



## Effects of Some Ethno Medicinal Plant Extracts on *Botryodiplodia theobromae* the Causal Organism of Yam (*Dioscorea rotundata* Poir) Rot

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### Authors' contributions

This work was carried out in collaboration among all authors. Author CAE designed the study and performed the statistical analysis. Author ORN wrote the protocol and the first draft of the manuscript. Author ACA managed the analysis of the study. Authors AJU and EHN managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

Post-harvest deterioration and rot caused by diverse microorganisms is the single most important factor militating against yam production in Nigeria. In an approach towards the development of ecofriendly antifungal compound in controlling yam rot, ethanol and aqueous extracts of six commonly available plants: *Vernonia amygdalina*, *Ocimum gratissimum*, *Azadirachta indica*, *Cymbopogon citratus*, *Carica papaya* and *Citrus sinensis* were tested in combination for their synergistic effect against *Botryodiplodia theobromae*. Four different extract concentrations (2.5%, 5.0%, 7.5% and 10%) were obtained from each extract mixture. Cold solvent extraction method was used for the extraction of plant materials while food poisoning technique was used for *in vitro* screening of plant extracts against rot inducing fungal organism. *Botryodiplodia theobromae* was tested to be pathogenic on healthy yam tubers with rot incidence of 80 mm. The synergistic effects of the combined extract varied with the plants combined, solvent of extraction, concentration of

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extract and test fungi. Ethanol extract showed greater effect in the inhibition of the entire organism than aqueous. All the extract combinations that had Neem as a component did better than other combinations in aqueous extracts. Inhibition of fungal growth was best observed at 7.5% and 10% extract concentrations. The highest inhibitory effect on the test organism (*Botryodiplodia theobromae*) was by the combination of Neem/Bitter leaf and Orange/Scent leaf, with values of 98.40±0.095% and 94.24±0.583% respectively for aqueous while the highest inhibition for ethanol extract was observed from Scent leaf/Bitter leaf and Pawpaw/Lemon, with values of 99.80±0.000% and 98.83±0.619% respectively these were significantly ( $P<0.05$ ) better than other combinations. With respect to the synergistic activity between the plants materials combined in this study, all the combinations showed great synergism against the test organisms. This is likely to be a means of achieving pragmatic, effective control and prevention of food spoilage, since the development of new classes of antifungal agents is of paramount importance. The crude aqueous and ethanolic extracts of all the six plant extracts showed potential synergy on organisms responsible for yam rot, hence they are recommended for the control of rot inducing microbial organisms.

**Keywords:** Yam; rot; *Botryodiplodia theobromae*; *Vernonia amygdalina*; *Azadirachta indica*; *Occimum gratissimum*; *Cymbopogon citrates*; *Carica papaya* and *Citrus sinensis*.

## 1. INTRODUCTION

Yam (*Dioscorea* spp) belongs to the family *Dioscoreaceae* and is a perennial herbaceous vine cultivated mainly for the consumption of its starchy tubers [1,2]. It is one of the most important staple foods in the world, especially in some parts of tropics and subtropics [3]. The most cultivated species in Nigeria are *Dioscorea rotundata* (white yam), *Dioscorea cayenensis* (yellow yam) and *Dioscorea alata* (water yam) [4]. These edible varieties of yam are important food crops and serve as an important carbohydrate staple for millions of people in Sub-Saharan Africa, the Carribeans, the Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania [5]. More than 90% of the yam consumed in the world is produced in West Africa. Yam production has increased steadily in the last decades from 18 million metric tonnes in 1990 to a recent estimate of over 39 million metric tonnes [6]. Nigeria alone produces three quarter of the world total output of yams [1,7,8].

One of the most pressing problems facing the countries of the third world is food scarcity. It is reported that nearly 1 billion people are challenged by severe hunger in these nations of which 10% die from hunger-related complications [9]. During the past four decades, food production has failed to keep pace with population growth in many African countries. One group of commodities that holds much potential for reversing this trend is the roots and tubers. But in Africa, data compiled by researchers show that more than 40% of these root and tuber crops are lost to rot annually [10,11]. Hence a substantial part of this problem from hunger

stems from inadequate agricultural storage and produce preservation from microbes-induced spoilages. According to Anukwuorji et al. [12], of all losses caused by plant diseases, those that occur after harvest are the costliest.

