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Antifungal Activities Of Four Plant Extracts Against Cercospora Papayae Casual Pathogen Of Black Spot Disease Pawpaw In Ado-Ekiti South Western Nigeria

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Abstract ***Cercospora black spot of pawpaw caused by Cercospora papayae is responsible for 50-70% yield loss of pawpaw when not controlled. Based on the above, laboratory studies were conducted to evaluate the effect of hot water leaf extracts of four plants: Castor oil leaf (Ricinus communis), African eggplant (Solanum macrocarpon), scent leaf (Ocimum gratissium) and Tobacco leaf (Nicotiana tabacum) in the management of the disease. Three concentrations of the extracts (15, 30 and 45) were prepared and applied in-situ to determine their efficacy of control on cercospora black spot disease of pawpaw. The result of the studies shows statistically significant (P ≤ 0.05) variation in C. papayae growth depending on the extracts and concentration. N. tabacum at 45% concentration was the most effective in reducing the growth of the fungus and this was closely followed by S. macocarpon and R. communis while that of O. gratissimum was the least. Growth rate induced by N. tabacum at 45% concentration was 2.38 mmday-1 while that of S. macrocarpon and R. comumis were 2.92 and 3.15 mmday-1 respectively. There was 60-84% inhibition of conidia germination irrespective of plants extracts and concentration but the extracts do not have effects on sporulation. The study concluded that the active compounds in the leaf extracts can be developed into synthetic fungicide for the management of cercospora black spot disease of pawpaw.***

Keywords: *Pawpaw, Cercospora papayae, Plant extract, Inhibition*

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INTRODUCTION

Pawpaw (*Carica papaya*) is a member of the family caricacea, class dicotyledonac and order brassicales. It's widely cultivated in both the tropical and sub tropical regions as a source of food (ogwulumba *et al,* 2010). Pawpaw contains a high amount of vitamin C and E needed for growth and as immune boosters essential for protection of the body against illness. Apart from this, it is useful as an antioxidant preventing oxidation of cholesterol. In addition, it helps to reduce the risk of heart disease (FAOSTAT, 2021) Pawpaw originated from south Mexico and is widely grown or cultivated all over the world. World production of pawpaw in 2020 was estimated at 6 million metric tonnes with India being the largest producer accounting for about 3 million metric tonnes. This is closely followed by Dominican Republic and Brazil with production of 1.7 and 1.2mm tonnes respectively. Nigeria is the fourth largest producer of pawpaw with over 800,000 metric tonnes (Agbowuo, 2012).

Pawpaw is attacked by different fungal diseases like anthracnose (*Colletotrichum gloeosporioides*), charcoal rot, black rot, phytophthora rot, fusarium wilt all of which impair the growth, vigour and photosynthesis with direct effect on yield or productivity. Pawpaw is propagated by seeds and since most of the pathogens are seed borne, they are likely to spread easily thus increasing production cycle from year to year which will adversely contribute to build up of inoculum (Parveen, *et. al.,* 2014). Black spot disease of pawpaw is caused by the pathogen *Cercospora papayae*, the pathogen is capable of infecting wide variety of plants. The disease affects all parts of the plant. Symptoms of the disease are irregular dark brown measuring about 1/16 to ¼ on lower leaf tiny black dots that eventually enlarge to about 3mm in

diameter, this is visible on fruits thus reducing quality and marketability. The upper surface of the infected plant is slightly sunken. In some cases, black spots have also been observed on the surface of fruits but not as much as those on the foliage. Spores of the fungus are released when relative humidity is high, lesion size depends on severity of the disease and his lesion may drop out of leaf during dry weather (Muritala *et. al.*, 2021).

Various approaches have been used in Nigeria to reduce the effect of this disease such as the use of resistant varietis where they exist, intercropping of susceptible and non susceptible crops, pruning of dead leaves from tree, removing and destroying infected plants, use of plant extracts and selective fungicides (Parveen *et. al*., 2014). However, due to increased awareness of the side effects of synthetic pesticides, attention is now being focused on alternative methods that are safe, cheap, less costly and in addition compartible with the farming practices of the farmers for pest and disease control (Falade, 2022).

The antifungal effects of castor oil (*Ricinus communis*) (Na2 and Bano, 2012), scent leaf (*Ocimum gratissimum*) (Okoi *et. al*., 2013), African eggplant (*Solanum macrocarpon*) and tobacco leaf (*Nicotiana tabacum*) (Qian-lichen *et. al.*, 2020) are well known but their use in the management of black spot disease of pawpaw have not been exploited. Based on the above, the present study was carried out to compare the effects of extracts of these plants at various concentrations on the mycelia growth. Conidia germination and sporulation of the fungus using invitro trials.

