

SHORT COMMUNICATION

FUNGITOXIC EFFECTS OF *Carica papaya* AND *Piper guineense* EXTRACTS AGAINST *Colletotrichum destructivum* IN THE GLASSHOUSE

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ABSTRACT

Cowpea is the second most important grain legume in Africa. A major limiting factor to its production both in the savannas of the North and the humid rainforest agro-ecological zones of Southern Nigeria is anthracnose (*Colletotrichum destructivum* O'Gara). Previous studies have established the potentials of extracts of higher plants for its management. In this present study, the fungitoxic effects of *Carica papaya* roots and seeds and *Piper guineense* seeds extracts (0, 25, 50, 75 and 100% w/v) against *Colletotrichum destructivum* were evaluated in the glasshouse. The experiment consisted of potted cowpea seedlings (2 seedlings/pot) in 4kg heat sterilized top soil. At four weeks after sprouting, the seedlings were spray-inoculated to run-off with 10^5 spores/mL suspension of the fungus. Four days after inoculation, the seedlings were sprayed with respective concentrations of the test plant extracts. The experiment was laid out in a randomized complete block design (RCBD) consisting of 16 treatments and five replications and was repeated twice. All the extracts, regardless of concentration of application effectively minimized the incidence and severity of anthracnose on the potted cowpea plants 21 days after spray inoculation with spore suspension (10^5 spores /mL) of the pathogen. Irrespective of type and dosage of application, the parameters of height, leaf area and dry matter content of the cowpea plants compared well with that of benomyl treated plants. However, cowpea plants treated with *C. papaya* seeds extract at 25% concentration had slightly greater dry matter content than other treatments. Hence, crude extracts of *C. papaya* roots and seeds and *P. guineense* seeds can be used to manage anthracnose of cowpea in low-input agriculture for greater productivity.

KEYWORDS: *Colletotrichum destructivum*, fungitoxic natural pesticides, plant extracts

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) (Fabaceae) is the second most important pulse crop in Africa after common bean *Phaseolus vulgaris* (Purseglove, 1976; Gibbon and Pain, 1991), with Nigeria producing 2 million metric tonnes of its grains annually (Singh *et al.*, 2002). The gains rich in proteins and other nutrients supplement for scarce animal protein for 110 million low income consumers of the crop in the tropics and subtropics (SADAF, 2009), while the crop trash are fed to livestock. Generally, its yield is low in Africa occasioned by high disease pressures at all stages of its growth (Awurum, 2000; Ajibade and Amusa, 2001).

Anthracnose characterized by black lesions is incited by fungi of the Genera *Colletotrichum* and is among the most important diseases of pulses, fruits and cereals in Europe and Africa. It has wide host range affecting egg plants, citrus, cotton, tomato, coffee, banana, water melon, wheat, yam, onion, avocado and legumes (Awurum, 2000; GPP, 2009; Infonet-Biovision, 2011). *Colletotrichum destructivum*, O'Gara is the incitant of anthracnose of cowpea in Nigeria. The organism is thrash and seed-borne and seed transmitted. In affected plants up to 50% grain yield reduction occurs (COPR, 1981, Aveling, 2007). Control of this disease depends on use of synthetic



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fungicides. However, the negative environmental impacts, mammalian toxicity and high costs are making their use discouraging prompting research for alternatives such as natural plant-based chemicals (Asawalam, 2006). We previously evaluated the impact of *C. papaya* and *P. guineense* against *C. destructivum* under *in vitro* conditions (Enyiukwu and Awurum, 2011). Based upon their performance, this present study was undertaken to assess the *in vivo* fungicidal effects of *P. guineense* seeds and *Carica papaya* roots and seeds used widely in ethnomedicine on *Colletotrichum destructivum* causal agent of anthracnose of cowpea in Nigeria.

MATERIALS AND METHODS

Sources of seeds and plant materials

The experiment was conducted at the Plant Health Management laboratory of Michael Okpara University of Agriculture, Umudike and in the glasshouse of the University. The environmental parameters were 2162 mm of rainfall, temperature 20–30°C and relative humidity 51–87% per annum while the soil type was sandy clay with organic carbon 6.8% and pH of 3.85 – 4.48. The seeds of cowpea (variety IAR –48) obtained from the Research and Training Unit of the University. *Carica papaya* (roots and seeds) and *Piper guineense* (seeds) obtained from the neighborhoods were used for the study.

Preparation of plant extracts

Carica papaya roots and seeds and *P. guineense* seeds were washed thoroughly in tap water, rinsed in sterile distilled water and air-dried on the laboratory bench for 20 days. Thereafter, they were milled separately into fine powder using a hand milling machine (Model Corona Lavesch 250) to obtain 300 g of each specimen which were stored separately in air-tight bottles. Each powder was weighed out separately into 25, 50, 75 and 100g into different 250 mL conical flasks, to which 100 mL of sterile distilled water was added and the flask stoppered with foiled stoppers. These were allowed to stand for 12 hrs thereafter they were strained through 4-folds of sterile cheese cloth to obtain filtrates of 25, 50, 75 and 100% strengths of the extracts.

