

Host Grain Preference, Reproductive Biology, and Botanical-Based Management of *Callosobruchus* spp. Infesting Stored Pulses

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Abstract— The imperative role of subsistence grain products in sustaining expanding populations underscores their significance as primary sources of nourishment. In the realm of grain storage, insect pests pose a formidable threat by directly consuming stored items, leading to weight loss, compromised quality, and diminished viability. Among the prominent storage-dwelling pests, *Callosobruchus* sp. emerges as a key adversary. The aim of the present investigation was to ascertain the fecundity of *Callosobruchus* sp. and identify a natural remedy for mitigating this pest menace. The experiment aimed to discern the most efficacious natural insecticides against *Callosobruchus* sp. infestations on stored grains, encompassing cowpea (*Vigna unguiculata*), corn kernel (*Zea mays*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*). Notably, cowpea and green gram grains exhibited a higher incidence of pest population during the fecundity study. This observation was attributed to the unique characteristics of pulse seeds, including their forms, colors, sizes, as well as the curvature, texture, and thickness of the seed coat, all of which influence egg-laying behavior. In the context of botanical treatments, all examined plant substances significantly reduced pest populations compared to control treatments. Nevertheless, treatments with neem, garlic, and chili extracts at a 50% concentration emerged as superior. Neem extract, in particular, demonstrated a mortality range of 73% to 80% across all tested grains. Chili extract exhibited a mortality rate of 62% to 74%, while garlic extract displayed mortality rates ranging from 68% to 71%. This underscores the potential of nature to furnish effective biological insecticides as a sustainable remedy against pest populations, obviating the need for chemical pesticides. The study highlights the promising avenues offered by natural solutions in addressing agricultural challenges and underscores the importance of further research in this domain.

Keywords— Fecundity, Stored grains, *Callosobruchus* sp, Botanical insecticides, Mortality range

I. INTRODUCTION

Grain products are crucial for populations that are expanding. Because they are a good source of proteins and provide a remedy for malnutrition, they are the primary food source. (Ojo *et al.*, 2013). Unfortunately, a variety of insect pests have made a large infestation of the grain. These pests attack stored goods directly, causing loss of weight and quality as well as poor germination and decreased viability.

The most notable storage pest of common legumes is *Callosobruchus maculatus*, widely known as the cowpea weevil (Onyido *et al.*, 2011), which belongs to the main order of coleopterans (Pandy *et al.*, 1997). Numerous varieties of beans, including cowpea (*Vigna unguiculata*), rice bean (*Vigna umbellata*), and groundnut (*V. subterranea*), serve as hosts. Peas, lentils, chickpeas, and peanuts also contain them. On the

seeds, these insects are depositing their eggs, and as the larvae grow, they eat the seeds. They develop from the seeds into adults, producing significant losses in the field and storage (Gupta *et al.*, 2011; Thakur 2012); Durairaj, 2008; Park, *et al.*, 2003; Talukder & Howse, 1994).

Due to their high reproduction rate and brief life cycle, cowpea weevils are the most adaptive form of life, with a total number significantly exceeding that of any other animal group. The female deposits one egg straight on the surface of the legume before moving to another area of it. Typically, the eggs hatch after 3 to 5 days and are transparent, orange cream in color, eventually becoming greyish white. When the first instar larva emerged from the eggs, it had two pro-thoracic plates as a distinguishing feature. The larva may enter the seeds vertically from close proximity because of the ability of these plates to cut the tough seed covering (Augustine & Balikai, 2019). They were fleshy, curved, creamy white in color, and had a black mouth portion as larvae in their second and third instars after entering the seeds and growing by feeding on embryo grains. The bean is chewed through by the larvae until they are ready to pupate. Under the seed coat, in a pupal cell that has been prepared, pupation occurs. After biting a clean, circular exit from the pod, the full adult finally emerges from the bean. After emerging from the pupa, the adult beetles can survive for up to two weeks. They are just 3 to 4 millimeters in length, are brownish-gray in color, have long, noticeable serrated antenna, and a distinctive pattern of raised ivory-like dots at the center of the dorsal side.

