Comparative effectiveness of synthetic fungicide and Neem seeds aqueous extract on *Colletotrichum gloeosporioides*, yam anthracnose causative disease agent

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Abstract

Anthracnose is economically the most damaging yam disease in the course of its growth against which no fungicide is approved. The objective of this work is to compare the effectiveness of synthetic fungicide against neem seeds aqueous extract on *Colletotrichum gloeosporioides*, yam anthracnose causative agent. The only stock solution of synthetic fungicide (SF) was compared with three stock solutions of neem aqueous extract (NS1, NS2 and NS3) macerated for 24 and 48 hrs. Three concentrations were separately prepared by incorporating 10; 25 and 50 % of each stock solution in the culture medium for in vitro testing. 45 µL of fungicide and neem extract were applied separately to fungi inoculum used to infect yam detached leaves. Fungi mycelial growth and conidial germ tube elongation were completely inhibited by fungicide. However, they were gradually inhibited according increasing neem aqueous extract concentrations. Neem stock solution NS3 macerated at 48 h showed similar effect with the fungicide in vitro (P > 0.05). Fungi inoculum treated with synthetic fungicide and 25 and 50 % of NS3 did not induce any necrosis symptoms, five days after inoculation. This experiment constitutes a basic study for future yam anthracnose management on yam farms.

Key words: Anthracnose, aqueous extract, yam

I. INTRODUCTION

Yam (*Dioscorea* spp.) is a staple food in a number of world tropical countries. It is mainly cultivated for its tubers endowed with exceptional nutritional qualities because they are rich in starch (important source of energy), fiber, vitamins (C, B1, and B3), minerals (iron), carbohydrates, proteins and antioxidant substances [1]. This tuber constitutes the basic diet for more than 2/3 of the Ivorian population. Indeed, out of 6 million tons of yam produced in 2008, more than 70% were self-consumed [2]. A survey conducted by [3] found that yam is the most consumed food in households after rice. This high demand coupled with year-round availability could position yams as a substitute for relatively expensive imports. [4] Meanwhile, global demand for yam tubers will grow stronger in the coming years and reach more than 60 million tons of fresh tubers in 2020 [5].

But, yam knows today, a decline in yield although the cultivated areas evolve. Indeed, a study carried out by the Ecole Normale de Statistique et d'Economie Appliquée [6] showed that the yield per hectare of yam rose from more than 88,000 to less than 69,000 t / ha from 2000 to 2010. This low production of yam results from several constraints, one of the most important of which is the strong fungal pressure due to the intensification of the crop.

Anthracnose caused by the fungus *Colletotrichum gloeosporioides* is the main fungal disease of yams in all growing areas [7]. It attacks all parts of the plant during its cropping cycle, reducing the photosynthetic surface, causing yield losses of up to 90% in more susceptibilities *D. alata* varieties [8].
Several control methods have been deployed against this pathology; namely: the use of improved varieties [2], more elaborate cultivation techniques and synthetic fungicides. However, this latter method presents enormous disadvantages due to the lack of expertise of the producers in the handling of the products and cases of resistance of the pathogen were also reported [9]. Nowadays, there are no approved fungicides for yam anthracnose, and the damage caused by this disease is constantly increasing in yam farms. A control involving the use of new active ingredients and plant extracts could help to fight the causative agent and reduce disease incidence.

Moreover, the combination of active ingredients Chlorothalonil and Carbendazime has been used successfully by [10] against causative vegetable diseases fungi in the farms. The effectiveness of these molecules has also been demonstrated in fungi of the plant pathogenic Ascomycetes class [11, 12]. Similarly, [13] revealed the antifungal properties of neem seed extracts. Neem seed oil was also used by [14] to reduce considerably the incidence of cocoa brown rot pods. [15] also revealed the antifungal properties of neem extract on C. gloeosporioides infecting Jatropha curcas.

