

## Efficacy of Biological Fungicide Control on Cashew Nuts Lost caused by *Colletotrichum gloeosporioides* Penz. in the North of Côte d'Ivoire

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**Abstract: Context and Objective:** Anthracnose, caused by *Colletotrichum gloeosporioides*, represents the main disease of cashew in the north of Côte d'Ivoire. This study was conducted to assess the toxicity of three synthesis fungicides, Azoxystrobin, Carbendazim and Carbendazim+Chlorothalonil and two essential oils extracted from *Cymbopogon nardus* and *Ocimum gratissimum* against *Colletotrichum gloeosporioides*. **Material and Methods:** Fungicidal properties of these products were evaluated *in vitro* on mycelial radial growth of *C. gloeosporioides* strain, through six concentrations added to a PDA medium (Potato Dextrose Agar). At the laboratory test, an essential oil, *Ocimum gratissimum* and the synthetic fungicides based on Azoxystrobin and Carbendazim+Chlorothalonil were selected and tested *in situ* against anthracnose disease. **Results:** After 16 days of incubation at  $30 \pm 2$  °C, the mycelial radial growth of *C. gloeosporioides* was completely inhibited from 1 µL/L by Carbendazim, 5 µL/L of Carbendazim+Chlorothalonil, and 1500 µL/L by essential oil of *O. gratissimum*. After reeling mycelial disks, the minimum fungicidal concentration obtained was 25 µL/L for Carbendazim, 100 µL/L for Carbendazim+Chlorothalonil and 2000 µL/L for *O. gratissimum*. The study *in situ* showed a highly significant reduction of the incidence and the severity of anthracnose on the leaves by essential oil of *O. gratissimum*. **Conclusion :** The essential oil of *Ocimum gratissimum* can be used in biological control of the pathogen *Colletotrichum gloeosporioides* in cashew plantation.

**Keywords:** *Anacardium occidentale*, Toxicity, Fungicides, Essential Oils, Côte d'Ivoire

### 1. Introduction

*Anacardium occidentale* L. is a perennial plant grown in several countries of the world, particularly in the tropics in America, Asia and Africa. The cultivation area of cashew is estimated in 2002 to 7.5 million ha. Its culture was introduced in Côte d'Ivoire in the 1960's to contribute to the protection of the ecosystem severely affected by deforestation. It then expanded into the whole savanna area with "every seed". Cashew nut production grew very rapidly, reaching 711,236 tons in 2017. However, the walnut yield per hectare of cashew orchard remains low in Côte d'Ivoire, in the order of 350 to 500 kg.ha<sup>-1</sup> compared to the standard global yield of 1 t.ha<sup>-1</sup> (Djaha *et al.*, 2017). This production deficit could be attributed on the one hand to poor seed quality but

also to the lack of management of farms but, above all, to the presence of pests and diseases. Like diseases, anthracnose, the main disease of cashew orchard in Côte d'Ivoire, caused by *Colletotrichum gloeosporioides* causes a lot of damage to cashew (Koné *et al.*, 2015 ; Soro *et al.*, 2015). Damage from the attack of this pathogen may occur through necrosis on cashew nuts (Koné *et al.*, 2015, Silué *et al.*, 2017 b), thus affecting the quality of nuts and the yield of orchards. Unfortunately at the national level no data appear to exist on the direct loss of cashew yields caused by this polyphage fungus. In this context, the Council of Cotton and Cashew (CCA) established the National Cashew Research Program (PNRA) in 2016 in collaboration with FIRCA and PSAC, which involved all National Universities and

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the National Center of Agronomic Research (CNRA). This study is part of the overall objective of the Program to contribute to improving the productivity of cashew and specifically, it was :

- to determine *in vitro* the efficacy of synthetic fungicides and biofungicides (essential oils) on the mycelial growth of *C. gloeosporioides* ;
- to compare *in situ* the efficacy of the best biological fungicide and the synthetic fungicides on anthracnose disease on the cashew leaves ;
- to evaluate *in situ* the efficacy of the fungicides on the harvest of cashew nuts.

## 2. Material and methods

### 2.1. Material

#### 2.1.1. Plant material

Plant material consists of cashew plants in production with an age of between 10 and 20 years. Two

aromatic plants, *Ocimum gratissimum* and *Cymbopogon nardus* were used to extract essential oils that were used in biological control.

