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Efficacy of Plant Derivatives in Protecting Mungbean Grains against Cowpea Weevil (*Callosobruchus maculatus*) under Storage Conditions in Southern Bhutan

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ABSTRACT

Food grains infestation by insect pests in stores is a severe challenge in food production around the globe, especially in wet and humid regions. In Bhutan, mung bean is commonly grown for consumption as a superior source of protein. However, severe cowpea weevil infestation is observed while in storage condition. Controlling with synthetic pesticides is associated with health risks due to toxic residues which intervene in safe and healthy protection methods. Protecting the grains in-store through organic approach is imperative for consumption and seed purposes. Some botanical plant extracts are known for their protective properties which need location-specific studies based on availability and suitability. Therefore, this study was conducted to evaluate the efficacy of eight treatments (Acorus calamus rhizome powder, mustard oil, garlic cloves, turmeric rhizome powder, wood ash, Vitex negundo leaf powder, super grain bag including untreated control) against cowpea weevil (Callosobruchus maculatus) in mungbean under storage condition. The experiment was laid in a randomized complete block design with three replications. The result revealed that the lowest mean number of grains perforated and percent grain perforated were recorded in grains treated with Acorus calamus rhizome powder (0.46 & 0.20 %) followed by mustard oil (1.05 & 0.47 %) and grains stored in super grain bag (5.74 & 2.49%), which were significantly lower (P<0.001) as compared to other treatments. The lowest number of adult cowpea weevils was also recorded in grains treated with Acorus calamus and mustard oil followed by grains stored in super grain bag. Germination percentage was found highest in wood ash followed by mustard oil, Acorus calamus and garlic-treated grains while the lowest was in super grain bag. Therefore, Acorus calamus rhizome powder and mustard oil were found to be effective in managing cowpea weevil without affecting seed germination and vigour.

Keywords: *Cowpea weevil; Mung bean; Management; Treatment*

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1 Introduction

Insect infestation and damage to stored grains are severe challenges in food production around the globe. The stored food grain damage ranges from 5% to 30% of the total production globally (Pugazhvendan, Elumalai, Ronald Ross, and Soundararajan, 2009). The reasons for the widespread of insects are due to their evolutionary adaptation in terms of morphological and physiological behaviours created by human actions providing suitable habitat within the food stores. These insects are mostly found in storage, processing, packaging and other post-harvest processes. The storage insects commonly cause substantial damage to the stored grains due to their ability of high reproductive potential especially in warm areas due to the conducive environment (Ahmad et al., 2021).

Many insects such as cowpea weevil (*Callosobruchus maculatus*), lesser grain borer (*Rhyzopertha dominica*), granary weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*), angoumois grain moth (*Sitotroga cerealella*), rust-red flour beetle (*Tribolium castaneum*), confused flour beetle (*Tribolium confusum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*), flat grain beetle (*Cryptolestes spp.*), warehouse moth (*Ephestia spp.*), Indian meal moth (*Plodia interpunctella*), warehouse beetle (*Trogoderma variable*), broad horned flour beetle (*Gnatocerus cornutus*), cadelle beetle (*Tenebroides mauritanicus*) coffee bean weevil (*Araecerus fasciculatus*) and others are responsible for the infestation and damage of food grains in storage (Banga, Kumar, Kotwaliwale, & Mohapatra, 2020; Deshwal et al., 2020). Among these, the Cowpea weevil (*Callosobruchus maculatus*) is one of the major pests affecting economical legume crops such as cowpea, lentils, green gram and black gram in storage condition (Devi & Devi, 2014).

Cowpea weevil (*Callosobruchus maculatus,* Fabricius, 1775) is taxonomically classified under Domain: Eukaryota, Kingdom: Metazoa, Phylum: Arthropoda, Subphylum: Uniramia, Class: Insecta, Order: Coleoptera, Family: Bruchidae, Genus: *Callosobruchus* and Species: *maculatus*. This insect is globally called by different names such as Cowpea seed beetle, Four-spotted bean weevil, Southern cowpea weevil or Spotted cowpea bruchid. The adult weevils are about 2.0 to 3.5 mm long having slightly serrated antennae in both sexes. Female adults have strong markings on the elytra with two large lateral dark patches at mid-way along the elytra and smaller patches at the anterior and posterior end leaving a pale brown area resembling a cross. Males are less distinctly marked on their elytra as compared to females. The doomed-shaped egg has an oval and flat base which attaches to the surface of the pulses grain and measures about 0.47mm long and 0.12mm wide. Fully grown larvae size is about 3.64 mm long and 2.00 mm wide and the size of the male and female pupa is about 4.07 mm long, 2.23 mm wide and 4.57 mm long and 2.60 mm wide respectively (Devi & Devi, 2014).

