



In Vitro* Evaluation of Antifungal Activities of Six Plant Extracts Against *Colletotrichum lindemuthianum* *Sensu-lato

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To cite this article:

Falade M. J. *In vitro* Evaluation of Antifungal Activities of Six Plant Extracts Against *Colletotrichum lindemuthianum* *Sensu-lato*. *American Journal of Plant Biology*. Vol. 2, No. 2, 2017, pp. 61-65. doi: 10.11648/j.ajpb.20170202.13

Received: March 10, 2017; **Accepted:** March 29, 2017; **Published:** April 19, 2017

Abstract: This study examined the effect of six plant extracts on growth, conidial germination and sporulation on *C. lindemuthianum* *in vitro*. Six plant extracts namely; *Tridax procumbens*, *Jatropha gossypifolia*, *Sida acuta*, *Blighia sapida*, *Ricinus communis* and *Datura stramonium* were tested against *Colletotrichum lindemuthianum*. Three concentrations (30, 50 and 65%) of the extracts were tested to determine their effects on conidia germination, growth and sporulation of *C. lindemuthianum* *in vitro*. Results showed that all the plant extracts had no significant inhibitory effect on conidia germination and sporulation but growth rates reduced significantly compared to the control. The rate of inhibition of growth was concentration dependent, being higher at 65% for all the extracts. *D. stramonium* was the most effective extract followed by *R. communis* and *J. gossypifolia* while *B. sapida* caused the least inhibition of growth. At 30, 50 and 65% concentrations, the rates of inhibition of growth were 10, 16 and 33% for *D. stramonium* and 2, 8 and 10% for *B. sapida* respectively. The study showed that the plant extracts has the potential for the inhibition of the pathogen.

Keywords: *Colletotrichum lindemuthianum*, Plant Extract, Growth, Conidia, Germination

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a member of the family Fabaceae (Leguminosae) which is widely grown in the tropical and sub-tropical regions of West Africa. It is a major source of dietary protein for man and livestock and more than 600 million people in Africa and Asia depend on it for food security, poverty reduction and income generation (FAOSTAT, 2012). The estimated world cowpea production in 2000 was 3.32 million metric tonnes (MMT) with Africa accounting for 75% of the output (FAOSTAT, 2000).

The annual output in Nigeria of 2.1 MMT fall short of domestic demand by about 0.52 million (MMT) that is partly met by importation from neighbouring countries, mainly Niger and Burkina-Faso. This deficit is attributed to myriads of production constraints such as drought, low yield of local unselected cultivars, lack of good planting materials, pests and diseases [2].

Cowpea anthracnose disease is the most important fungal disease of field grown cowpea capable of causing 75% reduction in yield if left uncontrolled [6]. The disease affects

all stages of the plant but more often in the reproductive stages [13]. The symptoms are most visible on leaves and ripe fruits but the disease also produces cankers on petioles and stems thereby causing defoliation and rotting of fruits and infected fruits have water-soaked and sunken circular spots [5].

This disease can be controlled effectively by the use of resistant varieties where they exist and culturally by removing sources of inocula. The use of synthetic fungicides like Benomyl (Benzimidazole) and Mancozeb (Dithiocarbamate) had proven very effective. However, due to the increased awareness of environmental side effects of these synthetic pesticides, development of resistant strains of pathogens and toxicity to non-target organisms, attention is being focused on alternative methods of control such as the use of plant extracts, which are considered relatively cheap and compatible with the farming practices [10]. Extracts of many plants have been reported to be toxic to phytopathogenic fungi. However, efficacy of botanicals in plant disease management has been shown to vary with the strain of the fungus and the active ingredients in the plant

extract. Therefore, there is need to evaluate the efficacy of indigenous plants that have considerable toxicity to *C. lindemuthianum*. This report is a preliminary study on antifungal activity of hot water extracts of six tropical plants against *C. lindemuthianum* *in vitro*.

2. Materials and Method

2.1. Collection of Plant Leaves and Preparation of Extracts

Leaves of *Datura stramonium*, *Jatropha gossypifolia*, *Blighia sapida*, *Tridax procumbens*, *Ricinus communis* and *Sida acuta* were collected from 12-15 month old plants and air-dried at ambient temperature ($24 \pm 2^\circ\text{C}$) for 14-21 days. The dried leaves were turned into powder using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C for two weeks. Thereafter, 65, 50 and 30 grams of the powder of each plant was weighed into 250 ml standard flask and 100 ml of distilled water at 70°C was poured into each flask. The flasks were maintained at this temperature in hot water bath-shaker for 30 minutes. Thereafter, the liquid extract was separated by vacuum filtration and poured into standard bottles which were refrigerated at 4°C and subsequently used as the stock solution.

