ASN 53rd Annual Conference Proceedings (Sub-Theme: Crop Protection)

Antifungal Efficacy of three Botanical Extracts on Red Rot Pathogen (Colletotrichum falcatum) of Sugarcane (Saccharum officinarum)

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Abstract.
Botanical extracts have shown appreciable achievement in controlling plant diseases. Based on this premise the antifungal potency of *Azadirachtaindica, Lawsoniainermis*and *Khaya senegalensis*leaf extracts were tested on the mycelia growth of *Colletotrichum falcatum,* the pathogen of red rot disease of sugarcane. The ethanolextracts were prepared at different concentrations; 25%, 50%, 75% with distilled water and 0.5g/l cloth as the negative and positive control respectively. The prepared extracts and controls were tested for antifungal effects against *Colletotrichum falcatum.*Phytochchemical analysis extracts revealed that these botanicals contained alkaloids, saponin, saponnin, flavonoids, phenol, steroids, tannins, terpenoids, anthraquinones and cardiacglycosides. The results also revealed that the maximum inhibitory effects on mycelia growth was due to 75% concentration with the meanvalue of from 100% (*Lawsoniainermis*), 53% (*Azadirachtaindica*) and 60.60% (*Khaya senegalensis*). The values were significantly different ($p \leq 0.05$) from all other treatments, except for 50% concentration of *L.inermis.*Therefore, it can be deduced 75% concentration of these could be effective in the control of the of the pathogen and further field trial of these botanical on red rot disease pathogen should be carried out authentic confirmation of their efficacy and eradication of the disease.

Introduction

Sugarcane (*Saccharum officinarum*), is a perennial grass of the family Poaceae, primarly cultivated for its juice from which sugar can be processed. It is an important cash crop cultivated in tropical and sub-tropical regions of the world (Dacosta*et al*., 2011). It is valuable because of its ability to store high concentrations of sucrose in the stem and more recently for the production of ethanol, which is an important renewable biofuel source (Menossi*et a*l., 2008 and Dacosta*et al*., 2011). However, this crop is face with biotic constraint such as fungal attack leading to reduction in its production. Red rot of sugarcane caused by *Colletotrichum falcatum* (Went) infects the sugarcane stalk at both the initial and mature stages of growth, causing discoloration, while the pathogen produced invertase enzyme which causesinversion of the sucrose in addition to drying of the cane stalk as well ashalting and the overall vegetative growth of the plant (Sharma and Tanta, 2015).It can reduces cane weight to 29%, loss in sugar by 31%, sucrose content by 75% and juice yield by 90% (Husaini and Afghan, 2006). This dual loss of juice content and quality result in great losses for both cane grower and the sugar factories. Annual loss of revenues by *C. falcatum* infection is estimated to be between 500 -100 million USD (Hussain and Afghan, 2006). Various methods such as chemicals, biological and tissues culture have been adopted in managements of the disease. However, these methods are not effective enough with high cost implication. Therefore, there is need to source for alternative means of managing the disease that will be effective, available, eco-friendly and less cost effective. Adebola *et al. (* 2019) reported that plants contain extensive variety of secondary metabolites, such as tanins, terpenoids, alkaloids, and flavonoids, with antimicrobial properties. Plant extracts such as *Azadirachta indicia*, *Lawsoniainermis* and*Khaya senegalensis* has been usedto inhibit the growthof some fungi pathogen (Hafiz *et al*., 2016). In view of the above promises,

this study aimed at evaluating the efficacy of *Azadirachtaindica, Khaya senegalensis* and*Lawsoniainermis*on mycilia growth of *Colletotrichum falcatum* causing red rot disease of sugar cane.

Materials and methods

Collection of plant materials: Infected stalks of sugarcane were collected from sugarcane farm centre at National Cereal Research Institute (NCRI) Baddegi , Niger state. Fresh healthy leaves of *Azadirachtaindica, Lawsoniainermis* and*Khaya senegalensis* were collected in sterile polythene bags from the wild. The plants were authenticated in the herbarium of the Department of Plant Biology, Federal University of Technology, Minna, Nigeria.

Preparation of Potato Dextrose Agar (PDA): Two hundred grams (200g) of peeled, washed Irish potato and boiled for 20 minutes in 500ml of sterilized distilled water. The supernatant was drained in to conical flask (1000ml) and make up to the mark. Twenty grams (20g) of agar and 20g of glucose powder were weighed and added. The conical flask was plugged and sterilized in autoclave at 121°c for 15 minutes. The sterilised mixture was allowed to cool at room temperature for ten minutes and 0.5ml of 100% concentration of chloramphenicol was added to prevent bacteria growth (Adebola *et al*., 2014).