Because of their ovipositional activity, grains lose their suitability for human eating, their market value drops, and seeds lose their potential to germinate (Oluwafemi, 2012; Raja *et al.*, 2001). Numerous techniques and control methods have been developed, and more are still being created, in order to lessen the serious losses that can occur during storage. Chemical pesticides are crucial for controlling the pests that attack cowpea seed storage. Insecticides, however, also harm non-target creatures, humans, and the environment.

It is necessary to create low-cost, secure, and simple strategies to guard stored cowpeas from cowpea weevil. Alternatives to synthetic pesticides that are sustainable, safe, and kind to the environment include plant-based solutions. Therefore, the purpose of this study was to determine how fertile these insects were under laboratory conditions and also this study aims to determine the impacts of various plant items on cowpea weevil reproduction, which may aid in evaluating

population dynamics for controlling the large population. Additionally, it will raise awareness of the value of using plant products to control cowpea weevils.

II. MATERIALS AND METHODS

Investigate the fecundity of Callosobruchus sp in rearing cage:

The experiment was conducted in the Zoology special degree laboratory. By using an electronic scale, 50 g of each of the following were weighed: cowpea (*Vigna unguiculata*), corn kernel (*Zea mays*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*). Those were taken into four same sized labelled beakers separately. *Callosobruchus* weevils were taken and each was observed under stereomicroscope for differentiate male and female separately. Males have brown plates that are smaller and devoid of stripes, while females have bigger plates that are darker overall and have darkened stripes on both sides. Following that, each beaker contained 20 rice weevils (10 identically sized males and 10 identically sized females), whose upper surfaces were then netted with twin ropes. In triplicate samples, this experiment was evaluated. The experiment setups were kept into normal environmental condition inside the laboratory with ensuring oxygen intake. The amount of eggs, larvae, and adult weevils in each beaker were counted after 5 weeks had passed. The fecundity of *Callosobruchus* in various hosts was determined through egg counts and larval emergence.

Preparation of botanical extracts:

Neem leaves (*Azadirachta indica*), garlic bulb (*Allium sativum*), chilies (*Capsicum frutescens*), and tulsi leaves (*Ocimum tenuiflorum*) were chosen as four commonly accessible botanicals. The neem leaves (50g) were grinded by using water as solvent with the help of motor and pistol and their concentration was 100 percentage. Then these concentration was diluted with distilled water in order to get 50% concentration of extraction sample. Likewise, garlic (50%), tulsi (50%), Chili (50%), concentrations were prepared.

Experimental setups for Botanical treatments against the Callosobruchus sp:

To ensure the absence of insects, mites, and disease-causing bacteria, 125 grams of seeds from each variety—cowpea, corn kernel, green gram, and chickpea—were carefully extracted, cleaned, and subjected to a drying process in an oven set at 60°C for approximately 6 hours. Each seed batch was then treated separately with a distinct diluted botanical insecticide (50% concentration), including extracts from tulsi leaves (*Ocimum tenuiflorum*), chili leaves (*Capsicum frutescens*), garlic bulbs (*Allium sativum*), and neem leaves (*Azadirachta indica*). The botanical insecticides were diluted to a concentration of 25 milliliters and evenly sprayed over the respective seed lots until all seeds were adequately moistened. A similar procedure involving water spraying was carried out for the untreated control treatment. Following the treatment, the seed batches were air-dried in a shaded environment. This same treatment process was applied to other seed varieties. Each seed lot was further divided into equal portions of five seeds, resulting in 25

grams of grains per seed lot. Subsequently, five pairs of male and female cowpea beetles were selected from the rearing cage and placed into each seed lot. The seed containers were securely sealed after the introduction of the pests to prevent their escape. After a period of five weeks, the number of hatched eggs, surviving offspring, and deceased insects were recorded and tallied.

Statistical Analysis

For all statistical calculations, SPSS statistical software and Microsoft Excel 2019 were utilized. The mean, standard deviation, maximum, and lowest values were used in descriptive analyses. There was also a Pearson correlation analysis. For all statistical tests in this scenario, the significance level was set at 0.05%.

III. RESULTS AND DISCUSSION

The quantity of viable offspring an organism produces over time is measured by its fecundity. It is also known as an organism's reproduction rate. It counts how many viable offsprings are produced from a seed, an egg, or a female herself and have the capacity to continue reproducing.

