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Fungicidal Effect of Some Plant Extracts against Tuber Dry Rot of White Yam (*Dioscorea Rotundata Poir*) Caused by *Aspergillus Niger*

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Abstract

In vitro study was carried out to test the efficacy of Azadirachta indica A. Juss. (Neem), Nicotiana tabacum Linn. (Tobacco), rhizomes of Zingiber officinale Rosc. (Ginger) leaves of Carica papaya Lam. (pawpaw) and seeds of Piper nigrum Linn. (Black pepper) and a chemical fungicide (mancozeb) at three concentrations of plant extracts (30, 60 and 90 g/L) and mancozeb (4, 8 and 12 g/L). The concentrations were amended in potato dextrose agar (PDA). A. Niger was isolated from rotted tissues of yam tubers obtained from Kadarko in Keana Local Government Area of Nasarawa State, Nigeria. The research was conducted at Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria. Results revealed that P. nigrum and Z.officinale were the best in fungi toxicity against A. niger at their respective concentrations throughout the period of incubation. This was followed by C. papaya, A. indica and N. tabacum respectively. Mancozeb gave 100 % inhibition at all concentrations tested throughout the period of incubation. Though all the extracts at all concentrations produced significant inhibitory effect ($P \leq 0.05$) on mycelial growth of A. niger; the concentrations of 60 g/L and 90 g/L of the plant extracts and 4 g/L of mancozeb were considered more effective and are therefore, recommended for the control of A. niger. This has shown that there is high potential in these natural plant products for the control of yam disease if properly harnessed to replace chemical fungicide which are often harmful to the environment, toxic to man and very costly to purchase.

Keywords: A. Niger; Concentrations; Fungitoxic; Inhibition; Mancozeb; Plant extracts;

Introduction

Yams (Dioscorea spp.) are among the most important staple foods in the world, especially some parts of the tropics and subtropics [21]. The most cultivated species in Nigeria are the D. rotundata (white yam), D. cayenesis, (yellow or guinea yam) and D. alata (water yam). Yam has very high food value and is a major source of carbohydrate, minerals such as calcium, phosphorus, iron and vitamins including riboflavin, thiamine and vitamins B and C [39, 41]. Nigeria, in West Africa, is the largest producer of the crop, producing about 38.92 million metric tonnes annually [20, 33]. The major yam producing states in Nigeria are Benue, Taraba, Adamawa, Nassarawa, Ekiti, Kwara, Kaduna, Ogun, Oyo, Delta, Plateau, Edo, Cross River, Imo, Ondo, and Osun [3, 4].

Many genera of fungi have been reported in association with storage decay in yam tubers [42, 25]. The major micro organisms causing diseases in yams are: Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Collectotrichum spp, Fusarium oxysporum, Fusarium solani, Geotrichum candidum, Penicillius chrysogenum, Pennicillium digatum, Rhizoctonig spp, [45, 44]. Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. Losses of yams in storage mostly due to rot are considered to be heavy in Nigeria; as a result the demand for yam tubers has always exceeded its supply [19]. It has been estimated that between 20 and 39.5 % of stored tubers may be lost to rot organisms [39]. Also an estimated 40% microbial postharvest losses in yam is reported [13].

Some control methods for yam rot have been researched and adopted by farmers in different part of the country. Chemicals have proved helpful in the control of yam diseases but one of the major problems is that frequent use of chemicals predisposes target organisms to resistance and also chemical control leaves behind residual effects which are not eco-friendly. Plant extracts have been used to control yam diseases [25, 26], Gwa et al. The advantages of these natural plant products include its local availability, little or no toxicity to humans, no residual effects on the environment and simple preparation procedures (Okigbo and Nneka, 2005). The research, therefore, focuses on some selected methods of control including the antifungal effects of some selected aqueous plant extracts such as seeds of Piper nigrum (Black Pepper), Rhizomes of Zingiber officinale (Ginger), leaves of Azadirachta indica (Neem), leaves of Carica papaya (Pawpaw) and leaves of Nicotiana tabacum (Tobacco) against Aspergillus niger, one of the fungi responsible for yam tuber dry rot in storage.

Materials and Methods

Experimental site

The experiment was conducted at the Advanced Plant Pathology Laboratory, Department of Crop and Environmental Protection, Federal University of Agriculture, Makurdi, Nigeria between January and April, 2015.

