3.2. Comparative Effect of Synthetic Fungicides

Figure 1 shows the inhibition rates of synthetic fungicides. The synthetic fungicide Banko Plus recorded a higher inhibition rate than Callicuivre at all equivalent concentrations (Figure 1). Callicuivre therefore had a very low efficacy (Table 6). Inhibition rates ranged from 0.75 ± 0.00 ppm at the lowest concentration to 6.58 ± 0.15 ppm at the highest (Table 5). On the other hand, Banko Plus was clearly effective on *S. rolfsii* isolates at concentrations of 10, 25, 50 and 100 ppm (Table 6), with inhibition rates ranging from 79.59 ± 0.5 to 98.05 ± 0.15 (Table 5).

3.3. Comparative Effect of Biopesticides

Statistical analysis showed a highly significant interaction between biopesticides and concentrations ($F = 13.752$ and $p = 0.00$). For each biopesticide applied, a dose-concentration effect was noted (Figure 2). At concentrations between 100 and 1000 ppm, the inhibition rates of the biopesticides NECO 50 EC and ASTOUN 50 EC were higher (Table 5) than those of the biopesticides FERCA 50 EC, BIOSAKINE 50 EC and NORDINE 50 EC (Figure 2). At concentrations of 100 and 200 ppm, all biopesticides were highly ineffective, with inhibition rates ranging from 1.27 ± 0.01% for FERCA 50 EC to 21.60 ± 0.10% for NECO 50 EC. Then, at 400 and 600 ppm, only the biopesticide NECO 50 EC induced average efficacy in isolates (Table 7), with a stable rate of 60%.

The efficacy of the biopesticides NECO 50 EC and ASTOUN 50 EC increased at 800 and 1000 ppm. NECO 50 EC was very effective (Table 7), with inhibition rates of 92.63 ± 0.85 and 96.26 ± 0.44% respectively. As for the biopesticide ASTOUN 50 EC, its efficacy went from good at 800 ppm to very good (Table 7) at 1000 ppm, with

respective rates of 79.37 ± 0.55 and 96.60 ± 0.40% according to the [20] scale.

At 2000 ppm, the extreme concentration applied, the biopesticide FERCA 50 EC remained ineffective. In contrast, the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC increased in efficacy. At this concentration, the inhibition rate of the biopesticide BIOSAKINE 50 EC rose from 52.31 ± 0.88 to 97.86 ± 0.62%, making it highly effective (Tables 5 and 7).

The biopesticide NORDINE 50 EC, on the other hand, was highly effective, reducing mycelial growth by 84.86 ± 0.82%, a statistically lower value than that of the biopesticide BIOSAKINE 50 EC (Table 5). The biopesticides NECO 50 EC and ASTOUN 50 EC stood out for their 100% inhibition of mycelial growth in *S. rolfsii* isolates. Subculturing the mycelial discs on unamended PDA medium for one week showed no mycelial growth, thus demonstrating the fungicidal (fungitoxic) capacity of these biopesticides at this concentration.

3.4. Classification of Isolates at Effective Concentrations of Each Fungicide

For the effective concentrations of each biopesticide, isolates from Songon (Song 73) and Korhogo (Korb 2) were the most sensitive. At the 800 ppm concentration of the biopesticides NECO 50 EC and ASTOUN 50 EC, isolates from Songon (Song 73), Daloa (Dalc 14) and Korhogo (Korb 2) were highly susceptible (Table 8). In contrast, isolates from Yamoussoukro (Yamb 22) and Séguéla (Sega 4) were resistant. All *S. rolfsii* isolates were highly susceptible to the 1000 and 2000 ppm concentrations of these two biopesticides.

At the 2000 ppm concentration of the biopesticide BIOSAKINE 50 EC, all isolates were highly susceptible, with an inhibition rate of over 90%, with the exception of the Séguéla isolate (Sega 4), which was resistant (Table 8).

The biopesticide NORDINE 50 EC induced the same effect as the biopesticide BIOSAKINE 50 EC at this concentration of 2000 ppm. For this product, the Korbho isolate (Korb 2) showed average resistance (Table 8).

At the lowest concentration of the synthetic fungicide Banko Plus, isolates from Séguéla (Sega 4) and Daloa (Dalc 14) were the most resistant. At concentrations of 25, 50 and 100 ppm, this product caused sensitivity in all isolates (Table 8).

