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Two plant extracts protect stored maize against infestation of *Sitophilus zeamais* in Northern Ghana

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ABSTRACT

Botanicals have been encouraged as substitutes for conventional pesticides to reduce the impact of the latter on the environment. In line with that, we studied the effects of two plant extracts in controlling *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) attacks on stored maize (*Zea mays* L. Poales: Poaceae). The experiment was conducted under a normal room temperature of 27ºC to 30ºC and relative humidity of 74–76 % in a completely randomized experimental design. We infested the stored maize seeds in glass jars with *S. zeamais* F_1 under a 12-hour day and 12-hour night within 24 hours period. This was after 50 g of the seeds were mixed with 2 g each of the two botanicals separately in different glass jars. There were 5 treatments and 10 replications in total. The data taken for this study were quantitative. To ascertain the efficacy of the botanicals we compared the number of dead and live weevils, of seeds damage and weight loss caused by weevil attacks under each of the different treatments by determining percentage mortality, percent weight loss, seed damage and progeny production. Our results showed that the different botanical formulations significantly controlled *S. zeamais* population resulting in reduced damage, better protection and improved storage of maize seeds. However, there were no significant differences in protections offered between shade dried neem seed powder and rice husk powder although the latter was the most efficacious with 85 % mortality compared to the former with 65 % with sundried neem seed powder being the least with a little above 40 % mortality. We concluded from this study that the formulated botanicals were very effective in controlling *S. zeamais* populations and therefore recommended that it be used as a cost effective and environmentally friendly protection of stored maize against *S. zeamais* attacks, especially in rural poor areas and places known for continuous seed production and storage, such as the case of the study community.

Introduction

Humans have been successful on earth partly due to the ability to maintain sustainable food supply system (Kuijt and Finlayson, 2009; Hammond et al., 2015; McClements et al., 2021). This success story is partially attributed to ensuring food security at the individual level and sometimes through governmental level (Hammond et al., 2015; McClements et al., 2021). However, some of the biggest threats to this success story are issues related to postharvest losses which are also problems confronting the food supply chain. This is because food production and supply are variable in both time and space and that all food items inherently deteriorate over time (Block et al., 2016; Shafiee-Jood and Cai, 2016). Due to the seasonality of agricultural production and the traditional dependency on rainfed agriculture especially in sub-Saharan Africa (Guido et al., 2020; Emediegwu et al., 2022), food preservation especially that of staple seeds such as maize (*Zea mays* L. Poales: Poaceae) becomes critical if we are to meet future demands especially during lean seasons within the year (Singh and Devi, 2020; Asogwa et al., 2021).

To minimize the variability in food production and supply and the inevitable deterioration over time, humans have developed various innovations and technologies to preserve food for future use especially in store houses to ensure food security (Mok et al., 2020; Singh and Devi, 2020; Asogwa et al., 2021; Firouz et al., 2021). Stored food items such as seeds are also major sources of insect pest attacks, causing up to 100 % food losses (Srivastava and Subramanian, 2016; Mesterházy et al.,

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2020). As a major pest control strategy, pesticides have been the most preferred means since the period of industrial revolution with high success rates (Umetsu and Shirai, 2020; Tudi et al., 2021; Hajam and Kumar, 2022). Despite the high benefits to food storage, pesticides use is also responsible for increased environmental contamination and chemical residues in food items (Phillips and Throne, 2010; Essuman et al., 2023). As a result, interests in alternative pest control methods have surged over the past few decades, with some of the most promising new control methods being biorational. Examples of some of the biorational pest control methods in the store houses are temperature manipulations, gas management, the use of botanicals, and natural enemies with some offering protection through pest development suppression (Kedir Shifa et al., 2014; Hamel et al., 2020; Mpumi et al., 2021; Stejskal et al., 2021; Guru et al., 2022; Neupane et al., 2022; Chuquibala-Checan et al., 2023; Adu-Acheampong et al., 2024; Murugesan et al., 2024). There are also new and promising tools in decision-making through computer assistance in sampling for insects within grain elevators and store houses for proper decision making (Phillips and Throne, 2010; Mankin et al., 2021). Several of the most interesting innovative storage methods have been used for maize preservation.

