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EFFECT OF SOME CRUDE PLANT EXTRACTS ON GROWTH OF *Colletotrichum capsici* (Synd) Butler & Bisby, CAUSAL AGENT OF PEPPER ANTHRACNOSE

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ABSTRACT
Objective: To study the effect of some plant extracts on the growth of *Colletotrichum capsici*.
Methodology and results: The crude extracts of leaf, stem bark and root bark of *Annona senegalensis* (Pers.), *Azadirachta indica* (A. Juss.), *Chromolaena odorata* (Linn.), *Citrus limon* (L.Burm. F.), *Cochlospermum planchonii* (Hook F.), *Hymenocardia acida* (Tul.), *Ocimum gratissimum* (Linn.) *Psidium guajava* (L.), *Ricinus communis* (Linn), *Tephrosia vogelii* (Hooks) and *Vernonia amygdalina* (Del.) were screened to determine their effect on the colony diameter and sporulation of *Colletotrichum capsici*. The potentially active components responsible for fungitoxicity were also assessed. All the leaf extracts did not inhibit colony diameter of *C. capsici* while stem bark and root bark extracts of *A. indica* and *V. amygdalina* exhibited strong fungitoxicity against the fungus. The stem bark extract of *Cochlospermum planchonii* completely inhibited sporulation of *C. capsici.* The groups of compounds present that are likely to be responsible for fungitoxicity were identified to be alkaloids, flavonoids, glycosides, saponins and tannins.
Conclusion and application of findings: The study demonstrate that crude extracts from stem bark and root bark of *A. indica*, *V. amygdalina* and *C. planchonii* exhibit strong fungitoxicity against *C. capcisi* and have potential for being formulated into products for the control of anthracnose of sweet pepper.

Key words: Plant extracts, *Colletotrichum,* anthracnose, pepper, *Capsicum* sp.

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INTRODUCTION

Pepper is one of the top five most important vegetable crops in Nigeria (Denton & Makinde, 1993; Olowu & Onyemelukwe, 2001). The production and sale of pepper provides income especially for women growers in many Nigerian communities where the crop is commonly used to flavor sauces, soups and stews (Amusa *et al.,* 2004).

Anthracnose caused by *Colletotrichum capsici* is the most destructive disease of pepper (Amusa *et al.,* 2004), and causes losses by pre- and post-emergence damping off, leaf spots, pre-mature fruit drop, mummification of unripe green pepper fruits and fruit rots (Agrios, 1988). In Nigeria, losses of about 95% have been recorded in farmers'

fields with several farmers abandoning pepper production as a result of the disease (Amusa *et al.,* 2004). Between 50 - 100% fruit loss has been reported in India, North America and tropical Africa due to anthracnose infection (Tindall, 1983; Amusa *et al.,* 2004).

Although plant based pesticides are cheep, locally available, non-toxic and easily biodegradable (Bajwa & Schaefers, 1998) limited efforts have been made to screen plants that are suspected to possess antimicrobial properties for effect against *C. capsici*. Higher plants may contain secondary compounds that could effectively control plant diseases, but which are yet to be exploited and used as pesticides (Kurucheve *et al.,* 1997). Although there is a growing interest in the use of medicinal plants to control plant diseases, only about 2,400 plant species among more than 250,000 higher plants have been screened for phytoactivity (Oluwalana & Adekunle, 1998; Oluwalana *et al.,* 1999; Khafagi & Dewedar, 2000). This paper reports on a study of several plant extracts for fungicidal activity against *C. capsici,* the causal agent of anthracnose of pepper.

MATERIALS AND METHODS

Pathogen isolation: Sweet pepper fruits with anthracnose lesions were collected from farmers' fields in Tarka Local Government Area of Benue State in September 2005. Sections of 3-5 mm^2 were cut from the margin of the infected lesions and sterilized for one minute in 1.0% sodium hypochlorite solution and rinsed in three changes of sterile distilled water (SDW). The sterile pieces were blotted dry using sterile filter papers and placed on Potato Dextrose Agar (PDA) in 9cm Petri dishes. The dishes were incubated at ambient conditions of light and temperature (30 ± 2°C) for 7 days after which cultures with salmon-pink sporulation typical of *Colletotrichum* spp were subcultured to obtain pure cultures. Culture identification was confirmed by microscopic examination and comparison with reference cultures (Baxter *et al*., 1983).

Plant materials: Leaf, stem bark and root bark of eleven plants consisting of *Annona senegalensis* (Pers), *Azadirachta indica* (A. Juss), *Chromolaena odorata* (L. Poit), *Citrus limon* (L.Bum.f), *Cochlospermum planchonii (*Hook F.*),* *Hymenocardia acida* (Tul.), *Ocimum gratissimum* (L.), *Psidium guajava* (Linn), *Ricinus communis* (L.), *Tephrosia vogelii* (Hooks) and *Vernonia amygdalina* (Del.) were collected from within the University of Agriculture, Makurdi and around the Makurdi metropolis in October 2005.

