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Full Length Research

Invitro Control of Cashew Leaf Spot (*Pestalotia heterocornnis*) with Use of Plant Extracts in Ado-Ekiti, South Western Nigeria

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ABSTRACT

Cashew leaf spot caused by *Pestalotia heterocornnis* is responsible for reduction in yield or productivity of cashew with serious economic implications. Based on the above, laboratory studies were conducted to evaluate the effect of hot water leaf extract of Coat button (*Tridax procubens* Linn), Iron weed (*Sida acuta* Burm.f) and Castor oil leaf (*Ricinus communis* Linn) in the management of the disease. Four concentrations of the extracts (10, 20, 30 and 40%) were prepared and applied in-situ to determine their efficacy of control on pestalotia leaf spot disease of cashew. The result of the studies shows statistically significant ($p < 0.05$) variation in *P. heterocornnis* growth depending on the extracts and concentration. *T. procumbens* at 40% concentration was the most effective in reducing the growth of the fungus and this was closely followed by *R. communis* and *S. acuta* was the least. Growth rate induced by *T. procumbens* at 40% concentration was 2.81 mmday^{-1} while that of *R. communis* and S. acuta were 2.91 and 3.10 mmday^{-1} respectively. All the extracts do not have effect on sporulation but reduced conidia germination. There was 61-87% inhibition of conidia germination irrespective of plant extracts and concentration. The study concluded that the active compounds in the leaf extracts can be developed into synthetic fungicide for the management of leaf spot disease of cashew.

Keywords: Cashew, Plant extracts, Inhibition, *Pestalotia heterocornnis*.

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INTRODUCTION

Cashew (*Anacardium occidentalis*), a member of the family Anacardiacea, Class Magniolopsidae and Order Sapindales is widely cultivated in both tropical and subtropical regions as a source of food. (*Jules et al*., 2021). Cashew is important in boosting human immune system, it is low in sugar, rich in fibre and plant proteins. Apart from this, it is a good source of Copper, Magnesium and Manganese necessary for bone formation (Issa *et al*., 2017). Cashew nuts are a source of food, they are useful in cosmetic and automotive industries. In addition, they are important in the conservation of biodiversity and improvement of impoverished land (FAOSTAT, 2020). World production of cashew in 2020 was estimated at 4.18 million metric tonnes (MMT) with Vietnam being the Leading producer accounting for about 44.9% (2.598MMT) of the total production, this is closely followed by India and Ivory coast with production capacity of 0.786 and 0.731MMT respectively. Nigeria is ranked thirteen producing about 0.097 MMT. Most of the cashew produced are consumed locally with little or no export hence the need to increase production (Adeigbe *et al*., 2015).

Cashew is attacked by different fungal diseases like anthracnose (*Colletotrichum gloesporiodes*), gummosis (*Lasiodiplodia theobromae*) that impair the growth, vigour, photosynthesis and abortion of flowers and fruits all of which reduces yield or productivity. Cashew is propagated by seed and since most of the

pathogens are seed borne, they are likely to spread thus increasing production cycle from year to year which will adversely contribute to build up of inoculum (Nasir *et al*., 2018)

Cashew leaf spot caused by the fungus *Pestalotia heterocornnis* is capable of infecting a wide variety of deciduous trees, shrubs and oarks. The disease affects all parts of the plants (Nasir *et al.,* 2018). Symptoms are visible on the leaves, twigs, petioles, flowers cluster and fruits, on leaves small angular brown to black spots are noticeable and this enlarge to form extensive dead area (Savadi *et al*., 2019). Older leaves have necrotic tissues, covered by black structures, which are spores of the fungus that has increased in size. The spores are released when relative humidity is high, lesion size depends on severity of the disease, the lesion may drop out of leaf during dry weather (Muntala *et al*., 2021).

The management of the diseases above in Nigeria can be achieved by the use of resistant varieties where they exist, intercropping of susceptible and non-susceptible crops, removing and destroying infected plants, pruning dead leaves from trees, use of plant extracts and control using selective fungicides (Parveen *et al*., 2014). However, due to increased awareness of the side effects of synthetic pesticides on the soil and beneficial microorganisms coupled with the need to produce crop with minimal residual pesticides, attention is now being focused on alternative methods that are safe, cheap, less costly and in addition is compatible with the farming practices of the farmers for pest and disease control (Falade, 2021).

The antifungal effects of coat button (*Tridax procumbens*) (Kumar and Sharma, 2009, Falade 2018), iron weed (*Sida acuta*) (Hoffnam *et al*., 2004) and castor oil (*Ricinus communis*) [Naz and Bano, 2012] are well known but their toxicity to *P. Heterocornnis* and its use in the management of cashew leaf spot have not been studied. Therefore, this study was carried out to compare the effects of the extracts of these plants on growth, conidia germination and sporulation of *P. heterocornnis* using invitro bioassay study.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Leaves and Source of Fungicide

The leaves of *P. procumbens, R. Communis* and *S. acuta* 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 (40, 30, 20 and 10) 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 40, 30, 20 and 10% concentrations were prepared (Collin and Michael, 2000).

2.3 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 and identification of *P. heterocornnis*

Infected cashew leaf showing symptoms of leaf spot were collected from the cashew 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 subcultured 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 *P. heterocornnis* 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;

$$PCG = \frac{No\ of\ germinated\ conidia}{Total\ counted\ conidia\ within\ field\ of\ view} X100$$

2.7 Evaluation of sporulation density *P. heterocornnis*

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 cm^{-2} colony area) = estimated number of spores/ colony area (Falade, 2018).

