International Journal of Ecosystems and Ecology Science (IJEES) **Vol. 13 (3):** 23-28 (2023)
https://doi.org/10.31407/ijees https://doi.org/10.31407/ijees13.3

PHYTOCHEMICAL ANALYSIS AND GROWTH INHIBITORY EFFECTS OF SOME BOTANICALS ON SEED BORNE FUNGI OF AVOCADO PEAR (*Persea gratissima)* FRUITS IN RIVERS STATE, NIGERIA

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Received May 2023; Accepted June 2023; Published July 2023;

DOI: https://doi.org/10.31407/ijees13.304

ABSTRACT

Phytochemical analysis and growth inhibitory effects of plant leaf extracts of *Ocimum gratisimum* and *Azadiracta indica* on seed borne fungi of Avocado Pear fruits in the rain forest ecosystem of Rivers State, was carried out. The experiment was laid out in a Completely Randomized Design (CRD) with eight treatments and three replicates. Results showed, the most frequently occurred fungus isolated from infected fruits of avocado Pear was *Fusarium pallidoroseum* (76.00%), followed by *Colletotrichum gloeosporoides* (36.00%), while *Botryodiplodia theobromae* (32.00%) was the least. Phytochemical analysis of leaf extracts of *O. gratisimum* and *A. indica* showed the presence of the following phytochemical constituents, essential oil (eugenol), flavonoid, quinones, tannins, saponins and terpenes. Significant differences ($P \leq 0.05$) existed among the various phytochemical constituents with quinones (25.0mg/g ± 0.00) having the highest quantity present in *O. gratissimum*, followed by flavonoid (15.00 mg/g ± 0.02) and tannins being the least (5.20mg/g ± 0.02). The most prevalent phytochemical constituent in *A. indica* was tarpenes (10.30mg/g ± 0.01), followed by flavonoid (10.25mg/g ± 0.01), Saponins (8.30mg/g ± 0.04) while tannins was the least (2.30mg/g ± 0.03). Radial/mycelial growth of test fungi in potato dextrose agar (PDA) amended with 20, 60, and 100% extracts of *O. gratissimum* and *A. indica* were significantly ($P \leq 0.05$) reduced when compared with control. *O. gratissimum* was most inhibitory against the test fungi compared to *A. indica*. The chemicals, Benlate (Beromyl) were also tested for their efficacy in controlling Avocado Pear rot organisms. Results showed a progressive reduction in growth of the fungi as chemical concentrations used increased particularly at 100ppm. Therefore, this research holds promise for the use of botanicals as an alternative means of synthetic fungicide. It is readily available, eco-friendly and cheaper than synthetic fungicides.

Key words: Botanical, inhibitory, fungi, Avocado Pear, Phytochemical.

INTRODUCTION

The avocado, also known as alligator pear, is a tropical and subtropical fruit, belonging to the family of Luraceae. The fruit is native to Central America from which it was taken to Southern Spain in 1601 and to Jamaica in 1650. The spread of avocado to the old world tropics was much later than most other new fruits. It was reported to be in Mauritus in 1980, avocado spread to Asia in the mid l9 Century. In 1833 and 1956 avocado was found in Florida

and California respectively for the first time (Chen *et al* 2008). Avocado is one of the most economically important fruits not only in Nigeria but also in the world (Schaffer *et al* 2013). It is a highly perishable commodity (Gamble *et al* 2010) and yet valued for export. Because of perishable nature and high moisture content, it is susceptible to post – harvest factors like rough handling, use of inadequate packaging materials, improper cooling, poor sanitation and improper harvesting method which leads to loss in quality and quantity. The losses are due to high moisture, high sugars (in fruits) and low pH that promote fungal growth leading to fruit deterioration and decay. All these reduce the quality and sometimes result in completely unmarketable produce that fail to meet the standards for exports. Besides, fungal pathogens can attribute to quality loss and health hazards through the production of toxins (Wills and Golding 2016). The use of synthetic chemicals in the preservation of post-harvest agricultural produce in storage has proven over the years to be very effective in controlling pathogenic fungi (Manczinger *et al.*, 2022). However, their use is increasing becoming undesirable because they are themselves carcinogenic, teratogenic, highly toxic with long degradation periods and are able to induce chemical poisoning, as well as fungal resistance (Adegoke *et al.*, 2022). As a result, the search for post-harvest control strategy has recently been directed towards the use and implementation effect on human health (WHO, 2002). Amongst natural preservatives, the use of natural essential oils obtained from plants, particularly medicinal plants have been promising. They have shown to reduce microbial and chemical spoilage amongst agricultural produce with no proven detrimental effect on human and the environment even at high concentration (Pessoa *et al*., 2002). These botanicals of medicinal importance have been proven to be very effective against fungal infection even where treatments with synthetic antibiotics failed. (Oshin *et al*., 2016). Among such proven botanicals are *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (neem). This study was aimed at isolating and identifying rot fungi of avocado pear, determine the phytochemical constituents and effects of plant leaf extracts of *Ocimum gratissimum* and *Azadirachta indica* on mycelium growth.

