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Effect of some botanicals on *Colletotrichum destructivum* O`Gara of cowpea

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**Colletotrichum destructivum** is the causal pathogen of cowpea anthracnose; botanical extracts and benlate fungicide were evaluated as Biopesticides/chemical control strategies in cowpea (*Vigna unguiculata* L.). Botanicals of four plants: *Azadiractha indica*, *Cymbopogon citratus*, *Ocimum gratissimum*, and *Xylopia aethiopica*, proved effective in reducing spore germination and colony growth *in vitro* and the growth of the pathogen *in vivo*. The extracts of *X. aethiopica* and *A. indica* more effectively reduced both the growth of the pathogen *in vitro* and the spread of the disease *in vivo*. Extracts and benlate applied both before and after pathogen inoculation of cowpea significantly reduced the size of pathogen induced lesion.

**Key words:** Anthracnose, diseases, biopesticides, botanical, benlate, *Colletotrichum destructivum*.

**INTRODUCTION**

Cowpea (*Vigna unguiculata* (L.) Walp) is an important source of protein and other essential nutrients for human and livestock consumption, particularly among the low income segment of the populace in the semi-arid regions of the tropics and subtropics (Adebanjo and Bankole, 2004; Sun and Zhang, 2009). The optimum production of the crop is hindered by several factors including the plant pathogenic organism like *Colletotrichum destructivum* O`Gara (Allen et al., 1998; Akinbode and Ikotun, 2008).

The pathogen *C. destructivum* is seed borne and causes anthracnose disease in cowpea (Fokung et al., 1997; Allen et al., 1998) where the inoculum sporulates readily at localized infection foci and produce symptoms within 96 h of susceptible crop inoculation (Latunde-Dada et al., 1996).

In a susceptible crop, all its parts are attacked by the fungus including: seedlings, hypocotyl, stems, peduncles, flowers, leaves and pods and can lead to yield loss of up to 50% in cowpea (Emechebe and Lagoke, 2002; Adebjanjo and Bankole, 2004).

Several control methods have been adopted including the application of chemical (fungicides) and integrated pest management (Emechebe and Lagoke, 2002), biocontrol (Adebjanjo and Bankole, 2004; Akinbode and Ikotun, 2008), host resistance (Amusa et al., 1994; Latunde-Dada et al., 1999), use of phosphorus fertilizers (Adebitan, 1996; Owolade et al., 2006) and cultural practices like spacing and plant pattern (Adebitan and Ikotun, 1996).

Resistance development in plant pathogens (Emechebe and Lagoke, 2002), and chemical build up both of which are hazardous to man and his environment (Airol and Critter, 1996; Jansch and Frische, 2009) have led to search for bioactive molecules in plants and plant parts as alternative and/or complements in the control methods of crop health issues (Alkhail, 2005; Win et al., 2007; Necha and Barrera, 2008). And just recently Mogle and Maske (2012) reported the leaf extracts of *Argemone*
**MATERIALS AND METHODS**

**Botanical materials**

Mature fruits from *A. indica* A. Juss (neem) were harvested, dehisced and then oven-dried for two days at 60°C. The seed coats were then split for the cotyledons which were subsequently washed in sterile distilled water and oven dried together with the sterile fruits of *X. aethiopica* (Dunal) A. Rich at 60°C for 24 h. The fruits and the seeds were separately ground in a sterile mortar to obtain 0.1 kg of dry powder from each material. Fresh leaves of *C. citratus* (DC.) Stapf. and *O. gratissimum* L., were washed thoroughly in sterile distilled water, air-dried at 26°C, weighed (0.1 kg) and ground separately in sterile mortar.

**Botanical extraction**

Oils were extracted from 80 g of each of the oven dried mortar-grounded seeds of *A. indica* and fruit of *X. aethiopica* in 500 mL (diethyl ether (TKM Pharma, Andhra Pradesh, 500020, India) in a Soxhlet extractor (Bionics Scientific Technolgies Pvt.Ltd, India) for six hours. The solvent was evaporated initially using a water bath and then left overnight at 26°C for evaporation of the remaining solvent. Hot water extracts (HWE) were obtained by infusing 100 g each of the four ground test materials separately with 100 mL sterile distilled water in a water bath at 80°C for 1.5 h, then filtering the extract through 4 layers of sterile cheesecloth. Cold water extracts (CWE) from *C. citratus* and *O. gratissimum* were obtained by adding 100 g to 100 mL sterile distilled water. The mixture was stirred vigorously and allowed to stand for one hour, then filtered through 4 layers of sterile cheese cloth.

