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Efficacy of Bitter Kola Seed Powder in Control of the Maize Weevils (*Sitophilus Zeamais*) Infesting Stored Maize (*Zea Mays L.*)

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Abstract:

This study investigated the efficacy of bitter kola seed powder in control of the maize weevils (*Sitophilus zeamais*) infesting stored maize. The experimental design adopted in this study was a completely randomized design (CRD), with one maize variety (Succharata), three treatment groups, and one (1) control group. All experimental treatments were replicated four times. Result showed that 120g of bitter kola seed powder gave the highest percentage mortality rate as well as the lowest percentage longevity rate of *Sitophilus zeamais*. Also, 120g bitter kola seed powder gave the least mean weight loss of 3.7g as well as the lowest mean number of punctures (13.4) on the maize grain with WPI of 2.2%. In addition to pest control, bitter kola seed is composed of valuable nutrients like dry matter (38.62%), carbohydrate (27.63%), fat (4.45%), fibre (0.16%), protein (0.011%), and ash (0.005%). It equally contains some bioactive substances such as alkaloids, tannins, flavonoids, saponins, terpenoids, and cardiac glycosides which could be responsible for the antimicrobial activities. Therefore, bitter kola seed powder can be considered as being safe for the control of *S. zeamais* infesting stored maize products in Nigeria. The study strongly recommended the use of bitter kola seed powder as repellents and plant-based preservatives for storage of maize (*Zea mays*) against maize weevil (*S. zeamais*).

Keywords:

Bitter Kola, Seed Powder, Sitophilus Zeamais, Stored Maize

1. Introduction

Bitter kola is an evergreen tree, with a heavy, spreading crown, that can grow up to 30 metres tall, but is more usually around 12 to 15 metres [1]. A multipurpose tree and an important plant in the local economy. The tree is often found cultivated around villages. The seeds are sold in local markets and occasionally exported to ethnic populations living abroad. Bundles of pencil-sized wood are commonly sold in local markets for use as chew sticks. The seeds are an important article of commerce being traded well beyond the distribution area of the tree [2]. Bitter kola is also known as African wonder nut. In Nigerian languages, it is commonly called Namijigoro in Hausa, Agbilu or Akilu in Igbo, and Orogbo in Yoruba. Garcinia kola has economic and cultural values across West and Central African countries where the nuts are commonly chewed and used for traditional ceremonies. The seeds are also used in folk medicine in many herbal formulations and have potential therapeutic benefits due largely to the activity of their flavonoids and other bioactive compounds [3].

Sitophilus zeamais Motschulsky is a serious pest of stored cereals. It attacks grains in the field and its infestation is carried into the store where it continues its destructive activities [4]. [5] reported that *S. zeamais* can infest non-cereals like yam and cassava chips. Owing to the economic importance attached to grains especially cereals in Nigerian agriculture, it became necessary to protect the grains and cereal grain products against damage by insect pests. The population dynamics of *Sitophilus* species is favoured in food materials that have more than 10% water content. They affect processed food materials and the nutritive content of the food materials by lowering their components [6]. Voracious feeding on whole grains by this insect causes product weight loss, fungal growth, quality loss through an increase in free fatty acids and it can even completely destroy stored grains in all types of storage systems/facilities. Invasion by this primary coloniser may facilitate the establishment of secondary and mite pests and pathogens. *S. zeamais* is an invader and enters packages (e.g. commercial rice or pasta) through existing openings that are created from poor seals, openings made by other insects or mechanical damage. This tarnishes brand image to the consumers, thus resulting in serious economic damage to food companies [4].

Pest control in Africa has mostly depended upon the use of synthetic chemical pesticides. However, fears concerning the effect on workers' health, increased pest resistance and the negative environmental impact of these chemicals have led to the development of safer, more environmentally acceptable and cost-effective control alternatives, especially the use of plant products. Most of these plants have known insecticidal properties or have been used against ectoparasites, and or as antihelmintics or vermicides [7].

In Nigeria, most farmers are unaware of the availability of numerous and easily available plant-based insecticides. Researchers have failed to take into consideration the field-to-store nature of most storage pests and the fact that most of these pests initiate infestation of the seeds in the field. It has been reported that most of the first-rate biopesticides had no answer to established infestations [8].