During the early stage of damage, the visible symptoms are not exposed except for the presence of eggs, as they are attached to the surface of grain enclosed by an egg case. The newly hatched larvae start feeding on the grain and perforate inside the grain. Upon completion of its life cycle, the adult emerges through a circular hole, the only observable symptom on the grain. The weevil can breed throughout the year and takes about 45 to 48 days to complete one life cycle under favourable conditions depending on the availability of food materials (Devi & Devi, 2014). The egg stage duration ranges from 6 – 7 days, the larvae stage ranges from 18 to 22 days, and the pupa ranges from 5 to 7 days (Devi & Devi, 2014). The adults do not feed on the stored grains and have a life span of about 12 days. During this short period, the female lays about 115 eggs on the surface of the grains with a firm glue-like substance (Devi &Devi, 2014). As per the study conducted by Moreno, Duque, De la Cruz, and Tróchez (2000), the average female oviposition period is about 10.2 days. The temperature range between 18.14^{o}C to 27.14^{o}C and humidity of 79.5% is suitable for oviposition (Devi &Devi, 2014).

There is much research conducted on the use of different insecticides for the management of cowpea weevil in stored grains (Braga et al., 2007; Visarathanonth, Khumlekasing, & Sukprakarn, 1990). Continuous and indiscriminate use of pesticides has not only led to the development of resistant strains but also the accumulation of toxic residues in food grains used for human consumption (Rajapakse, 2006; Said & Pashte, 2015). Globally, there are serious problems of pest resurgence, genetic resistance of insects, residual toxicity in crops, phototoxicity, vertebrate toxicity, environmental hazard, and increased cost of pesticides due to which there is a need for effective alternatives to synthetic pesticides (Rahman & Talukder, 2006; Uzair et al., 2018). Such issues have diverted pest control approaches from conventional towards the use of plant derivatives, which are eco-friendly and safer alternatives for seed storage and consumption.

Botanical extracts of many plants have antifeedant, repellant and ovicidal properties on insects and affect insect growth and development due to which they can be used as safer and eco-friendly alternatives for the management of storage insect pests (Haridasan, Gokuldas, & Ajaykumar, 2017; Rajapakse, 2006; Said & Pashte, 2015). Moreover, they are readily available to farmers and they can be prepared locally. Many botanical plants such as *Vitex sp.*, turmeric

(*Curcuma longa*), *Brassica compestris*, garlic (*Allium sativum*) & neem (*Azadiratchta indica*) have been found to be effective in the management of storage pests (Said &Pashte, 2015).

As per the RNR Census of Bhutan, (RSD, 2019), 14.33 percent of households are facing problems with crops damaged by pests and diseases which is inclusive of storage pests. Like in other countries, storage pest causes serious post-harvest losses for smallholder farmers of Bhutan, who use traditional storage methods and structures for grain storage. Storage pests not only damage the food grains but also reduce the quality of stored products with the presence of insects and their feedings in the products. There are about 49 storage insect pests recorded in Bhutan out of which *Sitotroga cerealella*, *Sitophilus zeamais* and *Sitophilus oryzae* were found to cause significant damage in major staple cereals like rice and maize (Devi & Devi, 2014). Similarly, the Cowpea weevil also causes significant damage in legume crops like mung beans in storage but no research has been done to quantify and validate the storage loss caused in legumes.

However, an assessment of storage losses in Maize by Dorji, Tshering, and Lhamo (2020) shows that insect infestation is responsible for storage losses up to 16.18% to 38.21% and causes the maximum storage losses in Bhutan. Mung bean is usually stored traditionally in polypropylene bag and jute sags after sun drying in Bhutan. Although modern storage techniques like Super Grain Bags (SGB) have been introduced in Bhutan by the National Plant Protection Centre (NPPC), their adoption is still almost negligible due to limited access to the product in rural farming communities. SGB is an important eco-friendly measure to protect grains in stores by reducing water and oxygen (from 21% to 5%) flow between stored grains and the outside atmosphere.