Isolation of causal Organism

Colletotrichum destructivum infected cowpea, *Vigna unguiculata* (L.) Walp pod with typical anthracnose symptoms were collected from the field. The pod was cut in bits using a sharp blade, sterilized in 70% ethanol and finally washed in several changes of 500 mL of sterile distilled water. The tissues were plated in Petri dishes containing moistened Whatman No 1 filter paper, and incubated for 7 days at 27 °C. 200g of fresh peeled potato was boiled in 500 mL of sterile water for 1 hr. The broth obtained by straining through 4-folds of cheese cloth was made up to 1L to which 20g agar and 15g glucose was added, stirred thoroughly with a glass stirrer in a 2L flask, then stoppered and autoclaved at 15 Psi for 30 minutes. The mycelial growth from the plated tissues was sub-cultured repeatedly to obtain pure culture of the organism *C. destructivum* which was maintained on PDA thus prepared. Slides of the organism were mounted and examined under a microscope. The organism's identity was confirmed to be *C. destructivum* by the aid of identification manual by Banett and Hunter (1979).

Preparation of Spore Suspension

The spores of the pathogen *C. destructivum* were collected from 10 day old culture-agar stock in Petri dishes by lifting 60cm² pieces into a beaker containing 200mL of sterile distilled water. This was sieved through 4-folds of sterile cheese cloth to remove agar and mycelial mesh and the filtrate centrifuged for 10 minutes. The spores suspension was then standardized using a haemocytometer counting slide to 10⁵ spores/mL. This was there after poured into a laboratory sprayer pump and used to spray the relatively disease-free seedlings of cowpea to run-off.

In vivo experiment

Cowpea seeds surface-sterilized in 0.5% sodium hypochlorite solution for 1 mint and then rinsed in 3 changes of 1L sterile water were sown 3/4kg heat sterilized top soil contained in 20 cm diameter pots. This was later thinned to 2 seedlings/pot. Four weeks after sprouting, the seedlings were wounded with sterile needle. They were then spray-inoculated to run-off with spore suspension of the pathogen (10⁵ spores/mL), and incubated in humid chambers comprising of transparent lightweight polythene bags for 48 hrs. Four days after inoculation, the seedlings were sprayed with suspensions of the different concentrations of the plant extracts. The controls were similarly set up but sprayed only with suspension of the fungus and sterile distilled water or benomyl. The potted seedlings were arranged in a randomized complete block design (RCBD) made up of 16 treatments and 5



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replicates. The whole experiment was repeated twice. The % incidence of the disease on the inoculated cowpea seedlings was assessed as:

$$\text{Per cent incidence} = \text{Number of plants with diseases} / \text{Total number of plants examined} \times 100/1$$

The % severity of the disease was taken by visual assessment of the test plants with typical symptoms of the disease anthracnose, using the descriptive scale outlined by Owolade and Osikanlu (1999): 0 = no diseases; 2 = 1 – 25% of seedling with anthracnose; 4 = 26 – 50% of seedling with anthracnose; 6 = 51 – 75% of seedling with anthracnose; 8 = 76 – 100% of seedling with anthracnose and 10 = death of seedling occasioned by stem breakage or girdling due to anthracnose. The fungitoxicity of the extracts was evaluated as reduction in severity of anthracnose disease on the plants sprayed with spore suspension and the extracts of varying concentrations compared to the controls (water or benomyl treated plants).

Table 1. Comparison of effects of benomyl and different concentrations of the plant extracts on the development and spread of anthracnose (*Colletotrichum destructivum* O’Gara) of cowpea and performance of the crop in the glasshouse

Extract	Disease Incidence (%)	Disease Severity	Leaf Area (cm ²)	Height (cm)	Dry Matter wt. (g)
Benomyl 3g L ⁻¹	18.40	0.8	51.00	54.92	3.42
<i>P. guineense</i> seed (100%)	20.00	0.4	45.4	57.12	3.28
<i>P. guineense</i> seed (75%)	30.00	0.8	38.0	58.14	3.26
<i>P. guineense</i> seed 50%	30.00	0.8	36.2	57.19	2.62
<i>P. guineense</i> seed 25%	30.00	0.8	37.8	58.25	3.42
<i>C. papaya</i> root 100%	20.00	0.4	44.8	57.46	3.40
<i>C. papaya</i> root 75%	20.00	0.8	52.4	55.98	3.32
<i>C. papaya</i> root 50%	30.00	0.8	42.2	62.80	2.92
<i>C. papaya</i> root 25%	30.00	1.2	39.2	50.60	2.56
<i>C. papaya</i> seed 100%	20.00	0.8	54.8	67.26	3.52
<i>C. papaya</i> seed 75%	30.00	0.8	53.8	69.05	3.58
<i>C. papaya</i> seed 50%	30.00	1.2	43.2	72.06	3.48
<i>C. papaya</i> seed 25%	40.00	1.4	43.2	58.53	4.60
Control (Sterile water)	91.40	5.16	31.8	44.33	2.18
LSD(0.05)	45.299	1.351	1.896	23.129	0.94

* Data are means of 5 replicates in two separate experiments.

