



ASSESSMENT OF INSECTICIDAL POTENCY OF EGGSHELL POWDER AGAINST MAIZE WEEVIL

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ABSTRACT: *Efficacy of chicken eggshell powder at concentrations 0, 0.25, 0.5, 1.0 and 2.0 g /100 g maize, for control of Sitophilus zeamais, were compared with permethrin at dosage rate of 0.167g / 100 g of yellow maize. Experiments were conducted at Biotechnology laboratory (NSPRI), Ilorin under ambient condition (26±3°C 65±5% RH). Mortality of S. zeamais increased with increasing dosages of the CESP as well as with days of exposure. CESP at 2.0g gives 96.67% mortality which is not significantly different from the positive control. The mean progeny emergence (0.00%), weight loss (1.11%), and percentage grain damage (19.68%) by S. zeamais was significantly ($P \leq 0.05$) decreased with increased dosage of CESP. However, CESP did not significantly affect seed germination. Total coliform count at 2.0 g (2.48×10^2) was significantly different from both positive and negative controls at $p < 0.05$. Therefore, CESP can serve as a good maize protectant which can be used as substitute for synthetic chemicals.*

KEYWORDS: Chicken eggshell powder (CESP), Permethrin, *Sitophilus zeamais*, Total Coliform



INTRODUCTION

Maize plant (*Zea mays*) is one of the oldest and the mostly cultivated world cereals. It belongs to the family Graminae and is one of the most important cereal crops in Nigeria. After wheat and rice, maize is the third most grown cereal (Lyon, 2000). The grain is utilized for human consumption and forms about 50% of livestock feeds (Longe, 2010). The grain is very nutritious, with about 70% carbohydrate, 4-5% fats and oils and 9.5 (Larger and Hills, 2001). It is an important source of protein ranking only after meat, fish and legumes in term of annual protein production. It also rich in vitamins and fats which makes the crop compare favorably as an energy source, with root and tuber crops per unit quantity (Dasbak *et al*; 2008). In developing world, about 50% of all maize is consumed by humans as food while 43% is fed to livestock and the remainder for industrial purposes (IITA, 2003). Though maize grain can be stored for a considerable period of time, preservation of quality during long term storage is a problem in many parts of the world (Gras *et al*; 2000). Degradation of grain quality is recorded as rodent and insect infestation, cracking of seeds due to over-drying, and contamination with mycotoxins caused by molds and bacteria (Boxall, 2001).

The maize weevil (*Sitophilus zeamais* Motschulsky) is a major pest of stored maize grains and its infestation causes severe postharvest losses of staple food crops in Nigeria (Oni and Ileke, 2008). Presence of the insects in the grain lowers its quality and value. It also brings about the establishment of mold infestations including *Aspergillus spp.* which produces aflatoxins making the grain unsuitable for food and feed (Kling, 2001). The destructive activities of this insect and other storage pests have been widely subdued by chemical control methods, such as fumigation of stored products with carbon disulphide and phosphine and dusting with pirimiphos methyl or permethrin (Adedire *et al.*, 2011). The problems of many synthetic insecticides however include high persistence in the soil after use, environmental pollution, high cost of application, and lethal effects on non-target organisms or directly toxic to users (Ofuya and Longe, 2009). There has been a steady increase however in recent times, in the use of naturally (plant and animal materials) available products as a cheaper and ecological safer means of controlling insect pests of stored grains, especially in the tropics with medicinal properties as grain protectants (Longe, 2010)

Chicken eggshell which form the outer crust of an egg is a non-edible product with limited use and value and is largely disposed as waste. It is necessary to find an alternative means which would transform the waste giving financial benefits to the egg processing company and also help to overcome the high disposal costs and environmental concerns (MacNeil, 2006) and serve as an alternative to synthetic chemicals. This study aims at evaluating the efficacy of chicken eggshell powder in controlling *S. zeamais* on maize grains and to assess the effect of the eggshell powder on adult mortality, seed damage, weight loss, and germination microbial load and progeny developments of the insects (*S. zeamais*).

Materials

Experimental site

The experiment was carried out in the Biotechnology laboratory of Nigerian Stored Products Research Institute (NSPRI), Ilorin, Kwara State.