Post-harvest loss of crops can be minimized by managing the storage insect pests using locally available botanical plant extracts besides adopting good management practices like proper drying to moisture content at 9 to 10% (Mbeyagala et al., 2017), maintaining clean storage facilities, improving storage facilities and use of modern storage technologies like super grain bags. Managing storage insect pests can enhance food security of our marginal farmers by preventing post-harvest loss of grains in storage conditions. Much research has been conducted in other countries for controlling storage pests of grains with many recommendations. There are limited studies and experiments on storage pest management conducted in Bhutan. Therefore, this study aims to evaluate the efficacy of different botanical plant extracts, traditional storage methods and modern storage technology in managing the cowpea weevil (*Callosobruchus maculatus*) in mung bean grains in storage conditions.

2 Materials and Method

2.1 Experimental design and materials

The study was conducted at the Agriculture Research and Development Center (ARDC) Samtenling (26º 54’ 17” N, 90º 25’ 51” E) located at 372 meters above sea level, from February to October, 2020. The experiment was carried out inside room conditions without any controlled environmental factors but with windows open for air circulation. The experiment was conducted using Randomized Complete Block Design (RCBD) with three replications and eight treatments. For each experimental unit, 3 kg mung bean grains were packed in polypropylene bags after the application of each treatment and stored for 195 days (6.5 months). Grains were thoroughly washed, and sun-dried up to 9% moisture content and all the damaged grains were discarded before the application of treatments and storage as per the safe grain storage guidelines by Sharon, Abirami, Alagusundaram, and Sujeetha (2015).

2.2 Preparation of treatments

Eight different treatments were used to evaluate its efficacy against Cowpea Weevil infestation as shown in Table 1. Locally available plant parts were collected from the nearby localities. Local mung bean (*Vigna radiata*) grains were harvested from the research field and used for the study. Polypropylene bags were used for storing the grains as it is commonly used by the farmers in Bhutan for grain storage. For treatments, sweet flag rhizomes (*Acorus calamus*), garlic cloves (*Allum sativum*), wood ash and Chinese chaste tree leaves (*Vitex negundo*) were collected from the locality in Sarpang district. Turmeric rhizome powder and mustard oil were purchased from local shops and super bags (GrainPro®, MSD-DR001-2) were used as per the technical recommendation.

Table 1. Treatment preparation and application

S.N.	Treatment	Application rate	Preparation and application of treatments	Reference
1	Sweet flag (*Acorus calamus*) rhizome powder	50g/Kg	Rhizomes were washed, cut into pieces, shed-dried and ground into powder and mixed with mung bean grains	(Khanal et al., 2021)
2	Mustard oil (Tulsi®)	16ml/Kg	Commercial Tulsi® brand mustard oil was mixed with mung bean grains	(Khanal, Alisha. Khadka, & Rameshwor. Pudasaini, 2020; Mbeyagala et al., 2017)
3	Garlic cloves (*Allium sativum*)	50g/Kg	Individual cloves were separated, sheath were discarded and crushed and mixed with mung bean	(Khanal, *et al*., 2021)
4	Turmeric (*Curcuma longa*) powder (BMC Haldi®)	33g/Kg	Commercial turmeric powder (BMC Haldi®) was mixed with mung bean grains	(Said & Pashte, 2015)
5	Wood ash	33g/Kg	Wood ash prepared from locally available wood (*Schima wallichai* and *Gmelina arborea*) was mixed	(Apuuli & Villet, 1996)
6	Chinese chaste tree (*Vitex negundo*) leaf powder	33g/Kg	Leaves were washed, sun-dried and crushed into powder and mixed with mung bean grains	(Khalequzzaman & Goni, 2009)
7	Super grain bag (GrainPro®)	NA	Standard super grain bag developed by IRRI (GrainPro®, MSD-DR001-2) was used as per technical recommendation	(Tivana et al., 2020)
8	Un treated Control	NA	Grains were stored in the same polypropylene bags without any treatments	NA

2.3 Seed germination test

After 195 days, 100 non-infested seeds from each treatment were selected and tested for seed germination following the paper towel method as per the International Seed Testing Association (ISTA) standard (FAO, 2018). Seed vigour was tested by sowing the non-infested seeds in plastic plug tray using a mixture of soil, compost and sand (ratio2:1:1) as growing media. The seed germination percent and seedling vigour index were calculated as

Equation 1

$$Germination\ Percent\ (\%) = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown} * 100$$

Equation 2

$$Seed\ Vigour\ Index = \frac{Germination\ percentage * Mean\ of\ Seedling\ length\ (cm)}{100}$$

Where; the length of seedlings was measured on the 15^{th} day after sowing.

