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Daily Mortality Responses of *Callosobruchus maculatus* and *Sitophilus zeamais* to Changes in the Concentrations of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens*

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Abstract: The powders of the leaves of *H. suaveolens*, *O. gratissimum* and *Azadirachta indica* were assessed for their daily effect against *Sitophilus zeamais* Mots infesting maize grains (*Zea mays*) and *Callosobruchus maculatus* Fab infesting cowpea seeds. The powders were tested at 1.5, 2.5 and 3.5 g/80 g of food materials. The results revealed that, daily mortality of *Sitophilus zeamais* was dependent on concentration with maximum kills occurring on day 3 of exposure. Mortality of *S. zeamais* was either absent or minimal on the first two days of exposure. This however, contrasted the pattern of mortality exhibited by *Callosobruchus maculatus* which showed a negative daily mortality with increased concentrations compled with maximum daily mortality occurring in the first two days of exposure.

Key words: Daily mortality, *Sitophilus zeamais*, *Callosobruchus maculatus*, concentrations

Introduction

The use of botanical insecticides is fast gaining ground in the effort to control if not to eradicate the incidence of pests both in field and storage in order to ensure food abundance in the fast growing world population, especially in the third world countries. In view of the numerous successes and near successes already recorded on the use of botanicals as alternative insecticides to chemical insecticides, the need to ascertain the daily responses of some notable insect pests of stored grains have necessitated this bioassay.

The toxicological effect of botanicals is traceable to the presence of secondary compounds (metabolites) present in the plants. According to Sallam (1999), the presence of secondary compounds, which do not have known function in photosynthesis, growth or other aspects of plants physiology, give plant materials or their extracts their anti- insect activity. KeAOEta *et al*. (2000), noted that these secondary compounds are volatile metabolites that plants produce for their own needs other than nutrition (i.e., protestant or attractants). In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. Secondary compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals. They can affect insects in several different ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagsostimulants, antifeedants or modify oviposition (KeAOEta *et al*., 2000). Bell *et al*. (1990) stated that, recent surveys of desert and semi-desert plants have revealed a range of sesquitepenes, benzopyrans chromenes and prenylated quinones that are repellent or cytotoxic.

Noting these secondary metabolites in plant materials, which confer their anti-insect activity on them, it is therefore imperative to demonstrate a pattern of mortality on range of insects. Many

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workers have developed the concept of LD_{50} and LC_{50} for most tested plant materials found effective against target insects, but the daily mortality effect seems to be elusive, the justification for this research.

The test organisms in this bioassay are cowpea weevil, (*Callosobruchus maculatus*) a bruchid and the maize weevil, (*Sitophilus zeamais*), a curculionid. The cowpea weevil infests both pods in the field and seeds in storage (Stoll, 1988). Egbuchua (1980) and Tanzubil (1991) found that this insect can damage 100% of stored seeds of cowpea causing weight losses of up to 60% infested after 3-5 months of storage in West Africa.

The maize weevil is a field to store pest (i.e., primary pest) which attack stored maize leaving circular holes on the surface of the grain and make tunnels below the seed coat (Macdonald, 1984). Kossou *et al*. (1993) stated that maize weevils are distinquishable from all other common storage pest by their long beak or rostrum. These are found in all warm and tropical parts of the world and have been found as causing heavy loses to farmers every year.

Promising biological insecticides for the control of *Sitophilus zeamais* have been reported by various workers (Lale, 1995). Su (1977) reported the efficacy of Piper nigrum on *Sitophilus zeamais*. Shikaen and Uvah (1992) reported the protection of maize grains against *S. zeamais* with burnt stem of pawpaw and attributed its efficacy to deference of oviposition and adult emergence. Sowumi and Akinnusi (1983) reported that kernels of *Azadirachta indica* to control *Callosobruchus maculatus* were attributable to the protectant effect of the presence of azadirachtin. Schmutterer (1990) stated that, the neem insecticide does not have an immediate knockdown effect on pests, but reduces feeding and death occurs within several days and the residual effect may persist for two to seven days. Fatope *et al*. (1995) started that 10% (w/w) died powder (shoot of *Hyptis suaveolens* admixed with cowpeas reduced damage by adult *C. maculatus* for a period of four months. Peerzada (1997) revealed the constituents of *H. suaveolens* to be mainly 32% 1, 8-cineole and 29% Caryophyllene. Aggarwal *et al*. (2001) had shown the toxicity of 1, 8-cineole towards three species of stored product Coleopterans of which *C. maculatus* is one. Despite these successes, the daily mortality response and the duration of activity of these constituents are yet to be elucidated.

Materials and Methods

Collection and Culturing of Specimen Insects
(*Sitophilus zeamais*; *Callosobruchus maculatus*)

The adult *Sitophilus zeamais* and *Callosobruchus maculatus* used were collected and cultured from infested stored maize and cowpea seeds obtained from Oba market in Benin City, Nigeria. These maize and cowpea weevils were introduced into uninfested maize grains and cowpea, in two separate 1 L kilner jar covered with muslin cloth which prevented the insects from escaping and also allowed for aeration. These were used as stock cultures.

