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Assessment of fungitoxic effects of botanicals on the management of growth in *Colletotrichum lindemuthianum*, the incitant of cowpea anthracnose

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Abstract
Antifungal activities of thirty six botanicals were assessed with an objective to find out the potential of botanicals against the mycelia growth of *Colletotrichum lindemuthianum in vitro.* Plant specimens were collected locally based on its medicinal, antimicrobial properties and extracted using aqueous means. The botanicals were tried in six different concentrations. *Strychnos, Rauwolfia, Pongamia, Azadirachta, Datura, Zingiber, Allium cepa, Allium sativum, Eucalyptus, Aegel and Momordica* plant extracts at 25% and 30% concentration arrested the growth of the mycelia to the tune of 100% which can be recommended for the use in field.

Keywords: botanicals, cowpea, *Colletotrichum lindemuthianum*, anthracnose

Introduction
The cowpea (*Vigna unguiculata* L.) also called black eyed pea is an important food legume and an essential component of cropping systems of the drier regions of the tropics covering parts of Asia, Middle East, Southern Europe, Africa, Southern USA and Central and Southern America. Besides being a fast growing crop, cowpea curbs erosion by covering the ground, fixes atmospheric nitrogen and its decaying residues contribute to the soil fertility. Cowpea diseases induced by different pathogens belonging to various pathogenic groups (fungi, bacteria, viruses and nematodes) constitute one of the most important constraints to the profitable cowpea production. Anthracnose is one of the most dreaded fungal diseases of cowpea incited by *C. lindemuthianum* (Sacc and Magn) Scribber affecting all parts of the plant throughout the growth season of the crop. This causes economic loss in tropical regions of Africa especially Nigeria, Latin America and Asia where conditions are wet and humid for the main part of the growing season (Latunde-Dada, 1990) [12]. Botanicals are emerging as safer alternatives to conventional fungicides for the control of plant diseases (Tripathi and Shukla, 2010) [24]. Natural product-based fungicides have the ability to decompose rapidly, thereby reducing their risk to the environment (Fokialakis *et al*. 2006) [9]. The antifungal activities of different plant species and the importance of plants as possible sources of natural fungicides are well established as they can synthesize aromatic secondary metabolites like phenols, phenolic acids, quinines, flavones, flavonoids, flavonols, tannins and coumarins. The components with phenolic structures like carvacrol,eugenol and thymol are highly active against pathogens. (Choudhary *et al*. 2017) [6] These group of compound really show the antimicrobial effect and serves as the defense mechanism against pathogenic microorganisms (Satpathy and Beura, 2021) [20]. The fungicidal potential of botanicals against *C. lindemuthianum* have been reported (Wilson *et al*. 1997; Peraza-Sánchez *et al*. 2005; Bosquez-Molina *et al*. 2010) [25, 19, 5]. But, there is a need for continued research effort so as to develop effective and economical alternative methods for the management of Cowpea anthracnose.

Materials and Methods
In order to evaluate the efficacy of some botanicals, thirty six plant species were selected on the basis of local availability, medicinal values and possession of number of secondary substances with antimicrobial properties which are toxic to phytopathogens (Tripathi and Shukla, 2010; El Sayed *et al*. 2000; Nduagu *et al*. 2008) [24, 7, 17]. To obtain the extracts of all these plants, the plant parts were cut into small pieces and weighed up to 100 gm and grinded in 100ml of distilled water in a homogenizer. The extract so obtained was filtered through muslin cloth and used as standard.

In order to study the effect of dilutions of these extracts, different concentrations at 5, 10, 15, 20, 25, and 30% were used against mycelia growth of *C. lindemuthianum*, by the filter paper disc-plate method (Loo *et al.* 1945) [14]. Sterilized filter paper discs of 10 mm diameter were soaked with botanicals. The filter paper discs were placed at the center of petriplates (one disc per plate) containing 20 ml of Richard's agar medium. Five inoculum discs cut from the margin of a 10 days old culture of the test fungus were placed equidistantly from each other as well as from the filter paper disc along the periphery of each petriplate. Each disc of 5mm diameter was cut with sterilized cork borer and aseptically transferred to the plates. All the petriplates were incubated at 28 ± 1^0 C for 8 days. Each treatment was replicated twice. A control check without any botanical was maintained for comparison. The activity was measured in terms of inhibitory zones appearing around the filter paper disc. The width of zone measured as an average of zone of inhibition taken along five different directions. The linear measurement of inhibitory zone of *C. lindemuthianum* in each concentration of individual plant extract was compared with control checks and the percent inhibition of mycelia growth of each concentration of the plant extract was worked out. For preparation of Richard's medium, ingredients such as Sucrose 50gm/l, Potassium nitrate 10gm/l, Potassium dihydrogen phosphate 5gm/l, Magnesium sulphate 2.5gm/l and Ferric chloride 0.01gm/l were dissolved in distilled water and molten agar-agar was added at the rate of 2% for solidification. Final volume of the medium was made to 1000ml by adding distilled water. The surface growth was measured basing on the diameter of growing colony. The linear growth of mycelium was measured in two directions at right angle to each other along the diameter of developing colony in millimeter(mm) in case of circular and regular colony (Lilly and Barnett, 1951) [13]. The measurement of each colony was taken separately and an average was worked out to denote the radial growth of the colony. As regards irregularly developed colonies in plates, the measurements were taken along the largest and shortest diameter and then average was worked out as the measurement of the growing colony.