2.0- MATERIALS AND METHODS

2.1 Collection of plant Leaves

The leaves of *Ricinus communis*, *Solanum macrocarpon*, *Ocimum gratissimum* and *Nicotiana tabacum* were collected from the Ekiti State University Teaching and Research Farm (Latitude 7 7212°N and longitude 5.2575°E) in the South western Nigeria. The leaves were air-dried at ambient temperature (28±2°C) for 4-6 weeks, powdered using a blender (Okapi®, Mixer Grinder), packaged into sealable nylon and refrigerated at 4°C until they were required for bioassay.

2.2 Preparation of plant extracts

Extracts were prepared by mixing equivalent grams of the prepared plant powder (15, 30 and 45) with 100 ml of distilled water in 500 ml flasks and kept in hot water bath-shaker at 70 °C for 2 hours. Thereafter, the extract was separated from the shaft by vacuum filtration and stored at 4 °C in McCartney bottles and used as the stock solutions from which 15, 30 and 45% concentrations were prepared (Collin and Michael, 2000).

2.3 Preparation of modified media

Preparation of Modified Media Standard Potato Dextrose Agar (PDA, E. Merck, Darmstadt Germany) was modified with different concentrations of the plant extracts and autoclaved. Thereafter, the agar was allowed to cool to 50 °C, amended with 30 µg/L streptomycin sulphate, poured into 9 cm sterile petridishes (Sterilin® Product, UK) inside a laminar flow cabinet and left for 20 minutes to solidify.

2.4 Isolation & Identification C. Papayae

Pawpaw leaf showing symptoms of leaf spot were collected from the pawpaw fields in the Teaching and Research Farms Ekiti State University, Ado Ekiti. The leaves were cut into approximately 1-2 cm sizes and surface sterilized with sterile distilled water containing 0.2 % hypochlorite solution followed by two rinses in sterile distilled water in a laminar flow cabinet. Three leaf cuttings were placed on standard PDA media containing 30 µg/L streptomycin sulphate to suppress bacteria growth. The plates were sealed with parafilm and incubated at 28 °C for 5-6 days. Single spores of developing colonies were isolated and sub-cultured to obtain pure cultures. The samples from the single spore cultures were used for morphological identification on Malt Extract Agar (MA) at x400 magnification of a compound microscope with Zivkovic *et. al.,* (2010).

2.5 Evaluation of growth

One-centimeter agar disk of the pure culture was transferred unto the prepared plant extract modified PDA media. After 24 hours, the colony diameter along pre-marked orthogonal axes at the bottom of the Petridishes was done and this continued until the surface of the plate was covered. The values of the colony diameter were averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each treatment relative to control.

2.6 Evaluation of conidia germination

Sterile PDA in 9 cm Petri dishes were inoculated with 10 ml of *C. papayae* conidia suspension measured with a micropipette and spread-plated using Drigalsky spatula, the lids were replaced and sealed with parafin. The incubation was carried out at ambient temperature (25±2°C) for 24 hours. Thereafter, sterile cover slip was placed on the spread-plated area and percentage germinated conidia was estimated for 100 conidia in the cover slip area under a compound microscope using x40 magnification. The conidium with

germ tube length longer than it's diameter was considered as germinated. The Percentage Conidia Germination (PCG) was calculated as; PCG = $No\ of\ germinated\ conid\ X\ 100$
$Total\ counted\ conidia\ within\ field\ of\ view$

2.7 Evaluation of sporulation density *C. papayae*

C. papayae Spores suspension was prepared from 7-days old culture by flooding the surface of the growing colonies in each petri-dish with 10ml sterile distilled water containing 0.05% Tween 80 (Polythelene glycol sorbitan monolaurate) and Drigalsky spatula was used to dislodge the spores carefully. Suspensions were serially diluted and spores counted using improved Neaubaur Haemocytometer. Sporulation density which was the estimated number of spores per colony area was calculated as; Sporulation density (spores cm2 colony area) = estimated number of spores/ colony area (Falade, 2018).

RESULTS

Table 1 shows the effects of hot water extracts of the four plants on growth of *C. Papayae.* The growth rate differed significantly in relation to plant extracts and their concentration. Result showed that the growth rate reduced as the concentration of the four extracts increased. At the highest concentration (45% w/v), the growth rate induced by *N._ tabacum* was 2.83mmday^{-1}While those of *S. macrocarpon*. *R. communis* and *O. gratissimum* were 2.93, 3.0 and 3.2 mmday^{-1} respectively.

Table 1: Effect of three concentration of hot water leaf extract of four plant on growth rate of *C. papayae* concentration in Ado-Ekiti, South Western, Nigeria.

