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Research Article

Antifungal Effect of Five Aqueous Plant Extracts on Mycelial Growth of *Penicillium Expansum* Isolated from Rotted Yam Tubers in Storage

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Abstract

Efficacy of medicinal plants such as *Zingiber officinale* Rosc., *Piper nigrum* Linn., *Azadirachta indica* A. Juss., *Nicotiana tabacum* Linn and *Carica papaya* Lam. as well as a synthetic fungicide (mancozeb) using three concentrations of plant extracts (30 g/L, 60 g/L and 90 g/L) and three concentrations of mancozeb (4 g/L, 8 g/L and 12 g/L) for the management of yam tuber rot fungal pathogen caused by *Penicillium expansum* isolated from rotted yam tubers in storage were carried out. Decayed and good tubers were got from farmers' barns and *P. expansum* was isolated and identified from the rotted yam in the laboratory at Federal University of Agriculture, Makurdi, Nigeria. Pathogenicity test was conducted, and the result revealed that *P. expansum* was pathogenic to yam tubers. The result showed that the test plants significantly ($P < 0.05$) inhibit growth of *P. expansum in vitro. P. nigrum* was the most effective extract among the extracts with growth inhibition of 60.38%, 71.32% and 76.29% at 30 g/L, 60 g/L and 90 g/L concentrations respectively; followed by *Z. officinale* with mean percentage growth inhibition of 57.37%, 63.85% and 72.06% at 30 g/L, 60 g/Land 90 g/L respectively. The least inhibited plant extract was *C. papaya* with mean percentage growth inhibition of 43.52%, 47.91% and 63.24% at 30 g/L, 60 g/Land 90 g/L respectively. There was 100% inhibition using mancozeb in spite of the concentration and duration of incubation. It is therefore concluded that all the plant extracts at different concentrations posses' antifungal compounds capable of inhibiting mycelial growth of fungal pathogens and can thus be used to control fungal rots of yam since they are eco-friendly, less expensive, easily available and simple to prepare.

Keywords: Antifungal; Rottening; Plant Extracts; Penicillium expansum; Yam Tubers

Introduction

Yams (*Dioscorea* sp) are major staple food and source of livelihood for most parts of West Africa, East Africa, the Caribbean, South America, India and South East Asia [1,2]. It has been reported that Nigeria is the largest producer of the crop, producing about 38.92 million metric tonnes annually [3,4]. Despite the high volume of production, pathogenic fungi continue to cause losses at different stages of growth including post-harvest. Losses in yam after harvest could be as high as seven million metric tonnes per year (Taiga, 2011). Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting.

Fungal pathogens constantly inciting rot in yam include *Fusarium oxysporum, F. solani Aspergillus flavus, A. niger, Rhizoctonia spp., Botryodiplodia theobromae, Penicillium chrysogenum, P. oxalicum, Trichoderma viride* and *Rhizopus nodosus* [5-8]. Several methods have been used to control these deteriorating fungal pathogens but many of which such as chemical method have been proven to have detrimental effects to the environment as they are non-biodegradable and extremely toxic [9,10]. Other disadvantages of using chemicals include genotoxicity, reproductive disorders immunosuppression and hepatotoxicity [11-14]. As a result of these effects, it is necessary to search for alternative control measures that are non-toxic, eco-friendly and cost-effective for the management of yam fungal pathogens. Extracts of plant origin are known to contain toxic free compounds such as, glycosides, flavonoids, phenols, saponins, alkaloids sterols etc [10]. The study therefore focuses on the use of selective aqueous plant extracts in the in vitro control of *P. expansum* isolated from yam tubers in storage.

Materials and Methods

Study area

The study was conducted at the Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria.

Source of yam tubers

Rotted yam tubers were collected from yam farmers from Zaki-Biam, Benue State, Nigeria. The location of the settlement lies between longitudes 9° 25' and 9° 28'E, and latitude 7° 32' and 7° 35'N respectively. The yam tubers were packaged in sterile polyethylene bags, taken to the laboratory for isolation and identification of pathogens two days after collection. The medium used in the isolation of *P. expansum* was Potato Dextrose Agar (PDA) while the healthy yams were used for pathogenicity test according to Koch's postulate.