3.0: RESULTS

Table 1 shows the effects of hot water extracts of the three plants on growth of *P. heterocornnis*. The growth rate differed significantly in relation to plant extracts and their concentrations. As the concentration of the extracts increased, the growth of *P. heterocornnis* reduced in all the three plant extracts. At the highest concentration (40% w/v), the growth rate induced by *T. Procumbens* leaves was 2.81mm day^{1} while those of *R. Communis* and *S. acuta* were 2.91 and 3.10 mm day^{1} respectively.

Table 1: Effect of three Concentration of hot water leaf extracts of three plants on growth rate of *P. heterocornnis* in Ado Ekiti, Nigeria

	Concentrations			
Plant extracts	**10**	**20**	**30**	**40**
T. procumbens	3.51^{c} (19.1) *	3.35^{c} (22.8)	3.01^{d} (30.6)	2.81^{d} (35.3)
R. communis	3.55^{c} (18.2)	3.48^{b} (19.8)	3.25^{c} (25.1)	2.91^{c} (32.9)
S. acuta	3.59^{c} (17.2)	3.50^{b} (19.4)	3.31^{b} (23.7)	3.10^{b} (28.6)
Control	4.34^{a}	4.34^{a}	4.34^{a}	4.34^{a}

Values in parenthesis are GRI (Growth Rate Inhibition %)
Means with the same letter in each column are not significantly different (P<0.05) (Turkey' HSD)

Table 2 shows the effects of the plant extracts on conidia germination *P. heterocornnis*. There was 61-87% germination of conidia irrespective of the plant extracts or concentrations. At the highest concentration, conidia germination was low but as the concentrations reduced, conidia germination was high.

Table 2: Germination of Conidia of *P. heterocornnis* after 12 hours incubation on modified PDA at three concentrations of the plant extracts in Ado-Ekiti.

Extracts			
Concentrations (w/v)	***T. procumbens***	***R. communis***	***S. acuta***
0	100^{a}	100^{a}	100^{a}
10	82^{b} (18) *	85^{b} (15)	87^{b} (13)
20	76^{c} (14)	82^{c} (18)	83^{c} (17)
30	69^{d} (31)	76^{d} (24)	79^{d} (21)
40	61^{e} (39)	69^{e} (31)	72^{e} (28)

Values in parenthesis are % reduction in conidial germination
Means with the same letter in each column are not significantly different (P<0.05) (Turkey' HSD)

Table 3 shows the effects of the three plants extracts on the sporulation density of *P. heterocornnis.* There was no significant difference (P≤0.05) in the conidia density in the media modified with the different concentrations of all the three plant extracts

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Table 3: Effect of three Concentration of hot water leaf extracts of three plants on sporulation rate of *P. heterocornnis*in Ado Ekiti, Nigeria

Plant Extract	**Sporulation density/ Concentration/ (%w/v)**			
(Treatment)	10	20	30	40
T. procumbens	6.3	6.2	6.2	6.3
R. communis	6.3	6.2	6.2	6.3
S. acuta	6.3	6.2	6.3	6.3
Control	6.2	6.2	6.2	6.2

The sporulation values are $\log_{10}$ of conidia cm^{-2}
Means with the same letter in each column are not significantly different (p≤0.05) (Turkey HSD).

DISCUSSION

Pestalotia leaf spot disease undermine cashew production in Nigeria, the fungus is capable of producing spores that can travel thousands of kilometres to infect plant thus causing reduction in yield or productivity of cashew (Mange and Shomari, 2016). In this study, hot water leaf extract of the plant: *T. procumbens*, *R. communis* and *S. ccuta* reduced mycelia growth of *P. heterocornnis* and the rate of inhibition was concentration dependent. Higher inhibition of growth occurred at relatively higher concentration of the plant extract and there was 17-35% inhibition irrespective of extract or concentration. The inhibitory activity of the extract was probably due to increase availability of antifugal chemicals in the medium.

Mathukumar *et al.,* (2010) evacuated 66 medicinal plants belonging to forty-one families for their antimicrobial activities against the myceha growth of *Pythium aphamidermatum,* the causative organisms of rust disease in chilli plants and found out that 23 of the plant extracts had inhibitory effect on the mycelia growth of the pathogen with extracts of *T. Procumbens. Allium cepa* and *Allium satium* as the most effective which are in agreement with the current study.

In the study, spore suspension of *P. heterocornius* prepared from 7-day old culture was flooded with Tween80 to dislodge spores and the sporulation density was evaluated. The result of which shows that all the three extracts at the tested concentrations have no effect on sporulation of the fungus. The result of the present study is in agreement with the work of Tegene *et al.,* (2008) who reported crude extracts of various Agapanthus Africana plant parts that was screened against eight economically important plant pathogenic fungi. The study showed high degree of tolerance. It has been established that when mycelia growth is inhibited, it is a form of stress to which a fungus would respond to by producing large number of Conidia. Obi and Bariuso vargas (2013) reported that sporulation of *Colletotrichium lindemuthianum* the pathogen causing anthracnose disease of cowpea decreased as the concentration of the active ingredients in the plant extracts increased which is in contrast to the present study.

The susceptibility of phytopathogenic fungi to botanicals are controlled by a number of factors which include the chemical constituents of the plants, strain of fungus, mode of exposure to fungitoxic constituents, mode of extraction of active ingredients, these and, many others may be responsible for the result obtained in this study.

Conclusion

This research provided information that cashew anthracnose can be controlled with the use of plant extracts in the laboratory. The three leaf extracts used in the study are readily available in homesteads and the method of extraction is simple, this can easily be adapted by peasant farmers for the control of cashew anthracnose disease, thereby replacing the conventional fungicide that are costly alongside the attendant side effects.

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