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# *In vitro* effects of some pesticides on pathogenic fungi associated with legumes

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**Abstract**

An *in vitro* effect of three pesticides commomly used in the southern Guinea savanna namely Benomyl (fungicide), Galex® (herbicide) and Karate® (insecticide) on the growth of three pathogenic fungi of legumes, *Fusarium oxysporum*, *Aspergillus flavus* and *Fusarium monilliforme,* was investigated to determine the effectiveness of the pesticides in reducing the growth of these fungi . Each fungicide was assayed with each fungus at 0, 100, 200, 400 and 500 mg $L^{-1}$ rate in Potato Dextrose Broth (PDB) and incubated at 30°C for five days. Each treatment was replicated four times. Data were collected on the mycelia weights of the fungi under each treatment. Mycelia weights of the three fungi were significantly reduced at 100 mg $L^{-1}$ by all the pesticides except that of *A. flavus* under Benomyl treatment where some resistance was observed. Significant reduction of mycelia weights of the three fungi was observed at 500 mg $L^{-1}$ of Galex® application compared to other application rate of Galex. Application of Benomyl and Karate® significantly reduced mycelia weight of *F. oxysporum* irrespective of the rate applied. Growth of the two *Fusarium* species was completely inhibited by Benomyl at the application rate of 500 mg $L^{-1}$. Galex® and Karate® which are herbicide and insecticide respectively, had detrimental effect on the tested pathogenic fungi of legumes and therefore may reduce incidence of diseases caused by these fungi in legume production in the southern Guinea savanna of Nigeria. We suggest that application of Karate or Galex can be effectively used to control some important pathogenic fungi of legumes.

***Keywords:*** *Aspergillus flavus*; fungicide *Fusarium monilliforme*; *Fusarium oxysporum*; herbicide; insecticide; mycelial weight

**Abbreviations:** PDA_Potato dextrose agar; PDB_Potato dextrose broth; a.i_active ingredient; EC_Emulsifiable concentrate

## Introduction

Pesticides come in contact with both target and non-target organisms when applied on crops and have been implicated to have some effects, inhibitory or stimulatory, on the development of non-target organisms (Rodriguez Kabana and Curl 1980; Stratton, 1983; Schustrer and Schroder, 1990; Digrak and Ozcelik, 1998).The growth of some organisms has been inhibited by herbicides which are meant to destroy weeds while some fungi have been affected by application of insecticides. (Chen *et a*l., 2001; Das *et al*., 2003; Sanyal and Shrestha, 2008).

Legumes, especially cowpea, soybean and groundnut are important food crops cultivated in Nigeria. They are consumed by humans and livestock, and incorporated as secondary crops in most farming systems for sustenance and maintenance of soil structure and fertility. (Alegbejo, 2001; Randall et al., 2006). One of the major constraints to legume production is pests and diseases. Many legume diseases are caused by fungi such as *Fusarium, Collectrichum, Aspergillus, Rhizoctonia* species and some others fungal pathogens. *Fusarium oxysporum* and *F. monilliforme* which are soil borne pathogens have been identified as either primary or secondary causal agents of *Fusarium* wilt and root rots of cowpea and groundnut

***Table 1.*** Effect of Galex® on the mycelial growth of *F. oxysporum*, *A. flavus* and *F. monilliforme* under laboratory conditions

| Rate (mg $L^{-1}$ ) | mycelial weight (mg) | | |
|---|---|---|---|
| | *Fusarium oxysporum* | *Aspergillus flavus* | *Fusarium monilliforme* |
| 0 | 1.03a | 1.37a | 0.82a |
| 100 | 0.29b | 0.83b | 0.36b |
| 200 | 0.24b | 0.81b | 0.30b |
| 400 | 0.16c | 0.58c | 0.13c |
| 500 | 0.07d | 0.39d | 0.05d |

Column values followed by the same letters were not significantly different (P<0.05, Duncan test)

in Nigeria (Emechebe and Shoyinka 1985; Bunting et al., 1985). *Aspergillus flavus*, also a soil borne pathogen is known to cause storage rot of groundnut in Nigeria and many cases of aflatoxin in legume products are associated with the pathogen (Ihejirika et al., 2005).