Isolation and identification of *P. expansum*

The diseased yam tubers were cut at interphase between

healthy and disease tissues. Yam pieces were sterilized in 5% Sodium hypochlorite solution for 2 minutes. The pieces were then rinsed in three changes of sterile distilled water and four pieces were incubated on acidified sterile potato dextrose agar per Petridish at ambient room temperature (30 ± 5°C) for 192 hours before observing for fungal colonies. Pure cultures of each fungal isolate were maintained by aseptic transfer to a freshly prepared medium. Identification of *P. expansum* was done with the aid of microscope and cultural characteristics growth [11,12].

Pathogenicity test

Fresh looking healthy yam tubers were washed with tap water, before sterilization in 5% Sodium hypochlorite solution for 30 seconds. The chemical was rinsed off in three successive changes of sterile distilled water before drying for about 1 hour. A sterile 5 mm diameter cork borer was used to remove discs from the yam tubers. [13]. A disc of a five days old culture of *P. expansum* isolated from yam tubers was transferred into holes created in the healthy tubers; petroleum jelly was used to completely seal the remaining portions of the holes created in the yam tissues [14]. The control experiment had only discs of uninoculated PDA placed in the holes instead of the mycelia of the *P. expansum* [14]. The inoculated tubers were incubated for 14 days at room temperature (30 ± 5°C) under sterile condition. After incubation period of 14 days at room temperature, the tubers were examined for infection and disease development.

Preparation of plant extracts

The different plant parts were prepared using the method of Taiga [15] and Tijjani., *et al*. [16] with little modifications as described by Gwa and Akombo [8]. The procedures of preparation of the 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 as described by Gwa and Akombo [8]. About 30 g/L, 60 g/L and 90 g/L of the powder of each plant extracts was added to 1 litre of sterile distilled hot water (100oC) separately in 1000 ml Pyrex flask. The concentrations were left for 24 hours and subsequently filtered using fourfold of sterile cheese cloth. The filtrates that were collected from each concentration were used as the plant extracts in the experiment. Concentrations of 4 g/L, 8 g/L and 12 g/L were used for mancozeb in the inhibition of *P. expansum.* The efficacy of the extracts from plant origin and the chemical were tested in culture for their potency in inhibiting the mycelia growth of *P. expansum* at different concentrations.

Antifungal activity of some plant extracts on growth of *P. expansum*

The method of Amadioha and Obi [17] was used to determine the effect of selected plant extracts and the chemical fungicide on fungal mycelia growth on Potato Dextrose Agar (PDA) medium. This involves drawing two perpendicular lines at the bottom of the plate; the point of intersection indicates the centre of the plate where the pathogen will be inoculated. Sterilized Petri dishes were used to pour the medium into and 5 ml of each plant extracts and chemical fungicide at respective concentrations were poured into Petri dishes containing 15 ml of the media separately [18]. The plates containing the extract and medium in a ratio of 1:3 were mixed thoroughly and allowed to solidify after some time. The inoculation of the plates was done at the intersection of lines drawn at the bottom of the plates after the solidification of the medium using a 5 mm disc diameter of one-week old culture of *P. expansum* [19]. Three plates were treated with extract of each plant extract. 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 [20] and incubated for 120 hours at ambient room temperature (30 ± 5°C). Measurement of growth as radius of a growing fungal colony of P. expansum was determined after 24 hours interval for 120 hours with a transparent ruler. The potency of the extracts and synthetic fungicide were determined as absence of growth in any of the plates inoculated with *P. expansum*. Toxicity against *P. expansum* was calculated as percent growth inhibition (PGI) as determined by Korsten and De Jager [21].

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

Where,

PGI = Percent Growth Inhibition

R = distance (measured in mm) of *P. expansum* growth from the point of inoculation to the colony margin in control plate,

R1 = distance of *P. expansum* 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 $P \leq 0.05$. Fishers least significance difference was used for means separation (F-LSD) [22].