**Evaluation of botanical extracts in vitro on pathological activities of Colletotrichum destructivum**

The bioassay was on the pathological activities of spore germination, colony extension and sporulation density. The effect of botanicals on fungal growth was determined by growing *C. destructivum* on a PDA (potato dextrose agar) media containing extract in Petri plates. A 50% concentration of crude extract in PDA was prepared by adding 50 mL of the oil or hot water extract to 50 mL molten PDA (primed by dissolving 3.70 g PDA in 50 mL sterile distilled water). One hundred percent botanicals in PDA were primed by smearing one milliliter of each botanical (full strength) on the surface of the solidified PDA-botanical medium contained in Petri dishes and PDA without botanicals served as standard. With a cork borer within a laminar flow chamber, a disc (3 mm in diameter) of ten day old *C. destructivum* culture was aseptically transferred to the center of the solidified PDA-extract medium in the Petri plates. Treated cultures were incubated at 28°C for seven days. Colony growth of the *C. destructivum* was measured, with a line gauge on each Petri dish. The experiment was replicated five times per treatment.

Suspension of the virgin cultures of *C. destructivum*, were prepared for evaluation of spore germination using a disc (3 mm diameter) in 1 mL each of the undiluted (100% concentration) and diluted (50% concentration) extracts in test tubes. Similar spore suspensions were prepared in sterile distilled water as control. The contents of the tubes were subsequently centrifuged at one hundred revolutions per minutes for ten minutes and then filtered through four folds of cheese cloth.

With a Pasteur Pipette (Ningbo Mflab Medical Instruments Co., LTD., Zhejiang, China Mainland), a drop (0.05 mL) of each spore suspension (10 × 10³ spores/mL) was placed on triplicate sterile slides inside Petri dish moistened chambers and incubated at 26°C for 24 h. Further spore germination was then stopped by adding a drop of a biological stain, the Lactophenol Cotton Blue (LPCB) (Thomas Baker (Chemicals) Pvt Ltd, Mumbai India) to each spore suspension on the slide and 100 spores observed at random with a microscope (x10) and values used to determine the percentage extract inhibition of spore germination.

The culture plates used for the study on colony extension were employed in the study on the effect of Botanical extracts on sporulation density. Five millimeters of sterile distilled water was added to each of four replicate plates per treatment. Spores from each Petri plate were washed into suspension with the aid of a sterile scalpel and filtered through three layers of muslin cloth into a test tube. Spores counts per replicate spore suspension were made using Hemacytometer slide (Ningbo Mflab Medical Instruments Co., LTD., Zhejiang, China Mainland) for each treatment.

Number of spores per treatment was calculated using the formula: 

\[ (A+B+C+D+E) \times 50 \]  

where \( A, B, C, D \) and \( E \) represent spore counts in 5, 1 mm² rulings of the Hemacytometer. The final unit is 0.1 mm². The sporulation density data were divided by related colony areas, for differences in colony compensation, using the formula: 

\[ \text{m}² \times r = \text{radial growth} \]  

\[ \text{and} \quad \pi = 3.142 \]

**Evaluation in vivo of botanical extracts and benlate effectiveness on disease development**

With a susceptible *V. unguiculata* the inhibitory effect of botanicals on disease development in vivo were evaluated. The seeds were disinfected using a solution of sodium hypochlorite (The Stutz Company 4450W Carroll Ave. Chicago, IL 60624, USA) at 0.5% concentration for a minute and rinsed in sterile distilled water. The sterile seeds were sown in 4 kg of Metham Sodium (SPE Chemicals Co., Ltd. Shanghai, China Mainland) sterilized humus soil in 22.5 cm diameter clay pots at three seeds/pot randomly arranged into sets in a green house. The crops in the first set were inoculated with a spore load of 1 × 10⁵ spores/mL filtered water of the pathogen two days before treatment application of botanicals or benlate (3 g/L). Crops in the second set were inoculated two days after application of botanicals or benlate. Crops in the third collection were treated with the botanical extracts or benlate when anthracnose symptoms became evident (21 days after inoculation). Control crops were treated with sterile water.
Twenty five (25) days after crop treatment with botanical extracts and benlate four lesions per pot were selected at random and cut out, using a surgical blade, into test tubes containing 5 mL distilled water.