Isolation and identification of pathogen from infected sugarcane: Samples of 1.0 - 1.5 cm cut segments of an infected portion of cut open sugarcane stalks were wash thoroughly under running tap water and surface sterilized with 15% sodium hypochlorite for 30 seconds and washed properly with sterile distilled water. The surface sterilized samples were inoculated on Petri dish containing PDA and incubated at room temperature 28±2°C in an incubating chamber. After seven (7) days of incubation, subcultures were done to obtain pure culture of the fungi isolate and identifiedusing morphologicaland microscopic features as described ina standard identification manual of soil fungi (Gillman, 1957).

Preparation and preservation of plant extracts: Leaf samples collected were properly washed and disinfected with sodium hypochlorite (0.5%) for 5 minutes. The samples were rinsed thoroughly with distilled water and then blunt dried with whatman No. 1 filter paper. The samples were dried under room temperature for two weeks and ground to powder using sterile mortar and pestle. Twenty gram (20g) of each samples were weighed and extracted usingsoxhlet apparatus for 24 hours at a temperature of 55°C. More grounded plants were extracted until a substantial amount was obtained. The liquid extracts were concentrated and then preserved in airtight containers for further analysis (Gnannasekaran*et al*., 2015).

Qualitative photochemical screening of the plant extracts: The plants extracts were screened for the presence of Alkaloids,Flavonoids,Tanins,steroids, terpenoids,Ancthroquinones,Phenols,saponins and glycosides using the methods of Association of Official Analytical Chemists (AOAC, 1995).

Pathogenicitytest: Pathogenicity test for the organismwas carried out fresh and apparently healthy sugarcane collected sugar stalk. The plants stalks were inoculated with 2ml of freshly prepared spore suspension of *C.falcatum* using a 2ml needle and syringe, and the control were inoculated with sterile distilled water. The samples were observed for symptoms development after 15 days.

Antifugal efficacy of plant extracts on mycelia growth of *Colletotrichum falcatum*: The plant extracts were evaluated for antifungal activities using the food poison techniques (Balouiri*et al*., 2010). Three(3) ml of the extracts at different concentrations (75, 50 and 25%) were thoroughly mixed with 15ml of sterile PDA and chloramphenicol in a Petri dish. After gelling of the medium, 5mm disc of active growing, seven days old of *C. falcatum* was inoculated at the centre of the each plate using cork borer. The Petri plates were incubated at 28±2°C for 7 days with three replicates for each treatment. Radial mycelia growth of the pathogen was measured using transparent meter rule (mm) and percentage inhibition was calculated after seven days using the formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter of (Control-Treatment)}}{\text{Colony Diameter of Control}} \times 100$$

Data Analysis: Data obtained from both *in vitro*measurements were subjected to statistical analysis of variance (ANOVA) to determine the significant differences among means. Duncan multiple range test (DMRT) was used to separate the means where there were differences using statistical package for social sciences (SPSS) version 20 at 5% level of significance.

Results and discussion

Identification of *Colletotrichum falcatum*: The colony turf of the isolate is light loose, cottony, and floccose, almost white in young cultures, becoming light ashy gray with broad white magins in 7 to 14 days as shown in plate three (3) below. The conidia is curve or falcate shape with hyaline having two septate.

Phytochemical screening: The phytochemical analysis revealed the presence of alkaloids in all the three tested plants. Tannins, saponins and terpenoids were only present in *A. indica and L. inermis.* Flavonoids, was persent in *A.*

*indica*and *K. senegalensi.* Phenols and steroid were in *L. inermis*and *K. senegalensis* but absent in *A. indica.*Cardiacglycosides and anthraquinoneswere only found in *A. indica*and *L. inermis*respectively.

Pathogenicity test: The results from the pathogenicity test showed that the dissected sugarcane tissue became red in colour after fifteen (15) days of inoculation. This confirmed that *Colletotrichum falcatum* is the actual causative organism of the red rot disease of the sugarcane while the (sterile distile water) had no symtom of red rot disease.

In vitro* Assessment of the three (3) Plants Extracts on Mycelia Growth of *C. falcatum: The results of the different concentration (75, 50 and 25%) of the three (3) plant extracts after seven (7) days inoculation in PDA of *C. falcatum,* have proved effective on mycelia growth inhibition of the tested organism (table 4.2). The results of statistical analysis showed that there was significant different in the inhibitory effect of all the extract with increased in antifungal activity of the extracts with increased in concentration. The antifungal efficacy of the extract revealed that the highest mycilia inhibition was recorded at 75% concentrations for all the extracts with the value of 53%, 100% and 100% for *A. indica*, *L. inermi*s and *K. senegalensis* respectively. However, total mycelia inhibition (100%) was recorded at 50% concentration of *L. inermis*. The negative control showed no inhibitory effects against the fungi with 100% inhibition of the mycelia growth recorded for positive control.