Results and Discussion

There were significant but varied inhibition of mycelia growth of the test fungus at different concentrations of the botanicals included under the investigation. Impact analysis of individual concentration of botanicals used was given in the Table-1.

Table 1: Effect of different concentrations of botanicals on the mycelia growth inhibition of *Colletotrichum lindemuthianum*.

Treatments	Botanical name of the Plant	Plant part used	Concentration of Botanicals					
			5%	10%	15%	20%	25%	30%
T_1	*Strychnos nux-vomica*	Leaf	45.9 (42.6)*	68.3 (55.7)	72.5 (58.3)	70.4 (59.1)	100.0 (90.0)	100.0 (90.0)
T_2	*Azadirachta indica*	Leaf	42.6 (40.5)	66.7 (54.7)	88.7 (70.3)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
T_3	*Rauwolfia serpentiana*	Leaf	39.5 (38.9)	52.5 (45.8)	67.5 (55.2)	80.4 (63.7)	92.3 (73.9)	100.0 (90.0)
T_4	*Vitex trifolia*	Leaf	35.8 (36.7)	48.5 (44.1)	54.5 (47.5)	70.5 (57.0)	83.5 (66.0)	95.7 (78.1)
T_5	*Pongamia pinnata*	Leaf	35.6 (36.6)	51.2 (45.6)	52.1 (51.9)	82.6 (64.8)	89.4 (70.9)	100.0 (90.0)
T_6	*Adhatoda vasica*	Leaf	28.6 (32.3)	36.6 (37.2)	51.4 (45.7)	68.7 (55.9)	73.5 (59.0)	81.7 (64.6)
T_7	*Aegel marmelos*	Leaf	34.6 (36.0)	48.7 (44.2)	61.0 (51.3)	85.6 (67.6)	90.0 (71.5)	98.4 (82.7)
T_8	*Terminalia arjuna*	Leaf	16.4 (23.8)	28.3 (32.1)	42.7 (40.7)	50.4 (45.2)	62.5 (52.2)	74.5 (59.6)
T_9	*Cassia tora*	Leaf	22.0 (27.9)	38.2 (38.1)	49.5 (44.6)	68.0 (55.5)	73.0 (58.6)	86.5 (68.4)
T_{10}	*Eucalyptus spp.*	Leaf	35.8 (36.7)	44.0 (41.5)	56.3 (48.5)	88.3 (70.0)	92.3 (73.9)	97.3 (80.7)
T_{11}	*Calotropis procera*	Leaf	36.6 (37.0)	48.3 (44.0)	59.3 (50.3)	72.7 (58.4)	77.8 (61.9)	86.4 (68.3)
T_{12}	*Datura stramonium*	Leaf	36.3 (37.0)	52.4 (46.3)	75.4 (60.4)	91.1 (72.8)	92.9 (74.5)	100.0 (90.0)
T_{13}	*Ocimum sanctum*	Leaf	31.4 (64.1)	48.4 (44.0)	62.8 (52.4)	84.7 (66.9)	84.7 (66.9)	98.1 (82.1)
T_{14}	*Chrysanthemum coronarium*	Leaf	26.6 (31.0)	41.0 (39.8)	56.5 (48.7)	61.6 (51.6)	76.2 (60.8)	78.1 (62.1)
T_{15}	*Tagetes patula*	Leaf	27.0 (31.2)	44.0 (41.5)	52.5 (46.4)	64.4 (53.3)	76.4 (60.9)	79.9 (63.3)
T_{16}	*Bougainvillea glabra*	Leaf	24.0 (29.3)	35.5 (36.5)	48.3 (44.0)	68.1 (55.5)	70.5 (57.0)	82.5 (65.2)
