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Efficacy of plant leaf extracts on the mycelial growth of kolanuts storage pathogens, *Lasiodioplodia theobromae* and *Fusarium pallidoroseum*

S. O. Agbeniyi[1]* and M. S. Ayodele[2]

[1]Cocoa Research Institute of Nigeria, P. M. B. 5244, Ibadan, Oyo State, Nigeria.
[2]Department of Biological Sciences, University of Agriculture, Abeokuta, Ogun State, Nigeria.

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The efficacy of leaf extracts of five plant species namely: *Glyricidia sepium* (Jacq.) Linn, *Tectona grandis* Linn. *Ocimum gratissimum* Linn. *Anacardium occidentales* Linn. and Carica *papaya* Linn. against storage fungi *Lasiodioplodia theobromae* and *Fusarium pallidoroseum* was evaluated. The potency of these leaf extracts after storage at ambient temperature for 15 and 30 days, respectively was also tested on the radial growth of *L. theobromae* and *F. pallidoroseum*. The results indicate that leaf extracts from *O. gratissimum* and *A. occidentales* are effective in inhibiting the radial growth of *L. theobromae* and *F. pallidoroseum,* respectively. *O. gratissimum* even at 2.5% concentration gave 35.89% mycelial growth inhibition of *L. theobromae* and 10% concentration gave 50.3% mycelial growth inhibition after five days. The extract of *C. papaya* exhibited less antifungal activity than either *O. gratissimum* or *A. occidentales.* Generally, with the exception of *C. papaya* leaf extract, there was no significant difference (P = 0.05) between the fresh leaf extract and the stored extracts in the inhibition of the mycelial growth of either *L. theobromae* or *F. pallidoroseum and the* potency of the leaf extracts was retained even after 30 days of storage at ambient temperature.

Key words: Leaf extracts, kolanuts, storage pathogens, mycelial growth.

INTRODUCTION

Kolanuts are widely cultivated in West Africa, where they are used as stimulants to counteract fatigue, suppress thirst and hunger, and are believed to enhance intellectual activity (Nickalls, 1986). In addition the nuts are exported to Europe and North America, where they are used chiefly as flavouring agents (Oludemokun, 1982). Disease incidence during storage is a major post harvest problem that farmers and kolanut traders seek to solve. The major post harvest pathogens in west Africa for the nut are *Lasiodiplodia theobromae* and *Fusarium* pallidoroseum (Agbeniyi, 2004). *L. theobromae* is a ubiquitous pathogen of tropical woody trees reported to cause shoot blight and dieback of many plant species including black branch and dieback disease of cashew. It has also been reported to cause gummosis of *Jatropha podagrica* (Fu et al., 2007).

F. pallidoroseum has also been implicated as causative pathogen of brown rot disease of kolanuts. Presently, the only control strategy practice by farmers is to remove diseased nut at intervals during the storage period. The use of chemical fungicides is not desirable due to health hazard on the consumers. Plants extracts have previously been used successfully to control other plants diseases in plants (Alkhail, 2005) and they could as well

*Corresponding author. E-mail: remiagbeniyi@yahoo.com.

be employed in the control of kolanuts storage rot. This strategy has however not been explored. The present study was initiated to elucidate the efficacy of *Glyricidia sepium* (Jacq.) Linn, *Tectona grandis* Linn. *Ocimum gratissimum* Linn. *Anacardium occidentales* Linn. and *Carica papaya* Linn. leaf extracts on suppression of the the mycelial growth of kolanut storage pathogens, *L. theobromae* and *F. pallidoroseum in vitro.*

MATERIALS AND METHODS

The sources of the plant leaf extracts was *G. sepium* (Jacq.), *T. grandis* Linn, *O. gratissimum* Linn. *A. occidentales* Linn. and *Carica papaya* Linn. Fresh leaves of *G. sepium, T. grandis, O. gratissimum, A. occidentales* and *C. papaya* were washed with tap water and surface sterilized by soaking them for 60 s in 1% sodium hypochlorite (NaOCl) and later rinsed with sterile distilled water. The leaves mere separately crushed with mortar and pestle in distilled water (w/v 25 g/100 ml) and filtered through muslim cloth (Pandey et al., 1982). The crude extract of each plant leaf was then stored in the laboratory at ambient temperature 28 ± 2°C.

