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Effects of three aqueous plant extracts in the control of fungi associated with post-harvest rot of yam (*Dioscorea alata)*

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Abstract

Yam is the first food crop in Cote d'Ivoire. However yam postharvest losses due to fungi especially at storage are a major challenge. The use of chemicals has helped but the constant use can induce resistance of organisms. Biological method of control has been preferred in some cases because it is selective with no side effect and cheap. The objective of this work was to evaluate the effect of three aqueous plant extracts in the control of two fungi associated with post-harvest rot of yam. Aqueous plant extracts of *Aframomum melegueta* leaves, *Ricinus communis* seeds and *Zingiber officinale* rhizome on mycelial growth of *Aspergillus niger* and *Penicillium oxalicum* isolated on yam (*Dioscorea alata)* at different concentrations (150, 200, 250 et 300 g/L) were investigated. The ability of plants extract to protect yam tuber was also study. Our findings showed that the three plant extracts at various concentrations inhibited the mycelial growth of the two fungi *in vitro*. However, the inhibitory effects of the plant extract of *R. communis* increase with the higher concentrations. The mycelial inhibition percentage caused by *A. melegueta, Z. officinale* and *R. communis* ranged respectively from 11.63%-17.5 %; 44.57%-46.79%; 85.36%-100% for *A. niger*. For *P. oxalicum*, it ranged respectively from 72.91%-79.7%; 94.4%-96.8% and 89.72%-100%. In yam, the plant extracts used showed antifungal activity on the two isolated fungi. The extract of *R. communis* showed interesting potential for controlling the fungal agents causing rot in yam.

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Introduction

The yam (*Dioscorea spp*.) is one of the most important food crops in the tropical countries of Asia, South America, Africa and more particularly West Africa (Kouakou *et al*., 2012). According to FAO (2012), world yam production was estimated at 60.2 million tons from which 38 million tons are produced by Nigeria. Nigeria is therefore the first producing country and alone accounts for 65% of world production. Côte d'Ivoire ranks third in the world after Ghana, with a production of 5.7 million tons (FAO, 2012). The main crops grown are *Dioscorea alata* and the complex *Dioscorea cayenensis-rotundata* (Doumbia *et al*., 2004). The *Dioscorea alata* species (60% of the production) is mainly intended for family consumption whereas *Dioscorea cayenensis-rotundata* (40%) is preferably intended for marketing (Stessens, 2002). The yam constitutes the basis of the food of more than 2/3 of the Ivorian population. It contributes to the improvement of food security and poverty reduction in production areas (FAO, 2012).

Despite its importance, the yam undergoes many losses during storage. One of the main causes of these losses is the rotting of yam tubers caused by fungi. The most damaging fungi in yam storage are generally *Aspergillus niger* and *Penicillium oxalicum* (Assiri *et al*., 2009; 2015). They can cause losses of between 25 and 60% (Okigbo, 2004). These losses constitute a real shortfall for the farmers because this activity is a source of income for most populations of the cultivated areas (Babajide *et al*., 2006).

To control fungi responsible for post-harvest yam rots, different control methods are used. The most commonly used control method is the chemical control method.

The use of chemicals such as borax, captan, thiabendazole and benomyl significantly reduced the rot of yams in storage (Okigbo and Ogbonnaya, 2006). However, lack of expertise in the handling of chemical pesticides in most farmers immediately results in environmental pollution, persistence and product accumulation in the food web (Okigbo and Ogbonnaya, 2006). Public health problems and the development of resistant strains of chemical pesticides used can also be observed (Okigbo, 2004). Face to this situation, new non-chemical methods of control are recommended. This is the use of plant-based pesticides that are specifically biodegradable, cheap, and available with respect to the environment. Thus, *Xylopia aethiopica, Zingiber officinale, Ocimum gratissimum , Aframomum melegueta* have been tested for their antifungal action against yam rot fungi (Okigbo and Nmeka, 2005; Okigbo and Ogbonnaya, 2006). The objective of this study is to improve the conservation of yam tubers by the use of local plant extracts.

Material and methods

Plant material

The plant material was composed of yam tubers, leaves of *Aframomum melegueta*, rhizomes of *Zingiber officinale* and seeds of *Ricinus communis*. Yam tubers were used for the *in vivo* efficiency test. As for the leaves of *Aframomum melegueta*, to the rhizomes of *Zingiber officinale* and the seeds of *Ricinus communis*, they were used for the preparation of aqueous extracts.

The leaves of *Aframomum melegueta* and the rhizomes of *Zingiber officinale* were purchased at the market while the seeds of *Ricinus communis* were collected near the Banco forest (Abidjan, Côte d'Ivoire).

Fungal material

The fungal material consisted of two strains of fungus isolated from yam rots in storage. The strains were provided by the Plants Health Unit of the Plant Production Research Center of Nangui Abrogoua University. These are *Aspergillus niger* and *Penicillium oxalicum*.