MATERIALS AND METHODS

Isolation and Identification of Fruit-Borne Pathogen

Infected avocado pears were surface sterilized with 70% alcohol and cut through using a sterile knife. The infected parts were cut and plated on 100 Petri-dishes and incubated at 25°C for 3-5 days as described by Chukunda (2014).

Pathogenicity Test

In order to ascertain that the isolated micro-organism caused rot, cylindrical cores (10mm) long was used to remove surface sterilized healthy fruits by means of 5 mm sterile Cork borer disc (5 mm) of 7 days old fungal culture creatures growing on Petri-dishes were introduced into the holes and sealed by means of sterile Vaseline while the controls were without fungi. Inoculums (not containing fungal mycelium) incubated at 25°C for 2 weeks in sterile polyethene bags. At the end 50 fruits of avocado were cut through and examined for rot (Chukunda, 2014).

Analyzing the Active Ingredient in the Plant Leaf Extract of Ocimum gratissimum and Azadirachta indica

The screening of phytochemical constituents of *Ocimum gratissimum* and *Azadirachta indica* plant extract was to identity the active ingredient. This was done using standard procedures as described by Kokate (2001) at the Biochemistry laboratory of Rivers State University, Nigeria. Plant materials were washed three times with sterile distilled water and dried at room temperature. Hundred grams (100g) of plant parts were separately crushed to powder using sterilized mortar and pestle. These crushed materials were extracted sequentially into 300ml sterile distilled water. The extracts, thus obtained were subjected to preliminary phytochemical screening, following the methodology of A.O.A.C (2004) and Kokate (2001).

Quantitative Analysis. Determination of Saponin

Two grammes of the samples were mixed with Diethyl ether using a Soxhlet apparatus for hrs. Each sample was ground and 20g of each were put into a conical flask and 1.00cm3 of 20% ethanol. The combined extracts were

reduced to 40m1 over water bath at about 90°C. Then, the concentrate was transferred into a 250m1 separating funnel. 10mi diethyl ether was added to the funnel and the mixture shaken vigorously. The aqueous layers were recovered while the ether was discarded. The purification process was repeated. In addition, 30m1 of N-butanol was added. The combined N-butanol extract was washed twice with 5nnl of 5% aqueous sodium chloride. The remaining solution was then heated in a water-bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage. (Obadoni and Ochuko, 2001). It was also determined by comparing the absorbance of the fruit extracts after 30mins with the standard at 380mm (Makkar and Beckar, 1996).

Determination of Quinone

Two grammes of plant sample were defatted with 200m1 of Diethyl ether using Soxhlet apparatus for 5hrs. The fat samples were boiled with 300m1 of chloroform for the extraction of the quinone component for 30mins. Upon cooling the reaction mixture were basify with a saturated aqueous solutions of Sodium hydrogen carbonate. The reaction mixture were extracted into 50m1 Dichiotromethane and the organic layer were dried over Na_2SO_4 and evaporated under reduce pressure. The extraction efficiency was calculated in percentage Pakulski *et al*, (1996)

Determination of Terpenoid

Fifty grammes (50g) of the plant. extract were extracted with solvent combination of methanol and water at room temperature for 24hr. The solution were filtered using whatman filter paper and the filtrate were then evaporated to 1/10 volume at 40°C. The evaporated filtrate was acidified with 2ml Sulphuric acid (pH 0.89) followed by chloroform extraction (three times volume), stirred and allowed to stand in a separate funnel. Out of the two layers formed, the non-aqueous layer was taken and evaporated till dryness. The dried extract contained terpenoids which were further weight and calculated as percentage (Majaw *et al.,* 2009).

Determination of Total Flavoniods

Hundred grams (100g) of the crushed leaves of *O. gratissimum* were mixed with ethanol solvent in suitable ratio. The extract obtained was filtered by using musclin cloth. The content of flavonoids was determined by the addition of NaNO2, Al (NOj) 2-NaOFI colourimetric assay (XU, 2007; Wang *et al.,* 2008; Wei *et al.,* 2009).