The poisoned techniques described by Nene and Thaphiyal (1979) and Tewari and Nayak (1991) were adopted to study the effect of plant leaf extracts on the radial growth of *L. theobromae* and *F. pallidoroseum.* Each plant extract was evaluated at varying concentration: 2.5, 5.0, 7.5 and 10% concentrations (v/v). 2 ml of each of the extract was added to 15 ml sterilized cooled potato dextrose agar (PDA) in 9 cm Petri dishes. Mycelial discs of 8 mm diameter were cut from the periphery of 5-day-old actively growing cultures of *L. theobromae* and *F. pallidoroseum* using sterile cork borer. Each disc was placed in the centre of Petri dishes containing the treated medium. Three replications were maintained for each concentration. Plates without plant extracts were set up to serve as negative controls. The inoculated plates were incubated at 25°C. Radial growth of the colony in each plate was recorded on the third, fifth and seventh day after inoculation by measuring the diameter of the colony along two perpendicular axes. The average of two measurements was taken as the colony diameter (Raghu and Mohanan, 1997). The percent inhibition of *L. theobromae* and *F. pallidoroseum* was calculated by the equation given by Raghu and Mohanan (1997):

$$I = \frac{C-T}{C} \times 100$$

Where, I = Inhibition of fungal growth; C = growth in control, and T = growth in treatment.

Evaluation of storage duration of leaf extracts and inhibitory effect on mycelial growth of isolates

The plant leaf extracts were stored in round-bottom flask at ambient temperature (28±2°C). Each plant extract was stored in two set of flasks, one set stored for 15 days and another set for 30 days at room temperature. After the end of storage period, the extracts were tested for their effect on the mycelia growth of *L. theobromae* and *F. pallidoroseum.*

Three replications were maintained for each concentration 2.5, 5.0, 7.5 and 10.0%. The plates without leaf extracts served as control. The inoculated plates were incubated at 25°C. Radial growth of the colony in each plate was recorded on the third, fifth and seventh day after inoculation along two perpendicular axes. The average of two measurements was taken as the colony diameter. The percentage inhibition of mycelia growth of *L. theobromae* and *F. pallidoroseum* was calculated as described subsequently.

RESULTS AND DISCUSSION

All the plant leaf extracts evaluated inhibited the mycelial growth of the fungi which proves the antifungal property of the leaf extracts even at lower concentration of 2.5%. For instance, the mycelial growth inhibition of *L. theobromae* at 2.5% for each of the leaf extract ranged between 17.8 to 43.5% after three days, 9.4 to 28.3% after five days and 17.6 to 35.8% after seven days (Table 1). The percentage inhibition of *L. theobromae* in the presence of each of the fresh leaf extract is given in Table 1. Similarly the percent inhibition of *F. pallidoroseum* in the presence of each of the fresh leaf extract at different concentrations is presented in Table 2. Among the leaf extracts of five plants screened, extract of *O. gratissimum* and *A. occidentales* were very effective in inhibiting the growth of *L. theobromae* and *F. pallidoroseum,* respectively. *O. gratissimum* extract even at five percent concentration caused 33.8% mycelial growth inhibition of *L. theobromae* and at ten percent concentration gave 59.3% mycelia growth inhibition in *L. theobromae* after five days (Table 1). This study revealed that antifungal compounds were present in the five leaf extracts screened since they were able to suppress the growth of the microorganisms tested.

The performance of *A. occidentales* was better than that of *O. gratissimum* in the mycelial inhibition of *F. pallidoroseum*. Whereas *O. gratissimum* gave the highest percent mycelial inhibition in *L. theobromae* culture, the mycelia of *F. pallidoroseum* were more sensitive to the extract of *A. occidentales.* For example, at five percent concentration, *A. occidentales* extract caused 34.4% mycelial growth inhibition in *F. pallidoroseum* compared to 24.0% mycelial inhibition obtained in *O. gratissimum* extract after five days (Table 2). There was no significant difference (P = 0.05) between the leaf extract of *T. grandis* and *O. gratissimum* in the inhibition of *F. pallidoroseum* in culture (Table 2).

The extract of *C. papaya* exhibited less antifungal activity than either *O. gratissimum* or *A. occidentales.* For instance, *C. papaya* extract at 5 percent concentration gave 19.7% mycelial growth inhibition of *L. theobromae* and 12% inhibition of *F. pallidoroseum* after five days. Amadioha (1998) also reported that leaf extracts of *C. papaya* was effective in inhibiting the growth of powdery mildew fungus (*Erysiphe cichoracerarum*) *in vitro.* When *C. papaya* extract was tested at 10% concentration, there was no significant difference (P = 0.05) between its performance and *A. occidentales* in the mycelial inhibition of *L. theobromae* (Table 1). However, there was a significant difference between the performance of *C. papaya* extract and *A. occidentales* in the mycelial

Table 1. Percentage inhibition of mycelial growth of *L. theobromae* at different concentrations of leaf extract.