Preparation of PDA medium

The PDA (Potato Dextrose Agar) medium was used for fungi culture as well as for *in vitro* tests. The preparation of 1 L of PDA medium supplemented with

chloramphenicol was made as follows: two hundred grams (200 g) of peeled potato were cut into dice. These were put in one liter of water and were boiled for 1 hour. The dice were crushed and the crushed material was then filtered through the broth. The filtrate was collected in an Erlenmeyer flask, to which 20 g of glucose and 20 g of agar-agar were added. The mixture was adjusted to 1 L by adding distilled water. This medium was sterilized in an autoclave at 121 ° C for 30 minutes under a pressure of 1 bar and then 0.5 g of chloramphenicol was added to the PDA medium. The obtained medium was distributed in Petri dishes 9 cm in diameter under a laminar air flow hood, in the presence of a flame.

Fungal strain cultivation

The two fungal strains were cultived into the Petri dishes containing PDA medium. To do this, a fungal inoculum with a diameter of 5 mm was placed in the center of each Petri dish containing the PDA medium. This culture was carried out with the aim of preparing 7 days old pure strains in order to evaluate the antifungal effect of the various plant extracts. These fungal strains were also use for the pathogenicity test.

Pathogenicity test

Apparently healthy tubers were disinfected with 10% hypochlorite sodium for 5 to 10 minutes. These tubers were then dried on blotting paper. They were first cut into slices 4 cm thick. Then a 5 mm diameter cookie cutter was used to make a 1 cm deep hole in the center of each yam puck. Then a fungal inoculum in the form of a disc taken from a week of mycelia colony culture was introduced into the whole made on the slice. The inoculum was placed so that the mycelial fragments were in contact with the bottom of the hole. Finally it was closed with the cylinder of yam. For the controls, the yam slices were inoculated with discs of the PDA medium containing no fungi. The yams, thus, treated were stored in sterile plastic containers containing blotting paper soaked in sterile distilled water to maintain high relative humidity. These plastic containers were finally stored in the laboratory at room temperature from 27°C to 30°C. For each fungus and for the control, 4 yam slices were used. Symptoms caused by the two fungal strains used were described after 10 days of incubation. The description was made after refreshing the infested surface.

Evaluation of the in vitro effect of the three plants aqueous extracts on fungi

Preparation of plant aqueous extract

The leaves of *Aframomum melegueta*, the seeds of *Ricinus communis* and the rhizomes of *Zingiber officinale* were disinfected with hypochlorite sodium 12 ° diluted to 10%, for 5 minutes. Then they were rinsed and dried in the shade for two weeks and crushed with a blender. Then 500 g of these different organs were macerated in 500 mL of distilled water for 3 days. The whole was agitated daily for homogeneous impregnation of the crushed material. After the maceration period, the solution obtained was transferred to another jar through a 3 µm diameter sieve. The filtrate was then sterilized through a ten-centimeter thick hydrophilic cotton column (Ackah *et al*., 2008).

Effect of aqueous extracts on the radial growth of mycelial colonies

To obtain the 150, 200, 250 and 300 g/L concentrations, respective volumes of 45, 60, 75 and 90 mL of the various extracts were incorporated in volumes of 105, 90, 75 and 60 mL on PDA medium to obtain a final volume (extract + PDA medium) of 150 mL. Each Petri dish containing the amended PDA medium of extract was gently agitated to obtain a homogeneous mixture. The PDA medium without extract was used as a control. A fungal inoculum of 0.7 cm diameter of each fungal strain was placed in the center of the medium culture. Two perpendicular axes have been drawn on the back of the Petri dishes for measurements. Six replicates (Petri dishes) were made for each concentration. A total of 24 replicates per plant extract. This experiment was repeated three times. Daily measurements of the growth diameter of the mycelial colonies were made until the mycelial colonies in the control Petri dishes were filled. The efficiency of extracts on mycelial growth of fungal strains was expressed by the inhibition rate (Ti) and calculated according to the formula of Kumar *et al.*

(2007) bellow:

$$\text{Ti (\%)} = \frac{Dt-De}{Dt} \times 100$$

Ti (%) = Inhibition rate of mycelial growth
Dt = Average diameter of mycelial growth of control
De = Average diameter of the mycelial growth of the treatment

Evaluation of the effect of plants aqueous extracts on rot yam tubers

To evaluate the ability of *A. melegueta, R. communis* and *Z. officinale* extracts to protect yam against *A. niger and P. oxalicum*, yam discs of about 13 cm diameter and 5 cm thick were punched out of the yam tubers. Then, there soaked in the aqueous extracts of plants for 15 minutes at the concentration that showed the most significant inhibitory effect in the *in vitro* tests. Then a 5 mm mycelium diameter disc was taken with a punch and placed in the center of each yam puck. These artificially inoculated slices were stored in sterile plastic containers containing blotting paper soaked in sterile distilled water to maintain high relative humidity. These bins were finally stored in the laboratory at room temperature. Rot volume of yam discs was determined according to the formula of Mascher and Défago (2000).

rot volume (cm^3) = $\pi r^2 \times h$

r = radius (cm)
h = height of the rot (cm)

From these rot volume, the inhibitory percent of the rot volumes was then calculated with respect to the inoculated yam discs which had not undergone any treatment, according to the formula of kumar *et al.* (2007).

$$Pip = \frac{(Vt - Ve)}{Vt} \times 100$$

Pip: percentage of rot inhibition
Vt: rot volume of the control
Ve: rot volume of the treatment

Statistical analysis

Analysis of variance (ANOVA) was employed in all numerical data to test for significance in thetreatment and Least Significant Difference (LSD) test was used to separate the means. The statistica 7.1 software was used.

