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MANAGEMENT OF ANTHRACNOSE DISEASE OF COWPEA WITH THREE PLANT LEAF EXTRACTS FOR ENHANCED GRAIN YIELD IN ABEOKUTA, NIGERIA

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ABSTRACT
The antifungal activity of aqueous three plants leaf extracts were tested against anthracnose disease of cowpea caused by *Collectotrichum lindemuthianum*. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates. Extracts of *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* were sprayed on cowpea (Ife brown) foliage. A total of four foliar sprays were applied: three weeks after planting, flowering stage, at the initial podding stage and at the full podding stage. Benlate served as positive controls while distilled water was negative control. Results showed the disease incidence and severity as 23.67-46.67% and 1.07-1.93, respectively. Combination of the extracts resulted in significantly ($p \leq 0.05$) low incidence of anthracnose (66.67% reduction) and improved the yield of cowpea by 1.63 tons/ha (77.25% increase) compared with untreated control plots. *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* extracts, singly or in combinations, reduced incidence and severity of anthracnose which translated to yield increase.

Keywords: Anthracnose, botanicals, *Collectotrichum lindemuthianum*, cowpea, and management

Introduction

Cowpea, *Vigna unguiculata* L. (Walp.), is cultivated around the world primarily for its seeds but also as a vegetable, a cover crop and for fodder (Claudius-Cole *et al*., 2014). Its high protein content, adaptability to different types of soil and intercropping systems, resistance to drought, ability to improve soil fertility and prevent erosion make it an important economic crop in many developing countries (Claudius-Cole *et al*., 2014). The seeds are important in diets for the provision of protein to rural as well as the urban dwellers as a substitute for the animal protein (Wakili, 2013). The crop's haulms are also valuable source of livestock protein (Owolade *et al*., 2006).

This plant encounters a number of constraints, including pests and diseases that limit its production and yields, from seedling to harvest (Adebanjo and Bankole, 2004; Akinbode and Ikotun, 2008) and often provoking grain yield loss of over 35% (Amadioha, 2003). Major pathogenic groups associated with cowpea diseases, include: fungi, bacteria, viruses and nematodes (Emechebe and Lagoke, 2002). The major fungal infection is anthracnose caused by *Collectotrichum lindemuthianum*. Infection of a susceptible cultivar in favourable conditions leading to an epidemic may result in 100% yield loss (Fernandez *et al*., 2000).

Despite the availability of management practices like seed and foliar treatment with fungicides, crop rotation, use of certified seeds and genetic resistance etc, bean anthracnose is still of regular occurrence in most areas (Padder *et al*., 2010) in Nigeria. Best strategy to manage the disease is planting resistant cultivars, which is most effective, least expensive and easiest for farmers to adopt. However, high pathogenic variability present in the pathogen population (Mahuku and Riascos, 2004; Padder *et al*., 2007; Sharma *et al*., 2007) renders their use ineffective due to continuous breakdown of the resistance mainly in recommended cultivars with good agronomic and marketability traits (Sharma *et al*., 1994).

Anthracnose symptom appears on leaves as small, dark brown to black lesions (Falade, 2016). The infected tissues show minute rust-colored specks. The specks gradually enlarge and form sunken lesions or eye-spots. This disease causes huge losses in temperate and subtropical zones. It infects seedlings (Smith *et al*., 1999; Aveling and Adandonon, 2002); leaves (Emechebe and Lagoke, 2002; Amadi, 1995; Santos *et al*., 1997); pods and fruits (Munoz and Tamayo, 1994; Roy and Ratnayake, 1997) and whole plant parts (Akinbode and Ikotun, 2008). Anthracnose affects yield, seed quality and marketability of the crop (Adegbite and Amusa, 2008).

Available management techniques for anthracnose disease of cowpea include the use of bio-control (bio-agents), pesticides (synthetic chemicals), cultural observations (clean seeds/hygienic fields and practices), Host plant resistance (HPR) and botanicals (Biopesticides) (Colpas *et al*., 2009). Some, though seem effective, are enlaced with residual and often negative and indelible impressions (Jansch *et al*., 2009). Current position in the use of botanicals to combat agricultural pests and disease is 7% of the total cowpea disease management options (Obi and Barriuso-Vargass, 2014). Biological control of plant disease through the use of antagonistic micro organism (Akinbode and Ikotun, 2008) and botanical control of plant disease through the use of plant extracts are two major ways in the control of plant disease with respect to natural agro biological balance (Popoola *et al*., 2016).