Media amendment with plant extracts: The stem bark and root bark samples of each of the plants were peeled off with a scalpel blade, washed with distilled water and cut into smaller sizes of about 1-3cm long. The leaf, stem bark and root bark samples were air-dried at room temperature for two weeks, then pounded using a mortar and pestle and subsequently ground using a potable mechanical grinder (model DFH 48) (Wokocha & Okereke, 2005).

Crude plant extracts were obtained by separately infusing 2.5g of each plant material in 50ml distilled water to give 5% w/v in a 250ml conical flask for 72 hours. The infusion was filtered afterwards through double-layered cheesecloth. Two grams of PDA (Oxoid CM 139) was added to each extract and the flasks were autoclaved at 121°C for 15 minutes (Onekutu *et al.,* 2001). After autoclaving each medium Stretomycin sulphate (100mg/litre) was added to prevent bacterial contamination. Media were allowed to cool to about 40°C (Obagwu *et al.,* 1997).

Fungitoxic effect of plant extracts: The media amended with plant extracts were inoculated with mycelial discs (1mm diameter) taken from the advancing edges of 10 day-old pure cultures of *C. capsici.* The factorial set of treatments consisting of three plant parts (leaf, stem bark and root bark) of each of the eleven plants and a control (where no extract was added) were arranged in a completely randomized design replicated three times. The inoculated media were incubated at ambient conditions of light and temperature (30 ± 2°C).

The diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri plate 7 days after inoculation. Sporulation was

determined by adding 10ml SDW to each plate and gently scraping with a sterile glass rod to dislodge the spores. The spore suspensions obtained were filtered through sterile cheesecloth into a sterile 50ml glass beaker and homogenized by manual shaking. The spores were then counted using a haemocytometer. The percentage reduction (M_r) or stimulation (M_s) of colony diameter and sporulation by each extract was computed using the formula

(1) $Mr. = \frac{M_1 - M_2}{M_1} \times 100$

Where M_r. = % reduction in colony diameter or sporulation; M_1 = colony diameter or sporulation on the untreated medium (control); M_2 = colony diameter or sporulation on the treated medium.

(2) $Ms = \frac{M_2 - M_1}{M_2} \times 100$

Where M_s = % stimulation in colony diameter or sporulation; M_2 = colony diameter or sporulation on treated medium; M_1 = colony diameter or sporulation in untreated medium (Enikuomehin *et al.,* 2002).

Phytochemical analysis: Phytochemical analysis of stem and root bark extracts of plants that showed antifungal activity against *C. capsici* was carried out using 5% w/v concentrations. The plant extracts used were those of *A. indica, V. amygdalina, C. limon, O. gratissimum and A. senegalensis.* The extracts were evaluated for the presence of alkaloids, flavonoids, glycosides, saponins, steroids and tannins as follows:

Alkaloids: One milliliter of 1% HCl was added to 3ml of the water extract of each of the five plants which showed antifungal activity against *C. capsici* in separate test tubes. Each extract was then treated with two drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids (Hassan *et al*., 2004).

*Flavonoids (*Shinoda Test*):* One gramme of $MgSO_4$ powder and two drops of concentrated HCl was added to 3ml of each water extract. A red coloration indicates the presence of flavonoids (Hassan *et al.,* 2004).

Glycosides: To 1ml of the water extracts of individual plants in a test tube, 10ml of 50% H_2SO_4 was added. The mixture was heated in boiling water for 15 minutes. Fehling's solution (2ml) was added and the mixture was boiled. A brick red precipitate indicates the presence of glycosides (Okerulu and Ani, 2001).

Saponins (Frothing Test): Two milliliters of the extracts in separate test tubes were vigorously shaken for two minutes. An observation of frothing in the extract indicates the presence of saponins (Okerulu & Ani, 2001; Hassan *et al.,* 2004).

Steroids (Liebermann – Burchard Reaction): One milliliter of concentrated H_2SO_4 was added to 1ml of each extract. No red colouration indicates the presence of steroids (Hassan *et al.,* 2004).

Tannins: Two drops of 5% $FeCl_3$ were added to 1ml of each extract. A dirty-green precipitate shows the presence of tannins (Okerulu & Ani, 2001; Hassan *et al.,* 2004)

Data analysis: Data on colony diameter and sporulation were analyzed using Genstat 5 statistical package. Means were compared using Fishers Least Significant Difference (FLSD) at 5% level of significance (Obi, 2002).

