Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 311-316 https://dx.doi.org/10.4314/sa.v22i1.26
© Faculty of Science, University of Port Harcourt, Printed in Nigeria **ISSN 1118 – 1931**

ANTIMYCOTIC EFFECTS OF SYNTHETIC FUNGICIDES AND PLANT EXTRACTS ON *ASPERGILLUS FLAVUS*

Ndifon, E.M.[a] and Lum, A.F.[b*]
[a]Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Abakaliki, Nigeria
[b*]The Byrd Research Center, ICT-U, Baton Rouge, Louisiana, USA
*Corresponding Author: lumfontem@yahoo.com

Received: 08-03-2023
Accepted: 28-04-2023

ABSTRACT

Aspergillus flavus contaminates the seeds of cowpea (Vigna unguiculata) and produces mycotoxins. This study was carried out to proffer solutions to the infection caused by A. flavus on cowpea seeds. The in vitro *trial was set up using two rates (50 and 100%) of each potential control agent (mancozeb, COPMET (Copper-I-oxide + metalaxyl), and aqueous extracts of Eucalyptus and neem) and a control (0%). The botanical extracts and pesticides inhibited the fungus growth significantly (P≤0.05) at different times. Both concentrations of mancozeb gave 100% inhibition of the fungus at 24–168 hours after incubation (HAI). At 24 HAI, Eucalyptus extract (50 and 100%) completely inhibited the fungus growth similar to mancozeb. Eucalyptus extract was more effective than neem extract and COPMET throughout. Though the extracts of both botanicals inhibited the growth of* A. flavus *at 168 HAI, Eucalyptus (83.8–89.2%) was more potent than neem (20.3–28.4%). At 48–168 HAI, both concentrations of neem extract and COPMET had similar effect on the fungus. The early stage of the antimycotic intervention is critical to the control of the fungus. This can be achieved by dressing cowpea seeds with mancozeb or Eucalyptus. However,* ***d****ue to the high cost and toxicity of synthetic chemicals, Eucalyptus extract could be used as an alternative for management of the fungus or incorporated into integrated disease management programmes for* A. flavus*. Research on the effects of long-term storage of cowpea using these botanicals is highly admonished.*

Keywords: *Aspergillus flavus*, control, cowpea, aqueous plant extracts, seed treatment

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a low-input legume crop that fixes nitrogen and yields well in most soils. The crop is rich in protein, minerals and vitamins; and serves as foodstuff for humans and livestock (Davis et al., 1991; Quinn, 2014). Because of this rich profile, its cultivation has been adopted globally. Ninety percent of the global production is in sub-Saharan Africa (Omoigui et al., 2019). The crop is mainly cultivated by small-scale farmers who generally obtain low yields due to numerous production constraints. The constraints include weeds, insect pests, diseases, inadequate spacing and environmental stresses (Adigun et al., 2014; Lum and Ekpo, 2004; Lum et al., 2018; Omoigui et al., 2019).

Fungi and insects constitute a serious problem in cowpea production and storage. Additionally, post-harvest problems result ultimately in a decrease in percentage germination, an increase in the discoloration of seeds, heating and mouldiness, and a change in the taste of the beans (Isleib, 2012; Yakubu et al., 2012). Seed-borne diseases cause decay and rotting of seeds that may lead to a severe reduction (22–63%) in percentage germination (Khare et al., 2016); and an increase in the production cost. Symptoms of the seed-borne diseases include changes in the colour, shape and functioning of the plant. It is common knowledge that numerous fungi species are associated with seed-borne diseases of cowpea, among which are *Aspergillus spp* (*Aspergillus flavus, A. niger* and A. *fumigatus*) (Embaby and Abdel-Galil, 2006; Mogle and Maske, 2012; Yakubu et al., 2012; Iyanyi and Ataga, 2014; Shahnaz et al., 2015; Khare et al., 2016). *Aspergillus flavus* is a major producer of aflatoxin. A lot of mycotoxins are produced when the number of fungi species associated with a seed lot is high. When this number is high, there is a reduction in the viability, nutritive quality and germination of seeds (Agarwal and Sinclair, 1996; Rahim et al., 2013). The presence of aflatoxins in cowpea seed samples from West Africa has been reported (Houssou *et al*., 2009).