The objective of this study is to compare the in vitro effectiveness of aqueous neem seeds extract and synthetic fungicide on C. gloeosporioides, and the ability of both products to reduce spots caused by this fungus on yam detached leaves, for future yam anthracnose management in yam farm using neem seeds aqueous extract.

II. MATERIALS AND METHODS

2.1. Fungal Material

Three isolates of Colletotrichum gloeosporioides responsible for yam (D. alata) anthracnose were used. There were isolated from the leaf necrosis of yam from the experimental sites of the Nangui Abrogoua University (Abidjan) and Robert porte (Soubré).

2.2. Plant material and Fungicide

The plant material used consisted of neem seeds almonds and apparently healthy yam leaves. Neem seeds almonds were obtained from the mature seeds collected from Yamoussoukro district in the central region of Côte d'Ivoire. They were first pulped and then kept under at room temperature at 25 ± 2°C for 30 days. The dry seeds were finally husked and the almonds were used to prepare the aqueous extract.

The apparently healthy mature yam leaves between positions 4 to 6 from the apex of the yam plant were used to perform the fungicide and aqueous neem extract efficacy tests on leaves.

A binary liquid formulation fungicide having two active ingredients [Chlorothalonil (550 g/L) and Carbendazime (100 g/L)] was used. As per manufacturers’s indications, this synthetic fungicide is capable of controlling tomato and eggplant rot. It is homologated in Côte d'Ivoire.

2.3. Preparation of stock solutions and concentrations

The method of [16] was used for the preparation of the neem aqueous extract. Three different stock solutions were thus prepared by adding separately 100 mL of sterile distilled water to the finely ground powder derived from 10; 25 and 50 g of neem seeds almonds kernels using laboratory mortar and pestle.

The solutions obtained were vigorously homogenized ever 6 h and then left in dark glass bottles for two maceration times (24 and 48 hrs) at room temperature according to method used by [17]. After maceration, each solution was filtered through double layered muslin cloth according to method of [18]. The neem seeds aqueous extract solutions obtained [NS1 (100 mg/mL), NS2 (250 mg/mL) and NS3 (500 mg/mL)] were then followed by sterile hydrophilic cotton according to the method of [19] for sterilizing.

The synthetic fungicide is in liquid form, the only stock solution (5 mL/L or 5 g/L or 5 mg/mL) recommended by the manufacturer was prepared by solubilizing using sterile distilled water.
Three different concentrations were prepared by incorporating 10; 25 and 50 % of each stock solution in the culture medium according to the method of [20]. Twenty and ten ml of amended media were poured respectively into 90 mm diameter Petri dishes and test tubes.

2.4. Evaluation of antifungal activity of synthetic fungicide and neem seeds aqueous extract

2.4.1. Inhibition of mycelial growth

The in vitro effectiveness of the synthetic fungicide and neem seeds aqueous extract was evaluated by poisoned food technique using potato dextrose agar. To study the mycelial growth of isolates of *C. gloeosporioides* on these culture medium previously prepared, 5 mm diameter discs of the tested fungal culture from ten day-old, was deposited in the center of each Petri dish containing the solidified medium, amended with the both products. PDA medium without products served as control. Each treatment was replicated five times. Each Petri dish was sealed with parafilm and incubated in darkness at 25±2°C for 7 days. The diameters of the growing mycelium were measured every 24 hrs as soon as mycelial colonies appeared, according to two perpendicular lines drawn on the undersides of Petri dishes. The end of the experiment was marked by the colonization of the surface of the culture medium in the control Petri dishes by fungus mycelial colonies. The experiment was repeated three times for the fungicide and the neem seeds aqueous extract at each maceration period. All the Petri dishes were arranged in a complete randomized design. The inhibition rate of mycelial growth was then calculated using the means of the two growth diameters of each fungus for each synthetic fungicide or neem seeds aqueous extract concentration according to the slightly modified formula of [21]:

\[
I = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{Dc - Dt}{Dc} \times 100 \right)
\]

