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Insecticidal Potential of an Orally Administered Metabolic Extract of *Aspergillus niger* on *Chrysomya chloropyga* (Green bottle fly) Larvae

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ABSTRACT: The insecticidal activity of *Aspergillus niger* IHCS-4 metabolic extract against *Chrysomya chloropyga* larvae was examined *in vitro*. The toxicity test revealed that 0.04 mg/g and 0.08 mg/g extract concentration significantly (P>0.05) affected the insect larvae, inducing 20% and 65% mortality respectively, within 24 hours. Larval growth was inhibited only at concentrations of 0.04mg/g or higher. Survivors of the toxicity treatment at 0.04mg/g and 0.08mg/g recorded significant reductions in weight over time. These observations have indicated the potential of *A. niger* as a simple, inexpensive and accessible source of bioinsecticide @JASEM.

More recently attention has been turned to microorganisms as sources of insecticidal compounds with the overmectins being an example of insecticidal microorganisms derived compounds with significant commercial potential (Dowd *et al.,* 1992). Researches on neuroactive fungal metabolites, primarily indole-derived compounds, have demonstrated significant insecticidal activity (Dowd *et al.*, 1988, and 1992). Many of these compounds are tremorgenic, inducing tremors in vertebrates by their interactions with glutaminergic receptors (Gant *et al.*, 1987, Yao *et al.*, 1989 and Dowd *et al.*, 1992).

Another group of tremor inducing compounds, the territrems have been isolated from the fungus *Aspergillus terreus* (Peng *et al.*, 1985, and Dowd *et al.*, 1992). Other filamentous fungi with known insecticidal activity are *Fusarium* sp, *Metarrhizium anisopliae* and *Beauveria bassiana* (Okafor 1985 and Waage 1991). The insecticidal properties of compounds derived from these fungi have prompted the examination of moulds for new insecticidal compounds. The preponderance of moulds, most especially the aspergilli and penicllli in our environment provides an adequate means of their utilization as a cheap source of biological agent for studies on the production of bioinsecticides. In addition to their high sporulation rate, their ability to adapt to changing environmental conditions, and to utilize a wide range of substrates including hydrocarbons for growth is of uttermost importance to their utilization in the production of bioinsecticides. Nisbet and Fox (1991) reported that the accumulation of hydrogen compounds in the cell mass of microorganisms gives them greater potentials as sources of bioinsecticidal substances. In this investigation the toxicity of the growth extract of soil borne *Aspergillus niger* to *Chrysomya chloropyga* larvae was examined in order to assess its potential as an insect control agents or a model for new insecticides.

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MATERIALS AND METHODS

Isolation of Test Mould: The *Aspergillus niger* isolate used in this study was obtained from an oil impacted soil in Ibeno L. G. A. of Akwa Ibom State, Nigeria, using the spread plate technique (Fawole and Oso 1998) and Sabouraud dextrose agar (SDA) as the culture medium. The identity of the isolate was confirmed by the application of the taxonomic characterization procedures described by Domsch, *et al.,* (1980) and Barnett and Hunter (1986).

Cultivation of Aspergillus niger IHCS-4: The submerged culture technique was adopted. In this technique spore suspensions from a 4-day old slope culture of the mould maintained on SDA were prepared as described by Agina (1991). The conidia of the test mould were dislodged into the solutions using a sterile inoculating needle bent slightly at the tip. The spore suspensions were then filtered to remove mycelial fragments and clumps of spores using four layers of sterile cheesecloth. The spore suspensions were diluted to give spore concentration of about 1 to 1.2 x 10^6 spores per ml. The spore concentration was determined by direct counting on an haemocytometer. One-milliliter aliquots of the suspension were used to inoculate 100ml of Sabouraud dextrose broth (SDB) contained in 250ml capacity conical flasks. Four replicates of the cultures were prepared and incubated at room temperature (28 $\pm$ 2^0C) for 3 weeks.

Extraction of Growth Metabolites: Prior to extraction of the growth metabolites, the biomass of the cultures were carefully and aseptically harvested and oven dried at 80^0C. The metabolites in the filtrate were obtained by means of the Liquid-Liquid

extraction technique with benzene as the extraction solvent (Burks 1982). The solvent was recovered by distillation and the extract dried by evaporation in a draft oven at 40^0C. The extract powders contained in 50ml clean glass bottles were stored in the refrigerator until used.

Rearing of Insect Larvae: Chrysomya chloropyga, like other gnobiotic flies is a useful model for the bioassay of biocidal compounds. The insect larvae were reared on decayed goat meat medium. The medium consisted of moistened pieces of goat meat incubated for 48 hour at room temperature (28 ± 2^0C) to allow for sufficient bacterial decomposition of the meat components. Following the sterilization of the medium it was seeded with eggs of *C. chloropyga* obtained from unhygienic pit latrines under the supervision of an entomologist. The emergence and development of the larvae was rapid and the age of the cohorts tested was standardized using neonates within 12 hours of eclosion. The average weight of the neonates selected for bioassay was 0.082mg

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Bioassay: The insecticidal activity of the fungal metabolic extract was tested with decayed meat diets incorporated with metabolic extract at first instar larvae stage (Patana 1969, Dowd *et al* 1992, and Rodriguez- Saona *et al*., 1998). Four concentrations (0.08mg/g, 0.04mg/g 0.03mg/g, 0.02mg/g) of the mould metabolic extract were used for the bioassay. Treated diets were prepared by transferring lml of acetone solutions of the desired extract concentration into 50ml polypropylene centrifuge tubes, evaporating the acetone and adding 2ml of 0.1% Tween solution. The extract-Tween solution mixture was homogenized before the addition of 5g diet (decayed meat) to produce a final weight of 8g. The mixture was vortexed for 3 minutes. Control diets were prepared by mixing 2ml of Tween solution and 6g of the diet to produce a final weight of 8g.