Plant leaf extracts	3rd day				5th day				7th day			
	Concentration (%)				Concentration (%)				Concentration (%)			
	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
G. sepium	43.5	48.9	52.0	57.1	25.1	28.1	33.9	36.9	24.1	40.7	44.4	44.4
T. grandis	39.4	43.1	43.5	45.1	21.7	30.6	30.6	31.4	43.5	20.2	36.4	42.8
A. occidentals	17.8	20.3	21.3	44.5	9.4	10.2	16.4	21.4	17.6	19.3	24.9	32.2
O. gratissimum	35.4	51.9	51.7	62.1	29.3	33.8	36.9	59.3	35.8	35.8	44.8	53.3
C. papaya	28.9	46.6	46.6	48.9	9.7	19.7	25.3	33.1	24.1	32.66	33.0	33.7
LSD (0.05)	8.2				6.2				6.6			

Data are means of 3 replicates.

Table 2. Percentage inhibition of mycelial growth of *F. pallidoroseum* at different concentrations of leaf extract.

Plant leaf extracts	3rd day				5th day				7th day			
	Concentration (%)				Concentration (%)				Concentration (%)			
	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
G. sepium	12.6	16.9	23.5	28.9	22.8	23.2	27.2	32.4	25.7	32.4	38.2	39.8
T. grandis	10.9	27.9	28.9	31.1	8.0	15.2	33.2	34.8	12.5	22.3	33.7	38.5
A. occidentals	2.7	10.4	23.5	27.3	28.8	34.4	44.0	46.8	22.5	28.9	44.6	49.6
O. gratissimum	20.8	31.7	36.6	41.5	7.2	24.0	30.8	41.2	18.6	23.16	23.1	38.2
C. papaya	4.4	13.7	23.5	26.2	3.0	12.0	28	38	11.1	19.1	30.5	37.2
LSD(0.05)	4.3				7.3				4.3			

Data are means of 3 replicates.

inhibition of *F. pallidoroseum* (Table 2). Further study on the chemical composition of the leaf extracts is recommended.

There was no significant difference (P = 0.05) between the leaf extracts of *G. sepium* and *T. grandis* in mycelial inhibition of *L. theobromae* after seven days (Table 1). However, *A. occidentales* even at 2.5% concentration performed significantly better in the inhibition of growth of *F. pallidoroseum* compared to *T. grandis, G. sepium* or *O. gratissimum,* respectively (Table 2). This trend was observed at five percent and ten percent concentrations. The results presented in Tables 1 and 2 established the sensitivity of *L. theobromae* or *F. pallidoroseum* to the plant leaf extracts None of the extract was found to exhibit stimulatory effect on the mycelial growth of either *L. theobromae* or *F. pallidoseum. O. gratissimum* at ten percent concentration exhibited more than 50% inhibition of mycelial growth of *L. theobromae* after five days (Table 1). However, Shafique et al. (2007) reported that the toxicity of the extracts against a particular fungal species varied with the test plant species. This study also reported different results for each of the leaf extract. Chemical analysis of the leaf extracts will elucidate the differences in the performance of the leaf extracts.

Similarly, *A. occidentales* extracts at ten percent concentration caused 46.8% inhibition of mycelial growth of *F. pallidoroseum* after five days. The percentage mycelial growth inhibition of *F. pallidoroseum* at 2.5 percent for each of the leaf extract ranged between 2.7 and 20.8% after three days, 7.2 and 28.8% after five days and 11.1 to 25.7% after seven days (Table 2). *T. grandis* at ten percent concentration also caused 43.5% inhibition of mycelial growth in *L. theobromae* after five days.

All the leaf extracts of the five plants after fifteen days of storage at ambient temperature of 28 ± 2°C inhibited the mycelial growth of *L. theobromae* (Table 3) and *F. pallidoroseum* (Table 4). The same trend was observed when the leaf extracts were stored for 30 thirty days (Tables 5 and 6). The antifungal activity of the extracts did not decrease with the period of storage at either fifteen or thirty days. However, the antifungal activity of *A. occidentales* against *L. theobromae* decreased with the period of storage at either fifteen days (Table 3) or thirty days (Table 5). Thus if extract must be stored, refrigeration of the leaf extract is recommended to maintain their efficacy during storage. Similarly, antifungal activity of the extract of *C. papaya* against mycelial of *F. pallidoroseum* decreased after fifteen days (Table 4) and thirty days (Table 6) of storage.

The fresh extract of *C. papaya* at ten percent concentration caused 37.2% mycelial inhibition of *F. pallidoroseum* (Table 2), when stored for either fifteen days or thirty days, it caused 20.5% (Table 4) and15.9% (Table 6) mycelial inhibition, respectively.

Table 3. Percentage inhibition of mycelial growth of *L. theobromae* at different concentrations of leaf extract stored for 15 days.