Collection of infected and healthy yam tubers

Yam tubers of white yam varieties (Dioscorea rotundata) showing various degree of disease symptoms of dry rots were obtained from yam farmers from various storage barns in Kadarko, Keana local government area of Nasarawa State, Nigeria located between longitudes 8o 30' and 8o 35'E, and on latitudes 8o 10' and 80 14' N. The rotten yam tubers were packaged in sterile polyethylene bags and were taken to the laboratory for isolation and identification of pathogens. The tubers were protected using wire mesh to prevent rodent attack [18]. The healthy yam tubers were used for pathogenicity test. A. niger which was the most frequently isolated organism was selected as the test fungus.

Preparation of Potato dextrose agar (PDA)

Potato dextrose Agar (PDA) was prepared according to manufacturer's recommendations by dissolving 39g of dehydrated PDA in 1 litre of distilled water and autoclaved at 1210C for 15 min [30] and medium was allowed to cool to 45-500C. About 0.16 g/L Streptomycin sulphate powder was added to suppress bacterial contaminations [15]. 15ml of the molten PDA was poured into sterile 9 cm glass Petri dishes and were allowed to cool at room temperature before inoculation.

Isolation of fungi organisms

Small sizes of approximately 2x2 mm were cut out with sterile scalpel from yam tubers infected with rot at inter-phase between the healthy and rotten portions of the tubers. They were first surface sterilized by dipping completely in a concentration of 5 % Sodium hypochlorite solution for 2min; the sterilized sections to be inoculated were then removed and rinsed in four successive changes of sterile distilled water (SDW) [46]. The yam pieces were placed on sterile filter paper in the laminar Air flow cabinet (Environmental Air control Inc. USA) to dry for 2 minutes.

Inoculation

The bits of the rotten yam were aseptically transferred onto solidified sterile potato dextrose agar (PDA) medium in Petri dishes. Up to four pieces of the yam sections were placed on each PDA plate and incubated at ambient room temperature (30 ± 5oC) for 192 hours. The plates were examined daily for the development of fungal growth.

Characterization and identification

Sub-culturing of mycelia colonies from the inoculated plates was done to obtain pure cultures. Sterilized surgical blade was used to cut a section of mycelia and transferring the cut sections onto sterile PDA plates. The plates (inoculated) were then incubated at ambient room temperature (30 ± 5°C) for 192 hours. The purified isolates were kept in slants and stored for characterization and pathogenicity test. Microscopic examination and morphological characteristics were noted and compared with existing authorities [15].

Pathogenicity test

Fresh, healthy yam tubers were washed with tap water, rinsed with sterile distilled water and surface sterilized with 5 % Sodium hypochlorite solution. Cylindrical discs were removed from the tubers with a sterile 5 mm cork borer. A 5 mm disc from five days old culture of A. niger was transferred into holes created in the tubers, Petroleum jelly was used to completely seal the holes. The same procedure was used for the control except that discs of un inoculated PDA were placed in the holes created in the tubers [9]. After incubation period of 14 days at ambient room temperature (30 ± 5°C) the tubers were examined for infection and disease development.

Preparation of Plant extracts

The methods of [48, 50] were used with some modifications. Seeds of Piper nigrum (Black Pepper), Rhizomes of Zingiber officinale (Ginger), leaves of Azadirachta indica (Neem), leaves of Carica papaya (Pawpaw) and leaves of Nicotiana tabacum (Tobacco) were washed thoroughly with cold running tab water, air-dried and separately ground into fine powder using a mortar. Hot water (100°C) extraction was obtained by adding 30 g, 60 g and 90 g of the powder of each plant extracts to 1litre of sterile distilled water separately in 1000 ml Pyrex flask. These were left for 24 hours and subsequently filtered through four fold of sterile cheese cloth. The filtrates obtained were used as the plant extracts in the experiment. Mancozeb a multipurpose, preventive, contact broad spectrum fungicide was prepared in cold sterile distilled water at 4 g/L, 8 g/L and 12 g/L concentrations respectively. The efficacies of the aqueous plant extracts and chemical fungicide were tested in vitro for their fungicidal activity against tuber dry rot of white yam (Dioscorea rotundata) caused by A. niger.