Insect culture

Adults of *S. zeamais* was obtained from already existing culture in the Entomology laboratory of Nigerian Stored Product Research Institute, Ilorin, Kwara state Nigeria and was mass reared on yellow maize grains purchased from Ago-market in 1litre kilner jars covered with muslin cloth to serve as source of aeration and to prevent escape of the insects at temperature of $26\pm 3^{\circ}\text{C}$ and $65\pm 5\%$ relative humidity. The adult *S. zeamais* was sieved out after 14 days and the kilner jar was left undisturbed in the insectary. Newly emerged three days old insects were used for the bioassay (Odeyemi *et al.*, 2008).

Collection and preparation of grains

The purchased maize was sorted to separate the damaged grains from the wholesome grains and cold shocked for 14 days to ensure infestation free and were brought out thereafter to be equilibrated under atmospheric condition on the laboratory table before usage.

Preparation of eggshell powder

The eggshell used for this experiment was gathered from chicken eggs consumed at home. It was washed thoroughly under running water, blanched and air dried in the laboratory until crispy dried, it was grounded into powder with electric blender (LAPRIVA MODEL NO:LA-T4PN) and sieved 90 microns mesh size sieve to obtain fine powder. The powder was stored in an air tight container prior to use.



Plate 1: Chicken eggshell



Description of experimental set-up

Different dosages (0.25, 0.5, 1.0 and 2.0g) of the eggshell powder were weighed and was admixed separately to 100 g of wholesome maize in 360 ml cylindrical gas jars container and 100 g of another maize was weighed and admixed with permethrin 0.6% a.i (Rambo™) at dose rate of 0.167g / 100 g of maize for positive control and another lot of 100 g of maize that was not treated was placed in 360 ml jar for negative control. The containers with their contents (Maize and treatment) were gently shaken for 5 minute to ensure thorough coating of maize grains.

Ten 3-day old mixed sexed *S. zeamais* were introduced into containers containing treated maize and control and was covered with muslin cloth to allow aeration and prevent escape of insect. Three replicate of the treatment and controls (positive and negative) were laid out in completely randomized design (CRD) on the laboratory table. Adult mortality was assessed after 7 and 14 days of infestation. Dead insect was removed and live ones retained. The insect was confirmed dead when there was no response to probing with sharp pin at abdomen (Adedire *et al.*, 2011). The insects in the containers were sieved out at 14 days and the vials were closed and left undisturbed for 26 days (40 days of post treatment) and newly emerged adult insects were counted and the maize seeds were reweighed, the percentage loss in weight was determined and recorded using the method described by Odeyemi and Daramola (2000).

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

After re-weighing, the number of damaged grains were evaluated by counting wholesome and seed with weevil emergent holes. Percentage seed damaged was also calculated according to

The percentage seed damaged were also evaluated as follows:

$$\% \text{ seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total no of seeds}} \times 100$$

The percentage insect mortality was calculated according to Niber, (1994) as

$$\text{Mortality \%} = \frac{\text{Number of dead insect}}{\text{Total number of insect}} \times 100$$

Viability Assessment

Initial and final (40-day post treatment) seed germination was determined. 10 grains were randomly picked from each sub-sample per treatment after F1 progeny count. The seeds were placed on moistened cotton wool in 9 cm diameter disposable Petri dish which was humidified every 3 days. Germination count was taken on the 10th days (Rao *et al.*, 2006). Percentage seed germination was calculated using previously described method (Adedire and Akinkulere 2005)

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seed planted}} \times 100$$



Microbial Analysis

Microbiological analysis was done using pour plate method described by Society for General Microbiology (SGM, 2006)

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and where significant differences existed, treatment means were compared at 0.05 significant level using the New Duncan's Multiple Range Test (Zar, 1984).

RESULTS

Table 1: Effect of the eggshell powder on mortality, protection, Reproductive parameters of *Sitophilus zeamais*, and germinability of maize seed

Treat	Dosage	% Mortality		Percentage	Percentage	F1	Germination
ment	g	7	14	grain damage	weight loss	Emergence	
	/100g						
Co		14.44±6.26 ^a	24.44±8.18 ^a	73.33±16.11 ^b	4.44±5.27 ^{ab}	0.88±1.05 ^b	70.00±20.62 ^a
	0.25	58.89±30.59 ^b	80.00±37.08 ^b	43.52±12.30 ^{ab}	2.22±4.41 ^{ab}	0.00±0.000 ^a	65.56±26.03 ^a
E.G.S	0.5	86.67±16.58 ^c	92.22±13.02 ^b	52.32±11.10 ^{ab}	3.33±5.00 ^{ab}	0.00±0.000 ^a	65.56±30.05 ^a
	1.0	95.56±8.82 ^c	95.56±7.26 ^b	41.19±13.81 ^{ab}	3.33±7.07 ^{ab}	0.00±0.000 ^a	72.22±29.91 ^a
	2.0	96.67±7.07 ^c	96.67±5.00 ^b	19.68±8.21 ^a	1.11±3.33 ^a	0.00±0.000 ^a	75.56±26.98 ^a
RAM	0.167	96.67±5.00 ^c	98.89±3.33 ^b	38.78±7.05 ^{ab}	7.78±6.67 ^b	0.11±0.333 ^a	53.33±25.50 ^a