	Concentration/ (Growth rate mmday^{-1})		
Plant extracts	**15**	**30**	**4.34**
N. tabacum	3.34* (23.0)	3.01 (30.6)	2.83 (34.80)
S. macrocarpon	3.46 (20.3)	3.22 (25.81)	2.92 (32.70)
R. communis	3.50 (19.4)	.30 (24.0)	3.00 (30.90)
O. gratissimum	3.57 (17.7)	3.37 (22.4)	3.20 (26.30)
Control	4.34	4.34	4.34

Values in parenthesis are GRI (Growth rate inhibition %).
Means with the same letter in each column are not significantly different (R< 0.05) Tukeyhsk

Table 2 shows the effect of the four plant extracts on conidia germination of C. papaya. There was 64-84% inhibition of conidia germination in respective of the plant extract of concentration. Conidia germination was relatively low at the highest concentrations of the extract but as the concentrations reduced. Condia germination was high
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Table 2: Germination or condia of *C. papaya* after 12 hours incubation on modified PDA at the concentrations of the extract in Ado – Ekiti**.**

	Plant Extracts/Conidia germination (%)			
Conc. extracts	*N. tabacum*	*S. macrocarpon*	*R. communis*	*O. gratissimum*
0	100^{a}	100^{a}	100^{a}	100^{a}
15	69^{d} (31	76^{d} (24)	80^{d} (20	84^{d}(16)
30	64^{c} (36)	67^{c} (33)	69^{c} (31)	74^{c} (26)
45	60^{b} (40	63^{b} (37)	65^{b} (25)	68^{b} (32)

Means with the same letter in each column are not significantly different (R< 0.05) Tukeyhsk

Table 3 shows the effects of the three plants extracts on sporulation destiny of *C. papayae*. There was no significant difference in the conidia destiny in the media modified with the different concentrations of all the four-plant extract

Table 3: Effect of three concentrations of four plant extracts on sporulation density of *C. papayae* in Ado-Ekiti, South Western, Nigeria**.**

Plant Extract	Sporulation density/Conc. (W/V)			
	0	***15***	***30***	***45***
N. tabacum	6.1[a]	6.2[a]	6.1[a]	6.1[a]
S. macrocarpon	62[a]	62[a]	62[a]	62[a]
R. communis	62[a]	62[a]	62[a]	62[a]
O. gratissmum	63[a]	61[a]	61[a]	62[a]
Control	63[a]	63[a]	63[a]	63[a]

Means with the same letter in each column are not significantly different (R< 0.05) Tukeyhsk

DISCUSSION

Cercospora black spot of pawpaw undermines its production in Nigeria because the pathogen is capable of surviving in soils and plants debris thus increasing inoculum load. In this study, control of the disease was achieved with the use of plant extracts as against the conventional approach involving the use of fungicides. The extracts of the four plants reduced mycelia growth of C. Papayae and inhibition was concentration dependent. Higher inhibition of growth occurred at relatively higher concentration of the plant extract and there was 18-35% inhibition irrespective of plant extracts or concentration. The inhibitory activity of the extracts was probably due to increased availability of antifungal chemicals in the medium thus surpressing the growth of *C. Papayae.* Hubert *et al.,* (2021) evaluated the aqeous extracts of *Annona Muricata* seed extracts against mycelia growth cercospora leaf spot of okro. The study shows that the plants extract reduces the mycelia growth of the fungus which is in agreement with the current study, similarly Kone *et. al.,* 2018 reported the ageous and organic extracts of *jatropha curcas* seeds being inhibitory to the growth of *cercospora malayensis* the causal agent of sigatoka disease of okra leave. In the study, all the four extracts at the tested concentration inibit conidia germination irrespective of plant extracts or concentration when modified on PDA after 24 hours incubation at ambient temperature. This finding is in agreement with that of Falade, 2022 who reportedly that extracts of *Tridax procumbens, Ricinus Communi* and *Sida acuta* successfully reduced Conidia germination of *Pestalotia heterocornnis,* the causal agent of cashew leaf spot. This finding contrasted some earlier reports on the effect of botanicals on condia germination, Falade, 2017 reported the antifungal activities of six plant extracts against Colletorichum lindemuthianum, the causal agent of cowpea anthracnose, the study showed that all the extracts had no effect on conidia germination.

In the study, all the four extracts at the tested concentration do not have any effect on sporulation o *C. papaya*. This finding however contrasts the report of Falade 2018, who reported the effect of extracts of some indigenous plants on the growth of *Colletotricum lindemuthianum* the pathogen causing anthracnose disease of Cowpea. The study showed that the extracts had significant effect on sporulation. The mechanism of some plant extracts having effect on Conida germination without significant deterrent on sporulation is not fully understood.

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