

RESEARCH PAPER

## Efficacy of powder formulations of some tropical flora and clay against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) on maize in storage

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### ABSTRACT

A study was carried out to determine the efficacy of three plant materials; pawpaw (*Carica papaya*) seed powder, kapok tree (*Ceiba pentandra*) leaf powder, soursop (*Annona muricata*) leaf powder as well as clay and Permethrin dust against *Sitophilus zeamais* (Mots.) under laboratory conditions. The experiment was laid out in 5×4 factorial with 5 replicates in Completely Randomized Design in the laboratory. Maize grains weighing 50 g were treated with the plant powders, clay and the synthetic Permethrin at the rate of 0.00 g, 1.5 g, 3.5 g and 5.0 g by weight and then infested with 10 adult weevils in each vial for assessments of adult mortality, F1 emergence and damage. The comparative effectiveness of each of the treatments were assessed by counting weevils' mortality at 14 days, 28 days, 42 days and 56 days post treatment and progeny emergence at 28 days, 42 days and 56 days post-treatment. The results were subjected to statistical analysis after transforming the data using mean percentage method and the ANOVA, the significant means were separated using the least significant differences test at 5% level. The Permethrin, a synthetic insecticide proved to be very effective followed by clay among all the treatments. Among the botanicals, *C. papaya* was the most effective followed by *A. muricata*, but were concentration dependent while *C. pentandra* was the least or not effective. In all trials, percentage mortality among adult *S. zeamais* reared on maize treated with Permethrin powders was observed to be the highest (100%) followed by those treated with *C. papaya* seed powder (56.6%), clay (55.5%) and *A. muricata* seed powder (38.8%). However, grains treated with powdered leaves of *C. pentandra* exhibited a low mean percentage mortality of 0.00, 0.20, 0.57 and 0.63, respectively, at different intervals post treatment which was not significantly different when compared with the untreated (control). These percentage mortality among adults were significantly ( $P < 0.05$ ) different from the untreated (control). Thus, all the treatments were found to significantly affect the survival of the *S. zeamais* at different concentration except in *C. pentandra*. Effect of plant powder on the emergence of adult *S. zeamais* was significantly ( $P < 0.05$ ) different and reduced the F1 progeny emergence. The result indicated that *C. pentandra* had no significant effect on mortality and F1 emergence of adult *S. zeamais* ( $P < 0.05$ ) resulting in severe damage of the grains and weight loss.

**Key words:** botanical insecticides, maize, maize weevil, plant extracts, storages losses

### INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop following wheat and rice in the world (Souki et al., 2011). It is one of the major staples consumed in Nigeria (Chukwu & Enyiukwu, 2016; FAO, 2022). It has a lot of industrial uses including its use as feed for domestic animals (Shiferaw et al., 2011). Maize is grown in most agro-ecological areas including the Niger Delta region where oil exploration and industrial activities are predominant. It is utilized for human consumption and forms about 50–70% of the livestock feed (Abdulrahman & Kolawole, 2006; Souki et al.,

2011). Similarly, maize which accounts for about 11.2% of grains produced in Nigeria is a staple diet for humans (Agricdemy, 2022). Between 60 and 70% of grains in Africa are reported to be stored at farm level, generally to provide a food reserve as well as seed for planting (Mobolade et al., 2019). However, maize storage conditions in developing countries are inappropriate and farmers experience post-harvest grain losses due to fungal diseases and insect pests attacks (Asawalam & Arukwe, 2004; Chukwu & Enyiukwu, 2016).

The maize weevil, *Sitophilus zeamais* Motschulsky, is a key pest of cereal grains whose infestations start in the field before harvest and extend throughout the storage period (Likhayo et al., 2014; de Araújo et al., 2019). In recent years, postharvest losses to storage insect pests such as the maize weevil (*S. zeamais* (Mots.)) and the cowpea weevil (*Callosobruchus maculatus* (Coleoptera: Bruchidae)) have been recognized as constraints to food security in

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Africa (Madugu et al., 2020). The use of insecticides is considered most effective in controlling *S. zeamais* in storage. Presently, insect control in stored food products relies heavily on the use of toxic gaseous fumigants, dusts and residual contact insecticides (Adejumo et al., 2014) known to have organ and CNS toxicity amongst other drawbacks (Likhayo et al., 2014).