With production estimates of over 1 billion tons per year, maize is one of the leading crops supplying humans and their livestock with nutrition and livelihoods (Dowswell, 2019; Serna-Saldivar, 2023). The crop is a very important seed crop which is widely cultivated in sub-Saharan Africa as a staple food for both man and livestock and plays a huge role in ensuring food security. There are over 500 maize cultivars grown in Africa which are mostly derivatives of the main maize varieties in the world namely dent, flint, pod, sweet, flour and popcorn (Abate et al., 2017). Currently, the leading field pests of maize in the world are *Spodoptera frugiperda*, *Phyllophaga* spp, *Agrotis ipsilon*, *Frankliniella* spp, *Thrips tabaci*, *Rhophalosiphum maidis Tetranichus urticae*, *Diabrotica* spp, *Helicoverpa zea*, *Sitobion avenae*, *Epitrix* spp, *Spodoptera exigua* and *S. frugiperda* are known to be the most destructive of them all especially in the study area. The main control methods for these maize pests are the use of synthetic pesticides and biological control (Assefa and Ayalew, 2019; Blanco et al., 2014; Koffi et al., 2020a; Koffi et al., 2020b).

Stored product pests have been reported to cause food losses estimated between 9 % and 100 % (Phillips and Throne, 2010; Nayak and Daglish, 2018; Mesterházy et al., 2020). For instance, in a review, Nukenine (2010), reported that farmers in Africa lose about 50 % of their cereals and up to 100 % of their pulses through pest attacks with grain borers, weevils, moths and beetles being the most destructive of them. A related review also reported that weevil attacks could cause up to 40 % maize loss worldwide (López-Castillo et al., 2018). The main destructive stored product pests of maize are *Sitophilus oryzae*, *Sitophilus zeamais*, *Sitophilus granaries*, *Rhizopertha dominica*, *Trichoderma granarium*, *Tribolium castaneum*, *Tribolium confusum*, *Oryzaephilus surinamen*, *Cryptotests ferrugineus*, *Cryptotests pusillus*, *Cadra contella*, *Sitotroga cerealella*, and *Plodia interpurutella* (Abd El-Aziz, 2011; Hagstrum, 2016).

Currently, insect pests of stored maize are mainly controlled with dichlorvos and methylbromide and their derivatives while vertebrate pests are controlled with anticoagulant rodent baits. These groups of chemicals are however losing their efficacy due to insect resistance, and as a result, new seed protection methods such as the use of biological control, modified atmospheres, low pressure, hydrogen cyanide and biorational or plant extracts (botanicals) are becoming more common (Stejskal et al., 2014; Baributsa et al., 2020; Koffi et al., 2020a; Ahmad et al., 2021).

Along the line of searching for efficacious and environmentally friendly biorational control methods, this study sought to evaluate the inhibitory effects of rice (*Oryza sativa* L, Poales; Poaceae) husk and neem (*Azadirachta indica* A. Juss, Sapindales; Meliaceae) seed powders as cheap and easy to make biorational control methods for maize weevils (*Sitophilus zeamais* Motsch, Coleoptera: Curculionidae) especially in the northern parts of Ghana. Although there are few studies that have reported that they are effective in controlling weevils (Nukenine, 2010; Dufera et al., 2019; Neupane et al., 2022) there is still much to be done in evaluating these plant extracts especially in different formulations and in different preparations compared to those reported in literature. Published records of cost benefit ratio analysis between conventional pesticides use and botanicals range from 1:1.26–1:30 showing that the application of botanicals is a very cost-effective way of controlling pests (Amoabeng et al., 2014; Kamei, 2021; Divekar et al., 2024).

Our hypothesis for this study is that rice husk and neem seed powders are effective in controlling *S. zeamais* and so they can be used for maize seeds protection in store houses especially in Ghana. Based on our hypothesis, we evaluated the effect of neem seed extracts and rice husk ash in controlling *S. zeamais* in stored maize where we measured their efficacy on the mortality and their potential to reduce pest attacks on stored seeds. This study was conducted as a confirmatory test to other similar studies in other parts of the world with some modifications regarding the preparation of the neem seed powder, especially the sun dried powder.

Materials and methods

Study Area

Our study took place at the entomology laboratory of the Savannah Agricultural Research Institute (SARI) at Manga in the Upper East Region of Ghana. The relative humidity (RH) of Upper East Region ranges from 30 % to 75 %. Upper East Region extends from latitude 10°30'N to 11°8'N and longitude 1°15'W and 0°5'E and located in semi-arid Sudan savanna agro-ecological zone of Ghana respectively (Haruna et al., 2018).

