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Effect of Age of *Eugenia aromatic* Powder on the Control of *Callosobruchus maculatus* and *Sitophilus zeamais*

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The shelf life of *Eugenia aromatica* in the control of *Callosobruchus maculatus* and *Sitophilus zeamais* was considered from its formulated dusts that was prepared and preseved for 5 years. Dry fruits of *E. aromatic* was obtained, pulverized and sieved to a particle size of 150 µm which was preserved in a air-tight plastic container under ambient laboratory conditions. To determine the efficacy of the powder, it was applied at one month later at 12 month ınterval for 5 years on a culture of cowpea storage beetles, *C. maculatus* and maize weevils, *S. zeamais* in No. 1 Kilner jars in the laboratory. The efficacy of the plant powder against *C. maculatus* and *S. zeamais* was measured by beetle mortality after 48 and 96 hours post-treatment respectively during which high insect mortality was observed at all stages of treatment applications. Also the number of eggs laid by introduced females, number of F1 beetles and seed holing after removal of F1 individuals were also determined for the control of *C. maculatus* while the number of F1 beetles and grain weight loss after removal of F1 individuals were determined for *S. zeamais*. General observations showed that there was no significant difference in the efficacy of *E. aromatic* with respect to all the parameters obtained on the two insect pests studied throughout the experiment. Thus, this study

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gave an insight to the proficient use of *E. aromatic* in storage pest control and well as the shelf life of its active ingredient.

Keywords: Formulated dusts; Insecticidal plants; maize; cowpea; powder; shelf life.

1. INTRODUCTION

Stored products are attacked by many pests that cause serious damages to the products. Amongst these are storage pests which are often considered to be field-to-store pests. Rawsley [1] reported that *S. zeamais* has been identified as the most primary pest of stored maize in Ghana where it causes about 35% grain loss within or less than eight weeks of storage in the Euro-bran of most small scale farmers while Gallo et al. [2], 2002 reported that losses in warehouses reach figures of 10%; in Brazil and the losses are around 20% since the storage conditions in the countryside are poor. The maize weevil, *S. zeamais* is described as one of the most destructive pests in tropical and sub-tropical regions, Caswell [3]. This pest causes direct damage to grains, causing loss of viability and contamination of produce with excrements, lowering the quality and price of produce Agboola [4], Ivbijaro et al. [5], Lale [6]. Dick [7] also reported the mode of infestation of *Sitophilus* weevils where they infest grains only after the moisture content had dropped to 50%.

Mason [8] reported *S. zeamais* as a major pest of maize and can be found in numerous tropical areas around the world, including the United States. The maize weevil has been observed to infest other types of stored, processed cereal products such as pasta, cassava and various coarse, milled grains and also known to attack fruit while in storage, such as apples, Meikle [9].

S. zeamais occurs throughout warm, humid regions around the world, especially in locations where maize is grown Danho et al. [10]. The maize weevil commonly attacks standing crops in particular, maize before harvest Peng et al. [11]. It can as well breed in crops with a moisture content of a much wider range than *S. oryzae.* Although the maize weevil cannot readily breed in finely processed grains, it can easily breed in products such as macaroni and noodles, and milled cereals that have been exposed to excessive moisture, Nardon and Nardon [12].

According to Nardon and Nardon [12], early detection of infestation is difficult but becomes evident when *S. zeamais* larvae feed on the interior of individual grains, leaving a flour-like grain dust, mixed with frass and holes through which adults emerge. Nardon and Nardon [12], also indicated that damaged maize grains can be detected by floatation method. Considering the extent of damage and losses encountered from the infestation of *S. zeamais*, the control of the pest becomes inevitable.