The widespread use of synthetic chemicals has led to some serious problems including development of pest resistance, toxic residues on stored grains and health hazards to grain handlers (Enyiukwu et al., 2014; Emeasor et al., 2022a; Emeasor et al., 2022b). Currently, botanicals constitute 1% of the world's insecticide market, despite the knowledge that plants constitute a rich source of bioactive chemicals and may provide alternatives to regular insect control agents (Enyiukwu et al., 2016). Many plant powders have been found to be very effective in the control of *S. zeamais* attacking maize grains in storage (Asawalam & Emosaiure, 2006). There is need to develop and adopt alternative and sustainable crop protection technologies which are eco-friendly and devoid of the obvious negative effects on non-target organisms (Asawalam & Arukwe, 2004).

The objectives of this research was to evaluate and compare the efficacy of selected plant materials clay and permethrin against *S. zeamais*, and to access the level of damage caused by the weevil to stored maize grains within a period of 60 days.

## MATERIALS AND METHODS

**Research Site** This experiment was conducted at the Crop Science Teaching and Research Laboratory of Michael Okpara University of Agriculture Umudike. The prevailing ambient environmental conditions within the laboratory include:  $\pm 28$  °C and  $75 \pm 20\%$  relative humidity (RH). Umudike lies on latitude  $05^{\circ}29'N$  and Longitude  $07^{\circ}33'E$  with an altitude of 122 m above sea level. It has an average rainfall of 2200 mm per annum distributed over an eight-month period (March–November) with bimodal peaks in June–July and September with a short dry spell in August. It lies within the tropical rainforest zone where the mean daily temperature is above 29 °C all through the year but rarely exceeds 35 °C.

Table 1. Plants evaluated for insecticidal properties

Scientific Name	Common name	Family	Part used
<i>Annona muricata</i>	Soursop	Annonaceae	Seeds
<i>Carica papaya</i>	Paw Paw	Caricaceae	Seeds
<i>Ceiba pentandra</i>	Silk cotton tree	Malvaceae	Leaves

**Insect Rearing.** The maize weevils *S. zeamais* (Mots.) used for this study were obtained from naturally infested maize which was reared and kept in a transparent 3-litre plastic bucket whose lid was perforated and the openings was securely covered with muslin cloth and rubber band to prevent the beetles from escaping and also to disallow entrance and infestation from other storage insects. Thereafter, the bucket thus covered was placed inside a cupboard in the laboratory. A clean healthy uninfested maize grain (Variety: Bende White) was purchased from Ndoru market in Ikwuano L.G.A. Abia State, Nigeria, and sundried to avoid moldiness.

**Plant Materials and Experimental Design.** The treatments in Table 1 are three plant materials. The sour sop (*Annona muricata*) and pawpaw (*Carica papaya*) seeds were sourced from Ikot Akai village, Ukanafun Local Government of Akwa Ibom state, Nigeria. Leaves of the silk cotton tree (*Ceiba pentandra*) were obtained from Umudike. The experiment was laid out in  $5 \times 4$  factorial in a Completely Randomized Design replicated 5 times set out in the laboratory.

**Preparation of Sample Materials.** The matured fruits (paw-paw and soursop samples) were sliced opened using sterilized stainless-steel knife and the seeds collected separately. The seeds were washed in distilled water, sun-dried and later oven dried for four days and grinded with a (Phillips Harris model) laboratory blender passed through a 2 mm sieve and the powder stored in polythene bags. The matured leaves of *C. pentandra* was plucked and oven-dried for four days and subsequently pulverized with a milling machine (Retsch Mucile, Germany) into fine powder and Permethrin (synthetic insecticide powder 100 mg/g) which was incorporated into the study for comparison was obtained from an agro-chemical store. The clay used was purchased from the local market was milled into powder and placed in an air tight container.

**Application of Plant Treatments Materials.** About 50 g of the susceptible local maize variety (Bende White) obtained from a local market were introduced in a deep freezer for 72 hours, and later dried under the sun to prevent moldiness and were placed in a transparent 12

cm diameter vial (1 L) after which the botanicals were introduced at different levels; 1.5 g, 3.5 g and 5.00 g. The grains and the treatments were thoroughly admixed to allow effective stickiness with the plant materials to the maize seeds. A control treatment in which no powder 0.0 g was added was also set up with 5 replicates.