Each value is a mean ± standard error of three replicates. Mean followed by the same letter in a column are not significantly different (P<0.05) from each other using new Duncan multiple range test.

Co- Control, E.G.S – Eggshell powder, RAM-Rambo powder,

DAT-Days after treatment

Effect of the Chicken eggshell powders on the mortality of adult *S.zeamais*

Table 1 shows the percentage mortality count of adult *S. zeamais* exposed to the different concentrations of the chicken (*Gallus gallus*) eggshell powder. The different concentrations of the test plants exhibited varying degrees of insecticidal activities killing adult *S. zeamais* more than the control (p < 0.05). There were significant differences in the mortalities of *S. zeamais* with treated experiment compared to the untreated control.

There was no significant difference (0.05) in maize weevil mortality at 0.5, 1.0, 2.0 g /100 g of maize grain and the positive control (Rambo™ at dose rate 0.167g /100 g of maize) but are significantly different from the maize treated with 0.25 g/100g of maize (58.89%,) and



untreated control at 7 DAT. At 14 DAT there were significant differences in mortalities of *S. zeamais* with treated compared to untreated control. The chicken eggshell powders tested at various level showed the various bioactivity against *Sitophilus zeamais* and significantly ($P < 0.05$) reduce longevity of adult on treated maize grains. All the dosages of the powders showed weevil mortality ranging from 58.89 to 95.56% and 80.00 to 96.67% at 7 and 14 days after treatment respectively. Adult mortality increased as the dosages increases. The chicken eggshell powder was most effective against *S. zeamais* at dosage level of 2.0 evoking 96.67% mortality, after 7 and 14 days of exposure. This followed by dosage level of 1.0, 0.5 and 0.25 with mortality of 95.56%, 86.67%, 58.89% and 95.56%, 92.22% and 80.00 at 7 and 14 days after treatment respectively.

Progeny Development

The various dosages of the eggshell powder significantly ($P < 0.05$) reduced the progeny of *S. zeamais*. All dosages significantly prevent the emergence (0.00%) of *S. zeamais* compared to the control (0.88%) and positive control (0.11%). The percentage adult emergence in the untreated maize seeds was significantly different ($P < 0.05$) from emergence in the treated maize. The eggshell powder completely inhibited the development of *S. zeamais*.

Damage Assessment

Maize seeds treated with chicken eggshell powder showed significant difference ($P < 0.05$) in reduction of damage caused by *S. zeamais*. Compared to untreated control of highest damage of (73.33 %) damage occurred as revealed by the emergent holes.

Weight Loss Assessment

Maize treated with 2.0 g of the powder significantly reduced the weight loss compared to the control and positive control but there was no significant difference between dosage 0.25, 0.5 1.0 and control.

Viability Assessment

The effect of chicken eggshell powder on the viability of treated grains showed that none of the dosage level of the powder used adversely affected the viability of the maize grains when compared with the untreated control and the positive control after 1 months of storage (Table 3). Almost all the treated seeds germinated. There was no significantly different t ($P > 0.05$) in the mean percentage viability of control (untreated), positive control and the seed treated at four dosage levels of the chicken egg powder.

The high mortality of adult mortalities observed on maize treated with chicken eggshell powder could be due to the toxic effect of the product on *S. zeamais*. The analysis of the result showed that chicken eggshell powder at the tested dosages is good in managing *S. zeamais* in storage. In all dosages, Chicken eggshell powder at 2.0g/100g seed dosage or concentration had the highest potency of insecticidal properties, its effect was clearly observed on the mortality rate of the insect, oviposition deterrence, seed weight loss and damage and even its suppressive effects on the emergence of F1 progeny. From the mortality studies, the insect mortality increased with increasing application rate or dosage After 7 and 14 days of exposure, the efficacy of the chicken shell powder is very close to conventional insecticide, permethrin at dosage of 0.5, 1.0 and 2.0 in treated maize grain.