3.5. Fungistatic and Fungitoxic Effects of Biopesticides on *Sclerotium rolfii* Isolates

The fungitoxic/fungistatic effect of each biopesticide was assessed one week after transplanting onto

unamended medium. The aim was to show the most toxic concentration for each product. The observation showed the fungitoxicity of the biopesticide NECO 50 EC at a concentration of 2000 ppm on all *S. rolfii* isolates (Table 9). The biopesticide ASTOUN 50 EC was fungitoxic at the same concentration on 80% of the isolates used in this study. The Séguéla isolate (Sega 4) resumed its growth after subculturing on the new, fungicide-free PDA medium. The biopesticide ASTOUN 50 EC was therefore fungistatic for the Séguéla isolate (Sega 4) at a concentration of 2000 ppm. The biopesticide BIOSAKINE 50 EC was fungitoxic on 2 isolates (Dalc 14 and Korb 2), while the biopesticide NORDINE 50 EC destroyed only one isolate (Dalc 14).

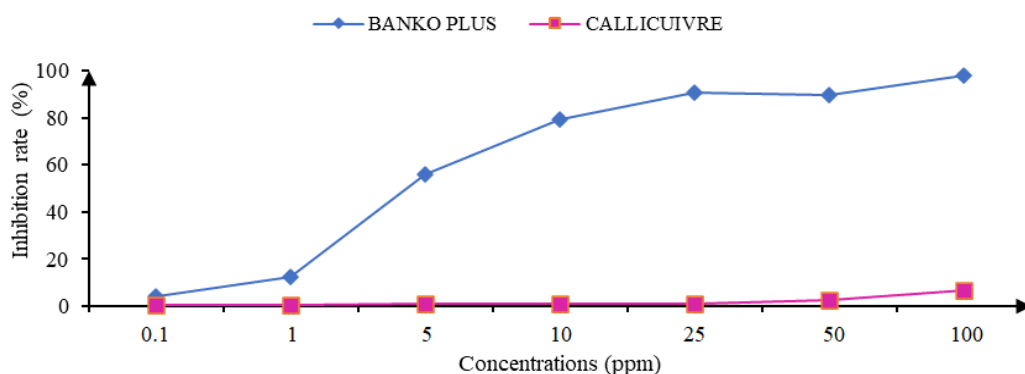


Figure 1. Evolution of inhibition rates depending to synthetic fungicide concentrations