**I**: Inhibition rate of mycelial growth (%); **n**: Repeat number; **Dc**: Diameter of fungal colony in the control (mm); **Dt**: Diameter of fungal colony in the treatment

2.4.2. Inhibition of conidia germ tube elongation

Conidia of the *C. gloeosporioides* isolates of ten day-old on PDA medium were used to carry out the germination and germ tubes elongation test according to the modified method of [22]. The conidia were first harvested in sterile distilled water and then adjusted to 10⁷ conidia per mL using the haemocytometer, under 40 X magnification. The different concentrations 10; 25 and 50 % of each stock solution of the fungicide and neem seeds aqueous extract were incorporated into the liquid medium (distilled water + glucose) to test the sensitivity of the conidia. The liquid medium containing neither fungicide nor aqueous extract served as control. 100 μL of the conidia suspension of each fungal isolate were added to each preparation. For each concentration of the fungicide or aqueous extract, three test tubes were used. After 20 h of incubation, 10 μl of each preparation were deposited between plate and slide and in case of emission, germ tubes length were measured using a micrometer incorporated into the objective of the microscope. Conidia were considered germinated if the length of the germ tube (μm) is greater than its smallest diameter. The experiment was repeated three times. The inhibition percentage of germ tube elongation (I) was then calculated for each fungal isolate in the presence of each concentration of fungicide and aqueous extract according to the formula [21]:

\[
I = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{GTL_c - GTL_t}{GTL_c} \times 100 \right)
\]

**I**: Inhibition rate of germ tubes elongation (%); **n**: Repeat number; **GTLc**: Germ tubes length in the control (mm); **GTLt**: Germ tubes length in the treatment

The resistance/sensitivity of *C. gloeosporioides* isolates to the synthetic fungicide and neem seeds aqueous extract was deduced from inhibition percentage of both mycelial growth and germ tubes elongation, according to the scale proposed by [21] as given below: 1) Highly sensitive (>
90% inhibition); 2) Sensitive (>75-90% inhibition); 3) Moderately resistant (>60-75% inhibition); 4) Resistant (>40-60% inhibition) and 5) Highly resistant (<40% inhibition).

2.5. Antifungal properties of synthetic fungicide and neem seeds aqueous extract

Mycelial discs (inoculum) for which no mycelial growth has been observed were re-cultured on new PDA media containing neither fungicide nor aqueous extract. Similarly, the glucose water medium containing the synthetic fungicide and the neem aqueous extract in which no germ tube was observed; 100 μL of each of these media was transferred to media free of aqueous extract and fungicide. After 14 days and 72 hrs respectively for mycelial discs and conidia, the antifungal properties of the synthetic fungicide and the neem seeds aqueous extract were noted.

2.6. Evaluation of antifungal activity of synthetic fungicide and neem seeds aqueous extract on yam detached leaves

On the basis of the outcome of mycelial growth and germ tube elongation, the only stock solution of the synthetic fungicide (SF) recommended by the manufacturer and the stock solution NS3 of neem seeds aqueous extract macerated for 48 hrs were selected for the efficacy tests on yam detached leaves. Thus, the most recent mature leaves of the healthy D. alata plants (most susceptible yam variety) of the same age were taken from the experimental plot of the Nangui Abrogoua University. The detached leaves were washed first in running tap water, then sterile distilled water and were finally surface-sterilized using 70% alcohol to remove epiphytic microbes as per the method of [23]. A hard inoculation was carried out on the underside of the leaves with a mycelial disc of 5 mm diameter (10 days old on PDA medium) according to the method of [24]. A volume of 45 μL of each of the three concentrations of the stock solution of the fungicide or the NS3 stock solution was separately applied to the wound followed immediately by the deposition of the inoculum according to the method of [25]. For each concentration of the fungicide or aqueous extract, five leaves carrying each a treated inoculum. Five other leaves with inocula treated with 45 μL of sterile distilled water served as controls for each fungal isolate. All the leaves were placed in petri dishes (90 mm in diameter), with the top side placed on blotting paper. These blotting papers were soaked in 1.5 mL of sterile distilled water in order to maintain a saturated moisture. These Petri dishes were incubated for 5 days at room temperature (25 ± 2 °C). The five days of incubation mark the period at the end of which the necrosis symptom caused by the fungus on at least one control leaf has reached an edge of the leaf blade. The size of the necrosis (in mm) was measured at the end of the experiment following two perpendicular lines drawn on the reverse side of the boxes. The experiment was repeated three times. The percentage of the leaf area occupied by the necrosis symptom was then calculated according to the different concentrations according to the following formula:

\[
\text{Percentage of leaf area occupied by necrosis} = \frac{\text{Area occupied by necrosis}}{\text{Total leaf area}} \times 100
\]

2.7. Statistical analysis

Statistical analysis of results was performed using Statistica 7.1 software. One way ANOVA (Analysis of Variance) was made to compare average inhibition rate of mycelial growth and germ tubes elongation according to concentrations and stock solutions of synthetic fungicide and neem seeds aqueous extract at each maceration time. In case of significant difference at 5% probability level, Least Significant Difference (LSD) test of Fischer has been used. Then, the percentage of leaf area occupied by necrosis was compared according to concentrations of both products followed by Kruskal-Wallis variance analysis test and Mann-Whitney test with p < 0.05 was used to determine the significant differences.

III. RESULTS AND DISCUSSION

3.1. In vitro antifungal activity of synthetic fungicide and neem seeds aqueous extract

3.1.1. Sensitivity of fungus mycelial growth

✓ According to the concentrations

Synthetic fungicide and the three stock solutions of neem seeds aqueous extract showed varying levels of antifungal activity expressed as the reduction of tested fungus mycelial growth (P <
0.05). The mycelial colonies were strongly reduced on the PDA media amended with 10 % and 25 % of stock solution NS3 macerated for 24 h. Interestingly, on PDA media amended with all the concentrations of the synthetic fungicide and stock solution NS3 macerated for 48 h, no mycelial colony was observed. While the control PDA media were fully colonized by mycelial colonies (Figure 1).

Thus, all synthetic fungicide concentrations completely inhibited the mycelial growth of the tested fungus. However, the inhibition rate of each stock solution of neem seeds aqueous extract varied according to the increasing concentrations in the culture medium and also to the maceration time.

At 24 h of maceration, the three stock solutions of neem seeds aqueous extract, at 50 % concentration exhibit highest inhibitory activity (100 %). These inhibition rates were statistically identical of those of the synthetic fungicide. However, the lowest inhibition rates were observed at the 10 % concentration of the three stock solutions of neem seeds aqueous extract (Figure 2A).

According to scale of [21], isolates of C. gloeosporioides were highly sensitive to aqueous neem extract at the concentration 25% NS3 and 50% of the three stock solutions of the neem extract as well as to the synthetic fungicide. The Dunnett test also revealed that the antifungal activity of the fungicide was similar to that of these four concentrations of neem seeds aqueous extract.

At 48 h of maceration, all the concentrations of the stock solution NS3 and the concentrations 50% SN1 and 50% SN2 of neem seeds aqueous extract exhibit the highest inhibitory activity (100 %). These inhibition rates were statistically identical of those of the synthetic fungicide (Figure 2B).

According to scale of [21], isolates of C. gloeosporioides Penz. exhibited high sensitivity to all the concentrations of the synthetic fungicide in the same way as the neem seeds aqueous extract except for the concentrations 10% and 25% of NS1 and NS2. The mycelial growth inhibitory activity of the synthetic fungicide was also similar to that of the neem seeds aqueous extract concentrations for which the isolates were sensitive.