Control and treated diets were separately poured into 20-well bioassay trays (15.9mm x 15.9mm capacity). One neonates was added per well, and trays were incubated at room temperature (28 ± 2^0C). Twenty neonates were tested for each extract concentration and control. The mortality rate was recorded after every 4 hours for one day. Survivors (larvae) of the 24 hour-mortality test were examined for larvae weight after every 12 hours. Treatments were analysed for significant differences using chi-square analysis for mortality and linear contrast analysis of variance for weight (Sokal and Rohlf 1969).

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RESULTS AND DISCUSSION

The percent mortality of *Chrysomya chloropyga* fed on diet augmented with *Aspergillus niger* IHCS-4 metabolic extract is presented in Table 1. The results showed that the extract exhibited a moderate toxicity or low killing effect on the insect larvae. The toxic effect however increased with increase in the concentration of extract and duration of exposure to the treated diets. At the end of the test period (24 hours) a mean mortality rate of 65%, 20%, 10% and 5% were recorded for larvae fed with diets augmented with 0.08, 0.04, 0.03 and 0.02 mg/g respectively, of the metabolic extract. This showed that the mould growth extract possessed a significant insecticidal potential when orally administered at 0.08mg/g, and precisely caused 15% and 65% larvae mortality after 12 and 24 hours exposure times respectively.

These observations are in agreement with the report that fungi used in the biological control of pests have to be able to elaborate metabolites harmful to the pests (Zahner *et al.*, 1983). Although the exact models of toxicity was not determined in the present study, earlier investigations have shown that fungi, particularly the aspergilli posses the ability to elaborate harmful metabolites which can induce acute and chronic toxicological effects on insects and man (Whitten and Oakeshott 1990, Dowd *et al.*, 1992). The killing effect of *A. niger* IHCS-4 growth extract on *C. chloropyga* larvae may be attributed to the mould ability to elaborate toxic chemicals such as aspergillic acid and probably territrems, a neuroactive, tremorgenic substance commonly associated with the related species *A. terreus* (Peng *et al*., 1985, and Dowd *et al.*, 1992).

Many fungal metabolites are potent inhibitors of the insect head cholinesterase *in vitro* (Dowd *et al.*, 1988), and recent work with the tremorgens and several active site inhibitors also suggest that fungal metabolites can bind to a hydrophobic site remote from the catalytic site (Chen and Ling 1991). Similarly Lacey *et al.*, (1994) observed a sharp increase in mortality when adult Japanese beetle *Papillia japonica* was treated with extracts of *Metarrhizium anisopliae* and *Beauveria bassiana*

Table 1: Killing effect (percent mortality rate) of *A. niger* IHCS-4 metabolic extract of *Chrysomya chloropyga* larvae.

Incubation period (hrs)	Control	0.02	0.03	0.04	0.08
12	0	9	9	9	10
24	0	0	0	5	10
36	0	0	0	5	15*
48	0	0	15*	50*	0
60	0	0	5	15*	55*
72	0	5	10*	20*	65*

Mortalities are expressed as mean percentages of four replicates per treatment.
Values followed by an asteric are significantly different at p>0.05 from control by chi-square analysis.

Survivors (insect larvae) of the killing effect of the mould metabolic extract at 0.04mg/g and 0.08mg/g concentrations recorded significant reductions in weight over time (Figure 1). The loss in weight of the *C. chloropyga* larvae fed with diets impregnated with concentrated metabolites of *A. niger* IHCS-4 may by attributed mainly to the poison effect of the diet, although the failure of the larvae to metabolize the "poisoned" diet could cause similar effect. These observations and a change in the metabolic pathways of the neonates have earlier been reported by Dowd *et al.,* (1992) on the toxicity and anticholinesterase activity of a group of fungal metabolites, territrems to eelworm (*Helicoverpa zea)* larvae. They observed that the growth rate of the larvae was significantly inhibited by 40% at 250ppm and by 89% at 250ppm.

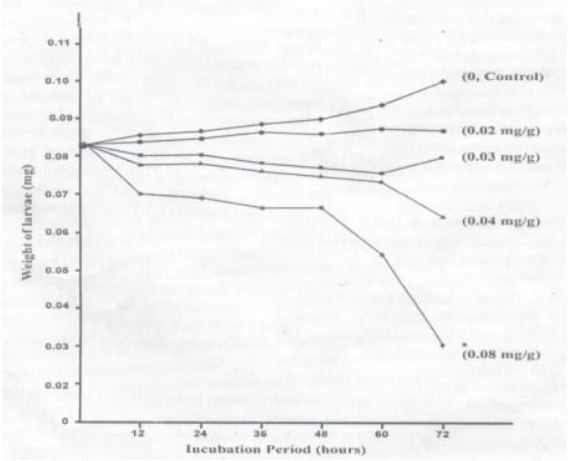

Fig 1: The effect of different concentrations of *Aspergillus niger* growth extract on the weight of *Chrysomya chloropyga* larvae incubated at room temperature. Significant at P>0.05*

It is also possible that the metabolites may accumulate in the insect larvae blocking its enzymatic pathway and or inhibits an enzyme. Chanto and Ulate (1996) equally observed that a cell free supernatant and ethanolic growth extract of a 3-day old culture of *Bacillus* VCR – 236 inhibited growth of *Mycena citricolor*.

This study has further confirmed the insecticidal potential of metabolic compound produced by the aspergilli. However the accessibility for large scale use is hindered by lack of detail information on the chemical stability, photostability, phytotoxicity and non-target of the active compounds in the metabolites. To enhance our knowledge of these factors routine analytical studies on the active properties and their specific toxicity are necessary.

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