EVALUATION OF ANTIMICROBIAL POTENTIALS OF SELECTED BOTANICALS AGAINST KOLANUT STORAGE ROT PATHOGEN (*LASIODIPLODIA THEOBROMAE)* IN NIGERIA

[1]Ogundeji, B. A., [1]Orisajo, S. B., [1]Olorunmota, R. T., [2]Oduwaye, O. F., [1]Oyedokun, A. V. and [1]Agbeniyi, S. O.
[1]Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Nigeria.
[2]Institute of Agricultural Research and Training, Ibadan, Nigeria.

SUMMARY

Stored kolanuts are predisposed to infection by *Lasiodiplodia theobromae* sequel to infestation and/or attack by kolanut weevils (*Sophrorhinus* spp. and *Balanogastris* sp.) from field to store. The effectiveness of varying aqueous/ethanolic leaf extracts concentrations of *Azadirachta indica, Piper guineense, Lantana camara, Eucalyptus sp.* And fruit peels of *Citrus sinensis* against mycelia growth and pycnidia production of *L. theobromae* (causative agent of kolanut storage rot) was determined using poisoned food technique. Percentage mycelia inhibitions by extracts of *A. indica, P. guineense, L. camara, Eucalyptus* and *Citrus* peels within 24 hours of incubation ranged between 26.09-33.05%, 33.05-51.31%, 45.22-60.87%, 55.65-64.35%, and 52.18-67.83%, respectively. Contrary to observations in other extracts, higher percentages of the pathogen's mycelia inhibition were generally recorded for concentrations of *Eucalyptus, Citrus* and the standard check/positive control (mancozeb) after 48 hours of incubation. However, the mycelia inhibitions noticeably reduced at 72 hours of incubation. The study also revealed a decrease in the number of pycnidia produced by the pathogen as the extracts' concentrations increased. The reduction in the number of pycnidia produced by the pathogen varied with individual treatments in the following order, *Eucalyptus*>*Citrus* peel>*Lantana*>*Piper*>Neem. *Lantana*, *Eucalyptus* and *Citrus* extracts competed favourably with the standard check in the inhibition of the pathogen's mycelia growth. *P. guineense* and *L. camara* extracts which were comparable to mancozeb, induced higher inhibitory effects on both mycelia growth and pycnidia production. Hence, could be used for effective control of kolanut storage rot.

Keywords: Kolanut, Storage rot, Incubation, Inhibition, Pycnidia, Extracts

COLA species (Family: *Sterculiaceae*) are native to the tropical rainforests of Africa, West Indies, and Brazil. The kola tree reaches heights of between 40-60 feet. Out of the twenty-five species known to exist, *Cola nitida* and *C. acuminata* are the two most commonly cultivated and suitable for human consumption (Opeke, 1992). *C. nitida* is considered to be indigenous to the forest area of Cote d' Ivoire and Ghana, where it was originally distributed along Africa's West Coast from Sierra Leone to Dahomey. Cultivation of this kola specie in Nigeria must have started long before 1900.The original distribution area of *C. acuminata* stretches from Nigeria to Gabon. It is still cultivated in South Eastern Nigeria, the South South and in the Middle Belt states. However, in Southwestern Nigeria, its cultivation has been replaced by that of *C. nitida* (Ndubuaku *et al.*, 2015) Nuts of these two *Cola* spp. are commercial export commodities for the production of kola-chocolate, liquors and laxatives. The presence of alkaloids such as caffeine, kolanin and theobromine make kolanuts useful for pharmaceutical purposes (Atanda *et al*., 2011). The caffeine containing nut is used also as a flavouring ingredient in beverages and that is the origin of the term *cola* (Greenwood, 2016).

Maturity of kola fruits usually takes place 4-5 months after pollination, with characteristic change in colour from deep green to a paler tint (at a time the fruit should be harvested), as the follicles will start to dehisce thereafter. Exposed seeds are more prone to insect attack and subsequently predispose the nuts to fungal infection. Numerous toxic metabolites are produced in mould-infected stored kola nuts as can be found in other mould-contaminated foods. When such nuts are consumed, they pose a huge health risk to the consumers (Opeke, 1992; Ndubuaku, 2015; Jimenez, 1991).

Due to the warm, humid rainforest zone in which cultivation, processing and storage of the kola nuts take place, there is high risk of mould infection. Most traders as a result, labour assiduously to maintain the freshness of the nuts, prevent fungi growth and insect attack that predisposes *Cola* to rot infection in storage. This requires removal of infested nuts at intervals during the storage period, but this does not control fungi which spread rapidly in the nuts. Most traders and consumers do not discard fairly mouldy nuts during storage, leading to infection of more hitherto healthy nuts and a huge loss in the long-run (Atanda *et al*., 2011).