The search for effective plant-based insecticides must therefore be taken to ascertain the feasibility of storage pest management. Bitter kola, the test plant, is one of the most useful multi-purpose plants known to man. It has been found to be useful in the treatment of medical conditions like coughs, hoarseness, and bronchial and throat troubles, gonorrhoea, etc. [9]. Bitter kola seeds have also been presumed to prevent or scare insects and reptiles in the house. However, there has been no

practical proof or empirical suggestions to such cases. Against this backdrop, the present study tends to ascertain the efficacy of Bitter kola seed powder in the control of storage pests with unique emphasis to stored Maize weevil.

This study seeks to elucidate the efficacy of bitter kola seed powder in control of maize weevils infesting stored maize. The result of this study will reveal the insecticidal properties and potency of bitter kola for maize weevil infestation.

2. Materials and Method

2.1. Study Area

This research work was carried out in the Biology laboratory, School of Biological Sciences, Federal University of Technology, Owerri. The mean temperature of the laboratory ranges from 27°C-30°C and the mean relative humidity 81%- 90%. The Federal University of Technology Owerri lies between latitude 05° 21' and 05° 42'North and longitude 07° 48' and 06° 53'East. Owerri consists of tropical rainforest zone with average annual rainfall distribution of 2,250-2800mm. This region produces many agricultural products [10].

2.2. Collection of Experimental Materials

This study was carried out using: Maize grain, variety-Succharata (sweet corn), Bitter kola seed (*Garcinia kola*), White plastic plates, Masking tape, Permanent marker, Maize weevil (*Sitophilus zeamais*), White netting material, Rubber band, Weighing balance, Thermometer and Hygrometer.Maize grains which has not been treated with insecticide and maize weevil were procured from Ekeonunwa market in Owerri, Imo state; whereas bitter kola seed was purchased from Mami market at Obinze in Owerri, Imo State.

2.3. Preparation of Bitter Kola Seed Powder

Good seeds of the procured bitter kola (*Garcinia kola*) were selected, separated from the husks, and washed. The seeds were chopped into tiny pieces, air dried for 30 (thirty) days, and ground into powder using an electric blender into various grams of 40g, 80g, and 120g of bitter kola seed powder and placed in air tight containers for use.

2.4. Experimental Design

The experimental design adopted in this study was a completely randomized design (CRD), which is made up of one maize variety (succharata), three (3) treatment groups, and one (1) control group. All experimental treatments were replicated four (4) times with the following treatments; T_0 = 0g control, T_1 = 40g bitter kola seed powder, T_2 = 80g bitter kola seed powder, and T_3 = 120g bitter kola seed powder.

Treatments	R_1		R_2		R_3		R_4
T_0= 0g BKSP	T_0R_1		T_0R_2		T_0R_3		T_0R_4
T_1= 40g BKSP	T_1R_1		T_1R_2		T_1R_3		T_1R_4
T_2= 80g BKSP	T_2R_1		T_2R_2		T_2R_3		T_3R_4
T_3= 120g BKSP	T_3R_1		T_3R_2		T_3R_3		T_3R_4

Note: BKSP = Bitter kola seed powder

2.5. Experimental Procedures

Seed powder of *Garcinia kola* was applied to different containers, each containing 250g of maize grains weighed using an electronic weighing balance in the laboratory at the rates 40g, 80g, and 120g respectively. The plastic white plates were vigorously shaken for optimum coverage of the grain surfaces. Seed powder of *Garcinia kola* was not applied to the control plate. Thirty (30) males and thirty (30) females of maize weevil (*Sitophilus zeamais*) was introduced into each treatment group as well as the control group. Each treatment and control were replicated four (4) times. The containers were covered with white netting material and held in place with rubber bands. The plastic containers were labeled using masking tape and permanent marker accordingly. After every forty-eight hours, the mortality of *Sitophilus zeamais* in both treated plates and control place were observed and recorded. The sexes of *Sitophilus zeamais* was determined by examining the snout. The snout of females is longer and thinner while that of males are shorter and fatter. Also, females have smooth textured bodies while that of the males are rough [11]. (Figure 1)

Figure 1. *Experimental set-up.*

2.6. Parameters to Measure

2.6.1. Concentration of Bitter Kola Seed Powder

Bitter kola seed powder was introduced at the rate of 0g, 40g, 80g and 120g into different plastic containers with 250g of maize grain each with 30 maize weevils. After 8 weeks of observation, the concentration that showed more death of weevils in each treatment groupwere observed and recorded.