Postharvest deterioration and rot caused by diverse microorganisms is regarded as the single most important factor militating against commercial yam production in Nigeria apart from lack of research for development and capacity building in yam-based researches [13]. Most post harvest spoilage of yam tubers are caused by fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Rhizoctonia* spp., *Penicillium oxalicum*, *Trichoderma viride* and *Rhizopus nodosus* [14,15]. These microorganisms cause huge losses in the quantity of production; they also create a lot of other consequences on production quality [16]. Some workers reported that spoilage organisms produce extra-cellular enzymes such as amylases, cellulases, polygalacturonases and pectin-methyl esterases which degrade cell wall components of susceptible produce resulting in the emission of foul odor and water [17,18]. Hence rots reduce the market value of affected production, hamper the addition of value to them and prevent production to complete their roles in the food chain. In order to keep these organisms under check, controls are employed which increase the cost of production.

There are myriads of reports by researchers on the antimicrobial potentials of plant materials but there is a dearth of information on their synergistic effects on rot producing organisms in

root and tubers crop. Hence the need to carry out this research in order to develop the right combination of these plant materials for effective inhibition of microbial growth. The inference of this study will offer valuable information on the need to use different mixtures of plant materials at certain concentrations to control microbial rot of yam.

## 2. MATERIALS AND METHODS

### 2.1 Sources of Materials

The rot and healthy yam (*Dioscorea rotundata* Poir) tubers were obtained Umuariaga market at Umudike Abia State Nigeria. The neem leaves (*Azadirachta indica* L.), bitter leaves (*Vernonia amygdalina* L.) and scent leaves (*Ocimum gratissimum* L.) were collected from a home garden in Abia state, while Paw-paw (*Carica papaya*) leaves, Lemon grass leaves (*Cymbopogon citratus* L.) and orange bark (*Citrus sinensis* L.) were obtained from the staff quarters of The National Root Crops Research Institute, Umudike. The plants were identified and authenticated by a specialist in the Department of Botany, Nnamdi Azikiwe University, Awka.

### 2.2 Isolation of Fungal Pathogens

The method of Okigbo et al. [19] was used for fungal isolation. The yam tubers with rot symptoms were washed with water and surface was sterilized with 70% ethanol solution for 1 minute and rinsed with sterile distilled water. Then a sterile kitchen knife was used to cut open the yam tubers to reveal the rotten and healthy parts. Carefully, portions (about 3 mm in diameter) were taken at the boundary area of the healthy and infested part of the incised yam tuber and inoculated on the solidified Potato Dextrose Agar (PDA) medium in culture plates. The plates were sealed and incubated in an incubator at 25°C for 3-5 days. The plates were examined daily for the presence of fungal growth. Pure cultures were obtained by sub-culturing on a freshly prepared PDA for 3-5 days. The pure cultures were identified using a microscope and identification guide (atlas) to confirm the isolated organisms.

### 2.3 Pathogenicity Test and Identification of Isolates

The needle injection inoculation method of Suchitra and Shavannor [20] was used to test the

pathogenicity of the isolated organism (*Botryodiplodia theobromae*). The fungal isolate obtained from the rotten yam tubers was tested for its ability to cause the same rot condition in healthy yam tubers. The healthy yam tubers were washed with sterile water and the surface sterilized with 70% ethanol solution. With the aid of a sterile syringe, the pure culture of isolate was inoculated into a healthy yam tuber at different points. The inoculated tuber was kept in a micro humidity chamber at room temperature and examined daily for rot development for 7 days. On establishment of rot condition, re-isolation was carried out to obtain pure cultures of the inoculated isolate which was compared with original isolates before being characterized and identified as the casual organism.

The pathogenic organism was subjected to microscopic examination during which its structural features were observed under the hand lens before being mounted on a slide mount and stained with cotton blue lacto-phenol and viewed under the microscope. The characteristic features observed were compared with those contained in Barnett and Hunter [21] and identified accordingly. A pathogenicity test of the isolate was replicated three times. On appearance of symptoms, the area of infection was measured in millimeters using a metre rule and the mean percentage infection (disease severity) was calculated using the formula cited in Umana et al. [22].

