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Full Length Research Paper

Invitro and Invivo Control of Cowpea anthracnose caused by *Colletotrichum lindemuthainum* using extracts of Indigenous Plants.

Falade, M.J.

Department of Crop, Soil and Environmental Sciences, Ekiti State University, Ado-Ekiti, Nigeria.

Corresponding Author's Email Address: faladem osesjimoh@yahoo.com

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Laboratory study was conducted to evaluate the effects of hot water extracts of six indigenous plants: *Tridax procumbens, Jatropha gossypifolia, Sida acuta, Blighia sapida, Ricinus communis* and *Datura stramonium* on the growth of *Colletotrichum lindemuthianum* in the laboratory and subsequent control of the disease on the field. Result from the experiment shows that all the plants extracts reduced the growth rate of *C. lindemuthianum*. *D. stramonium* was the most effective followed by *R. communis* and *J. gossypifolia* while *B. sapida* caused the least inhibition of growth. Similarly, the extracts reduced the incidence and severity of the disease on the field. The study showed that the plant extracts have the potentials for the control of anthracnose disease of cowpea.

Keywords: *C. lindemuthianum, Plant extracts, Growth rate, Disease Incidence and Severity.*

INTRODUCTION

Cowpea anthracnose disease caused by the fungus, *Colletotrichum lindemuthianum* is an important fungal disease of field grown cowpea capable of 75% yield reduction in Nigeria (Enyiokwu *et al.,* 2014).The disease affects all stages of the plant but more often in the reproductive stages. Its symptoms include round brownish or purple specks which become darker and enlarge into lesions (about 2 cm in diameter). (Sharon and Douglas, 2011). Control of the disease rely solely on the use of synthetic fungicides like Benomyl and Mancozeb which often in time past are effective. (Swapan and Chakraborty, 2012).

However, due to increased awareness of side effects of these synthetic pesticides, (pollution of environment, toxic effect on non-target organism, development of resistance to diseases) much attention is being focused on the use of indigenous plants for the control of diseases (Shilpa and Gokulapan, 2014). In addition, disease problems are expected to be more severe in most agro-ecological regions of Sub-Saharan Africa which have been predicted as potential hot-spots for climate change. (Apata and Adeola, 2009) While climate change will have dire consequences on agriculture, the impact of agrochemicals used in agricultural productions on global warming is also significant. In view of these, there is need for development of environment friendly approach, such as the use of botanicals.

Studies have shown the importance of these botanicals as possible source of easily biodegradable and non-phytotoxic substances which can inhibit the growth of many pathogenic organisms (Gideon and Anita, 2013). Apart from this, pesticides of plant origin are usually cheap, readily available and compatible with the farming practices of peasant farmers for disease control (Lowell, 2004).

Leaf extracts of plants such as Purple princess (*Cyathula prostrata*), *Diodia scandens,* Black pepper (*Piper nigrum*), Clove basil (*Ocimum gratissimium*), Lemon (*Citrus limon*), Lemon grass (*Cymbopogon citratus*) have been tested against fungal infections in cowpea with varying degrees of success (Gideon and Anita 2013; Amadioha and Obi, 1999; Amadioha, 2003). The potentials of botanicals for disease control have been recognized such that there is the need for research into other botanicals for the control of anthracnose in cowpea.

The anti-fungal effects of physic nut (*Jatropha gossypifolia*) (Igbinosa *et al.,* 2009), akee apple (*Blighia sapida*) (Pakinson, 2007), castor oil (*Ricinus communis*)

(Naz and Bano, 2012),jimson weed(*Datura stramonium*) (Usha et al., 2009),iron weed (*Sida acuta*)(Hoffman et al., 2004) and coat button (*Tridax procumbens*) (Sharmar and Kumar, 2009) are well known but their use for the management of anthracnose in cowpea has not been studied. The study is carried out to evaluate the effect of leaf extracts of these plants on growth of *Collectotrichum lindemuthanum* in the laboratory and the effect of the extracts on disease incidence and severity on the field.

MATERIALS AND METHOD

Collection of plant leaves and preparation of extracts.

Leaves of *D. stramonium, J. gossypifolia, B. sapida, T. procumbens, R. communis* and *S. acuta* were collected from 12-15 month old plants and air-dried at ambient temperature (24±2°C) for 14 -21 days. The dried leaves were turned into powder using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C. Thereafter, 65 grams of the powder of each plant was weight into 250 ml standard flask and 100 ml of distilled water at 70°C was poured into each flask. The flasks were maintained at this temperature in hot water bath-shaker for 30 minutes. Thereafter, the liquid extract was separated by vacuum filtration and poured into standard bottles which were refrigerated at 4°Cand subsequently used as the stock solution.

