Short Research Article

Assessment of bacterial biocontrol agents formulations against anthracnose of cashew (*Anacardium occidentale* L.) in Côte d'Ivoire

ABSTRACT

The cashew tree (*Anacardium occidentale* L.) is of vital importance to the Ivorian economy. Côte d'Ivoire is first world producer. However, its cultivation faces several constraints linked to anthracnose. The aim of this study was to assess the efficacy of two formulated bacterial biocontrol agents against anthracnose of cashew. To this end, *in vitro* confrontation tests were carried out against *Colletotrichum gloeosporioides* with two previously formulated bacterial biopesticides. Biocontrol tests were then carried out against anthracnose in greenhouses and cashew plantations. The results showed that the two bacterial biocontrol agents tested controlled *in vitro* the proliferation of *Colletotrichum gloeosporioides* responsible for anthracnose and significantly reduced the disease severity index on cashew seedlings in the greenhouse. *In vitro*, inhibition rates ranging from 70.16±6.9 to 72.65±6.5% were observed on the mycelial growth of *Colletotrichum gloeosporioides*. In the greenhouse and in cashew plantations, a sharp reduction in the anthracnose severity index was observed. In the greenhouse, anthracnose reduction rates by the two bacterial biocontrol agents ranged from 62.80 ± 5.2% to 85.95 ± 2.8%. In cashew plantations, the reduction rates varied from 42.24 to 41.05%. . In view of the above, these two bacterial biocontrol agents could be used for biological control of cashew anthracnose in Côte d'Ivoire.

Key words: *Cashew, Anthracnose, Bacterial biocontrol agents, formulations, Côte d'Ivoire*

1. INTRODUCTION

Cashew is a crop who takes an important role in the Ivorian economy because of its cashew nut and is a particular strategic resource that generates income for farmers in the north, south, centre and east of the country **(Soro, 2012)**. Unfortunately, its cultivation is subject to several phytopathological problems that compromise the qualitative and quantitative yield of cashew nuts **(Silué *et al.*, 2017)**. Indeed, in addition to insect pests, several diseases caused by fungi and bacteria have been detected in cashew orchards in Côte d'Ivoire. These include downy mildew, leaf rust, bud desiccation, gum blight, bacterial blight and anthracnose. Anthracnose is one of the main diseases found in cashew orchards in Côte d'Ivoire **(Soro *et***

***al*., 2022)**. This disease is thought to be one of the reasons for the low yield per hectare, estimated at 414.3 kg/ha **(FIRCA, 2018)**. Anthracnose is caused by the fungal *Colletotrichum gloeosporioides*. It generally attacks all parts of the plant, with an average severity index of 28.48%. It causes brown spots on the leaves and premature fruit drop **(Soro *et al*., 2022)**. Yield losses due to this disease were around 40% in Brazil, 50-70% in Mozambique and 40-56% in Uganda **(Mathur et Kongsdal, 2003; Kiwuso *et al*., 2013**).

This disease is generally combated by adopting good cultivation practices and using chemical products. However, the application of these cultivation techniques is still very time-consuming. Chemical products, when used incorrectly by growers, have harmful effects on their health and the plants, and even on the environment **(Kouassi, 2012)**.

To mitigate the harmful effects of chemical pesticides, biological control agents are emerging as promising alternatives for managing crop pathogens. Among these biological agents, microbial biopesticides (bacteria, fungi, viruses) are the most suitable. They offer the advantages of greater selectivity and lower toxicity than conventional chemical pesticides. Recent studies have shown their importance in the biocontrol of diseases **(Pérez-Garcia *et al*., 2011)**. Over the last decade, numerous studies on greenhouse and field trials have demonstrated the potential interest of rhizosphere bacteria, particularly *Pseudomonas fluorescens* and *Bacillus subtilis*, as biological agents for controlling plant pathogens **(Akram, 2008)**. Work by **Koua*et al.* (2020)** has shown that *B. subtilis* strains isolated from the rhizosphere of cocoa trees in Côte d'Ivoire are effective bioinoculants for controlling greenhouse cocoa diseases such as swollen shoot. The aim of this study was to assess the efficacy of two formulations of bacterial biocontrol agents based on bacteria isolated from the rhizosphere of cashew trees against cashew anthracnose in Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1 Material

The material used in this study consisted of two biocontrol agents already formulated and named Biobact 1 for the *Pseudomonas fluorescens* based formulation and Biobact 2 for the *Bacillus subtilis* based formulation. These two biocontrol agents were formulated during the work of **Tehua *et al*. (2022)**. Greenhouse cashew seedlings were also used for the greenhouse biocontrol trial. For the cashew plantation trials, disease assessment sheets, sprayers and a cooling cooler were used.

2.2 Méthods

2.2.1 *In vitro* mycelial growth inhibition test for *Colletotrichum gloeosporioides*

This test was carried out using the method described by **Silué *et al*. (2018)**. Thus, for each formulation of bacterial biocontrol agents, concentrations of 0.0625; 0.125; 0.25; 0.5; and 1% corresponding to concentrations C5, C4, C3, C2 and C1, respectively, were prepared. PDA (Potato Dextrose Agar) culture media were prepared and autoclaved at 121°C for 15 minutes. Once the medium had cooled to 45°C, the various solutions containing the formulated concentrations of bacterial biocontrol agents were each incorporated into the PDA medium contained in an Erlenmeyer flask. The media thus prepared were homogenised and dispensed into 90 mm-diameter Petri dishes, at a rate of 20 ml per dish. Next, 7 mm diameter mycelial fragments of the pathogenic fungi were cut from 7-day-old cultures and placed in the centre of the Petri dishes containing the PDA medium with added formulation.

The cultures were incubated in a culture chamber at 30°C for fourteen (14) days. Five Petri dishes were used for each treatment and the experiment was repeated 3 times. The radial growth of the mycelium of the

fungal colonies was measured every three days using a graduated ruler, until total coverage of the surface of the culture medium in the control Petri dish, i.e. fifteen days after cultivation. The radial growth of the mycelium was measured along two perpendicular lines drawn at the bottom of each Petri dish and intersecting at a point in the middle of the mycelial disc. The effect of biopesticides on fungal growth was determined from the inhibition rate (Ic) of mycelial growth calculated by the following formula **(Hmouni *et al.*, 1996)**.

$$\text{Ic } (\%) = \frac{D0 - Dc}{D0} \times 100$$

Ic (%) = Inhibition rate
Do = Radial growth of fungi without formulated product
Dc = Radial growth of fungi with formulated product

2.2.2 Assessment of the *in vivo* antifungal effect of formulations of bacterial biocontrol agents against anthracnose of cashew in the greenhouse

2.2.2.1 Preparation of the fungal inoculum

Ten millilitres (10 mL) of sterilised distilled water was added to 28-day-old mycelial cultures of *Colletotrichum gloeosporioides*. The surface of these cultures was scraped with a sterilised metal spatula. The resulting solution was homogenised in a test tube using a vortex. A few drops (200 µL) of this suspension were used to fill the wells of a Malassez cell in order to estimate the number of spores. This number was adjusted to 10^8 spores/ml by dilution or by increasing the spore concentration of the solution by re-suspending the spore solution on another mycelial culture. One millilitre (1 mL) of a 1% glucose and agar solution was added to the spore suspension prior to inoculation. The role of this solution is to facilitate adhesion and germination of the spores on the leaves **(Silué *et al.*, 2018)**.

