Antimicrobial Activities of Ethanolic Leaf Extracts of S. alata Linn on Post-Harvest Yam (Dioscorea rotundata Poir) Tuber Rot Pathogens

Tedela[#1], P.O., *Ijato, J Y*[*2]
[#]Department of Plant Science, Faculty of Science, Ekiti State University,
Ado – Ekiti, P.M.B 5363, Ekiti State, Nigeria.
patosateddy@yahoo.com; GSM: 08033968087
[*]Department of Plant Science, Faculty of Science, Ekiti State University,
Ado – Ekiti, P.M.B 5363, Ekiti State, Nigeria.
jamesyeni@yahoo.com

ABSTRACT

The Antimicrobial effect of ethanolic leaves extract of *Senna alata* was determined *in vitro*. It was found that all the varied concentrations of the extracts were effective in inhibiting the mycelia growth of *Botryodiplodia theobromae, Aspergillus flavus, Aspergillus niger* and *Aspergillus glaucus,* 40and 50g in 10% ethanol inhibited the radial mycelia growth of *A. niger* and *A glaucus* by 96.43%, 30 and 40g in 20% ethanol reduced the radial thrive of mycelia of *B. theobromae* by 97.10 and 97.07 % respectively, 20g in 30% ethanol, 30 and 40g in 40% ethanol reduced *A. flavus* by 100%, all the concentrations of extracts in 50% ethanol reduced the radial mycelia extension of *A. flavus* by 100%. This finding revealed that ethanolic leaves extract of *S. alata* had significant potential for the control of fungal rot of yam tubers.

Key words: *in vitro*, *Senna alata*, rot pathogens, yam tuber rot

INTRODUCTION

*S*enna *alata* commonly referred to as ringworm Senna belongs to the family FabaceaeIt is a Pan tropical, ornamental plant that is widely distributed from America to India [4]. It is erect, 1.5 to 3 meters high, branched shrub and has coarse bark. The leaves are pinnate with orange rachis on stout branches with 16 to 28 leaflets, 5 to 15 centimeters in length, broad and rounded at the apex, inflorescence 10 to 50 centimeters long, in simple or panicle racemes, terminal and at the axils of the leaves. Flowers yellow, about 4 centimeters in diameter. The pod is straight, dark brown or nearly black, containing 50 to 60 flattened triangular seeds.

Rot pathogens which are majorly fungi often penetrate the tuber through wounds which are caused by insects, nematodes as well as poor handling before, during and after harvest. Insects that predispose yam to rot include mealy bug (*Plarococcus citri*), storage beetle (coleoptera), as well as scale insect (*Aspidiella harti*). References [1]-[3], reported the association of many fungi such as *Asperigillus niger, Botryodiplodia theobromae, Aspergillus flavus Rosellinia bunodes, A. tamari, Fusarium* species as causal organisms of yam tuber diseases. Medicinal values of *S. alata* leaf extracts against ringworm, itching, eczema, pruritis, scabies and ulcer have also been reported by [5],[7].

Therefore, the aim of this experiment is to investigate the effect of ethanolic leaf extracts of *S. alata* on post harvest rot pathogens of yam tubers *in vitro*.

MATERIALS AND METHODS

Collection of materials and preparation

Healthy and Infected yam tubers with symptoms of rot were purchased at Oba market in Otun-Ekiti, placed separately in a sterile polythene bags and taken to the laboratory of Plant Science, Ekiti State University, Ado-Ekiti for authentication in the herbarium and for analysis. Leaves of *Senna alata* were collected from the premises of Enterprise Bank within Ekiti State University, Ado-Ekiti campus, taken to herbarium unit of the University in the Department of Plant Science for identity authentication, air dried at room temperature and stored at 24^{0}C until ready for use

Preparation of plant extracts

Leaves of *S. alata* were dried, ground and weighed into 10, 20, 30, 40 and 50g. Each sample was added to 100ml of varied concentrations of ethanol: 10, 20, 30, 40 and 50%. The mixtures were filtered with a four-fold cheese cloth and the filtrates were used to poison the Potato Dextrose Agar (PDA) prior to inoculation.