The tubes were subjected to centrifugation at 100 rpm for 5 min to release conidia from lesions into suspension. Three replicate drops of each spore suspension were placed on a Hemacytometer slide and sporulation density evaluated as earlier described under evaluation in vitro. The mean sporulation density of three replicate tubes was then divided by four to obtain the mean sporulation density per lesion for per extract treatment.

RESULTS

Treatments effect of botanical extracts in vitro on three pathological activities of Colletotrichum destructivum

The in vitro inhibitory potentials of the extracts of A. indica, C. citratus, O. gratissimum and X. aethiopica on three pathological activities of spore germination, colony growth and sporulation density is as indicated in Table 1. The comparative effect of treatment as indicated in Figure 1 showed the oil extract of A. indica (Aoe) and X. aethiopica (Xoe) to inhibit spore germination of C. destructivum by up to 100% at full strength fat extract concentration. At half dose concentration C. citratus water extract (Cwce) had 93% inhibitory effect contra spore germination. This is also the least control value among the screened plants on C. destructivum.

Though there was inhibitory effect of over 60% from A. indica oil (Aoe), O. gratissimum cold water (Ocwe) and X. aethiopica hot water (Xhwe), the extracts of A. indica and C. citratus at different forms and concentration induced or supported sporulation of the test pathogen. While A. indica extracts (hot water and oil at half dose) aided pathogen sporulation with well over 150% intensity, all forms of C. citratus extracts assisted pathogen sporulation, between 86 and 222%, at all levels of treatment forms and concentration (Figure 1).

Extracts and benlate effect on Vigna unguiculata diseases development

The in vivo effect of extracts and benlate on C. destructivum is shown in Table 2. Disease development was inhibited at various degrees in all cowpea crops treated with either extracts or benlate in all three levels of evaluation. Comparatively, treatment at two days after crop infection (2dai) indicated O. gratissimum hot water (Ohwe) treatment to be the most effective with 70.4% disease inhibition.

The highest disease inhibition, of 22.2% on 21 days treatment after crop inoculation (21dai) was by X. aethiopica oil (Xoe). Disease inhibitory effect of 37.8% was exhibited by both A. azadirachta oil (Aoe) and X. aethiopica oil (Xoe) on crops treated two days before pathogen inoculation (2dbi) as shown in Figure 2.

DISCUSSION

The advocacy for the use of plant extracts with antifungal property in agriculture, particularly in organic farming system where synthetic pesticides are restricted, is documented (Wang et al., 2004; Albiter et al., 2007). This work adds to the list of plants screened for antifungal activity significant to crop protection. The four plants’ extract indicated inhibitory effects on C. destructivum. The extracts of A. indica and C. citratus however stimulated sporulation in the C. destructivum at some different levels of concentration. For example in the in vitro evaluation the water and oil extracts of A. indica stimulated up to 140 and 184% sporulation in C. destructivum respectively. This suggests the suitability of A. indica and C. citratus for substrate base/components in the culture of phytopathogens as C. destructivum.

The observed variation in the inhibitory effect of the test plants may be due to qualitative and quantitative differences in antifungal principles. For example the leaf extracts of Ricinus communis (Linn), Tephrosia vogelli (Hooks) and Psidium guajava (L) were reported in the work of Nduagu et al. (2008) to stimulate instead of inhibit the growth of Colletotrichum capsici (Synd.) Butler and Bisby, pathogen of Pepper anthracnose. However, the same Ricinus communis effectively inhibited growth of C. destructivum (Akinbode and Ikotun, 2008).

Anthracnose lesions were observed on both the leaves and stems of the inoculated cowpea crops. This tends to contradict the work of Frayssinet (2008) who, though on different crop types, reported that lesions of C. destructivum were observed only on the leaves and petioles but non on the stems of Medicag sativa (Lucern) test plant.

It is possible that the antifungal property exhibited by the C. citratus in the study was due to the Citral content which corroborates the work of Palhano et al. (2004), who successfully inactivated spores of C. gloeosporioides using high hydrostatic pressure separate and combined with Citral essential oil.

The presence of Xylopic acid in X. aethiopica fruit is documented (Woode et al., 2012). This diterpenes might be responsible for the antifungal property exhibited on the test pathogen in this work.

In the induction of plant defense responses by O. gratissimum leaf extracts, Colpas et al. (2009) reported the effective defense response in sorghum and soybean against the pathological activities of Colletotrichum lagenarium and attributed such feat to the several polar compounds and or essential oils, such as geraniol and nerol. These components could as well be responsible in the effect exhibited against C. destructivum in this evaluation.