Effects of plant extracts on A. Niger mycelia growth

Direct medium treatment

The isolated test fungus, A. Niger obtained from rotten yam tissues were used in this experiment. This method involved direct treatment of Potato Dextrose Agar (PDA) medium with the plant extracts before inoculation of the fungus. To evaluate the fungi toxic effect of the plant extracts and the chemical fungicide on fungal mycelia growth, four equal sections on each plate was created by drawing two perpendicular lines at the bottom of the plate [5]. The point of intersection indicates the centre of the plates. These were done before dispensing PDA into each of the plates. The prepared medium was poured into sterilized Petri dishes and 5ml of each plant extracts and chemical fungicide at the different levels of concentrations were poured into Petri dishes containing the media separately [34], mixed well and allowed to solidify. The solidified medium was inoculated

centrally at the point of intersection of the two perpendicular lines drawn at the bottom of the plate with discs (5 mm diameter) which was obtained from one-week-old cultures of the test fungus. Three plates were treated with extract of each plant. The control experiments had 5 ml of distilled water added to PDA in place of plant extracts respectively; the treatments and control were completely randomized [22] and incubated for 120 hours at ambient room temperature (30 ± 5oC) and measurement of growth as radius of a growing fungal colony were undertaken at intervals of twenty four hours for five times using a transparent ruler. The absence of growth in any of the plates was indicative of the potency of the extract and the chemical fungicide against the test fungus. Fungi toxicity was determined as percent growth inhibition (PGI) according to the method described by [32] as:

$$PGI\left(\%\right) = \frac{R - R_1}{R} \times 100$$

Where,

PGI = Percent Growth Inhibition

R = the distance (measured in mm) from the point of inoculation to the colony margin in control plate,

R1 = the distance of fungal growth from the point of inoculation to the colony margin in treated plate.

Data Analysis

Test of variance was calculated using Analysis of variance (ANOVA) and statistical F-tests were evaluated at P ≤ 0.05. Mean separation was done using fishers least significance difference (F-LSD) [16].

Results

Sample collection, isolation and pathogen Identification

The fungal organisms isolated from the samples of rotten white yam were identified as Aspergillus niger, Aspergillus flavus and Fusarium solani. Colony characteristics growth of A. niger on PDA was rapid. Colonies were black or dark brown, smooth, or faintly brownish (Plate 1). Two series of conidia-bearing cells (supporting cells and phialides) were produced, but in some heads only phialides were present. Phialides were more uniform in length. Conidia were typically spherical at maturity, often very rough or spiny, mostly and very dark in colour or with conspicuous longitudinal striations (Plate 2).

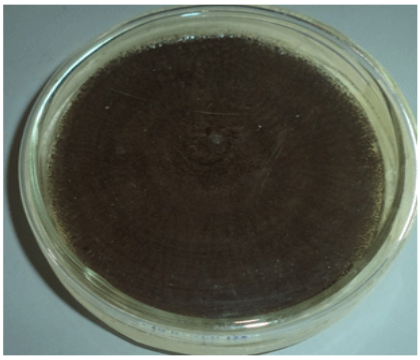

Plate 1: Culture of *A. niger* on potato dextrose agar

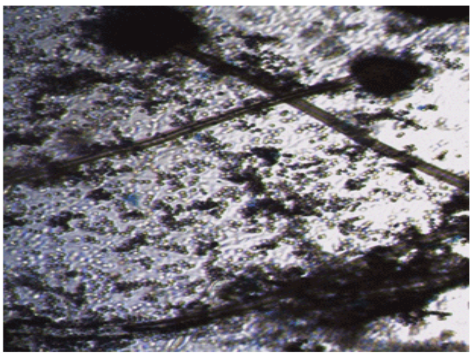

Plate 2: Micrograph of *A. niger* showing conidia

Pathogenicity test

The pathogenicity test using A. niger when inoculated into the healthy white yam tubers showed that A. niger was able to induce rot on white yam (D. rotundata) tubers and was associated with dry tuber rot of the yam. Symptoms of rot were seen on the re-inoculated yam tubers as dry black or brown rot. Morphological characteristics and growth patterns similar to those earlier observed on pure cultures were exhibited which was a confirmation of pathogenicity test (Plate 3) of the isolate.