2.2.2.2 Inoculation and treatment of seedlings with formulations of bacterial biocontrol agents

Twenty-seven (27) two-month-old cashew seedlings were used for the experiment, with nine (9) seedlings for each treatment. The treatments involved inoculated and untreated control seedlings (T0), seedlings inoculated and treated with the *Pseudomonas fluorescens* formulation (T1) and seedlings inoculated and treated with the *Bacillus subtilis* formulation (T3). Using a sprinkler with a flow rate of 500 µl/jet, the undersides of the leaves of cashew seedlings were sprayed with 1 mL of fungal inoculum. Six (6) leaves were inoculated per cashew seedling, i.e. 6 ml of fungal inoculum per seedling. A total of 162 ml of fungal inoculum was used for the twenty-seven (27) cashew seedlings. Twenty-four hours after inoculation, both sides of the inoculated leaves were sprayed separately with the biopesticide formulations, with nine (9) inoculated seedlings for each formulated product. After treatment, the seedlings were placed in a greenhouse. The experiment was repeated three (3) times. The greenhouse was regularly humidified (2 times a day) by watering with a watering can to keep the humidity level high (95 - 100%). The purpose of the humidification was to facilitate germination of the fungal pathogen spores. The development of anthracnose symptoms was monitored regularly for three (3) months **(Silué *et al.*, 2018)**.

2.2.2.3 Assessment of the efficiency of treatments against anthracnose

The evolution of disease symptoms was monitored after each treatment. The ability of the two formulated bacterial biocontrol agents to protect against or reduce infection by the fungal pathogen was assessed. This was done by determining the infection rate, the severity index and the anthracnose reduction rate.

Determination of anthracnose infection rate after treatment

The infection rate of cashew seedlings after treatment was assessed by the ratio of the number of leaves showing symptoms of the disease to the total number of leaves inoculated **(Issa *et al.*, 2017)**.

$$\mathbf{Ti\ (\%)} = \frac{n}{N} \times 100$$

Ti (%) = Disease infection rate
n = Number of inoculated leaves showing disease symptoms after treatment
N = Total number of inoculated and treated leaves

Determination of anthracnose severity index after treatment

The anthracnose severity index after treatment was determined using a visual rating scale from 0 to 9 (**Groth *et al.*, 1999; Cardoso *et al.*, 2004**): 0 = No symptoms; 1 = 1-5%; 3 = 6-10%; 5 = 11-25%; 7 = 26-50%; 9 > 50% of leaf area infected. The anthracnose severity index was determined for each trial using the formula of **Kranz (1988)**.

$$IS\ (\%) = \left(\frac{\sum(xi \times ni}{NZ}\right) X\ 100$$

IS= severity index (%); Xi = disease score; ni = number of leaves with score xi; N = total number of leaves inoculated and then treated with the bacterial biopesticide and Z = highest score.

Determination of reduction rate post-treatment anthracnose

The rate of reduction of anthracnose after treatment was determined by the ratio of the difference between the severity index of the control plants inoculated with the pathogen and untreated and the severity index of the trials over the severity index of the control plants inoculated with the pathogen and untreated multiplied by one hundred.

$$\mathbf{Tr\ (\%)} = \frac{Io - I}{Io} \times 100$$

Tr (%) = Reduction rate

Io = Severity index of control plants infected with the pathogen and not treated with biopesticide ;

I = Severity index of plants infected with the pathogen and treated with biopesticides.

2.2.3 Assessment of the efficiency of biocontrol agent formulations against anthracnose in cashew plantations

2.2.3.1 Choice of experimental sites

The experiments were carried out in three (3) agro-ecological cashew nut production zones, namely Bouaké, Korhogo and Bondoukou, corresponding to the Central, Northern and Eastern zones respectively. In each

zone, experimental sites were selected according to several criteria. These criteria included the age of the plot to be treated (10-15 years), the ability to reach cashew tree foliage, the accessibility of the plots, the homogeneity of cashew plants in the plots to be treated, the spacing between plants in the plot, and the significant presence of bacterial blight and anthracnose. According to these criteria, one (1) cashew plot was selected in each zone.

2.2.3.2Plantation experiments

Evaluation of the efficacy of bacterial biopesticides formulated against anthracnose in cashew plantations was carried out using the method described by **Tonon *et al.* (2017)**. Thus, the treatment of the selected experimental plots was carried out with an automated sprayer. The Fisher experimental set-up was used. Three Fisher blocks were used for three treatments (T0 = Untreated control plants T1 = Plants treated with Biobact 1 formulation at 2.5 L/ha and T2 = Plants treated with Biobact 2. formulations at 2.5 L/ha). Cashew seedlings were randomly selected to form the three blocks. The experimental unit consisted of five (5) cashew trees for each treatment, i.e. forty-five (45) cashew trees per 1 ha plot, with fifteen cashew trees per Fischer block. The formulated bacterial biopesticides were diluted in the sprayer with tap water. The concentration chosen was 2.5 L/ha. After mixing the products in the sprayer, the cashew foliage and trunks were treated according to each Fischer block. Each cashew plant was treated with 2 L of spray, i.e. 200 L of spray for one hundred (100) cashew trees. Three (3) treatments were carried out during the vegetative phase of the cashew plants. The interval between each treatment was three weeks.

2.2.3.3Assessment of the efficiency of the formulations

Assessment of the efficacy of the bacterial formulations in cashew plantations was carried out every fifteen (15) days after the three (3) treatments until the end of the campaign (four months after treatment). It was carried out on the old and new leaves of the treated cashew trees. A total of twenty-seven (27) cashew plants were evaluated out of forty-five (45), with nine (9) plants for each Fisher block. This assessment focused on measuring a number of phytopathological parameters, including infection rate, severity index and anthracnose severity index reduction rate. The assessment of these development parameters was carried out in four 1 m2 frames placed on the four north, east, west and south sides of the foliage of treated cashew plants **(Tonon *et al.*, 2017).**

Determination of the rate of infection of plants by anthracnose after application of the formulations

To assess the rate of infection of plants by anthracnose, a count of the total number of buds and the number of infected buds was carried out in each $1m^2$ frame. The anthracnose infection rate was determined as the ratio of the number of buds showing disease symptoms to the total number of buds in the frame.