Results

Pathogenicity of the two fungal strains

The two fungi used induced different types of symptoms (Fig. 1).

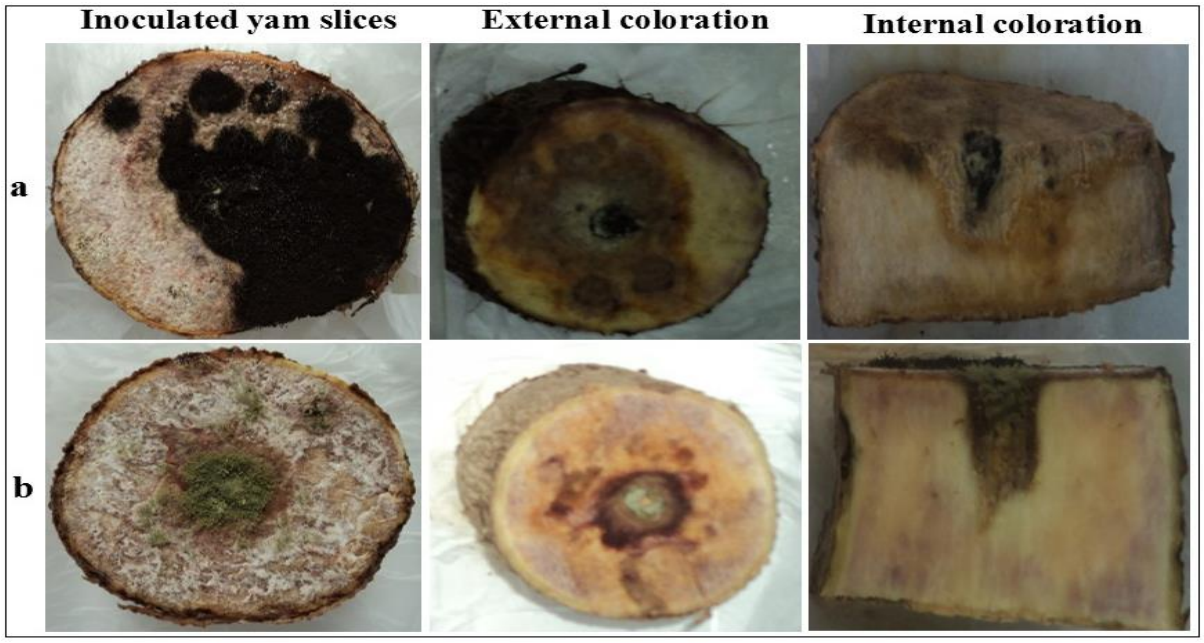

Fig. 1. Symptoms caused by two fungi on yam (*Dioscorea cayenensis-rotundata*) variety "kponan", a : *Aspergillus niger ;* b : *Penicillium oxalicum*.

Dry rot with brown coloration caused by *A. niger* and dry rot with green coloration induced by *P. oxalicum*.

In vitro effect of plant aqueous extracts on fungi mycelial growth

Effect of Aframomum melegueta

The extract of *A. melegueta* caused an inhibition of the mycelial growth of the different fungi (Fig. 2). However, this inhibition varied with the concentration of the extract and the fungi (fig. 3). Indeed, the rates of mycelia inhibition growth of fungi varied from 12 to 80%.

