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BIO-EFFICACY OF LEAF EXTRACTS OF *AZADIRACHTA INDICA* AND *VERNONIA AMYGDALINA* AND THEIR MIXTURE AGAINST *COLLETOTRICHUM FALCATUM*

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ABSTRACT
Chemical control of red rot disease of sugarcane with synthetic fungicides, though effective, enables the pathogen to produce new pathological races or strains that are resistant to the available synthetic fungicides in the market. The synthetic fungicides, in addition, have harmful side effects on humans, other animals and the environment. This study evaluated the in vitro antifungal activity of leaf extracts of Azadirachta indica and Vernonia amygdalina against Colletotrichum falcatum, the pathogen of red rot disease of sugarcane. Pour Plate method was used to determine the effect of 10, 20 and 30 g/ 100 ml concentrations of aqueous and ethanolic leaf extracts of A. indica and V. amygdalina and their integration on mycelial radial growth of C. falcatum. The aqueous and ethanolic leaf extracts of the botanical species and their integrated leaf extracts inhibited the mycelial growth of C. falcatum in vitro, with the ethanolic extracts being more potent at some of the concentrations tested against the pathogens. The gradient of efficacy of the 3 types of leaf extracts tested for antifungal efficacy against C. falcatum was: mixed leaf extracts leaf > neem leaf extracts > bitterleaf leaf extracts, regardless of the extractant at all the concentrations used in this study.
KEY WORDS: *Antifungal, aqueous, bitterleaf, concentrations, ethanolic, extracts, extractant, mixed, neem, red rot.*

INTRODUCTION

Colletotrichum falcatum Went is a homothallic fungus, whose sexual stage is named *Glomerella tucumanensis* (Abbott & Hughes, 1961). It is highly variable in nature; hence it causes the frequent breakdown of resistant varieties of its host (Sharma & Tamta, 2015). *C. falcatum* is the pathogen of red rot disease of sugarcane, with its inoculum being disseminated by wind, rain, heavy dews or irrigation water. Infected cane setts can readily spread or cause secondary infections, while crop debris or subtle may also provide inoculum to infect a new crop (Raid, 2012).

The recent research interest in the use of some medicinal plant parts for control of some plant and animal diseases can be related to presence of secondary metabolites which have antimicrobial properties such as alkaloids, anthraquinones, cardiac glycosides, cyanogenic glycosides, tannins, polyphenols, etc. in them (Iwu, 2000). Non-effectiveness, development of resistance and phytotoxicity of synthetic fungicides for the control of plant diseases call for the search for natural products as alternatives.

Popular among these natural products are plant species of family Meliaceae (*Azadirachta indica* A. Juss), Neem tree and family Asteraceae (*Vernonia amygdalina*), Bitterleaf. *Azadirachta indica* is characterized by 20–40 cm long leaf, with 20 to 31 light to dark green leaflets which are about 3–8 cm long, while leaves of *V. amygdalina* are lanceolate to oblong shaped and usually about 10–15 by 4–5 cm.

MATERIALS AND METHODS

Fresh leaves of neem (*A. indica*) with voucher number UILH/002/2019/613 and bitterleaf (*V. amygdalina*) with voucher number UILH/003/2019/507, were harvested and washed properly under a running water. The leaves were air-dried separately on a laboratory bench for fourteen days and then grinded into powder using an Electric Blender (SMB 2977 model). To obtain the three concentrations (10, 20 and 30%) assayed in this study, 10, 20 and 30 grams of each of the powdered botanical species was soaked separately in 100 ml of 70% ethanol (ethanolic extraction) and 100 ml of sterile distilled water (aqueous extraction) in different 250 ml conical flasks. For the mixed leaf extracts, powdered leaves of neem and bitter leaf were measured in equal proportion to make the 3 concentrations into separate 250 ml conical flasks containing 100 ml of 70% ethanol and 100 ml distilled water respectively. Each of the conical flasks was covered with aluminum foil and allowed to stand for 48 hours on the laboratory bench. The extracts concentrations were sterilised and filtered into labelled sterile bottles with cork and then stored at 10 °C prior to use.