$$\mathbf{Ti\ (\%)} = \frac{n}{N} \times 100$$

Ti (%) = Disease infection rate

n = Number of buds showing disease symptoms

N = Total number of buds in the $1m^2$ frame

Determination of the anthracnose severity index after application of the formulations

The anthracnose severity index was determined on the first five (5) leaves of three buds in the four quadrants of $1m^2$ showing characteristic symptoms of each disease to be assessed. It was determined by taking into account the rate of leaf surface infected by this disease. Scores were then assigned to each diseased leaf, taking into account the scoring scale from 0 to 9 in **Cardoso *et al.* (2004**). 0 = No symptoms; 1 = 1-5%; 3 = 6-10%; 5 = 11-25%; 7 = 26-50%; 9 > 50% of leaf area infected. The disease severity index was determined for each trial using the following equation **(Kranz, 1988)**.

$$Is\,(\%) = \left(\frac{\sum(xi \times ni)}{NZ}\right) X\ 100$$

IS (%) = Severity index; Xi = Disease score; ni = Number of leaves with score xi ;

N = Total number of trees treated and Z = Highest score.

Determination of the rate of reduction of the anthracnose severity index after application of the formulations

The rate of reduction in the anthracnose severity index before and after treatment was determined using the following formula:

$$Tr\,(\%) = \frac{Io - I}{Io} \times 100$$

Tr (%) = Reduction rate

Io: Severity index before treatment ;

I: Average severity index after the 3 treatments.

2.2.4 Statistical analysis

The data collected was recorded using Excel 2016 and analysed using Statistica version 7.1 software. Analyses of variance (ANOVA) were performed. The normality of the residuals and the homogeneity of the variances were checked. Comparisons between means were made using the Newman-Keuls test with a threshold of 5%.

3. RESULTS

3.1*In vitro* effect of bacterial biocontrol agent formulations on the growth of *Colletotrichum gloeosporioides*

The ability of bacterial formulations to inhibit the mycelial growth of *Colletotrichum gloeosporioides* was studied *in vitro*. In general, these formulations effectively reduced the mycelial growth of *Colletotrichum*

gloeosporioides at different concentrations (**Figure 1**). The average inhibition rates for the different formulations were 72.65 and 70.16% for Biobact 2 and Biobact 1 respectively. However, these inhibition rates were significantly different at the 5% threshold (**Figure 2**). For both formulations, concentrations of 0.5 and 0.25% did not completely inhibit the mycelial growth of the pathogenic fungus. However, the 1% concentration (C1) showed complete inhibition of fungal growth for all formulations after an incubation period of one (1) month. The average inhibition rates as a function of concentration for the different formulations were 100, 82.55, 73.71, 60.66 and 48.10% respectively at concentrations 1, 0.5, 0.25, 0.125 and 0.0625%, corresponding to concentrations C1, C2, C3, C4 and C5. Statistical analysis showed a significant difference between the average inhibition rates of the different concentrations of formulations at the 5% threshold **(Figures 3 and 4)**.

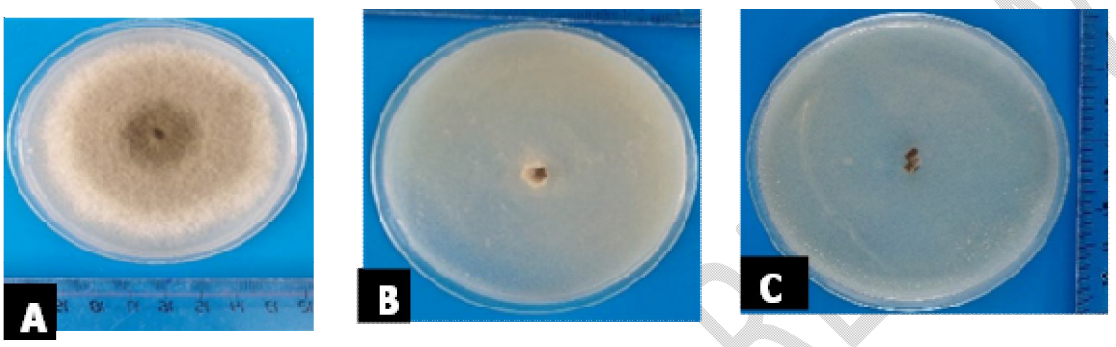

Figure 1: Inhibition of mycelial growth of *Colletotrichum gloeosporioides* by Biobact 1 and Biobact 2 products at a concentration of 1%.

A- Mycelial growth of the control fungus on PDA medium without formulated products

B- Mycelial growth of Colletotrichum gloeosporioides on PDA medium with Pseudomonas fluorescens formulation

C- Mycelial growth of *Colletotrichum gloeosporioides* on PDA medium with *Bacillus subtilis* formulation

Biobact 1: Treatment with *Pseudomonas fluorescens* formulation

Biobact 2: Treatment with *Bacillus subtilis* formulation

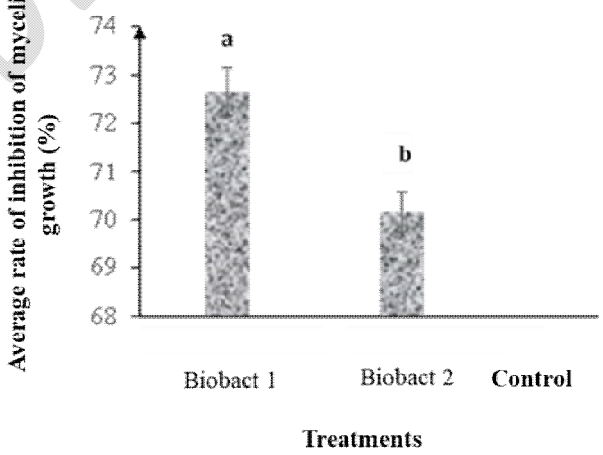

Figure 2: Growth inhibition rate of *C. gloeosporioides* by biopesticide formulations after one month's incubation

Histograms with the same alphabetical letters are not statistically different ($p \leq 0.05$) (Newman and Keuls)