### Data Collection

**Experimental evaluations of *S. zeamais* damage assessment.** Damage caused by the weevil to the maize grain was assessed at 60 days post treatment by visual counting of the damaged grains in the control and treatments. Records of the damage due to the weevil both in the treated and control experiments were taken and recorded at 14 days, 28 days, 42 days and 56 days post treatment. The effect of the plant extracts on *S. zeamais* was thus observed fortnightly and assessed in-situ for a period of 60 days which the experiment lasted.

**Assessment of Perforated Grains.** Damage to the maize grains was assessed in each trial after mortality of the adult *S. zeamais*. Samples of 10 grains were randomly taken from treated and untreated grains and the number of insect-damaged grains (perforations) was counted. Damage by the weevils to the maize grains was assessed at 60 days post treatment by re-weighing the maize grains in each vial to determine the mean percentage loss in weight from the final weight.

**Mortality and Progeny Assessment.** The treatment remained under the same conditions after the removal of the live and dead adult *S. zeamais* to assess  $F_1$  progeny emergence. The  $F_1$  progeny of *S. zeamais* adults were removed and counted as soon as they emerged. This was continued up to 56 days according to Emeasor et al. (2022b). At the end, weight loss of grain and number of damaged kernels were recorded by separating the admixture by sieving in each treatment. Insects subsequently emerging were counted to estimate  $F_1$  progeny production as counting was stopped at 56 days to avoid overlapping of generation.

**Data Analysis.** The data were subjected to two-way analysis of variance (ANOVA) using Genstat level of significance and significant means were separated by Fisher's Least Significant Difference (F.L.S.D) at 5% probability level.

## RESULTS AND DISCUSSION

**Mortality of Adult Insects.** Table 2 showed the mean percentage mortality of the maize weevil at 14 days

post treatment. All treatments except the control and *C. pentandra* showed significant difference within the first two weeks of the experiment. Permethrin, at all levels was highly significant as it recorded the highest mean percentage mortality (2.14). Also, the levels of significance increases with the levels of concentrations among the treatment means at 1.5 g, 3.5 g and 5.0 g which recorded 0.82, 0.94, and 1.29, respectively. However, there was no significant difference between those treated with *C. pentandra* leaf powder when compared with the control, as no mean mortality was recorded at their various concentrations. The synthetic Permethrin giving the best result attested to the findings of Chukwu et al. (2022) that synthetic insecticides are usually more effective and superior to the botanicals. This attribute of synthetic pesticides could be explained by their greater ability to persist longer in the environment than botanicals whose active principles are easily broken down by heat and UV-radiation (Enyiukwu et al., 2016).

Table 2 also showed the mean percentage mortality of maize weevil at 28 days post treatment. All the treatments except the control and *C. pentandra* at 1.5 g and 3.5 g caused a significant mean percentage mortality. The interaction showed that Permethrin had the same mean mortality (3.16) of the maize weevil at all dosages ( $P < 0.05$ ) and was the highest among all the treatment means (2.37) followed by clay (1.60), *C. papaya* (1.50) and *A. muricata* seed powder (1.474). However, the mortality rate increased in concentration. Therefore, it is ascertained that the levels of significance is concentration dependent. A view also held by several workers (Oyededeji et al., 2020; Gitahi et al., 2021a; Gitahi et al., 2021b; Chukwu et al., 2022).

At 42 days post treatment, there was no significant difference ( $P < 0.05$ ) in all the treatment means at all levels except in *C. pentandra* and the control. Numerically, Permethrin had the best results followed by clay, *C. papaya*, and *A. muricata* with percentage mean of 2.45, 1.79, 1.69, and 1.53, respectively, following increased concentration. From the interaction, the highest mean mortality was recorded in all the treatments at 5.0 g concentration (2.20), followed by 3.5 g (2.05) and 1.5 g (1.83). This confirms that the rate of mortality increases as concentration increases and were all significant at ( $P < 0.05$ ). The ability of the plant powders to cause mortality of the weevil can be attributed to contact toxicity to maize weevil (Gitahi et al., 2021a) as well as its repellent pungent odour (Gariba et al., 2021; Gitahi et al., 2021b) or fineness of its powder which makes it unpalatable and unattractive to the maize weevil and this could lead to starvation, asphyxiation and death (Oboho et al., 2017; Noudem et