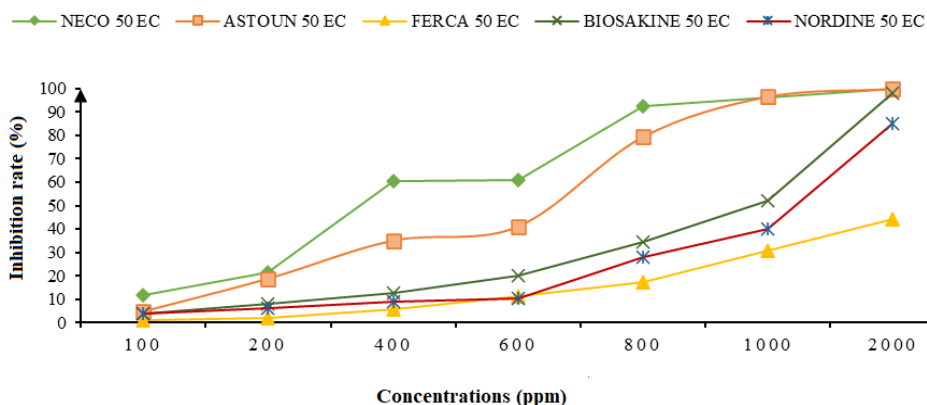


Figure 2. Evolution of inhibition rate depending of biopesticide concentrations

Table 5. Fungicide efficacy on all isolates

BIOPESTICIDES	CONCENTRATIONS						
	C1	C2	C3	C4	C5	C6	C7
NECO 50 EC	11.57 ± 0.37 ^{mp}	21.60 ± 0.10 ^{km}	60.29 ± 0.91 ^f	61.02 ± 0.94 ^f	92.63 ± 0.85 ^{bd}	96.26 ± 0.44 ^{bc}	100.00 ± 0.00 ^a
ASTOUN 50 EC	4.69 ± 0.05 ^{ps}	18.75 ± 0.07 ^{lo}	35.04 ± 0.07 ^{ik}	41.02 ± 0.12 ^{sj}	79.37 ± 0.55 ^e	96.60 ± 0.40 ^{bc}	100.00 ± 0.00 ^a
FERCA 50 EC	1.27 ± 0.01 ^s	1.83 ± 0.03 ^{rs}	5.91 ± 0.14 ^{ps}	11.42 ± 0.15 ^{mp}	17.53 ± 0.20 ^{lo}	30.56 ± 0.32 ^{il}	44.31 ± 0.14 ^{gi}
BIOSAKINE 50 EC	3.72 ± 0.03 ^{ps}	8.12 ± 0.04 ^{or}	12.85 ± 0.04 ^{mp}	20.19 ± 0.11 ^{ln}	34.51 ± 0.39 ^{ik}	52.31 ± 0.88 th	97.86 ± 0.62 ^{ab}
NORDINE 50 EC	3.89 ± 0.09 ^{ps}	6.16 ± 0.05 ^{ps}	8.98 ± 0.11 ^{nr}	10.33 ± 0.09 ^{mq}	27.79 ± 0.18 ^{jl}	40.07 ± 0.68 ^{bj}	84.86 ± 0.82 ^{de}
BANKO PLUS	4.19 ± 0.14 ^{ps}	12.60 ± 0.61 ^{mp}	55.86 ± 0.59 ^{ig}	79.59 ± 0.50 ^e	90.78 ± 0.27 ^{cd}	89.56 ± 0.30 ^{ce}	98.05 ± 0.15 ^{ab}
CALLICUIVRE	0.75 ± 0.00 ^s	0.75 ± 0.00 ^s	1.00 ± 0.02 ^s	1.00 ± 0.02 ^s	1.09 ± 0.02 ^s	2.53 ± 0.08 ^{ps}	6.58 ± 0.15 ^{ps}

In the same column and row, numbers topped by the same letters are statistically identical at the 5% threshold according to Duncan's test. $F = 13.752$ and $p = 0.00$ Biopesticides : C1 : 100 ppm, C2 : 200 ppm, C3 : 400 ppm, C4 : 600 ppm, C5 : 800 ppm, C6 : 1000 ppm and C7 : 2000 ppm Synthetic fungicides: C1 : 0.1 ppm, C2 : 1 ppm, C3 : 5 ppm, C4 : 10 ppm, C5 : 25 ppm, C6 : 50 ppm and C7 : 100 ppm

Table 6. Assessment of the efficacy of synthetic fungicides according to the Kumar *et al.* [20] and Yao *et al.* [21] scale

Synthetic fungicides	Concentrations (ppm)						
	0.1	1	5	10	25	50	100
Banko Plus	--	--	-	++	+++	++	+++
Callicuivre	--	--	--	--	--	--	--

Efficacy scale from Kumar *et al.* [20] and Yao *et al.* [21]

-- : Very low efficacy; - : Low efficacy; + : Average efficacy; ++ : Good efficacy; +++ : Very good efficacy

Table 7. Assessment of biopesticides efficacy on the Kumar *et al.* [20] and Yao *et al.* [21] scale

Biopesticides	Concentrations (ppm)						
	100	200	400	600	800	1000	2000
NECO 50 EC	--	--	+	+	+++	+++	+++
ASTOUN 50 EC	--	--	--	-	++	+++	+++
FERCA 50 EC	--	--	--	--	--	--	-
BIOSAKINE 50 EC	--	--	--	--	--	-	+++
NORDINE 50 EC	--	--	--	--	--	-	++

Efficacy scale from from Kumar *et al.* [20] and Yao *et al.* [21]

-- : Very low efficacy; - : Low efficacy; + : Average efficacy; ++ : Good efficacy; +++ : Very good efficacy

Table 8. Sensitivity/resistance of *Sclerotium rolfsii* isolates

Fungicides	Concentrations (ppm)	ISOLATES				
		Song 73	Yamb 22	Sega 4	Dalc 14	Korb 2
NECO 50 EC	800	HS	R	MR	HS	HS
	1000	HS	HS	S	HS	HS
	2000	HS	HS	HS	HS	HS
ASTOUN 50 EC	800	HS	R	R	S	S
	1000	HS	S	S	HS	HS
	2000	HS	HS	HS	HS	HS
BIOSAKINE 50 EC	2000	HS	HS	R	HS	HS
NORDINE 50 EC	2000	HS	S	R	HS	MR
BANKO PLUS	10	HS	S	R	MR	S
	25	HS	HS	S	S	S
	50	HS	S	S	S	HS
	100	HS	HS	S	HS	HS

HR : Highly resistant; MR : Moderately resistant; R : Resistant; S : Sensitive; HS : Highly sensitive