Figure 1 shows that *Callosobruchus* sp.'s preference for oviposition and its growth on four different leguminous seeds, including cowpea, green grams, corn kernels, and chickpea seeds, with cowpea and green grams seeds emerging as the most preferred oviposition substrates. The maximum number of eggs (253.33 ± 4.51) were laid in cowpeas, and the majority of those eggs hatched into larvae and most pupae were molted to form the greatest number of adults (73.0 ± 2.0). From green grams host stored products, roughly comparable outcomes were obtained, and this finding was consistent with that of Singh, (1976), and Mainali, (2015).

The ascending order of fecundity rate of *Callosobruchus* sp. is cowpea>green grams>corn kernel>chickpea. Therefore, cowpea and green grams had a higher fecundity rate than other store products. Due to the tiny number of eggs (81 ± 2) and larvae (22 ± 1.73), the chickpea was found to have a very low fecundity rate. The fertility of corn kernels is modest, with 150 ± 4.36 eggs and 38.67 ± 1.15 larvae present. (See Figure 1).

When taking into account the percentage of damaged seeds, Chick peas had the fewest damaged seeds (28.6%), whereas maize kernels had the highest percentage (44.6% of damaged seeds). On the other hand, cowpea and green grams had the greatest percentages of damaged seeds (61% and 75.1% respectively). This has implications for the seeds' fecundity behavior as well. As it was clearly demonstrated, seeds with low percentages of damage have less availability of ovipositional behavior, whereas seeds with large percentages of damage may have more availability of fecundity behaviors. The Pearson correlation between the quantity of damaged seeds and the quantity of larvae was positive (0.708) and statistically significant ($P < 0.05$).