Results

Isolation of *P. expansum*

P. expansum was isolated and identified from the rotted yam tubers. The characteristics growth of the colony of P. expansum on PDA was fast covering the entire plate within 7 days of incubation (Figure 1). The mycelia of the fungus produced were powdery bluish grey colour with a clear zone around it. Colonies were often dominated by copious, clear to yellow or brown exudates at the centres (Figure 1). Microscopic examination of the mycelia showed that conidia were spherical to sub-spheroidal, with walls smooth or very finely roughened, typically borne in long, well defined columns, one per metula, arranged in a characteristic whorl on each conidiophores (Figure 2).

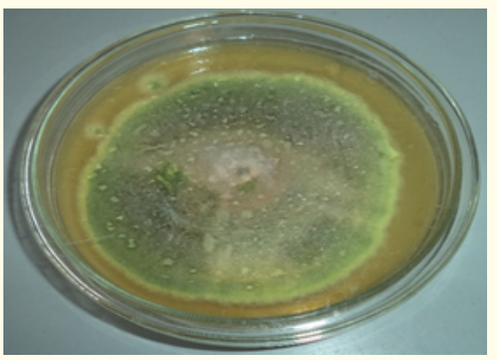

Figure 1: Culture of *P. expansum* on Potato Dextrose Agar.

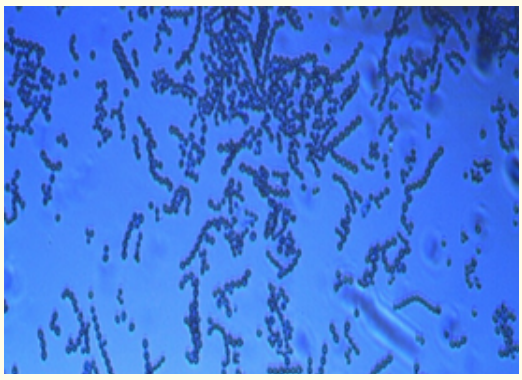

Figure 2: Micrograph of conidia of *P. expansum* (×10).

Pathogenicity test

The result of the pathogenicity test established the susceptibility of the healthy yam tubers and invasion by rot inducing *P. expansum* (Figure 3). Yam tubers treated without *P. expansum* mycelia showed no symptom of rot (Figure 4).

Figure 3: Rotted yam by *P. expansum.*

Figure 4: Control

In-vitro effect of plant extracts on the mycelia growth of *P. expansum* isolated from rotted white yam tubers

Table 1 shows that *P. nigrum, Z. officinale, N. tabacum, C. papaya* and *A. indica* had antifungal properties against *P. expansum* at all the levels of concentrations tested. *Z. officinale*, there were no significant differences at concentration 30 g/L using *C. papaya* and N. tabacum across period of incubation; however, *P. nigrum* and *A. indica* showed significant difference at all the levels of concentrations. At concentration II and III, all the extracts showed significant differences in inhibiting the mycelia growth of *P. expansum* across the period of incubation. There was no significant difference ($P \leq 0.05$) at 24 hours of incubation but varied significantly for all the plant extracts in the remaining period of incubation (Table 1). Mean percentage growth inhibition of *P. expansum* after 120 hours of incubation showed an increase in the performance of the extracts from the lowest concentration to the highest concentration. The result showed higher inhibition of *P. expansum* using extracts of *Z. officinale, N. tabacum* and *P. nigrum* compared *A. indica* and *C. papaya* extracts (Table 2). Mancozeb was however, observed to show no variation in inhibiting the growth of *P. expansum* in both concentration and duration of incubation. Mean inhibition of three concentrations (30 g/L, 60 g/L and 90 g/L) of plant extracts on growth of *P. expansum* throughout the period of incubation showed that increase in the duration of incubation resulted to decrease in the performance of the extracts (Figure 5).