2.6.2. Mortality Rate of Maize Weevil

Mortality of *Sitophilus zeamaiswas* determined from weekly counts for dead adults for 8 weeks of observation,after which all surviving adults were removed as described by [10].The number of maize weevils that died in each treatment and controlwere counted and recorded as mortality rate for each plate. The percent mortality was calculated by the formula used by [12].

$$\text{Percent mortality} = \frac{\text{No. of dead insects}}{\text{Total No. of insects}} \text{x} \frac{100}{1}$$

2.6.3. Longevity of Maize Weevil

Alternative to mortality rate determination, longevity of maize weevil will be determined from weekly counts for surviving adults for 8 weeks of observation, after which all dead adults will be removed, the number of maize weevils that survived in each treatment and control will be counted and recorded as longevity rate for each plate. The percent longevity can be calculated as thus:

$$\text{Percent longevity} = \frac{\text{No. of survived insects}}{\text{Total No. of insects}} \text{x} \frac{100}{1}$$

2.6.4. Weight Loss of Maize Grain

The weight loss of maize grain was determined by measuring the initial weight of the maize grain before infestation minus the final weight of the maize grain after infestation by *Sitophilus zeamais* in each plate as described by *Nwachukwu et al*. (2020). The formula for weight loss can be stated thus:

$$\text{Weight loss} = (W_1 - W_2)$$

Where:

W1 = Initial weight of maize grains before infestation

W2 = Final weight of maize grains after infestation [11].

2.6.5. Punctures and Perforation Index on Maize Grain

The extent of weevil punctureswas assessed using the Weevil Perforation Index (WPI) and was recorded as a measure of damage to the grains. Grains that were riddled with punctures were counted. The weevil perforation index (WPI) to the grains was calculated the following formula as used by [12]:

$$\text{WPI} = \frac{\text{\% of treated grains perforated}}{\text{\% of control grains perforated + \% of treated grains perforated}} \text{x} \frac{100}{1}$$

2.7. Nutritional Analysis of Bitter Kola Seed Powder

Standard methods of the Association of Official Analytical Chemist were used to determine the crude protein, crude fat, total ash and crude fibre contents of the samples bitter cola seed powder. The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogen x 6.25) was also determined by the Kjeldahl method, using 2.0g samples, crude fat was obtained by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum boiling point range 40-600C as the extract. Ash content was determined by the incineration of 10.0g samples placed in a muffle furnace maintained at 5500C for 5hrs. Crude fibre were obtained by digesting 2.0g of samples with H2S04 and NaOH and incinerating the residue in a muffle furnace maintained at 5500C for 5hrs, total carbohydrate was obtained by different method.

2.8. Phytochemical Analysis of Bitter Kola Seed Powder

Phytochemical analysis of bitter kola seed powder for alkaloids, tannins, flavonoids, saponins, terpenoids and cardiac glycosides was carried out according to the procedures of [13].

2.9. Alkaloid

A few ml of seed powder was prepared; two drops of Mayer's reagent were added along the side of the test tubes. 1.0ml portion was treated similarly with Dragenduff's reagent. Appearance of white creamy precipitate indicates the presence of alkaloids.

2.10. Tannins

0.5g of powered sample of the seeds were boiled in 20ml of distilled water in a test tube and filtered 0.1 % $FeCl_3$ was added to the filtered sample and observed for brownish green or a blue-black colouration which shows the presence of tannins.

2.11. Flavonoids

Exactly 20mg of bitter cola seed powder were dissolved in 1ml of distilled water. Precisely 0.5ml of dilute ammonia solution was added to it and concentrated sulphuric acid was added later. A yellow colour indicated the presence of flavonoids. The yellow colour disappeared on allowing the solution to stand.

2.12. Saponins

Closely 2g each of powered sample seed was boiled together with 20ml of distilled water in a water bath and filtered 10ml of the filtered sample was mixed with 5ml of distilled water in test tubes and shaken vigorously to obtain a stable persistent forth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicated the presence of saponins.

2.13. Terpenoids

Precisely 20mg of seed powder was dissolved in l ml of chloroform and 1 ml of concentrated sulphuric acid was added to it. A reddish brown discolouration at the interface showed the presence of terpenoids.

2.14. Cardiac Glycosides

Exactly 20ml of the seed powder was dissolved in lml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. Closely 0.5ml of concentrated sulphuric acid was slowly added along the side of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides, or cardiac glycoside constituent.

2.15. Phlobatannins

An aqueous extract of the seed samples of bitter kola was boiled with 1% aqueous hydrochloric and deposition of a red precipitate was taken as evidence for the presence of phlobatannin.

