

Full Length Research Paper

Effects of three plant extracts (*Ocimum gratissimum*, *Acalypha wilkesiana* and *Acalypha macrostachya*) on post harvest pathogen of *Persea americana*

Erute Magdalene Ogbo* and A. E. Oyibo

Department of Botany and Microbiology, Delta State University, Abraka, Nigeria

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Disease assessment of *Persea americana* (Avocado) in a local market in Delta State Nigeria showed that only 7% of the fruits were healthy. 36% of the avocado fruits had *Cercospora* spot; 28% had *Diotherella* rot and 29% had anthracnose. Plant extracts of three different species; *Ocimum gratissimum*, *Acalypha wilkesiana* and *Acalypha macrostachya* were assessed for inhibitory effect on the radial growth of *Cercospora purpurea*. Ethanol extracts of the three plants caused total inhibition of *C. purpurea*. Crude extract of sterilized and un-sterilized water significantly reduced the radial growth of the fungus. However, the fungistatic effect of *O. gratissimum* was highest and was followed by *A. macrostachya* and then *A. wilkesiana*.

Key words: Plant extracts, inhibition, cercospora spot, antifungal, disease assessment.

INTRODUCTION

The avocado tree is an ever green tree that has economic value worldwide. It is widely cultivated and can be productive for a very long time. Fifty seven countries are involved in avocado production but Mexico is the major producer. It is highly nutritious. The fruit finds use in salads, sauces and marinades. The avocado oil is used in skin products as cleansing creams, sunscreen lotions, moisturizers and hair conditioners (Anthony et al., 1992). Farmers in Nigeria cultivate the plant around them as a good source of income since the fruit are highly sought after and are expensive. They, however, incur losses due to post harvest diseases with very high disease incidents. Although there are field diseases of this plant the post-harvest diseases are a more serious problem for the sellers. Post harvest diseases associated with this plant are Anthracnose (*Glomerella unguilata*), *Cercospora* spot (*Cercospora purpurea*), *Dothiorella* rot caused by *Botryosphaeria ribis* and also *Verticillium* (Darvas et al., 1987). The most serious field disease of avocado is root rot caused by *Phytophthora cinnamom*. It affects small roots thereby causing leaf all, dieback of branches and tree collapse (Samson, 1980). There are also surface contaminants like *Aspergillus*, *Fusarium* and *Rhizopus* species that could become opportunistic and could ren-

render 90% of a harvest unfit for consumption. Disease assessment of post harvest fruits indicates the presence of the three postharvest diseases in the local market in Delta State Nigeria.

Natural plant products and their analogues are an important source of new agricultural chemicals (Cardelina, 1988, Gulter, 1988). Pesticides of plant origin are preferred in countries where the plants are readily available and the synthetic pesticides are expensive and difficult to obtain. Many workers have reported antimicrobial activities of plant extracts (Maji et al., 2005). The use of plant extracts and phyto-products is gaining attention due to their proven nature specificity, biodegradability, low toxicity, and minimum residual toxicity in the ecosystem (Adityachaudhury, 1991; Pan and Debb, 1997). There is evidence from earlier works that several plant species possess antifungal and antibacterial properties (Ali-Shtayeh and Abu-Ghdeib, 1999; Ficker et al., 2003; Erdogru, 2002, Amadioha, 2002; Maji et al., 2005; Kowalski and Kedzia, 2007; Sardari et al., 1998). There is new interest in the antifungal properties of plants known to have medicinal value to the people of Nigeria. The extracts of *Ocimum gratissimum* and *Acalypha* spp. have been reported to have antimicrobial properties (Amuchi, 1989; Ejechi and Akpomedaye, 1999; Owolade and Osikanlu, 1999). Secondary metabolites found in *O. gratissimum* include alkaloids, saponins and terpenoids and

*Corresponding author. E-mail: erute70@yahoo.com

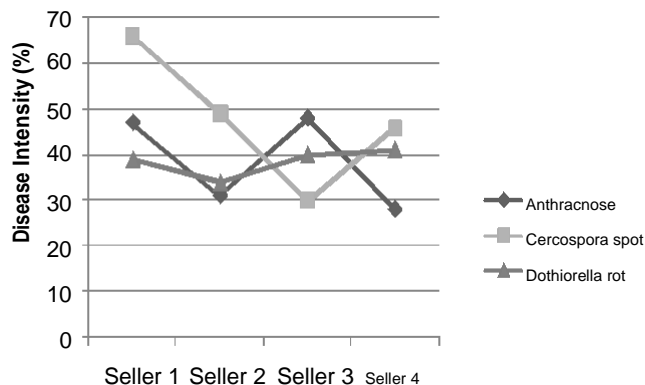


Figure 1. Disease incidence of avocado in Igbudu Market, Warri, Nigeria.