Isolation and morphological identification of *C. lindemuthianum*.

Cowpea plants showing distinct symptoms of anthracnose disease were collected from fields at Ekiti State University Teaching and Research farm. Ado – Ekiti, Nigeria. The leaves were cut into pieces of about 1-2cm and surface sterilized by immersion in 0.2 % NaOCl for two minutes. This was followed by two rinses in sterile distilled water and spraying with 70% isopropanol. The sterilized leaves were kept inside a laminar flow cabinet for 20-30 minutes to dry. . Five sterilized leaf cuttings were appressed unto the surface of Potato Dextrose Agar (PDA) (Sigma-Aldrich) containing 0.05% chloramphenicol inside 9 cm Petri-dishes and removed. For the isolation of the anthracnose pathogen, three of the surface sterilized leaf cuttings were placed on PDA-modified with chloramphenicol. The plates were sealed with parafilm and incubated separately at ambient temperature for 5-6 days. . There was no growth on the plates unto which leaves were appressed and this confirmed that the surface of the leaves were sterile. Single conidium from developing colonies in the isolation plate was transferred into prepared standard PDA media to obtain a pure culture. Agar plugs from single conidia cultures were used for morphological identification on Malt Extract Agar (MEA) at x400 magnification of a compound microscope (OLYMPUS Binocular) (Zivkovic*et al*, 2010).

Effect of plant extract on growth

In order to evaluate the effect of the extracts on growth, standard PDA media (control) and plant extract-modified PDA based media were prepared as described previously. The plates were inoculated at the centre with 1µl of conidia suspension containing 1 x 10^2 conidia ml^{-1}using micro-pipette (Eppendorf 1-10µl).They were sealed with parafilm and incubated at 20°C for eight days. The treatments and the control were replicated three times. Daily measurement of the colony diameter along two orthogonal axes which were marked on the plates was commenced at 24 hours after inoculation and this continued for 5-10 days. The values of the growth rates were averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each treatment and compared with the control:

$$\text{PIMG} = \frac{(R1-R2)\,100}{R1}$$

Where, R1= Radial extension of colony in the control plate And R2 =Radial extension of colony in sample plate.

Effect of Extract on Disease Incidence and Severity

Four cowpea seed susceptible to anthracnose infection (Ife brown) were surface sterilized in 0.5% sodium hypochloride solution for 1 minutes later rinsed twice with sterile distilled water and sown in 10cm2 diameter plastic rubbers containing 5kg of topsoil which has been previously heated at a temperature of 1200c for 3 hours in an oven to kill all microorganism. The soil was allowed to cool overnight before planting the seeds. After germination, the seeds were thinned to 3 per stand and the plastic rubbers arranged randomly in three groups in a glasshouse and watered every 48 hours.

Two weeks after germination. Plants in the first category were inoculated with spore suspension of C. lindemuthianum, 48 hours after which it was sprayed with different concentrations of the extract. Plants in the second category were sprayed with the different extracts and after 49 hours inoculated with spore suspension of the organism. Plants in the third category were inoculated with spore suspension followed by simultaneous spraying of all the different concentration of extracts. In all the sprays was used. The control plot in each category was sprayed with distilled water. Observation on ease development and spread were recorded and the data analyzed. Disease incidence was determined using the formula

$$\%\ \text{disease incidence} = \frac{\text{No plants Infected X 100}}{\text{Total No of Plants}}$$

Disease severity was recorded on a scale of 0 – 4 with zero representing no infection and 4 denoting plants that are completely infected three replicates were done for each treatment using a completely randomized design.

RESULTS

The effect of hot water extracts of the six plants on growth of *C. lindemuthianum* is shown in Table 1. Growth inhibition rates varied significantly in relation to the plant extracts and their concentrations. The rate of growth in the control was significantly the highest (P<0.05). At 65% concentration of *D. stramonium* extract, there was 32.6% inhibition of growth while *R. communis* and *J. gossypifolia* caused 16.4% and 10% reduction in the rates of growth respectively. Sixty five percent concentration of *S. acuta*, *T. procumbens* and *B. sapida* caused 16.4%, 12.4% and 10.0% inhibition respectively. Lower rates of growth inhibition were recorded at 50% and 30% concentrations.