Diseases associated with stored kolanuts include dry rot, grey mould and black rot. Storage rot caused by *Lasiodiplodia theobromae* is a serious post-harvest disease of kolanut (Agbeniyi 1998). This fungus, with some other storage moulds, causes discolouration, shrinking, rottening and physiological alterations in kolanut. These subsequently cause defects which seriously depreciate the commercial value of the nuts (Agbeniyi, 2014).

In a bid to control the incidence of storage-induced kolanut rot/spoilage, most of the individuals trading in the commodity have resorted to the use of synthetic chemicals, which though very effective, is costlier and constitute some high level health hazards to both the handlers and consumers of the commodity (Mokwunye and Oluyole, 2017). There is therefore, the need to shop for much safer, ecologically friendly and cheaper alternatives for the management of kola nut storage disease.

MATERIALS AND METHODS

The effectiveness of leaf extracts of *Azadirachta indica*, *Piper guineense*, *Eucalyptus camaldulensis*), *Lantana camara* and *Citrus sinensis* peel against mycelia (vegetative) growth and pycnidia production of kolanut rot pathogen, *L. theobromae,* was determined using poisoned food technique.

One hundred (100) grammes of air-dried leaf samples of *Azadirachta indica*, *Piper guineense*, *Eucalyptus camaldulensis* and *Citrus sinensis* peel were separately ground and soaked in 1000ml of sterile distilled water for about 24 hours. The liquid extracts of each of the botanicals were then filtered from the soaked samples using sterile muslin clothes and collected into sterile conical flasks to serve as stock solutions. Also, air dried leaves of *Lantana camara* were ground, and about 50g of the ground leaves were extracted with 333ml absolute ethanol for 24hours on a rotary shaker at 25rpm and then allowed to stand for 5hours. The extract was filtered and the residue re-extracted with ethanol as before. The two filtrates were combined and the extractant (ethanol) completely evaporated. Twenty (20) grammes of the extract (paste) were re-constituted in 200ml of sterile distilled water in sterile conical flask as the aqueous extracts (Snehali and Mohammed, 2014). Ten (10), 20, 30 and 40% concentrations of each of the five extracts were prepared from their stock solutions and pour-plated with freshly sterilized but cooled (45^{o}C) potato dextrose agar (PDA). Synthetic fungicide (mancozeb 80WP) solution was prepared at the manufacturer's recommended rate of 0.5g/100ml and separately pour-plated with PDA to serve as standard check/positive control. The freshly prepared PDA was also poured into another set of sterile Petri dishes containing neither extracts nor chemicals to serve as untreated/negative control.

The poisoned as well as control plates were inoculated with agar discs (8mm diameter) of the kola rot pathogen cut with the aid of a sterile cork borer. All the plates were incubated at 28-32^{o}C and the mycelia growth diameter of the inoculated fungus in each of the plates was measured every 24hours using transparent ruler until the negative control plates were completely covered. Percentage inhibitions of the pathogen's mycelia growth were calculated using the formula:

Percentage inhibition (%) = $\frac{Dc-Dt}{Dt} x100$

Where: D_c = Mycelia growth diameter in control

D_t = Mycelia growth diameter in treatment

Each of the treatments was replicated thrice in a completely randomized design.

At the tenth day of incubation, pycnidia structures produced on each of the treated plates and their controls were counted and recorded. Data obtained were subjected to analysis of variance using Statistical Analysis Software (SAS) 9.1 package.

RESULTS

Percentage mycelia growth inhibitions produced by extracts of *A. indica, P. guineense, L. camara, Eucalyptus* and *Citrus* peels within 24 hours of incubation ranged between 26.09-33.05%, 33.05-51.31%, 45.22-60.87%, 55.65-64.35%, and 52.18-67.83% respectively (Table 1).