Thus:

$$\text{Disease severity (Area)} = \frac{\text{Area of plant tissue affected}}{\text{Total area}} \times \frac{100}{1}$$

### 2.4 Preparation of Plant Leaf Extracts

The method of Anukwuorji et al. [10,11] was adopted. Fresh leaves of neem (*Azadirachta indica*), bitter leaf (*Vernonia amygdalina*), paw paw (*Carica papaya*), lemon grass (*Cymbopogon citratus*), scent leaf (*Ocimum gratissimum*) and bark of orange (*Citrus sinensis*) respectively were thoroughly washed in running tap water and rinsed with sterile distilled water, air dried in the laboratory and milled with a milling machine (CNC Germany) to obtain a powdery form. Seven extract combinations of the milled part of the plant namely leaves of *A. indica* and *V. amygdalina*, *C. papaya* and *C. citratus*, *C. sinensis* and *O. gratissimum*, *A. indica* and *C. citratus*, *O. gratissimum* and *V. amygdalina*, *V. amygdalina* and *C. papaya* and *C. sinensis* and

*A. indica* were used for the study. Serial dilution method according to Doughari et al. [23], was adopted by infusing 25 g, 50 g, 75 g and 100 g of each of the botanical mixtures in 100 mls of sterile distilled water in 500 ml conical flask, they were thoroughly mixed together using sterile glass rod and left for 24 hours before being filtered into a fresh 500 ml flask using the four fold cheese cloth as described by Wokocho and Okereke [24]. These preparations: 25 g of solute/100 ml of solvent, 50 g of solute/100 ml of solvent, 75 g of solute/100 ml of solvent and 100 g of solute/100 ml of solvent represent 25%, 50%, 75% and 100% of aqueous-extract concentrations respectively of all the extracts. The same procedure was also done for ethanol extract of all the plant materials.

### 2.5 *In vitro* Screening of Plant Extracts against Radial Fungal Growth

Effect of plant extract on mycelia growth of the test fungi was studied using the food poisoning techniques [25]. One milliliter of each plant extract concentrations (25%, 50%, 75% and 100%) was dispensed in each Petri dish and 9 ml of molten PDA was added to each of the Petri dishes containing extract and carefully spread evenly over the plate, this gave rise to PDA-extract mixture with corresponding 2.5%, 5.0%, 7.5% and 10% extract concentration. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the centre with a 4 mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of the test fungi.

After inoculation, all the plates were incubated at 25°C for 5 days and examined at the 5<sup>th</sup> day for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Okigbo et al. [26].

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_2} \times \frac{100}{1}$$

Where  $R_1$  is the farthest radial growth of Pathogen in control plate while  $R_2$  is the farthest radial growth of pathogen in extract incorporated agar plates.

## 2.6 Analysis of Data

Data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan at 0.05 probability level to indicate the level of significance between values.

## 3. RESULTS

### 3.1 Pathogenicity Test

The test fungus (*Botryodiplodia theobromae*) was pathogenic and caused rot in healthy yam tubers after seven days of inoculation with rot incidence of 80 mm.

### 3.2 Effects of Aqueous and Ethanol Extracts of Plant Materials on the Inhibition of Test Organisms

The effects of plant extracts on the inhibition of the test fungus isolated varied with extraction medium and concentration. Most inhibitory effects were recorded at 7.5% and 10% extract concentrations than 2.5 and 5% concentrations while ethanol extract proved to be more potent than the aqueous extract.

Ethanol extracts of *A. indica* gave the highest inhibitory values of 45% at 7.5 and 10% extract concentrations and inhibition zone of 38% at 5.0% concentration. *V. amygdalina* at 7.5% and 10% concentrations gave inhibitory values of 43% and 40% respectively. With respect to aqueous medium, *A. indica* and *V. amygdalina* recorded a relatively higher values of 38% and 36% at 7.5% and 10% concentrations. The least inhibitory value was obtained from aqueous extract of *C. papaya* at 7.5% and 10% concentration, while no inhibition was observed at 2.5% and 5.0%. The inhibition zone of 85% was significantly higher than values obtained from other interactions (Fig. 1).