Plate 3: Rot caused by *A. niger* (left) and controls without rot (right)

Effect of plant extracts on the mycelia growth of A. niger.

The different plant extracts showed variations in inhibition of mycelia growth of the test fungus. The rate of growth was influenced by the type of extract used and this also increased with increase in concentrations of the extracts used. There was no significant effect ($P \leq 0.05$) between Piper nigrum and Zingiber officinale at all the levels of concentrations and throughout the period of incubation. The rest of the extracts at their different levels of concentrations showed significant differences indicating that period of incubation and concentration influenced the efficacy of the plant extracts. The synthetic fungicide, mancozeb which showed the highest percentage growth inhibition was more effective than the plant extracts and also showed significant difference with the plant extracts at all the levels of concentrations and throughout the period of incubation (Table 1).

Table 1: In vitro Percentage Growth Inhibition of A. niger of some plant extracts and chemical fungicide at different concentrations after 120 hours of incubation

	Period of Incubation (Hours)				
Plant Extract	**24**	**48**	**72**	**96**	**120**
Concentration I					
Azadiracta indica	53.81±9.27^{bcd}	29.96±6.67^{d}	30.15±3.13^{c}	28.39±0.43^{d}	31.39±0.62^{c}
Carica papaya	44.29±2.97^{cd}	39.68±5.20^{cd}	49.03±2.41^{b}	36.26±2.07^{c}	29.59±1.32^{c}
Nicotiana tabacum	33.17±8.30^{d}	37.20±6.11^{cd}	36.79±4.36^{c}	32.77±3.05^{cd}	21.74±2.70^{d}
Piper nigrum	65.20±10.60^{bc}	58.93±4.49^{b}	59.17±5.32^{b}	63.58±1.46^{b}	55.14±1.81^{b}
Zingiber officinale	66.03±3.31^{b}	50.99±8.96^{bc}	52.06±4.24^{b}	58.83±4.48^{b}	50.31±2.54^{b}
Mancozeb	100.00±0.00^{a}	100.00±0.00^{aa}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LSD	21.34	18.2	11.32	7.55	5.51
Concentration II					
Azadiracta indica	61.27±2.82^{c}	47.22±2.78^{cd}	43.76±3.81^{cd}	43.06±3.02^{c}	40.88±1.19^{c}
Carica papaya	43.49±7.09^{d}	49.60±4.47^{cd}	52.39±1.32^{cd}	41.90±3.09^{c}	38.91±2.87^{c}
Nicotiana tabacum	43.39±7.09^{d}	46.23±8.59^{d}	41.57±6.56^{d}	39.53±4.44^{c}	32.08±5.60^{c}
Piper nigrum	83.02±1.66^{b}	68.95±1.38^{b}	67.76±0.55^{b}	70.41±1.34^{b}	64.63±2.08^{b}
Zingiber officinale	77.46±5.64^{b}	59.33±1.62b^{c}	53.67±5.04^{c}	62.36±2.76^{b}	61.77±2.17^{b}
Mancozeb	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LSD	15.05	12.95	11.6	8.7	8.9
Concentration III					
Azadiracta indica	60.48±6.19^{cd}	46.23±8.59^{c}	47.13±5.31^{cd}	46.51±4.32^{c}	44.65±4.12^{cd}
Carica papaya	65.20±10.60b^{cd}	54.17±4.17^{c}	55.76±1.87^{cd}	51.04±1.96^{c}	47.73±1.90^{c}
Nicotiana tabacum	50.95±4.97^{d}	50.10±7.22^{c}	47.56±8.61^{d}	44.22±6.61^{c}	36.12±5.32^{d}
Piper nigrum	83.02±1.66^{ab}	81.05±1.38^{b}	72.88±0.43^{b}	75.02±0.48^{b}	70.47±0.51^{b}
Zingiber officinale	77.46±5.64^{bc}	76.19±1.19^{b}	60.78±2.62^{bc}	67.00±1.40^{b}	66.53±3.17^{b}
Mancozeb	1000.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LSD	18.26	15.23	13.36	10.4	9.68

Means on the same column (for each concentration) with different superscript are statistically significant ($P \leq 0.05$). (**Conc I** = 30 g/L of Plant extract, 4 g/L of Mancozeb; **Conc II** = 60 g/L of Plant extract, 8 g/L of Mancozeb; **Conc III** = 90 g/L of Plant extract, 12 g/L of Mancozeb)

