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Short Communication

ANTIFUNGAL EFFICACY OF LEAF EXTRACTS OF NEEM, BITTERLEAF AND THEIR MIXTURE AGAINST *USTILAGO SCITAMINEUM*

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ABSTRACT

This study was aimed at evaluating the antifungal activity of leaf extracts of neem, bitterleaf and their mixture against *Ustilago scitamineum* (Syd.), the pathogen of sugarcane smut disease. A suspension of mancozeb, carbendazim+maneb, matalaxy l+ carboxin + flurathiocarb, pyroquilon, benomyl, chlothalonil and flutriafol are effective as a dip in controlling the disease. However, the listed synthetic fungicides led to emergence of resistant strains of the pathogen and also cause environmental pollution. Hot water treatment of the cane setts at 52° C for 30 minutes before planting is also effective, but this can cause serious injuries on sugarcane farmers if not carefully practiced. Pour Plate method was used to determine antifungal effect of 10, 20 and 30 g/ 100 ml concentrations of aqueous and ethanolic leaf extracts of neem, bitterleaf and their mixture on radial growth of *U. scitamineum* for 6 days. The tested leaf extracts inhibited mycelial growth of *U. scitamineum* *in vitro*, with the ethanolic leaf extracts being more potent at some of the concentrations tested in this study. In conclusion, gradient of antifungal efficacy of the tested leaf extracts against *U. scitamineum* was: mixed leaf extracts > neem leaf extracts > bitterleaf leaf extracts at all the concentrations used.

Keywords: Antifungal, aqueous, cane setts, smut, sugarcane).

INTRODUCTION

Ustilago scitamineum (Syd.), the pathogen of smut disease of sugarcane belongs to the Kingdom Fungi, Division Eumycota, Sub division Basidiomycotina, Class

Ustilaginomycetes, Subclass Ustilaginomycetidae, Order Ustilaginales, Family Ustilaginaceae, Genus *Ustilago* and Species *scitamineum*. Synonyms of are *U. scitaminea*, *Sporisorium scitamineum* and *S.*

scitaminea (Sharma and Tamta, 2015). It is spread by wind currents over great distances, with the “smut whip,” infected cane setts and plant debris serving as inoculum sources (Gana, 2010). Wind- and insect-borne spores may settle on the soil of cropped or newly prepared fields; disease-free sugarcane setts may become infected if planted in such soil containing viable spores (Rott and Comstock, 2015).

The recent research interest in the use of some medicinal plant parts for the control of some plant diseases is as a result of the presence of secondary metabolites which have antimicrobial properties such as alkaloids, anthraquinones, cardiac glycosides, cyanogenic glycosides, tannins, polyphenols, etc in them. Non-effectiveness and toxicity of some synthetic fungicides for the control of these diseases, as well as their adverse effects on the environment, call for search for natural products, e.g. botanicals to be tested for controlling plant diseases. Popular among these are the leaves of *Azadirachta indica* A. Juss (Neem tree) belonging to the family Meliaceae, and *Vernonia amygdalina* (Bitterleaf tree) belonging to the family Asteraceae (Iwu, 2000). Neem leaves are 20–40 cm long, with 20 to 31 medium to dark green leaflets which are about 3–8 cm long, while bitterleaf leaves are lanceolate to oblong shaped; usually about 10–15 by 4–5 cm (Aiyelaja, 2006).

MATERIALS AND METHODS

Fresh leaves of neem (*Azadirachta indica* with voucher number UILH/002/2019/613) and bitterleaf (*Vernonia amygdalina* with voucher number UILH/003/2019/507) were harvested and washed properly under a running tap water. They were air-dried separately for fourteen days and then ground into powder using an Electric Blender (SMB 2977 model). To get the three concentrations tested in this study, i.e. 10, 20 and 30 gm/

100 ml, 10, 20 and 30 grammes of each of the powdered leaves was soaked separately in 100 ml of 70% ethanol (for ethanolic extraction) and 100 ml of sterile distilled water (for aqueous extraction) inside separate 250 ml conical flasks. For the mixed leaf extracts, powdered leaves of neem and bitterleaf were measured (in equal proportion to make the 3 concentrations) into separate 250 ml conical flasks containing 100 ml distilled water and 70% ethanol respectively. Each of the conical flasks was covered with aluminium foil and allowed to stand for 48 hours. Each of the different concentrations of the leaf extracts was sterilised using the methods of Fawole and Oso (2007). Each of the sterilized leaf extracts was filtered into separate labelled bottles and then stored at 10° C prior to their usage.