### 3.3 Synergistic Effects of Plant Extracts on Growth of Pathogenic Organism

The investigations as shown in Table 1 depicts that all the antifungal agents displayed varying degrees of inhibitory effect on the phytopathogenic fungal spores. Colony diameter of the inhibition increased as the concentration of the extract increased with 7.5% and 10% extract concentrations being more effective than 2.5% and 5.0% extract concentration.

Table 1. Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Botrydioplodia theobromae*, 7 days after inoculation

Solvents	Extracts	Concentrations (%)						$\bar{x}$ extract
		0	0.5	2.5	5.0	7.5	10.0	
Aqueous	N+ L			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	37.50±0.000 <sup>b</sup>	70.00±0.000 <sup>d</sup>	26.88±0.000 <sup>c</sup>
	B + P			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	12.50±0.000 <sup>b</sup>	3.125±0.000 <sup>b</sup>
	O + N			0.00±0.000 <sup>a</sup>	5.00±0.000 <sup>b</sup>	65.00±0.000 <sup>d</sup>	75.00±0.000 <sup>d</sup>	36.25±0.000 <sup>d</sup>
	S+ B			0.00±0.000 <sup>a</sup>				
	N + B			98.75±0.354 <sup>d</sup>	98.85±0.212 <sup>d</sup>	98.00±0.000 <sup>e</sup>	98.00±1.414 <sup>e</sup>	98.40±0.095 <sup>e</sup>
	O + S			93.50±0.707 <sup>d</sup>	93.15±0.212 <sup>d</sup>	95.30±0.000 <sup>e</sup>	95.00±1.414 <sup>e</sup>	94.24±0.583 <sup>e</sup>
	P + L			6.25±1.839 <sup>b</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	1.56±0.460 <sup>b</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		47.06±6.908 <sup>a</sup>					
	$\bar{x}$ Conc.			28.36±0.414 <sup>c</sup>	28.14±0.061 <sup>c</sup>	42.26±0.000 <sup>c</sup>	39.36±0.404 <sup>c</sup>	
Ethanol	N+ L			70.00±3.536 <sup>e</sup>	85.00±3.536 <sup>f</sup>	98.65±1.626 <sup>d</sup>	99.85±0.071 <sup>d</sup>	88.38±2.192 <sup>c</sup>
	B + P			66.25±5.303 <sup>e</sup>	73.75±1.768 <sup>e</sup>	98.55±0.354 <sup>d</sup>	99.30±0.000 <sup>d</sup>	84.46±1.856 <sup>c</sup>
	O + N			0.00±0.000 <sup>a</sup>	21.25±5.303 <sup>b</sup>	98.00±0.000 <sup>d</sup>	98.70±0.000 <sup>d</sup>	54.48±0.758 <sup>b</sup>
	S+ B			99.80±0.000 <sup>f</sup>	99.80±0.000 <sup>g</sup>	99.80±0.000 <sup>d</sup>	99.80±0.000 <sup>d</sup>	99.80±0.000 <sup>d</sup>
	N + B			7.15±1.112 <sup>b</sup>	17.65±5.486 <sup>a</sup>	57.10±0.000 <sup>a</sup>	55.20±2.687 <sup>a</sup>	34.28±2.321 <sup>a</sup>
	O + S			29.3±1.597 <sup>c</sup>	57.55±7.000 <sup>c</sup>	75.00±0.000 <sup>b</sup>	67.75±3.253 <sup>b</sup>	57.40±2.962 <sup>b</sup>
	P + L			97.70±0.849 <sup>f</sup>	98.85±0.778 <sup>g</sup>	99.55±0.212 <sup>d</sup>	99.25±0.636 <sup>d</sup>	98.83±0.619 <sup>d</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		40.18±7.196 <sup>a</sup>					
	$\bar{x}$ Conc			52.89±1.771 <sup>d</sup>	64.84±3.410 <sup>d</sup>	89.41±0.313 <sup>c</sup>	88.55±0.949 <sup>c</sup>	