#### 2.1.2. Biological material

The biological material used consists of a strain of *Colletotrichum gloeosporioides* from the Laboratory of Plant Physiology at Felix Houphouët-Boigny University of Abidjan in Côte d'Ivoire. This strain was isolated from the leaves reached from the locality of Séguéla in the western of Côte d'Ivoire and was the most virulent among several strains.

#### 2.1.3. Fungicides

Three fungicides and two essential oils were tested in the control of *C. gloeosporioides* in this study (Table 1).

**Table 1** : Fungicides and essential oils used

Name of active substance	Dose	Chemical family
Carbendazim+Chlorothalonil	100 g/L + 550 g/L	Benzimidazole + adjuvant phthalique
Carbendazim	500 g/kg	Benzimidazole
Azoxystrobin	250 g/L	Strobilurine
<i>Ocimum gratissimum</i>	30 g/L	Essential oil
<i>Cymbopogon nardus</i>	30 g/L	Essential oil

## 2.2. Methods

### 2.2.1. Preparation of culture medium and isolation of *Colletotrichum gloeosporioides* strain

The efficacy *in vitro* of the fungicides and biofungicides to inhibit growth of *C. gloeosporioides* was determined on a PDA medium. The synthetic fungicides were tested at concentrations of 1 ; 5 ; 25 ; 50 ; 100 and 150 µL/L. Each concentration was directly incorporated into a previously prepared PDA culture medium and cooled at 45 °C (Silué *et al.*, 2017a). Essential oils were tested each at concentrations of 500 ; 1000 ; 1500 ; 2000 ; 2500 and 3000 µL/L. Each concentration has been added to the PDA medium in addition to a few drops of tween 20 (Soro *et al.*, 2010 ; Tuo, 2013). The preparation of the PDA medium consisted of weighing 20 g of potato, 20 g of D-glucose and 20 g of Agar to which the distilled water was added to 1 liter. The mixture was then prepared for autoclave at the temperature of 121 °C and the pressure of 1 bar for 30 min. The products were added in Petri dish of 90 mm diameter at 5 dishes per concentration and let solidify under the culture hood. After the media were solidified, each Petri dish was sown with a 7 mm mycelial disk from a 20-day strain, then sealed and incubated at the oven at 30 ± 2 °C.

### 2.2.2. Rate of inhibition *in vitro*

The mycelial growth of the fungal colony was assessed by measuring on two opposite diameters drawn on the reverse of the Petri dish (Soro *et al.*, 2010 ; Tuo, 2013 ; Silué *et al.*, 2017a). Measurements were made by 48-hour intervals until mycelial filaments reach the periphery of the Petri dish in the lot of the control over sixteen (16) days. At the end of the 16 days, when mycelial growth is not observed, it is sown in a new Petri dish containing a simple PDA medium and maintained under the same conditions. After seven (7) days of incubation, the concentration is known as fungistatic if the growth of mycelium has resumed or fungicide if no growth is found (Soro *et al.*, 2010 ; Tuo, 2013). On the basis of this experiment, the rate of inhibition (Ti) of each fungicide at a given concentration is determined from equation 1 (Soro *et al.*, 2010).

$$Ti = \frac{D_{co} - D_{cx}}{D_{co}} \times 100 \quad (1)$$

**Dco** : Average diameter in the control ;

**Dcx** : Average diameter in the essay.

The inhibitory concentrations at 50 % (CI<sub>50</sub>) and 90 % (CI<sub>90</sub>) were determined graphically from the linear relationship between the decimal logarithm of the fungicide concentration in abscissa and the probits

values from the inhibition percentages of the mycelial growth in ordered (Attrassi *et al.* 2007 ; Soro *et al.*, 2010).

### 2.2.3. Experimental sites

Three parcels were selected from prospecting on the basis of criteria such as phytosanitary problems and accessibility of the plot to carry out the tests. The parcels are located in the localities of Napié (9°18'00" latitude, -5°35'00" longitude) in the Southeastern of Korhogo, Nabrémkaha (9°24'34" latitude, -5°42'55" longitude) in the west of Korhogo and Tortiya (8°46'00" latitude, -5°41'00" longitude) in the Department of Niakara.