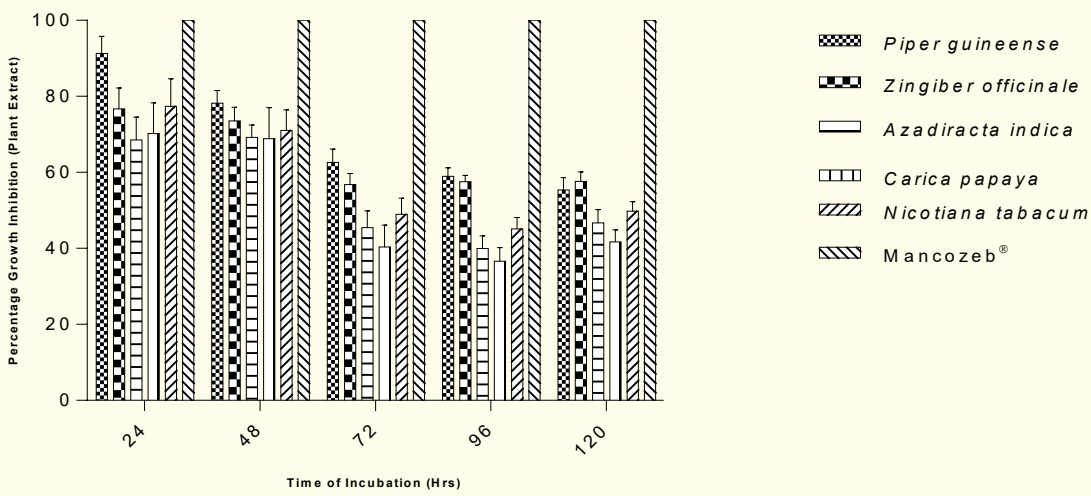

Figure 5: Mean percentage growth inhibition of three concentrations of plant extracts (30 g/L, 60 g/L and 90 g/L) and mancozeb (4 g/L, 8 g/L and 12 g/L) of mancozeb on the mycelial growth of *P. expansum.*

Table 1: *In vitro* effect of different filtrate concentrations of some plant extracts and chemical fungicide at different concentrations on percentage growth inhibition of *P.expansum* after 120 hours of incubation.

Plant Extract	Concentration (g/L)	Period of Incubation (Hours)					LSD
		24	*48*	*72*	*96*	*120*	
Piper nigrum	Conc I (30)	85.00 ± 7.64^{a}	68.25 ± 1.59^{b}	51.28 ± 1.28^{c}	51.85 ± 0.93^{c}	45.50 ± 2.43^{c}	*11.73*
	Conc II (60)	88.90 ± 11.10^{a}	80.42 ± 6.94^{ab}	69.42 ± 6.94^{abc}	59.62 ± 2.59^{bc}	57.74 ± 4.48^{c}	*21.05*
	Conc III (90)	100.00 ± 0.00^{a}	85.98 ± 1.61^{b}	67.07 ± 5.08^{c}	65.42 ± 2.59^{c}	62.97 ± 4.53^{c}	*10.51*
Zingiber officinale	Conc I (30)	62.80 ± 14.80	66.40 ± 8.40	52.89 ± 6.57	55.57 ± 2.80	49.19 ± 1.75	*26.12^{ns}*
	Conc II (60)	73.89 ± 3.89^{a}	71.96 ± 3.22^{a}	56.23 ± 3.36^{b}	57.65 ± 0.75^{b}	59.50 ± 1.82^{b}	*8.73*
	Conc III (90)	93.33 ± 3.12^{a}	82.33 ± 8.67^{a}	61.17 ± 5.77^{b}	59.29 ± 4.67^{b}	64.23 ± 3.12^{b}	*15.09*
Azadirac-ta indica	Conc I (30)	56.10 ± 12.20^{a}	62.70 ± 6.50^{a}	44.80 ± 9.16^{a}	36.01 ± 5.70^{b}	42.56 ± 4.59^{a}	*25.53*
	Conc II (60)	67..22 ± 4.34^{a}	68.25 ± 1.59^{a}	44.43 ± 7.88^{b}	36.09 ± 5.55^{b}	44.78 ± 7.95^{b}	*18.77*
	Conc III (90)	82.22 ± 9.69^{a}	76.72 ± 5.52^{a}	47.18 ± 9.21^{b}	47.79 ± 5.10^{b}	52.79 ± 5.86^{b}	*23.13*
	LSD	*32.28*	*17.33*	*30.36*	*18.88*	*21.75*	
Carica papaya	Conc I (30)	56.10 ± 12.20	58.99 ± 6.42	35.40 ± 12.70	32.17 ± 5.13	34.92 ± 4.97	*28.29^{ns}*
	Conc II (60)	62.80 ± 14.80^{ab}	66.40 ± 8.40^{a}	36.15 ± 8.74^{b}	34.05 ± 7.02^{b}	40.16 ± 3.49^{ab}	*29.13*
	Conc III (90)	91.67 ± 8.33^{a}	81.22 ± 5.15^{a}	49.56 ± 5.86^{b}	43.65 ± 6.40^{b}	50.10 ± 4.89^{b}	*22.64*
Nicotiana tabacum	Conc I (30)	69.40 ± 19.40	60.80 ± 13.90	47.00 ± 8.55	41.69 ± 6.18	43.84 ± 4.18	*37.27^{ns}*
	Conc II (60)	82.22 ± 9.69^{a}	76.72 ± 5.52^{a}	47.18 ± 9.21^{b}	44.18 ± 4.58^{b}	51.62 ± 2.92^{b}	*21.77*
	Conc III (90)	80.60 ± 10.00^{a}	75.66 ± 6.76^{ab}	52.71 ± 7.03^{c}	49.45 ± 5.14^{c}	53.92 ± 4.69^{bc}	*22.51*
Mancozeb	Conc I (4)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-
	Conc II (8)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-
	Conc III (12)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-