Both cultures were maintained at ambient temperature of between 28-30°C at a relative humidity of 70-80% Egbuchua (1980). New generations of both cultures were obtained and sustained by the replacement of devoured grains with fresh undevoured ones. These were maintained for sometime and the new emergences from the subcultures were then used for the experiment in the Postgraduate Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City.

Collection of Seeds

The maize grains and the bean seeds used were purchased from Oba Market in Benin City. Both the maize grains (local variety) and the bean seeds (Mala local variety from Kano) were white in colour. These were properly hand-picked and sieved. This ensured that only wholly and uninfested grains and seeds were used. These were then kept in a deep-freezer for two weeks to kill any immature stages

Table 1: Name of plants and parts used

Scientific name	Common name	Family	Part used
Azadirachta indica	Neem	Meliaceae	Leaves
Hyptis suaveolens	Curry leaf	Lamiaceae	Leaves
Ocimum gratissimum	Scent leaf	Lamiaceae	Leaves

of the insects if any, followed by air-drying in the laboratory for 7 h. This drying exercise helped to prevent moldiness in the products. Disinfested maize grains and cowpea seeds were then weighed using digital weigh balance model TS400D (precision standard) into 80 g in triplicate for each concentration and then stored in cooled dried place.

Plant Materials: Collections and Preparations Collection

The fresh leaves of *Azadirachta indica* (Dongoyaro) and *Ocimum gratissimum* (Scent leaf) were collected from Odigie Street,G.R.A. Benin City. Fresh leaves of *Hyptis suaveolens* (Curry leaf) were obtained from cultivation of seeds collected from Kogi State, Nigeria (Table 1).

Preparations of Plant Materials

Matured leaves of the plant species were sun-dried for three days. The sun-dried leaves were ground using a domestic electric blender into powder form. The ground fom of the different plant species were then weighed into portions of 1.5, 2.5 and 3.5 g and replicated thrice per plant type, per concentration using a digital weigh balance model TS400D (precision standard). These were then used for admixture with disinfested maize grains and bean seeds.

Method of Examination of Plant Materials: Direct Admixture of Plant Materials with Disinfested Maize Grains and Cowpea Seeds

1.5 gram ground plant materials was replicated 3 times for each plant type in 80 g of disinfested maize grains and also for 80 g disinfested cowpea in a plastic dish with lid. This was repeated with concentrations of 2.5 and 3.5 g. The mixtures were thoroughly mixed using a glass rod. Ten newly emerged adults of *S. zeamais* were introduced into each plastic dish and covered. Adult mortality in each treatment was recorded at 1, 2, 3 and 7 days post treatment according to duration in FAO bulletin No. 137 (1999).

For all plant types at the different concentrations, a similar procedure was also carried out using *C. maculatus*. Three controls each were set up for both but lacked plant materials.

Statistical Analysis

All data collected were subjected to One-way analysis of variance (ANOVA) procedure of Little (1978) at 5%. Where there were differences, Duncan Multiple Range Test (DMRT) was used to determine whether there were significant differences within the source of variation used. Also, for easy deduction of results, the use was made of bar charts.

Results and Discussion

The use of indigenous plant products and locally available materials to protect stored cereals and legumes has been reported by many workers (Golob *et al*., 1982; Lale, 1995).

This present research revealed that the plant materials (powders of leaves) exerted mortality on the insects under considerations. It also showed that concentrations and plant types have affected the daily kills that were recorded in course of the study.

The powders of leaves of the three plants; *H. suaveolens*, *O. gratissimum* and *A. indica* did not cause any death of *S. zeamais* within the first two days of treatment at 1.5 g concentration. At days

3 and 7 however, considerable deaths were recorded with more occurring at day 3. As concentration increased to 2.5 g plant materials *O. gratissimum* effected mortality first day while *H. suaveolens* and *A. indica* caused death from day 3. The death pattern exhibited by the three plant species in powder forms at concentration of 3.5 g followed the same pattern as in 2.5 g. This suggests that there is a concentration of plant material that must probably be present for the test organism to be able to take up dosages that cause death. When such concentration is inadequate or exceeded, death may not necessarily occur to target organism as exemplified by *S. zeamais* in Fig. 1. Also, the number of target organisms that dies daily on exposure to the plant materials are characteristic inherent in the tested plants.

The performance of the tested plants: *H. suaveolens*, *O. gratissimum* and *A. indica* against *S. zeamais* as indicated in Fig. 1 showed that *O. gratissimum* was more effective in causing the death of *S. zeamais* in all considered concentrations and its mortality ability is optimum on day 3 of exposures. This however was different with *H. suaveolens* which exerted optimum mortality against *S. zeamais* at 1.5 and 2.5 g on day 3 with no record of death in days 1 and 2. The mortality effect of *A. indica* against *S. zeamais* on the other hand, was optimal at days 3 and 7 which to a large extent corroborates with Schmutterer (1990).