### 2.2.4. Experimental device and application of fungicides on cashew trees

At the laboratory test, three fungicides were selected for orchards, including Azoxystrobine, Carbendazim+Chlorothalonil and *O. gratissimum* essential oil. Synthetic fungicides were tested at the concentration of 500 mL.ha<sup>-1</sup> and the biological fungicide at the concentration of 300 mL.ha<sup>-1</sup>. On each site, the experiment was performed according to a device consisting of 4 Fisher blocks completely randomized to 4 elementary repetitions. Each elementary repetition consists of 5 cashew trees arranged on one line and spaced between them 10 m is a basic plot of 500 m<sup>2</sup>. A total of 20 cashew trees are selected for each treatment on a plot. Ribbons of different colors (yellow, blue, green and white) were used to mark trees to treat and differentiate treatments between them (Tonon *et al.*, 2017). The treatments are composed of control (T1) Azoxystrobine (T2), Carbendazim+Chlorothalonil (T3), biological fungicide, essential oil of *O. gratissimum* (T4). After installation of the device, application of the fungicides was carried out using a Cifareli brand atomizer with capacity of 15 L. The treatments were repeated 3 times per month in every orchard.

### 2.2.5. Indice of severity of anthracnose in plantation

The products efficacy were assessed through the mean incidence and the indice of severity of anthracnose from necrotic stains observed on leaves (Issa *et al.*, 2017 ; Tonon *et al.*, 2017). The incidence was calculated from the ratio of the number of diseased trees to the total number of trees for each treatment in the orchard. The severity assessment was done on two opposite sides of the crown of each tree. On each side, a quadrat of 1 m<sup>2</sup> was delineated at the foliage level and a representative bud was chosen for the assessment that was made on the last 5 leaves from the tip of the bud to its base. Then the severity of the disease was assessed using a rating scale ranging from 0 to 9 (Groth *et al.*, 1999 ; Cardoso *et al.*, 2004) as follows : 0 = Absence of symptoms ; 1 = 1-5 % ; 3 = 6-10 % ; 5 = 11-25 % ; 7 = 26-50 % ; 9 <

50 % of the infected leaf area. At the end, the indice of severity of anthracnose was determined in each orchard from equation 2 (Kranz, 1988) :

$$Is = \frac{\sum (xi \cdot ni)}{N \cdot Z} \times 100 \quad (2)$$

**Is** = Indice of severity of anthracnose ; **xi** = Severity *i* of the disease on the tree ; **ni** = Number of trees with the severity *i* ; **N** = Total number of trees ; **Z** = Highest scale of severity.

### 2.2.6. Measuring of economic impact of anthracnose in orchard

At the production level, the assessment consisted of determining the daily mass of nuts falling under trees by treatment. The evaluation began with a collection of nuts under the 5 trees forming the treatment in each block. The harvested nuts were then sorted into 2 lots, a lot of good nuts and the second batch of bad nuts. The mass of the 2 batches was determined by weighing using an electronic weighing scale with the capacity of 50 kg. This evaluation was conducted over a week more precisely from March 23-30, 2018. Finally, the economic impact was determined through the economic loss of performance (P) using equation 3 (Judenko, 1972).

$$\begin{cases} W = \frac{100 \times RT}{100 - L} \\ L = \frac{x \times p}{100} \end{cases} \quad \begin{matrix} P = W - RT \\ \alpha = \frac{(RTx - RT0) \times 100}{RTx} \end{matrix} \quad (3)$$

With

**P** : Economic loss of performance ; **W** : Relative loss ; **RT** : Total return on the parcel ; **Rtx** : Total performance for treatment x ; **Rto** : Total tree yield in control ; **L** : Percentage of loss ; **α** : Coefficient related to nut loss ; **p** : Percentage of trees attacked in treatment.

### 2.2.7. Data analyse

The data collected were analyzed for variances (ANOVA) at the level of 5 % using the Statistica 7.1 software. When the difference was significant, averages were classified as homogeneous by the Newman-Keuls test. This test was used for the mycelia radial growth inhibition, the incidence of anthracnose and the harvest of cashew nuts. Fisher LSD test was used for the severity of anthracnose. The graphics were made using the Excel software 2013.