2.2. Isolation and Morphological Identification of *C. lindemuthianum*

Cowpea plants showing distinct symptoms of anthracnose disease were collected from fields at Ekiti State University Teaching and Research Farm, Ado -Ekiti, Nigeria. The leaves were cut into pieces of about 1-2 cm and surface sterilized by immersion in 0.2 %NaOCl for two minutes. This was followed by two rinses in sterile distilled water and spraying with 70% isopropanol. The sterilized leaves were kept inside a laminar flow cabinet for 20-30 minutes to dry. Five sterilized leaf cuttings were appressed to the surface of Potato Dextrose Agar (PDA) (Sigma-Aldrich) containing 0.05% chloramphenicol (company purchased) inside 9 cm sterile Petri dishes and removed. For the isolation of the anthracnose pathogen, three of the surface sterilized leaf cuttings were placed on PDA containing chloramphenicol to prevent growth of bacteria. The plates were sealed with parafilm and incubated separately at ambient temperature for 5-6 days. There was no growth on the plates unto which leaves were appressed and this confirmed that the surface of the leaves was sterile. Single conidia from developing colonies in the isolation plate was transferred into prepared standard PDA media to obtain a pure culture. Agar plugs from single conidia cultures were used for morphological identification on Malt Extract Agar (MEA) at x400 magnification of a compound microscope (OLYMPUS Binocular) [15].

2.3. Effect of Hot Water Extract on Conidia Germination

One mL of different concentrations (30, 50 and 65% w/v) of the extracts was added to 9 ml molten PDA. The plant extract-modified PDA was poured into 9cm Petri dishes and allowed for 1 hour to solidify. The media for the control

treatment consisted of standard PDA media alone. The media were inoculated with 10 μL of *C. lindemuthianum* conidia suspension, 1.0×10^2 conidia mL^{-1} prepared from 21 days old culture and spread-plated using spatula. The Petri dishes were sealed with parafilm to prevent evaporation of moisture from the agar surface and incubated at ambient temperature for 12 hours. Thereafter, sterile coverslips were placed in three positions on the surface of the agar and viewed under x40 objective of compound microscope. A conidium with the germ tube length which was longer than its diameter was considered as germinated. One hundred conidia were randomly counted in each of the coverslip field and the percentage germination was calculated as:

$$\% \text{ germination} = \frac{\text{Germinated conidia}}{\text{Total counted conidia}} \times 100$$

2.4. Effect of Plant Extract on Growth

In order to evaluate the effect of the extracts on growth, standard PDA media (control) and plant extract-modified PDA based media were prepared as described previously. The plates were inoculated at the centre with 10 μL of conidia suspension containing 1×10^2 conidia mL^{-1} using micro-pipette (Eppendorf 1-10 μL). They were sealed with parafilm and incubated at 20°C for eight days. The treatments and the control were replicated three times. Daily measurement of the colony diameter along two orthogonal axes which were marked on the plates was commenced at 24 hours after inoculation and this continued for 5-10 days. The values of the growth rates were averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each treatment and compared with the control [16]:

$$\text{PIMG} = \frac{(R1 - R2) 100}{R1},$$

Where, R1= Radial extension of colony in the control plate and R2 =Radial extension of colony in sample plate.

2.5. Effect of Plant Extract on Conidiation

Agar plugs were taken from three positions on 14 days old culture into a McCartney bottle using 1cm cork borer and 10 ml of sterile distilled water containing 0.05% Tween-80 (surfactant) was poured into each bottle. The bottle was vortexed for 1-2 minutes to dislodge conidia. The concentration of conidia in the suspension was estimated using a haemocytometer and the density of conidia (conidia cm^{-2} of the colony) was calculated.

3. Results

3.1. Effect of Hot Water Extract on Conidia Germination Rates

Figure 1 shows the effect of different concentrations of six plant extracts on germination rates of *C. lindemuthianum* conidia. The extracts had no significant inhibitory effect on conidia germination when compared with each other. There was 92-100% germination of conidia irrespective of the plant

extracts and their concentrations and these values were not significantly different from the control where 100% germination was recorded.

