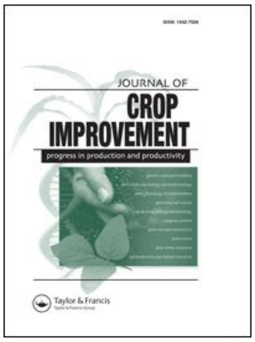

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Control of common bacterial blight disease of cowpea (*Vigna unguiculata* [L.] Walp) with certain plant extracts in Abeokuta, Nigeria

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ABSTRACT

Common bacterial blight disease of cowpea, caused by *Xanthomonas axonopodis* pv *phaseoli*, has been identified as the most important biotic constraint to cowpea production worldwide. Continuous and indiscriminate application of chemical pesticides has necessitated the search for an environment friendly method of control. *Azadirachta indica* (neem), *Acalypha wilkisiana* (red acalypha) and *Carica papaya* (pawpaw) extracts were field-tested against common bacterial blight disease. The experiment was laid out as a randomized complete-block design (RCBD) with three replicates. Cold extracts of 6.67% concentration of the plant leaves were sprayed on cowpea (variety 'Ife brown') foliage. Streptomycin sulfate of 0.2% concentration was applied as a positive control and sterile distilled water as a negative control. Incidence of common bacterial blight ranged from 20.00 to 43.74% and severity from 1.02 to 2.00 on a 1–6 scale, where 1 = no symptoms of common bacterial blight and 6 = necrotic lesions on more than 75% of the leaf area. A combination of extracts of pawpaw, neem and red acalypha reduced disease incidence by 73.68% and improved yield by 1.58 tons/ha (a 73.49% increase) compared with untreated control. *Azadirachta indica*, *Acalypha wilkisiana* and *Carica papaya* extracts, both singly and in combination (Pawpaw + neem + *Acalypha*, Pawpaw + neem, Pawpaw + *Acalypha* and neem + *Acalypha*), reduced incidence and severity of common bacterial blight disease and increased yield of cowpea. Thus, these plant extracts could serve as viable alternative to synthetic chemicals to control *Xanthomonas*-caused diseases in cowpea.

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Introduction

Cowpea, *Vigna unguiculata* L. (Walp.), is cultivated around the world primarily for its seeds but also as a vegetable, as a cover crop and for fodder. 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

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(Claudius-Cole, Ekpo, and Schilde 2014). The seeds provide dietary protein to rural and urban dwellers as a substitute for animal protein (Wakili 2013). The crop is highly valued for both its grain and forage and therefore often has dual use (Latunde-Dada 1993).

Intensive cultivation of crops often results in an increase in pest pressure, especially in the humid tropics. The major economic diseases of cowpea in the humid agroecological regions of south-western Nigeria include brown blotch, anthracnose, cercospora leaf spot, web blight, sclerotium stem blight (Adegbite and Amusa 2008), cowpea aphid-borne mosaic virus and cowpea mosaic virus (Ittah et al. 2010), root-knot nematodes (Atungwu et al. 2011) and common bacterial blight and bacterial pustule (Okechukwu and Ekpo 2008).

Common bacterial blight initially appears as water-soaked spots on the underside of leaves. The spots then enlarge irregularly and adjacent lesions coalesce (Okechukwu and Ekpo 2008). Common bacterial blight adversely affects yield, seed quality and marketability of the crop (Adegbite and Amusa 2008). It causes huge losses in temperate and subtropical zones. It infects all stages of cowpea plant: seedling, vegetative, flowering and podding stages and all plant parts, including leaves, pods and seeds (Adegbite and Amusa 2008).

Synthetic pesticides have some disadvantages, such as toxic residue, development of resistant pathogen strains, increased cost of production and toxicity to mammals. Hence, there is a need for alternatives to chemical pesticides in the management of the disease. Applications of *Azadirachta indica* (neem) extract have been shown to reduce the number of lesions on infected leaves, protect flowers and capsules from infection, thereby curtailing disease development on sesame (Enikuomehin 2005).