Figure 1: Growth of mycelial colonies of Colletotrichum gloeosporioides on PDA media amended with different concentrations of synthetic fungicide and neem aqueous extract, 7 days after incubation at 25 ± 2°C

a: Synthetic Fungicide (FS); b: NS3 macerated for 24 h and c: NS3 macerated for 48 h

Figure 2: Inhibition rate of mycelial growth of Colletotrichum gloeosporioides on PDA media amended with different concentrations of fungicide and neem aqueous extract, 7 days after incubation

A: Solutions of neem extract macerated for 24 h   B: Solutions of neem extract macerated for 48 h
According to the stock solutions

The mycelial growth inhibition rates of the three isolates of *C. gloeosporioides* varied from a stock solution of the neem aqueous extract to another for the two maceration times (P < 0.05). On the other hand, the synthetic fungicide completely inhibited mycelial growth of all isolates of *C. gloeosporioides* to the stock solution recommended by the manufacturer.

At the neem seeds aqueous extract, the highest inhibition rate was obtained for the NS3 (500 mg/mL) stock solution for both maceration times. At 48 hrs of maceration, antifungal activity of this aqueous extract stock solution NS3 was statistically identical to that of the fungicide (Figure 3).

According to the scale proposed by [21], *C. gloeosporioides* isolates were highly sensitive to the synthetic fungicide and the stock solution NS3 of neem aqueous extract macerated for 48 h. These isolates were then sensitive to the two other neem aqueous extract stock solutions macerated for 48 h and to the NS2 and NS3 solutions macerated for 24 hrs. However, they were moderately resistant to NS1 stock solution macerated for 24 hrs. The Dunnett test further revealed that the stock solution of the synthetic fungicide exhibited the same antifungal activity as the only neem seeds stock solution NS3 macerated for 48 hrs.

![Histograms marked by the same letter do not differ statistically according to the Fischer LSD test (P < 0.05)](image)

**Figure 3**: Comparative effect of inhibition rates of stock solutions on the mycelial growth of *Colletotrichum gloeosporioides* isolates, 7 days after incubation at 25 ± 2°C.

A : Neem aqueous extract macerated for 24 hrs

B : Neem aqueous extract macerated for 48 hrs.

3.1.2. Sensitivity of elongation of the germ tubes of conidia

According to the concentrations

The length of the germ tubes emitted by the conidia of the three isolates of *C. gloeosporioides* has varied depending on the treatments and concentrations of each stock solution (P < 0.05). In the control medium, the germ tubes were very long. But, in the presence of concentrations of the synthetic fungicide and the stock solution NS3 of neem aqueous extract macerated for 48 h, no germ tube was emitted by the conidia (Figure 4).

Thus, the germ tubes of conidia of the three fungi was completely inhibited (100 %) by the synthetic fungicide. In the other hand, the inhibition rates of the elongation of germ tubes emitted by conidia has varied according to the concentrations and the maceration time of neem seeds aqueous extract.

At 24 h of maceration, the concentrations 25% NS3, 50% NS2 and NS3 as well as the three concentrations of the synthetic fungicide gave the highest percent inhibition (Figure 5A). Analysis of the variance revealed that these different concentrations induced statistically identical percent inhibition.

According to the scale described by [21], conidia of tested fungal were highly sensitive not only to the synthetic fungicide but also to neem seeds aqueous extract at concentrations 50% NS1, 25% and 50% of NS2 and NS3. The antifungal activity of the various concentrations of the synthetic fungicide was similar activity to that of these neem seeds aqueous extract concentrations (P > 0.05).

At 48 h of maceration, all the concentrations of stock solutions NS2 and NS3 of neem seeds aqueous extract and that of the synthetic fungicide completely inhibited the elongation of germ tubes of conidia (100%) of the tested fungus (Figure 5B).
Conidia showed a high sensitivity to different concentrations of the same synthetic fungicide and to all the concentrations of neem aqueous extract except 10 and 25% of NS1. These concentrations of synthetic fungicide and neem aqueous extract showed similar antifungal activity (P > 0.05) according to the Dunnett test.

