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Combined Antifungal Effects of Extracts of *Jatropha curcas* and *Chromolaena odorata* on Seed Borne Fungi of *Solanum gilo* Raddi

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ABSTRACT
Ethanolic and aqueous extract of Jatropha curcas and Chromoleana odorata were investigated for their antifungal effect at three levels of concentration (5, 8 and 10%) on seed borne fungi (Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus and Fusarium oxysporium) isolated from Solanum gilo. Ethanolic extracts of C. odorata when compared with J. curcas recorded inhibitory effect on A. flavus, A. fumigatus and A. terreus at highest concentration of extracts being 10%. This result was significantly (P<0.05) higher than that recorded by aqueous extract of both plant materials; though aqueous extract of C. odorata had significant inhibitory impact on A. fumigatus. However, a combination of 2mls each of the ethanolic plant extracts of both plants material recorded best results of fungal inhibition on all test fungi. Effect of combination of aqueous extract of both plants recorded a significant inhibition only on A. fumigatus and A. flavus at 10% concentration. Combined ethanolic extract of these plant materials are environmentally safe, and promising for protecting S. gilo seeds against major seed-borne fungi.
Keyword*: Antifungal effect, Seed-borne fungi, J. curcas, C. odorata, Solanum gilo.*

INTRODUCTION

Jatropha curcas L is a species of flowering plant in the family, Euphorbiaceae, and is thus closely related to other important cultivated plants like rubber tree and castor. The plant, *J. curcas* is a small tree or large shrub which can reach a height of up to five meters [12].
The seeds have been substituted for castor oil and are sometimes called "Larger Castor oil". The oil is widely used for skin diseases and to soothe pains such as that caused by rheumatism. The seeds are also used in the treatment of syphilis [9]. Aiyelaagbe [2] reported that latex of *Japtropha curcas* has antibiotic properties against *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Strephococcus pyogens*. It also has coagulating effects on blood plasma.That is why it juice or latex is applied directly to wounds and cuts as a styptic.
It is widely cultivated in the tropics as a living fence in fields and settlements.
Chromolaena odorata (L.) R. M. King and H. Robinson is one of the World's worst tropical weed. It is a perennial scrambling shrub of about 4m tall. It is a member of the sunflower family Asteraceae [13]. The weed goes by many common names including siam weed, devil weed, French weed, communist weed, hagonoy, cohoy [8]. The plant is commonly called "Awolowo" in Nigeria.
It is widely believed that this plant is a soil improver and an indicator of a good and fertile soil. Wherever it grows, crops (especially sweet yam, maize, fluted pumpkin) thrive very well. It is also used as a mulch material [15].
In the southern part of Nigeria and in Vietnam, fresh leaves or a decoction of *C. odorata* are used for the treatment of leech bite, soft-tissue wounds, burn wounds, skin infection, dento-alveolitis, and to stop bleeding [13]. It is being used traditionally for its many medicinal properties, especially for external uses, such as inflammation. The phytochemical studies have revealed the presence of a wide range of chemical entities in the plant. These studies underline the significance of treating the widely occurring flora on this planet as potential sources of new drug entities and not only as harmful weeds [10]. It can grow rapidly and form infestations that can affect agriculture, pastures and biodiversity, as *C. odorata* interferes with the functions of natural ecosystems.

In recent years, much attention has been given to non-chemical systems for seed treatment to protect them against seed-borne pathogens. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds [14].

Plants generally produce many secondary metabolites which constitute an important source of fungicides, pesticides, and many pharmaceutical drugs and still remain the principal source of pharmaceutical agents used in traditional medicine. The inhibitory activity of plant extracts generally depends upon the concentration, type of parts used and microbes tested. The accumulation and concentration of secondary metabolites which are responsible for inhibitory activity is varied according to the plant parts [2].

The leaf and bark extracts of *J. curcas* have high concentration of tannin, saponin, flavanoid, steroid, alkaloid, cardiac glycoside, anthraquinone and terpenoid. The ability of the ethanolic extracts of the leaf and bark of *J. curcas* to inhibit growth of the tests bacteria is an indication of its antimicrobial potency which may be employed in treatment of microbial infections [2].

Aiyelaagbe [2] reported the anti-parasitic and anti-fungal activity of the sap and crushed leaves of *J. curcas*. A study reveals that the seed extracts of *J. curcas* is very effective against *Aspergilus fumigatus A. flavus, C. albicans, A. niger, Staphylococcus aureus*, and *K. pneumoniae* than the other strains tested [7].