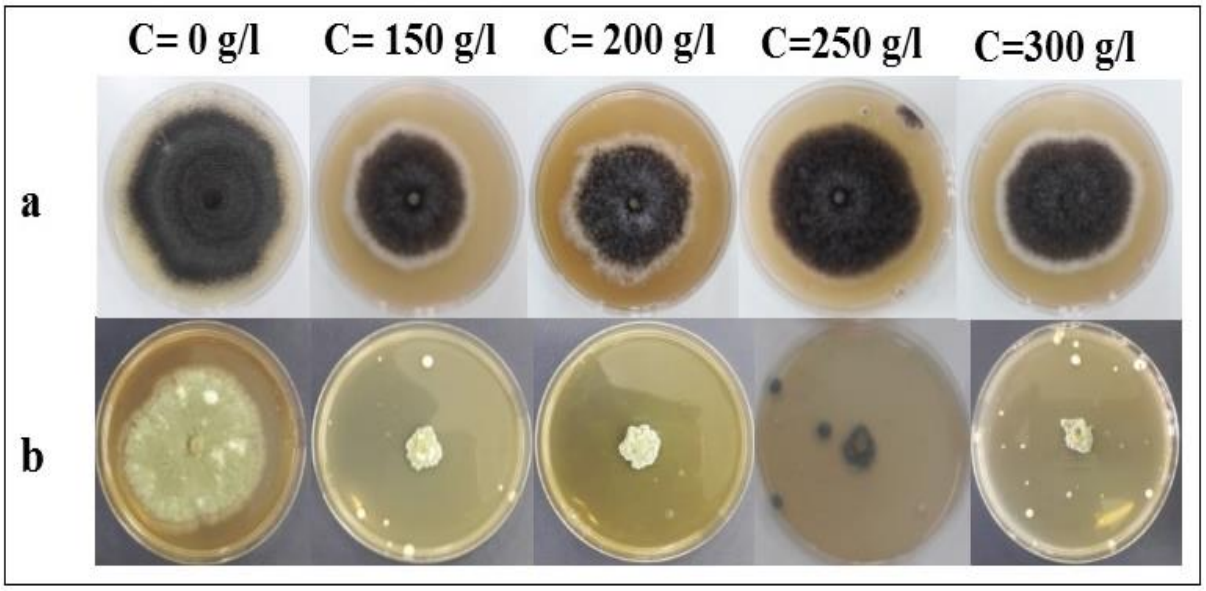

Fig. 2. Antifungal activity of *Aframomum melegueta* on mycelial growth of two fungi isolated from yam rot in storage, a : *Aspergillus niger ;* b : *Penicillium oxalicum*.

The lowest level of inhibition was obtained on the fungus A. *niger* at a concentration of 150 g/L. On this fungus, the 4 concentrations plant extracts had significantly different inhibitory effects. The highest levels of inhibition were observed on *P. oxalicum*. They varied from 75% to 80%. No significant difference was observed between the 4 extract concentrations on the rate of mycelial growth inhibition of this fungus.

Effect of Zingiber officinale

The extract of *Z. officinale* had an inhibitory effect on the mycelial growth of the two fungi (Fig. 4). The inhibition levels generated by this extract varied from 44.57 to 96.75%. The highest levels of inhibition were observed on *P. oxalicum* (Fig. 5).

The statistical analyzes carried out, revealed no significant difference between the rates of inhibition of the mycelial growth of the two fungi at different concentrations.

Effect of Ricinus communis

Ricinus communis extract showed an inhibitory activity on the two fungi. Depending on the extract concentrations of the culture medium, different diameters were observed (Fig. 6). The rates of fungi mycelia inhibition growth varied from 85.36% to 100%. The highest level of inhibition was observed at 250 g/L and 300 g/L on *A. niger* and 300g/L for *P. oxalicum*. At these concentrations, the mycelial growth of the two fungi was totally inhibited (Fig. 7). Statistical analyzes revealed a significant difference in the rates of fungi mycelia inhibition growth at different concentrations.

Comparative efficiency of the three plants aqueous extracts on the two fungi mycelia growth

The plant aqueous extracts used revealed an inhibitory activity on the mycelial growth of the fungal strains. However, this activity varied according to the extracts and the fungi (Fig. 8). On *A. niger*, the inhibition rates were lowest.

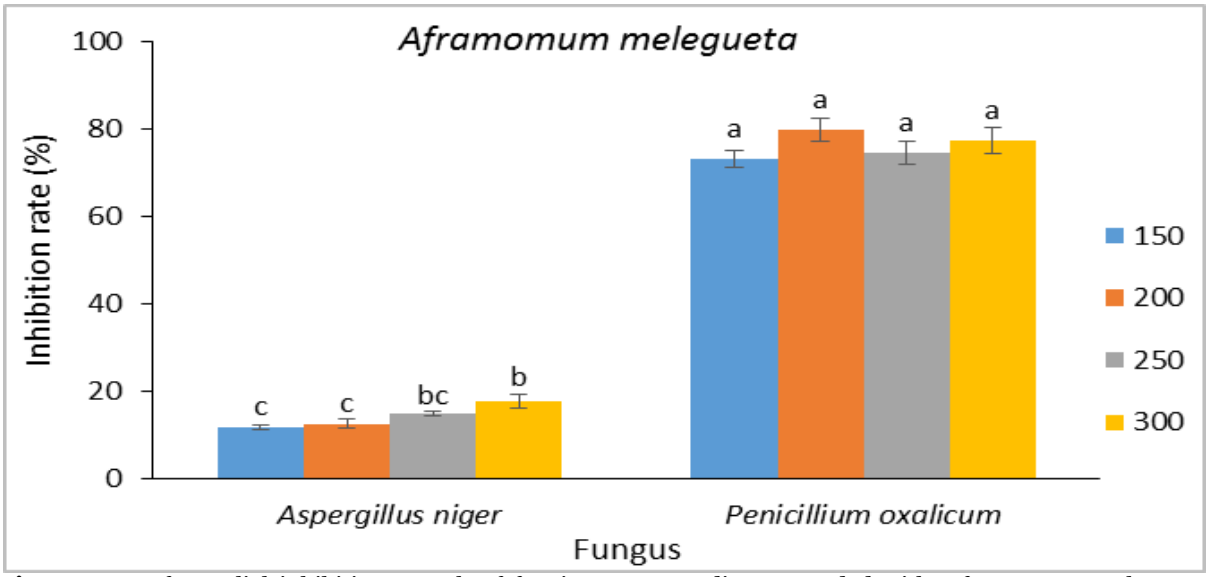

Fig. 3. Rate of mycelial inhibition growth of fungi on PDA medium amended with *Aframomum melegueta* extract.