Key: NCNTRL is control,

PCNTRL Synthetic fungicide,

N+L means mixture of Neem leaves and Lemon grass leaves

B+P means mixture of Bitter leaves and Pawpaw leaves

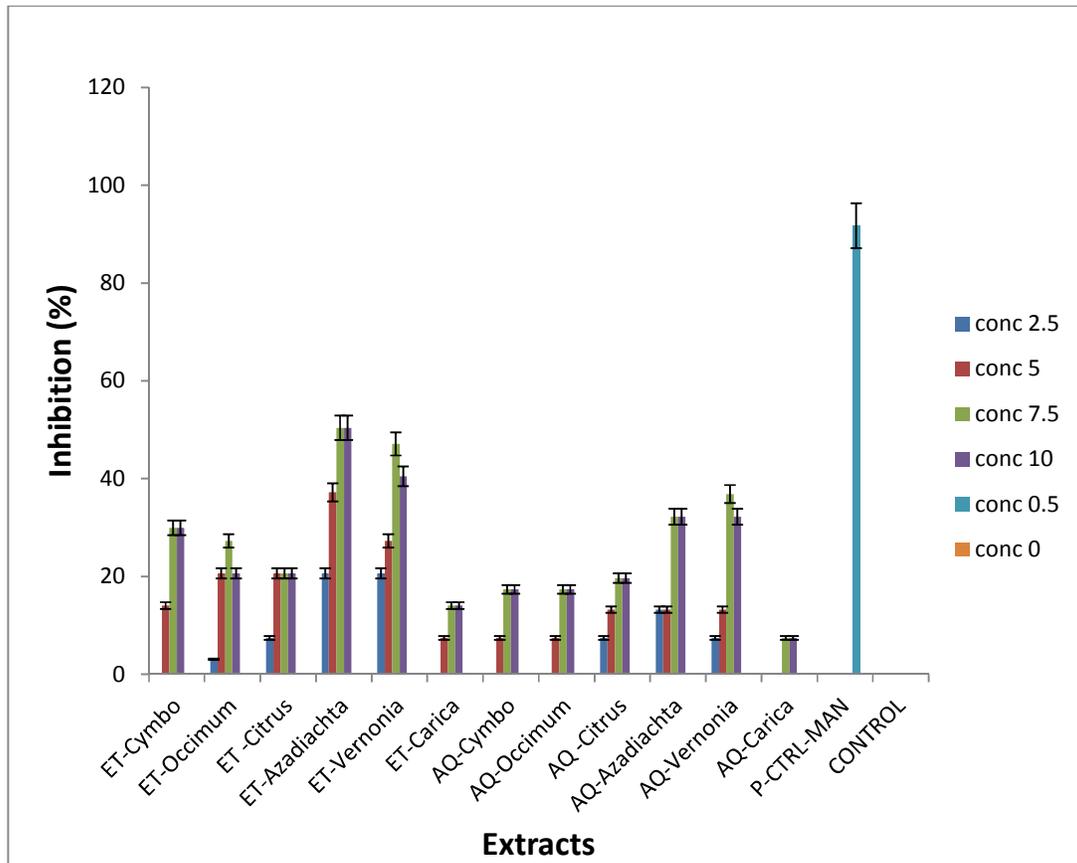
O+N means mixture of Orange bark and Neem leaves

S+B means mixture of Scent leaves and Bitter leaves

N+B means mixture of Neem leaves and Bitter leaves

O+S means mixture of Orange bark and Scent leaves

P+L means mixture of Pawpaw leaf and Lemon grass leaves



**Fig. 1. Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Botrydiplodia theobromae***

Key: ET-Cymbo means ethanol extract of *Cymbopogon citratus*; ET-Occimum means ethanol extract of *Occimum gratissimum*, ET-Citrus means ethanol extract of *Citrus sinensis*, ET-Azadirachta means ethanol extract of *Azadirachta indica*, ET-Vernonia means ethanol extract of *Vernonia amygdalina*, ET-Carica means ethanol extract of *Carica papaya*, AQ-Cymbo means aqueous extract of *Cymbopogon citratus*; AQ-Occimum means aqueous extract of *Occimum gratissimum*, AQ-Citrus means aqueous extract of *Citrus sinensis*, AQ-Azadirachta means aqueous extract of *Azadirachta indica*, AQ-Vernonia means aqueous extract of *Vernonia amygdalina*, AQ-Carica means aqueous extract of *Carica papaya*, P-CTRL-MAN is Mancozeb (Commercial fungicide)