MATERIALS AND METHODS

The seeds of *Solanum gilo* were obtained from a market in Itu Local Governmant Area of Akwa Ibom State. While the plant materials (*Chromolaena odorata* and *Jatropha curcas*) were collected from the University of Uyo Phamacognosy farm, these were stored in sterile polythene bags and transferred to the department of Botany and Ecological studies laboratory.

Isolation and identification of seed-borne fungi

The standard blotter method was used for the isolation of the seed-borne fungi. Twenty-five seeds were plated per Petri dish (9cm diameter) on three layers of blotter soaked in sterile distilled water after seed were pretreated with 1% commercial bleach (Sodium hypochlorite) for three minutes and rinsed with two changes of sterile distilled water.The plates were then incubated at 28+. After seven (7) days, the seeds were examined under a stereobinocular microscope (X50) for fungal growth. Sub-culturing of fungi that grew out of the seeds was done on successive potato dextrose agar (PDA) media to obtain pure cultures of the fungi. Fungi were identified based on their spore characteristic with the aid of fungi identification manual by Barnett and Hunter [5].

Determination of the Effect of the Extracts on the Fungal isolates *in vitro*

Three different concentrations (5%, 8% 10%) were prepared from the ethanolic aqueous extract of both *J. curcas* and *C. odorata* . The combined antifungal test was carried out by pipetting 2mls of 5% concentration each of ethanolic extract of *J. curcas* and *C. odorata* aseptically into 12 milliter of cool molten PDA medium in each of the Petri-dishes. Each medium was thoroughly homogenized by gentle circular rotations in order to achive uniform dispersal of the extract. The media was allowed to solidify, and each plate was inoculated with fungal isolates by placing a 5mm disc taken from the advancing edges of 7 day old cultures. These method was repeated for 8 and 10% concentration of both plant materials. The same concentrations of aqueous extract of *J. curcas* and *C. odorata* was repeated following the same method. Same test was carried out separately for the extract of each plant material. All plates were incubated at 28±2^{0}C for 36hrs. Mycelia growth of the fungus were measured in milimeters. Control plates containing test organism without any extract were also incubated. Each examination was carried out in triplicate for all isolates.

Statistical Analysis

Analysis of variance (ANOVA) was employed in all numerical data to test for significance in the treatment and Least Significant Difference (LSD) test was used to separate the means.

RESULTS

Fungi associated with the seed samples were identified as *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus* and *Fusarium oxysporium*.

From the results obtained, the inhibitory effect of the extracts were dependent on percentage concentration, solvent used for extraction, plant materials and combined effect of the plants extract. Performance of ethanolic extract on the inhibition of fungal growth was better than aqueous extracts. Generally, ethanolic extract of *C. odorata* had significantly ($P<0.05$) inhibitory effect on all the fungi when compared to the effect recorded by *J. curcas*. (Fig.1).

For ethanolic extract of *J. curcas*, the effect at 10% concentration was greater on *A. flavus* (56.3%), *A. terreus* (52.5%) and *A. fumigatus* (51.3%). *Fusarium oxysporium* recorded the least inhibition (42.3%), which was not significantly different from that of *A. niger*(44.9%). *Chromoleana odorata* recorded the highest inhibitory effect in *A. fumigatus* at all concentration (50.8, 72.2 and 81.2%). Inhibitory effect of aqueous extract of *J. curcas* and *C. odorata* was least at 5% concentration but highest at 10%. *A. fumigatus* was more susceptible to *J. curcas* recording 66.4% inhibition at 10% concentration. Effect was least on *F. oxysporium* (37.3%) at same concentration. The performance of aqueous extract of *C. odorata* was better when compared to that of *J. curcas*. The highest percentage inhibition was recorded on *A. fumigatus* (71.6%) at 10% concentration. Least effect was recorded on *F. oxysporium* (43.5%), this was not significantly ($P<0.05$) different from performance on *A. terreus.* Inhibitory effect of extracts on fungi increased with increase in concentration of extract (Fig. 2).

The combined ethanolic extract of both plant material have recorded significant activity against all test fungi where as the combined aqueous extract did not show reasonable antifungal activity on *A. flavus* and *A. fumigatus* (Fig.3).