Histograms with the same letter on standard deviations are statistically equal according to the Fischer LSD test at the threshold of = 0.05.

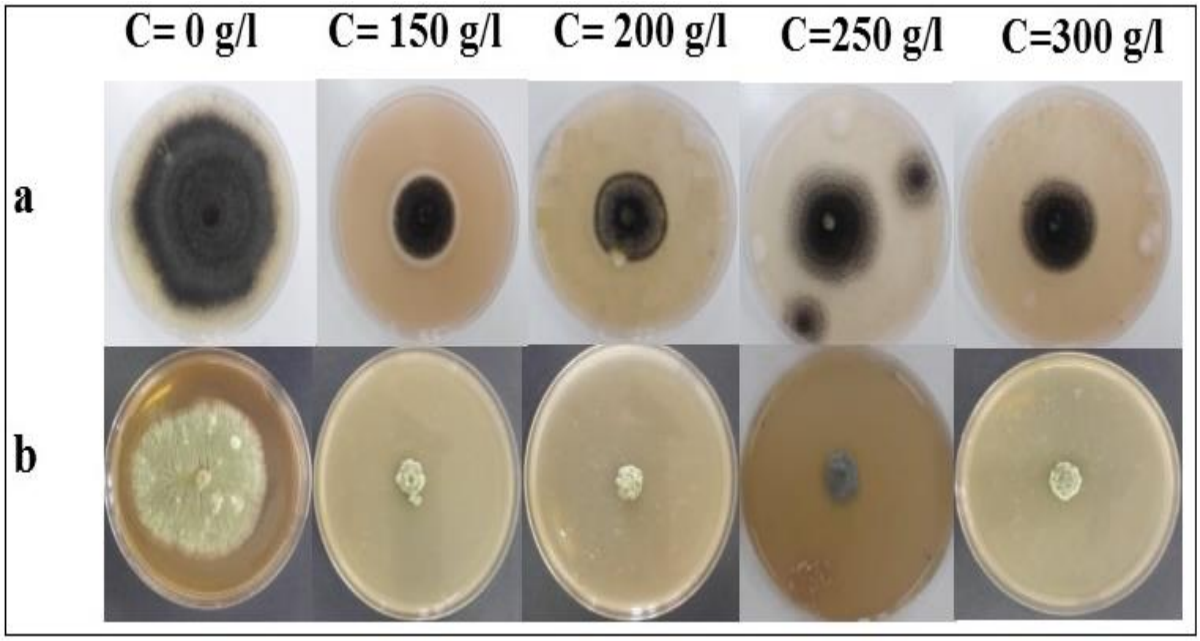

Fig. 4. Antifungal activity of *Zingiber officinale* on the mycelial growth of two fungi isolated from yam rots in storage, **a**: *Aspergillus niger ;* **b** : *Penicillium oxalicum.*

They ranged from 13.99 to 92.9. Statistical analyzes revealed a highly significant difference between the effect of these three extracts. Thus, the aqueous extract of *R. comminus* was the most effective, followed by that of *Z. officinale* and *A. melegueta*.

Concerning *P. oxalicum*, the inhibition rates caused by the three extracts were above 75%. The aqueous extract of *Z. officinale* and *R. comminus* had an inhibitory effect statistically identical but superior to that of *A. melegueta*. By comparing the three extracts on the two fungi, *R. comminus* extract was statistically the most effective.

Effect of plant aqueous extract on yam tuber rot

The aqueous extracts of the three plants inhibited the

rot caused by fungi on the yam discs. The rot volume recorded after treatment of yam tubers with plant extracts varied from 3.42 to 11.75 cm^3 for those inoculated with *A. niger* and from 8.90 to 31.12 cm^3 for *P. oxalicum* (Fig. 9). The yam tubers treated with the extract of *R. comminus* showed the lowest rot volumes in relation to the other two extracts. It has thus proved to be the most effective in controlling the rots caused by both fungi. It is followed by the extract of *Z. officinale* and *A. melegueta*. Statistical analyzes show a significant difference in the antifungal activity of these three extracts on the rot volume caused by the fungi.