it belongs to the family Labiateae which are characterized by strong fragrant smell and slight pungency (Gill, 1992). Similarly, *Acalypha wilkesiana* and *Acalypha macrostachya* contain secondary metabolite but belong to Euphorbiaceae family that causes only low toxicity if eaten but severe skin irritation. They are both made up of diterpenoid esters that cause nausea, vomiting and diarrhea as well as acute dermatitis on contact with the milky sap of stems and leaves. This study was, therefore, designed to assess whether the aqueous and ethanolic extracts of *O. gratissimum*, *A. wilkesiana* and *A. macrostachya* will be active against *C. purpurea* a known fungal pathogen of *Persea americana*.

MATERIALS AND METHODS

Samples

The major sellers of the avocado fruit in the market were approached and a count of the healthy fruit fruits and the unhealthy ones were made. The disease symptoms were separated and the incidences of the various post harvest diseases were assessed using the formula:

$$\frac{\text{Number of infected fruits}}{\text{Total number of infected and healthy fruits}} \times 100$$

The fruits were also taken to the laboratory and the surface contaminants were isolated and identified. *C. purpurea* was isolated from avocado with typical brown spots that had fissures/ symptoms collected from the field. Pure cultures of the pathogen were prepared through aseptic transfer of spores to PDA in Petri dishes.

Plant extracts

Fresh leaves of *A. macrostachya*, *A. wilkesiana* and *O. gratissimum* from a privately owned garden in Abraka, Ethiope East Local Government Area Delta State. They

were washed thoroughly in tap water and sterile distilled water, air-dried at room temperature, weighed (100 g) and ground in a sterile mortar. The paste was added to 100 ml of sterile distilled water and non-sterile water in 250 ml beaker stirred vigorously and then allowed to stand for 1 h (Amadioha, 2002). The crude extract was then filtered with four folds of cheese cloth. The ethanol extract was prepared from leaves dried at 45°C in the oven and ground. One hundred grams of the dry powder was extracted with 100 ml of with absolute ethanol. The mixture was allowed to stand for one hour and the supernatant passed through Whatman No. 1 filter paper to obtain the alcohol extract.

The effect of the plant extracts on the radial growth of *C. purpurea* was determined using the method described by Amadioha (2002). The extract-PDA medium was prepared by spreading 1ml of the extracts separately on the surface of the solidified PDA contained in the Petri dishes. The control was 1ml of sterile distilled water. Five millimeters discs were cut from the pathogen grown on PDA and each placed in the centre of a Petri dish with three replicates for each treatment. The plates were incubated at room temperature (28 ± 2°C). The radial growth of fungus for each treatment was measured after five days, when the fungal growth in the control experiment reached the periphery of the Petri dishes.

Determination of percentage inhibition of various concentrations of leaf extracts on the radial growth of isolated most prevalent fungus. This is done using the formula:

$$\text{Inhibition (\%)} = 100 - \left\{ \frac{\text{Growth of fungus in extract}}{\text{Growth of Fungus in control}} \times 100 \right\}$$

RESULTS AND DISCUSSION

Disease assessment of post harvest disease investigated in a local market showed that disease incidence for *Cercospora* spot was highest (36%). Only 7% of the fruits assessed were healthy. The disease intensity among the sellers varied (Figure 1). The results obtained showed that (Figure 2) *Cercospora* spot were the most prevalent. The occurrence of fungal isolates associated with *P. americana* surface was *Aspergillus niger* (40%), *Rhizopus stolonifer* (29.85%), *Fusarium* sp. (17.9%) and *Penicillium* sp. with the lowest percentage occurrence of 11.94% (Table 1).

The problem of *Cercospora* infection was addressed and a cheap and accessible method was sought after. The uses of plant extracts that are found in the community and are known to have medicinal values were investigated. All ethanol extracts of the three test plants (*O. gratissimum*, *A. macrostachya* and *A. wilkesiana*) had 100% inhibition while sterilized and unsterilized aqueous extracts recorded over 70 and 60% inhibition, respectively (Table 2). There was no significant difference bet-

Table 1. Occurrence of fungi associated with *Persea americana* surface.