Popoola et al. (2016) reported that water extract of *Lawsonia inermis* could be a substitute for chemical pesticides to control bacterial blight of cotton seedlings. Ganiyu et al. (2008) and Popoola et al. (2011) reported on bactericidal properties of neem, mango (*Mangifera indica*) and siam (*Chromolaena odorata*) leaves on tomato plant.

Common bacterial blight disease of cowpea has been identified as the most important biotic constraint to cowpea production worldwide. In the tropical and sub-tropical areas, it can be severe because of high temperatures and alternating wet and dry conditions. It affects the foliage and pods of cowpeas and causes significant yield losses and adversely affects seed quality. Continuous and indiscriminate application and cost of chemical pesticides, with their attendant health hazards, make it difficult for many growers, especially small landholders, to produce beans profitably and have necessitated the search for an environment friendly method of control. Therefore, the objective of this study was to evaluate the effects of application of leaf extracts of *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* on common bacterial blight and, consequently, on yield of cowpea.

Materials and methods

Source of seeds

Seeds of cowpea (variety 'Ife brown') were obtained from Institute of Agricultural Research and Training (IAR&T), Ibadan, Oyo State. This variety of cowpea is popular among farmers in south-west Nigeria.

Experimental site and design

The experiment was conducted on DelPHE-5 Project Research field, Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria during the late planting season (10th of August) in 2012. The experiment was arranged in a randomized complete-block design (RCBD), with three replicates. Each plot was 2.5 m × 2.5 m, with a pathway or border row of 1 m; the planting space was 0.7 m × 0.4 m. The inter row spacing was 0.7 m, while the intra row spacing was 0.4 m. Allowances of 0.2 m and 0.05 m were left from the edge of each plot, respectively, to ensure stability of plants on beds. Each plot consisted of 28 plants.

Isolation of Xanthomonas axonopodis pv *phaseoli*

Cowpea leaves showing symptoms of common bacterial blight disease were collected from previous experiments conducted on the research field. The leaves were placed in plastic bags and taken to the laboratory. The leaves were surface sterilized with 0.5% NaOCl, rinsed three times with sterile distilled water and air dried at room temperature. A portion of leaves (1–2 mm) with bacterial infection was placed on nutrient agar (NA) [15.00 g/L agar, 5.00 g/L peptone, 5.00 g/L NaCl, 2.00 g/L yeast extract, 1.00 g/L beef extract] produced by Sisco Research Laboratories Pvt. Ltd., India. The plates were incubated at 28°C for 48–72 hours. Sub-culturing was performed to obtain pure cultures. The isolate was stored on NA slants for further use.

Pathogenicity test

Pathogenicity test was conducted in a screen house using steam-sterilized soil in plastic containers. A total number of eight plastic containers were used: four plastic containers for the tested organism, *Xanthomonas axonopodis* pv *phaseoli*, and four plastic containers for the negative, sterile distilled water, control. Five kg of soil was placed in each of the plastic containers. Three (3) seeds of the cowpea were planted in each container. Two weeks after planting, inoculum prepared from the isolate (10^6 cfu/ml) was sprayed on young, healthy cowpea seedlings at the upper and lower surfaces of the leaves till run-off and incubated for14 days at 25–28°C and 90% humidity. The presence or absence of characteristic symptoms was observed.

Preparation and application of plant extracts

Fresh mature leaves from *Azadiracta indica, Carica papaya* and *Acalypha wilkisiana* were collected from plants within the university premises. In the laboratory, the 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 through a 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, whereas positive control was streptomycin sulfate (0.2% w/v). A total of four foliar sprays were applied in the field as follows: three weeks after planting, at flowering stage, at the initial podding stage and at the full podding stage.

Data collection and analysis

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

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

where NPSWS = number of plants 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 1–6 rating scale provided by Okechukwu and Ekpo (2004) after making some modifications (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-seed weight (g) and yield (tons/ha). Data were subjected to analysis of variance (ANOVA), and means were separated via the least significant difference (LSD) at $p \leq 5\%$ using GenStat Discovery Edition 4.