Table 9. Fungitoxic and fungistatic effect of fungicides

Fungicides	Concentrations (ppm)	Fungal isolates				
		Song 73	Yamb 22	Sega 4	Dalc 14	Korb 2
NECO 50 EC	800	ft	X	X	ft	ft
	1000	ft	fs	X	ft	ft
	2000	ft	ft	ft	ft	ft
ASTOUN 50 EC	800	fs	X	X	X	X
	1000	ft	X	X	fs	fs
	2000	ft	ft	fs	ft	ft
BIOSAKINE 50 EC	2000	fs	fs	X	ft	ft
NORDINE 50 EC	2000	fs	X	X	ft	X
BANKO PLUS	10	fs	X	X	X	X
	25	ft	fs	X	X	X
	50	ft	X	X	X	fs
	100	ft	ft	X	fs	fs

ft : fungitoxic; fs : fungistatic; x : Resumption of mycelial growth (isolate for which growth was not totally inhibited at the indicated concentrations).

4. Discussion

The various *Sclerotium rolfsii* isolates used in this study were distinguished by their morphological appearance and staining. The general appearance of the different isolates was the downy type, which was either shaved or bristly. These morphological types were also observed by Sarma *et al.* [23] and Tariq *et al.* [24] on a collection of isolates from various cultures in India.

The fungal isolates of *S. rolfsii* used in this study did not differ in daily growth rate from one another. However,

they filled the entire 90 mm diameter control Petri dish within 72 hours (3 days). In their study of *S. rolfsii* isolates from different localities in Ghana, Tortoe and Clerk [25] showed that this growth rate characterizes fast-growing isolates. Intermediate-growth isolates cover the agar plate in 84 hours (3.5 days), while slow-growth isolates completely cover the plate in 96 hours (4 days). However, it should be noted that the growth of fungal strains is mainly influenced by the composition of the culture medium. Indeed, in three (3) days, growth is maximal (90 mm) on PDA medium, the substrate used in this study. According to Tariq *et al.* [24], growth rate is an important marker of strain morphological diversity. It is

correlated with the virulence of strains and their ability to produce sclerotia. However, the different isolates considered did not differ in growth rate, but produced different quantities of sclerotia.

This study evaluated the *in vitro* effect of the biopesticides NECO 50 EC, ASTOUN 50 EC, FERCA 50 EC, BIOSAKINE 50 EC and NORDINE 50 EC on the mycelial growth of five (5) isolates of *S. rolfsii* in comparison with that of two reference synthetic fungicides (Banko Plus and Callicuivre).

The synthetic fungicide Banko Plus was more effective than Callicuivre at all concentrations. This could be explained by the difference in the active molecules making up these synthetic fungicides. In fact, the synthetic fungicide Banko Plus is composed of a combination of carbendazim (100 g/l) and chlorothalonil (550 g/l), while Callicuivre is composed of the copper oxychloride molecule (75 g/15L). Yao *et al.* [21] have shown that combining two or more active ingredients leads to synergy, as it broadens the spectrum of antifungal activity against several diseases that can attack crops simultaneously. The two synthetic fungicides (Banko Plus and Callicuivre) are systemic and contact fungicides which destroy the fungus by inhibiting its respiration and energy production. While the synthetic fungicide Banko Plus has clear antifungal activity against several phytopathogenic agents, its use is not without consequences.

The ability of each biopesticide to inhibit the mycelial growth of *S. rolfsii* was demonstrated in this study. Indeed, all the biopesticides evaluated showed an inhibitory action on the mycelial growth of *S. rolfsii* isolates. However, this efficacy varied from one biopesticide to another. Indeed, at equal concentrations, differences in activity between biopesticides were noted. In general, all *S. rolfsii* fungal isolates showed sensitivity to increasing doses, with a progressive increase in the rate of inhibition. This inhibitory action of the various biopesticides would therefore be "dose-dependent". However, Traore *et al.* [26] noted that with *Eucalyptus citriodora* essential oil, antimicrobial activity did not always increase with increasing deposit quantity. For these authors, antimicrobial activity does not appear to be dose-dependent. The variability in efficacy between biopesticides is thought to be linked to the active ingredients they contain in their chemical composition. Indeed, Silue *et al.* [27], the antifungal activity of a biopesticide depends as much on its composition in aromatic compounds as on its structure. All the biopesticides used were formulated from essential oils produced from aromatic plants, which contain volatile compounds and have antifungal properties.