2.16. Statistical Analysis

The data obtained weresubjected to analysis of variance (ANOVA),procedure using SAS package 20 and the effectiveness of the treatment means will be compared using the least significant difference (LSD);where significant differences existed, treatment means will be compared at 0.05 significant level using the New Duncan's Multiple Range Test [12].

3. Results and Discussion

3.1. Results

3.1.1. Nutritional Composition of Bitter Kola Seed Powder

Results from the proximate nutritional composition of bitter kola seed powder were presented in Table 1. The result showed that bitter kola seed is made up of 38.62% of dry matter, 27.63% of total carbohydrate, 4.45% of crude fat, 0.16% of crude fibre, 0.011% of crude protein, and 0.005% of ash.

Table 1. *Proximate Composition.*

S/N	Constituents	Bitter Kola
1	Dry matter (%)	38.62
2	Crude Protein (%)	0.11
3	Crude Fibre (%)	0.16
4	Ash (%)	0.005
5	Crude Fat (%)	4.45
6	Total Carbohydrate (%)	27.63

3.1.2. Phytochemical Composition of Bitter Kola Seed Powder

Results of phytochemical composition of bitter kola seed powder were presented in Table 2. The result indicated the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, and cardiac glycosides. However, the result indicated that phlobatannin was absent.

Table 2. *Phytochemical Composition.*

S/N	Parameters	Bitter Kola
1	Alkaloids	+
2	Tannins	+
3	Flavonoids	+
4	Saponins	+
5	Terpenoids	+
6	Cardiac glycosides	+
7	Phlobatannins	-

3.1.3. Effect of bitter kola powder on Sitophilus zeamais

The effect of bitter kola seed powder against *Sitophilus zeamais* infestation on maize grain is shown in Table 3.The result shows that 120g of bitter kola seed powder had 100% mortality of *S. zeamais* at 4 weeks after application, while 80g of bitter kola seed powder had 100% mortality of *S. zeamais* at 6 weeks after application. The control treatment (0g) recorded no mortality effect at 2 weeks, 4 weeks, 6 weeks and 8 weeks respectively (Table 3).

Table 3. *Effect of bitter kola seed powder on Sitophilus zeamais infesting maize grain.*

Bitter kola seed powder	Number of Weevils introduced	Mortality in Weeks				Mortality (%)
		2	4	6	8	
0g	30	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
40g	30	7 (23)	9 (30)	5 (17)	2 (2)	23 (77)
80g	30	13 (43)	11 (37)	6 (20)	Nil	30 (100)
120g	30	17 (57)	13 (43)	Nil	Nil	30 (100)

3.1.4. Effect of Bitter Kola Seed Powder on Longevity of Sitophilus Zeamais Infesting Maize Grain

The result in Table 4 shows the effect of bitter kola seed powder on percentage longevity of Sitophilus zeamais at 2 weeks, 4 weeks, 6 weeks and 8 weeks after application of bitter kola seed powder. The result shows that 120g of bitter kola seed powder gave the lowest percentage longevity rate at 2 weeks (43.3%), 80g (56.7%), and 40g (76.7%) and 0g (100%) life expectancy respectively. Similar trend was observed at 4 weeks while 6 weeks and 8 weeks after application of 120g bitter kola powder, there were no life expectancy (Table 4). The overall mean longevity shows that 120g of bitter kola seed powder (25%), 80g (50%), 40g (81%) and 0g (100%) life expectancy.

Table 4. *Effect of bitter kola seed powder on longevity of Sitophilus zeamais.*

Bitter kola seed powder	Number of Weevils introduced	Percentage Longevity in weeks				Mean Longevity (%)
		2	4	6	8	
0g	30	100	100	100	100	100
40g	30	76.7	70	83.3	93.3	81
80g	30	56.7	63.3	80	Nil	50
120g	30	43.3	56.7	Nil	Nil	25

3.1.5. Effect of Bitter Kola Seed Powder on Weight Loss of Maize Grain, Number of Punctures and Weevil Perforation Index

Table 5 showed the results of mean weight loss, mean number of punctures and weevil perforation index (WPI) against bitter kola seed powders at 0g, 40g, 80g and 120g respectively at p = 0.05 level. The results reveal that the mean weight loss was high on control treatment (0g) followed by 40g bitter kola seed powder (57.4g), 80g bitter kola seed powder (37.3g), and the least was 120g bitter kola seed powder (3.7g). Similarly, the mean number of punctures on the maize grain and weevil perforation index (WPI) showed the save trend of which 120g of bitter kola seed powder gave the lowest mean number of punctures (13.4) with WPI of 2.2% (Table 5).