Means on the same row (for each Plant Extract) with the different superscript are statistically significant ($p<0.05$) by period of incubation, ns = not significant.

Table 2: Mean Percentage Growth Inhibition of *P. expansum* at different concentrations of plant extracts and chemical fungicide after 120 hours of incubation.

Plant Extract	Concentrations		
	Conc I	Conc II	Conc III
Azadiracta indica	48.44 ± 4.00^{cd}	52.16 ± 4.16^{cd}	61.32 ± 4.83^{c}
Carica papaya	43.52 ± 4.59^{d}	47.91 ± 5.08^{d}	63.24 ± 5.84^{c}
Nicotiana tabacum	52.56 ± 5.32^{bcd}	60.38 ± 4.98^{bc}	62.46 ± 4.39^{c}
Piper nigrum	60.38 ± 4.11^{b}	71.32 ± 4.08^{b}	76.29 ± 4.05^{b}
Zingiber officinale	57.37 ± 3.56^{bc}	63.85 ± 2.26^{b}	72.06 ± 4.02^{bc}
Mancozeb	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
LSD	11.18	10.87	12.03

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

Rot of yam tubers is linked with various fungal pathogens both in field and in storage. This has always made demand for yam tubers to exceed its supply (FAO 2013). The work revealed that *P. expansum* is one of the fungal pathogens associated with storage rot of different white yam cultivars in Zaki-Biam, Benue State, Nigeria. These pathogenic organisms were previously reported to caused rot in yam tubers in Nigeria [6,23,24]. Test of pathogenicity revealed that *P. expansum* initiated rot in the healthy yam by utilizing the nutrients in the yam as substrate for growth. The control tubers were however not infected suggesting the absent of inoculum in the bored yam tissues.

The result revealed that the plant extracts and the chemical fungicide possess antimicrobial compounds capable of inhibiting mycelia growth of *P. expansum.* Growth reduction in *P. expansum* was a function of the type of plant extract, concentration of extract used and as well as duration of incubation. This is in agreement with earlier report by Gwa and Akombo [8]; Banso and Adeyemo [25] and Bobbarala., *et al* [26]. According to Banso and Adeyemo [27] the actions of the antifungal substances present in the plant ex-