In the evaluation of some botanicals against *C. destructivum* (Akinbode and Ikotun, 2008) the growth of the pathogen *in-vitro* using *Nicotiana tabacum* plant extract was inhibited. Crude botanical extracts from stem back and root bark of *Azadiractha indica*, *Vernonia amygdalina* and *Cochlospermum planchonii* exhibited strong fungi toxicity against *Colletotrichum capsici* (Nduagu *et al*., 2008). Applications of plant extracts have been shown to reduce the number of lesions on infected leaves, protect flowers and capsules from infection thereby curtailing disease development (Enikuomehin, 2005). Azher (2009) reported some plant extracts to be effective against seed-borne pathogenic fungi, these include the seeds of bittergourd (*Memoridica charanta* L.), cardomom (*Elettaria cardamonium* Maton), coriander (*Coriandrum sativus* L.), and fruits of chilli (*Capsicum annum* L.), radish (*Raphanus sativus* L.) and brassica (*Brassica compestris* var. *rapa*. L.). Cold-water extracts of *Azardiracta. indica* (neem), *Garcinia cola* (bitter kola) and *Zingiber officinale* (ginger) at various concentration posses fungicidal activity against the mycelial growth and sclerotial germination of soil fungus (*Sclerotium rolfsii*) (Wokocha and Okereke, 2005). Leaves of pawpaw (*Carica papaya)* and neem (*Azadirachta indica)* possess antifungal properties that inhibit alternaria mycelial growth at various concentrations (Suleiman, 2010). The objective of this study was to evaluate the effects of the application of aqueous leaf extracts of *Azadirachta indica*, *Acalypha wilkisiana* and *Carica papaya* on anthracnose disease of cowpea for grain yield increase.

Materials and Methods

Source of Seeds

Seeds of cowpea (Ife-brown) were obtained from Institute of Agricultural Research and Training (IAR&T), Ibadan, Oyo State.

Experimental site and design

The experiment was carried out on DelPHE-5 Project Research field, Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria during the late planting seasons of 2012. The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates. Each plot was 2.5 m x 2.5 m, with border row of 1 m; the planting space was 0.7 m x 0.4 m.

Isolation of Colletotrichum lindemuthianum

Infected cowpea leaves showing symptoms of anthracnose disease were collected from the research field, placed in paper bags and isolation was done in the laboratory. The leaves were washed with tap water, surface-sterilized with 0.5% NaOCl, rinsed in three changes of sterile distilled water and air dried at room temperature. Portion of leaves with fungal infections were cut (1-2 mm^2) at the border of the infection. Three leaf cuttings were placed on Potato Dextrose Agar (PDA) inside Petri plates. The plates were incubated at 28±2^oC for 7 days. Single spore of developing colonies was isolated and sub-cultured for purification. The isolate was stored on PDA slants for further use.

Pathogenicity test

Pathogenicity test was conducted in the screen house using steam-sterilized soil in plastic containers. Five (5) kilogram of sterilized soil sample was placed in each of the plastic containers. Three (3) seeds of cowpea cultivar (Ife-Brown) were planted in each container. Two weeks after planting, inoculum prepared from the isolate (10^7 spores/ml) was sprayed on young, healthy cowpea (Ife Brown) seedlings at the upper and lower surfaces of the leaves till run-off and incubated for 14 days at a temperature range between 25 and 28^oC with relative humidity of 90%. Presence or absence of characteristic symptoms of *Colletotrichum lindemuthianum* was monitored.