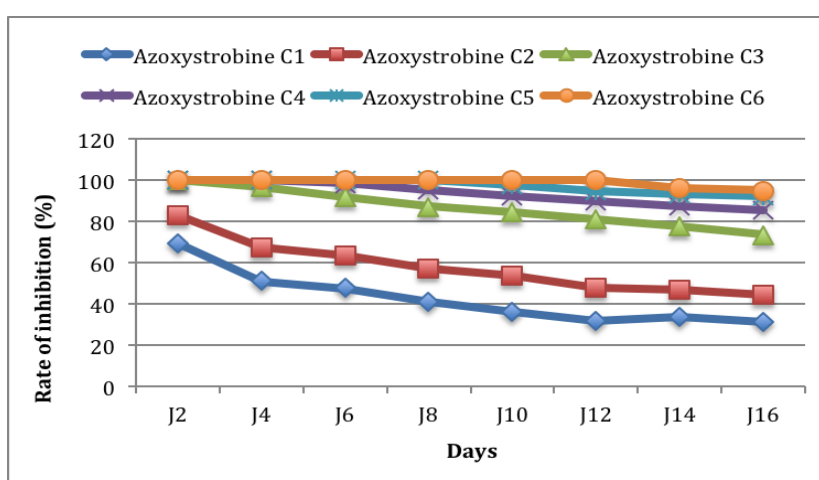
### 3. Results

#### 3.1. *In vitro* efficacy of fungicides on mycelial growth of *C. gloeosporioides*

*In vitro* tests showed that efficacy in terms of inhibition of mycelial growth depends on the nature of the chemical compound or essential oil. The fungicide Azoxystrobin was moderately fungitoxic at 1  $\mu\text{L/L}$  and 5  $\mu\text{L/L}$  with respective inhibition rates of 31.08 % and 44.49 % in the sixteenth day. It was very fungitoxic from 25  $\mu\text{L/L}$  with an inhibition rate of 73.42 % in the sixteenth day. However, no tested concentration inhibited the growth of strain mycelium throughout 16 days of incubation (Figure 1) at 100 %. Carbendazim was very fungitoxic on *C. gloeosporioides* strain. Throughout the sixteen days of incubation, this fungicide completely inhibited

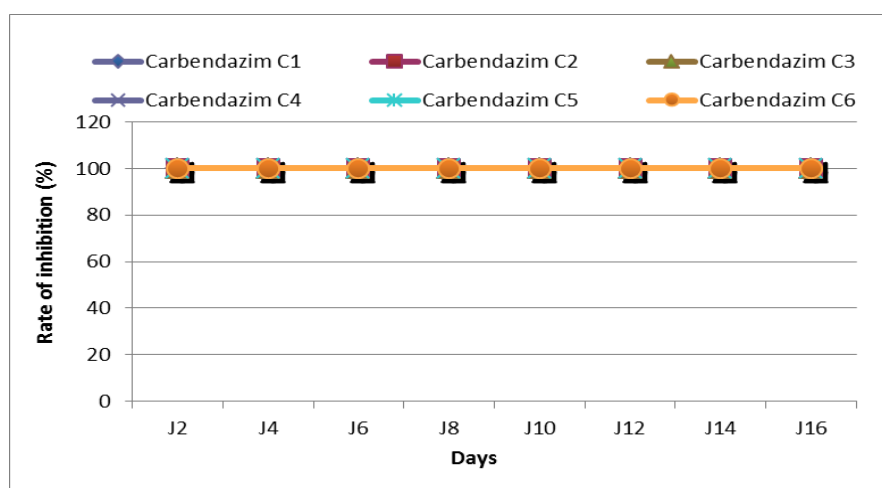
mycelial growth of the fungus from 1  $\mu\text{L/L}$  (Figure 2). By adding Chlorothalonil to Carbendazim, the product loses efficiency at 1  $\mu\text{L/L}$ . Indeed, with fungicide Azoxystrobin, total inhibition of mycelial growth was observed from 5  $\mu\text{L/L}$  (Figure 3).

Two essential oils were also cause the inhibition of the mycelium of *C. gloeosporioides* strain. The essential oil of *O. gratissimum* was more effective by inhibited the total mycelium growth from 25  $\mu\text{L/L}$  while with the essential oil of *Cymbopogon nardus*, up to 150  $\mu\text{L/L}$  total inhibition of mycelium was not observed throughout the sixteen days of incubation (Figures 4 and 5). Statistical analysis revealed that the difference between these two essential oils was very significant ( $P < 0.001$ ).



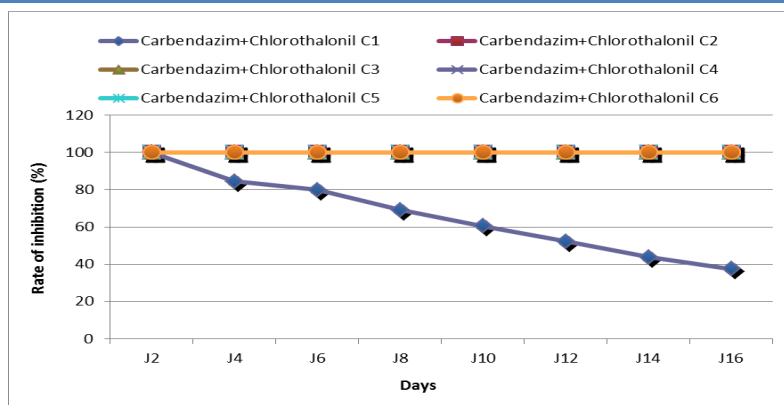
C1 : 1  $\mu\text{L/L}$  ; C2 : 5  $\mu\text{L/L}$  ; C3 : 25  $\mu\text{L/L}$  ; C4 : 50  $\mu\text{L/L}$  ; C5 : 100  $\mu\text{L/L}$  and C6 : 150  $\mu\text{L/L}$