Biobact 1: Treatment with *Pseudomonas fluorescens* formulation

Biobact 2: Treatment with *Bacillus subtilis* formulation

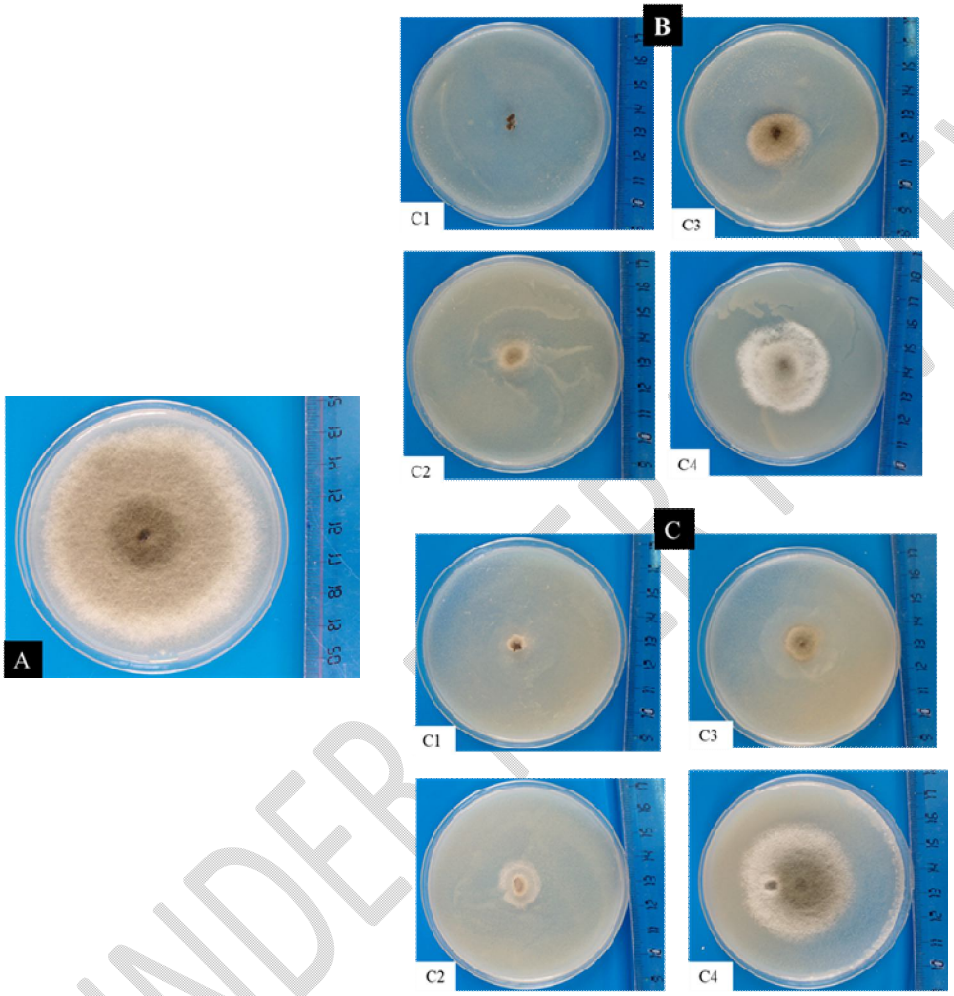

Figure 3: *In vitro* inhibition of *C. gloeosporioides* growth as a function of the concentration of formulated biocontrol agents

A- Mycelial growth of the control fungus on PDA medium without formulated products
B- Mycelial growth of *Colletotrichum gloeosporioides* fungus on PDA medium with *Pseudomonas fluorescens* (Biobact 1) formulation
C- Radial growth of the fungus *Colletotrichum gloeosporioides* on PDA medium with *Bacillus subtilis* formulation (Biobact 2).
C1: 1% concentration
C2: 0.5% concentration
C3: Concentration 0.25
C4: Concentration 0.125

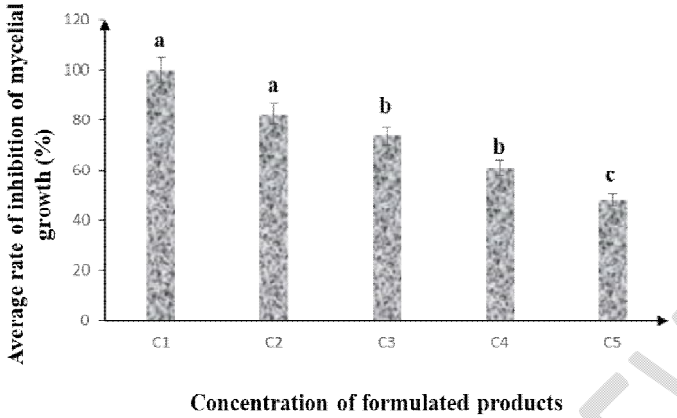

Figure 4: Average cumulative inhibition rate of the concentrations of the two formulations on the mycelial growth of C. gloeosporioides after one month's incubation

C1: Concentration 1%, C2: Concentration 0.5%, C3: Concentration 0.25%, C4: Concentration 0.125% and C5: Concentration 0.0625%.

Histograms with the same alphabetical letters are not statistically different ($p \leq 0.05$) (Newman and Keuls).

3.2 Pest control activity of formulated biocontrol agents on anthracnose and bacterial blight of cashew under controlled conditions

3.2.1 Development of anthracnose symptoms on young cashew seedlings

One month after inoculation, uninoculated and untreated plants and plants treated with the formulation showed no symptoms of anthracnose **(Figure 5 A)**. Those inoculated with *Colletotrichum gloeosporioides* spores and untreated showed typical symptoms of the disease, which manifests itself as leaf necrosis beginning at the edge of the leaf blades (**Figure 5 B**). Inoculated and treated plants showed no leaf necrosis. Normal development of the plants was also observed, with the appearance of new leaves and an increase in the green colour of the leaves **(Figure 5 C)**.

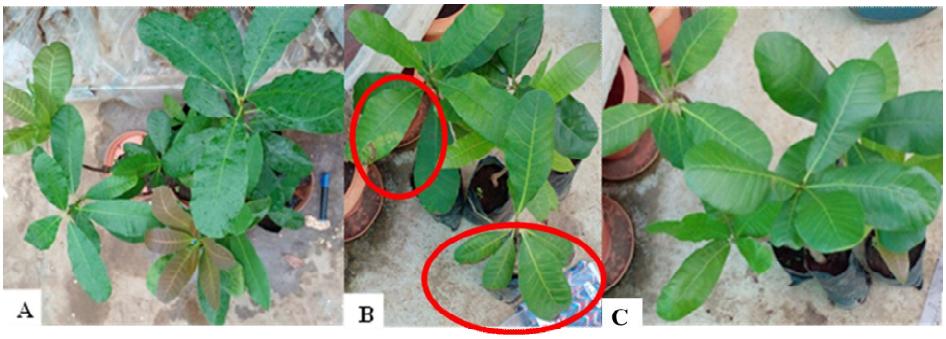

Figure 5: Anthracnose symptoms on cashew seedlings in the greenhouse after application of formulations one month after treatment

A- Uninoculated and untreated control plants showing no symptoms of anthracnose on the leaves

B- Control plants inoculated with *colletotrichum gloeosporioides* and not treated with formulated products showing anthracnose symptoms

C- Plants inoculated with *Colletotrichum gloeosporioides* and treated with formulated products showing no anthracnose symptoms

3.2.3 Infection rate of anthracnose-treated plants

Three months after treatment, a 100% infection rate was observed with plants inoculated with *Colletotrichum gloeosporioides* spores and not treated with the formulations. Plants inoculated with the fungus spore suspension and treated with the formulations recorded infection rates of 44.44 and 57.42% respectively for the *Pseudomonas fluorescens* (Biobact 1) and *Bacillus subtilis* (Biobact 2) formulations. These results show that treatments with the *Pseudomonas fluorescens* formulation had the lowest infection rate **(Figure 6)**.