2.4 Data collection and analysis

In this experiment, the data collection on adult cowpea weevil and its infestation on grains was conducted at regular intervals of 15 days starting from the date of treatment application as conducted by Uddin Ii and Sanusi (2013). On each observation date, 10 grams of grains from each experimental unit were weighed using a high-precision electronic digital weighing balance (WENSAR®) and observed for grain infestation and the number of adult cowpea weevils. The percent grain perforated and Insect Perforation Index (IPI) was calculated as per the methods of Fatopee*et al*., (1995) as mentioned by Krishnappa, Lakshmanan, Elumalai, and Jayakumar (2011) (2011) and Ojiako and Adesiyun (2013) (Equation 3 & 4).

Equation 3

$$Percent\ Grain\ Perforated\ (\%) = \frac{Total\ No.of\ grains\ perforated}{Total\ No.of\ treated\ grains} X100$$

Equation 4

$$IPI = \frac{\%\ of\ treated\ grains\ perforated}{\%\ of\ control\ grains\ perforated + \%\ of\ trated\ grains\ perforated} X100$$

If the insect perforation index (IPI) value is above 50, it is an indication of negative protectant ability. Seed germination percent and Seed Vigor Index was calculated as per the method of Shahrajabian, Khoshkharam, Sun, and Cheng,(2019) as per equation 5 & 6, respectively;

Equation 5

$$Germination\ \% = \frac{Number\ of\ germinated\ seeds}{Total\ numberof\ seeds\ sown} X100$$

Equation 6

$$Seed\ Vigour\ Index\ = \frac{Germination\ percentage\ X\ Mean\ of\ seedling\ length}{100}$$

The data was first entered and processed in Microsoft Excel 2007 spreadsheet. Further, it was analyzed using Statistical Tools for Agriculture Research (STAR) version 2.0.1. Both descriptive and inferential statistical analysis was done using the software. One-way Analysis of Variance (ANOVA), pairwise comparison and Pearson correlation coefficient were tested on the effect of treatments at a significance level of 0.05.

3 Results and Discussion

3.1 Grain damage assessment of Mung bean treated with seven different treatments

The number of grains perforated and percent grain perforation in Mung bean grains treated with eight different treatments is presented in Tables 2 & 3. The results show that there were no significant differences ($P\leq 0.05$) in the number of grains perforated and percent grain perforated between the treatments initially from the 15th to the 45th day after treatment application. However, after 45 days, *Acorus calamus* rhizome powder and mustard oil treated grains followed by the grains stored in super grain bag recorded significantly lesser ($P\leq 0.05$) number of grains perforated and percent grain perforated throughout the storage period. There was significant differences in the mean number of grains perforated ($P<0.001$) and percent grain perforated ($P<0.001$) between the treatments. The highest mean number of grains perforated and percent grain perforated was recorded in grains treated with *Vitex negundo* leaf powder (106.77 & 39.85 %) followed by grains treated with *Curcuma longa* powder (91.62 & 35.75 %) which were not significantly different ($P\leq 0.05$) as compared to the mean number of grains perforated (98.39) and percent grain perforated (37.80 %) in the untreated control. The lowest mean number of grains perforated and percent grain perforated was recorded in grains treated with *Acorus calamus* rhizome powder (0.46 & 0.20 %) followed by grains treated with mustard oil (1.05 & 0.47 %) and grains stored in super grain bag (5.74 & 2.49%) which were significantly lower ($P<0.001$) as compared to all other treatments.

This result is consistent with the findings of Said and Pashte (2015) who also found that Sweet flag rhizome powder treatment had significantly lower insect infestation at the end of the 10th month of storage besides having higher germination percent and seed vigour index. Similar research by Rajapakse (2006) also found a reduction in oviposition, emergence and the overall population of *Callosobruchus chinensis* with the use of *Acorus calamus* rhizome powder treatment in storage. Various vegetable oils such as sesame oil and mustard oil against *Callosobruchus maculatus* and *Bruchidius incarnates ("CABI Compendium,")* palm kernel oil and groundnut oil against *Callosobruchus maculatus* (Uddin Ii & Sanusi, 2013) were found significantly effective in suppressing various storage pests and provided a promising reduction of oviposition, deterrence and toxicity, protecting legumes in storage condition. Mustard oil was found effective for the management of cowpea weevil with minimum mean adult emergence (25) and affected grains (23.33) and higher adult mortality (40) at 90 days (Khanal, Alisha. Khadka, & Rameshwor. Pudasaini, 2020).