Table 2. Effects of plant materials, clay and permethrin on adult mortality of *S. zeamais* 14, 28, and 42 days post-treatment

Treatments	Mean percentage mortality, days of treatment assessment and dosages of treatment per 50 g of maize grains dosages per 50 g seeds of maize																							
	14 Days						28 Days						42 Days						56 Days					
	0.00	1.50	3.50	5.00	Mean	0.00	1.50	3.50	5.00	Mean	0.00	1.50	3.50	5.00	Mean	0.00	1.50	3.50	5.00	Mean				
<i>A. muricata</i>	0.00	0.33	0.67	1.00	0.50	0.67	1.28	1.79	2.16	1.47	0.67	1.63	1.69	2.15	1.53	0.80	1.63	1.95	2.23	1.65				
<i>C. papaya</i>	0.00	0.47	0.47	0.67	0.40	0.00	1.72	2.06	2.23	1.50	0.00	1.96	2.37	2.44	1.69	0.00	1.96	2.50	2.49	1.74				
<i>C. pethandra</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.79	0.20	0.33	0.33	0.80	0.80	0.57	0.33	0.33	0.33	0.80	0.63				
Clay	0.00	0.67	0.67	1.72	0.76	0.00	2.06	2.03	2.31	1.60	0.47	2.06	2.20	2.44	1.79	0.47	2.06	2.50	3.16	1.87				
Permethrin	0.00	2.64	2.89	3.05	2.14	0.00	3.16	3.16	3.16	2.37	0.33	3.16	3.16	3.16	2.45	0.33	3.16	3.16	2.27	2.45				
Mean	0.00	0.82	0.94	1.29		0.13	1.64	1.88	2.07		0.36	1.83	2.05	2.20		0.39	1.83	2.19	2.520					
	LSD (0.05) Treatment (T) = 0.38						LSD (0.05) Treatment (T) = 0.28						LSD (0.05) Treatment (T) = 0.373						LSD (0.05) Treatment (T) = 0.39					
	LSD (0.05) Dosages (D) = 0.37						LSD (0.05) Dosages (D) = 0.25						LSD (0.05) Dosages (D) = 0.33						LSD (0.05) Dosages (D) = 0.36					
	LSD (0.05) Treatment × D = 0.75						LSD (0.05) Treatment × D = 0.56						LSD (0.05) Treatment × D = 0.75						LSD (0.05) Treatment × D = 0.79					

al., 2021; Chukwu et al., 2022).

Also, Table 2 compares the maize weevil mortality in all the five treatments. All the plant powders except *C. pentandra* had significant effect on adult weevil mortality when compared with the control. However, the level of significance was highest in Permethrin followed by clay, *C. papaya* seeds and *A. muricata* seed powders respectively when compared with other treatments as they caused 2.45, 1.87, 1.74, and 1.65 mean percentage mortality, respectively. Consequently, their effect increases with the increase in concentration as shown in mean concentration of the treatments at 1.5 g, 3.5 g and 5.0 g recorded 1.83, 2.19, and 2.28, respectively.

**Weight Loss.** Weight loss suffered by the maize grains due to attack by *S. zeamais* 60 days post treatment is shown in Table 3. Percentage weight loss of maize treated with the various plant powders followed a similar trend as the percentage mortality. Permethrin showed the highest potency in reducing percentage grain weight loss at all concentrations by 3.68% equivalent of 46.32 followed by clay (40.27) and *C. papaya* seed powder (39.75) when compared with the control (36.59%) equivalent of 13.41%. Greater reduction in grain damage in Permethrin treated grains and subsequent decrease in evapo-transpiration from the grains could account for the superior performance of Permethrin over the botanicals. Weight loss recorded highest in units treated with *A. muricata* (37.06) and *C. papaya* (38.08) when compared with the control. The interaction showed that weight loss reduces at increased concentration given by 1.5 g (40.07), 3.5 g (42.22) and 5.0 (43.27). However, all the treatments were significantly different at (P> 0.05).