A few researchers have attempted the management of these seed-borne diseases using agrochemicals with limited success. Kumar *et al*. (2004) observed that seed treatment with a mixture of tetramethylthiuram disulfide and carbendazim, carbendazim alone, tetramethylthiuram disulfide alone, mancozeb 75% WP, metalaxyl and thiophanate methyl effectively controlled fungi associated with cowpea seeds and improved germination. Khare *et al.* (2016) also reported similar results using dithane M45 (mancozeb). The fungi incidence reduced when the cowpea seeds were treated with captan, dithane M45, benlate and chlorothalonil by 5.3, 2.3 and 5.3 and 4.3% respectively, compared to the control which suffered 62% disease incidence.

Yakubu et al. (2012) reported that **s**everal plant materials effectively controlled insect pests of stored cowpea seeds. So far, the plant materials utilized include neem (*Azadirachta indica* A. Juss.) kernel, Eucalyptus (*Eucalyptus globulus* Labill.), lemon grass (*Cymbopogon citratus (DC.) Stapf.*) and guava (*Psidium guajava L)* leaves, orange (*Citrus sinensis*) and grape (*Vitis vinifera* L.) peels, Sesame (*Sesamum indicum L.), bird pepper (Capsicum frutescens L.), basil (Ocimum basilicum L.), g*roundnut (*Arachis hypogaea L*.) oil. It seems that not much research has been carried out on the application of plant extracts against fungi associated with cowpea seeds compared to their application against insect infestations. This study was conceived to proffer some solutions to the consistent problem of *A. flavus* associated with cowpea seeds using synthetic pesticides and plant extracts.

MATERIALS AND METHODS

Experimental site

This experiment was conducted at the Faculty of Agriculture Laboratory complex, Alex Ekwueme Federal University Ndufu-Alike, Abakaliki in Ebonyi State, Nigeria. Ebonyi State experiences a tropical climate with two seasons (rainy season and dry season) per annum; it is an agrarian State and crops are cultivated by subsistence farmers.

Collection of seed samples, isolation and identification of *A. flavus*

Four samples of cowpea seeds were obtained from the main market in Abakaliki and from the University Teaching and Research farm. They were packaged in manila envelopes, sealed and taken to the Laboratory. Seeds (400) were randomly taken from each of the four samples and processed to isolate the seed-borne fungus. There were four replicates and each had 100 seeds.

Potato dextrose agar (PDA) was prepared as recommended by the manufacturer, then streptomycin sulphate (1 g/L) was added to prevent the growth of bacteria contaminants. The cowpea seeds were surface sterilized in

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 311-316 https://dx.doi.org/10.4314/sa.v22i1.26
© Faculty of Science, University of Port Harcourt, Printed in Nigeria ISSN 1118 – 1931

1% sodium hypochlorite solution for 2 minutes; then they were placed aseptically on each Petri dish containing PDA at the rate of 10 seeds per plate. The plates were incubated for 7 days at room temperature (Shahnaz et al., 2015; Khare et al., 2016**)**. The fungus was sub-cultured on PDA to obtain pure cultures. It was identified by microscopy (ZEISS compound microscope), literature and manuals on fungi (Barnett and Hunter, 1972).

Preparation of test materials and experimental design

The synthetic pesticides utilized in this study were weighed using a mettler balance and dissolved in sterile distilled water at the recommended rates: 5.0 g Mancozeb®/L and 2.0 g Tandem®/L. These rates were considered as 100% concentrations. Tandem is a contact and systemic wettable powder formulation made from Copper-I-oxide (60%) + metalaxyl (methyl N-(2-methoxyacetyl)-N-(2, 6-xylyl)-D-lalaninate) (12%) (henceforth Tandem will be referred to as COPMET); Mancozeb (Zinc Manganese ethylene bisdithiocarbamate) (80% WP) is a contact wettable powder.

Aqueous extracts (100% concentration) of Eucalyptus and neem leaves were prepared using 333.3 g/L of distilled water. The plant leaves were blended using a Warrington blender and filtered using a double-layer sterile muslin cloth in a funnel over a beaker. The treatments were 50 and 100% concentrations of the two pesticides (mancozeb and COPMET) and aqueous extracts of the two plants (Eucalyptus and neem), and a control (0%) without pesticides or extracts. The experiment was carried out in Petri dishes which were laid out using a completely randomized design with nine treatments, and replicated three times.