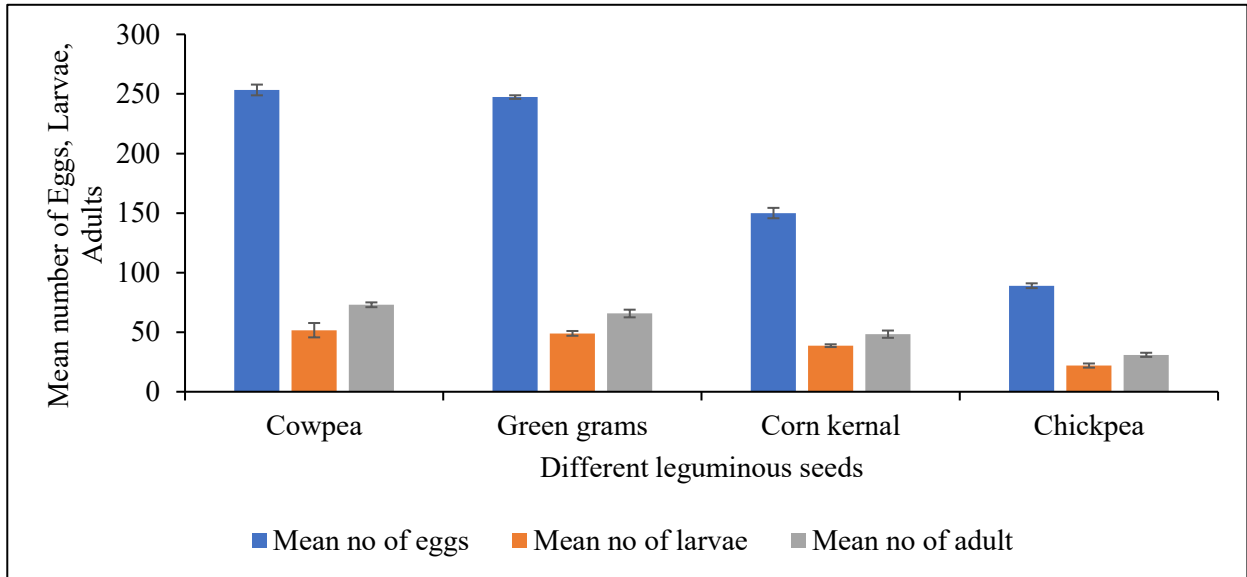


Figure 1: Mean number of Eggs, Larvae, Adults of from Different leguminous seeds

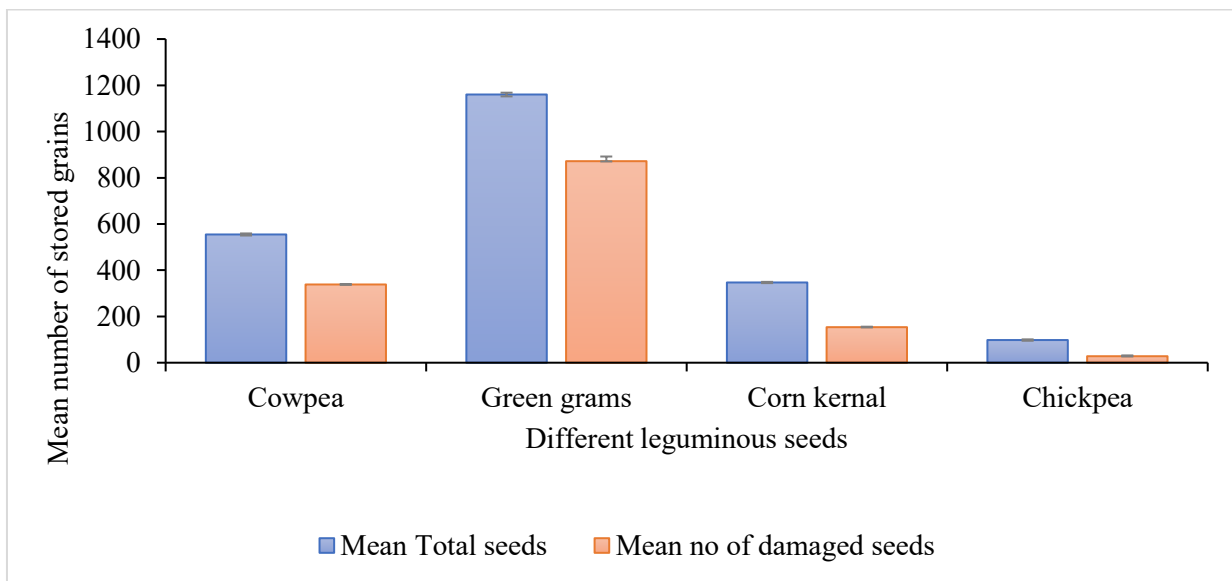


Figure 1: Mean number of total leguminous seeds and damaged seeds

Variations in egg deposition on various pulse species are influenced by the physical and morphological traits of the seeds. Factors such as seed-coat curvature, texture, and thickness, as well as seed shape, color, and size, all play a role in determining egg-laying behavior (Nwanze et al., 1975). The fertility of cowpea weevils is affected by the size and quality of seeds, particularly those with smooth surfaces and fully-filled interiors (Teotia and Singh, 1968). When smooth, well-developed seeds are available for oviposition, the weevils tend to produce more offspring. These findings suggest that cowpeas exhibit the highest fecundity due to their thin seed covering and uniform margins, which provide a large surface area for egg laying and facilitate larval entry through easily created holes.

Since green grams also have a thin seed coat, they were found to be closely related to cowpeas in terms of fecundity, but there is some variation due to the green grams' smaller granule size than cowpeas, which automatically reduces the surface area

for egg laying. Due to their polished, slick, and hard surface, maize kernels and chickpeas have lower fecundity than other examined stored items. As a result, fewer adults are generated from chickpeas than from the other evaluated stored products.

There will be various limitations that might impact the life cycle process based on the overall findings. The present study's results may differ from one another due to a variety of factors. The humidity, temperature, kind of legume, population density, and degree of inbreeding all affect how quickly insects grow. Beans that are excessively dry or damp may have fungus growing on them, and beans that are too moist will be impossible for the larva to burrow into. The survival and development of the young are influenced by the female's age at oviposition. Less young larvae survive to adulthood, and there is a lower likelihood that an older female's eggs will hatch. It's possible that this explains why there are fewer larvae than eggs. Because females typically lay fewer eggs when there are fewer

hosts, the limited number of hosts also limits their ability to lay eggs.

Plant extracts on ovipositional behavior

According to Abdullahi and Muhammad (2004) and Gupta & Srivastava (2008), plant materials are crucial in the

management of insect pests. Red cowpea, green gram, maize kernels, and chickpea extracts from four indigenous plants as well as an untreated control were tested for their ability to repel cowpea beetles from stored grains. Below is a presentation and discussion of the experiment's findings.