With respect to the effects of aqueous extracts of plant materials on the inhibition of *Botrydiplodia theobromae*, the highest inhibitory percentages were recorded from the mixture of Neem/Bitter leaf and Orange/Scent leaf with values of  $98.40 \pm 0.10\%$  and  $94.24 \pm 0.58\%$  respectively, these values were significantly ( $P < 0.05$ ) better than the mixture of other plant materials, the mixture of Scent leaf/Bitter leaf did not inhibit the growth of *Botrydiplodia theobromae*, while the mixture of Bitter leaf/Pawpaw and Pawpaw/Lemon showed a very slight inhibition with values of  $3.125 \pm 0.00\%$  and  $1.56 \pm 0.46\%$  respectively. For concentration, 7.5% extract concentration gave the highest inhibitory value of  $42.26 \pm 0.00\%$  while the least value was recorded

from 5% extract concentration. For their interaction, the mixture of Neem/Bitter leaf and Orange/Scent leaf almost inhibited completely the growth of this organism at all concentrations, the mixture of Neem/Lemon did not show any inhibition at 2.5% and 5.0% extract concentration, the mixture of Bitter leaf/Pawpaw did not show any inhibition at all the concentrations except at 10% while the mixture of Pawpaw/Lemon did not show any inhibition at all the concentrations except at 2.5% (Table 1).

With respect to the effects of ethanol extracts of plant materials on the inhibition of *Botrydiplodia theobromae*, the mixture of Scent leaf/Bitter leaf and the mixture of Pawpaw and Lemon showed

the highest inhibition on this organism with values of  $99.80 \pm 0.00\%$  and  $98.83 \pm 0.62\%$  respectively, these values were significantly ( $P < 0.05$ ) higher than values gotten from the mixture of other plant materials, the mixture of Neem/Lemon and the mixture of Bitter leaf/Pawpaw also inhibited the growth of this organism at a relatively reasonable value with values of  $88.38 \pm 2.20\%$  and  $84.46 \pm 1.86\%$ , the least inhibitory percentage was recorded from the mixture of Neem/Bitter leaf ( $34.28 \pm 2.32\%$ ), for the effects of concentrations of ethanol extracts on the inhibition of *Botryodiplodia theobromae*, the highest inhibitory value was observed at 7.5% extract concentration with value of  $89.41 \pm 0.31\%$ , the next high value observed was at 10% extract concentration ( $88.55 \pm 0.95\%$ ) while the least inhibition was observed at 2.5% ( $52.89 \pm 1.77\%$ ). For their interaction, the mixture of Pawpaw/Lemon and the mixture of Scent leaf/Bitter leaf at all concentrations showed a very high inhibition ranging from  $99.25 \pm 0.64\%$  and  $99.80 \pm 0.00\%$ , the mixture of Orange/Neem at 2.5% extract concentration did not inhibit the growth of this organism while the mixture of Neem/Bitter leaf at 2.5% and 5.0% inhibition showed the least inhibition with values of  $7.15 \pm 1.112\%$  and  $17.65 \pm 5.49\%$  respectively, the commercial fungicide gave an inhibitory percentage of  $40.18 \pm 7.20$ , this is less than values recorded from most interactions (Table 1).

#### 4. DISCUSSION

The result of this research showed that *A. indica* and *V. amygdalina* inhibited the growth of the test organism more than other extracts screened for their inhibition against the fungus responsible for post-harvest spoilage of yam. This is similar with the findings of Owolabi et al. [27] who reported that *Azadirachta indica* stem bark (ethanolic extract) and *Azadirachta indica* leaf water extract were effective against all four test organisms. The findings of this work slightly differ with the result of Okpara et al. [28] who reported that *O. gratissimum* is more active than *V. amygdalina* in the control of pathogenic microorganisms of yam rot.