Statistical Analysis

Data were analyzed by ANOVA using the general linear model procedure in SAS system (2008 version) at significant level of 5%. Means were separated and compared using Fishers LSD at probability of 0.05.

RESULTS

The results presented on Table 1 show that the extracts had significant effects on the incidence and severity of anthracnose disease of cowpea in the glasshouse. Regardless of concentration, all the extracts used in the study performed well in minimizing the incidence and severity of anthracnose disease of cowpea which compared very ($P \geq 0.05$) well with benomyl, a standard fungicide but differed from the control. The results of the effects of the plant extracts on the leaf area, height and dry matter content of the plants are prevented in Table 1. At concentration of 75% and 100%, *C. papaya* seeds extracts had leaf areas of 53.80cm² and 54.80cm², respectively. These were significantly ($P \leq 0.05$) superior to values obtained from benomyl. In terms of plant height, the best heights were obtained from *C. papaya* seeds treatment at 50% and 75% concentrations respectively. *Carica papaya* seeds extracts also gave the best dry matter weight of the plants 4.60grams which was significantly ($P \leq 0.05$) greater than that of benomyl (Table 1).



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DISCUSSION

The result indicates the presence of antifungal ingredients in the plant extracts which effectively checked the development and spread of anthracnose incited by the pathogen *C. destructivum* on the cowpea plants regardless of dosage of application. This goes to say that all the extracts at low doses could be used as prophylactics against anthracnose of cowpea since they minimized the development and expression of symptoms of the disease on the plants 21 days after inoculation with the pathogen *C. destructivum* in the glasshouse. However, higher doses could serve as therapeutic interventions in the event of disease outbreaks. Various workers have emphasized the advantages and potential use of ingredients of higher plants in controlling plant diseases. Amadioha (2003) showed that leaf extracts of *Piper nigrum* and *Ocimum sanctum* effectively checked the development and spread of anthracnose of cowpea in the field. In the same vein earlier finding from Amadioha and Obi (1998), demonstrated the fungitoxic activity of *Azadirachta indica* and *Xylopia aethiopica* on *C. lindemuthianum* causing anthracnose of cowpea. The present findings from this study harmonize with theirs.

These extracts acted by inhibiting mycelial growth and spore germination of the organism (Olufolaji, 1999). This implies that the extracts contain potent *phytochemicals* with systemic activity against *C. destructivum* in the seedlings. For instance alkaloids such as Piperine and Piperine in *P. guineense* seeds have been implicated by Scott *et al.* (2005) for their pesticidal activity and carpaine and pseudocarpaine in *C. papaya* may be the fungicidal compounds which inhibited the growth and development of the pathogen on the test cowpea plants. The fungitoxic effects of the extracts may have been enhanced by the presence of other phytochemicals such as terpenoids, glycosides, saponins as well as flavonoids contained also in the plant materials which are known toxic compounds impairing cell membrane integrity and inhibiting fungal energy production channels (Adjaye-Gbenwonjo *et al.*, 2010; Echerrigaray *et al.*, 2010). In like manner, Reulas *et al.* (2005) implicated benzyl isothiocyanate a glycoside, as powerful fungicides in the seeds of *C. papaya* and this may inform its superior performance as evidenced in this study. Hence agreeing with similar findings by Wokocha and Nwaogu (2008) on *Phytophthora palmivora* the incitant of black pod-disease of cocoa where seeds extract of *C. papaya* was superior to ridomil (metalaxyl), a standard fungicide. In the overall, the superior performance of the extracts of *C. papaya* seeds treatments in this study may also indicate that these extract may be the most photo and thermo stabile. In general, all the treatments similarly inhibited the incidence and severity of anthracnose of cowpea *in vivo* and compared favourably with benomyl but differed statistically ($P \leq 0.05$) from sterile water treated plants. This implies that the fungitoxic constituents of the plant tissue extracts were plant-safe and did not adversely affect the growth and yield parameters of the test crops. However, *C. papaya* seeds extracts treated plants at 25% dose had a slight statistical edge over benomyl and other extracts in terms of dry matter yield (4.6 g) of the test crops.

In conclusion therefore, phytochemicals from *Carica papaya* roots and seeds and *P. guineense* seeds have considerable anthracnose control potentials and can suitably be exploited as potent fungicides. They all are possible substitutes for synthetic chemicals in controlling anthracnose disease of cowpea caused by *C. destructivum* O’Gara in low-input agriculture.

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