Table 5. *Effect of bitter kola seed powder on mean weight loss of maize grain, number of punctures and weevil perforation index (WPI).*

Bitter kola Seed powder	Initial weight of maize Grain (g)	Finial Weight (g)	Mean weight Loss (g)	Mean number of punctures	WPI (%)
0g	250	102.9^{d}	147.1 ^{a}	441.9 ^{a}	71.1
40g	250	192.6^{c}	57.4 ^{b}	179.3 ^{b}	28.9
80g	250	212.7^{b}	37.3 ^{c}	112.6 ^{c}	18.1
120g	250	246.3^{a}	3.7 ^{d}	13.4 ^{d}	2.2

3.2. Discussion

Table 1 result showed that bitter kola seed is made up of 38.62% of dry matter, 27.63% of total carbohydrate, 4.45% of crude fat, 0.16% of crude fibre, 0.011% of crude protein, and 0.005% of ash. Contrary to the study of Mazi*et al*. (2013), bitter kola is made up of carbohydrate (70.31%), crude protein (11.27%), moisture content (9.28%), ash (4.17%), crude fiber (3.94%) and ether extract/fat (1.03%).

Table 2 result indicated the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, and cardiac glycosides. However, the result indicated that phlobatannin was absent. [14] noted that the seed of *G. kola* contains some bioactive substances such as bioflavonoids triterpenes, tannins, saponins, glycosides and alkaloids which could be responsible for the antimicrobial activities.

Table 3 result displayed that bitter kola seed powder 80g and 120g each had 100% mortality of *S. zeamais* at 4and6 weeks after application respectively, as against the control treatment which recorded no mortality effect between 2 to 8 weeks of the trial. This could be due to the presence of phytochemicals and bioactive substances in the bitter seed powder. Similarly, the study of [15] revealed that use of bitter kola were found to be active in suppressing the growth and development of maize weevil.

Table 4 result shows that 120g of bitter kola seed powder gave the lowest percentage longevity rate at 2 weeks (43.3%), 80g (56.7%), and 40g (76.7%) and 0g (100%) life expectancy respectively. However, the overall mean longevity shows that 120g of bitter kola seed powder (25%), 80g (50%), 40g (81%) and 0g (100%) life expectancy. In the same way, the study of [16] found that the use of bitter kola decreased the growth and longevity of maize weevil.

Table 5 result revealed that the mean weight loss was high on control treatment (0g) followed by 40g bitter kola seed powder (57.4g), 80g bitter kola seed powder (37.3g), and the list was 120g bitter kola seed powder (3.7g). Similarly, the mean number of punctures on the maize grain and weevil perforation index (WPI) showed the save trend of which 120g of bitter kola seed powder gave the lowest mean number of punctures (13.4) with WPI of 2.2%. This is also in line with Arannilewa et al. (2006) who reported that bitter kola has the potential of bio-insecticide for protecting maize grains from *S. zeamais*.

4. Conclusions

The evaluation for the efficacy of bitter kola seed powder in control of the maize weevils infesting stored maize showed that 120g of bitter kola seed powder gave the highest percentage mortality rate as well as the lowest percentage longevity rate of *Sitophilus zeamais*. Also, 120g bitter kola seed powder gave the least mean weight loss of 3.7g as well as the lowest mean number of punctures (13.4) on the maize grain with WPI of 2.2%.

In addition to pest control, bitter kola seed is composed of valuable nutrients like dry matter (38.62%), carbohydrate (27.63%), fat (4.45%), fibre (0.16%), protein (0.011%), and ash (0.005%). It equally contains some bioactive substances such as alkaloids, tannins, flavonoids, saponins, terpenoids, and cardiac glycosides which could be responsible for the antimicrobial activities.

Therefore, bitter kola seed powder can be considered as being safe for the control of *S. zeamais* infesting stored maize products in Nigeria.

5. Recommendation

The results of this study strongly suggest the possibility of using bitter kola seed powder as repellents and plant-based preservatives for storage of maize (*Zea mays*) against maize weevil (*S. zeamais*).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author Contributions

This work was carried out in collaboration among all authors. Author NMO and UAA designed the study, performed the statistical analysis, wrote the protocols, managed the literature searches, interpretation and wrote the manuscript. Authors NMO, JNA, ALA and OPC managed the analysis of the study and supervised collection of data from the field. All authors read and approved the final manuscript.

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