**Figure 1 :** Inhibition of mycelial radial growth of *C. gloeosporioides* by the fungicide Azoxystrobin



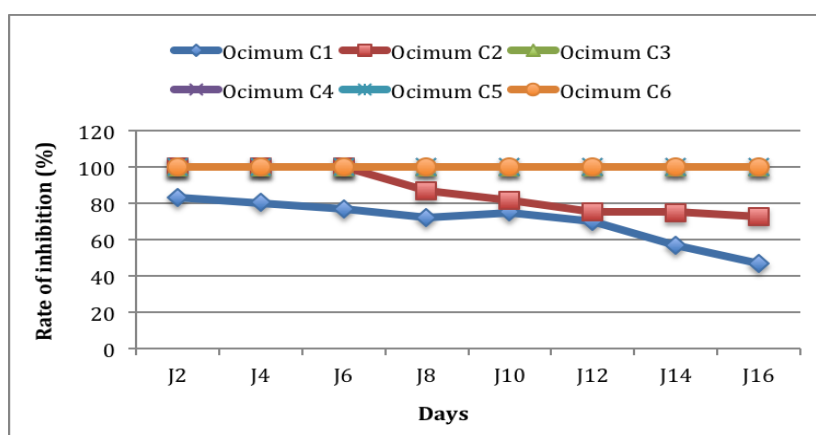
C1 : 1  $\mu\text{L/L}$  ; C2 : 5  $\mu\text{L/L}$  ; C3 : 25  $\mu\text{L/L}$  ; C4 : 50  $\mu\text{L/L}$  ; C5 : 100  $\mu\text{L/L}$  and C6 : 150  $\mu\text{L/L}$

**Figure 2 :** Inhibition of mycelial radial growth of *C. gloeosporioides* by the fungicide Carbendazim



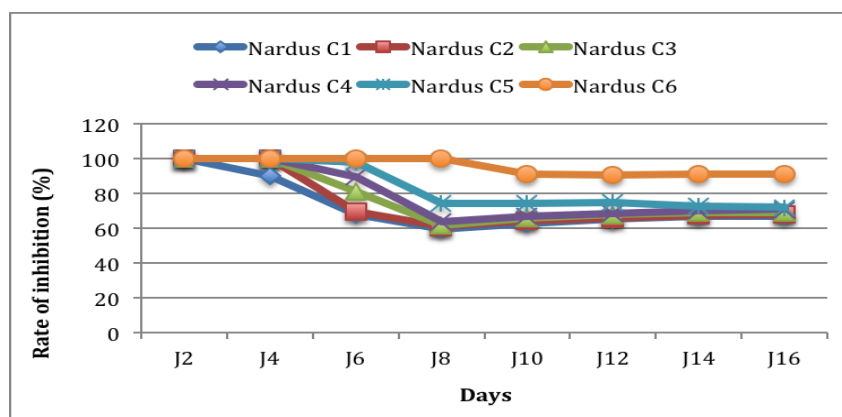
C1 : 1 µL/L ; C2 : 5 µL/L ; C3 : 25 µL/L ; C4 : 50 µL/L ; C5 : 100 µL/L and C6 : 150 µL/L

**Figure 3 :** Inhibition of mycelial radial growth of *C. gloeosporioides* by the fungicide Carbendazim+Chlorothalonil



C1 : 500 µL/L ; C2 : 1000 µL/L ; C3 : 1500 µL/L ; C4 : 2000 µL/L ; C5 : 2500 µL/L and C6 : 3000 µL/L

**Figure 4 :** Inhibition of mycelial radial growth of *C. gloeosporioides* by essential oil of *Ocimum gratissimum*



C1 : 500 µL/L ; C2 : 1000 µL/L ; C3 : 1500 µL/L ; C4 : 2000 µL/L ; C5 : 2500 µL/L and C6 : 3000 µL/L