Due to the diseases caused by these fungi which lead to reduction or total loss of yield in legumes, farmers in the southern Guinea savanna of Nigeria often applied some pesticides. Galex 500 EC is a common pre-emergent herbicide applied to control weeds while Karate is a common systemic insecticide applied to control leaf, fruit and soil dwelling insect of cowpea, groundnut and soybean in Nigeria (Dugje *et al*., 2008). Benomyl is used for wide range of crops including legumes (Oladiran, 1990). The aim of this research is to investigate *in vitro* effect of commonly used pesticides by farmers in the southern Guinea savanna of Nigeria on three pathogenic moulds of legumes

## Materials and methods

### *Isolation of moulds*

The three pathogenic moulds investigated were *F. oxysporum* f sp *tracheiphilum*, *Aspergillus flavus* and *F. monilliforme*. *Fusarium oxysporum* was isolated from diseased leaves and roots of cowpea using Potato Dextrose Agar (PDA). These were incubated for five days at 30°C. *Aspergillus flavus* and *F. monilliforme* were isolated from soil by pour plate technique. Ten-fold serial dilution of soil suspension was made and 1ml of $10^{-3}$ was plated using PDA media and incubated at 30°C for five days.

### *Description of pesticides*

The three pesticides used were Galex® 500 EC (herbicides), Benomyl (fungicide) and Karate® 2.5 EC. obtained from the Department of Crop Production, University of Ilorin, Nigeria. Galex® 500 EC is the registered trade name for the selective herbicide used for cultivation of soybeans, cowpeas, and sweetpotatoes. Its active ingredients (a.i) are metalochlor (250 g/a.i) and metobromuron (250 g/a.i). Benomyl (methyl 1-(butylcarbomoyl benzimidazol 1-2-ly carbamate) is a systemic fungicide available as a wettable powder (WP) containing 50% active ingredient. It is usually applied as a foliar treatment at low rates for control of a wide range of fungus diseases affecting fruits' vegetables, field and ornamental crops. Karate® is a registered trade name for an insecticide belonging to the group pyrethroids. It contains 25 g λ-cyhalothrin (lambda-cyhalothrin). It is used for the control of insect pests of cowpea and soybean.

### *Fungi proliferation*

Potato dextrose broth (PDB) was the culture medium for the growth experiment and was prepared by heating 24 g of Difco™ potato dextrose broth in 1 litre of distilled water. The mixture was allowed to boil for 1 minute. The medium was then dispensed into 125 ml conical flasks at the rate of 50 ml per flask and were sterilized by autoclaving at 121°C, for 15 minutes. After sterilization, the media were allowed to cool down (40 - 50 °C) before chloraphenicol (1%) was added aseptically to suppress bacterial growth. Galex®, Benomyl and Karate® were introduced separately into PDB inside flasks at the rate of 100, 200, 400 and 500 mg $L^{-1}$. Experimental set up of medium containing no pesticides was used as control. Each of the pathogenic moulds were introduced directly from the inoculated plate at the rate of 100 µg and incubated at 30°C for six days. At the end of incubation, mycelia weights were recorded.

***Table 2.*** Effect of Benomyl on the mycelial growth of *F. oxysporum*, *A. flavus* and *F. monilliforme* under l aboratory conditions

| Rate (mg $L^{-1}$ ) | mycelial weight (mg) | | |
|---|---|---|---|
| | *Fusarium oxysporum* | *Aspergillus flavus* | *Fusarium monilliforme* |
| 0 | 0.83a | 0.96a | 0.78a |
| 100 | 0.07b | 0.94ab | 0.04b |
| 200 | 0.03b | 0.87bc | 0.02bc |
| 400 | 0.03b | 0.84c | 0.02bc |
| 500 | 0.00b | 0.82c | 0.00c |

Column values followed by the similar letters were not significantly different (P<0.05, Duncan test)

***Statistical Analysis***

All data were subjected to analysis of variance using PROC GLM of Statistical Analysis System (SAS, 2003). Means were separated using the Duncan's Multiple Range Test at 5 % significant level.

**Results**

Galex® significantly (P<0.05) reduced the growth of *F. oxysporum*, *A. flavus* and *F. monilliforme* mycelia at different concentrations when compared to the control treatment (Table 1). The three pathogenic moulds did not show any significant difference in mycelial growth at 100 mg $L^{-1}$ and 200 mg $L^{-1}$. However, the least growth of mycelia which was significantly lower than others was recorded at 500 mg $L^{-1}$ (Table 1). Among the three fungi, the weight of mycelia observed in A. flavus was higher than any of the *Fusarium* at any of the rate.