Also, Table 3 showed the damage caused by *S. zeamais* to the maize grains 60 days post treatment. Permethrin recorded the lowest grain perforation with a mean value of 0.511 while *C. pentandra* recorded the highest perforation mean of 1.37. Among the different concentration used, 5.0g of the treatments recorded the lowest mean percentage grain perforation (0.84) as compared to the control (1.27). The results suggest that weevils would avoid maize grains treated with any plant powders to varying degrees. It further showed that all the treatments had varying degrees of insecticidal activities. The insecticidal property of any plant material would depend on the active constituents of the plants such as flavonoids, alkaloids, tannins, terpenoids and phenolic compounds (Kouame et al., 2018; Brügger et al., 2019; Yadav et al., 2019; Wifek et al., 2021). In addition to the direct toxicity of these chemical compounds to the weevils, colour and smoothness of the seed coat also discourage their feeding habits. The fineness and colour

of the treatments could in the opinion of Chukwu et al. (2022) add to the overall antifeeding effects, starvation or asphyxiation of the target insects.

The number of progenies produced by *S. zeamais* in untreated grains and grains treated with different concentrations of plant extracts, clay Permethrin are shown in Tables 4. Relatively lower number of F<sub>1</sub> progeny and mean number of damaged grains were recorded from higher rates of application compared to the untreated control in all the treatments except in *C. pentandra*.

The highest number of F<sub>1</sub> progeny was recorded from grains treated with *C. pentandra* and control while the lowest number of progenies was recorded from grains treated with Permethrin powder, *C. papaya*, clay and *A. muricata* powders, respectively. Significantly, higher number of F<sub>1</sub> progenies was produced by the *S. zeamais* in the untreated grains compared with the grains treated with the plant extracts. All the levels of the essential plant extracts significantly reduced the number of F<sub>1</sub> progeny produced by the weevil except *C. pentandra*. No progeny was produced in grains treated with the highest dose of Permethrin powder.

All the test powders except *C. pentandra* were observed to have potentials of reducing progeny emergence. Least adult emergence (4.8) was observed when the maize grains were treated with Permethrin followed by *C. papaya* (19.2), clay (23.3) and *A. muricata* (35.1), while *C. pentandra* gave the highest emergence (65.3).

The effect of these plant powders on adult emergence of *S. zeamais* grown on maize grains was

significantly (P < 0.05) different among the treatments and the control. Adult emergence decreased with increase in the amount of the test powders used except in *C. pentandra*.

All the tested powders except *C. pentandra* were found to be effective in killing the adult weevils. Permethrin gave 2.454 mean percentage mortality in all the dosages used (1.5, 3.5 and 5.0 g per 50 g grains) except in control from 28 days post treatment agreeing with the findings of Danjuma et al. (2009), Parwada et al. (2018) and Chukwu et al. (2022). This also agrees with the findings of Asmanizar & Idris (2008) who reported 100% adult mortality of *S. zeamais* when treated with seed crude plant extract. Similarly, the increase in mortality in levels of treatment indicates that the effect is directly proportional to the amount used. The mortality effect of this plant powder increased from when the dose was increased from 1.5 to 5.0 g. Numerically, Permethrin had the best results followed by clay, *C. papaya* and *A. muricata* with percentage mean of 2.45, 1.79, 1.69, and 1.53, respectively, following increased concentration. From the interaction, the highest mean mortality was recorded in all the treatments at 5.0 g concentration (2.20), followed by 3.5 g (2.05) and 1.5 g (1.83). This confirms that the rate of mortality increases as concentration increases and were all significant at (P < 0.05).

The results have also revealed that clay is effective in killing adult *S. zeamais* growing in maize grains. Seeds of *C. papaya* and *Annona muricata* were also found to be very promising and increased adult mortality as the concentration was raised. This agrees

Table 3. Effects of plant materials, clay and permethrin on grain weight loss and grain perforatidue to attacks of *S. zeamais* on treated maize grains at 56 days post-treatment