3.2. Effect Extracts on Growth Rate

The effect of hot water extracts of the six plants on growth of *C. lindemuthianum* is shown in Figure 2. Growth inhibition rates varied significantly in relation to the plant

extracts and their concentrations. The rate of growth in the control was significantly the highest. At 65% concentration of *D. stramonium* extract, there was 32.6% inhibition of growth while *R. communis* and *J. gossypifolia* caused 16.4% and 10% reduction in the rates of growth respectively. Sixty five percent concentration of *S. acuta*, *T. procumbens* and *B. sapida*.

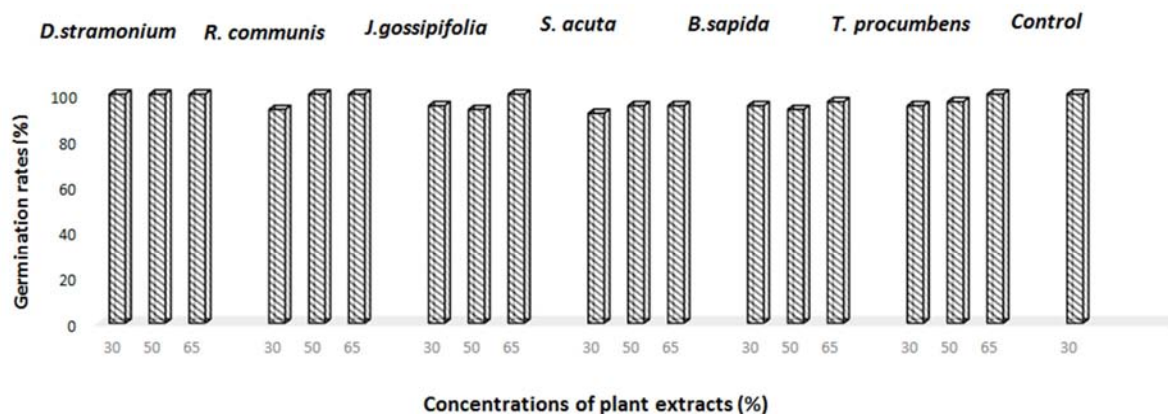


Figure 1. Effect of six plant extracts at different concentrations on germination rate of *C. lindemuthianum* conidia.

Caused 16.4%, 12.4% and 10.0% inhibition respectively. Lower rates of growth inhibition were recorded at 50% and 30% concentrations.

3.3. Effect of Extract on Conidiation

The effects of the six plant extracts on conidiation of *C. lindemuthianum* shown in Figure 3. There was no

significant difference in conidia per colony area on substrates containing 35% of extracts of all the six plants. *D. stramonium* and *B. sapida* stimulated higher conidia production at 50% concentration. At 65% concentration of *D. stramonium*, *R. communis* and *S. acuta* extracts, conidia density also increased significantly ($P < 0.05$).