In an experiment by Tivana et al. (2021) who compared the effectiveness of high-density polyethylene container, super grain bags and polypropylene bag for cowpea grain storage, the use of super grain bag and polypropylene bag resulted in damaged grain of up to 13% and 52% respectively. Further, it also stated that after 4 months of storage, the super grain bag was perforated by insects, compromising its hermeticity.

Table 2. Mean number of grains perforated in store after treating with different treatments at different days after treatment

Treatment	Number of grains perforated in mung bean treated with different treatments at different days after treatment (DAT)													
	15	30	45	60	75	90	105	120	135	150	165	180	195	Mean
Acorus calamus rhizome powder	1.33	1.00	2.00	0.33^{c}	0.00 ^{c}	0.00 ^{d}	0.33 ^{d}	0.33 ^{b}	0.00 ^{c}	0.00 ^{b}	0.67 ^{d}	0.00 ^{d}	0.00 ^{d}	0.46^{d}
Mustard oil	1.33	1.33	1.67	0.67 ^{c}	1.00 ^{bc}	1.00 ^{cd}	0.67 ^{cd}	0.67 ^{b}	1.67 ^{c}	0.33 ^{b}	0.33 ^{d}	1.00 ^{d}	2.00 ^{d}	1.05^{d}
Allium sativum cloves	2.33	1.33	2.33	2.67 ^{b}	1.33 ^{bc}	3.00 ^{abc}	2.67 ^{cd}	11.33 ^{b}	61.67 ^{bc}	41.00 ^{b}	103.67 ^{c}	166.00 ^{c}	232.67 ^{ab}	48.62^{c}
Curcuma longa powder	2.67	1.00	2.33	3.67 ^{b}	2.33 ^{ab}	1.33 ^{bcd}	28.00 ^{ab}	86.33 ^{a}	125.00 ^{ab}	166.67 ^{a}	295.67 ^{a}	212.67 ^{bc}	263.33 ^{a}	91.62^{ab}
Wood ash	1.67	3.67	0.67	3.33 ^{b}	1.33 ^{bc}	1.33 ^{bcd}	18.67 ^{bc}	60.33 ^{ab}	112.00 ^{ab}	190.33 ^{a}	200.33 ^{b}	238.67 ^{bc}	198.00 ^{b}	79.26^{b}
Vitex negundo leaf powder	2.33	3.00	1.00	3.67 ^{b}	1.67 ^{b}	3.33 ^{ab}	18.33 ^{bcd}	72.00 ^{a}	191.67 ^{a}	216.33 ^{a}	327.33 ^{a}	337.67 ^{a}	209.67 ^{ab}	106.77^{a}
Super grain bag (GrainPro®)	2.67	4.33	1.67	2.67 ^{b}	1.33 ^{bc}	3.00 ^{abc}	3.33 ^{cd}	3.67 ^{b}	7.67 ^{c}	11.67 ^{b}	5.67 ^{d}	10.33 ^{d}	16.67 ^{d}	5.74^{d}
Un treated Control	2.67	2.00	3.00	5.33 ^{a}	3.33 ^{a}	4.33 ^{a}	36.33 ^{a}	101.00 ^{a}	193.67 ^{a}	160.67 ^{a}	265.00 ^{ab}	253.33^{b}	248.33 ^{ab}	98.39^{a}
P-value	0.771	0.660	0.780	<0.001	0.006	0.006	0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SD	1.26	2.54	1.63	1.72	1.14	1.71	15.40	48.99	86.81	97.56	138.86	131.34	116.07	44.82

Note: Means in the column with different letters are significantly different at $P \leq 0.05$ by Duncan`s post-hoc test

Table 3. Percent Grain damaged in mung bean after treating with different treatments at different days after treatment