**Figure 5 :** Inhibition of mycelial radial growth of *C. gloeosporioides* by essential oil of *Cymbopogon nardus*

### 3.2. Minimal fungicidal concentration and inhibitory concentrations IC<sub>50</sub> and IC<sub>90</sub>

The table 2 shows the fungicidal minimum concentrations (FMC) and inhibitory concentrations at 50 % (IC<sub>50</sub>) and 90 % (IC<sub>90</sub>) of the various fungicides used. These concentrations vary from product to product. The fungicide Azoxystrobin and

the essential oil of *C. nardus* were not fungicides at concentrations tested. Also the lowest inhibitory concentrations are observed only with Carbendazim (IC<sub>50</sub> < 0.5 and IC<sub>90</sub> = 0.6). This fungicide appeared to be the most effective fungicide against the *C. gloeosporioides* strain *in vitro*.



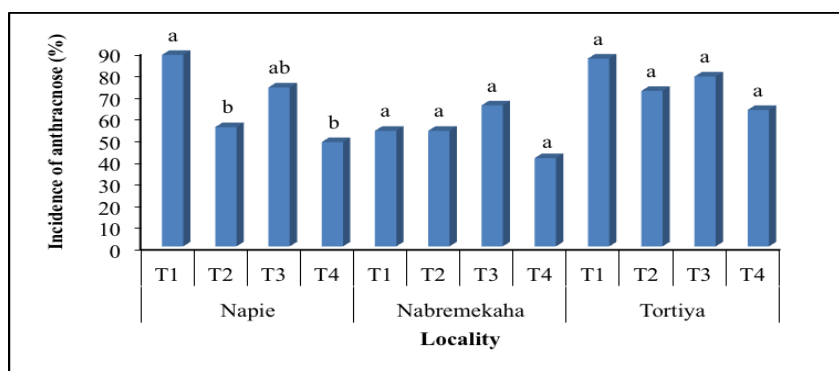
**Table 2 :** Fungicidal minimum concentrations and inhibitory concentrations IC<sub>50</sub> et IC<sub>90</sub>

Fungicides	FMC (ppm)	IC <sub>50</sub> (ppm)	IC <sub>90</sub> (ppm)
Azoxystrobin	> 150	6,60	75,86
Carbendazim	25	< 0,5	0,60
Carbendazim + Chlorothalonil			
	100	1,38	3,98
<i>O. gratissimum</i>	2000	549,54	1288,25
<i>C. nardus</i>	> 3000	> 500	2985,38

### 3.3. Efficacy of fungicides tested in orchard on anthracnose disease

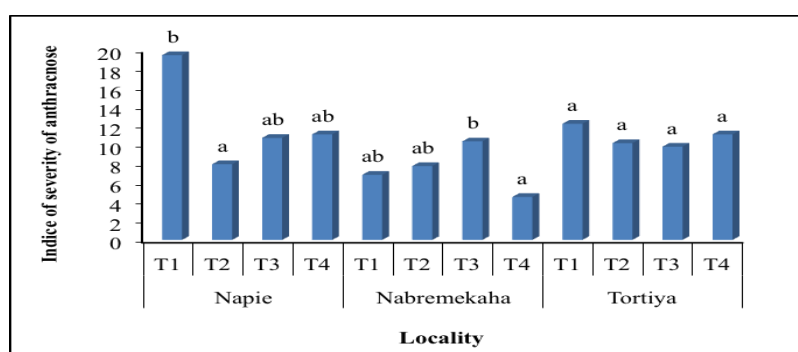
The incidence of anthracnose varied from one locality to another while remaining between 40 and 90 %. On the Napié site, treatments reduced the incidence of anthracnose by more than 38 % ; 17 % and 45 % respectively on foliage treated with Azoxystrobin, Carbendazim+Chlorothalonil and essential oil of *O. gratissimum* compared to control. This reduction is also obtained with each product on Tortiya site but with lower percentages. On the Nabremekaha site, however, only the essential oil of *O. gratissimum* reduced the incidence of 23 % (Figure 6). At the level of anthracnose severity, at Napié site, it has been reduced by more than 59 %, 45 %, and 43 % respectively on the foliage treated with Azoxystrobin, Carbendazim+Chlorothalonil and essential oil of *O. gratissimum* compared to control. This reduction was also obtained on Tortiya site. On

this site, the percentage of reduction varies according to the products. The Carbendazim+Chlorothalonil fungicide appeared to be the most effective with a reduction rate of 20 % while the one with Azoxystrobin and the essential oil of *O. gratissimum* reduced severity by 17 % and 9 % respectively. However, on Nabremekaha site, only essential oil of *O. gratissimum* was effective by reducing severity by 33 % (Figure 7). The aggregated statistical analysis of localities revealed that the fungicide Azoxystrobin had a significant effect at 5 % on the incidence of anthracnose while essential oil of *O. gratissimum* was very significant ( $P < 0.001$ ). Also, in severity, treatments with Azoxystrobin and essential oil *O. gratissimum* had a significant effect at 5 %. From the incidence or severity of anthracnose, the differences observed at the survey levels were highly significant ( $P < 0.001$ ).