Seeds preparation and addition of weevils

We acquired Obaatanpa maize variety from the maize section of SARI at Manga for this research. The is because it was the most preferred variety for cultivation by farmers in the study area. The study was conducted under laboratory conditions of normal room temperature of 27°C to 30°C and relative humidity of 74–76 % under 12-hour daytime and 12-hour night within 24 hour period. The maize seeds were cold treated to about −20°C prior to the experiment for seven days as prescribed by (Khaeim et al., 2022) for such experiments. We mixed 50 g of the maize seeds with 2 g of each of the botanicals separately in 130 mm by 85 mm sterilized plastic jars. We covered the jars with mesh cloths and held them (mesh) in place with tight rubber bands.

Preparation of botanicals

We collected ripe and fresh undehusked neem seeds in the study area, sun-dried half of the seeds and dried the remaining under shade in the open. After drying (both types) for fourteen (14) days, the neem seeds were grounded into powders and later sieved with a fine mesh (1 mm in size) to obtain powders for the experiment. Like the neem seeds, rice husks were collected from SARI and burnt to ashes. The ashes were further grounded and sieved to obtain fine particles with 0.25 mm mesh after it had cooled to room temperature. Shade dried neem seeds dried under low humidity and high temperature conditions is a slight modification to the preparation of neem seed powders are prepared in similar studies (Aweke et al., 2020; Karakaş, 2023).

Experimental design and data collection

The study was conducted using a one-way ANOVA in a Completely Randomised Design with five (5) treatments and ten (10) replications giving a total of fifty (50) experimental units. The study treatments were sun dried neem seed powder (NSP1), shade dried neem seed powder (NSP2) and rice husk ash (RHA) and two controls; seeds without any treatment, and without any insects as control one (CTR1); and seeds without any treatment plus insects as control two (CTR2).

We filled fifty (50) separate jars with fifty grams (50 g) each of the maize seed and subsequently added two grams (2 g) of the three (3) different prepared botanical powders separately at a rate of ten (10) jars per treatment (making 30 jars for the treatments) and the rest of the twenty (20) jars filled with the two (2) controls (i.e. 10 jars each with the two controls respectively). Each jar was hand shaken for an average of 7 minutes for the powders to mix thoroughly with the maize seeds. We selected ten (10) 0–7 day-old cultured *S. zeamais* at random and placed them separately (i.e. 10 in each jar) for each of the respective treatments. We recorded live and dead insects from day 1 to day 7 and removed all dead insects on the last day. The experimental set ups (maize plus treatments and or insects) were left for six (6) weeks after the bioassay test to monitor for a possible *S. zeamais* progeny production. Natural mortality was corrected using Abbott's formula of Percent control $= \frac{A-B}{A} * 100$ where A denotes total live insects in the control jars while B denotes the total live insects in treated jar (s), and A-B equals dead insects (Abbott, 1925; Quitco and Quindoza, 2023).

Seed damage

We randomly sampled 100 seeds from each treatment, separated and counted the number of damaged and undamaged seeds. We then applied the formula: Percent damaged seeds $= \frac{ND}{(ND+NU)} * 100$, Where ND = the number of damaged seeds and NU = the number of Undamaged seeds (Ileke et al., 2020.; Taddese et al., 2020; Gariba et al., 2021).

Seed weight loss

Weight loss caused by *S. zeamais* infestation on maize seeds was measured after six weeks of experiment. Sampled seeds were separated into damaged and undamaged seeds and weighed after being sorted and counted. The percentage weight loss was calculated using the formula: $\%WL = \frac{TW-(DW+UW)}{TW} \times 100\%$. Where, TW = (DW+UW+WL), DW = (WD × ND) and UW = (WU× NU) and WL = TW − (DW + UW). DW = Weight of total damaged seeds, UW = Weight of total undamaged seeds, TW = Initial total weight of seeds, WL = Weight loss of seeds, %WL = Percentage weight loss of seeds, WD = Weight of damaged seeds, ND = Number of damaged seeds, WU = Weight of undamaged seeds, NU = Number of undamaged (Sori and Ayana, 2012; Taddese et al., 2020; Gariba et al., 2021).

Statistical analysis

We used a one-way analysis of variance (ANOVA) in GenStat Statistical package 12th Edition to analyze our data after the data (count) was log transformed and subjected to a Shpairo-Wilk test of normality (W= 0.91, $p > 0.05$).