At 24 hours after incubation, the four extract concentrations of *Eucalyptus* as well as 20-40% concentrations of *Citrus* extract and 10% *Lantana* gave significantly highest mycelia inhibitions (P≤0.05) against *L. theobromae*and better than the standard check (mancozeb).These were closely followed by 20% *Lantana,* Mancozeb*,* 10% *Citrus* and 40% *P. guineense* which produced 53.74, 53.48, 52.18, and 51.31% mycelia growth inhibitions, respectively, while the four extract concentrations of *Azadirachta* and 10% *P. guineense* gave the least inhibitions (Table 1). The situation was more or less the same at 48 hours after incubation, but with the standard check (Mancozeb) producing the highest inhibition at this instance. The chemical also gave the significantly highest mycelia inhibition, 53.69% (P≤0.05),at 72 hours of incubation, followed by 20-40% *Citrus* extracts, 30 and 40% *Eucalyptus*, 10 and 30% *Lantana* and 40% *P. guineense.* The four extract concentrations of *Azadirachta* as well as 10-30% *P. guineense*, 20% *Lantana*, 10 and 20% *Eucalypus* and 10% *Citrus* produced significantly lowest inhibition values (P≤0.05) (Table 1).

Virtually all the extract concentrations with the exemption of 10 and 20% *Azadirachta*, 10-40% *P. guineense,* 30% *Lantana* and 30 and 40% *Eucalyptus* showed noticeable reductions in the mycelia growth inhibitions at 48 hours after incubation when compared with their respective values, 24 hours earlier. A similar trend was noticed in the positive control plates (Table 1).

Average number of pycnidia induced by *Azadirachta, P. guineense, Lantana*, *Eucalyptus* and *Citrus* ranged between 10-24, 3-28, 9-34, 8-51, and 8-48, respectively, while an average of 36 pycnidia were observed in the negative control plates (Table 1). The number of pycnidia produced by the pathogen decreased as the concentration of each of the extracts increased. *Eucalyptus* at 10%concentrationinduced the highest average number of pynidia (51), followed by 20% concentration of the extract (48), and 10% *Citrus* (48). The positive control plates showed no pycnidia growth, while *P. guineense* at 40% concentration closely followed with an average number of 3 pycnidia (Table 1).

Highest overall inhibitions (58%) were produced by mancozeb, and closely followed by *Citrus* (49.17%), *Eucalyptus* (49.13%) and *Lantana* (42%), while *Azadirachta* (26.6%) gave the least inhibition against the pathogen (Figure 1).

Table1: Inhibitory effects of plant extracts on mycelia growth and sporulation of *L. theobromae*

Extract Conc. (%)	Mycelia growth inhibition (%) at: 24HAI	48HAI	72HAI	Average no. of pycnidia
NL				
10	29.59fg	34.93f-h	23.93c	24.00
20	26.09g	26.83h	21.57c	21.00
30	33.05e-g	28.60gh	22.94c	17.00
40	26.96g	23.54h	21.18c	10.00
IY				
10	33.05e-g	38.98fg	24.71c	28.00
20	40.87d-f	47.34c-f	26.28c	23.00
30	37.39e-g	44.81d-f	23.34c	20.00
40	51.31b-d	58.23a-c	29.23b-c	3.00
LT				
10	45.22c-e	40.00e-g	24.71c	34.00
20	46.09c-e	41.52ef	25.69c	31.00
30	53.74b-d	46.83c-f	30.80bc	21.00
40	60.87ab	56.96a-d	31.59bc	9.00
EU				
10	55.65a-c	44.81d-f	24.32c	51.00
20	64.35ab	52.40b-e	23.34c	48.00
30	62.61ab	64.05ab	38.07b	36.00
40	64.35ab	64.05ab	31.59bc	8.00
OR				
10	52.18b-d	43.29ef	23.93c	48.00
20	57.39a-c	51.90b-e	30.41bc	24.00
30	62.61ab	61.77ab	38.66b	16.00
40	67.83a	60.00ab	40.04b	8.00
MCB	53.48b-d	66.83a	53.69a	0.00
CTR	-	-	-	36.00

Means with same letters in the same column are not significantly different at P≤0.05 using Fisher's LSD Test

Key: NL- *Azadirachta indica* IY- *Piper guineense* LT- *Lantana camara*
EU- *Eucalyptus camaldulensis* OR- *Citrus sinensis* (peel) MCB- Mancozeb
HAI- Hours after incubation