Benomyl significantly (P<0.05) reduced the growth of *F. oxysporum* and *F. monilliforme* when pesticides amended treatments were compared with the control (Table 2). There was no significant difference in the mycelia growth of *F. oxysporum* between 200 and 500 mg $L^{-1}$ pesticide rates and of *F. monilliforme* between 200 and 400 mg $L^{-1}$ pesticide rate. Complete inhibition of mycelial growth of the two *Fusarium* spp was observed at 500 mg $L^{-1}$. Unlike the *Fusarium* species, inhibition of mycelial growth of A flavus was not observed at 100 mg $L^{-1}$ of Benomyl. There was no significant difference between the control and 100 mg $L^{-1}$ and between 100 and 200 mg $L^{-1}$ pesticide rates.

In fact, Benomyl effects at 200 and 500 mg $L^{-1}$ pesticide rate also did not differ significantly (Table 2). Mycelial weight of *A. flavus* was marginally reduced even at higher rate of Benomyl application.

Application of Karate® insecticide at different rates significantly (P<0.05) reduced mycelial growth in all the fungi when compared with the control (Table 3). The reduction observed in the mycelia growth of *F. oxysporum* at 100 mg $L^{-1}$ pesticide rates was not significantly differ compared to 500 mg $L^{-1}$ pesticide rates. However, 400 mg $L^{-1}$ of Karate® significantly reduced mycelial growth of *A. flavus* and *F. monilliformes* when compared to 100 or 200 mg $L^{-1}$ (Table 3).

**Discussion**

The application of the three pesticides had detrimental effect on the growth of the three pathogenic fungi in this study. The inhibitory effects of some pesticides on non-target organisms have already been confirmed (Rodriguez Kabana and Curl 1980). Galex and Karate, herbicide and insecticide respectively, were able to reduce the mycelial weight of all the three fungi even at 100 mg $L^{-1}$. According to Sanyal and Shresta (2008), activity of herbicides can extend beyond their target organisms and inhibit spore germination or mycelial growth of fungus in plant. Incidences of *F. oxysporum, A. flavus* and some other pathogens were decreased after application of some chemicals to the field soil and growth media (Das *et al*., 2003; Tubajika and Damann, 2002; El Khadem *et al*., 1984). Reports have shown that lambda cyhalothrin (active ingredient in Karate) insecticide significantly reduced *Fusarium* ear rot of maize caused by *F. verticilloides* (formerly known as *F. monilliforme*) (Blandino et al., 2008).

The high sensitivity of the *Fusarium* species to Benomyl at 100 mg $L^{-1}$ was an indication that lower rates of Benomyl application in our study effectively inhibit mycelial growth of these fungi. Benomyl has effectively control *F. oxysporum* and *F. monilliforme* in some other studies (Smit et al 1989; Allen et al 2004). However, *A. flavus* was tolerant to Benomyl at 100 mg $L^{-1}$ rate and less sensitive at higher rates compared to *Fusarium* spp. The reason why Benomyl, a fungicide, was less fungitoxic on

***Table 3.*** Effect of Karate® on the mycelial growth of *F. oxysporum*, *A. flavus* and *F. monilliforme* under laboratory conditions

| Rate (mg $L^{-1}$ ) | mycelial weight (mg) | | |
|---|---|---|---|
| | *Fusarium oxysporum* | *Aspergillus flavus* | *Fusarium monilliforme* |
| 0 | 1.85a | 1.98a | 0.81a |
| 100 | 0.13b | 0.28b | 0.18b |
| 200 | 0.06b | 0.23bc | 0.15b |
| 400 | 0.06b | 0.17cd | 0.07c |
| 500 | 0.04b | 0.12d | 0.05c |

Column values followed by the same letters were not significantly different (P<0.05, Duncan test)

*A.flavus* than Galex and Karate was not very clear. However, resistant to Benomyl have been reported in *A. flavus* and could be due to some strains of *A. flavus* that carry Benomyl resistant β tubulin gene (Seip et al 1990). Except for application of Benomyl and Karate on *Fusarium oxysporum* where the effect at lower rate was not significantly different to higher rate, inhibition of mycelial growth of other fungi increased with higher rate of application.

**Conclusion**

Our findings have shown that the pesticides tested in this study caused reduction in the mycelial growth of *F. oxysporum*, *F. monilliforme* and *A. flavus in vitro*. Application of Galex® and Karate® can be exploited by farmers in southern Guinea savanna of Nigeria for the control of some pathogenic moulds. Exploiting the fungi toxicity of insecticides and herbicides on disease pathogens could minimize the use of fungicides and consequently reduce environmental pollution hazards. If Galex, a pre emergent herbicide, can reduce incidence of some fungi diseases, there would be no need to apply fungicides and this leads to reduction in pesticide use. However, more studies are needed to study the effect of these pesticides on the field as some external or internal factors may influence the outcome of this study.

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