The Pour plate method as described by Olahan & Amadi (2006) was used to determine the efficacy of each of the sterile ethanolic and aqueous leaf extracts on the radial growth of *C. falcatum* on Potato Dextrose Agar (PDA) plates. Four equal sectors were created on the cover of each of sterile disposable Petri dishes by drawing two perpendicular lines running through its centre. About 2 ml of 70% ethanol was introduced separately into sterile disposable Petri dishes with a new 5 ml hypodermic syringe and labelled as control E. (controls for the ethanolic leaf extracts). The same volume of sterile distilled water was also introduced separately into another sterile Petri dishes with a new 5 ml hypodermic syringe and labelled control aq. (controls for the aqueous leaf extracts). The 2 ml of each type of leaf extracts to be assayed for each of the concentrations were separately inoculated into different sterile disposable Petri dishes for *in-vitro* antifungal efficacy trials.

All inoculated Petri dishes were arranged in a Completely Randomized Design (CRD) on the work bench and served with 15 ml of molten PDA culture medium, amended with 1 ml of streptomycin BP under aseptic condition. The Petri dishes were labelled appropriately and swirled gently to homogenize its contents and then allowed to solidify on the bench.

Mycelial plugs (4 mm in diameter each) were picked from advancing edges of 5-day old pure culture of *C. falcatum.* A plug of *C. falcatum* was placed in an inverted

position at the centre of each disposable Petri dish containing PDA amended with different concentrations of the extracts (treatment plates) as well as control plates using a sterile inoculating needle. All the inoculated plates were incubated at 25 °C for 6 days. The diameter of the mycelium on each plate was measured daily with a 30 cm transparent plastic ruler and recorded. The mycelial extension of the pathogen was determined using the formula:

$$D_{(mm)} = \frac{dx + dy}{2}$$

Where: D = Diameter of the mycelium,
d*x*= Diameter along the horizontal line
d*y* = Diameter along the vertical line on each of the PDA plates.

The percentage growth inhibition of each of the treatments and concentrations was determined using the formula cited by Okigbo & Nmeka (2005), i.e.

$$\text{Growth inhibition by a leaf extract (\%)} = \frac{D_c - D_t}{D_c} \text{ x } 100$$

Where D_c = diameter of the test microorganism on the control plate
D_t = diameter of the test microorganism on the experimental plate.

Data for the percentage growth inhibition of the leaf extracts were subjected to Analysis of variance (ANOVA) and the treatment mean percentage growth inhibition values were separated by Duncan's Multiple Range Test (DMRT) at P≤0.05 using software IBM SPSS Statistics Package Version 25.

RESULTS AND DISCUSSIONS

The mycelial diameters of *C. falcatum* increased slowly but progressively throughout the incubation period. There were reductions in the mycelia growth of *C. falcatum* in the treated Petri dishes compared to mycelial diameters in the control plates and these applied to both aqueous and ethanolic leaf extracts. The aqueous and ethanolic mixed leaf extracts were more active in retarding the mycelial radial growth of *C. falcatum in-vitro* than the neem or bitterleaf leaf extracts across all the concentrations assayed, and this effect increased with the period of incubation (Table 1). For the aqueous extracts, all the tested concentrations of the mixed leaf extracts had the same efficacy on *C. falcatum*. The percentage growth inhibition of *C. falcatum* by the 10, 20 and 30 g/ 100 ml concentrations of these extract were 48.9, 48.1 and 48.8, respectively (Figure 1). This was closely followed by the effect of the 30 g/ 100 ml ethanolic leaf extract of neem leaf. Bitterleaf leaf extracts (both aqueous and ethanolic) had the least antifungal effect on the radial growth of *C. falcatum* (Figure 1).

There were no significant differences in the growth inhibitory effects of each of the 3 concentrations of the aqueous and ethanolic extracts of the mixed leaf extracts on *C. falcatum* (Figure 1). There were significant differences in the growth–inhibitory effects of the 3 aqueous extracts (neem, bitterleaf and mixed leaf extracts) on *C. falcatum* at P <0.001 (Figure 1). There were no statistical differences in the growth–

inhibitory effects of the 3 concentrations of ethanolic bitterleaf leaf extracts on *C. falcatum* (Figure 1).