Treatment	Percent grain damaged (%) in mung bean treated with different treatments at different days after treatment (DAT)													
	15	30	45	60	75	90	105	120	135	150	165	180	195	Mean
Acorus calamus rhizome powder	0.72	0.43	0.87	0.15^{c}	0.00^{c}	0.00^{c}	0.14^{c}	0.14^{b}	0.00^{d}	0.00^{b}	0.28^{d}	0.00^{c}	0.00^{c}	0.20^{d}
Mustard oil	0.77	0.62	0.89	0.28^{c}	0.42^{bc}	0.43^{bc}	0.29^{c}	0.31^{b}	0.80^{d}	0.14^{b}	0.15^{d}	0.42^{c}	1.01^{c}	0.47^{d}
Allium sativum cloves	1.09	0.57	0.96	1.09^{bc}	0.57^{bc}	1.33^{ab}	1.17^{bc}	5.16^{b}	33.33^{cd}	15.15^{b}	36.03^{c}	60.11^{b}	70.58^{b}	19.73^{c}
Curcuma longa powder	1.57	0.41	0.97	1.54^{b}	0.99^{ab}	0.57^{bc}	10.44^{a}	38.34^{a}	59.21^{abc}	50.64^{a}	82.55^{a}	71.75^{ab}	94.07^{a}	35.75^{a}
Wood ash	0.76	1.61	0.27	1.43^{b}	0.56^{bc}	0.57^{bc}	7.13^{ab}	23.07^{ab}	50.30^{bc}	58.51^{a}	60.83^{b}	69.09^{ab}	58.42^{b}	29.64^{b}
Vitex negundo leaf powder	1.27	1.23	0.41	1.60^{b}	0.72^{b}	1.40^{ab}	7.38^{ab}	27.71^{ab}	80.27^{ab}	62.92^{a}	90.06^{a}	84.77^{a}	92.35^{a}	39.85^{a}
Super grain bag (GrainPro®)	1.36	2.12	0.69	1.13^{b}	0.57^{bc}	1.3^{ab}	1.49^{bc}	1.72^{b}	3.50^{d}	4.54^{b}	2.33^{d}	4.20^{c}	6.82^{c}	2.49^{d}
Un treated Control	1.36	0.85	1.21	2.30^{a}	1.40^{a}	1.77^{a}	13.25^{a}	4.91^{a}	89.09^{a}	52.22^{a}	85.54^{a}	74.72^{ab}	91.09^{a}	37.80^{a}
P-value	0.765	0.555	0.799	0.000	0.006	0.008	0.001	0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SD	0.70	1.13	0.69	0.75	0.48	0.72	5.68	21.25	38.28	29.12	39.16	36.60	41.50	16.91

Note: Means in the column with different letters are significantly different at $P \leq 0.05$ by Duncan`s post-hoc test

3.2 Insect Perforation Index (IPI)

There was highly significant difference (*P*<0.001, SD=22.09) observed on the mean Insect Perforation Index (IPI) between the treatments. The lowest IPI was recorded in grains treated with *Acorus calamus* rhizome powder (0.54) followed by mustard oil treatment (1.24) and grains stored in super grain bag (6.13) which were significantly lower (*P*<0.001) as compared to all other treatments. The highest IPI was recorded in grains treated with *Vitex negundo* leaf powder (51.29) which was higher than 50 indicating an index of negative protectability (Ileke, Idoko, Ojo, & Adesina, 2020). The mean IPI in mung bean grains treated with *Acorus calamus* rhizome powder reached zero after 75 days of treatment application and remained below 1 till the end of the storage period.