Table 1: Growth rate of *C. lindemothianum* at different concentrations

Sources of variation	SS	DF	MS	F	Sig
Extracts	3.212	5	.642	60.257	.000
Error	.512	2	.011		
Total	16.969	6			
Corrected Total	13.245	6			

a. R Squared =.961 (Adjusted R Squared = .943)

Table 2: Effect of Extracts on Incidence of *Colletotrichum lindemuthianum*

Leaf Extract	2db inoculation	2d after inoculation	Simultaneous Inoculation
D. stramonium	20.4[a]	24.3[a]	26.7[a]
T. procumbens	25.2[b]	26.3[b]	30.8[c]
S. acuta	26.4[c]	28.2[c]	31.7[c]
B. sapida	27.0[c]	29.2[c]	31.7[c]
J. gossypifolia	24.8[a]	28.1[c]	30.4[c]
R. communis	23.1[a]	27.2[b]	28.4[b]
Control water	100[0d]	100[0d]	100[0d]

In table 2. Values for average of 3 replicates in two separate experiment. Values in the same column followed by similar letter are not significantly different by LSD (P=0.05)

Table 3: Effect of Extracts on Severity of Anthracnose Disease

Spray leaf extract	2db inoculation	2d after inoculation	Simultaneous Inoculation
D. stramonium	1[a]	2[b]	2[b]
T. procumbens	2[b]	3[c]	3[c]
S. acuta	2[b]	3[c]	4[c]
B. sapida	3[c]	3[c]	4[c]
J. gossypifolia	2[b]	3[c]	3[b]
R. communis	1[a]	2[b]	3[b]
Control water	4[d]	4[d]	4[d]

In Table 3, values for average of 3 replicates in two separate experiment. Values in the same column followed by similar letter are not significantly different by LSD (P=0.05)

DISCUSSION

Despite the increasing importance of cowpea in the diet of man as a source of protein, maximum yield potentials of the crop has not been revealed due to incidence and severity of anthracnose disease (Masangwa *et al.,* 2013). Extracts of all the six plants used in this study reduced the mycelia growth of *C. lindemuthianum,* this was probably due to chemicals released by the extracts which caused inhibition of the pathogen in the laboratory.

Similar results were reported by Enyiukwu and Awurum (2012) who used extracts of *Carica papaya* roots and seeds, and also seed of *Piper quineense* to control the radial growth of *Colletotrichum destructivum* O. Gara the causal agent of anthracnose of cowpea. The findings show that *P. quineense* seeds were the best in inhibiting the growth of the fungi irrespective of the

concentrations and this was closely followed by that of C. papaya roots while that of C. papaya seeds were the least in inhibiting the pathogen. In another experiment Ambika and Sujatha (2015) used extracts of *Sargassum myricocystum* (brown algae) and *Gracilaria edulis* (red algae) to control the mycelia growth of *Colletotrichum falcatum* the pathogen causing red rot of sugarcane, the result revealed that extract of *S. myrococystum* showed significant antifungal activity against the pathogen and this was closely followed by that of *G. edulis*.

All the plant extracts reduced the incidence and severity of anthracnose on the field. *D. stramonium* was the best and this was closely followed by *P. communis* and *J. gossypifolia* while B. sapida was the least. This finding is in agreement with work of Alves et al., 2015 who used extracts of sixteen indigenous plants for the control of anthracnose in bell pepper fruits. The result obtained from the findings shows that ageous garlic, mallow and ginger extracts reduce severity and incidence of the disease by 97%. The study also shows that application of the extracts two days before inoculation gave the best result in reducing the incidence of the disease suggesting the fact that the time of application plays a major role. All the treatments also check the spread of the disease when compared to the control. This suggests the presence of fungi-toxic substances in all the extracts which is in agreement with the work of Amadioha A.C (2000) who used extracts of *Azadirachita indica* to control blast disease of rice invitro and also minimize the spread of the disease on the field.

The extracts used in the study showed varying degree of toxicity, this may be as a result of varying solubilities of the active compounds in it or possibly due to the presence of active inhibitors to the fungitoxic principle.

The present study has contributed to a list of plants that can be used by peasant farmers for the control of anthracnose disease in cowpea. All the plants readily available in their homesteads at no cost (Ngowi, *et al.,* 2007*).* Apart from this, the procedure for its preparation are easy to adopt and its use will reduce the huge amount of money spent on the purchase of fungicide. Above all, they have no residual effect on man and the new environment (Mwine *et al.,*2011*)*

However, there is the need to investigate the efficacy of all the extracts using different extraction methods both in the laboratory and on the field.

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