T_{17}	*Lanatna camara*	Leaf	36.4 (37.0)	48.4 (44.1)	62.8 (52.3)	67.1 (54.9)	74.3 (59.5)	82.8 (65.5)
T_{18}	*Ipomoea purpurea*	Leaf	20.7 (26.9)	43.3 (41.1)	52.2 (46.2)	71.2 (57.5)	82.4 (65.2)	87.0 (67.2)
T_{19}	*Zingiber officinale*	Rhizome	42.5 (40.6)	64.8 (53.5)	86.6 (68.5)	92.6 (74.3)	100.0 (90.0)	100.0 (90.0)
T_{20}	*Allium cepa*	Bulb	30.6 (36.5)	52.6 (46.4)	72.5 (58.3)	94.5 (76.4)	100.0 (90.0)	100.0 (90.0)
T_{21}	*Allium sativum*	Bulb	42.4 (40.6)	60.8 (51.1)	71.9 (57.9)	90.4 (71.9)	94.5 (76.4)	100.0 (90.0)
T_{22}	*Carica papaya*	Leaf	32.8 (34.8)	47.7 (43.9)	61.2 (51.4)	72.1 (58.0)	88.3 (69.9)	92.7 (74.2)
T_{23}	*Momordica charantia*	Leaf	26.4 (30.8)	42.5 (40.6)	66.1 (54.3)	81.7 (64.6)	92.6 (73.2)	100.0 (90.0)
T_{24}	*Tamarindus indica*	Leaf	22.5 (27.6)	62.5 (34.7)	43.5 (41.0)	64.7 (53.5)	72.9 (58.6)	79.2 (62.8)
T_{25}	*Terminalia chebula*	Seed	31.7 (34.2)	46.0 (42.6)	53.7 (47.1)	68.3 (55.7)	81.3 (64.3)	91.5 (82.2)
T_{26}	*Eugenia jambolana*	Seed	32.8 (34.9)	41.5 (40.1)	55.2 (47.9)	71.0 (57.3)	82.6 (69.9)	87.7 (69.5)
T_{27}	*Thevetia peruviana*	Leaf	15.1 (22.8)	24.4 (29.6)	37.8 (37.8)	54.6 (47.6)	63.3 (52.7)	73.3 (58.8)
T_{28}	*Tridax procumbens*	Leaf	27.5 (31.5)	41.6 (40.1)	56.7 48.8)	72.1 (58.1)	82.1 (64.9)	83.8 (66.3)
T_{29}	*Catharanthus roseus*	Leaf	18.8 (25.6)	29.2 (32.6)	44.0 (41.5)	62.2 (52.0)	71.0 (57.4)	79.1 (62.8)
T_{30}	*Hibiscus rosasinensis*	Leaf	15.2 (22.9)	31.6 (34.2)	48.4 (44.1)	64.1 (53.1)	72.3 (58.2)	82.6 (65.3)
T_{31}	*Psidium guajava*	Leaf	25.8 (30.4)	32.9 (35.0)	48.3 (44.0)	78.5 (62.4)	82.2 (64.5)	87.4 (69.2)
T_{32}	*Diospyros melanoxylan*	Leaf	29.6 (32.8)	56.3 (48.5)	61.0 (51.3)	82.0 (66.1)	87.8 (69.5)	92.1 (73.6)
T_{33}	*Nicotiana tabaccum*	Leaf	31.6 (34.1)	41.5 (40.6)	53.8 (47.1)	75.5 (60.3)	79.3 (62.9)	83.6 (66.0)
T_{34}	*Citrus limon*	Leaf	22.7 (28.4)	41.9 (40.3)	52.7 (52.1)	81.0 (64.1)	85.7 (67.2)	89.2(70.7)
T_{35}	*Emblica officinalis*	Fruit	18.4 (25.3)	32.9 (35.8)	54.5 (47.5)	68.6 (55.8)	76.6 (61.1)	80.9 (64.1)
T_{36}	*Tabernamontana coronaria*	Leaf	19.3 (26.0)	32.1 (33.9)	47.7 (43.7)	67.7 (55.2)	74.1 (59.4)	83.8 (66.3)
S.Em±			(0.727)	(0.634)	(0.553)	(2.907)	(0.937)	(0.961)
C.D.(0.05)			(2.089)	(1.822)	(1.590)	(8.353)	(2.69)	(2.763)