Different concentrations of the leaf extracts of neem, bitter leaf and their mixture affected the radial growth of mycelia of *C. falcatum* on amended PDA plates significantly compared with the controls. Antifungal efficacy of the leaf extracts was concentration dependent with the 30 g/100 ml showing the highest growth inhibitory effect of each of the extracts. Different concentrations of each of the ethanolic leaf extracts were more effective than the same concentrations of each of the aqueous extracts of the same plant. These observations corroborated the findings of previous studies on the *in-vitro* antifungal efficacy of some medicinal plant extracts on some plant pathogens. Nwachukwu & Umechumba (2001) observed that the leaf extracts of basil, bitter leaf, neem, lemon grass and pawpaw were effective in controlling the incidence of *Aspergillus flavus, Botryodiplodia theobroma* and *Fusarium oxysporum* compared with the control, with the neem leaf extract being the most effective while lemon grass leaf extract was the least effective.

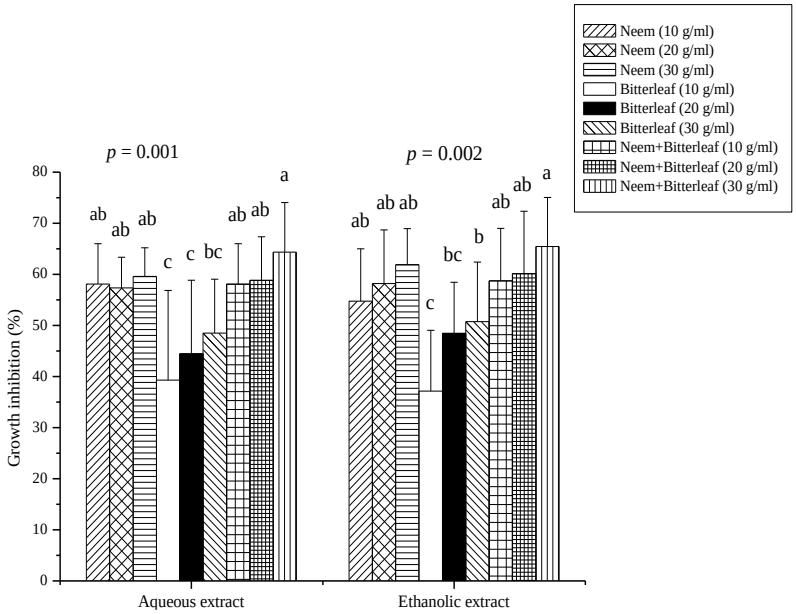

FIGURE1. Inhibitory effect of botanical extracts on the growth of *Colletotrichum falcatum*
Bars with the same letter(s) are not significantly different at P≤0.05 for each extractant.

Mondali *et al.* (2009) reported that different concentrations of aqueous and methanolic leaf extracts of neem significantly inhibited the growth of species of *Aspergillus* and *Rhizopus*, seed borne fungal pathogens *in-vitro*, with the methanolic extract being the more effective compared to the aqueous extract at all concentrations

of the neem extract used. Moslem & El–kholie (2009) also reported that different concentrations of ethanolic, hexanolic and methanolic extracts of neem seeds and leaves were effective as antifungal agents against *Alternaria solani*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, with *F. oxysporum* and *R. solani* being the most sensitive fungi. Javaid & Rehman (2011) reported that leaf extracts of *Azadirachta indica* L. and *Melia azedarach* L. regardless of the medium of extraction, had the highest antifungal activity against *Macrophomina phaseolina* (Tassi) Gold, the pathogen of charcoal rot disease in many plant species.