The Pour Plate method as described by Olahan and Amadi (2006) was used to determine the antifungal activity of each of the sterile aqueous and ethanolic leaf extracts on radial growth of *U. scitameum* on PDA plates. Four equal sectors were created on the cover of each of about forty (40) sterile disposable Petri dishes by drawing two perpendicular lines running through its centre. About 2 ml of 70% ethanol was introduced separately into two of the sterile disposable Petri dishes with a new hypodermic syringe (5 ml) and labelled as Control E. (for the ethanolic leaf extracts). The same volume of sterile distilled water was also introduced separately into another two sterile Petri dishes with a new hypodermic syringe (5 ml) and labelled Control aq. (for the aqueous leaf extracts). The remaining thirty-six (36) sterile disposable Petri dishes were divided into three, representing the three types of leaf extracts to be tested in this study.

About 2 ml of each of the three concentrations of each of the aqueous and ethanolic leaf extracts was introduced into

sterile disposable Petri dishes (40) separately with new hypodermic syringes (5 ml). All the Petri dishes (40) were arranged orderly on the work bench and each of them was served with 15 ml of molten PDA culture medium (already amended with 1 ml of streptomycin BP) in an aseptic environment. The lid of each of the Petri dishes was labelled to reflect the type and concentration of the leaf extract. Each labelled and covered Petri dish was swirled gently to homogenize its contents.

Mycelial discs (4 mm diameter each) were caught from advancing edges of 5 day old pure culture of *U. scitamineum* into a Petri dish. A disc of *U. scitamineum* was placed in an inverted position at the centre of each disposable Petri dish containing PDA amended with each concentration of the aqueous and ethanolic leaf extracts as well as the 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. This was determined using the formula:

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

Where: D = Diameter of the mycelium, dx= Diameter along the horizontal line and dy = Diameter along the vertical line on each of the PDA plates. The growth inhibition (%) of each of the leaf extracts was determined using the formula of Pandey *et al.* (1982) as cited by Okigbo and Nmeka (2005), i.e.:

Growth inhibition by a leaf extract(%)

$$= \frac{D_c - D_t}{D_c} \times 100$$

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

Analysis of variance of the mean percentage growth inhibition of the leaf extracts at $p \leq$

0.05 and Duncan's Multiple Range Test (DMRT) were determined using IBM SPSS Statistics Package Version 25.

RESULTS

Diameters of the mycelia of *U. scitamineum* inoculated on PDA plates amended with 10, 20 and 30 g/100 ml concentrations of the leaf extracts of neem, bitterleaf and their mixture increased slowly, but progressively throughout the incubation period, with diameters of the mycelia of *U. scitamineum* in the treatments being lower than those for the controls (Table 1). The extracts of the mixture of neem and bitterleaf leaves were more active in retarding the radial growth of mycelia of *U. scitamineum in vitro* than the leaf extracts of neem; with the bitterleaf leaf extracts having the least effect in most of the concentrations of the extracts tested in this study (Table 1).

Diameters of mycelia of *U. scitamineum* on the controls after 24 hours of incubation were 25.0 mm (Control aq.) and 15.0 mm (Control Ethanolic); diameters of mycelia of *U. scitamineum* inoculated on PDA plates amended with 10 g/ 100 ml neem leaf extract after same period were; 8.0 mm (aqueous extract) and 6.0 mm (ethanolic extract); diameters of mycelia of *U. scitamineum* on PDA plates amended with 10 g/100 ml concentration of bitterleaf leaf extract were 13.0 mm (aqueous extract) and 8.0 mm (ethanolic extract); while diameters of mycelia of *U. scitamineum* on PDA Plates amended with 10 g/ 100 ml of the mixture of extracts of neem and bitterleaf were the same with those for the same concentration of the neem leaf extract, 8.0 mm (aqueous extract) and 6.0 mm (ethanolic extract) (Table 1).