The conventional control methods earlier adopted in the control of storage insect pests had gained attention in the last three decades and several plant parts had been adopted. Lale [6,13] demonstrated repellency and oviposition deterrence of powdered dry chilli pepper fruits to adults of *C. maculatus.* Ofuya [14] also reported that powders made from *Nicotiana tabaccum* L., *Erythrophleum suaveolens* Brenan and *Ocimum gratissinum* L. significantly reduced oviposition and egg hatch in *C. maculatus*. Consequently, pulverized plant parts have also been found effective in the control of storage pests of cowpea. Aku et al. [15] reported that annonacin obtained from the root bark of *Annona senegalensis* L. and applied as powder, significantly reduced oviposition and adult emergence in *C. maculatus,* and seed weight loss caused by the pest. Several other research works had also consolidated the assertion of efficiency of different plant plants in various forms in the control of both field and storage pests. For more efficiency, the conventional approach to insect pest control had focused on the extraction and formulation of the active ingredients. Although most insecticidal plant powders have generally been recommended for use as undiluted active materials but there have been considerable efforts to formulate active ingredients in inert materials as synergists. This approach had been reported to be highly effective in terms of activity and cost.

Researches have further shown that insecticides age over time with a concomitant reduction in efficacy. This concept informed this research work which was then focused on the determination of the shelf life and efficacy of formulated dusts of *E. aromatica* powder in the control of *C. maculatus* and *S. zeamais*, storage pests of Cowpea and maize respectively.

2. MATERIALS AND METHODS

2.1 Study Site

The research work was conducted at the Laboratory of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

2.2 Insect Culture and Crop Varieties

The cowpea storage beetle, *C. maculatus* and the maize weevil, *S. zeamais* were cultured using Ife Brown cowpea, a well-known susceptible variety for culturing *C. maculatus* and local white susceptible maize was used for *S. zeamais* culture. These seeds were used as substrates in No. 1 Kilner jars. 20 males and 20 females of each insect pest were selected and introduced into the substrates and covered with meshed lids in an open laboratory throughout the period of the study. This set up was in conformity with [13] standard procedure for culturing seed beetles. Any batch to be used was first disinfested by deep-freezing for two weeks and acclimatized to the open laboratory conditions for 24 hours.

2.3 Preparation of Insecticidal Plant Powders

Dry fruits of *E. aromatic* were obtained from "Oja Oba", Ikare-Akoko, Ondo State, Nigeria. The identity of the plant material was confirmed at Obafemi Awolowo University Herbarium, Ile-Ife, Nigeria. The plant parts were further dried in an oven at 80ºC for 24 hours, pulverized and sieved to obtain a particle size of 150 µm. The plant powder was put in a plastic bucket with lid, and kept under ambient laboratory conditions.

2.4 Affirmation of Insecticidal Action of *Eugenia aromatic*

E. aromatica powder was tested at the reportedly effective rate of 0.4 g/20 g of grain i.e. 20 g/1 kg of grain [16]. Ten freshly emerged adults (5 males and 5 females) of the pest beetles were used to infest grain in plastic plates (8.5 cm diameter) with lids. There were five replicates per treatment including the control (uninfested grain). Efficacy of plant powder against *C. maculatus* was measured by beetle mortality after 48 hours post-treatment; number of eggs laid by introduced females; number of F_1 beetles and seed holing after removal of F_1 individuals. Efficacy of plant powder against *S. zeamais* was measured by beetle mortality after 96 hours post-treatment; number of F_1 beetles and grain weight loss after removal of F_1 individuals.

2.5 Efficacy of Differently aged *E. aromatica* Insecticidal Powders in the Control of *C. maculatus* and *S. zeamais*

Differently aged powders of *E. aromatica* prepared at different times have been kept at the Research Laboratory of Department of Plant Science and Biotechnology and were tested for contact toxicity at 0.4 g of material per 20 g of seed in separate plastic Petri plates (8.5 cm diameter). Ten couples (twenty individuals) of *C. maculatus* (aged 1 - 2 days old) and twenty unsexed individuals of *S. zeamais* (aged < 7 days) were introduced into each plate that was subsequently shaken.

For *C. maculatus*, adult mortality was monitored in 48 hours and thereafter all insects were removed. The number of eggs laid by the female beetles on the seeds was then counted. The number of adults that emerged from these eggs was also counted as from three weeks after introducing the beetle couples unto the seeds.

For *S. zeamais* adult mortality was monitored for 7 days after which all the beetles were removed. F_1 adult emergence was then observed and the number counted. There was a control treatment involving no addition of plant powder onto seeds. Each treatment including the control was replicated three times.