Mean percentage growth inhibition of three concentrations (I, II and III) of A. niger by period of incubation showed a statistical significant ($P \leq 0.05$) among plant extracts from 24 hours to 120 hours incubation period. Z. officinale showed the highest level of inhibitions among the plant extracts throughout the period of incubation followed by P. nigrum, the lowest percentage growth inhibition was recorded by N. tabacum for the same period under review (Table 2). Mean percentage growth inhibition of A. niger after 120 hours of incubation showed an increase in percentage growth inhibition from the lowest concentration to the highest concentration. P. nigrum had 60.41 % inhibition on the growth of A. Niger followed by Z. officinale which reduced the growth of A. niger by 55.64 %. The least inhibition of 32.33 % was recorded by N.tabacum at concentration I (30 g/L). There was no significant

difference (P ≤ 0.05) between Z. officinale and P. nigrum, A. indica and C. papaya and A. indica and N. tabacum. The same trend was observed when concentration II (60 g/L for plant extracts; percentage growth inhibition by P. nigrum on A. niger mycelia growth was found to be 70.95 % while Z. officinale inhibited the growth of A. niger by 62.92 %. N. tabacum increased its potency in inhibiting the growth of the fungus but was still found to be the least in activity. There was no significant difference (P ≤ 0.05) between A. indica and C. papaya and C. papaya and N. tabacum in the activity of the extracts while the rest showed significant differences (P ≤ 0.05). Concentration III (90 g/L plant extract and 12 g/L mancozeb) showed the highest mycelia growth reduction in culture of A. niger with P. nigrum at 79.49 % while Z. officinale showed a reduction in mycelia growth of A.niger at 69.59 %. There was an increase in percentage growth inhibition of the test fungus by 54.79 % when C. papaya extract was used. A. indica was second least (49.00 %) effective extract after the struggling N. tabacum increased it potency by 45.79 %. There was however, no significant difference (P ≤ 0.05) between A. indica and C. papaya and A. indica and N. tabacum in the activities of the extracts. All the extracts showed significant differences (P ≤ 0.05) on the mycelia growth of A. niger at different concentrations throughout the period of incubation. There was however, no significant difference in the performance of mancozeb in the inhibition of mycelia growth of A. niger irrespective of concentration levels used and was found to be highest in effectiveness as shown in (Table 3).

Table 2: Mean Percentage Growth Inhibition of *A. niger* at three concentrations (I, II and III) of Plant extracts by Period of Incubation (Hours)

	Period of Incubation (Hours)				
Plant Extract	**24**	**48**	**72**	**96**	**120**
Piper nigrum	77.09±4.32^{b}	69.64±3.50^{b}	66.60±2.53^{b}	69.67±1.76^{b}	63.41±2.38^{b}
Zingiber officinale	73.65±3.14^{b}	62.17±4.56^{b}	55.50±2.45^{c}	62.73±1.97^{c}	59.54±2.75^{b}
Azadiracta indica	58.52±3.52^{c}	41.14±4.28^{c}	40.35±3.33^{d}	39.32±3.17^{d}	38.97±2.34^{c}
Carica papaya	51.01±5.20^{cd}	47.82±3.15^{c}	52.39±1.37^{c}	43.07±2.47^{d}	38.74±2.83^{c}
Nicotiana tabacum	42.54±4.32^{d}	44.51±4.15^{c}	41.98±3.71^{d}	38.84±2.97^{d}	29.98±3.19^{d}
Mancozeb	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LSD	10.8	10.28	7.26	6.56	7.04

Means on the same column with the different superscript are statistically significant (P ≤ 0.05).