**Figure 4**: Conidia and germ tubes of Colletotrichum gloeosporioides on liquid medium, 20 hrs after incubation at 25 ± 2°C (G X 400)

- a : control
- b : liquid medium amended with fungicide
- c : liquid medium amended with neem aqueous extract macerated for 48 h
- d : measurement of the germ tube

**Figure 5**: Inhibition rate of conidia germ tubes elongation on the liquid medium amended with fungicide and neem aqueous extract, 20 hrs after incubation at 25 ± 2°C

- A: Neem extract macerated for 24 h
- B: Neem extract macerated for 48 h

**According to the stock solutions**

Stock solutions NS1, NS2 and NS3 of neem seeds aqueous extract macerated twice inhibited more than 60% of conidia germ tubes elongation. However, the highest inhibition were observed with stock solutions macerated for 48 hrs. In addition, the germ tubes emission was completely inhibited by the stock solutions NS2 and NS3 of neem seeds aqueous extract macerated for 48 hrs as well as the fungicide (Figure 6).

Conidia of the three isolates of *C. gloeosporioides* tested showed a high sensitivity to the stock solutions NS2 and NS3 of aqueous extract macerated for 48 hrs then to that of the fungicide. These stock solutions of neem aqueous extract developed the same inhibitory activity as the synthetic fungicide (P > 0.05) according to the Dunnett test.
3.2. Detached leaf antifungal assay of synthetic fungicide and neem aqueous extract

On the yam leaves served as a control, necrosis symptoms appeared on the second day after incubation. However, inocula treated with neem seeds aqueous extract did not start necrosis symptoms until the fourth day after inoculation. In addition, the tested fungus treated with synthetic fungicide did not developed necrosis symptoms on yam detached leaves, five days after incubation (Figure 7).

Thus, after five days of incubation, the percentage of leaf area occupied by necrosis symptom varied from one treatment to another (P < 0.00). The necrosis symptom covered an average of 75.07% of the surface of the control leaves while they were slight on the leaves containing the inocula treated with neem seeds aqueous extract. However, no symptom was observed on the surface of leaves containing inocula treated with the synthetic fungicide. Analysis of variance, however, did not reveal any significant difference between the percentage of leaf area occupied by necrosis produced by the inocula treated with the concentrations 25 and 50% of NS3 aqueous extract and those treated with the synthetic fungicide (Table 1).

Table 1 : Percentage of leaf area occupied by necrosis symptom induced by inocula of Colletotrichum gloeosporioides treated with fungicide and neem extract, 5 days after inoculation at 25 ± 2°C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations (mg/mL)</th>
<th>Leaf area occupied by necrosis symptom (%)</th>
<th>H</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>75.07 ± 1.62a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic fungicide</td>
<td>10 % FS</td>
<td>0.00 ± 0.00c</td>
<td>63.12</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>25 % FS</td>
<td>0.00 ± 0.00c</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 % FS</td>
<td>0.00 ± 0.00c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neem seeds aqueous extract</td>
<td>10 % NS3</td>
<td>6.20 ± 1.11b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 % NS3</td>
<td>0.92 ± 0.28c</td>
<td></td>
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<tr>
<td></td>
<td>50 % NS3</td>
<td>0.16 ± 0.06c</td>
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Values affected with the same letter are statistically similar at 5 % probability level. H: value of Kruskal-Wallis statistic. p: value of probability.
IV. DISCUSSION