A market survey on the shelf-life of synthetic dusts was conducted in three different towns of Ondo State, Nigeria. These were Akure, Owo and Ikare-Akoko. Available synthetic dusts in the market were sampled for their active ingredients as well as shelf life as provided by the manufacturing or trading companies. This information was obtained from the labels on the different containers.

Data were subjected to analysis of variance (ANOVA) procedures. Where necessary, data were transformed before analysis. Where the ANOVA test indicated significant differences between treatments, Fisher' protected LSD and Tukey's honestly significant tests were used to separate treatment means of parameters obtained on the age of powders.

3. RESULTS AND DISCUSSION

3.1 Affirmation of Insecticidal Action of *Eugenia aromatica*

It was observed that *E. aromatica* produced 100% mortality of *C. maculatus* adults within 48 hours which was significantly higher than the zero mortality in the control as well as in *S. zeamais* within 72hr. It was also observed that in the control experiment where there was no treatment application, there was a significant devastation of the seeds with a great number of eggs laid by the insect pests, number of punctures in grain, high percentage of holed cowpea seeds after F_1 adult emergence as well as F_1 adult emergence from eggs laid. This observation shows the correlation between insect pest threshold and extent of damage.

Also, Table 2 which shows that the mean of *S. zeamais* F_1 adult emergence was significantly higher in the control than in other treatments further corroborates the correlation between insect population and economic damage.

3.2 Efficacy of *Eugenia aromatica* Insecticidal Powders Prepared at Different Times in the Control of *Callosobruchus maculatus* and *Sitophilus zeamais*

The efficacy of insecticides could be attributed to the presence of its active ingredient. This also explains the reasons behind the significantly higher number of insect eggs laid by *C. maculatus* in the control than in the treatments involving the use of *E. aromatica* powders where the its active ingredient had prevented crop seed devastation. In the experimental set up, it was observed that the mean percentage adult emergence from *C. maculatus* eggs laid on seeds treated with the differently aged *E. aromatica* powders was zero and was significantly lower than adult emergence in the control. Consequently, the mean percentage of holed seeds in treatments involving the differently aged *E. aromatica* powders was zero which was significantly lower than seed holing in the control. These significant differences in the parameters studied between the treated and untreated seeds (control) were in no doubt attributable to the efficacy of *E. aromatica* in controlling storage pests which corroborates other documentations on the adoptive use of *E. aromatica* in the control of storage pests.

Tables 1 and 2 show that all the differently aged *E. aromatica* powders caused 100% mortality in *C. maculatus and S. zeamais* within 48hr and 72hr respectively. This shows a significant difference compared to the zero mortality observed in the control. Significantly, more *S. zeamais* F_1 adults emerged in the control in comparison with zero adult emergences in treatments involving the use of the differently aged *E. aromatica* powders. In the same vein, there were significantly more *S. zeamais* punctures in grains in the control than in treatments involving the use of the differently aged *E. aromatica* powders. Also, values obtained on the mean percentage weight loss in grain treated with the differently aged *E. aromatica* powders showed a significantly lower percentage than in the control. The devastating effect of insects based on weak crop protection culminates into reduction in yield and yield parameters.

Table 1. Efficacy of *E. aromatica* insecticidal powders prepared at different times in the control of *C. maculatus* attacking stored cowpea

Age of powder	Mean % adult mortality in 48hrs (n = 20)	Mean no of eggs laid on treated seeds	Mean % of adults emerged from eggs laid	Mean % of holed seeds after F_1 emergence
> 1 month	100.0±.00b	15.7±2.19a	0±.00a	0±.00a
12 months	100.0±.00b	18.7±2.03a	0±.00a	0±.00a
24 months	100.0±.00b	17.0±2.08a	0±.00a	0±.00a
36 months	100.0±.00b	18.7±0.88a	0±.00a	0±.00a
48 months	100.0±.00b	18.3±1.45a	0±.00a	0±.00a
60 months	100.0±.00b	21.0±1.53a	0±.00a	0±.00a
Control	0±.00a	558.3±33.72b	62.3±2.96b	86.7±2.40b