TABLE 1: Average number of hatched eggs, progeny and mortality (Mean ±Standard deviation) against the Plant extract treatments

Host	Treatment	Mean No of hatched eggs	Mean number of progeny	Mean number of mortality
Cowpea	Neem	68.2	17.8	49.8
	Garlic	68.2	19.2	43.8
	Chili	75.6	24.2	39.2
	Tulsi	99.8	66.4	33.2
	Control	156.8	95.6	15.2
Green grams	Neem	66	17.2	46
	Garlic	67.8	17.8	38.8
	Chili	69.2	20.4	34
	Tulsi	98.6	70.2	26.2
	Control	150.4	93.4	13.8
Corn kernel	Neem	62	12.2	56.8
	Garlic	69.2	13.6	50.8
	Chili	73.8	17.2	49
	Tulsi	83.8	45	27.4
	Control	115.6	81.4	14.2
Chickpea	Neem	47.8	11.2	48.6
	Garlic	52.6	17.4	43.2
	Chili	54.6	21.2	42.6
	Tulsi	68	39.8	20.8
	Control	92.8	66.6	8.8

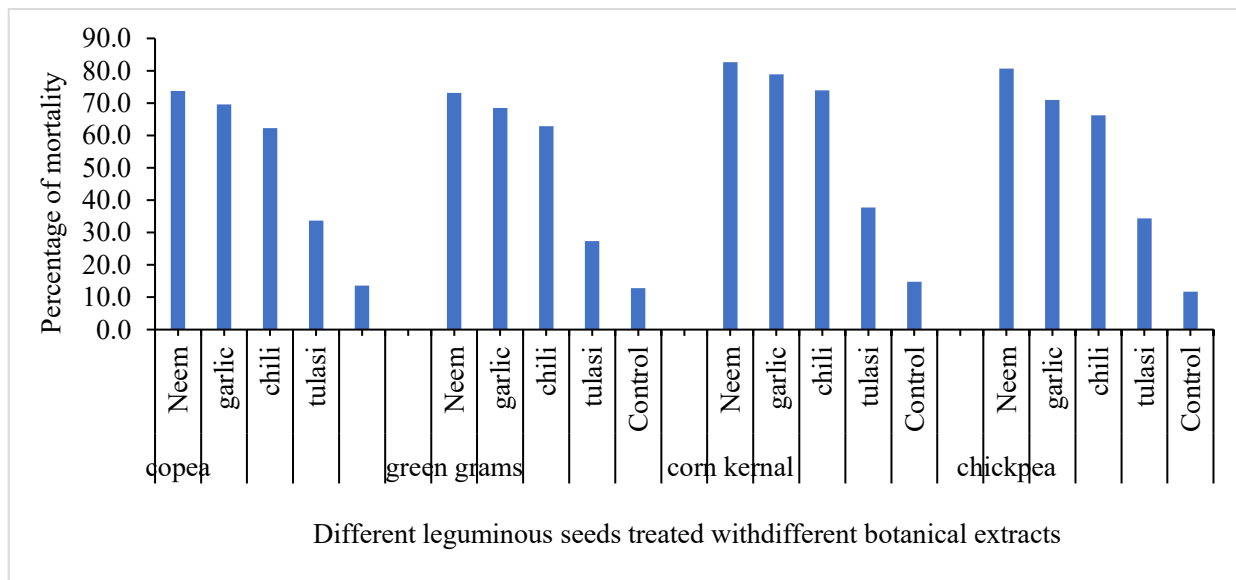


Figure 3: Mortality of Callosobruchus sp in percentage during the Plant extracts treatments in different leguminous seeds

Table 1 clearly shows that as compared to the control treatments, all plant products considerably decreased the quantity of cowpea weevil eggs deposited on the seeds. The cowpea host had 17.8 ± 2.95 hatched offspring in the Neem leaf extract treatment versus the store items, 17.2 ± 4.38 progeny in the green gram, and 12.2 ± 3.90 and 11.2 ± 3.03 progeny in the corn kernel and chickpeas, respectively. However, the garlic and chilli treatments produced roughly comparable results with

little variation (See Table 1). As a result, fewer eggs were deposited on the neem, garlic, and chili treatment settings as compared to the control setups.

While comparing tulsi treatment setups to other treatment setups, it was found that cowpea had 66.4 ± 11.19 progeny, green gram had 70.2 ± 6.72 progeny, corn kernel had 45.4 progeny, and chickpea had 39.8 ± 5.63 progeny. As a result, this treatment proved less effective in protecting the store's

merchandise than other botanical extracts due to its very poor protective efficacy.