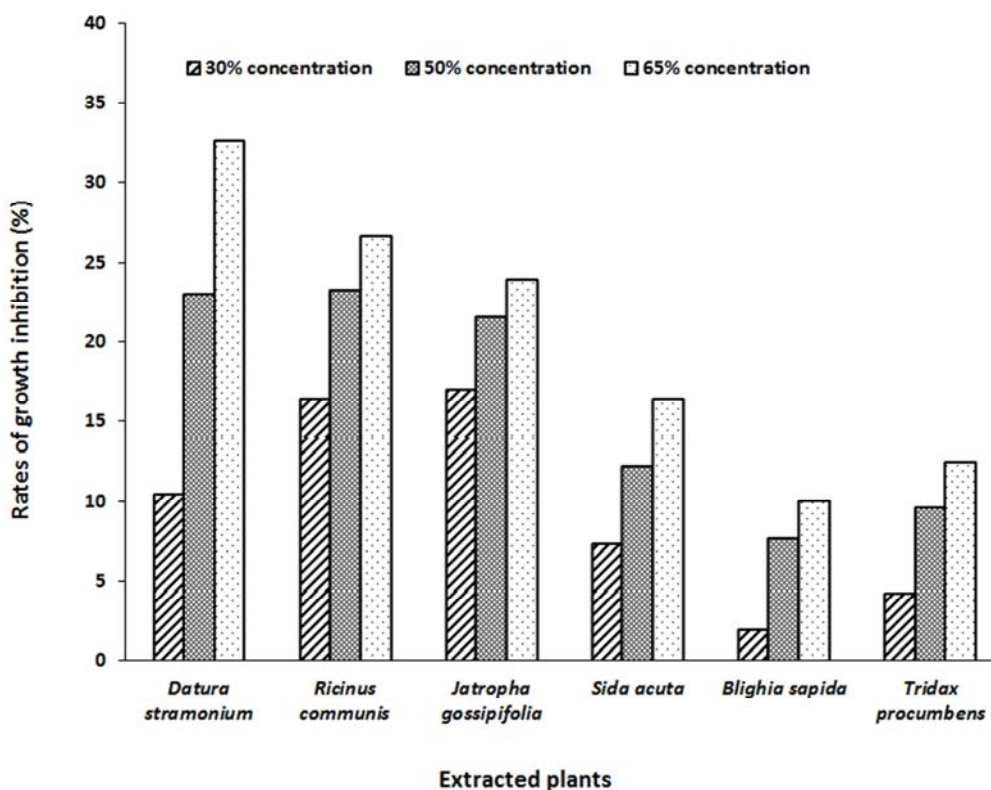


Figure 2. Rate of inhibition of growth (%) by different concentrations of hot water extracts of six plants.

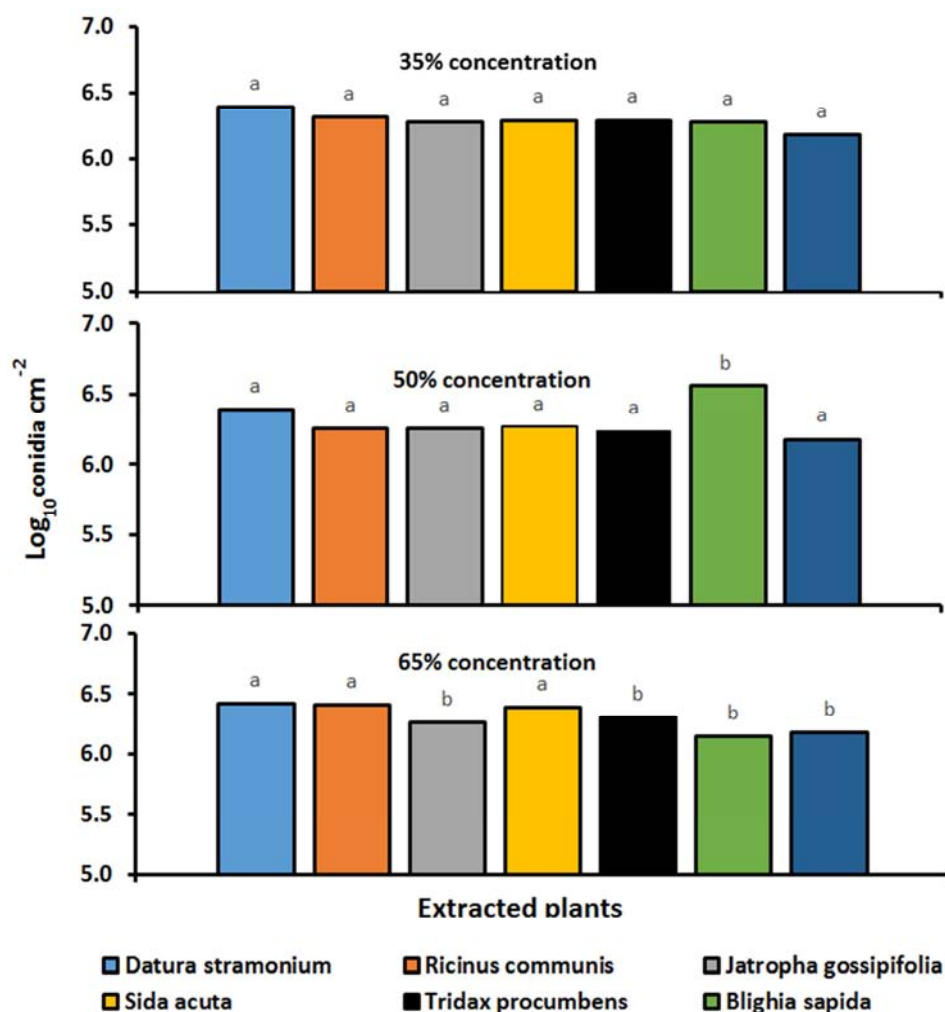


Figure 3. Effect of different concentrations of plant extracts on sporulation density of *C. lindemuthianum*.