Results of the Study

Effect of botanicals on weevil mortality

Mortality of *S. zeamais* recorded for the three (3) treatments was statistically significant from day 1–7 ($P < 0.05$). Consistently, RHA recorded the highest mortality of *S. zeamais* from days 1–7, with about 85 % mortality followed by NSP2 with 75 %, NSP1 with about 64 %, CTR2 with 40 %. However, NSP1 recorded a significantly higher cumulative mortality on the 5th and 6th days of experiment compared to the other treatments with about 46 % mortality (Fig. 1). The results also showed that maize treated with botanicals recorded significantly higher overall mortality of *S. zeamais* (85 % mortality) compared to the controls (18 %) in the experiment ($P < 0.05$). Among the three botanicals, there was a significantly higher mortality rate for RHA treated seeds compared to NSP1 treated seeds, while the mortality for NSP2 treated seeds was in between RHA and NSP1 ($P < 0.05$).

Number of damaged seeds and the corresponding weight between treatments

There was a statistically lower damage inflicted on the average number of seeds out of 100 seeds treated with RHA (4) and that of NSP2 (4 seeds) on one hand and the average number of seeds treated with NSP1 (6) on the other hand ($P < 0.05$), however, there was no statistically significant difference in seed weight of damaged seeds between all treatments after the exposure ($P < 0.05$). Control one (CTR1) had statistically higher number of damaged seeds and the corresponding higher damaged seed weight compared to the rest of the treatments (Table 1).

Progeny production of S. zeamais after the experiment

The highest number of live (24.00±2.52) *S. zeamais* representing 80.0 % of the progenies produced were recorded in control two (CTR2) in week 6 after the 7-day exposure and was statistically higher than either of the botanical treatments (RHA, NSP1, NSP2) with 6.7 %, 10.0 %, 3.3 % of the progenies produced respectively and (CTR1) ($P > 0.05$). There was however, no statistically significant difference between the three treatments ($P > 0.05$) (Fig. 2).

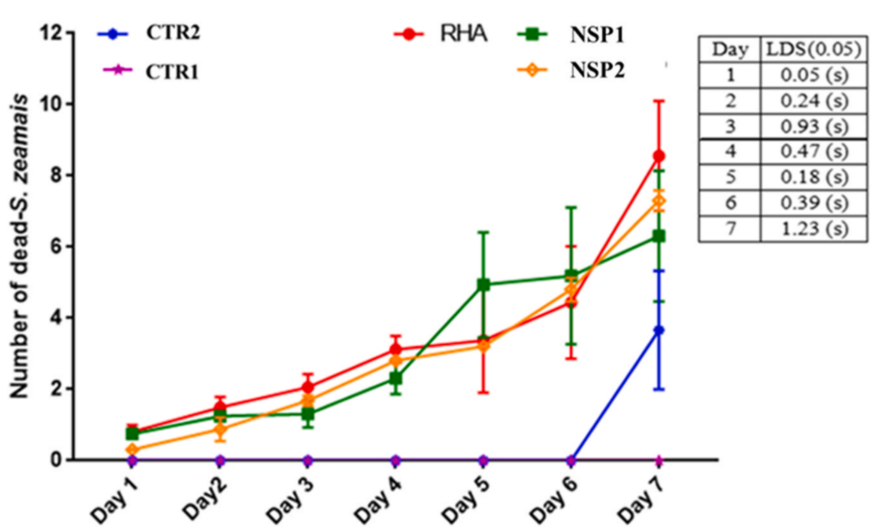

Fig. 1. Mortality of infested *Sitophilus zeamais* in 7 days experimental exposure of maize grains under botanicals treatment.

Table 1
The number of damaged and undamaged maize seeds and their corresponding weights under *Sitophilus zeamais* attack at 6 weeks after exposure to rice husk ask, sundried and shade dries neem powders.