Results

Soil properties and weather conditions

Soil texture data for the experimental location are presented in Table 2. Sand accounted for 76% of the texture, 9% silt and 15% clay, confirming

Table 1. Disease severity scale for common bacterial blight of cowpea.

Scale	Disease severity
1	No symptom of common bacterial blight
2	The visible necrotic lesions with chlorotic haloes on less than 10% of the leaf area
3	Necrotic lesions on 10–30 % of the leaf area
4	Necrotic lesions on 31–50% of the leaf area
5	Necrotic lesions on 51–75% of the leaf area
6	Necrotic lesions on more than 75% of the leaf area

Source: Okechukwu and Ekpo (2004).

soil's moderate porosity and sandy loam nature. Organic carbon also indicated some inherent level of porosity of the soil. During the planting season, relatively low rainfall was received in August (36.30 mm), November (49.60 mm) and December (1.30 mm) (Table 3). Rainfall was much higher 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 maximum temperature ranged from 28.40°C to 34.80°C and minimum from 22.10°C to 23.30°C. Sunshine hours per day ranged between 2.70 and 6.10 hr, and soil temperature ranged between 25.4 and 29.8°C in 2012.

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

Soil characteristics	Value
Clay (%)	15.00
Silt (%)	9.00
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.

		Temperature (ºC)			
Month	Rainfall (mm)	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

Pathogenicity test

The results showed initial appearance of water-soaked spots on the underside of leaves. The spots then enlarged irregularly, and adjacent lesions frequently coalesced. The re-isolated pathogen from inoculated plants was found to be identical to the isolate used for inoculation. Control plants treated with sterile distilled water did not show any symptom of common bacterial blight disease.

Effects of treatments on growth parameter of cowpea

Results on the effects of botanical extracts on growth parameters of cowpea are shown in Table 4. Plant heights ranged from 34.30 to 54.70 cm. Plant height (cm), number of leaves/plant and number of branches/plant were not significantly affected by the treatments.

Effects of treatments on incidence and severity of common bacterial blight

Treatments significantly ($p < 0.05$) affected incidence and severity of common bacterial blight of cowpea (Table 4). The disease incidence for the positive control (12.01%) was the lowest and was significantly different ($p < 0.05$) from single and combined (pawpaw + neem + *Acalypha*, pawpaw + neem, pawpaw + *Acalypha* and neem + *Acalypha*) application of leaf extracts. The same trend was applicable to disease severity (0.70) for the positive control. Pawpaw + neem + *Acalypha* combination performed best among the extract treatments (20.00% incidence).

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

Treatment	Plant height (cm)	Leaves/plant	Branches/plant	% Incidence	Severity[§]
Pawpaw + neem + *Acalypha*	54.70	49.30	5.60	20.00	1.07
Pawpaw + neem	46.03	41.83	3.87	26.67	1.60
Pawpaw + *Acalypha*	34.30	39.40	3.67	33.33	1.02
Neem + *Acalypha*	38.30	41.40	4.40	33.00	1.57
Pawpaw	49.03	39.67	4.93	33.33	2.00
Neem	42.83	43.90	4.20	43.74	1.07
Acalypha	48.13	40.97	5.10	39.33	1.64
Streptomycin (0.2%)	47.01	41.95	5.16	12.01	0.70
SDW[†]	47.10	39.67	4.67	76.00	4.62
$LSD_{(0.05)}$	ns[‡]	ns	ns	19.89	0.71

[†]SDW: Sterile distilled water.
[‡]ns: not significant.
[§]Severity scale: 1 = no symptom of common bacterial blight; 2 = visible necrotic lesions with chlorotic haloes on less than 10% of the leaf area; 3 = necrotic lesions on 10–30 % of the leaf area; and 4 = necrotic lesions on 31–50% of the leaf area; 5 = necrotic lesions on 51–75% of the leaf area; 6 = necrotic lesions on more than 75% of the leaf area.

Table 5. Effects of botanicals on the yield of cowpea.