Isolation of fungal organisms from rotten yam

Infected yam tubers were washed in a running tap water, rinsed in sterile distilled water, sterilized with 70% ethanol to remove external contaminant. Sections of yam tubers were cut with sterilized scalpel, surface sterilized with 30% ethanol, rinsed with several changes of sterile distilled water and were plated on (PDA), the plated dishes were incubated at room temperature and observed daily for 5 days for fungal growth after which pure cultures were taken and identified according to [4] and stored in slant using Mc Cartney bottles for pathogenicity test and antifungal studies

Pathogenicity test

Healthy yam tubers were surface sterilized with 0.1M of Mercuric chloride ($HgCl_2$) for 1 minute and washed in five changes of distilled water. Five millilitres cork-borer was punched to a depth of 4mm into the healthy yam tubers and the bored tissues were removed. Five (5) mm diameter disc of pure isolate was cut and placed back into hole created in the yam tuber. The wound was sealed with prepared paraffin wax according to the method of [6]. The control was set up in the same manner except that sterile agar disc was used instead of the fungal inoculum. The inoculated yam tubers were placed in four replications at room temperature (28±20C) under sterile condition. The pathogens were re-inoculated into yam tubers, observed for disease development, identified on the basis of morphology and observed culture characteristics were compared with structures in Snowdon, (1990).

Effect of *S. alata* ethanolic leaves extract on mycelia extension of isolated rot fungi of yam tubers

The method of [9] was adopted to determine the effect of leaves extract on mycelia extension of the fungi. This was obtained by placing one disc (3mm diameter) of 5-days-old culture of the pathogens in each of three Petri dishes (1cm diameter) with 170ml PDA medium and 1ml leaf extract. The control experiments were set up with 1ml of sterile distilled water. Three replicates per isolate were incubated at room temperature (28±2 0C) for 7days. Daily measurements of the mycelia extension of the cultures were determined along two diameters. Mycelia growth inhibition was taken as growth of the fungus on the ethanolic leaf extract expressed in percentage of growth. Fungitoxicity was determined in form of percentage growth of colony inhibition and calculated according to this formula:

$$\text{Growth inhibition (\%)} = \frac{dc-dt}{dc} \times \frac{100}{1}$$

Where dc = Average diameter of colony with control
dt = Average diameter of colony with treatment

RESULTS

Forty grams of *S. alata* in 10% ethanolic concentration had 95.83% inhibition on *B. theobromae* while the least inhibition was 50g (93.23%), 40g of 10% ethanolic extracts concentration of *S. alata* had 94.90% inhibition on *A. flavus* while the least being 50g (79.03%). Also, both 30 and 40g of 10% ethanolic extracts concentration of *S. alata* had 95.23% inhibition on *A. glaucus* and the least was 50g (92.10%). The result indicated that concentrations of 30 and 40g in 10% ethanol had high inhibitory value against *B. theobromae*, *A. flavus,* and *A. glaucus* (Table 1).

Table I: Percentage inhibition of mycelia radial growth (mm) of rot organisms grown on poisoned PDA with varied concentrations of 10% ethanolic extracts of *S. alata*

10% Ethanolic extract (g/100ml)	% inhibition of mycelia growth (mm)			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10g	95.70a	94.33b	93.87a	91.77a
20g	94.00a	93.43b	93.77a	96.83b
30g	95.17a	93.50b	95.23a	93.13ab
40g	95.83a	94.90b	95.23a	96.43ab
50g	93.23a	79. 03a	92.10a	96.43ab
Control	0.00	0.00	0.00	0.00

DMRT was used to separate means. Means followed by the same alphabet(s) in the same column are not significantly different (p=0.05)

Table 2 shows that thirty grams of 20% ethanolic extracts concentration of *S. alata* had 97.10% inhibition on *B. theobromae* and the least was 50g (95.47%), 40g of 20% ethanolic extracts concentration of *S. alata* had 89.63% inhibition on *A. flavus* and the least being 10g in 20% ethanolic (82.03%). Also, 50g of 20% ethanolic extracts concentration of *S. alata* had 95.97% inhibition on *A. glaucus* with the least being 30g (84.07%), 50g in 20% ethanolic extracts concentration of *S. alata* had 96.27% inhibition on *A. niger* and the least being 10g (92.27%). The result indicated that a concentration of 50g in 20% ethanolic was the most inhibitive on *A. glaucus* and *A. niger* while *B. theobromae* and *A. flavus* was most inhibited by 30 and 40g in 20% ethanolic respectively.

Table II: Percentage inhibition of mycelia radial growth (mm) of rot organism grown on poisoned PDA with varying concentrations of 20% ethanolic extracts of *S. alata.*

20% Ethanolic extract (g/100ml)	% inhibition of mycelia growth (mm)			
	B. Theobromae	*A. Flavus*	*A. Glaucus*	*A. Niger*
10g	96.77a	82.03a	94.47b	92.27a
20g	95.63a	85.17ab	95.43b	95.77a
30g	97.10a	82.93ab	84.07a	93.27a
40g	97.07a	89.63b	94.87b	94.53a
50g	95.47a	84.53ab	95.97b	96.27a
Control	0.00	0.00	0.00	0.00

DMRT was used to separate means. Means followed by the same alphabet in the same column are not significantly different (p=0.05)

Twenty grams of 30% ethanolic extracts concentration of *S. alata* had 96.03% inhibition on *B. theobromae* and the least being 50g (90.83%.), 20g in 30% ethanolic had 100% inhibition on *A. flavus* and the least being 50g (92.60%). Also, both 30 and 50g in 30% had 96.57% inhibition on *A. glaucus* and the least being 20g (94.67 %). The result indicated that concentration of 20g in 30% ethanol had the highest inhibitory effect on the fungi but with slight change in *A. glaucus* (Table 3).