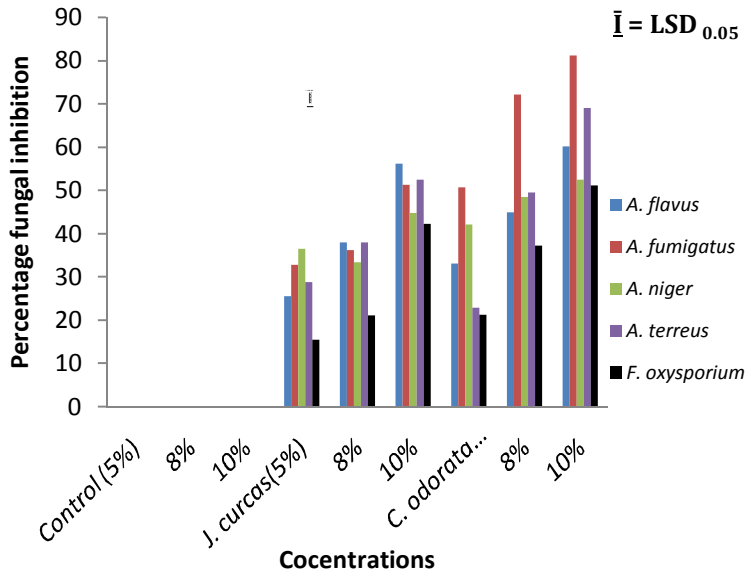

Figure 1: Effect of different concentrations (5, 8 and 10%) of ethanolic extract of *J. curcas* and *C. odorata* on isolated fungi.

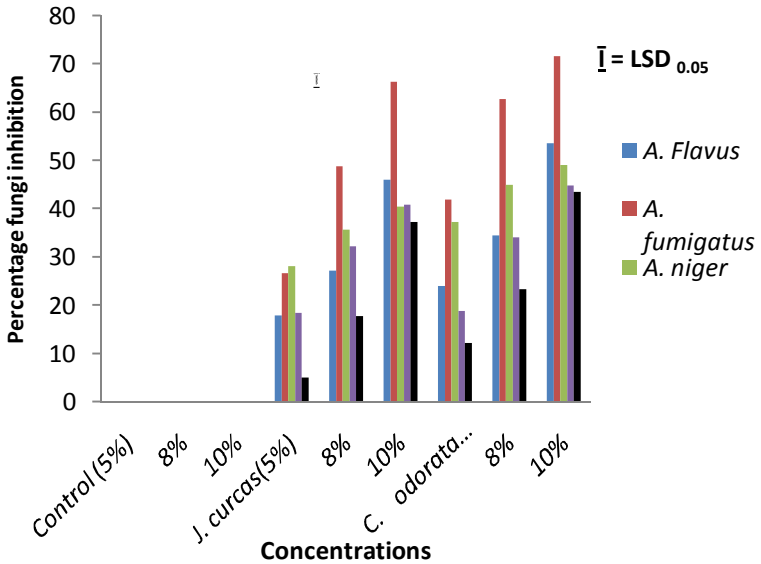

Figure 2: Effect of different concentrations(5, 8 and 10%) of aqueous extract of *J. curcas* and *C. odorata* on isolated fungi.

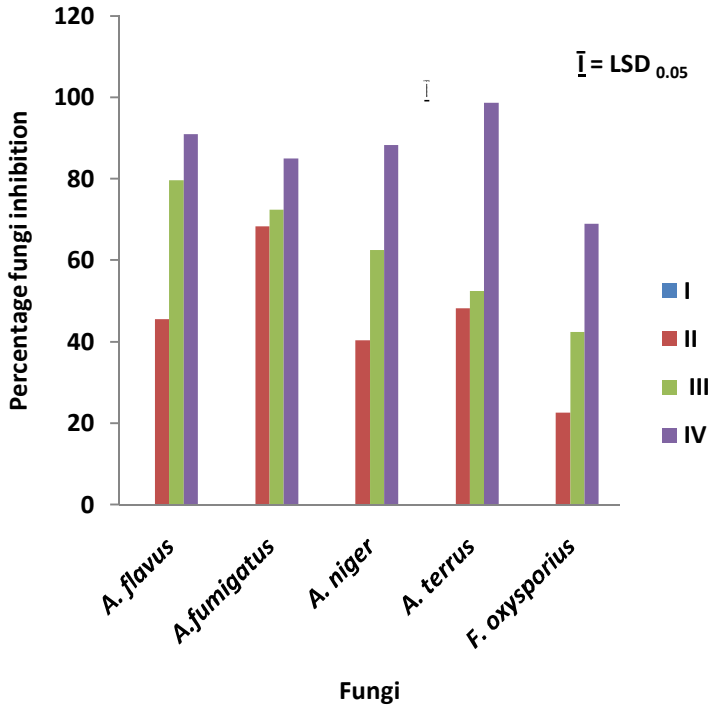

Figure 3: Combined effect of different concentrations (I=0, II=5, III=8 and IV=10%) of ethanolic extract of *J. curcas* and *C. odorata* on isolated fungi.