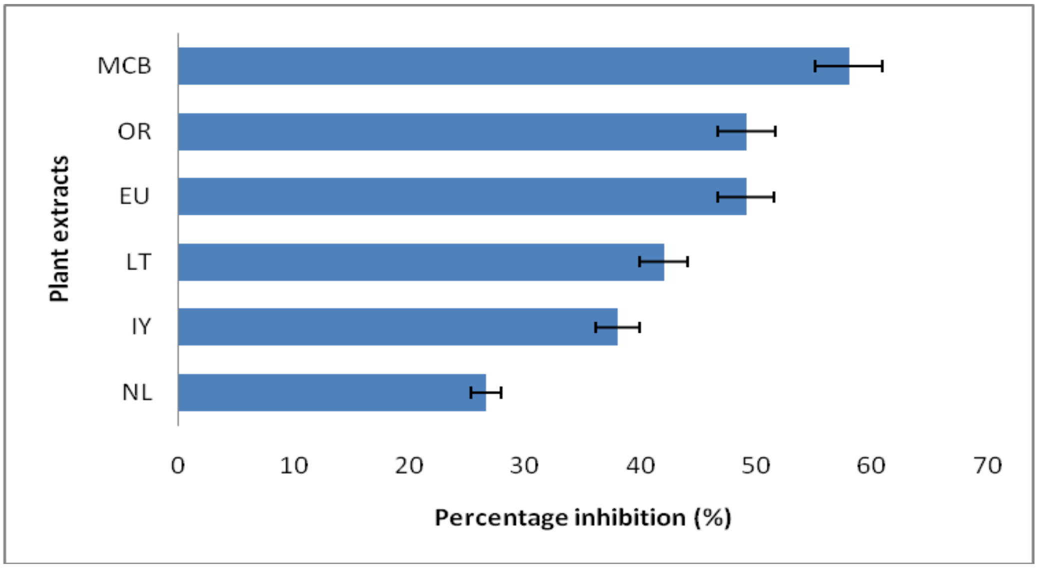

Figure 1: Overall effects of plant extracts on the mycelia growth of kola rot fungus, *L. theobromae*

Key: NL- *Azadirachta indica* IY- *Piper guineense* LT- *Lantana camara*
EU- *Eucalyptus camaldulensis* OR- *Citrus sinensis* (peel) MCB- Mancozeb

DISCUSSION

The inhibitory effects of the plant extracts used in this study against the kolanut storage pathogen, *L. theobromae* was due to the presence of some phytochemicals, which are secondary metabolites synthesized by plants and often sequestered in tissues to protect them against microbial attacks (Nweke, 2015).

Findings from this research showed a decrease in the number of pycnidia produced by the kola pathogen as extract concentrations of all the botanicals used in this study increased, indicating an increase in extract inhibitory effects on the pathogen's sporulation. All the extracts used in this study (with the exception *A. indica* and *P. guineense*) also clearly produced increasing inhibitory effects against the pathogen's mycelia growth with increasing concentrations all through the incubation periods. These findings clearly agree with the discovery of Nweke (2015) who reported that the inhibitory effect of the extract of *C. aurantifolia* on the mycelia growth and spore germination of some plant pathogens increased with increasing concentration of the extracts. The findings of Ogundeji *et al.* (2018) which indicated that the percentage inhibition exhibited by freshly prepared aqueous extract of *P. guineense* against *P. megakarya* consistently increased with extract concentration also partly agrees with the results of this study. Mycelia inhibitions produced by the *P. guineense*in this study however does not agree with the findings of the authors.

Noticeable reductions in the effectiveness of the extracts at 48 to 72 hours after incubation agrees with the findings of Babalola *et al.* (2017) which opined that the antimicrobial potencies of some plant extracts including *P. guineense* decreased as incubation period increased. This discovery also seems to agree with the findings of Ogundeji *et al.* (2018) which indicated a reduction in the effectiveness of some selected botanicals against cocoa black pod disease pathogen, *Phytophthora megakarya* with storage time. The increase noticed in the pathogen's mycelia inhibition between 24-48 hours of incubation however disagrees with these facts.

The very low average percentages of inhibition produced by *Azadirachta* extracts against the kola rot pathogen disagrees with the findings of Adeniyi and Joseph (2014) which explained that the botanical, among a few others, could be used to effectively control strains of *L. theobromae* affecting cashew. Findings from this study also partly disagree with Sahi *et al.* (2012), who in an *in vitro* evaluation discovered that *Eucalyptus camaldulensis* and *A. indica* were effective against mycelia growth of *L. theobromae* strain causing quick decline of mango. These disparities may be an indication to a stronger aggressive nature on the part of the strain of *L. theobromae* affecting kola, when compared to others isolated from both cashew and mango. The differences may most likely be brought about by inherent and/or acquired genetic variations among the different strains. Further research however needs to be carried-out to substantiate this possibility.

Differences in the number of pycnidia induced by the various extracts used in this study would most likely be due to differences in the type and/or concentrations of anti-sporulation ingredients naturally present within each of the plant extracts. Also, the production of lesser number of pycnidia as the concentrations of each of the extracts increased is an indication of the proportional presence of compounds capable of inhibiting sporulation in the botanicals.