Table 4. Insect perforation index in grains treated with different treatments

Treatment	Insect perforation index (%) in mung bean treated with different treatments at different days after treatment (DAT)													
	15	30	45	60	75	90	105	120	135	150	165	180	195	Mean
Acorus calamus rhizome powder	36.50	33.68	44.94	5.90^{b}	0.00^{c}	0.00^{d}	0.76^{b}	0.43^{b}	0.00^{c}	0.00^{c}	0.36^{d}	0.00^{c}	0.00^{c}	0.54^{d}
Mustard oil	38.50	22.96	44.50	9.26^{b}	20.27^{bc}	18.46^{cd}	3.19^{b}	0.42^{b}	0.89^{c}	0.21^{c}	0.19^{d}	0.57^{c}	1.14^{c}	1.24^{d}
Allium sativum cloves	43.69	22.41	43.73	32.12^{a}	28.7^{ab}	42.79^{abc}	10.28^{b}	13.45^{b}	23.28^{b}	22.14^{b}	29.62^{c}	44.24^{b}	43.03^{ab}	34.06^{c}
Curcuma longa powder	51.75	19.49	37.66	40.03^{a}	41.33^{ab}	19.63^{bcd}	44.3^{a}	45.68^{a}	39.96^{ab}	48.65^{a}	48.67^{a}	48.77^{ab}	50.80^{a}	48.56^{ab}
Wood ash	36.81	54.97	22.17	37.93^{a}	26.56^{ab}	15.08^{d}	37.58^{a}	36.11^{a}	33.07^{ab}	53.50^{a}	41.10^{b}	47.98^{ab}	38.99^{b}	43.83^{b}
Vitex negundo leaf powder	36.84	37.66	30.85	40.46^{a}	31.22^{ab}	44.6^{ab}	38.31^{a}	42.07^{a}	47.20^{a}	52.90^{a}	51.33^{a}	53.17^{a}	50.32^{a}	51.29^{a}
Super grain bag (GrainPro®)	47.36	63.01	27.81	32.97^{a}	27.92^{ab}	42.11^{abc}	11.92^{b}	3.63^{b}	3.52^{c}	8.12^{c}	2.72^{d}	5.33^{c}	6.38^{c}	6.13^{d}
Un treated Control	50.02	49.93	49.94	50.00^{a}	50.00^{a}	49.97^{a}	50.00^{a}	50.00^{a}	50.00^{a}	50.00^{a}	50.00^{a}	50.00^{ab}	50.00^{a}	50.00^{ab}
P-value	0.963	0.515	0.945	0.000	0.008	0.002	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SD	19.18	29.26	27.60	16.86	17.47	20.53	22.24	23.12	21.74	23.62	22.51	23.55	22.54	22.09

Note: Means in the column with different letters are significantly different at *P*≤0.05 by Duncan`s post-hoc test

3.3 Correlation on number of adult insects against percentage of grains perforated

Person correlation coefficient was conducted among quantitative parameters (Table 5) to explore on the number of adult insects against percentage of grains perforated. It was observed that there was highly significant positive correlation (r=0.939, P<0.001) on number of adult insect with percentage of grains perforated. This reveals that the percentage of grains perforated will increase with the increase in number of adult cowpea weevils. The number of cow pea weevil remained zero till 60th day after treatment application and increased exponentially till 150th day and declined as shown in (Figure 1).

Table 5. Correlation on number of adult insect against percentage of grains perforated

Characters	No. of adult insects	% grains perforated	Insect Perforation index (IPI)
No. of adult insects	1	0.939**	0.937**
% grains perforated	0.939**	1	0.987**
Insect Perforation index (IPI)	0.937**	0.987**	1

*Correlation is significant at the 0.05 level (2-tailed)