Determination Tannin Content

This was determined by extracting the fruit pulps with a mixture of acetone and acetic acid for five hours, measured their absorbance and compared the absorbance of the extracts with the absorbance of standard solutions of tannic acid at 500mm on spectrometer (Griffiths and Jones, 1977).

Effects of Fungicides – Benlate, and Plant Leaf Extracts on Fungal Growth of Avocado Pear.

Solutions or suspensions of benlate, scent leaf and neem extract were prepared to give 20, 60, and 100ppm concentrations of their active ingredients. To 18ml of PDA placed in Petri dishes were added to 2ml of each fungicide concentration mixed and allowed to solidify. Each Petri dish of media was inoculated with a 5mm culture disc of the fungus incubated at 30ºC, pH 4.0 and 100% relative humidity. No fungicide was added to the control Petri dishes. The linear extensions of the fungi colony per each fungicide concentration was measured along transect in two directions at right angles to each other after 14 days of incubation and each values was a mean of three replicates (Elenwo, 2009). The mean diameter of fungi growth inhibition was determined using the fungi toxicity formula of Abdulrahman *et al*., (2004).

$$F_p = \frac{F_1 - F_2}{F_1} \text{ x } \frac{100}{1}$$

Where: F_p = % inhibition of fungal growth; F_1 = Fungal growth in the control experiment (PDA); F_2 = Fungal growth on Benlate, Plant leaf extracts of A. indica and O. gratissium

The treatments in each experiment were completely randomized design with three replicates per treatment per fungus. Data on radial growth was subjected to analysis of variance (ANOVA) to determine significant difference (P = 0.05) and means compared using least significant difference (LSD).

RESULTS AND DISCUSSION

The result of investigation on fungi incidence of fruit borne of Avocado pear sampled from local market in Rivers State, Nigeria are shown in Table 1. The results implicated the following fungi; *Aspergillus niger, Botryodiplodia theobromae, Fusarium pallidoroseum Rhizopus stolonifer, Pencillium expansum, Botritis cinerrea* and *Colletotrichum gloeosporoides*.

Table 1. Percentage Frequency of Occurrence in Infected Fruits of Avacado Pear (*Persea gratissima*)

S/N	Fungal Isolates	% Frequency in *P. gratissima*
1	*Botryodiplodia theobromae*	32.00
2	*Rhizopus stolonifer*	30.00
3	*Colletotrichum gloeosporoides*	36.00
4	*Fusarium pallidoroseum*	76.00
5	*Penicillum expansum*	20.00
6	*Botrytis cineerea*	24.00
7	*Aspergillus niger*	26.00
8	LSD (P0.05)	0.019

However, the commonly isolated and most frequent of abundant fungi among these were *F. pallidoroseum* (76.00%) followed by *C. gloeosporoides,(36.00%) B theobromae (32.00%), R. stolonifer (30.00%). A nig*er (26.00%), *B. cinerea* (24.00%) respectively. Table 1: Percentage frequency of occurrence in infected fruits of Avocado pear (*Persea gratissima*). Most of these fungal of pathogens identified from the test fruit have earlier been associated with Avocado pear fruits rot by several researchers. Kebede and Belay (2019) implicated some of this fungal isolates to be responsible for soft rot diseases of Avocado fruits. Therefore, the present findings agree with earlier researches on the fruit-borne fungal incidence of Avocado. In this study, *A. niger, R. stolonifer, F. pallidoroseum, B. theobromae, C. gloesporoides, P. expansum* and *B. cinerea* were pathogens when inoculated into a relatively healthy fruits of Avocado fruits apparently caused soft rot disease of the fruits.

Results from this work indicate that fruit-borne fungal disease occurrence increased as the fungal pathogens increased. It agrees with the findings. Emiri (2015), observed an increase in Cocoyam Decline Disease (CDC) as the fungal pathogens increased in soil in accordance with our results. The results on phytochemical analysis of leaf extracts *of O. gratissimum* and *A. indica* as seen in Table 2, shows the presence of the following phytochemical constituent: essential oil, (eugenol), flavonoid, quinones, tannins, saponins and terpenes. Significant differences existed among the various phytochemical constituents with quinones, having the highest quantity present in *O. gratissimum*, while terpenes was the most prevalent in *A. indica*.

Table 2. Quantitative Phytochemical Constituents of Leaf Extracts *O. gratissimum* and *A. indica*. Phytochemical Constituents (mg 110g).