TREATMENT	NUMBER OF DAMAGED SEEDS	WEIGHT OF DAMAGED SEEDS (g)	NUMBER OF UNDAMAGED SEEDS	WEIGHT OF UNDAMAGED SEEDS (g)	PERCENT WEIGHT LOSS
RHA	$^{\alpha}$ 4.00 ± 0.19 d	$^{\alpha}$ 1.15 ± 0.05 b	$^{\alpha}$ 96.00 ± 0.19	$^{\alpha}$ 27.59 ± 0.09	4.0
NSP1	$^{\alpha}$ 6.00 ± 0.37 bc	$^{\alpha}$ 1.72 ± 0.11 b	$^{\alpha}$ 94.00 ± 0.38	$^{\alpha}$ 27.09 ± 0.19	6.0
NSP2	$^{\alpha}$ 4.50 ± 0.19 d	$^{\alpha}$ 1.29 ± 0.05 b	$^{\alpha}$ 95.50 ± 0.19	$^{\alpha}$ 27.45 ± 0.09	4.5
CTR1	$^{\alpha}$ 0.00 ± 0.00 e	$^{\alpha}$ 0.00 ± 0.00c	$^{\alpha}$ 100.00 ± 0.00	$^{\alpha}$ 28.69 ± 0.03	0.0
CTR2	$^{\alpha}$ 64.67 ± 2.03 a	$^{\alpha}$ 18.58 ± 0.58 a	$^{\alpha}$ 35.33 ± 2.03	$^{\alpha}$ 10.17 ± 0.58	64.5
LSD (0.05)	2.23 (s)	1.14 (s)	5.62	1.21	

$^{\alpha}$ mean ± standard errors with different alphabets indicating significant difference at *P*-value < 0.05 with Post-hoc means separation via least significant difference (LSD), s = significant difference

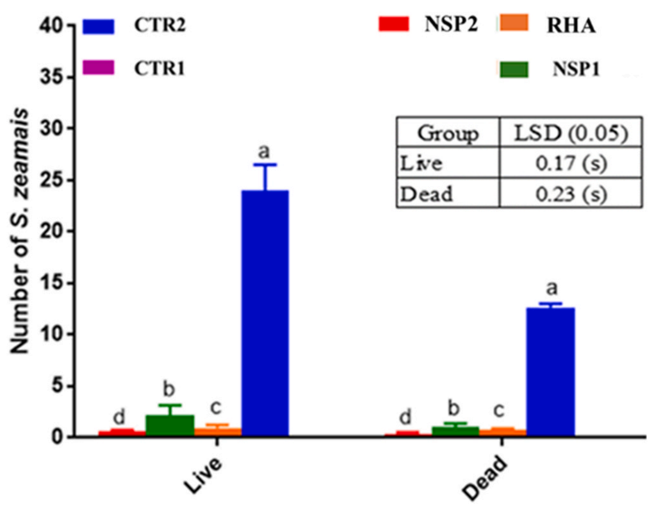

Fig. 2. Progeny production of *Sitophilus zeamais* 6 weeks after the 7 days experimental exposure.

Discussion

Weevil mortality in stored maize

The resultant mortalities recorded by RHA, NSP1 and NSP2, from day 1–7 agrees with the findings of such similar studies which reported that RHA has successfully been used to protect stored seeds against maize weevil (Atewoja et al., 2021; Yemisi et al., 2021; Neupane et al., 2022). There were, however, various degrees of protection (high, medium and low) offered by the RHA in those reported studies mainly due to the rice varieties used for their experiments (Atewoja et al., 2021; Yemisi et al., 2021; Neupane et al., 2022). Rice variety was not a factor considered for the current study. The high effectiveness of RHA in controlling *S. zeamais* could be attributed to the relatively dry nature and the presence of silica-like substances coupled with spine like projections found within the rice husk ash that have been reported to be toxic and could create wounds respectively on the weevils. This eventually exposes them to desiccation in dry conditions such as that of the present study location and promote infections that could lead to death and a resultant suppression of weevil population (Otitodun et al., 2017; Ertürk et al., 2020; Manivannan and Subramanyam, 2023).

The results further agree with the findings of other similar studies that used neem seed extracts to control stored product pests. For instance, Aweke et al. (2020) reported that neem powder prepared from neem seeds dried under ambient air temperature was effective in controlling weevils. This finding was corroborated by another report that neem seed powder was efficacious in managing weevil population in stored products (Karakaş, 2023). The results of these other studies show relatively high weevil mortalities compared to this current study, especially for the neem seed extracts. This may be attributed to the different environmental conditions under which the extracts were prepared and their effect on the active ingredient because of degradation over a period. For instance, studies have shown that temperature can have an impact on the bioactivity or degradation of the active ingredients within a plant extract or a botanical (Cawood et al., 2018; Onyebuchi and Kavaz, 2020). This is even more important because of the differences in annual temperature (which is higher in the present study location) between the current and the previous studies. The reasons for NSP1 recording the highest mortality after days 5 and 6 is not very clear. Low humidity could also play a part in the differences in efficacy. This assertion can be investigated further. Although neem products are known to be efficacious against stored product pests, there have been reports from other studies that, neem extracts leave bitter residues on seeds and so the overall taste may compromise the taste (Salako et al., 2008). This may compromise the practical use of neem extracts compared to rice husk ash.