Treatment	Number of Pods/Plant	Number of seeds/pod	100-seed weight (g)	Yield (tons/ha)
Pawpaw + neem + *Acalypha*	73.02	17.45	133.90	2.15
Pawpaw + neem	65.04	17.89	130.50	2.00
Pawpaw + *Acalypha*	59.30	17.25	125.00	2.12
Neem + *Acalypha*	59.23	16.54	121.01	2.01
Pawpaw	54.30	16.36	107.31	1.17
Neem	64.33	15.59	128.39	1.73
Acalypha	68.01	15.30	101.27	1.84
Streptomycin (0.2%)	75.98	18.34	135.22	2.59
SDW[†]	25.02	9.29	45.53	0.57
$LSD_{(0.05)}$	34.18	3.54	42.22	0.49

[†]SDW: Sterile distilled water.

Effects of treatments on the yield of cowpea

Number of pods/plant, number of seeds/pod, 100-seed weight (g) and yield (tons/ha) were all significantly affected by plant extracts (Table 5). The yield in plots treated with *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* leaf extracts increased significantly ($p < 0.05$) to 1.58 tons/ha, from 0.57 ton/ha in negative control plot, which represented an increase in 73.49% over the negative control. All the extracts significantly increased number of pods per plant, number of seeds per plant and seed weight (g) compared with the untreated plots.

Discussion

High rainfall and relative humidity in the early stages of development of cowpea increased its susceptibility to common bacterial blight, as a result of which high disease incidence and severity in plots not treated with plant extracts translated into significantly lower yield compared with plots treated with plant extracts (untreated control yield = 0.57 tons/ha). Nguyen and Ranamukhaarachchi (2010) reported the occurrence of a high level of disease intensity in years with above-average rainfall. In several other crop plants, weather factors also have been reported to play major roles in the development of various diseases.

Results further indicated the presence of antibacterial compounds in the plant extracts, which effectively controlled the development and spread of common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* on the cowpea plants. Any of the extracts tested can be used as prophylactics against the disease, as they minimized the development and expression of the disease symptoms, irrespective of the extract combination used. Cold-water extracts of *Azadirachta indica* were found to inhibit the growth of bacterial leaf spot and rot pathogens by 76.26 to 78.92% compared with hot-water extracts (Opara, Njoku, and Ogbonna 2013). Cold water extracts were used in this study. Thus, this and other similar works suggested that plant extracts had broad-spectrum anti-microbial activities and constituted a veritable tool in plant disease control.

The results further revealed that all the extracts had a significant effect on yield of cowpea. Combination of the three plant extracts (*Azadirachta indica, Acalypha wilkisiana* and *Carica papaya*) gave increased number of pods and seed weight, which eventually translated into yield increase. Hostettman and Wolfender (1997) stressed the role of green plants as reservoirs of effective chemotherapeutics, which can provide valuable sources of natural pesticides. Reports are available on the use of several plants with antimicrobial properties on pathogenic bacteria (Nahunnaro, Ayuba, and Tuti 2013). In this study, application of 6.67% concentration of *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* both singly and in combination reduced the incidence of common bacterial blight on cowpea compared with untreated control plots. In addition, Khan, Abdul, and Chohan (2000) reported that seed oil from *Azardirachta indica* at 2% significantly reduced bacterial blight of cotton. Water extract of *Lawsonia inermis* could be a substitute for chemical pesticides for controlling bacterial blight of cotton seedlings (Popoola et al. 2016). These are evidences that plant extracts can be adopted by resource-poor farmers to improve yield and minimize cost of production. Plant extracts are also environmentally friendly and they limit exposure of farmers to chemical hazards.

Conclusion

The present study has shown that aqueous leaf extracts of *Azadirachta indica, Acalypha wilkisiana* and *Carica papaya* extracts at 6.67% could significantly reduce incidence and severity of common bacterial blight disease of cowpea caused by *Xanthomonas axonopodis* pv. *phaseoli*. These plant extracts could serve as potential alternative to synthetic bactericides for use in the management of *Xanthomonas*-caused diseases in cowpea.

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