Test plants	Essential Oil	Quinores	Flavonoid	Tannins	Saponins	Tarpenes
O. gratissimum	10.25 ± 0.02	25.10 ± 0.01	15.00 ± 0.03	5.20 ± 0.02	12.00 ± 0.02	0.02
A. Indica	5.30 ± 0.04	3.20 ± 0.03	10.25 ± 0.01	2.30 ± 0.03	8.30 ± 0.04	10.30 ± 0.01
LSD (P≤0.05)	2.35	10.90	2.37	1.45	1.85	0.00

Results on plant leaf extracts and synthetic fungicide (benlate) on In-vitro control of Fungal rot isolates of Avocado Pear showed a significant reduction on the growth of fungal isolates. However, *O. gratissimum* performed better than *A. indica* with corresponding increase in concentration of the extracts. (table 3)

Table 3. Comparative Effects of Leaf Extracts and Fungicide (Benlate) on In-vitro Growth of Fungal Rot Isolates of Avocado Pear

Plant leaf Extract Fungal Isolates/ Fungicide Concentrations (%)							
Fungicide	*A. niger*	*R. stolonifer*	*F. pallidoroseum*	*B. theobromae*	*C.gloeosporoides*	*P. expansum*	*B. cinerea*
A. indica	Y= -0.07x+ 7.32	Y= -0.08x+ 8.34	Y= -0.06x+ 6.07	Y= -0.07x+ 8.01	Y= -0.08x+ 7.10	Y= -0.06x+ 5.8	Y= -0.06x+ 7.11
Regression (r)	r = 0.9097*	r = 0.9821***	r = 0.9973***	r = 0.9882*	r = 0.9711*	r = 0.9544*	r = -0.9386*
O. gratissimum	Y= -0.08x+ 7.26	Y= -0.08x+ 7.35	Y= -0.07x+ 6.40	Y= -0.08x+ 6.85	Y= -0.07x+ 6.8	Y= -0.05x+ 4.50	Y= -0.07x+ 7.0
Regression (r)	r = 0.9428**	r = 0.9213**	r = 0.9941**	r = 0.9638**	r = 0.9636**	r = 0.8721**	r = 0.9402**
Benlate	Y= -0.08x+ 7.6	Y= -0.09x+ 9.11	Y= -0.06x+ 6.3	Y= -0.09x+ 8.41	Y= -0.08x+ 7.0	Y= -0.06x+ 5.8	Y= -0.08x+ 7.4
Regression (r)	r = 0.9603*	r = 0.9965***	r = 0.9940***	r = 0.9882*	r = 0.9640*	r = 0.9477*	r = 0.9779***

*r Significant at P≤ 0.05, ***,**,**

The finding appears to indicate that these phytochemical constituents gave high inhibitory activity against the test fungi. Adeyeye and Olufolaji (2004), Onifade (2008), reported that *O. gratissimum* and *A. indica* have antifungal properties capable of checking the spread of many fungal disease of food crops. This presents work agrees with their findings. However, the fungi toxic potency or efficacy of *O. gratissimum* and *A. indica* could be attributed to the presence of essential oil such as ethyl chenotype and eugenol. Earlier, Afolabi, (2007) and Leylliance *et al* (2007), isolated eugenol essential oil from *O. gratissimum* which were used in the control of plant diseases. Their findings is consonance with the present work. The inhibitory effects of the leaf extracts were compared to synthetic fungicides (Benlate) (Table 3). It was observed, there was a progressive growth reduction of the test fungi when synthetic fungicides were applied. Many earlier workers have reported the effectiveness of Synthetic (chemical) fungicide to inhabit many pathogenic fungi. Ibiam *et al*, (2006) observed, Benomyl, Kocide 101, Captain, prevented fungal rot of sweet potato tubers. Similarly, Sahab *et al*, (2007) reported that Benlate and other consolidate materials have a positive effect on the resistance of the bio deterioration effects on papynus and linen papers. Nagendra *et al* (2010) reported the compounds on *Phomopsis azadiracta*, the causative agent of Die- back disease of Neem. This is in consonance with the present findings.

CONCLUSION AND RECOMMENDATIONS

The investigation into the post-harvest fungal disease of avocado pear implicated *A. niger*, *R. stolonifer* among others. The result obtained confirmed the anti-microbial potency of *O. gratissimum* and *A. indica* as alternative to toxic pesticide due to its biodegradability. It is therefore recommended to farmers to help achieve healthy fruits production.

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