Weevil damage to maize seeds

Several studies have reported that *S. zeamais* can potentially cause grain losses of approximately 20 %-50 %, although it may be as high as 80 % in weight loss under favourable conditions (Alam et al., 2018; Baributsa et al., 2020; Saeed and Laing, 2023). The control (CTRL2) recorded the highest seed damage and weight loss for the study compared to treated seeds that have minimal damages and reduced weight loss. This confirms previous reports that botanicals can potentially be of benefit to farmers in retaining the economic value of their stored grains (Otitodun et al., 2017; Duguma, 2020; Singh et al., 2021; Osei-Asibey et al., 2022). The results further revealed that seeds treated with NSP2 and RHA showed the least damage in the number of seeds and weight loss in agreement with studies by Otitodun et al. (2017); Aweke et al. (2020); Karakaş (2023). The application of powders prepared from shade dried neem seeds, however, offered better protection compared to powders prepared from sun dried neem seeds. The preservation of the bioactivity or the prevention of high degradation of the active ingredient within the neem seeds could be the reason (Cawood et al., 2018; Onyebuchi and Kavaz, 2020; Kolla et al., 2021; Zhang et al., 2022).

Effect of plant extracts on progeny production of weevils

The low number of *S. zeamais* progeny production in shade dried neem seed powder compared with sun dried neem seed powder may be due to the high residual effect because of the high biological activity retained from a reduction in degradation through high temperature compared to sun dried powder. This result agrees with other studies which reported that shade dried processed organic materials have a higher bioactivity compared to sun dried processed materials (Kolla et al., 2021; Zhang et al., 2022). Furthermore, limonoid, an active ingredient found in neem has proven to have a negative effect on insect development over time (Barik, 2021; Adusei and Azupio, 2022; Juma et al., 2022). This aligns with other studies which also reported that neem extracts suppressed progeny production (Joeniarti et al., 2020;

Liamngee et al., 2020; Ali et al., 2021; Tulashie et al., 2021; Adusei and Azupio, 2022). The rice husk formulation also suppressed progeny production of weevils better than the shade dried neem extracts and with a better protection than sun dried neem seed powder. This could be due to the earlier reason adduced as a reason for the high mortality for the RHA treatment at the previous section (relatively dry nature and the presence of silica-like substances coupled with spine like projections found within the rice husk ash) under weevil mortality in stored grain, but this may be subject for further investigation.

Conclusion

This study evaluated the efficacy of neem and rice husk powders for the protection of maize seeds in storage with the aim of seeking for a cheap and environmentally friendly pest control method especially for maize producing rural-poor communities in developing countries. The study revealed that the locally prepared botanical powders protected maize seeds against *S. zeamais* attacks. We arrived at this conclusion through testing the efficacy in a bioassay experiment which consisted of mixtures of these powders, maize seeds and cultured *S. zeamais* adults in a laboratory experiment under normal room temperature and laboratory conditions. The results showed that there were high insect mortalities, relatively low grain weight losses and damages and high suppression of *S. zeamais* progeny production after the experimental period. Although, the formulated botanicals effectively controlled weevil population in general, the level of control was slightly less than reported cases. This might have been because of environmental differences, especially temperature and or humidity for the preparations of the botanicals between this study and earlier ones despite the generally enhanced seed protection attributed to the botanicals. From the results, we can further conclude that RHA and NSP2 were the most effective botanicals to be recommended for promotion for use in store houses, especially in rural-poor communities. There were, however, limitations with equipment, and financial support for mass production of the most effective botanicals and lack of data to perform cost benefit analysis in relations to conventional pesticides use. Moving forward, we propose that there be investment on the part of government at the local level, especially at the rural level, in identifying the most effective local and indigenous plants that are known to have potential pesticidal effects, and work to isolate the active ingredients. The isolate could then be produced in commercial quantities for use to produce botanicals that could go a long way to offer cheap and environmentally friendly pest control in store houses in rural-poor communities as a way of ensuring food security in developing countries.

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CRediT authorship contribution statement

Abraham Akuoli Issaka Ndebugri: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Joseph Xorxe Kugbe:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Samuel Adu-Acheampong:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rosina Kyerematen:** Writing – original draft, Validation, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that there are no competing interests on this manuscript.

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Data Availability

Data will be made available on request.

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