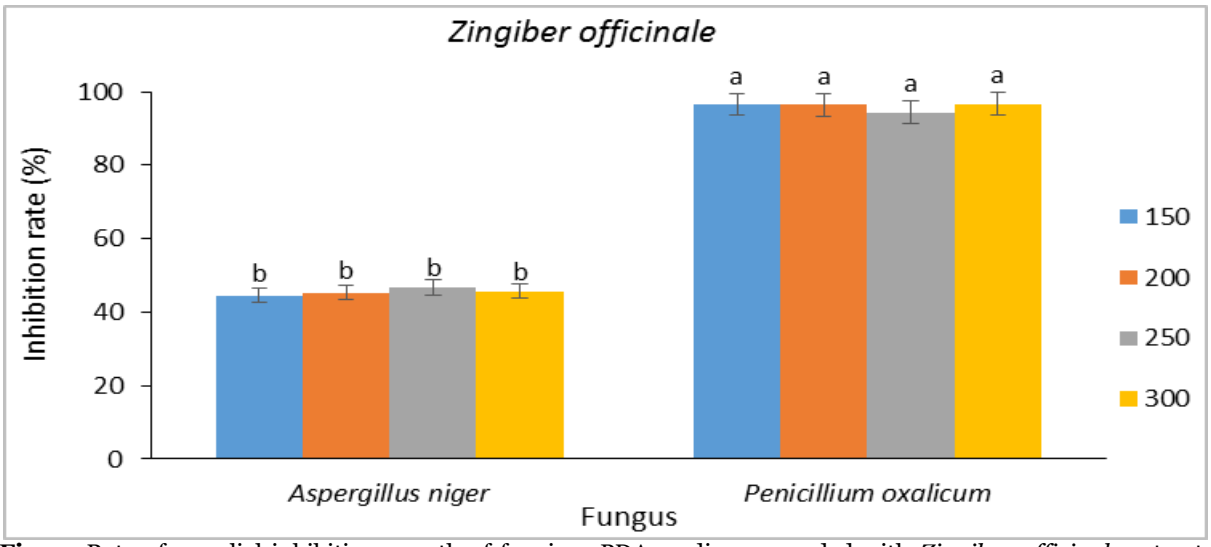

Fig. 5. Rate of mycelial inhibition growth of fungi on PDA medium amended with *Zingiber officinale* extract Histograms with the same letter on standard deviations are statistically equal according to the Fischer LSD test at the threshold of α = 0.05.

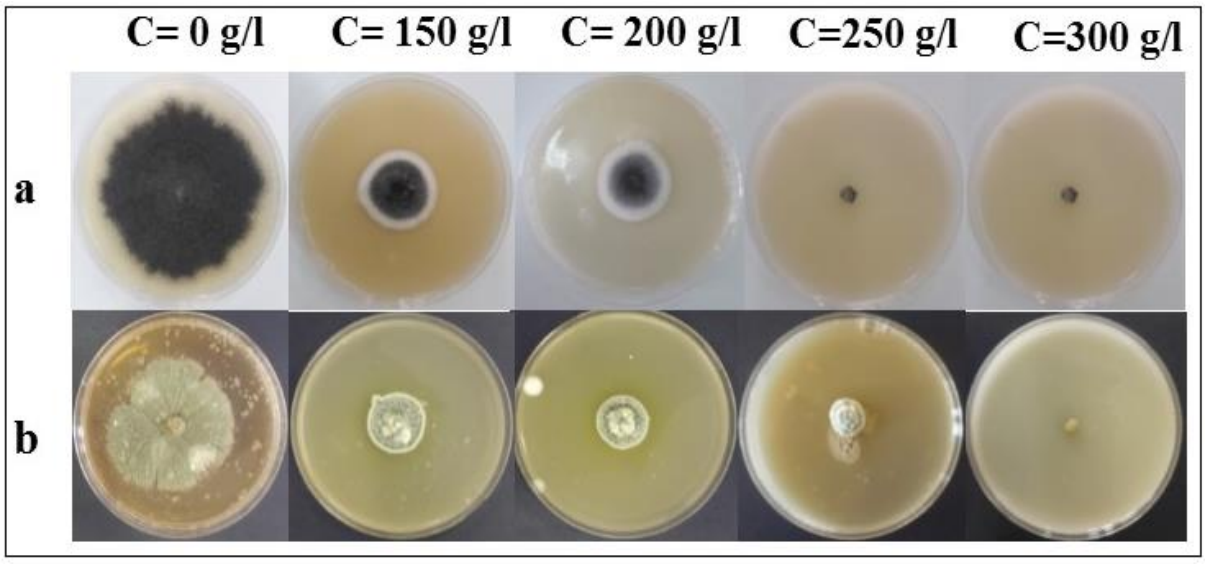

Fig. 6. Antifungal activity of *Ricinus communis* on the mycelial growth of two fungi isolated from yam rot in storage, a: *Aspergillus niger ;* b : *Penicillium oxalicum*

Discussion

The pathogenicity tests carried out with *A. niger* and *P. oxalicum* showed that both fungi cause rot on yam tuber. These results are consistent with those of Okigbo (2005), Yusuf and Okusanya (2008); Assiri *et al.* (2009 and 2015). Indeed these authors observed that *A. niger* and *P. oxalicum* are the most important fungi responsible for rot on yam in post-harvest.

Similarly Ezeibekwe *et al.* (2016) cited *Aspergillus* and *Penicillium* as one of the main fungi that are most commonly associated with post-harvest yam rots. Fungus rots demonstrate their ability to use the nutrients present in yam for their growth and development (Katoret *et al.*, 2015).