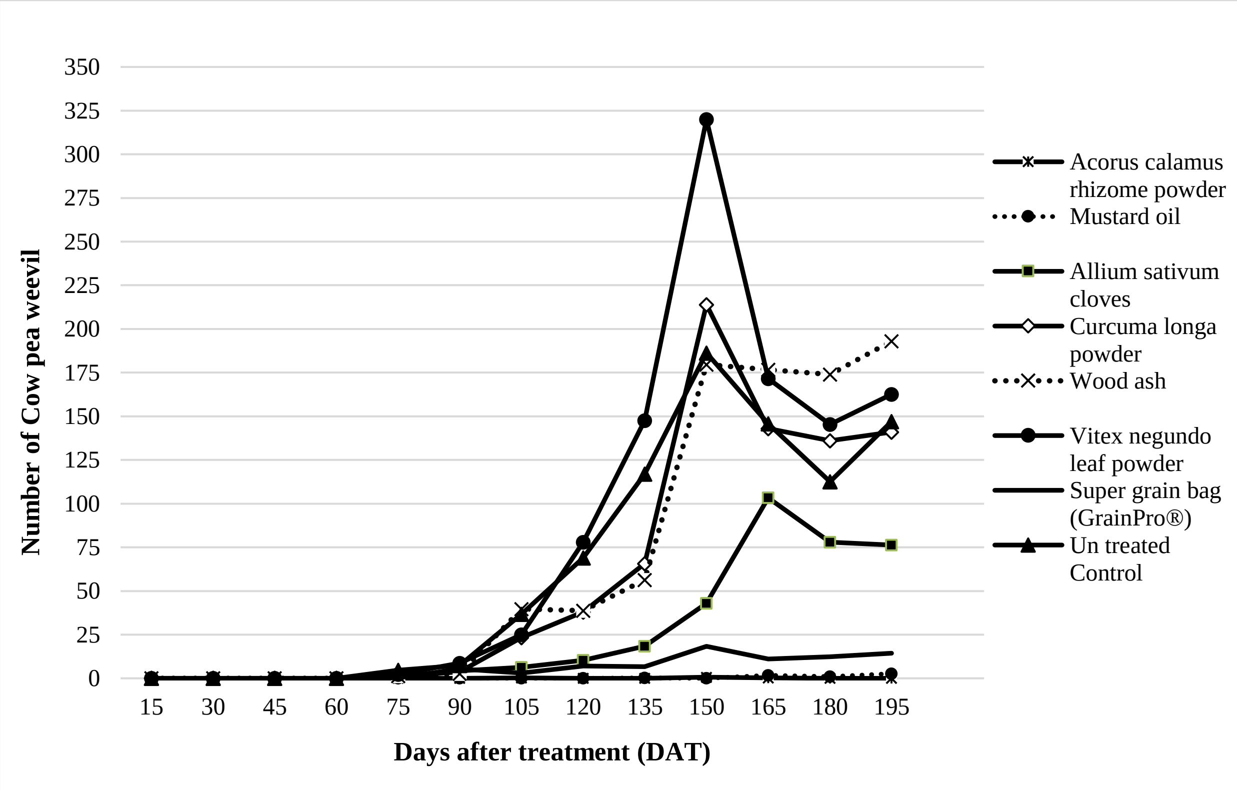

Figure 1. Number of adult Cow pea weevils recorded in grains treated with different treatments at 15 days interval

3.4 Seed germination percent and seed vigour index

The seed germination test result shows a highly significant difference in the mean seed germination percentage among all the treatments from the statistical analysis (*P*<0.013, *SD*=11.16) (Table 6). From pair wise comparison between treatments, highest germination percentage was in wood ash (98.67%) while the lowest was in super grain bag (70.67%). However, there were no significant differences (*P*=0.284) in seed vigour index among the treatments. The highest mean seed vigour index was in wood ash treatment (12.75) and lowest in super grain bag (9.46). So this study reveals that all the eight treatments do not have any significant effect (*P*=0.284) on the seed vigour index. However, seed germination percentage in grains treated with Wood ash (98.67%), Mustard oil (96.0%), *Acorus calamus* rhizome powder (95.0%), Garlic (95.0%) and untreated control (94.0%) were significantly higher (*P*=0.013) than germination percentage of grains stored in super grain bag (70.67%). Although the percent grain perforated (2.49%) and insect perforation index (6.13) was significantly lower in grains stored in super grain bag, significantly lower seed germination percentage (70.67%) indicates that super grain bag can be used preferably for grain storage but not for seed storage purpose.

Table 6. Percentage of seed germination and seed vigour index

Treatment	Seed germination (%)	Seed vigour index
Sweet flag rhizome powder (*Acorus calamus*)	95.00 [a]	9.76
Mustard oil	96.00 [a]	10.95
Garlic cloves (*Allium sativum*)	95.00 [a]	12.31
Turmeric rhizome powder (*Curcuma longa*)	90.67 [ab]	10.29
Wood ash	98.67 [a]	12.75
Chinese chaste tree leaves (*Vitex negundo*)	91.00 [ab]	11.67
Super grain bag	70.67 [b]	9.46
Control	94 .00[a]	11.01
CV (%)	8.32	15.65
p-value	0.013	0.284

4 Conclusion

From this study, it was found that the lowest percent grain perforated and insect perforation index was found in grains treated with *Acorus calamus* rhizome powder followed by mustard oil and grains stored in super grain bag as compared to other treatments. The lowest number of adult weevils was in grains treated with *Acorus calamus* rhizome powder followed by mustard oil and super grain bag throughout the storage period. The highest percent grain perforated was recorded in grains treated with *Vitex negundo* leaf powder followed by *Curcuma longa* powder which were found ineffective in managing cowpea weevil. However, the highest germination percentage was found in wood ash followed by mustard oil, *Acorus calamus* and garlic-treated grains while the lowest was in super grain bag. Therefore, *Acorus calamus* rhizome powder and mustard oil were found to be effective in managing cowpea weevil without affecting seed germination and vigour. Although the super grain bag was also found to be effective against cowpea weevil, it can be recommended for storing grains for consumption only but not for seed purposes. Further studies need to be conducted on the level of toxicity of the treated grains for human consumption.

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