TABLE 1. Effect of treatment types and concentrations on mycelial growth of *Colletotrichum falcatum (mm)*

Conc. of extract (g/100 ml)	Incubation period (hrs.)	Control Aqueous	Control Ethanolic	Neem Aqueous	Neem Ethanolic	Bitterleaf Aqueous	Bitterleaf Ethanolic	Neem & Bitterleaf Aqueous	Neem & Bitterleaf Ethanolic
10	24	15.0	10.0	10.0	8.0	10.0	10.0	8.0	8.0
10	48	23.0	17.0	15.0	11.0	17.0	12.0	12.0	10.0
10	72	32.0	24.0	18.0	13.0	23.0	16.0	16.0	12.0
10	96	40.0	30.0	22.0	14.0	28.0	18.0	20.0	14.0
10	120	51.0	35.0	28.0	15.0	34.0	20.0	27.0	16.0
10	144	64.0	40.0	33.0	15.0	40.0	24.0	31.0	18.0
20	24	15.0	10.0	10.0	10.0	10.0	10.0	10.0	6.0
20	48	23.0	17.0	13.0	11.0	15.0	12.0	12.0	8.0
20	72	32.0	24.0	19.5	12.0	20.5	14.0	16.0	10.0
20	96	40.0	30.0	24.0	13.0	24.0	16.0	20.0	12.0
20	120	51.0	35.0	27.0	14.0	30.0	18.0	25.0	13.0
20	144	64.0	40.0	30.0	15.0	33.0	20.0	28.0	15.0
30	24	15.0	10.0	10.0	8.0	10.0	10.0	6.0	6.0
30	48	23.0	17.0	12.0	10.0	18.0	12.0	12.0	7.0
30	72	32.0	24.0	19.0	12.0	20.0	13.0	16.0	8.0
30	96	40.0	30.0	22.5	13.0	25.0	14.5	20.0	10.0
30	120	51.0	35.0	25.0	14.0	28.0	16.0	22.5	12.0
30	144	64.0	40.0	28.0	14.0	30.0	18.0	26.0	13.0

Suleiman (2011) was of the opinion that neem leaf extracts at 20, 30, 40, 50 and 60% concentrations inhibited the vegetative growth of *Penicillium digitatum in-vitro*. Ilondu (2013) also reported that the ethanolic leaf extracts of *Vigna ambigua*, *V. amygdalina* and *V. cinerea* inhibited the radial mycelial growth of *Cercospora apersica* and *Curvularia lunatus*, isolates of groundnut leaf spot disease, different concentrations of aqueous and ethanolic leaf extracts of neem (*A. indica*) and pear (*Persea americana*) were tested against *Rhizoctonia solani*, the pathogen of rice sheath blight disease in *in-vitro* culture using the disc diffusion method by Jagessar *et al.* (2015). Both leaf extracts showed varying degrees of antifungal effect at different concentrations against the pathogen.

Arumugam *et al.* (2015) reported that growth of *Candida albicans* and *Aspergillus niger* was inhibited by both alcoholic and aqueous extracts of Malaysian neem leaf extract at the various concentrations tested *in-vitro*. Bazie *et al.* (2014) reported that *Acasia albida, Azadirachta indica, Argemone mexicana, Dovalis abyssinica, Prosopis juliflora* and *Vernonia amygdalina* showed high to moderate

antifungal activity against *Colletotrichum musae,* the pathogen of postharvest banana anthracnose. John *et al*. (2016) reported that *V. amygdalina* leaf extract has significant concentration–dependent *in-vitro* inhibitory effect on the growth of *Geotrichum candidum, Rhizopus stolonifer* and *Fusarium oxysporum*, while Okigbo & Emoghene (2018) reported an increased antifungal activity with a corresponding increase in concentration of aqueous leaf extracts of *V. amygdalina* Del., *A. indica* A. Juss and *Ocimum gratissimum* L. against conidiospore germination, mycelial extension and lesion development of *Mycosphaerella fijiensis* Morelet, the pathogen of black sigatoka disease of banana.

CONCLUSIONS

The efficacy of leaf extracts of *A. indica* and *V. amygdalina* were established against *C. falcatum in-vitro* at all concentrations assayed. There is need to proceed *in-vivo* evaluation of same against this pathogen of red rot disease of sugar cane.

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