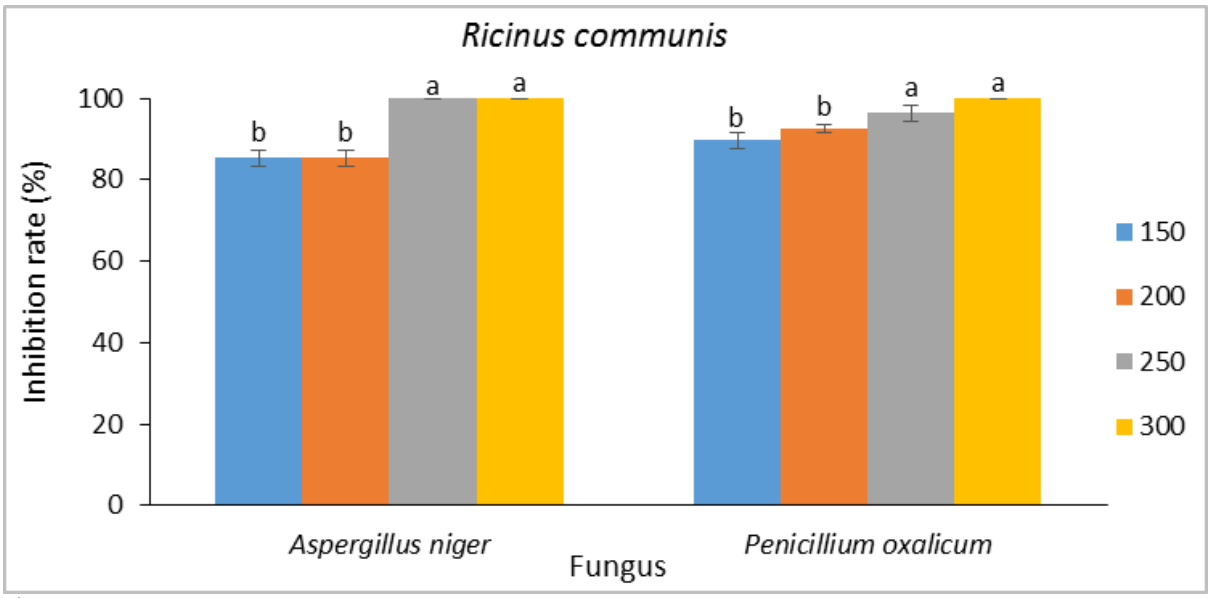

Fig. 7. Rate of mycelial inhibition growth of fungi on PDA medium amended with *Ricinus communis* extract
Histograms with the same letter on standard deviations are statistically equal according to the Fischer LSD test at the threshold of α = 0.05.

Aqueous extracts of *A. melegueta*, *Z. officinale* and *R. Communis* showed antifungal activity against *A. niger* and *P. oxalicum in vitro* at all concentrations. These plants would possess active substances which enable them to inhibit the development of these fungi. Such observations were made by Kator *et al.* (2015). They demonstrated the antifungal activity of three local plants in Nigeria on mycelial growth and spore germination of four fungi responsible for yam rot during storage. Similar results have been reported by Okigbo and Nmeka (2005), Okigbo and Ogbonnaya (2006), Taskeen *et al.* (2011).

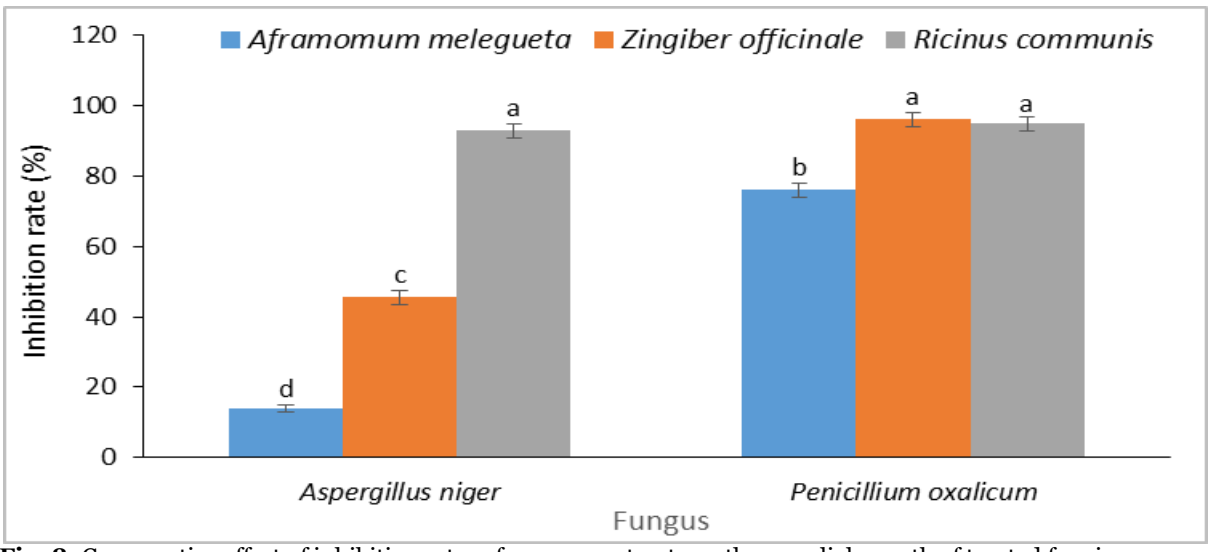

Fig. 8. Comparative effect of inhibition rates of aqueous extracts on the mycelial growth of treated fungi
Histograms with the same letter on standard deviations are statistically equal according to the Fischer LSD test at the threshold of α = 0.05.