4. Discussion

Anthraxnose disease undermines cowpea production in Nigeria. This pathogen is capable of surviving in soil for a long time, making crop rotation ineffective for the management. The effective control is by use of chemical fungicide which are sometime unaffordable to low income farmers. In addition, prolonged use often leads to development of resistant strains of pathogens.

In this study, hot water extracts of the six plants: *D. stramonium*, *R. communis*, *J. gossypifolia*, *S. acuta*, *B. sapida* and *T. procumbens* reduced mycelia growth of *C. lindemuthianum* and the rate of inhibition depended on the concentration of each extract. Higher inhibition of growth occurred at relatively higher concentrations of the plant extracts. This was probably due to increased availability of anti-fungal chemicals in the medium. [4] had observed reduced mycelia growth on potato leaves caused by the pathogen *Phytophthora infestans* using extracts of garlic and scouring rush at various concentrations. The results show that the extracts were effective in inhibiting the pathogen. In this experiment, the six extracts had no effect on conidiation of *C. lindemuthianum*. It has been established that when mycelial

growth are inhibited, it is a form of stress to which a fungus would respond by producing large number of conidia. [11] evaluated 66 medicinal plants belonging to forty one families for their anti-microbial activities against the mycelia growth of *Pythium aphanidermatum*, the causative organism of rust disease in chilli plants and found that 23 of the plant extracts had inhibitory effect on the pathogen, with the extracts of *Allium sativum*, *Allium cepa* and *Tridax procumbens* being the most effective. Similarly, [1] evaluated the effects of the extracts of mahogany, giant Indian milky weed, garlic and ginger at 30-70% concentrations on the growth and development of *C. gloeosporioides*. It was reported that garlic extract at 70% concentration was the most effective. [7] reported the effect of extracts of black pepper, (*Piper guineenses*) seed and (pawpaw) *Carica papaya* roots on growth of *C. destructivum*, the incitant cowpea anthracnose at five different concentrations (0, 25, 50, 75 and 100%). The study showed that effectiveness was concentration dependent. [12] reported that sporulation of *C. lindemuthianum* decreased as the concentration of the active ingredients in the plants increased, which is in contrast to the current study. The effect of active ingredients present in different plants on growth characteristics, mycelial proliferation and conidiation of

phytopathogenic fungi vary. [14] reported crude extracts of various *Agapanthus africana* plant parts that was screened against eight economically important plant pathogenic fungi and showed that *Phytium ultimum* and to a lesser extent, *Fusarium oxysporum* and *Alternaria alternate* showed high degree of tolerance to the extracts.

Susceptibility of phytopathogenic fungi to botanicals are controlled by a number of factors which include the chemical constituents of the plant, strain of the fungus, mode of exposure to the fungitoxic constituents and the mode of extraction of active ingredients. In this experiment, hot water extraction was used because the constituents in the six plants are water soluble and the method is relatively simple. Bioactive compounds occur naturally in plants and they have been reported to offer protection to plants against infections [17].

All the plant extracts caused no significant inhibition of germination, and there was 98 to 100% conidia germination on plant extracts modified-PDA media after 24 hours incubation at ambient temperature. This finding contrasted some earlier reports on the effects of botanicals on the germination of *C. lindemuthianum* conidia. [16] reported that different concentrations of the extracts of *Azadirachta indica* and *Xylopi aethiopica* inhibited conidia germination as well as the mycelia growth of *C. lindemuthianum*. [3] evaluated the effects of 19 different botanicals on mycelial growth and conidia germination of *C. gloeosporioides*, the pathogen causing *Papaya* anthracnose. The study showed that the plant extracts inhibited conidia germination. The mechanism of plant extracts and some fungicides causing inhibition of mycelial growth without significant deterrence to germination is not fully understood.

Different plant extracts have shown varying degree of antimicrobial effects to important pathogens of plants diseases in different studies. There is the need to evaluate the effect of composite mixture of plant extracts on plant pathogens. It can be suggested that a mixture of extracts that is capable of suppressing conidiation and that which prevents germination and mycelial growth would produce a more promising results if applied.

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