Data collection and analyses

The diameter of the fungus colony was measured using a transparent ruler at 24-hour interval from day 1 to 4 and 7 (24–96 and 168 hours after incubation (HAI)). The data were subjected to analysis of variance (ANOVA) and the means of the treatments were separated using Tukey's HSD test ($P \leq 0.05$). The SPSS Version 25 was used.

RESULTS AND DISCUSSION

The results of this study showed that there were significant differences ($P \leq 0.05$) among the treatments (Table 1). At both concentrations, the synthetic fungicide Mancozeb gave 100% inhibition of A. flavus throughout the study. COPMET (50 and 100%) provided significantly ($P \leq 0.05$) lower levels of inhibition compared to Mancozeb and Eucalyptus extract throughout. The extract of Eucalyptus also gave higher inhibition of the fungus than that of neem. At 24 HAI, both rates of the fungicides and plant extracts except 50% neem inhibited the growth of A. flavus significantly ($P \leq 0.05$) compared to the control. Neem extract caused 22.5% inhibition at the rate of 100% while both concentrations of eucalyptus gave 100% inhibition. The synthetic fungicide COPMET inhibited the fungus by 46.2% at a rate of 50% and by 76.9% at a rate of 100%. At 48 and 96 HAI, the fungus growth in plates with both concentrations of COPMET and 50% neem extract was comparable to that in the control treatment.

At 48–168 HAI, the diameter of the fungus was similar for each fungicide or plant extract tested at 50 and 100% concentrations. The concentrations of COPMET inhibited the fungus by <20%. Also, COPMET and neem extract had similar effect on the fungus. In addition, the percentage of inhibition of the fungus was 63.2–83.8% for 50% eucalyptus; 79.0–89.2% for 100% eucalyptus; 2.9–20.3% for 50% neem and 5.9–28.4% for 100% neem during this period.

Table 1. The effects of chemical and botanical agents on *Aspergillus flavus in vitro*

	Aspergillus flavus growth measured at 24–96 and 168 hours after inoculation and percentage of inhibition (%)									
	24		48		72		96		168	
Treatments	D	%	D	%	D	%	D	%	D	%
Mancozeb 100%	0.0a	100.0	0.0a	100.0	0.0a	100.0	0.0a	100.0	0.0a	100.0
Mancozeb 50%	0.0a	100.0	0.0a	100.0	0.0a	100.0	0.0a	100.0	0.0a	100.0
COPMET 100%	0.3b	76.9	1.8c	5.3	2.8cd	3.4	3.2c	5.9	6.1c	17.6
COPMET 50%	0.7c	46.2	1.9c	0.00	2.8cd	3.4	3.2c	5.9	6.4cd	13.5
Eucalyptus 100%	0a	100.0	0.4b	79.0	0.5ab	82.8	0.6b	82.4	0.8b	89.2
Eucalyptus 50%	0a	100.0	0.7b	63.2	0.7b	75.9	0.8b	76.5	1.2b	83.8
Neem 100%	1.0d	23.1	1.6c	15.8	2.2c	24.1	3.2c	5.9	5.3c	28.4
Neem 50%	1.3e	0.0	1.7c	10.5	2.6cd	10.3	3.3c	2.9	5.9c	20.3
Control	1.3e	0.0	1.9c	0.0	2.9d	0.0	3.4c	0.0	7.4d	0.0

Means followed by similar letter(s) are statistically similar using Tukey's HSD (*P*≤0.05); D=diameter of fungus (cm).

These results showed that the agrochemicals Mancozeb and COPMET, and extracts of Eucalyptus and neem had fungicidal properties against the growth of *A. flavus*. Mancozeb at 50 and 100% concentrations completely inhibited the fungus growth throughout the study period. This indicates that both concentrations of mancozeb were highly effective against the growth of *A. flavus*. Similar results were obtained for both concentrations of Eucalyptus extract only at 24 HAI. However, Eucalyptus also effectively inhibited the fungus growth at 48–168 HAI although this was not similar to Mancozeb. Eucalyptus extract and Mancozeb performed better than COPMET fungicide and neem extract throughout. Previous studies have also shown the effectiveness of Mancozeb in completely inhibiting the growth of several plant pathogenic fungi including *Aspergillus niger, Fusarium solani*, *Fusarium oxysporium*, *Phythophthora colocasiae* and *Athelia rolfsii* (Ekefan et al., 2018; Gwa and Ekefan, 2018; Lum et al., 2019; Lum and Takor, 2021; Ndifon and Lum, 2021; Ndifon, 2022). The performance of the aqueous extract of Eucalyptus at both concentrations was very impressive. Neem 50% did not inhibit the fungal growth at 24 HAI but this was considered normal for plant extracts, because the inhibition may not always set in immediately**.** Both concentrations of neem and COPMET were considered as slightly effective against the fungus. The performance of Eucalyptus rates against neem shows its superiority as a biopesticide.