Application of the eggshell powder achieved significant reductions in the microbial populace associated with the grains. Different doses of the eggshell powder attained comparable quality indices to the chemical pesticide Rambo.

Bacterial load ranged between $1.20\text{-}2.71 \times 10^4$ cfu/g, with the EGS dose of 2.0g presenting best quality (1.20×10^4 cfu/g) below presented RAMBO quality of 1.78×10^4 .

The presence of coliforms poses significant health risks. There was a steady reduction in the total coliforms as the dose of treatment increased with the 0.25g and 2.0g of EGS powder to 3.90×10^2 - $2. \times 10^2$ cfu/g.

Table 2: Shows the microbiological analysis of the grains over the study period.

	Bacteria	Total Coliform	Feecal Coliform	Fungi
Control	2.71×10^4 ^d	4.01×10^2 ^d	1.60×10^2 ^c	1.41×10^4 ^a
0.25	2.21×10^4 ^c	3.90×10^2 ^{cd}	1.01×10^2 ^b	1.28×10^4 ^a
0.5	1.91×10^4 ^{bc}	3.67×10^2 ^{cd}	5.0×10^1 ^a	1.23×10^4 ^a
1	1.53×10^4 ^{ab}	3.44×10^2 ^{bc}	3.8×10^1 ^a	1.53×10^4 ^{ab}
2	1.20×10^4 ^a	2.48×10^2 ^b	0.00 ± 0.00 ^a	8.58×10^3 ^a
Rambo	1.78×10^4 ^{bc}	1.61×10^2 ^a	1.39×10^2 ^{bc}	1.14×10^4 ^a
Significant value	0.00	0.00	0.00	0,763

Mean followed by the same letter in a column are not significantly different ($P < 0.05$) from each other using new Duncan multiple range test.

There was a total elimination of fecal coliform contamination at the dose of 2.0g.

The applied chemical pesticide RAMBO™ was observed to have less anti fungi activity compared to the eggshell powder as the doses increased. No fecal contamination was seen in the initial sample, this could be as a result of the good handling and storage processes

The bacterial contaminant which had 1.5×10^4 and 3.7×10^4 is within the acceptable range of $10^3 \times 10^5$ (ICMSF, 1986)

It was observed that as the dose of treatment increases the microbial load was lowered establishing the efficacy of animal waste as safe bio preservatives. The treatment serves as antagonizes to microbial activity.

DISCUSSION

From the result obtained in this research, it was observed that eggshell powder has considerable effect on the mortality of adult *Sitophilus zeamais* and microbial load. Mortality of adult insects increased as the period of exposure and dosage of the eggshell powder increases. Eggshell powder has been reported to inhibit locomotion of adult insects (Adedire



et al., 2011; Ileke *et al.*, 2013). The weevils were unable to move freely because of the powder and this might have affected mating activities and sexual communication (Ileke and Olotuah, 2012).

Reduction in progeny growth may be due to early mortality and partial or complete retardation of embryonic development (Dike and Mbah, 1992).

The effect of the powder on adult weevil emergence might be due to the poor egg-laying capacity of the weevil and physiological changes induced by the powder.

The chicken eggshell powder tested at various level showed different bioactivity against *Sitophilus zeamais* at $p < 0.05$ reduce longevity of adult insect on treated maize grains.

The effect of the eggshell powder on viability of treated grains showed that none of the dosage level of the treatment used adversely affected the viability of the maize grains when compared with the untreated control and positive control after one month of storage.

The physical abrasion of the epicuticle lipid layer of insect pest caused by eggshell powder and active components in eggshell powder might have been responsible for insecticidal activities (Nwaubani and Fasoranti 2008). Thapon and Bourgeois, (1994) stated that eggshell powder consist of 94% calcium carbonate, 1% phosphate, 1% magnesium carbonate and 4% organic substance. The bacterial contaminant is within the acceptable range of $10^3 \times 10^5$ (ICMSF, 1986).

It was observed that as the dose of the treatment increases, the microbial load reduced establishing the efficacy of the chicken eggshell powder as a safe bio preservative. The treatment serves as antagonist to microbial activity.

CONCLUSION

This study establishes the insecticidal potency of chicken eggshell powder as a good maize protectant by killing the insects *S. zeamais*, controlling microbial population and still maintaining grain quality and ultimately extends the shelf life of stored maize grain. This means it can be used as substitute for synthetic chemical.

Future Research

There is an ongoing research to compare and contrast between the efficacy of chicken eggshell powder with other bioactive pesticides in the control of storage insects.



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