The morphological characteristics of *C. falcatum* observed in this study were similar to those reported by (Vikash and Shukka, 2017) who stated that the colony colour ofpathogen is greyish white with falcate shape hyaline conidia.The presence of some bio-active components such as alkaloids, flavonoids, tannins, saponnin, steroids, cardiacglycosides and anthraquinones in the leaf extracts of *A. indica*confirmed the findings reported by Adebola *et al*. (2019). Also, the presence of phytochemical the leaf extracts of *L. inermis* (alkaloids, saponnins, tannins, glycosides and flavonoids) reported by Wangini *et al*. (2014) agreeds with the finding of this study. Bioactive compounds such as: flavonoids, carbohydrates, steroids and triterpenes obtained in the leaf extracts of *K. senegalens*correlate with that reported byElish*et al.* (2015) and Kurta, *et al*. (2015). The antifungal efficacy of the leaf extracts could be attributed to the presence of some important bioactive compounds. Earlier author had attributed the antifungal effects of plant extracts to the presence of phenolic compound (Abu-Taleb *et al*., 2011) flavonoids, terpenoids, and alkaloids Wangini *et al*. (2014). The increase in antifungal activity of all the extracts an increase in concentration, might be due to the increase in the quantity of the active secondary metabolites such as tannins, saponins, flavonoids and alkaloids etc. The decrease in inhibitory effects of the extract on the tested organism with decrease in concencentration confirmed the opinions Adebola, *et al*. (2016); who reported that antimicrobial activity of plant extracts is concentration dependent. The maximum antifungal efficacy of *L. inermis* leaves extracts observed agreed with the works of Zarrin*et al*. (2013), who tested several medicinally important plant and adjudged the plant to be the best.

Conclusion

This study has shown antifungal activities of the ethanolic leaf extracts of three medicinal plants. The efficacy of the ethanolic leaf extracts of these plants increased with the increase in concentration; 75% concentration showed the highest level of inhibition. The ethanolic leaf extract of *A. indica, L. Inermis, K. senegalensis* in that order proved to be effective in the inhibition of *C. falcatum*at the three concentrations. Since the plants are easy to be find and prepare, they can be used as substitute to control red rot disease of sugarcane.

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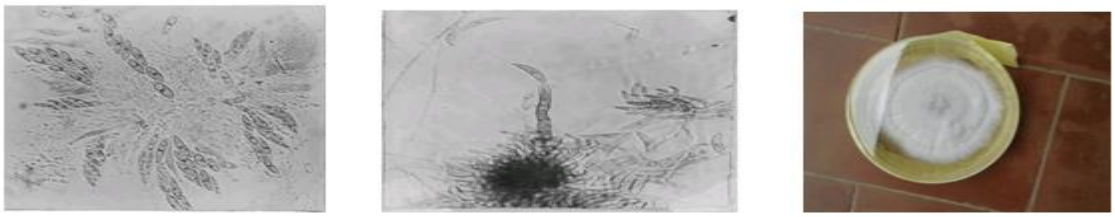

Plate 1A: Microscopic Features of *C. falcatum*

Table 1. Phytochemical Composition of leaf extracts of Threeplants.

Key: + = presence, - = absence.A= Alkaloids, F= Flavonoids, T= Tannins, Sp= Saponins, Tp= Terpenoids, P= Phenols, S= Steroids, C= Cardiacglycosides and An= Anthraquinones.

Plant Name	Phytochemicals								
	A	F	T	Sp	Tp	P	S	C	An
A. indica	+	+	+	+	+	-	-	+	-
L. inermis	+	-	+	+	+	+	+	-	+
K. senegalensis	+	+	-	-	-	+	+	-	-

Table 4.5. *In vitro* Assessment of *A. indica, K. senegalensis* and *L. inermis* plant leaf extracts on pecentage mycelia growth inhibition seven days after inoculation with the pathogen.

Treatment Extract used	CONTROLS SDTW	CLOT	EXTRACT CONCENTRATIION		
	0%(-ve)	0.5g/l(+ve)	75(%)	50(%)	25(%)
A. indica	0.00±0.00^{a}	53.00±1.15^{b}	33.00±0.00^{a}	15.30±.0.33^{a}	
K. senegalensis	0.00±0.00^{a}	100±0.00^{a}	60.60±0.00^{c}	40.00±1.15^{b}	25.00±0.00^{b}
L inermis	0.00±0.00^{a}	100±0.00^{a}	100.00±0.00^{e}	100.00±0.00^{d}	67.00±0.00^{e}

Values are means of three replicates ± standard error. Values followed by the same superscript across the columns are significantly different (p≤0.05).