RESULTS

The colony diameter of *C. capsici* was significantly ($P \leq 0.05$) lower on PDA amended with stem bark extracts of *A. indica, C. limon, V. amygdalina* and *O. gratissimum* (table 1). The reduction in colony diameter by each of these extracts was 40.8, 33.5, 22.5 and 17.2%, respectively. The stem bark extracts of *C. odorata, A. senegalensis, C. planchonii, T. vogelii* and *P. guajava* stimulated colony growth of *C. capsici* significantly ($P \leq 0.05$). Stem bark extracts of *H. acida* and *R. communis* had no significant effect on colony diameter of *C. capsici.* The root bark extract of *V. amygdalina* and *A. indica* significantly ($P \leq 0.05$) reduced colony diameter of *C. capsici* by 54.2 and 39.4%, respectively, but root bark extract of *A. senegalensis* had no significant effect. The root bark extracts of the other plants stimulated growth resulting in significantly ($P \leq 0.05$) higher colony diameter compared to the control. The leaf extract of *A. senegalensis, C. odorata, O. gratissimum and V. amygdalina* had no significant effect on the colony diameter of *C. capsici* while those of *C. planchonii, H. acida, R. communis, T. vogelii, P. guajava, C. limon* and *A. indica* stimulated growth.

Table 1: Effects of leaf, stem bark and root bark extracts of eleven plants on the colony diameter (cm) of *C. capsici* seven days after incubation.

Plant	Plant part			Plant type mean
	Leaves	Stem bark	Root bark	
A. senegalensis	5.13 (No effect)	6.47 (21.64 S)	4.40 (No effect)	5.33 (No effect)
C. planchonii	6.03 (14.93 S)	6.17 (17.83 S)	6.40 (30.78 S)	6.20 (21.29 S)
H. acida	6.47 (20.71 S)	5.47 (No effect)	6.03 (26.53 S)	5.99 (18.53 S)
C. odorata	5.33 (No effect)	7.50 (32.40 S)	5.00 (11.40 S)	5.94 (17.85 S)
R. communis	7.00 (26.71 S)	5.53 (No effect)	5.37 (17.50 S)	5.97 (18.26 S)
O. gratissimum	5.17 (No effect)	4.20 (17.16 R)	5.00 (11.40 S)	4.79 (No effect)
T. vogelii	5.87 (12.61 S)	6.07 (16.47 S)	5.73 (22.69 S)	5.89 (17.15 S)
P. guajava	6.63 (22.62 S)	5.93 (14.50 S)	5.37 (17.50 S)	5.98 (18.39 S)
V. amygdalina	4.93 (No effect)	3.93 (22.49 R)	2.03 (54.18 R)	3.63 (25.88 S)
C. limon	6.70 (23.43 S)	3.37 (33.53 R)	5.07 (12.62 S)	5.04 (No effect)
A. indica	6.73 (23.77 S)	3.00 (40.83 R)	2.67 (39.73 R)	4.13 (15.37 R)
Control	5.13	5.07	4.43	4.88

FLSD (P≤0.05) Plant type = 0.28; Plant part = 0.14; Plant type x Plant part = 0.49. Values in parenthesis represent % reduction (R) or stimulation (S) of colony diameter of *C. capsici* by the various plant extracts tested.

All the leaf and stem bark extracts tested significantly ($P \leq 0.05$) reduced sporulation except leaf extracts of *R. communis* and *T. vogelii* and stem bark extracts of *V. amygdalina* (table 2). The stem bark extract of *C. planchonii* completely inhibited spore production. The root bark extract of *C. planchnonii, H. acida, R. communis, C. limon and A. indica* significantly ($P \leq 0.05$) reduced sporulation compared to the control while the root bark extract of *O. gratissimum* significantly ($P \leq 0.05$) stimulated sporulation. There was no significant difference in sporulation of *C. capsici* when treated with the root bark extract of *A. senegalensis, C. odorata, T. vogelii, P. guajava* and *V. amygdalina.* The highest reductions in sporulation were obtained with leaf extract of *H. acida* (85. 9%) and the root bark extract of *C. planchonii* (78.9%).

Phytochemical analysis revealed the presence of glycosides, saponins, tannins, alkaloids and flavonoids in the plant extracts (table 3). All the stem bark extracts contained tannins except *C. limon* while steroids were absent in all the stem bark extracts screened. All the root bark extracts contained glycosides and saponins. Alkaloids were present in the root bark extracts of *A. indica* and *A. senegalensis* while tannin was found only in the root bark extract of *V. amygdalina*.

Table 2: Effects of leaf, stem bark and root bark extracts of eleven plants on the sporulation (x 10^6) of *C. capsici* seven days after incubation.