tracts were fungistatic at lower concentrations but became fungicidal at higher concentrations as described by Amadioha [28]. The results demonstrated that *P. nigrum, Z. officinale, A. indica* and the synthetic chemical, mancozeb were more potent compared with *C. papaya* and *N. tabacum* on inhibition of *P. expansum.* The differences in potencies may be attributed to presence of antimicrobial compounds or solubility of these compounds in water [29]. According to Sani and Gwa [30] dried leaf powder of A. indica and rhizomes of Zingiber Officinale extracts inhibited the growth of *Fusarium oxysporum* and *Rhizoctonia solani* on tomato (*Solanum Lycopersicum*) fruits. Oluma and Elaigwe [31] on the contrary, showed that A. indica extracts showed no antifungal effect on growth and sclerotial formation of *Macrophomina phaseolina*. According to Biu., *et al.* [32], antimicrobial compounds such as alkaloids, flavenoids saponins, tannins, glycosides and terpenes present in leaves of A. indica may be responsible in inhibiting mycelia growth of pathogens. Though *A. indica* (neem plant) has a lot of antimicrobial activities against different pathogens, it is also susceptible to other pathogenic organisms. Vedashree*., et al.* [33] used herbal extracts of 26 plants belonging to 20 different families of the plant kingdom and evaluated their antifungal activity against *Phomopsis azadirachtae,* a fungus causing destructive die-back disease in neem plant.

Okigbo and Nmeka [34] showed that *Z. officinale* suppresses the growth of rot fungi in culture and reduces rot development in yam tubers. Larhsini., *et al.* [35] and Sasidhran and Menon [36] demonstrated the antimicrobial activity of volatile oils of *P. nigrum* (black pepper) against *Bacillus subtilis*, *Pseudomonas aeruginosa, Aspergillus niger, Candida albicans* and *Saccharomyces cervisisae.* Chima [37] in his study on phytochemical compounds of *C. papaya* showed that post-harvest soft rot of yam caused by *Rhizopus nigricans* and *Mucor circinelloides* may be controlled by presence of tannins, glycosides, alkaloids, and flavonoids in *C. papaya*. Ijato (2011) studied the antifungal effects of *Allium sativum* (rhizome) and *Nicotiana tobacum* (leaf) extracts on rot causing organisms on yam against *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Botryodiplodia theobromae*, *Aspergillus flavus* and *Fusarium solani* and found out that both the aqueous and the ethanolic extract of the tested plants were effective as bio-killer on yam rot organisms. Studies conducted by Taiga., *et al.* [38] revealed that *N. tabacum* cold extract was able to reduce growth of *F. oxysporum* causal agent of yam rot. Antifungal compound, nicotine in *N. tabacum* was responsible in inhibiting the growth of *P. expansum* which is dose dependent [39,40]. Age of plant, method of extraction and time of harvesting plant materials may be responsible for the active substances in plants [17,34]. Antimicrobial activity of different extracts increased as the concentration increased. However, *P. nigrum, Z. officinale* and *A. indica* were generally more fungitoxic than *C. papaya* and *N. tabacum* at the same concentrations. The variation may be due to presence of more toxic compounds in the seeds of *P. nigrum*, rhizomes of *Z. officinale* and leaves of *A. indica* compared with leaves of *C. papaya* and leaves of *N. tabacum* extracts [41,42]. Though mancozeb a synthetic fungicide consistently gave 100% inhibition of *P. expansum* irrespective of the concentration used, it is advice that its use can only be when other methods prove ineffective due to its toxic effect on the environment [5,43-47].

Conclusion

The study revealed the potencies of *Z. officinale, P. nigrum, A. indica, C. papaya* and *N. tabacum* plant extracts in the control of *P. expansum in vitro*. The result proved that plant contained fungitoxic principles against *P. expansum* and also showed that *Z. officinale, P. nigrum, A. indica* were generally more effective compared with *C. papaya* and *N. tabacum* plant extracts at various concentrations. These plants could therefore be formulated and used as alternative to chemicals in the management of fungal pathogens of yam tubers since they have less adverse environmental effects, are easily available and less difficult to prepare compared to the use of synthetic fungicides which are very costly and harmful to the environment.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

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