The antifungal activity of *R. communis* aqueous extract on both fungi was higher at the highest concentrations. This would imply that the efficacy of the extracts evolves with increasing concentrations.

The efficiency of plant extracts depends on their nature and the concentration of their active substance in the culture medium (Yeni, 2011; Onyeke *et al.*, 2011).

In this study, *R. communis* extract was the most effective among the three plant extracts on the mycelial growth of the two fungi. This antifungal activity may be due to the presence of many antifungal substances (tannins, Terpernoids, flavonoids, steroids, sesquiterpenes, ricin) in the *R. communis* extract. The work carried out by Monisha *et al.* (2013) demonstrated the *in vitro* efficiency of *R. communis* against *A. niger* and *Botryodiplodia theobromae*. According to these authors, the extract reduced mycelial growth of these fungi to more than 80%. These antifungal properties of *R. communis* have also been demonstrated by Bayaso *et al.* (2013) in a study of early tomato browning. They showed that *R. communis* extract reduced by 60% the mycelial diameter of *Alternaria solani* responsible for this disease in tomatoes.

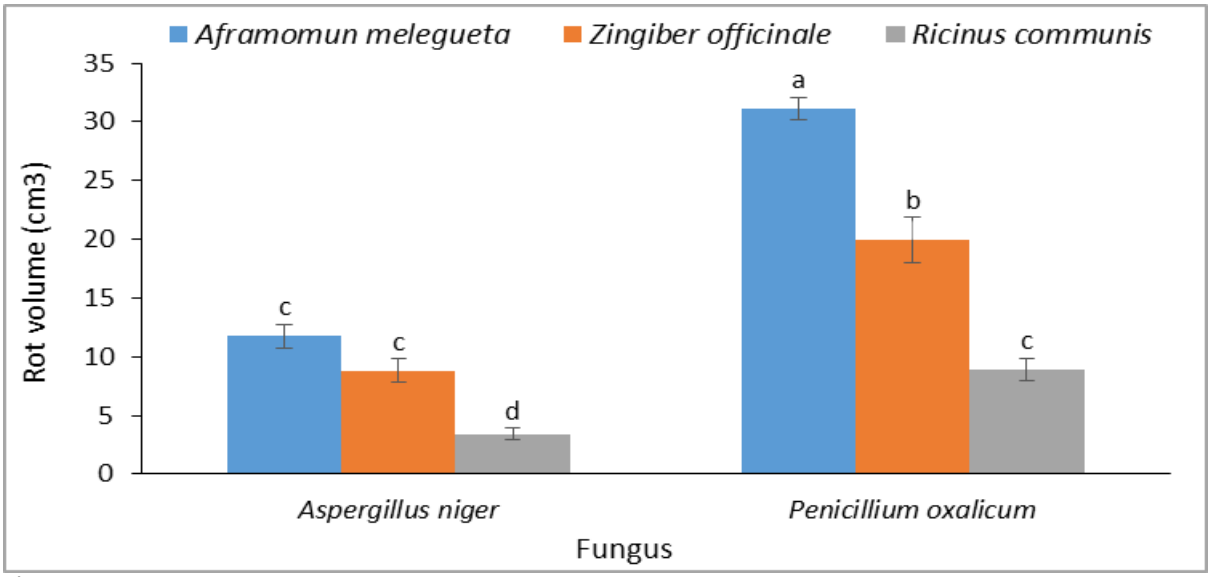

Fig. 9. Effect of aqueous extracts on yam tubers rot volume caused by the treated fungi, Histograms with the same letter on standard deviations are statistically equal according to the Fischer LSD test at the threshold of α=0.05.

The three plant extracts showed their ability to protect yam tubers against *A. niger* and *P. oxalicum* by reducing the volume of rot caused by these two fungi. These plant extracts possess an inhibitory effect against these fungi. Such result was obtained by Aisha *et al.* (2013). Indeed, these authors used extracts from two local plants in Nigeria to control fungi associated with post-harvest rots of *Solenostemon rotunifolius* tubers. Thus, they significantly reduced the incidence of these fungi on tubers. Tsado *et al.* in 2013 also showed that the yam seed treated with the extracts of *Allium sativum* and *Ocimum bacilium* and then inoculated with the fungi *Fusarium oxysporum* and *Rhizopus nigricans* were less susceptible to rot caused by the fungi and improved their germination rates.

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