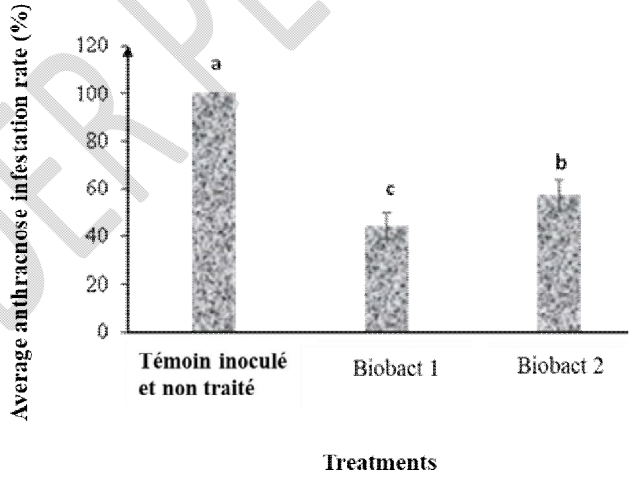

Figure 6: Anthracnose infection rate in cashew seedlings three (3) months after treatment

Histograms with the same alphabetical letters are not statistically different ($p \leq 0.05$) (Newman and Keuls)

Biobact 1: Plants inoculated and treated with the *Pseudomonas fluorescens* formulation

Biobact 2: Plants inoculated and treated with *Bacillus subtilis* formulation

3.2.4 Average anthracnose severity index as a function of plant treatments

An average severity index of 24.86% was observed for inoculated and untreated control plants. This severity was higher than that of plants treated with the formulations. The *Pseudomonas fluorescens* formulation showed a severity index of 5.55%, while the *Bacillus subtilis* formulation showed an average severity index of 9.25%. Plants treated with the *Pseudomonas fluorescens* (Biobact 1) formulation thus showed a lower severity index than those treated with *Bacillus subtilis* (Biobact 2). Statistical analysis also showed a significant difference between these two values **(Figure 7)**.

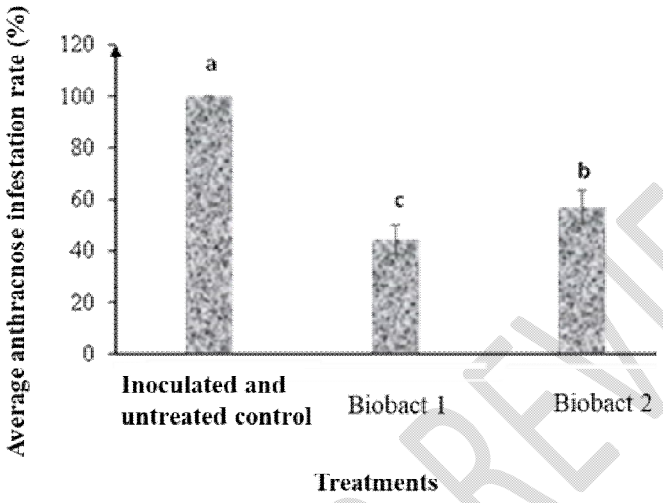

Figure 7: Average severity of anthracnose on cashew seedlings three (3) months after treatment.

Histograms with the same alphabetical letters are not statistically different ($p \leq 0.05$) (Newman and Keuls)

Biobact 1: Plants inoculated and treated with the *Pseudomonas fluorescens* formulation

Biobact 2: Plants inoculated and treated with *Bacillus subtilis* formulation

3.2.5 Reduction rate in anthracnose severity index after application of the formulations

Average reduction rates of over 50% were observed for both types of formulation. The reduction rates were 85.95 and 62.80% for the *Pseudomonas fluorescens* (Biobact 1) and *Bacillus subtilis* (Biobact 2) formulations respectively. These two values are statistically different at the 5% threshold **(Figure 8).**

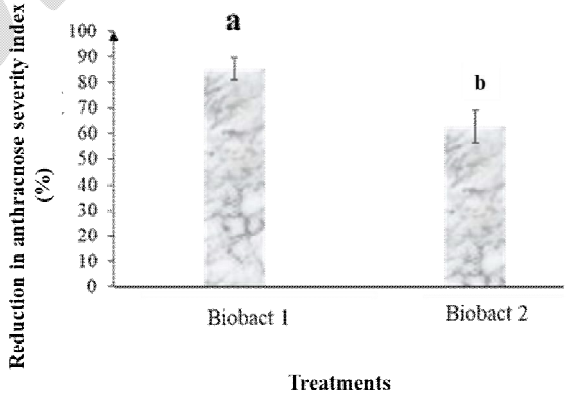

Figure 8: Rate of reduction in anthracnose severity index in young cashew seedlings three (3) months after treatment.

Histograms bearing the same alphabetical letters are not statistically different ($p \leq 0.05$) (Newman and Keuls)

Biobact 1: Treatment with *Pseudomonas fluorescens* formulation

Biobact 2: Treatment with *Bacillus subtilis* base formulation

3.3 Efficiency of bacterial formulations on anthracnose and bacterial blight symptoms in cashew plantations

3.3.1 Evolution of anthracnose symptoms on cashew plants after treatment

Cashew plants not treated with the formulated products showed a progressive increase in anthracnose symptoms, manifested by rounded necrotic black spots on the leaf surface (**Figure 9 A**). However, cashew plants treated with both formulated products (Biobact 1 formulation and Biobact 2 formulation) showed normal development. These plants were apparently healthy with the appearance of new leaves (**Figure 9 B and C**).

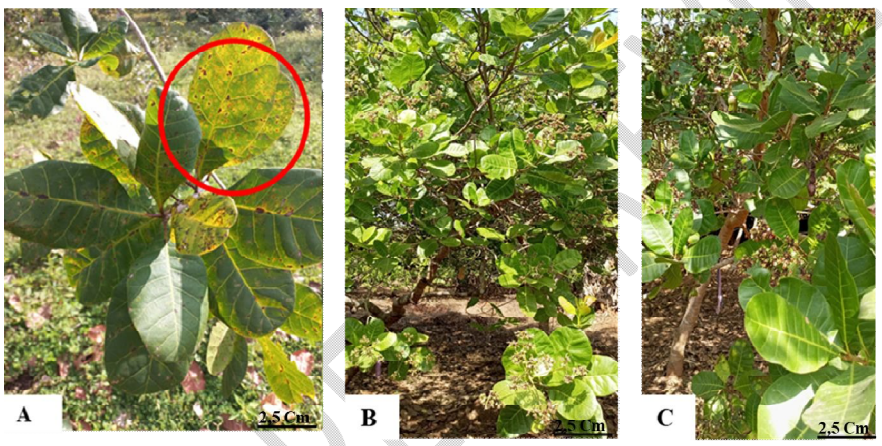

Figure 9: Level of development of anthracnose symptoms on cashew leaves after application of formulations one month after treatment