Treatments	Mean percentage weight loss, grain perforation, days of treatment assessment and dosages of treatment per 50 g of maize grains									
	Weight loss (%)					Grain perforation				
	Dosages per 50 g seeds of maize					Dosages per 50 g seeds of maize				
	0.00	1.50	3.50	5.00	Mean	0.00	1.50	3.50	5.00	Mean
<i>A. muricata</i>	34.24	34.04	39.71	40.20	31.06	1.278	1.221	1.023	0.880	1.100
<i>C. papaya</i>	35.07	40.17	37.87	45.90	39.75	1.288	1.151	0.914	0.914	1.045
<i>C. pethandra</i>	39.40	37.90	37.80	37.23	38.08	1.316	1.434	1.351	1.351	1.367
Clay	38.03	38.50	41.23	43.30	40.27	1.258	1.124	1.033	1.076	1.123
Permethrin	36.23	49.73	49.60	49.70	46.32	1.205	0.839	0.000	0.000	0.511
Mean	36.59	40.09	42.22	43.27		1.269	1.154	0.849	0.844	
	LSD (0.05) Treatment (T) = 2.89					LSD (0.05) Treatment (T) = 0.19				
	LSD (0.05) Dosages (D) = 2.58					LSD (0.05) Dosages (D) = 0.17				
	LSD (0.05) Treatment × D = 5.77					LSD (0.05) Treatment × D = 0.37				

Table 4. Effects of plant materials, clay and permethrin on adult progeny emergence of *S. zeamais* at 14, 28, and 42 days post-treatment

Treatments	Mean progeny emergence, days of treatment assessment application and dosages of treatment per 50 g of maize grains																		
	28 Days				42 Days				56 Days										
	Dosages per 50 g seeds of maize			Mean	Dosages per 50 g seeds of maize			Mean	Dosages per 50 g seeds of maize			Mean							
<i>A. muricata</i>	0.00	1.50	3.50	5.00	16.30	52.70	29.00	41.70	35.10	173.3	107.3	41.00	39.00	90.70	195.0	212.7	82.30	115.7	151.5
<i>C. papaya</i>	19.30	18.70	19.70	19.00	19.20	149.3	44.30	43.00	24.30	65.20	209.3	94.70	71.00	46.00	105.2				
<i>C. pethandra</i>	43.30	18.00	95.00	105.0	65.30	168.7	157.3	156.0	132.7	153.70	182.7	205.7	203.3	198.7	197.6				
Clay	28.30	28.00	18.70	18.30	23.30	119.7	44.00	23.70	25.30	53.20	170.7	146.3	53.00	59.30	107.3				
Permethrin	19.30	0.00	0.00	0.00	4.80	147.7	0.00	0.00	0.00	36.70	187.7	0.00	0.00	57.00	61.70				
Mean	25.30	23.50	32.60	36.00		151.9	70.60	52.70	44.30		189.5	131.9	81.90	95.30					
	LSD (0.05) Treatment (T) = 16.48				LSD (0.05) Treatment (T) = 23.19				LSD (0.05) Treatment (T) = 39.29										
	LSD (0.05) Dosages (D) = 14.74				LSD (0.05) Dosages (D) = 20.74				LSD (0.05) Dosages (D) = 35.15										
	LSD (0.05) Treatment × D = 32.95				LSD (0.05) Treatment × D = 46.37				LSD (0.05) Treatment × D = 78.59										

with the findings of Maduga et al. (2020) and Zewde & Jembere (2010) which reported that the plant powder was very promising.

### CONCLUSION

The plant powders screened for this study showed that all the plant powders except *C. pentandra* have insecticidal property. The ranking of the effectiveness of the plant powders with insecticidal properties are as follows: *C. papaya* > *Annona muricata* > *C. pentandra*. The powder's ability to kill or cause mortality of *S. zeamais* (Mots.) increases with exposure period and with concentration. Based on the result of this study, *C. papaya* seed powder at 5.0 g is recommended as a substitute to synthetic insecticide for the control of *S. zeamais* (Mots.) and to obtain excellent result, concentrations as well as days of exposure should be increased hence mortality increases with concentration and exposure period. The use of botanicals will be more acceptable to farmers because of their general safety and easy handling as well as being eco-friendly. More research should be carried out to ensure the efficacy of these plant materials in order to control other pest of cereal. Reports have necessitated the focus of attention on alternative insect pest control methods. The use of botanicals and their extracts which are believed to be relatively safe and biodegradable is one of such methods being explored.

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### AUTHORS' CONTRIBUTIONS

KCE and UEJ planned and designed the storage experiment. KCE interpreted the data on insect population and plant damage. UEJ set up the experiment in the laboratory, collected necessary data from the

study and analyzed them. All the authors played roles in preparing the manuscript. DNE interpreted phytochemical aspects of the work, arranged and edited the manuscript. All the authors have read and approved the manuscript.

### COMPETING INTERESTS

All authors declare that we have no conflicts of interest whatsoever in relation to the publication of this manuscript.

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