Fungal isolate	No of times isolated	Occurrence (%)
<i>Aspergillus niger</i>	54	40%
<i>Fusarium</i> sp.	24	17.9%
<i>Rhizopus stolonifer</i>	40	29.85%
<i>Penicillium</i> sp.	19	11.94%

Table 2. Inhibition (%) of *Cercospora purpurea* by extracts of three different plants.

Plant species	Ethanollic extract	Sterilized water extract	Unsterilized water extract
<i>Ocimum gratissimum</i>	100±0.00 ^a	75±3.17 ^b	64±5.71 ^b
<i>Acalypha macrostachya</i>	100±0.00 ^a	73±2.56 ^b	62±4.65 ^b
<i>Acalypha wilkesiana</i>	100±0.00 ^a	72±2.98 ^b	61±4.09 ^b

Means followed by standard error with same letter superscripts are not significantly different (P<0.05).

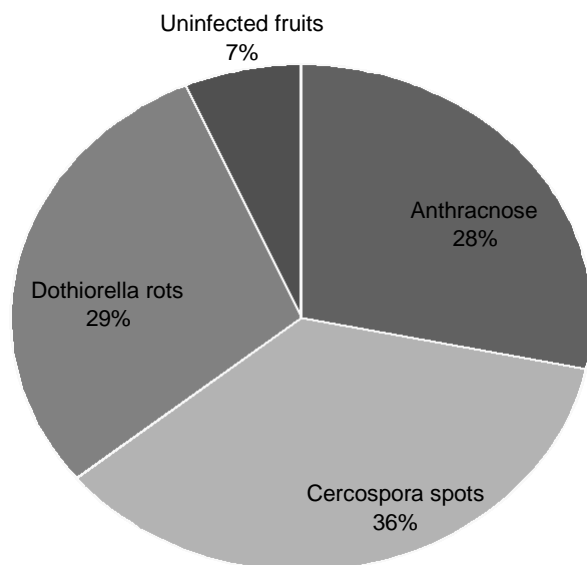


Figure 2. Prevalence of the post harvest diseases associated with avocado among sellers in Igbudu Market Warri, Nigeria.

between the different plants in their inhibitory effect but *O. gratissimum* recorded higher inhibition percentage.

The study shows that only small percentage of the fruits in the market was healthy. This situation could have arisen from poor storage conditions and facilities, which is common in developing countries. The treatment of post harvest fruits with prochloraz which is a practice in developed countries is not practiced in most developing countries. Pre-harvest spraying is done to prevent post harvest losses. The heavy losses incurred by farmers and sellers in the developing countries can be tackled by sourcing for cheaper compounds to be used as pre-harvest spray which are equally effective and less harm-

ful. The three plant extracts in this study were all active against the pathogen. The ethanollic extract showed a total inhibition of the pathogen, while for the aqueous extract, inhibition was over 60%. The use of unsterilized water for extraction also showed appreciable inhibition of over 60%. Amuchi (1989) reported that *O. gratissimum* reduced the radial growth of *Rhizopus* spp. which cause avocado rots. This report recorded reduction of radial growth of *Cercospora* sp. Other researchers like Ejechi and Akpomedaye (1999) reported that phenolic extracts of *A. hispida* inhibited *Gleophyllum sepiarum* and *Pleurotus* spp. displaying antimycotic properties. Owolade and Osikanlu (1999) observed that *O. gratissimum* and *A. ciliata* inhibited the radial growth of *Collectrichum* spp. and compared favorably with benlate.

The ethanollic extracts of the three test plants recorded 100% inhibition of the pathogen. Ethanollic extracts of other medicinal plants like *Mellissa officinalis*, *Capsicum annum* and *Piper nigrum* have also been shown to have antifungal properties (Erturk, 2006). Other plants like *Solanum deflexiflorum* have also recorded antimycotic activities against *Candida albicans* and *Fusarium solani* (Nino et al., 2006). The list of medicinal plants that have been recorded to be inhibitory to the growth of fungal pathogens of both animal and plant is quite much. There are still some plants that have not been investigated. The use of botanicals for the treatment of fungal diseases in both plants and animals has lot of promises. The 100% inhibition of the pathogen with the ethanollic extracts of the test plants is the base for further studies to isolate and elucidate the structure of the bioactive compounds in these plants.

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