Preparation and application of Plant extracts

Fresh mature leaves from *Azadiracta indica* (neem), *Carica papaya* (pawpaw) and *Acalypha wilkisiana* (red acalypha) were collected from plants within the

university premises. In the laboratory, leaves (1 kg of each species) were thoroughly rinsed in running tap water, air-dried at room temperature, blended in 15 L of sterile distilled water in an electric blender (Master Chef—®, China) and left for 24 hrs. The paste was filtered thereafter through clean cheese cloth to give 6.67% w/v stock filtrate. The stock filtrate was diluted accordingly to give the working concentration as required and made into the following treatment combinations: pawpaw + neem + acalypha, pawpaw + neem, pawpaw + acalypha, neem + acalypha, pawpaw, neem and acalypha. Negative control was sterile distilled water while positive control was benlate (0.5% w/v). A total of four foliar sprays were applied in the field as follows: three weeks after planting, flowering stage, at the initial podding stage and at the full podding stage.

Data collection and analysis

Disease incidence was calculated as percentage plants showing symptom of disease using the formula of Getachew et al. (2011):

$$\text{Disease Incidence (I)} = \frac{\text{NPSWS}}{\text{NPPT}} \times 100$$

Where NPSWS= number of plant showing wilt symptoms and NPPT= number of plants per treatment. Data were collected from eight plants from the middle of each plot. Disease severity was assessed using the table provided by Falade (2016) (Table 1). Agronomic data were collected on the number of leaves per plant, plant height (cm), number of branches per plant, number of pods per plant, 100-seeds weight (g) and yield (tons/ha). Data were subjected to analysis of variance (ANOVA) and means separated by LSD at p≤5% using GenStat Discovery Edition 4.

Results and Discussion

Soil texture data are represented in Table 2. Sand accounted for 76% of the texture, 9% silt and 15% clay confirming its moderate porosity and sandy-loam. Organic carbon also indicated some inherent level of porosity of the soil. In Table 3, low rainfall was observed in August (36.30 mm), November (49.60 mm) and December (1.30 mm). Rainfall was high in September (181.4 mm) and October (184.7 mm). High relative humidity was recorded in August (82.60%) and November (81.90%). Mean daily temperature ranged from 28.40°C-34.80°C (Maximum) and 22.10°C-23.30°C (Minimum). Sunshine hour ranged between 2.70 – 6.10 hr and soil temperature ranged between 25.4 -29.8°C in 2012. Kulkani and Benagi (2012) reported the influence of weather factors on the severity of anthracnose disease of greengram.

Excessive use of synthetic chemicals in plant disease management has resulted in a number of problems related to damage of non-target flora and fauna and other useful organisms along with hazardous effects of residues on environment (Singh, 2006). Therefore, the development of bio-pesticides has been focused as a viable pest control strategy in recent years. Results from Tables 4 showed the effects of botanicals on growth parameters of cowpea, incidence and severity of anthracnose disease of cowpea. Plant heights ranged from 27.53-39.45 cm. Growth parameters were not significantly affected by the treatments. The effects of plant extracts on incidence and severity of anthracnose showed significant differences (p≤0.05). The disease incidence for benlate was least in (10.25%) and this was significantly different (p≤0.05) from either single or combine application of leaf extracts. The same trend was applicable to disease severity (0.65). Pawpaw+neem combination performed best among the extract treatments with 23.67% incidence. Plant extracts and essential oils show antifungal activity against a wide range of fungi (Abd-Alla *et al*., 2001). Alkhail (2005) also found out that aqueous extracts of plants of *Allium sativum, Cymbopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia caryophyllus* had strong antifungal activity against *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani*. In this study, the results authenticated the presence of antifungal compounds in the tested plant extracts which effectively controlled the development and spread of anthracnose caused by *Collectotrichum lindemuthianum* on the cowpea plants.

Yield parameters measured were all significantly affected by plant extracts (Table 5). The results of the three plant extracts (*Azadirachta indica, Acalypha wilkisiana* and *Carica papaya*) suppressed the influence of anthracnose disease which eventually translated to grain yield increase. Grain yield was significantly increased (p≤0.05) to 1.63 tons/ha, representing an increase of 77.25% over the untreated plots. All the extracts significantly improved number of pods per plant, number of seeds per plant and seed weight (g) compared with the untreated plots. Claudius-Cole *et al*. (2010) observed that grain yield of cowpea plants treated with *Vernonia amygdalina* and *A. indica, Ocimum gratissimum* and *Moringa oleifera* were significantly higher than in untreated plants.