The percentage of eggs found in store products also followed a similar pattern, with the Neem treatment setup producing the fewest eggs hatched, followed by the garlic and chilies treatment, where the tulsi setups produced the most hatched eggs. A significant correlation was also found between the number of eggs present and the number of offspring.

Figure 3 showed that neem, garlic, and chili 50% concentration extract treatments were regarded as superior treatment options than other botanicals because neem treatment induced a range of mortality in all examined grains of roughly 73 to 80%. Chili produced 62-74% mortality at the same time that garlic showed mortality rates between 68 and 71%. Tulsi extract, however, had a lower mortality rate in all examined grains, ranging from 33% to 88%.

This experiment highlighted the significant negative impact of neem, chili, and garlic extracts on cowpea weevils, suggesting that these botanical compounds have stronger detrimental effects on the weevils compared to tulsi and control treatments. Consequently, neem, garlic, and chili botanical extracts can offer greater protection against cowpea weevil damage in stored seeds.

Neem, garlic, and chili are plant products known to reduce egg production, possibly due to their harmful effects on weevils rather than solely preventing oviposition. These plant materials caused the highest mortality rates and the lowest number of offspring, indicating their toxicity to the weevils. While these extracts didn't completely eliminate the presence of cowpea weevils on the seeds, they significantly reduced both the weevil population and their impact on the seeds compared to untreated seeds (control sets).

Neem extracts rich in azadirachtin (10–25%) act as potent anti-feedants, cuticle contact poisons, and regulators of insect development. Although the contribution of additional triterpenoids found in neem extracts to overall bioactivity is debated, most research attributes the potency to azadirachtin as the primary active ingredient. Neem plant azadirachtin exhibits deterrent, antifeedant, growth-disrupting, antiovipositional, and fecundity-reducing properties against various insects (Schmutterer, 1990).

According to Weissenberg et al. (1996), chillies contain the chemical capsaicin, which has also been shown to kill insects by disrupting their metabolism and causing damage to their membranes as well as by reducing their development (Gervais et al., 2014). Additionally, diallyl trisulfide and methylallyl disulfide, which are found in garlic, inhibit insect egg hatching and the subsequent emergence of offspring (Huang et al., 2000). Additionally, research has shown that garlic's active sulfur compounds, such as allicin and aliin, not only serve as potent pesticide repellents, but also kill fungus and discourage grazing rodents.

Tulsi extract demonstrated a substantial reduction in the quantity of eggs deposited and offspring discovered on seeds when compared with control settings, although having a less detrimental effect on weevils than garlic and chilies. Due to the presence of the insecticidal compounds eugenol, eugenol methyl ether, and carvacrol in tulsi leaves (Schmutterer, 1990).

This active ingredient is effective against bacterial and insect attacks.

Nature is always eager to provide these biological insecticides as a remedy for the petaurid without the need of chemical pesticides; chillies and neem are popular plants produced by residents in their backyards, making them easily accessible supplies for seed treatment. Additionally, compared to untreated seeds, garlic and chilies have shown a greater decrease in the percentage of eggs hatching. This implies that they do not have a significant negative impact on the seeds. The garlic treatment store products will create the best aroma can induce the conception pattern and also effectively reduce the store pest attack, making it the most suitable greenery option in storage legumes. However, the neem treated seeds also had some bitter taste that will reduce the conception interest and the chili treated seeds also spicy taste that will also reduce the conception behavior.

IV. CONCLUSION

Fecundity is a crucial life history characteristic for understanding population dynamics and the foundation upon which population variations are assessed in response to changes in environmental conditions. The frequency and necessity of pesticide treatment for maintaining the threshold level may be provided for pest control strategies. Based on our findings, it can be said that neem, chillies, and garlic were more effective biopesticides than tulsi extract and may be the most environmentally friendly alternatives. Given that these plants are simple to grow, using their products would be both affordable and environmentally friendly. Additionally, these products are risk-free for customers to use. Therefore, when compared to synthetic pesticides, the aforementioned botanical extracts are vastly superior at controlling cowpea weevils.

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Declaration of interest's statement:

The authors declare no conflict of interest.

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