CONCLUSION

Lantana, *Eucalyptus* and *Citrus* peel extracts used in this study, having shown reasonable efficacy, particularly within the first 48 hours of incubation, competed favourably with the positive control in the inhibition of kola rot pathogen, *L. theobromae.* Higher concentration (40%) of *P. guineense*, having shown some promise in inhibiting the pathogen's pycnidia production, could also be used in the suppression of the pathogen's sporulation. The use of extracts of *P. guineense* (aqueous) and *L. camara* (ethanolic) at 20-40% and 10-20% concentrations respectively, having shown some potential against the kola pathogen, is therefore recommended for an effective control of kola storage rot disease.

REFERENCES

1. **Adeniyi D. O. and Joseph A. 2014.** *In-vitro* evaluation of plant extracts against *Lasiodiplodia theobromae* causing cashew inflorescent blight. *African Journal of Biotechnology*, 14(13): 1139-1142.
2. **Agbeniyi S. O. 1998**. Efficacy of milton solution and wood ash in the control of storage moulds of kolanut (*Cola nitida*). A paper presented at the 26th Annual Conference of the Nigerian Society for Plant Protection (NSPP), held at Bida, 21-24 March, 1998.
3. **Agbeniyi S. O. 2014.** Incidence of *Lasiodiplodia theobromae* and other fungi in kolanuts (*Cola nitida* and *Cola acuminata*) in Nigeria. *American Journal of Experimental Agriculture* 4(12): 1764-1772.

4. **Atanda O. O., Olutayo A., Mokwunye F. C., Oyebanji A. O. and Adegunwa M. O. 2011.** The quality of Nigerian kolanuts. *African Journal of Food Science*, 5(17): 904-909.
5. **Babalolala E. A., Ogundeji B. A., Adio S. O. and Adeji A. O. 2017.** Effect of time of exposure on the antimicrobial potentials of some tropical plants against cocoa pod rot pathogen- *Phytophthora megakarya* (B & G) in Nigeria. *International Journal of Plant & Soil Science*, 20(4): 1-8.
6. **Greenwood V. 2016.** "The little-known nut that gave Coca-Cola its name". *BBC News-Future*. Retrieved 23 December, 2019. *www.bbc.com/future/article/20160922-the-nut-that-helped-to-build-a-global-empire*
7. **Jimenez M., Mateo R., Querol A., Huerta T. and Hernandez E. 1991.** Mycotoxins and mycotoxigenic moulds in nuts and sunflower seeds for human consumption. *Mycopathologia*, 115(1991): 121-127.
8. **Mokwunye F. and Oluyole K. 2017.** Postharvest processing and its effects on kolanut quality in southwestern Nigeria. *International Journal of Research in Agriculture and Forestry*, 4(11):1-4.
9. **Ndubuaku T. C. N., Asogwa E. U. and Hassan A. T. 2015.** Distribution of kolanut weevil (*Balanogastris kolae* (Coleoptera: Curculionidae) in *Cola nitida* stored in baskets. *African Journal of Plant Science*, 9(1), 13-16.
10. **Nweke F. U. 2015.** Effect of some plant leaf extracts on mycelia growth and spore germination of *Botryodiplodiatheobromae* causal organism of yam tuber rot. *Journal of Biology, Agriculture and Healthcare*, 5(8): 67-71.
11. **Ogundeji B. A., Babalola E. A., Adio S. O., Balogun S. T., Olorunmota R. T., Ogundeji F. O. 2018.** Effect of storage on the antimicrobial potencies of some botanicals against Phytophthora megakarya, causal pathogen of black pod disease of cocoa in Nigeria. *Advanced Research Journal of Microbiology*, 5 (3): 114-119.
12. **Opeke L. K.1992.** Tropical tree crops. Woye &Sons (Nig.) Ltd., Ilorin, Nigeria.

13. **Sahi S. T., Habib A., Ghazanfar M. U. and Badar A. 2012.** *In vitro* evaluation of different fungicides and plant extracts against *Botryodipldia theobromae*, the causal agent of quick decline of mango. *Pakistani Journal of Phytopathology*, 24:137-142.
14. **Snehali M. and Mohammed R. 2014.** Evaluation of antibacterial activity of ethanol extract of *Lantana camara* flowers against methicillin resistant *Staphylococcus aureus*. *World Journal of Pharmaceutical Research*, 3(9): 451-459.