A- Untreated control cashew plant foliage showing anthracnose symptoms

B- Cashew plant foliage treated with the *Pseudomonas fluorescens* (Biobact 1) formulation showing no anthracnose symptoms

C- Cashew plant foliage treated with the *Bacillus subtilis* (Biobct 2) formulation showing no anthracnose symptoms

3.3.2 Level of anthracnose infection in cashew trees after application of the formulations

The results concerning anthracnose infection levels indicate that, irrespective of the treatment zone, the untreated control plants showed the highest infection rates in the experiment. These infection rates were 94.06%, 97.24% and 92.64% for the control in the North (Korhogo), Centre (Bouaké) and East (Bondoukou) zones respectively. As for the infection rates of treated plants, those treated with the product Biobact 1 (*Pseudomonas fluorescens*) showed infection rates of 86.33, 82.20 and 72.44% respectively for the North, Centre and East zones. Meanwhile, plants treated with Biobact 2 (*Bacillus subtilis*) had infection rates of 81.13, 84.86 and 81.13% in the North, Centre and East zones respectively. Statistical treatments showed that regardless of the treatment zone, the infection rates observed with the

two biological products did not differ significantly ($p \leq 0.05$). However, these infection rates differed from those of the control plants **(Figure 10)**.

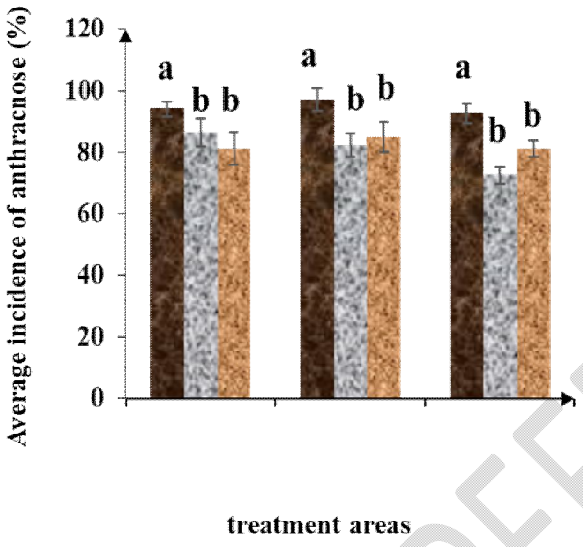

Figure 10: Anthracnose infection rate of cashew plants four (4) months after treatment

Biobact 1: Plants treated with the *Pseudomonas fluorescens* formulation

Biobact 2: Plants treated with the *Bacillus subtilis* formulation.

Histograms bearing the same alphabetical letters in each zone are not statistically different ($p \leq 0.05$) (Newman and Keuls)

3.3.3 Level of anthracnose severity on cashew plants after application of formulations

Untreated control plants showed average severities of 31.88, 16.17 and 40.89% respectively for the North, Centre and East zones. These severities were higher than those of plants treated with the formulations. Plants treated with the Biobact 1 (*Pseudomonas fluorescens*) formulation showed average severities of 16.94, 14.93 and 27.53% respectively in the North, Centre and East zones, while those treated with the Biobact 2 (*Bacillus subtilis*) product showed average severities of 15.43, 12.77 and 30.03% respectively in the North, Centre and East zones (Figure 11). In the central plots, the statistical treatments showed no significant difference between the average severity of the control plants and the plants treated with the two formulated products ($p \leq 0.05$). However, in the northern and eastern zones, a significant difference was observed between the severity of the disease in control plants and plants treated with the two formulations at the 5% threshold **(Figure 11)**.

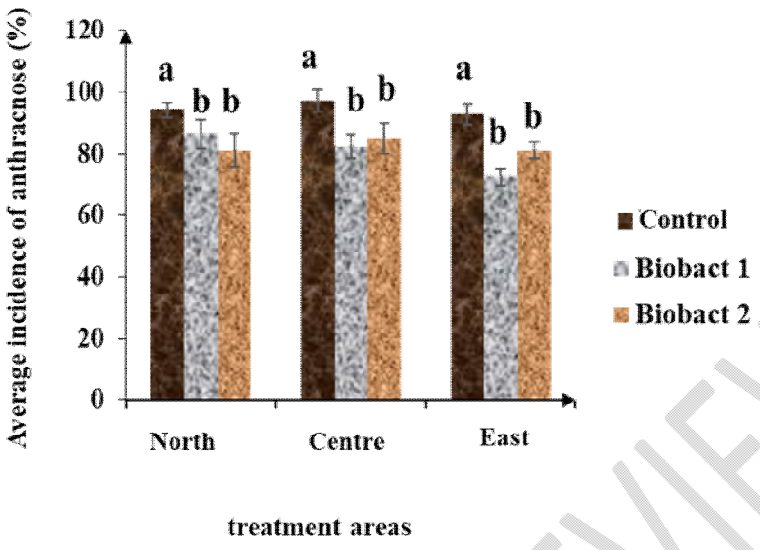

Figure 11: Average severity of anthracnose on cashew plants four (4) months after treatment

Biobact 1: Plants treated with the *Pseudomonas fluorescens* formulation

Biobact 2: Plants treated with *Bacillus subtilis* formulation

Histograms bearing the same alphabetical letters in each zone are not statistically different ($p \leq 0.05$) (Newman and Keuls)

3.3.4 Reduction rate of anthracnose severity index by bacterial formulations

Reduction rates of 42.24 and 41.05% were observed in the northern zone with Biobact 1 (*Pseudomonas fluorescens*) and Biobact 2 (*Bacillus subtilis*) respectively. These reduction rates did not differ significantly at the 5% threshold. In the Centre, a reduction rate of 4.35% was observed with the biopesticide Biobact 1, while the biopesticide Biobact 2 showed a reduction rate of 0%. In the Eastern zones, rates of 7.63 and 10.25% were obtained with Biobact 1 and Biobact 2 respectively (Figure 12).