* figures in the parentheses are angular transformed values

Effects of botanicals at 5, 10, 15, 20, 25 and 30 per cent concentrations were having a varied impacts. The above findings evident that there were sharp gradual increase in rate of inhibition of the mycelia growth of the test fungus as the concentrations of some botanicals increase. However there were some botanicals at 25% and 30% concentration proved fatal for the causative fungus and most effective to cease its growth. Bioassay studies with thirty six botanicals were conducted to find out efficacies in terms of per cent inhibition of mycelia growth against *C. lindemuthianum* (Table: 1). The data revealed that application of 20% concentration of the plant extract resulted in more than 80% inhibition of mycelia growth. The extracts of *Strychnos nux-vomica*, *Azadirachta indica*, *Eucalyptus* sp., *Datura stramonium*, *Ocimum sanctum*, *Zingiber officinale*, *Allium cepa*, *Allium sativum* and *Momordica charantia* proved effective in inhibiting the mycelia growth (more than 80% mean inhibition). Falade (2018) [8] while working on effect of six botanicals on conidia germination and growth rates of *C.lindemuthianum* observed that the botanicals did not had any inhibitory effect on conidia germination but the growth rates of the fungus were inhibited significantly. This inhibition was concentration dependent i.e. at 65% *Datura* caused highest inhibition followed by *Ricinus, Jatropha and Blighia* which is in support of the present finding. Choudhury *et al*. (2017) [6] applied botanicals to check the mycelia growth of *C. lindemuthianum* on *Vigna radiata.* They noted the antagonistic effects of botanicals on the mycelia growth at 10% concentration. *A. sativum* (80.56%), *A. indica* (78.83%), *Z. officinale* (74.38%) and *Datura* (70.91%) were proved quite efficacious in checking the growth of mycelia. *Allium sativum* and *Zingiber officinale* botanicals were found to be effective in controlling the mycelia growth of *C. gloeosporioides in vitro*.(Alam *et al*. 2004; *Nisa et al*. 2011; Mukherjee *et al*. 2011) [1, 18, 16]. Masangwa *et al*. (2013) [15] working on different botanicals found *Agapanthus, Carica, Syzygium* and *Allium* extracts were active against *Colletotrichum* spp. *in vitro*. This result is in confirmation with the present study where *Allium sativum* (20% and above) and *Carrica* (25% and above) botanicals were effective in controlling mycelia growth. Shinde and Gawai (2014) [22] while testing seven different medicinal plants on the *Colletotrichum capsici* observed that, the aqueous extracts of *A. indica* and *O. sanctum* showed strong fungitoxicity over the mycelia growth. However, they preferred alcoholic extracts over the aqueous extracts. The inhibitory effect of *Datura* plant extract was also studied by Gomathi and Kannabiran (2000) [10] where they observed that the mycelia growth of *Colletotrichum capsici* and *Gleosporium piperatum* were inhibited upto almost 70%. Shetty *et al.* (1989) [21] while working on *S. nux-vomica*, *A. indica*, *A. sativum* and *Zingiber officinale* reported that leaf extract of *S. nux-vomica* was found to be most effective leaf extract in inhibiting the mycelia growth. The efficacy of *A. indica* and *Ocimum sanctum* studied in banana against *C. musae* has been proved to be most effective by Bagwan (2001) [4] which is in agreement with the present investigation. The efficacy of *O. sanctum* has been earlier confirmed by Amadioha (1999) [2] against *C. lindemuthianum*. His finding was the alcohol and water extracts of *O. sanctum* found to be effective both *in vitro* and *in vivo* in checking the incidences and spread of the disease in culture and also in field. Amadioha (2003) [3] finally came to the conclusion that the pesticides of plant origin such as *Ocimum sanctum, Piper betel* and *Citrus limon* can be used to control the cowpea anthracnose pathogen in the field conditions. The present finding is also in agreement with the findings of the above workers. However, in the present investigation, *Citrus limon* leaf extract has not been proved effective against *C. lindemuthianum* which contradicts the findings of Amadioha (2003) [3]. Species of Citrus leaf extracts were tried to determine antifungal potentiality on *Colletotrichum capsici*, the causative of Chilli anthracnose by Harsha *et al*. (2014) [11] and reported more than 50% efficacy in *C.limon, C.reticulata* but *C.aurantium* was comparatively less efficient. However, they recommended the *Citrus* species for the use in control of mycelia growth of the *Colletotrichum*. Tasiwal *et al*.(2009) [23] tried different botanicals against the mycelia growth of *Colletotrichum gloeosporioides*, the causative of anthracnose in papaya where it was observed that the leaf extract of *Lantana camara* at 7.5 per cent found to be superior (45.54%) followed by turmeric at 7.5 percent (40.73%). This finding was in similarity with our findings as the efficacy of *Lantana camara* was more than 80% in reducing the mycelia growth of *C. lindemuthianum*. It is quite evident from this study that the above botanicals have been proved effective in inhibiting the growth of the fungus *in vitro*. Therefore, the recommended botanicals may be exploited in the field condition by farming community as a control measure which is environment friendly and involving low cost.

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