Table 1: Diameters of Mycelia of *Ustilago scitamineum* on Potato Dextrose Agar (PDA) Plates Amended with Different Concentrations of Leaf Extracts of Neem, Bitterleaf and Their Mixture

Conc. of the leaf extract (g/100 ml)	Incubation period (hrs.)	Diameters of Mycelia of <i>Ustilago scitamineum</i> (mm) on PDA Plates							
		Control (Aq.)	Control (Etha.)	Neem (Aq.)	Neem (Etha.)	Bitter leaf (Aq.)	Bitterleaf (Etha.)	Neem & Bitterleaf (Aq.)	Neem & Bitterleaf (Etha.)
10	24	15.0	10.0	8.0	6.0	13.0	8.0	8.0	6.0
	48	25.0	15.0	12.0	9.0	18.0	10.0	12.0	8.0
	72	37.0	20.0	18.0	10.5	25.0	14.0	16.0	9.0
	96	56.0	25.0	23.0	11.0	28.0	15.0	20.0	11.0
	120	64.0	30.0	28.0	13.0	30.0	16.0	25.0	11.0
	144	78.0	34.0	32.0	14.0	32.0	16.0	25.0	11.0
20	24	15.0	10.0	7.0	6.0	12.0	7.0	8.0	6.0
	48	25.0	15.0	13.0	7.0	16.0	8.0	12.0	7.0
	72	37.0	20.0	16.0	8.5	20.0	10.0	16.0	8.0
	96	56.0	25.0	21.0	9.0	24.0	12.0	20.0	9.0
	120	64.0	30.0	26.0	10.0	32.0	14.0	23.0	9.0
	144	78.0	34.0	28.0	11.0	33.0	14.0	24.0	9.0
30	24	15.0	10.0	6.0	5.0	10.0	7.0	8.0	5.0
	48	25.0	15.0	12.0	6.0	15.0	8.0	10.0	6.0
	72	37.0	20.0	17.0	8.0	20.0	10.0	12.0	7.0
	96	56.0	25.0	21.0	9.0	23.0	11.0	16.0	8.0
	120	64.0	30.0	24.0	10.0	29.5	12.0	20.0	8.0
	144	78.0	34.0	26.0	10.0	32.0	13.0	22.0	8.0

Keys: Aq. = Aqueous Extract; Conc. = concentration; Etha. = Ethanolic Extract.

From Figure 1 below, the higher the concentration of the leaf extract, the higher the mean percentage growth inhibition of *U. scitamineum* on the amended PDA plates. Ethanolic leaf extract of the mixture of neem and bitterleaf leaves at 30g/100ml concentration have the highest mean percentage growth inhibition of *U. scitamineum* on PDA plates (65.6), which was closely followed by the same concentration of aqueous extract of the same type of leaf extract (63.1%) (Figure 1). The neem leaf extracts at the concentrations tested in this study also inhibited the growth of *U. scitamineum* higher than bitterleaf leaf extracts (Figure 1).

There were significant differences in the growth inhibition (%) of *U. scitamineum* among the different leaf extracts for aqueous extract ($p = 0.001$) and ethanolic extract ($p = 0.002$), both p values are less than 0.05. For the ethanolic extracts, the 30 g/100 ml

mixture of neem and bitterleaf leaves leaf produced the highest growth inhibition (%) and this was statistically different from the growth inhibitory effects of the bitterleaf leaf extracts at 10 g/100 ml, 20 g/100 ml and 30 g/100 ml concentrations (Figure 1). The mean percentage growth inhibition of *U. scitamineum* on PDA plates by aqueous and ethanolic neem leaf extracts at all the concentrations tested in this study were not statistically different from those for the corresponding concentrations of the aqueous and ethanolic extracts of mixture of neem and bitterleaf leaves (Figure 1).

DISCUSSION

Different concentrations of the leaf extracts of neem, bitterleaf and their mixture affected the radial growth of mycelia of *U. scitamineum* on amended PDA plates significantly compared with the controls