Conclusion

In this study, application of 6.67% concentration of *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* either singly or in combination resulted in low incidence of anthracnose on cowpea plant compared with untreated plots. Extracts from the plant species

used in this study could be adopted by poor-resource farmers since these plants can be easily accessed around their homesteads. Cowpea farmers could therefore use *A. indica, A. wilkisiana* and *C. papaya* extracts to control anthracnose disease of cowpea to avoid the yield loss.

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Table 1: Disease severity scale for anthracnose on cowpea

Scale	Severity of anthracnose on cowpea
0	No symptom of anthracnose (no dark spots on leaves, petioles, stems and pods)
1	Dark spots on 0.5-20% of total surface area of plants.
2	Dark spots on 20.5-40% of total surface area of plants.
3	Dark spots on 40.5-60% of total surface area of plants.
4	Dark spots on 60.5-80% of total surface area of plants.
5	Dark spots on 80.5-100% of total surface area of plants.

Source: Falade (2016)

Table 2: Physico-chemical characteristics of soil at the experimental site in the year 2012

Soil characteristics	Value
Percent clay	15.00
Percent silt	9.00
Percent sand	76.00
Soil Texture	Sandy loam
pH	5.65
Organic carbon (%)	1.53
Total nitrogen (%)	0.13
Base saturation (%)	98.88
Available phosphorus (mg kg^{-1})	24.98
CEC (Cmol kg^{-1} soil)	8.91
Total Exchangeable Al (Cmolkg^{-1}soil)	0.10
Exchangeable bases (Cmolkg^{-1}soil)	
Ca^{2+}	4.40
Mg^{2+}	3.60
K^{+}	0.38
Na^{+}	0.43

Table 3: Agrometeorological data for the experimental site in the year 2012

Month	Rainfall (mm)	Temperatre (ºC) Mean maximum	Mean Minimum	Relative humidity (%)	Sunshine (hours)
August	36.30	28.40	22.60	82.60	2.70
September	181.40	29.60	22.70	76.00	4.00
October	184.70	32.20	22.10	77.50	5.70
November	49.60	33.00	23.30	81.90	5.40
December	1.30	34.80	22.70	78.50	6.10

Table 4: Effects of treatments on growth of cowpea, incidence and severity of anthracnose

Treatment	Plant height (cm)	Leaves/plant	Branches/plant	% incidence	Severity
Pawpaw+neem+*Acalypha*	39.45	48.46	5.82	26.67	1.67
Pawpaw+neem	33.87	45.90	5.93	23.67	1.73
Pawpaw+*Acalypha*	27.53	39.89	4.83	33.33	1.40
neem+*Acalypha*	29.31	42.76	5.67	30.00	1.07
Pawpaw	36.55	43.87	4.14	46.67	1.87
Neem	34.26	43.69	5.78	40.00	1.67
Acalypha	34.36	43.87	5.39	36.67	1.93
Benlate (0.5%)	35.49	43.69	5.80	10.25	0.65
SDW†	35.11	41.73	5.74	80.00	4.87
$LSD_{(0.05)}$	ns*	Ns	ns	12.40	0.63

*: not significant, †: Sterile distilled water

Table 5: Effects of botanicals on the yield of cowpea

Treatment	**Number of Pods/Plant**	**Number of seeds/pod**	**100seed weight (g)**	**Yield (ton/ha)**
Pawpaw+neem+*Acalypha*	77.00	18.33	137.33	2.11
Pawpaw+neem	69.33	16.67	133.00	1.99
Pawpaw+*Acalypha*	65.33	14.67	125.00	2.02
neem+*Acalypha*	61.67	15.33	111.00	2.08
Pawpaw	52.33	15.67	102.67	1.47
Neem	60.87	13.67	108.33	1.43
Acalypha	69.33	14.33	105.67	1.24
Benlate (0.5%)	79.35	19.63	136.20	2.51
SDW †	23.33	10.33	55.03	0.48
$LSD_{(0.05)}$	39.41	4.22	49.25	0.57

†: Sterile distilled water