This study indicated that the aqueous extract of Eucalyptus (50 and 100%) possesses fungicidal activity against *A. flavus*. In another study, Ndifon (2022) reported that Eucalyptus gum (50 and 100%) inhibited the growth of *Athelia rolfsii*. Satish et al. (2007) indicated that *Eucalyptus* had significant antifungal activity against *A. flavus*. Neem had slight activity against the fungus; this botanical has been recommended by various researchers as a biopesticide against several pests and it is even produced and used commercially. For

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 311-316 https://dx.doi.org/10.4314/sa.v22i1.26
© Faculty of Science, University of Port Harcourt, Printed in Nigeria ISSN 1118 – 1931

example, in earlier studies, the botanical inhibited the growth of plant pathogens such as *Alternaria solani* (Ravikumar and Garampalli, 2013), *Aspergillus niger* (Gwa and Ekefan, 2018).

CONCLUSION

Aspergillus species are frequently associated with crop seeds and some of them produce aflatoxins which are carcinogens. Thus, this research was carried out to proffer solutions to this contamination problem. Both concentrations of the synthetic fungicides (mancozeb and COPMET (Copper-I-oxide (60%) + metalaxyl (12%) and aqueous extracts of the plants (Eucalyptus and neem) had fungicidal activity against A. flavus. Mancozeb (50 and 100%) was the most effective followed by Eucalyptus (50 and 100%). For each fungicide or plant extract, both concentrations tested resulted in similar growth inhibition at 48–168 HAI. Eucalyptus was more potent than neem and COPMET. This trial revealed the feasibility of Eucalyptus extract for use in the management of A. flavus. Therefore, Eucalyptus extract could be recommended as a biopesticide for seed dressing and grain storage to replace the synthetic fungicides which are toxic and costly.

REFERENCES

Adigun, J., Osipitan, A.O., Lagoke, S.T., Adeyemi, R.O. and Afolami, S.O. (2014) Growth and yield performance of cowpea (*Vigna unguiculata* (L.) Walp) as influenced by row-spacing and period of weed interference in south-west Nigeria. Journal of Agricultural Science 6(4).

Agarwal, V.K. and Sinclair, J.B. (1996) Principles of seed pathology. 2nd Edn. Lewis Publishers, CRC Press, USA, pp. 539.

Barnett, H.L. and Hunter, B.B. (1972) Illustrated genera of imperfect fungi. 3rd edition, Burgess Publishing Co., Minneapolis, pp. 241.

Davis, D.W., Oelke, E.A., Oplinger, E.S., Doll, J.D., Hanson, C.V. and Putnam, D.H. (1991) Cowpea. Minnesota Extension Service, University of Minnesota, St. Paul, MN 55108.

Ekefan, E.J., Nwankiti, A.O. and Gwa, V.I. (2018) Comparative assessment of antimicrobial potency of some selected plant extracts against seed borne pathogens of germinating yam setts. Journal of Plant Pathology and Microbiology 9:444.

Embaby, E.M. and Abdel-Galil, M.M. (2006) Seed-borne fungi and mycotoxins associated with some legume seeds in Egypt. Journal of Applied Sciences Research 2(11):1064–1071.

Gwa, V.I. and Ekefan, E.J. (2018) Fungicidal effect of some plant extracts against tuber dry rot of white yam (*Dioscorea rotundata* Poir) caused by *Aspergillus niger*. International Journal of Horticulture and Agriculture 3(3):1–7.

Houssou, P.A., Ahohuendo, B.C., Fandohan, P., Kpodo, K., Hounhouigan, D.J. and Jakoben, M. (2009) Natural infection of cowpea (*Vigna unguiculata* (L.) Walp) by toxigenic fungi and mycotoxins contamination in Benin, West Africa. Journal of Stored Products Research 45:40–44.