Plant leaf extracts	3rd day				5th day				7th day			
	Concentration (%)				Concentration (%)				Concentration (%)			
	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
G. sepium	45.2	47.3	52.5	54.2	25.5	28.3	35.3	37.4	23.3	39.7	43.8	44.9
T. grandis	37.5	40.2	42.1	45.8	22.1	31.3	31.9	44.6	18.8	35.6	41.7	44.4
A. occidentales	18.8	18.8	20.4	43.1	11.0	14.3	18.1	38.6	16.9	18.8	24.4	30.7
O. gratissimum	52.7	65.2	69.5	71.8	25.9	34.1	40.7	56.0	30.7	34.8	43.0	54.8
C. papaya	29.2	31.5	41.7	44.4	4.9	18.8	23.1	29.7	16.5	29.0	29.6	34.8
LSD (0.05)	7.1				6.2				5.8			

Data are means of 3 replicates.

Table 4. Percentage inhibition of mycelial growth of *F. pallidoroseum* at different concentration of leaf extract stored for 15 days.

Plant leaf extracts	3rd day				5th day				7th day			
	Concentration (%)				Concentration (%)				Concentration (%)			
	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
G. sepium	15.3	24.7	26.8	36.3	14	23.2	35.6	40	27.7	35.1	38.5	41.0
T. grandis	13.1	30.0	33.7	35.8	12.4	17.2	30.8	37.2	16.4	24.9	33.3	38.5
A. occidentales	3.2	12.1	23.2	28.9	12.8	13.2	31.6	33.2	22.3	30.7	46.9	50.8
O. gratissimum	14.2	32.6	40.0	41.6	10.2	20.0	24	29.2	17.7	30.3	37.9	39
C. papaya	2.1	2.6	6.8	12.1	2.8	9.2	13.2	21.2	2.5	9.5	14.6	20.5
LSD (0.05)	6.4				5.5				4.9			

Data are means of 3 replicates.

Table 5. Percentage inhibition of mycelial growth of *L. theobromae* at different concentrations of leaf extracts stored for 30 days.

Plant leaf extracts	3rd day				5th day				7th day			
	Concentration (%)				Concentration (%)				Concentration (%)			
	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
G. sepium	43.6	44.7	50.6	56.4	25.1	32.5	34.1	36.7	27.8	38.9	43.8	45.1
T. grandis	36.2	37.2	39.4	45.3	20.2	30.5	32.3	40.5	21.9	37.4	42.6	44.8
A. occidentales	14.5	18.5	18.5	41.9	11.1	14.8	20.0	38.2	16.7	22.2	30.0	31.9
O. gratissimum	29.8	51.1	53.8	69.8	24.6	32.8	40.7	58.5	29.2	35.3	43.3	54.4
C. papaya	9.2	29.1	44.7	48.3	18.9	22.9	34.4	39.8	23.1	29.76	31.4	34.4
LSD (0.05)	11.5				6.8				6.2			

Data are means of 3 replicates.

Table 6. Percentage inhibition of mycelial growth of *F. pallidoroseum* at different concentrations of leaf extract stored for 30 days.

Plant leaf extracts	3rd day				5th day				7th day			
	Concentration (%)				Concentration (%)				Concentration (%)			
	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
G. sepium	17.1	24.5	27.5	40.9	17..9	28.8	41.6	43.2	25.1	32.9	37.3	42.6
T. grandis	15.5	29.0	31.1	37.8	11.7	21.0	27.2	40.8	15.1	20.9	29.5	38.6
A. occidentales	5.2	20.7	23.8	31.6	13.2	15.6	31.1	34.6	19.8	29.5	43.3	49.6
O. gratissimum	8.3	31.6	40.4	43.0	6.6	9.3	19.5	29.9	13.1	25.1	36.6	37.4
C. papaya	5.2	6.7	8.3	15.5	5.4	7.8	14.4	22.2	3.4	6.8	9.7	15.9
LSD (0.05)	7.6				4.8				6.4			

Data are means of 3 replicates.

Generally, with the exception of *C. papaya* leaf extract, there was no significant difference (P = 0.05) between the fresh leaf extract and the stored extracts in the inhibition of the mycelial growth of either *L. theobromae* or *F. pallidoroseum*.

The leaf extract of *O. gratissimum* demonstrated strong inhibitory effect even after thirty days of storage (Table 5). Similarly, *T. grandis* and *G. sepium* leaf extracts retained their antifungal properties against *L. theobromae* and *F. pallidoroseum* when stored for either fifteen or thirty days. It is evident from the results presented in Tables 5 and 6 that the concentration of each of the tested extract against *L. theobromae* was maintained during the period of storage.

Conclusion

The use of plant leaf extracts of *G. sepium* (Jacq.) Linn, *T. grandis* Linn. *O. gratissimum* Linn. *A. occidentales* Linn. and *C. papaya* Linn. for the control of kolanut storage disease would be seen as a practical solution to the problem encountered by kola farmers and traders during storage of nuts. Also it would be seen as a positive response to public concern about the adverse effects of the use of pesticides on human health and on the environment.

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