Table 3: Mean Percentage Growth Inhibition of *A. niger* at different Concentrations of Plant extracts after 120 hours of incubation

	Concentrations		
Plant Extract	**I**	**II**	**III**
Piper nigrum	60.41±2.39^{b}	70.95±1.78^{b}	79.49±1.34^{b}
Zingiber officinale	55.64±2.54^{b}	62.92±2.55^{c}	69.59±2.05^{c}
Azadiracta indica	34.74±3.25^{cd}	47.24±2.23^{d}	49.00±2.72^{de}
Carica papaya	39.77±2.13^{c}	45.26±2.08d^{e}	54.79±2.56^{d}
Nicotiana tabacum	32.33±2.51^{d}	40.58±2.81^{e}	45.79±2.92^{e}
Mancozeb®	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LSD	6.65	5.95	6.13

Means on the same column with the different superscript are statistically significant (P ≤ 0.05). (conc. I =30 g/L of Plant extract, 4 g/L of Mancozeb; conc. II = 60 g/L of Plant extract, 8 g/L of Mancozeb; Conc. = 90 g/L of Plant extract, 12 g/L of Mancozeb)

Discussion

The finding clearly showed A. niger as causal agent of storage rot of yam. The fungus had been previously linked with post harvest yam rot [35, 39, 49]. It has been found that most microorganisms infect tubers in the field and subsequently manifest in store [36]. The study showed that P. nigrum, Z. officinale, A. indica, C. papaya and N. tabacum, all possess fungi toxic substances and potentials to protect yam tubers against mycelia growth of A. niger. The susceptibility of A.niger to the tested plant extracts varied with duration of incubation, type of plant extract used as well as the concentrations of the extracts. Anti fungicidal activities of the plant extracts increased as the concentration increased against the test fungus. This supports earlier investigations [11,12]. The actions of the antifungal substances present in the plant extracts were fungi static at lower concentrations but became fungicidal at higher concentrations [10].

This work showed that A. niger was more susceptible to P. nigrum, Z. officinale, A. indica and the synthetic chemical,

mancozeb; but less susceptible to C. papaya and N. tabacum. Similar report by [5] demonstrated the fungi toxic activity of seed extract of Azadirachta indica (neem) and X. aethiopia against the anthraconose fungus (Collectotrichum lindemuthianum) of cowpea. Another study conducted by [48] showed that N. tabacum cold extract inhibited the mycelia of F. oxysporum yam rot organism. The same result also control yam rot with leaf of Zingiber officinale [38] and reduced the growth and sporulaton of fungal pathogens on sweet potato and yam with garlic (Allium sativum) (Udo et al., 2001). Other study by [2] showed that seed extract of P. nigrum inhibited the growth of Botryodiploidia theobromae and Fusarium oxysporum on two varieties of yam (D. rotundata and D. alata). [44] also reported the control of C. lingmuthianum using neem seed, leaf, bark and root extract, recording a 100% inhibition of spore germination and mycelia growth. Fungicidal effect of extracts may be due to the lysis of fungal cell wall and cytoplasmic membrane due to the liberation of antimicrobial products. It was also reported that plant lytic enzymes act on the fungal cell wall causing breakage of B-1,3 glycan, B-1,6, glycan and chitin polymer [14, 47].

Assessment of the effect of mancozeb on the percentage growth inhibition of the test fungus showed that increase in the concentration of the chemical does not affect growth inhibitions as the test fungus had already attained the highest level of inhibition at the lowest concentration. This result agreed with the work earlier on done by [17] which found out that mancozeb consistently gave 100 % inhibition (at concentrations of 250ppm, 500ppm and 1000ppm) of germination of conidia of Cercospora contraria and Didymosphaeria donacina which caused leaf spot diseases of cluster yam (Dioscorea dumetorum). On the other hand, it was observed that increase in the concentration of the chemicals positively correlated with the growth inhibitions [8, 33, 52]. Though all test plants were able to inhibit the growth of A. niger invitro; P. nigrum Z. officinale and A indica did better than the other plant extracts. This may be due to higher concentration of the active principle in these plants. The presence of fungicidal compounds such as nicotine (N. tabacum), Azadiractin (A. indica), piperine (P. nigrum), gingerol (Z. officinale) and papain (C. papaya) in these plant extracts which caused the inhibition of mycelia growth in vitro may be same as other reports [7,38, 45].

Conclusion

It was observed from the study that extracts of P. nigrum, Z. officinale, A. indica, C. papaya and N. tabacum all possess antifungal properties that were capable of inhibiting the growth of A. niger which caused tuber dry rot of yam in storage. It was however, observed that P. nigrum and Z. officinale were more potent than A. indica, C. papaya and N. tabacum. These extracts can therefore, be used as alternative to chemicals since they are eco-friendly, less expensive and easily accessible compare with chemicals which have been found to be harmful to the ecosystem.

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