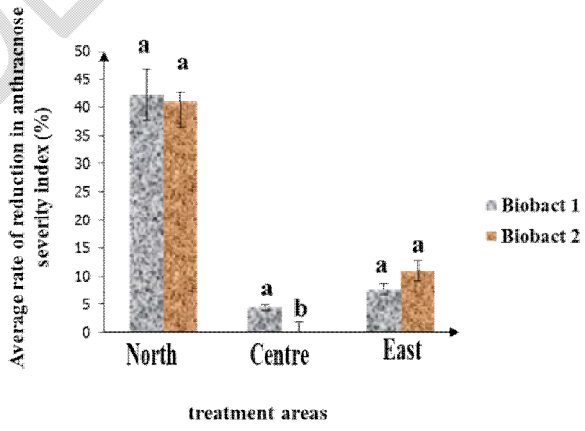

Figure 12 : Average rate of reduction in anthracnose severity index four (4) months after treatment

Biobact 1: *Pseudomonas fluorescens* formulation
Biobact 2: *Bacillus subtilis* formulation

Histograms with the same alphabetical letters in each zone are not statistically different ($p \leq 0.05$) (Newman and Keuls)

4. DISCUSSION

The general objective of this study was to evaluate the efficacy of two formulations of bacterial biocontrol agents based on bacteria isolated from the rhizosphere of cashew trees against anthracnose. *In vitro* antagonism tests were carried out on the fungal *Colletotrichum gloeosporioides* responsible for cashew anthracnose. The aim of these tests was to verify the effect of the two biocontrol agent formulations on the mycelial growth of the pathogenic fungi. The results showed remarkable efficacy of the formulations on the mycelial growth of *Colletotrichum gloeosporioide*s, with an average inhibition rate of 100% at the 1% concentration for all formulations. This would appear to be due to the fact that the bacterial isolates used in the formulation produce antifungal substances that inhibit the growth of pathogenic fungi in agricultural environments. These bacteria have broad-spectrum antagonistic activity against phytopathogenic agents. These activities include direct antibiosis, spatial or nutritional competition and the production of substances toxic to plant pathogenic microorganisms **(Showkat *et al*., 2012)**. These antifungal metabolites act by altering pathogen germination and growth. They may also distort the pathogen's hyphae and alter the appearance of colonies **(Campbell, 1989)**. As for the *Bacillus* sp genus, these bacteria produce antifungal substances such as fengycin and iturin, which inhibit the growth of plant pathogenic fungi **(Mora *et al*., 2011)**. Studies by **Tan *et al*. (2013)** also showed that *Bacillus subtilis* B25 possessing the bacyllomicin biosynthesis gene had antifungal activities against *Aspergillus niger*. Inhibition diameters of the supernatant of *B. subtilis isolates* greater than 8 mm showed that these isolates were capable of producing inhibitory substances against *Phytophtora palmivora*, *Lasidiplodia theobromae* and *Aspergillus niger***(Tan *et al*., 2013)**. The results of biocontrol tests for anthracnose on cashew seedlings in the greenhouse using the two formulations showed beneficial effects in protecting the seedlings. Application of the formulations to cashew seedlings conferred protection against the pathogen *Colletotrichum gloeosporioides*. Anthracnose reduction rates of 85.95 and 62.80% were respectively obtained with formulations based on *Pseudomonas fluorescens* (Biobact 1) and *Bacillus subtilis* (Biobact 2). These results show that the bacterial strains used in these formulations are suitable for biological control of cashew phytopathogenic fungi. This high rate of reduction and the strong growth of the plants compared with the controls are thought to be linked to their ability to produce indole-3-acetic acid and to solubilise phosphorus. Indeed, **Fatima *et al*. (2009)** also showed that the high germination rate and strong growth of the plants were linked to the synthesis of indole acetic acid (IAA). The ability of Bacillus sp. isolates to produce IAA is thought to improve chlorophyll synthesis in the leaves **(Fatima *et al*., 2009)**.This would explain the fact that the leaves of plants treated with the biopesticide formulations were greener than those treated with the phytopathogenic isolates. Similar results were observed with *Bacillus subtilis* strains BTP1 and BC25 against Botrytis cinerea in tomatoes and cucumbers **(Akram, 2008)**. The bacterial formulations demonstrated their ability to significantly reduce the anthracnose severity index in cashew plantations. This reduction in the anthracnose severity index in cashew by the two bacterial biocontrol agents formulated could be explained by the capacity of these bacteria involved in the formulation of biopesticides to produce substances that would induce systemic resistance in cashew trees against anthracnose and bacterial blight. Isolates of *Bacillus* sp. and*Pseudomonas fluorescens* are thought to possess two or three lipopeptide genes (srfAA, fenD and ituC), which are thought to play a major role in promoting plant growth **(Fatima *et al*., 2009)**. These strains could play antibacterial, antifungal, antiviral and

systemic resistance inducing roles in plants **(Stanković *et al*., 2012)**. Several *Bacillus* sp. species isolated from the plant environment have srfAA, fenD and ituC genes **(Mora *et al*., 2011)**. Studies by **Cawoy *et al*. (2015)**revealed that *B. subtlis* 98S strains possessed all 3 genes for surfactin, fengycin and iturin biosynthesis. However, B. subtlis 2504 used by **Ongena *et al* (2007)** in their study only possessed the fengycin biosynthesis gene. Similar results were observed by **Koua *et al*. (2020)**, who showed that *Bacillus subtilis* strains BL 1, BK 1, BL 2, BT 2, BT 5 and BT 10 could induce systemic resistance in cocoa trees against swollen shoot disease (CSSV). He also showed that these strains offered a good possibility of biological control against the swollen shoot virus and phytopathogenic cocoa moulds, and that induction of systemic resistance in cocoa seedlings by B. subtilis isolates reduced the severity of the cocoa diseases caused by *Phytophtora palmivora*, *Lasiodiplodia theobromae* and CSSV. Similarly, work by **Akram in 2008** showed the induction of systemic resistance in tomatoes and cucumbers by strains of *Bacillus subtilis* BTP1 and *Pseudomonas fluorescens* against *Botrytis cinerea* grey mould.

The variation in the efficacy of these two products formulated against anthracnose depending on the treatment areas could be explained by the fact that these three treatment sites are not located in the same agro-ecological zone. This could be explained by the effect of climatic parameters such as temperature and relative humidity, which are different in these three zones. These results corroborate those of **Fofana in 2021**. This author, after using the biofungicides NECO, ASTON and FERCA to combat brown pod rot in cocoa in the Agnéby-Tiassa region, observed variations in disease reduction rates depending on the treatment zones.

5. CONCLUSION

We can conclude that the two biocontrol agent formulations (Biobact 1 and 2) significantly inhibited the mycelial growth of the fungus *Colletotrichum gloeosporioidesin vitro*. These two formulations also significantly reduced the anthracnose severity index on cashew plants in the greenhouse and in cashew plantations. In view of the above, these two bacterial biocontrol agents could be used for biological control of cashew anthracnose in Côte d'Ivoire.

REFERENCES

Akram A. Elicitation de la résistance systémique induite chez la tomate et le concombre et activation de la voie de la lipoxygénase par des rhizobactéries non-pathogènes. Thèse de Doctorat, Université de Liège, Belgique. 2008 **:** 169 p.

Campbell R. Biological Control of Microbial Plant Pathogens. 1st Edn., Cambridge University Press, *Cambridge*. 1989 **:**12 p.