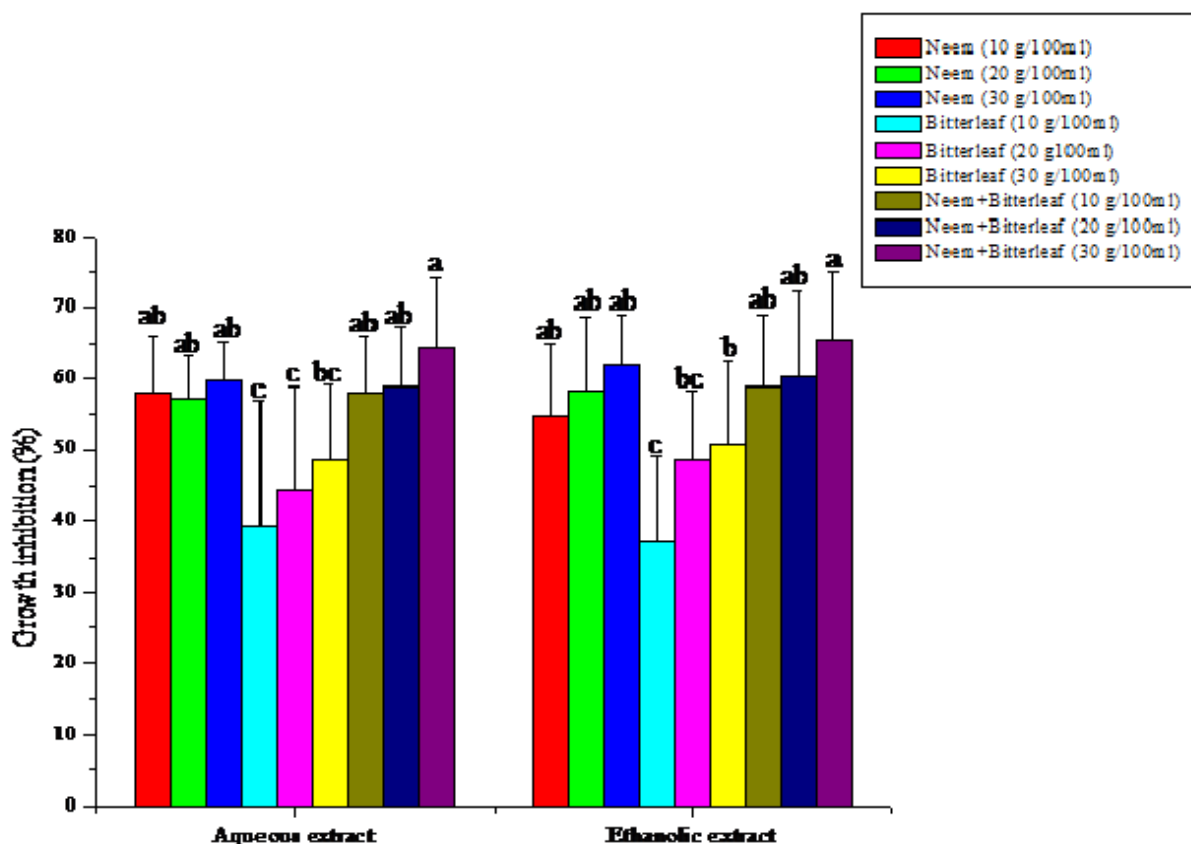


Figure 1: Percentage Growth inhibition of *Ustilago scitamineum* by different concentrations of extracts of neem, bitterleaf as well as mixture of neem and bitterleaf leaves. Bars with the same letter(s) were not significantly different at $p \leq 0.05$ for each extractant.

The antifungal efficacy of the leaf extracts of neem and bitterleaf is as a result of their secondary metabolite constituents (Agbogidi and Akpomorin, 2013; Saxena *et al.*, 2013). 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 on *U. scitamineum* compared with the controls. 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 in this study. Gradient of efficacy of the 3 types of leaf extracts tested for antifungal efficacy against the test organism in this study was:

mixed leaf extracts > neem leaf extracts > bitterleaf leaf extracts, regardless of the extractant at all the concentrations used. These observations corroborated the findings of previous studies on the *in vitro* antifungal efficacy of some medicinal plant extracts on some plant pathogens.

Javid and Rehman (2011) reported that leaf extracts of *Azadirachta indica* L. and *Melia azedarach* L. (Family Meliaceae), 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. 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 leafspot disease, while Bazie *et al.* (2014) reported that *Acacia 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.

Shrivastava and Swankar (2014) observed that different concentrations of methanolic and ethanolic neem leaf extracts have growth inhibitory effects against *Aspergillus flavus*, *Alternaria solani* and *Cladosporium* sp, with the methanolic extract being the most effective against *A. flavus*. 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. 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 and 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.

CONCLUSION

Neem and bitterleaf leaf extracts as well as their mixture are capable of inhibiting the radial growth of *U. scitamineum*, the pathogen of smut diseases of sugarcane *in vitro*.

RECOMMENDATION

It is therefore necessary to test the efficacy of these extracts on this pathogen *in vivo*, so as to be able to test them as a control agent against smut disease of sugarcane.

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