**Figure 6 :** Effect of treatments on the incidence of anthracnose according to different localities

The diagrams with the same letters are not significantly different according to the Newman-Keuls test at the level of 0.05.



**Figure 7 :** Effect of treatments on the severity of anthracnose according to different localities

The diagrams with the same letters are not significantly different according to the LSD of Fisher test at the level of 0.05.

### 3.4. Efficacy of fungicides on the economic impact of anthracnose in plantation

The table 3 presents daily cashew nuts production per treated tree. The mass of good quality nuts (NGN) remains between 0.95 and 10 Kg.ha<sup>-1</sup> with a maximum obtained with the essential oil of *O. gratissimum* treatment in Nabremekaha. The low quality nut (NLN) is between 0 and 1 Kg.ha<sup>-1</sup>. However, statistical analysis shows that the effect of treatments is not significant at the 5 threshold on this production. In contrast, locality\*treatment produces a

significant effect to 5 % in good quality nuts. This shows that good quality nut production is linked to the locality factor. Gains on the calculated economic loss, loss varies from treatment to treatment depending on the location. The highest gain is obtained with the treatments Carbendazim+Chlorothalonil and Azoxystrobin respectively in the localities of Napié and Tortiya followed by the essential oil of *Ocimum gratissimum* treatment in the locality of Nabremekaha.

**Table 3 :** Effect of treatments on cashew nut production by localities

Locality	Treatment	Average Performance GQN (kg.ha <sup>-1</sup> )	Average Performance LQN (kg.ha <sup>-1</sup> )	Economic loss of total return (kg.ha <sup>-1</sup> )
Napié	T1	5,25 ± 2,22 <sup>ab</sup>	0,15 ± 0,10	-
	T2	0,95 ± 0,57 <sup>a</sup>	0	0
	T3	8,00 ± 5,48 <sup>ab</sup>	0,35 ± 0,44	7,05
	T4	6,67 ± 1,67 <sup>ab</sup>	0,11 ± 0,20	1,55
Nabrémkaha	T1	1,90 ± 0,99 <sup>ab</sup>	0,1 ± 0,10	-
	T2	1,60 ± 1,08 <sup>ab</sup>	0,05 ± 0,10	0
	T3	1,50 ± 1,73 <sup>ab</sup>	0	0
	T4	10,0 ± 7,64 <sup>b</sup>	0,22 ± 0,20	5,65
Tortiya	T1	7,00 ± 3,27 <sup>ab</sup>	0,65 ± 0,25	-
	T2	9,00 ± 3,56 <sup>ab</sup>	1,05 ± 0,66	7,05
	T3	7,50 ± 3,11 <sup>ab</sup>	0,9 ± 0,74	2,65
	T4	6,11 ± 2,55 <sup>ab</sup>	0,78 ± 0,38	0
<i>P</i> value (Treatments x localities)		0,011410	0,589810	-
<i>P</i> value (Treatments)		0,083495	0,886547	-

GQN : Number of good quality nuts ; LQN : Number of low quality nuts.

Yields of the same letters are not significantly different according to the Newman-Keuls test at the level of 5 %.

**T1** : Control ; **T2** : Azoxystrobin (500 mL.ha<sup>-1</sup>) ; **T3** : Carbendazim+Chlorothalonil (500 mL.ha<sup>-1</sup>) ; **T4** : *Ocimum gratissimum* (300 mL.ha<sup>-1</sup>)