It has been established that certain constituents of essential oils can positively influence their overall activity [13]. The biopesticides NECO 50 EC and ASTOUN 50 EC showed a satisfactory level of efficacy at concentrations of 800, 1000 and 2000 ppm, while the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC were effective at the highest concentration of 2000 ppm. Of the five biopesticides tested, only the biopesticide FERCA 50 EC, characterized by the high presence of linear-structured molecules such as citronellal and citronellol, showed very low efficacy on all five *S. rolfsii* isolates at all concentrations. *S. rolfsii* isolates are said to be highly resistant to the antifungal compounds in this product. All the biopesticides tested showed very marked

efficacy on the mycelial growth of *S. rolfsii* isolates. Our results are in line with those of Doumbouya *et al.* [15], Yala *et al.* [28], Yarou *et al.* [29], Kasmi *et al.* [30] and N'Goran *et al.* [31], who have shown that essential oils have antimicrobial, insecticidal, fungicidal and bactericidal activities, as they have the capacity to stimulate plant defense reactions.

Bolou *et al.* [16], showed the fungicidal activity of *Xylopia aethiopica* fruit and leaf essential oils on the radial mycelial growth of *Sclerotium rolfsii*. Fruit essential oil showed the most significant inhibition rate (83.70%) on day 4 at a concentration of 500 µL/L. A study carried out in Côte d'Ivoire by N'Goran *et al.* [31] for the control of brown pod rot of cocoa caused by *Phytophthora katsurae* using the biofungicides NECO 50 EC, ASTOUN 50 EC and FERCA 50 EC also showed that the biological fungicide FERCA 50 EC was the least effective, inhibiting mycelial growth of *Phytophthora katsurae* by only half at the concentration of 2000 ppm.

Only the biopesticide NECO 50 EC was fungitoxic in this study on all *S. rolfsii* isolates at the 2000 ppm concentration. The efficacy of this biopesticide is thought to be due to the terpene compounds Thymol and Eugenol present in its formulation, which may have conferred on it this strong antifungal capacity. The antifungal activity of the biopesticide NECO 50 EC was demonstrated by Kassi *et al.* [15] against Black Leaf Streak Disease (BLSD) caused by the ascomycete fungus *Mycosphaerella fijiensis* Morelet on plantain (AAB) in Côte d'Ivoire. The results of their study indicated that the use of NECO 50 EC is therefore an effective and viable option in the control of BLSD, with the advantage of being economical and non-toxic for the farmer, the consumer and the environment.

Furthermore, the work of Yéo [32] in Côte d'Ivoire showed that the biopesticide NECO 50 EC completely reduced the mycelial growth of *S. rolfsii* at concentrations of 3000, 5000, 7000 and 10000 ppm. However, our work shows that the efficacy of the biopesticide NECO 50 EC can be seen at lower concentrations (800, 1000 and 2000 ppm) than those reported by Yéo [32]. These differences are thought to be due to the geographical origin of the isolates and their genetic characteristics, which determine the resistance of their biological structure [2]. The efficacy of the biopesticide ASTOUN 50 EC was close to that of the biopesticide NECO 50 EC. In fact, the biopesticide ASTOUN 50 EC contains the majority compounds Géraniol and Neral in its formulation.

The fungitoxicity of the biopesticides BIOSAKINE 50 EC and NORDINE 50 EC was less significant on isolates, but these biopesticides produced an effective inhibition rate on all *S. rolfsii* isolates at the 2000 ppm concentration. It should be noted that the BIOSAKINE 50 EC biopesticide formulation contains the majority compounds Alpha-zingiberene and arcurcumene, while the NORDINE formulation contains the majority compounds Carvacrol and 1.8-cineole.

5. Conclusion

This study demonstrated the antifungal activity of the biopesticides NECO 50 EC, ASTOUN 50 EC, FERCA 50 EC, BIOSAKINE 50 EC and NORDINE 50 EC on the

mycelial growth of five (5) isolates of *Sclerotium rolfsii* from different agroecological zones. The biopesticides NECO 50 EC and ASTOUN 50 EC showed good inhibitory potential on mycelial radial growth of *S. rolfsii* isolates at concentrations of 800, 1000 and 2000 ppm. The biopesticides BIOSAKINE 50 EC and NORDINE 50 EC were highly effective at 2000 ppm. The 2000 ppm concentration was fungistatic on the majority of *S. rolfsii* isolates. The biopesticide FERCA 50 EC was ineffective on all *S. rolfsii* isolates. The biopesticides tested need to be evaluated under controlled conditions and in the field in order to offer them to vegetable growers faced with sclerotinia in the field in order to offer them to vegetable growers faced with sclerotinia in their fields.

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