The antifungal activities of synthetic fungicide and neem seeds aqueous extract were tested against three isolates of *C. gloeosporioides* responsible for yam anthracnose disease. The result showed a high sensitivity of these fungi to synthetic fungicide at their different life stages (mycelial growth and conidia germination). This sensitivity is related to a synergistic activity of the two active ingredients including chlorothalonil and carbendazim. Indeed, these molecules would destroy the viability of conidia which are seeds of these fungi, thus preventing their development. Moreover, the efficacy of these molecules has already been proved on the plants pathogenic fungi of the Deuteromycetes and Ascomycetes classes. [26] reported that Carbendazim block cell division and elongation of mycelial hyphae by interfering with the formation and functioning of microtubules. As for chlorothalonil, it interferes with glutathion and coenzyme A, which are basic products in the metabolism of the fungus, thus disrupting the chemical constitution of the cells and thus the entire metabolism of the fungus. Similar result to our study have been obtained by several authors. [11] inhibited not only conidia germination but also mycelial growth of *C. gloeosporioides* responsible for anthracnose of *Euonymus fortunei* by chlorothalonil. They showed a low effectiveness of each active ingredient when used individually. [12] also inhibited the mycelial growth of the fungus *Colletotrichum capsici* by Carbendazim. However, the sensitivity of fungal isolates to neem seeds aqueous extract varied according to the increasing concentrations and especially the maceration time.

This extract developed similar antifungal properties to synthetic fungicide at high concentrations when it stayed longer maceration time. The strong antifungal activity of this aqueous extract is due to a synergistic action of the 18 constituents including azadirachtin identified by [27]. Moreover, the long maceration time would promote the release of antifungal compounds in water, which has a lower extraction potential. During this time, fermentation process also improved the antifungal properties of the various constituents; which would make the extract more effective. Our results corroborate those of [16]. This author, in his *in vitro* efficacy tests of aqueous extracts of five botanicals including neem; on *Fusarium oxysporum* and *Rhizoctonia solani* in Iran, showed that of the three different concentrations of aqueous extracts (25%, 50% and 100%) macerated for 48 hrs, only the most concentrated extract (100%) showed the high antifungal activity on fungi mycelial growth. According to this author, increasing neem aqueous extract concentration is proportional to its antifungal activity. Similarly, [28] showed that the kinetics of the inhibition of the development of fungal strains by the neem extract depends not only on its concentration but also on the maceration time. [17] also showed that aqueous extracts of *Portulaca oleracea* and *Cymbopogon citratus* inhibited the mycelial growth of *C. graminicola* more effectively when they were macerated at 48 hrs in contrast to those macerated at 6, 12 and 24 hrs.

The results obtained during this study also showed that the sensitivity of tested fungi to neem seeds aqueous extract varies from one stage of life to another. Conidia of the isolates were more sensitive than the mycelial colony. This could be explained, on the one hand, by a protective effect of the mycelial colonies since the fungal isolates emit conidia during their vegetative phase. Conidia freed from mycelial colonies find themselves in direct contact with the aqueous extract, thus accentuating their sensitivity. On the other hand, the neem extract would exhibit substances inhibiting the germination of conidia. Indeed, [20] showed that sporulation and conidia germination of *F. oxysporum* are relatively more sensitive to Callicuivre than is mycelial growth. They attributed these antifungal properties to copper mineral substances, which have the common property of inhibiting the spores germination of a multitude fungi. The inhibition of conidia germination and mycelial growth of fungi would then explain the absence of necrosis symptoms on yam detached leaves whose inocula were treated.

V. CONCLUSION

Life stages such as mycelial growth and conidia germination of *C. gloeosporioides* causative agent of yam anthracnose disease were highly sensitive to both synthetic fungicide and neem seeds
aqueous extract macerated for 48 hrs. Foliar necrosis symptoms were totally inhibited by these two products at all concentrations of the synthetic fungicide and at 25 and 50% NS3 aqueous extract. On the basis of the current study, this synthetic fungicide and neem seeds aqueous extract may be used for yam farm anthracnose control tests at 25% FS and NS3. Field experiment could better guide the possible substitution of synthetic fungicides by neem seeds aqueous extract in yam plantations in Côte d'Ivoire.

VI. ACKNOWLEDGEMENT

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