Plant	Plant part			Plant type mean
	Leaves	Stem bark	Root bark	
A. senegalensis	0.88 (77.83 R)	1.64 (70.61 R)	1.89 (No effect)	1.47 (59.62 R)
C. planchonii	2.05 (48.36 R)	0.00 (100.00 R)	0.71 (78.87 R)	0.92 (74.73 R)
H. acida	0.56 (85.89 R)	1.30 (76.70 R)	0.92 (72.62 R)	0.93 (74.45 R)
C. odorata	0.92 (76.83 R)	0.95 (82.97 R)	3.38 (No effect)	1.75 (51.92 R)
R. communis	4.36 (No effect)	3.33 (40.32 R)	1.47 (56.25 R)	3.06 (No effect)
O. gratissimum	1.09 (72.54 R)	0.61 (89.07 R)	5.03 (33.20 S)	2.24 (38.46 R)
T. vogelii	3.59 (No effect)	0.40 (92.83 R)	3.11 (No effect)	2.37 (34.89 R)
P. guajava	0.65 (83.83 R)	1.75 (68.64 R)	3.05 (No effect)	1.82 (50.00 R)
V. amygdalina	1.66 (58.19 R)	4.32 (No effect)	2.00 (No effect)	2.66 (26.92 R)
C. limon	1.36 (65.74 R)	0.34 (93.91 R)	1.55 (53.87 R)	1.08 (70.33 R)
A. indica	1.72 (56.68 R)	1.44 (54.19 R)	1.33 (60.42 R)	1.50 (58.79 R)
Control	3.97	5.58	3.36	3.64

FLSD (P≤0.05) Plant type = 0.88; Plant part = 0.44; Plant type x Plant part = 1.53. Values in parenthesis represent % reduction (R) or stimulation (S) of colony diameter of *C. capsici* by the various plant extracts tested.

DISCUSSION

The study showed that extracts from different parts of plants vary in their effect on the growth and sporulation of *C. capsici.* Extracts from some plant leaves effectively reduced sporulation but stimulated mycelial growth of the fungus, an indicator that plant extracts could have constituents with variable effects on pathogens. In a similar study, NRC (1992) reported that neem leaf extract failed to reduce growth of *Aspergilus flavus* but completely inhibited the production of aflatoxin in cotton seed, thus showing differential effects of active ingredients on pathogens. Srivastava and Lal (1997) also reported inhibition of spore germination of *Alternaria alternata* by the leaf extract of *Ocimum basilicum* and *Ocimum canum,* which are close relatives of *Ocimum gratissimum* that reduced sporulation of *C. capsici* in this study. Okigbo and Emoghene (2003) noted that the leaf extract of *V. amygdalina* was metabolized for the growth and spore germination of *Mycospharella fijiensis.* Similarly, Tewari and Dath (1984) reported the stimulation of growth and sporulation of *Drechslera oryzae* by the leaf extracts of *Oryza sativa.*

The leaf extracts that stimulate the growth of *C. capsici, e.g.* those of *R. communis, T. vogelii* and *P. guajava* could be used to develop a selective medium for isolating and culturing this fungus. Kurucheve *et al*. (1997) observed that the variation in the inhibitory effect of plant extracts may be due to qualitative and quantitative differences in antifungal principles. The strong fungitoxicity exhibited by the root and stem bark extracts of *A. indica.* and *V. amygdalina* and the stem bark extracts of *O. gratissimum* and *C. limon* can be attributed to their chemical constituents including tannins, glycosides, alkaloids, saponin and flavonoid that were found to be present. Okwute (1992) had previously related the potency of *A. indica* to the compound Azadirachtin.

The presence of alkaloids in the root bark extracts and its absence in the stem bark of *A. indica* may be due to variations of alkaloid distribution in the different plant parts. Harborne (1984) reported that alkaloids have about 9-10% distribution in vascular plants and are specific to a few related plants. Alkaloid detection in plants is dependent on factors such as age, climate, plant part, habitat, season, time of harvest, chemical races of plants and sensitivity of alkaloid (Farnsworth, 1966). Harborne (1984) further noted that alkaloid screening might fail to detect particular compounds due to the wide range of solubility of alkaloids.

We recommend that further work should focus on confirmation of the active ingredients in the plant extracts using chromatography or other spectral measurement techniques aiming towards application of the plant extracts to control *C. capsici*.

Table 3: Phytochemical components of stem and root bark extracts of plants with antifungal activity against *Colletotrichum capsici*.

Plant extracts	Alkaloids	Tannins	Glycosides	Saponins	Steriods	Flavonoids
Stem bark						
V. amygdalina	-	+	+	+	-	-
C. limon	+	-	+	+	-	-
A. indica	-	+	+	+	-	+
O. gratissimum	-	+	+	+	-	-
Root bark						
V. amygdalina	-	+	+	+	-	-
A. indica	+	-	+	+	-	-
A. sengalensis	+	-	+	+	-	-

Key: + = Present; - = Absent

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