It was noted from this research that ethanolic extract showed greater effect in the inhibition than aqueous extract, this suggests that water used in the extraction process was probably not able to dissolve all the principle compounds present in the plants which are contained in the ethanol extracts. In other words, it can be said

that alcohol is the better solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts. The higher inhibition of ethanol extracts observed in this research agrees with the result of Ekwenye and Elegalam [29] who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence it liberates the active principles required for fungal inhibition. This is also in tandem with the result of Anukwuorji et al. [10] and Okigbo et al. [26] who reported that ethanol extracts of *Allium sativum*, *Garcinia kola* and *Azadirachta indica* were more effective than aqueous extracts. The differences in the efficacy of the different extraction medium can also be linked to the susceptibility of each of the test fungi to different concentrations of the extracts, this also agrees with the findings of some workers using some plant materials on some microorganisms associated with the rot of cocoyam, potatoes and other root and tuber crops apart from yam [14,30] although the reports of several other researchers do not agree with the above axiom. For instance Tafesse et al. [31] reported that there was no significant difference in the effects of aqueous extracts and their ethanol counterparts.

The present observations showed that then test organism (*Botryodiplodia theobromae*) was sensitive to the type of solvent, concentration and mixture of plant materials. The aqueous mixture of Neem/Bitter leaf and Orange/Scent leaf inhibited the growth of *B. theobromae* more than the mixture of other plant materials. Generally, 7.5% extract concentration was more effective than other concentrations; this suggests that concentration more or below 7.5% will not produce the highest inhibitory effect. All or most ethanolic extract mixtures proved to be better than aqueous on *Botryodiplodia theobromae* except Neem/Bitter leaf and Pawpaw/Lemon grass, this does not completely agree with the results of Amienyo and Ataga [32] on the occurrence and control of fungal pathogens of potato with plant extracts. They stated that Pawpaw and lemon even at a very high concentration do not show any significant inhibition on the growth of *Botryodiplodia theobromae*. Synthetic fungicide used gave moderate inhibitory effect on *Botryodiplodia theobromae*, the efficacy was low than the effects of combined plant materials at 7.5% and 10% concentrations, this does not support the findings of so many researchers who reported that synthetic fungicides such as Mancozeb and

Grisovoid were more active in the inhibition of the mycelia growth of *Botryodiplodia theobromae* [26] that plant materials/botanicals.

In this research, the plant extracts had different synergistic ability in the inhibition of the growth of fungal organisms isolated from yams with symptoms of post-harvest rot depending on the extraction medium, concentrations of the extracts and the test organisms. Most of the extracts combined together showed better inhibition against the test organisms than when used singly. This result is in consonance with the earlier reports of several researchers but on different fungal organisms [2,26], hence the combination of these plant parts have the potential of protecting mechanically injured yam tubers against pathogenic fungal organisms. It has been documented that one of the effective means of overcoming microbial resistance is restoration of antimicrobial activity through the synergistic action of antimicrobial materials from natural and botanical agents [33]. Synergism between bioactive plant extracts is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) [34]. The differences in inhibition observed across the mixture of plant extracts can also be attributed to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process. The synergistic effect may be due to formation of certain complexes which become more effective in the inhibition of particular microorganisms when mixed together. Similar conclusions were drawn by Zafar et al. [35] who studied the synergistic effect of extracts on pathogenic organisms.

## 5. CONCLUSION

The inference of this research work depicted that all the combination of extracts that had *A. indica* and *V. amygdalina* as a component did better than others in aqueous. While relatively higher susceptibility of all the organisms was observed at 7.5% and 10% extract concentration. Comparably, ethanol extract was more efficacious with regards to the matrices of parameter studied. All the plant extracts depicted high degree of synergism against the test organism at varying degrees depending on extraction medium and concentration of the extract.

In addition, these locally sourced plant materials have fungitoxic potentials in preserving healthy yam tubers in storage, instead of the use of chemical fungicides which pose dangers to humans and crop plants involved. More so, these also have the advantage of being readily available and affordable because they are commonly grown in Nigeria. With regards to the synergistic activity between the plant materials combined, all the combinations showed great synergism against the test organism. Therefore, due to the emergence of multifungicides-resistant pathogens, control of rot induced pathogens with organic substances combinations, using two or more plant materials is vital and paramount. This is because synergistic interactions can potentially increase efficacy, prevent the emergence of resistance, and provide broader-spectrum of activity than the use of single plant material.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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