Isleib, J. (2012) Signs and symptoms of plant disease: Is it fungal, viral or bacterial? Michigan State University Extension - December 19, 2012.

Iyanyi, N.G. and Ataga, A.E. (2014) Fungal species associated with Vigna unguiculata (L.) Walp (cowpea) seed from parts of Enugu State, Nigeria. Scientia Africana 13(1).

Khare, K.B., Loeto, D., Wale, K. and Salani, M. (2016) Seed-borne fungi of cowpea [*Vigna unguiculata* (L.) Walp] and their possible control in vitro using locally available fungicides in Botswana. International Journal of Bioassays 5(11):5021–5024.

Kumar, K., Singh, J. and Ratan, V. (2004) Seedborne fungi of cowpea, their parasitism and control. Annals of Plant Protection Sciences 12(1):80–82.

Lum, A.F. and Ekpo, E.J.A. (2004) Effect of *Sclerotium rolfsii* on the performance of cowpea in engine oil- and spent oil-contaminated soil. Nigerian Journal of Ecology 6:20–25.

Lum, A.F., Ndifon, E.M., Mbong, G.A., Chi, S.J. and Ntsomboh, G.N. (2019) Anti-fungal activity of plant extracts for the management of *Fusarium oxysporum* F. sp. *elaiedis in-vitro*. International Journal of Biosciences 14(6):1–9.

Lum, A.F., Nji, G.F., Ndifon, E.M. and Neba, N.A. (2018) Agronomic performance of four cowpea (*Vigna unguiculata* L. Walp) varieties under different interrow spacings in Buea, Cameroon. Journal of Experimental Agriculture International 25(3):1–9.

Lum, A.F. and Takor, M.C. (2021) Taro Leaf Blight: Disease Assessment, Farmers' Knowledge and Management Potential of Goatweed Extract in South West Cameroon. Journal of Agriculture and Crops **7**(1):159–166.

Mogle, U.P. and Maske, S.R. (2012) Efficacy of bioagents and fungicides on seed mycoflora, germination and vigour index of cowpea. Science Research Reporter 2(3):321–326.

Ndifon, E.M. **(**2022) Inhibitory efficacy of microbial, botanical and synthetic fungicides against *Athelia rolfsii* (*Sclerotium* stem rot) of groundnut and Bambara groundnut. Tropical Agricultural Research and Extension 25(1):14–22.

Ndifon, E.M. and Lum, A.F. (2021) Assessment of White Yam Tuber Rot Disease and in vitro Management of *Aspergillus niger* in Ebonyi State, Nigeria. International Journal of Biosciences 19(4):32–40.

Omoigui, L.O., Arrey, M.O., Kamara, A.Y., Danmaigona, C.C., Ekeruo, G. and Timko, M.P. (2019) Inheritance of resistance to *Cercospora* leaf spot disease of cowpea [*Vigna unguiculata* (L.) Walp]. Euphytica 215:101.

Quinn, J. (2014) Cowpea. A versatile legume for hot, dry conditions. Jefferson Institute. http://www.hort.purdue.edu/newcrop/articles/ji-cowpea.html.

Rahim, S., Dawar, S., Tariq, M. and Zaki, M.J. (2013). Mycoflora associated with the seed samples of *Cucurbita pepo L.* collected from Pakistan. Pakistan Journal of Botany 45(6):2173–2179.

Ravikumar, M.C. and Garampalli, R.H. (2013). Antifungal activity of plants extracts against *Alternaria solani*, the causal agent of early blight of tomato. Archives of Phytopathology and Plant Protection 46(16):1897–1903.

Satish, S., Mohana, D.C., Ranhavendra, M.P. and Raveesha, K.A. (2007) Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology 3(1):109–119.

Shahnaz, D., Maimona, K. and Summiaya, R. (2015) Seed borne fungi associated with cowpea (V*igna unguiculata* (l.) Walp. International Journal of Biology and Biotechnology 12(4):565–569.

Yakubu, B.L., Mbonu, O.A. and Nda, A.J. (2012) Cowpea (*Vigna unguiculata*) Pest control methods in storage and recommended practices for efficiency: A Review. Journal of Biology, Agriculture and Healthcare 2(2):27–33.