The mortality pattern of *C. maculatus* exposed to the plant species showed a reversed trend as exemplified by *S. zeamais*. The daily mean mortality of *C. maculatus* exposed to the plant species are presented in Fig. 2. It shows that daily mortality decreased with days of exposure in all the concentrations with more occurring in days 1 and 2. Apart from *A. indica* which exerted it optimal mortality at day 3 when the concentration was increased from 1.5 to 2.5 g, increase in the concentrations of *H. suaveolens* and *O. gratissimum* did not result in corresponding increase in mortality of *C. maculatus* rather a decrease in mortality was observed when concentrations were increased from 2.5 to 3.5 g.

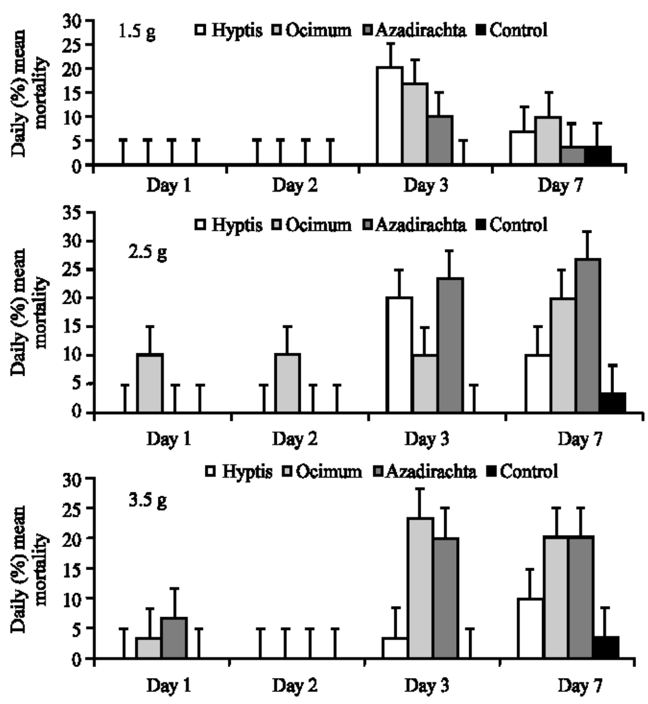

Fig. 1: Daily percentage mean mortality of *Sitophilus zeamais* at 1.5, 2.5 and 3.5 g per plant species

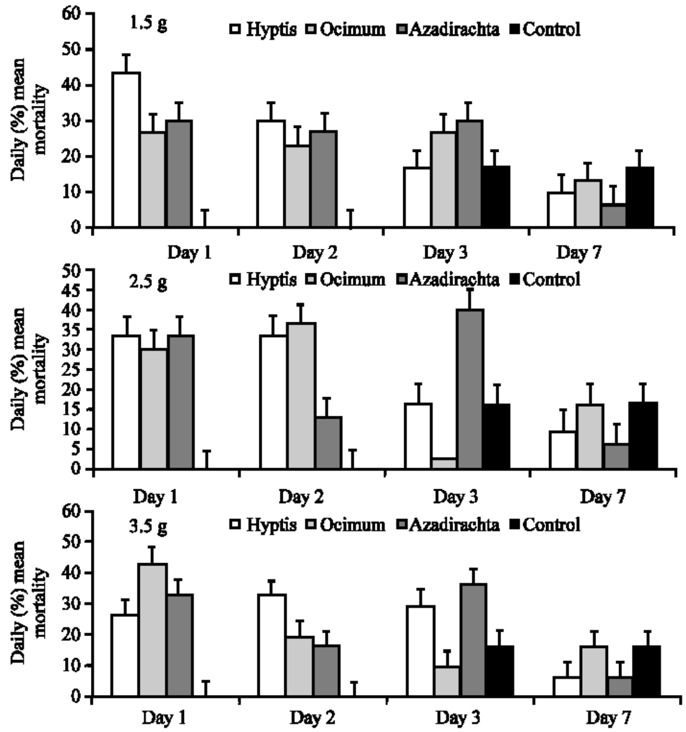

Fig. 2: Daily percentage mean mortality of *Callosobruchus maculatus* at 1.5, 2.5 and 3.5 g per plant species

The mortality exerted by the plant species considered in powder forms is adducible to a number of mechanisms. Firstly, being powders, they could block spiracles of the insects, thereby depriving adequate respiration. This would result in death by shocking of the insects. Secondly, while feeding, the adult weevil (*S. zeamais*) and the larvae of (*C. maculatus*) could pick up a lethal dosage, thus, resulting in stomach poisoning. Thirdly, the presence of azadirachtin in *A. indica* (Schmutterer, 1990) and 1, 8-cineole together with Caryophyllene (Peerzada, 1997) in *H. suaveolens* which exhibit deterrence and antifeedant properties could have prevented the insect pests from feeding. This would invariably lead to starvation, thus leading to death.

The result obtained in this study gives a simple daily expectation of an entomologist on exposures of these notorious insects to botanicals. It is expected therefore, that the daily responses of these insects to the main constituents of the considered plant species be experimented.

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