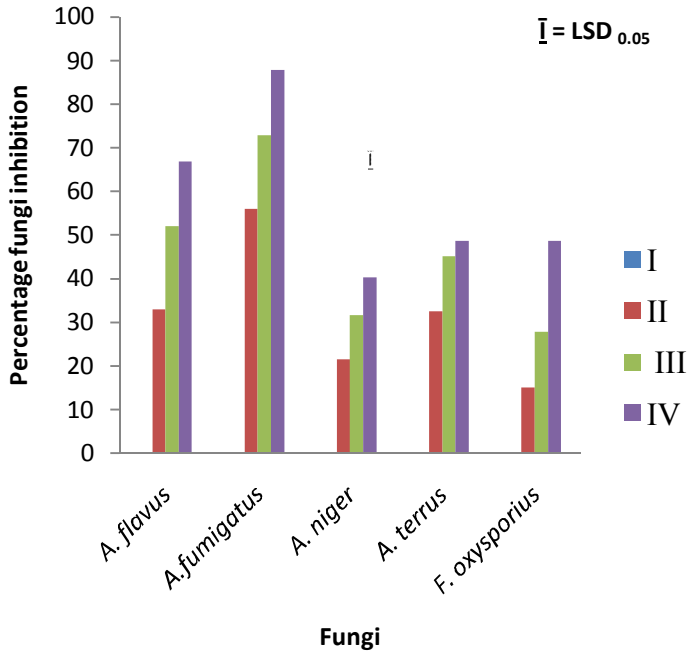

Figure 4: Combined effect of different concentrations (I=0, II=5, III=8 and IV=10%) of aqueous extract of *J. curcas* and *C. odorata* on isolated fungi.

Results obtained shows that combined performance of ethanolic extract of *J. curcas* and *C, odorata* recorded the best results. Performance was best at 10% concentration where *A. terreus* and *A. flavus* recorded 98.7 and 91.0% inhibition. The least percentage inhibition was on *F. oxysporium* at 68.9%. inhibition rate increased with increase in percentage concentration. Combined effect of aqueous extracts had least performance on the fungi when compared with the ethanolic extracts. The highest inhibitory effect here was recorded on *A. fumigatus* (56.0, 72.9 and 87.8%) at the different

concentrations. *Fusarium oxysporium* had the least percentage inhibition (48.7%) at 10% concentration. (Fig.4). The control plates recorded no inhibition.

DISCUSSION

Results obtained by several workers reported that *A. alternate*, *F. solani*, *F. oxysporium*, *A. flavus*, *C. lunata* were fungi associated with seed samples of eggplant. The presence of *A. niger*, *A. flavus* in bush mango seed, maize and groundnut was reported by Akano and Atanda [3, 1].

Isolation of variety of fungi, causing significant loss in seed quality have been reported by Koirala *et al.* [11]. Reports from researches reveal that plant based pesticides prove to be better alternatives because of the minimal environmental impact and danger to the populace because of consumption [6]. With these in mind these two plants were screened for antifungal activity against seed-borne fungi isolated from *S. gilo.*

In this study, the combined extract of *J. curcas* and *C. odorata* proved more effective on the test fungi suggesting that it had more potency with respect to antifungal activity than when the extracts were used individually. The fungicidal activity of some plant extracts in controlling different plant pathogens have also been reported by several workers [4].

The combination of the ethanolic extract of both plant material reduced the growth of all test fungi significantly. However, the combination of the aqueous extract of both plant materials did not have significant impact to inhibit fungal growth.

CONCLUSION

The combined ethanolic extract significantly inhibited the fungal growth of all the test fungi. This combination can be considered as a better method of application of plant extract for fungal inhibition. A further study is needed to find out other combination percentages of plant extracts for grater results and to elucidate their extract mode of action.

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