Table III: Percentage inhibition of mycelia growth (mm) of rot organisms grown on poisoned PDA with varying concentrations of 30% ethanolic extracts of *S. alata*.

30% Ethanolic extract (g/100ml)	% inhibition of mycelia growth (mm)			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10g	94.20ab	96.37abc	96.33a	93.77bc
20g	96.03b	100.00c	94.67a	96.03c
30g	91.13a	95.97abc	96.57a	96.00c
40g	92.40ab	97.83bc	95.53a	85.87a
50g	90.83a	92.60a	96.57a	91.00b
Control	0.00	0.00	0.00	0.00

DMRT was used to separate means. Means followed by the same alphabets in the same column are not significantly different (p=0.05)

Table 4 shows that fifty grams of 40% ethanolic extracts concentration of *S. alata* had 97.87% inhibition on *B. theobromae* while the least was 30g (68.57%.), both 30 and 40g of 40% ethanolic extracts concentrations of *S. alata* had 100% inhibition on *A. flavus* and the least being 20g (97.60%). Also, 20g of 40% ethanolic extracts concentration of *S. alata* had 95.80% inhibition on *A. glaucus* while the least being 30g and 40g (54.67%.), 20g of 40% ethanolic extracts concentration of *S. alata* had 97.47% inhibition on *A. niger* and the least was 30g (50.47%). The result indicated that a concentration of 20g in 40% ethanolic had high inhibitory value on *A. niger* and *A. glaucus* while *B. theobromae* and *A. flavus* were inhibited by 30, 40 and 50 g.

Table IV: Percentage inhibition of mycelia growth (mm) of rot organisms grown on poisoned PDA with varied concentrations of 40% ethanolic extracts of *S. alata.*

40% Ethanolic extract (g/100ml)	% inhibition of mycelia growth (mm)			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10g	91.50bc	97.63a	95.13b	97.03c
20g	89.20bc	97.60a	95.80b	97.47c
30g	68.57a	100.00a	54.67a	50.47a
40g	84.97abc	100.00a	54.67a	78.43b
50g	97.83c	99.63a	95.76b	79.70bc
Control	0.00	0.00	0.00	0.00

DMRT was used to separate means. Means followed by the same alphabets in the same column are not significantly different (p=0.05)

Table 5 shows that thirty grams of 50% ethanolic extracts of *S. alata* had 89.40% inhibition on *B. theobromae* while the least was 40g (60.60%.). All the varied weight of extracts in 50% ethanolic extracts concentration of *S. alata* had 100% inhibition on *A. flavus*. Also, 40g of 50% ethanolic extracts concentration of *S. alata* had 100.00% inhibition on *A. glaucus* while 20 and 30g inhibited the least (54.67%), 10g in 50% ethanolic extracts concentration of *S. alata* had 87.70% inhibition on *A. niger* while the least being 30g (60.77%).

Table V: Percentage inhibition of mycelia growth (mm) of rot organisms grown on poisoned PDA with varied concentrations of 50% ethanolic extracts of *S. alata*.

50% Ethanolic extract (g/100ml)	% inhibition of mycelia growth (mm)			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10g	75.30a	100.00a	76.33b	87.70a
20g	70.80a	100.00a	54.67a	65.63a
30g	89.40a	100.00a	54.67a	60.77a
40g	60.60a	100.00a	100.00b	73.10a
50g	84.20a	100.00a	87.43b	76.20a
Control	0.00	0.00	0.00	0.00

DMRT was used to separate means. Means followed by the same alphabet in the same column are not significantly different (p=0.05)

DISCUSSION AND CONCLUSION

Isolated organisms: *B. theobromae, A. flavus, A. niger and A glaucus* were found associated with rot of *D. rotundata* in the present study. Association of these organisms with post harvest rots of yam tubers had been reported by [8],[10]. Forty and fifty grams concentrations were most active in 10% ethanol while twenty grams in both 30 and 40% ethanol were found active as well as 10g in 50% ethanolic concentration rated as the most inhibitive concentrations. It can be established that *Senna alata* is potent against rot organisms of yam tubers, and it has been found very active in controlling post harvest dry rot of yam (*Dioscorea rotundata* Poir).

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