Cardosso J. E., Santos A. A., Rossetti A. G. & Vidal J. C. Relationship between incidence and severity of cashew gummosis in semiarid north-eastern Brazil. *Plant Pathology*. 2004 **:**53 (3) : 363 – 367.

Cawoy H., Debois D., Franzil L., De Pauw, E., Thonart P. & Ongena M. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis*/*amyloliquefaciens*. *Microbial Biotechnology*. **2015** : 8 : 281-295.

Fatima Z., Saleemi M., Zia M., Sultan T., Aslam M., Riaz-ur R. & Chaudhary M. F. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. African Journal of Biotechnology. 2009 : 8 : 219-225.

FIRCA. Fond Interprofessionnel pour la Recherche et le Conseil Agricole. Rapport annuel. 2018 : 64 p. https://firca.ci/ressources/pulications/rapports-annuels-et-bilans. consulté le 11/05/2023.

Fofana B. Caractérisation du verger cacaoyer, identification des parasites fongiques et stratégies de lutte durable contre *Phytophthora* sp. responsable de la pourriture brune des cabosses de cacaoyer dans la région de l'Agnéby-Tiassa au Sud-Est de la Côte d'Ivoire. Thèse de Doctorat de l'Université Félix HOUPHOUËT– BOIGNY, Abidjan, Côte d'Ivoire. 2021 : 187 p.

Groth J. V., Ozmon E. A. & Busch R. H. Respeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries. *Plant Disease*. 1999**:** 83: 1033 – 1038.

Hmouni, A., Hajlaoui M.R. & Mlaiki A. Resistance of *Botrytis cinerea* to benzimidazoles and dicarboximides in sheltered tomato crops in Tunisia. OEPP/ EPPO Bull. 1996 : 26 : 697 – 705.

Issa W., Drissa S., Ibrahima O., Abalo I. K., Issouf D., Léonard O. & Souleymane N. Diseases of cashew nut plants (*Anacardium Occidentale* L.) in Burkina Faso. *Advances in Plants & Agriculture Research.* 2017: 6 (3) :78–83.

Kiwuso P., Esegu J. F. O., Mujuni D & Epila-Otara J. Key Diseases and Insect Pests of Cashew Nut (Anacardium occidentales L.) in the Teso and Lango Farming Systems of Uganda. In Proceeding of the 2nd International Cashew Conference, Masawe PAL, Esegu JFO, Kasuga LJF, Mneney EE, Mujuni MD. (eds). Kampala, Uganda, 26-29 April 2010. CAB International: Wallingford UK. 2013 :. 68-70.

Koua S. H., N'golo D. C., Alloue-Boraud, W. M. & Konan F. *Bacillus subtilis* Strains Isolated from Cocoa Trees (*Theobroma cacao* L.) Rhizosphere for their use as Potential Plant Growth Promoting Rhizobacteria in Côte d'Ivoire. *Current Microbiology*. 2020 : 77 (9) : 2258-2264.

Kouassi A. O. 2012. Revue Nationale pour identifier les initiatives de valorisation non alimentaire de la mangue en Côte d'Ivoire. Revue Nationale no12/Coleacp Paepard-01/BNA12. 43 p.

Kranz P. Measuring plant disease : In : Kranz, J., Rostem, J (eds), Experimental technique in plant desaese epidemiology. *Springer Berlin*. 1988 : 35-50.

Mathur S. B. & Kongsdal O. Common Laboratory Seed Health Testing Methods for detecting fungi. Kandrups Bogtrykkeri, (1st ed.). **2003**. : 436 p.

Mora I. Cabrefiga J. & Montesinos E. Antimicrobial peptide genes in *Bacillus* strains from plant environments. *International Microbiology*. 2011: 14: 213-223.

Pérez-García A., Romero D. et de Vicente A.Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacilli* in agriculture. *Curr. Opin. Biotechnol*. 2011 : **22**(2), 187-193.

Showkat S., Murtaza I., Laila O. & Ali A. Biological Control of *Fusarium Oxysporum* and *Aspergillus* sp. By *Pseudomonasfluorescens* Isolated From Wheat Rhizosphere Soil Of Kashmir. *IOSR Journal of Pharmacy and Biological Sciences.* 2012 : 1: 24–32.

Silué N., Abo K., Johnson F., Camara B., Koné M. & Koné D. Evaluation *in vitro* et *in vivo* de trois fongicides de synthèse et d'un fongicide biologique sur la croissance et la sévérité de *Colletotrichum gloeosporioides* et de *Pestalotia heterocornis*, champignons responsables de maladies foliaires de l'anacardier (*Anacardium occidentale* L.) en Côte d'Ivoire. *Agronomie Africaine.* 2018 : 30 :107 – 122.

Silué N., Soro S., Koné T., Abo K., Koné M. & Koné D. Parasitical fungi in cashew (*Anacardium occidentale* L.) orchard of Côte d'Ivoire*. Plant Pathology Journal*. 2017 : 16 : 82-88.

Soro A. N., Soro S., Yéo G., Kouman A. M. N., Tehua A. A., Silue N., Abo K. & Kone D. Severity and distribution udapted of anthracnose on cashew trees (*Anacardium Occidentale* L.) In Côte D'Ivoire. *Agronomie Africaine* Sp. 2022**:** 34 (1) : 49 – 58.

Soro D. Couplage de procédés membranaires pour la clarification et la concentration du jus de pomme de cajou : performances et impacts sur la qualité des produits. Thèse de doctorat. Institut des régions chaudes, Montpellier SUPAGRO, France. 2012 : 156 p.

Stanković S., Mihajlović S., Draganić V., Dimkić I., Vukotić G., Berić T. & FIRA Đ. Screening for the presence of biosynthetic genes for antimicrobial lipopeptides in natural isolates of *Bacillus* sp. *Archives of Biological Sciences*. **2012**: 64 (4) : 1425-1432.

Tan Z., Lin B. & Zhang R. Novel antifungal protein of *Bacillus subtilis* B25. *Springer Plus*. 2013 : 2 : 543-549.

Téhua A. A.., Soro S., Kouman A. M. N., Yao K. F., Soro N. A., Waze A. M. A-B& Koné D. Efficacy of microbial biopesticide formulations in the control of *Xanthomonas citri* pv. *Mangiferaeindicae* in cashew (*Anacardium occidentale* L.) in Côte d'Ivoire. *International Journal of Agronomy and Agricultural Research*. 2022 :21 (4) : 18-25.

Tonon D., Sikirou R., Adomou A. C., Zinsou V., Zocli B., N'djolosse K. & Bello S. 2017. Efficacité des fongicides Mancozèbe 80 WP et Chlorothalonil-Carbendazime 65 SC contre *Colletotrichum gloeosporioides* agent causal de l'anthracnose de l'anacardier au Bénin. *International Journal of Biological and Chemical Sciences*. 2017 **:** 11(5) : 2093-2105.