The plant tissue extracts and the standard fungicide ex-
Table 1. Treatments effect (WE and OE) in vitro on three pathological activities of *Colletotrichum destructivum*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spore germination</th>
<th>Colony growth</th>
<th>Sporulation density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water extract</td>
<td>Oil extract</td>
<td>Water extract</td>
</tr>
<tr>
<td></td>
<td>A^1</td>
<td>B^1</td>
<td>C^1</td>
</tr>
<tr>
<td>Azadiractha indica</td>
<td>56.0</td>
<td>45.7</td>
<td>100</td>
</tr>
<tr>
<td>Cymbopogon citratus</td>
<td>20.3</td>
<td>9.3</td>
<td>31.3</td>
</tr>
<tr>
<td>Ocimum gratissimum</td>
<td>39.3</td>
<td>19.7</td>
<td>48.7</td>
</tr>
<tr>
<td>Xylopia aethiopica</td>
<td>78.7</td>
<td>59</td>
<td>100</td>
</tr>
</tbody>
</table>

1^Extracts were water (WE) or oil (OE) with data means of 5replicates. 2^Inhibition measured as a reduction on number of spore germination, extent of colony spread and quantity of inocula available. 3^Treatment at full extract concentration. 4^Treatments at diluted (50%) extracts concentration.

Figure 1. Comparative inhibitory effect of treatments, *in vivo*, on pathological activities of *C. destructivum*. 1 = Water extract at full concentration, 2 = water extract at diluted concentration, 3 = fat extract at full concentration, 4 = fat extract at diluted concentration.

Table 2. Effect of four botanical extracts and Benlate treatments on cowpea anthracnose disease development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment form</th>
<th>2 days before inoculation (2dbi)</th>
<th>2 days after inoculation (2dai)</th>
<th>21 days after inoculation (21dai)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azadiractha indica</td>
<td>Ahwe</td>
<td>29.6</td>
<td>23</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>Aoe</td>
<td>37.8</td>
<td>30.4</td>
<td>20</td>
</tr>
<tr>
<td>Xylopia aethiopica</td>
<td>Xhwe</td>
<td>32.6</td>
<td>16.3</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>Xoe</td>
<td>37.8</td>
<td>25.2</td>
<td>22.2</td>
</tr>
<tr>
<td>Ocimum gratissimum</td>
<td>Ohwe</td>
<td>8.9</td>
<td>70.4</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Ocwe</td>
<td>21.5</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>Cymbopogon citratus</td>
<td>Chwe</td>
<td>10.4</td>
<td>10.4</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Ccwe</td>
<td>25.9</td>
<td>11.9</td>
<td>8</td>
</tr>
<tr>
<td>Benlate</td>
<td>Bn</td>
<td>25.9</td>
<td>12.6</td>
<td>12.6</td>
</tr>
</tbody>
</table>

1^Inhibition measured as a reduction in lesion spread as compared with those on control crops, means of 10 lesions of 5 replicates. 2^Extracts in the form of Azadiractha hot water (Ahwe), Azadiractha oil (Aoe), Xylopia hot water (Xhwe), Xylopia oil (Xoe), Ocimum hot water (Ohwe), Ocimum cold water (Ocwe), Cymbopogon hot water (Chwe) and Cymbopogon cold water (Ccwe) treatments at full concentration.
Figure 2. Comparative effect of treatment in vivo on disease spread based on periods of pathogen inoculation on Vigna unguiculata. 1Ahwe = Azadiractha hot water extract; Aoe = Azadiractha oil extract; Xhwe = Xylopia hot water extract; Xoe = Xylopia oil extract; Ohwe = Ocimum hot water extract; Ocwe = Ocimum Cold water extract; Chwe = Cymbopogon hot water extract; Ccwe = Cymbopogon cold water extract; Bn = Benlate.

Non-standard abbreviations: HWE, Hot water extracts; CWE, cold water extracts; LPCB, lactophenol cotton blue; 2dai, two days after crop inoculation; 2dbi, two days before pathogen inoculation; 21dai, twenty one days after crop inoculation; Ahwe, Azadiractha hot water extract; Aoe, Azadiractha oil extract; Xhwe, Xylopia hot water extract; Xoe, Xylopia oil extract; Ohwe, Ocimum hot water extract; Ocwe, Ocimum cold water extract; Chwe, Cymbopogon hot water extract; Ccwe, Cymbopogon cold water extract; WE, water extracts; OE, oil extracts; Bn, Benlate.

REFERENCES