Means in each column bearing the same letter are not significantly different at the 5 % level of probability by Tukey's test

Table 2. Efficacy of *E. aromatica* insecticidal powders prepared at different times in the control of *S. zeamais* attacking stored maize grain

Age of powder	Mean % adult mortality in 72 hr. (n = 20)	Mean no of F_1 adults emerged	Mean no of punctures in grains	Mean % weight loss after F_1 adult emergence
> 1 month	100.0±.00b	0±.00a	0±.00a	0.3±.00a
12 months	100.0±.00b	0±.00a	0±.00a	0.3±.03a
24 months	100.0±.00b	0±.00a	0±.00a	0.3±.06a
36 months	100.0±.00b	0±.00a	0±.00a	0.3±.03a
48 months	100.0±.00b	0±.00a	0±.00a	0.4±.03a
60 months	100.0±.00b	0±.00a	0±.00a	0.4±.03a
Control	0±.00a	403.7±11.46b	400.0±10.58b	73.3±3.53b

Means in each column bearing the same letter are not significantly different at the 5 % level of probability by Tukey's test

Insects are generally believed to be the most prolific organisms, Gasogo [17], Kozár et al. [18] This was observed in the experiment as *C. maculatus* was able to reproduce throughout the long period of the study. Several cases had been reported on the efficacy of active ingredients of plant parts in pest management. Such documentations which include the activity of geraniol against the larvae of the caterpillar *Lymantria dispar*, devastator of oak forests, [19], the insecticidal activity (insect repellent and fungicide) of citronellol, citronellyl formiate and citronellyl acetate [20], nematicidal activities of geraniol and citronellol against *Caenorrhabditis elegans* and *Pratylenchus penetrans* respectively Tsao et al. [21] Reeves et al. [22] and antioxidant activities of isomenthone and acaricidal activity of citronellol against *Psoroptes cuniculi* which was observed using linalool and citronellol (Keszei et al. [23] Perruci et al. [24]) are all in agreement with the efficacy of eugenol, the active ingredient of *E. aromatica*, in the control of storage pest of cowpea. Conversely, the shelf life of most of these active ingredients has not been documented. More importantly, it was observed that *E. aromatica* powder was still effective ın the control of *C. maculatus* and *S. zeamais* five years after the dry flower buds were pulverized. This efficacy shows that with proper storage for five years, Eugenol, the active ingredient of *E. aromatica* could still be effective ın the control of storage pests. Thus, this study had made a significant contribution in unraveling the shelf life of *E. aromatica* powders which hitherto appeared to either not to have been earlier investigated nor documented. Similarly, an important factor in the retention of insecticidal action of these powders may be attributed to proper packaging in plastic containers with tightly fitted lids in the laboratory which might have prevented the volatility of the active ingredient, Eugenol. This corroborates the earlier assertion of Pedigo and Rice [25], that good packaging and storage have long been known to be vital in preserving and maintaining activity of chemical insecticides. Consequently, it has also been reported that through cultural practices of cleaning the store between harvests, removing and burning infested residues, fumigating the store to eliminate residual infestations and the selection of only uninfested material for storage, the severity of a maize weevil infestation can be reduced by good store hygiene. Similarly, harvesting the maize as soon as possible after it has reached maturity will reduce the chances of attack by maize weevil and other storage pests and the use of resistant cultivars may also reduce the severity of an infestation. These are suggested ways of reducing the devastating effects of field-to store pests rather than expending a huge capital on their control.

Since eugenol had been found effective for five years in thıs study, ın comparison to patented synthetic pesticides in Nigeria through the manufacturer labels on dates of manufacture and expiration dates, ıt was observed that the active material in most of the insecticides also expired in three to five years. This study gives a head up on the adopted use of *E. aromatica* in storage pest control sınce it is readily available, cheap, bıodegradable coupled with its efficacy over a long period of time with good packaging and storage techniques.

4. CONCLUSION

The adopted use of *E. aromatica* in pest management had been consolidated but the determination of its shelf life is within laboratory evaluation. This experiment was reported to be within laboratory evaluation so its findings could serve as a corridor for further research works which could be extended to the packing and patenting of *E. aromatica* for storage pest control.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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