### 4. Discussion

The study *in vitro* showed fungitoxic activity of the tested products. Synthetic fungicides had an inhibitory effect at all concentrations tested on mycelial growth of the *C. gloeosporioides* strain. Carbendazim and Carbendazim+Chlorothalonil were more effective by fully inhibited mycelium growth at certain concentrations. Carbendazim inhibited mycelium growth from 1 µL/L with an IC<sub>50</sub> below 0.5 µL/L at 100 %, while the fungicide effect was observed from 25 µL/L. As for the association Carbendazim+Chlorothalonil, the IC<sub>50</sub> is obtained at 1.38 µL/L, the total inhibition was observed from 5 µL/L and the fungicidal effect from 100 µL/L. The strong fungitoxic of these two products could be explained by the fact that active material, Carbendazim, is a compound interfering with the formation and/or operation of microtubules, blocking cell divisions and elongation of mycelial hyphae (Leroux, 2013). These results are consistent with those of Chand *et al.* (2013) and Silué *et al.* (2017a),

which showed the effectiveness of Carbendazim in the control of the fungal *Colletotrichum gloeosporioides*. However, the fungicide based on Azoxystrobin was less effective compared to the two fungicides. Azoxystrobin inhibitory activity on the mycelial growth of *Colletotrichum gloeosporioides* was previously revealed by Chand *et al.* (2013) and Silué *et al.* (2017a). The two essential oils tested *in vitro* were also inhibited the fungus strain. The maximum inhibition threshold (100 %) was reached at 1500 µL/L for essential oil of *O. gratissimum* while essential oil of *C. nardus* was reached at 3000 µL/L only on the first eight days of incubation. The non-resumption of mycelial growth of the fungus from 2000 µL/L after transfer on simple PDA medium reflects a fungicide activity of essential oil *O. gratissimum*. Camara *et al.* (2007), Soro *et al.* (2010) and Tuo (2013) showed that essential oils have fungicidal activity. The biological activity of an essential oil is to relate to its chemical composition and possible synergistic effects between its

components. Its value takes into account its constituents and not only its majority compounds (Lahlou, 2004).

The inhibition of anthracnose activity in cashew field was confirmed with the Carbendazim+Chlorothalonil, Azoxystrobin and essential oil of *O. gratissimum*. The impact of anthracnose on cashew field was significantly reduced on foliage treated with Azoxystrobin ( $P < 0.05$ ). However, it was very small ( $P < 0.001$ ) on foliage treated with essential oil of *O. gratissimum*. This indicates that the active substance of Azoxystrobin and essential oil of *O. gratissimum* applied on cashew foliage have been very effective against the fungus *C. gloeosporioides*. The efficacy of these products could be explained by an increase in plant resistance to the pathogen. The resistance of plants treated with the essential oil of *O. gratissimum* would be explained by an activation of the plant defense system. The increase in chitinase and peroxidase activity or an increase in enzyme activity in leaves resulting in systemic resistance in these plants (Colpas *et al.*, 2009). The incidence of anthracnose on leaves of treated trees, remains below 50 % in all localities while the severity level remains below 60 %. In its study on the efficacy of the Carbendazim and Carbendazim+Chlorothalonil against *C. gloeosporioides* causal agent of anthracnose in Benin, Tonon *et al.* (2017) obtained a rate of reduction between 80 and 98 % in the incidence of anthracnose by Carbendazim+Chlorothalonil used at the dose of 2 L.ha<sup>-1</sup> while for severity it is between 89 and 91 %.

The different treatments carried out in the field to control the fungus have been effective on nuts production and their quality. The amount of nuts produced varies from the localities. The reduction of the incidence and the anthracnose severity by treatments gains on the economic loss.

## Conclusion

This study assessed the antifungal activity *in vitro* of *Ocimum gratissimum* and *Cymbopogon nardus* essential oils compared with that of synthetic fungicides based on Azoxystrobin, Carbendazim and Carbendazim+Chlorothalonil. Synthetic fungicides from Carbendazim and Carbendazim+Chlorothalonil and essential oil of *O. gratissimum* had a significant inhibitory effect on mycelial growth of the fungus. This growth was inhibited at 100 % for most concentrations on the second day of incubation and subsequently decreased for low concentrations. However, these products had a fungicidal effect at some concentrations from 25 µL/L for Carbendazim, 100 µL/L for Carbendazim+Chlorothalonil, and 2000 µL/L for essential oil of *Ocimum gratissimum*. Also, this study showed that synthetic fungicides based on

Azoxystrobin, Carbendazim+Chlorothalonil and essential oil of *Ocimum gratissimum* used in field reduce the symptoms of anthracnose disease on leaves in cashew. Substance active of Azoxystrobin was significantly effective on incidence while the essential oil of *O. gratissimum* was very significant. Also, both treatments were significantly effective in the severity of anthracnose. The products increase cashew nut production. The essential oil of *Ocimum gratissimum* was effective as a biological control of the pathogen *Colletotrichum gloeosporioides* in cashew orchard.

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