

The effectiveness of powdery formulations of long pepper (*Piper longum* L.) and sweet flag (*Acorus calamus* L.) to combat the cowpea weevil (*Callosobruchus maculatus* F.) on stored mung beans

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Abstract

The study aimed to evaluate the efficacy of *Piper longum* L. fruit and *Acorus calamus* L. rhizome powders in counteracting the *Callosobruchus maculatus* F. attacks on the mung bean. People frequently consume mung beans; thus, the insecticides, which are used to prevent seeds from infestations by storage pests, should be formulated in such a way that they can be readily removed from grains prior to cooking; for that reason, powdery formulations were selected. Neem plant (*Azadirachta indica* A.) gum was pulverized and incorporated as adjuvant according to weight. 100 g of mung bean seed lots were individually

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exposed to powdery formulations of 80%, 90% and 100% of *P. longum* and *A. calamus*, respectively and 100% of adjuvant, along with control. Consequently, five pairs of unsexed *C. maculatus* were introduced into eight treatments, with three replicates and laid out in the Completely Randomized Design. This experiment was designed to evaluate the adult survival, ovipositional capacity, adult emergence of the F1 and F2 generations and developmental period of *C. maculatus*, and weight loss in mung bean seeds. Simultaneously, qualitative analysis was done to determine the presence of alkaloids, flavonoids, phenol, tannin, steroids, cardiac glycosides, saponin, and anthraquinone phytochemicals in selected botanicals. Data were subjected to the analysis of variance aided by SAS 9.4 version and means were separated by using the Duncan Multiple Range Test at the 5% significance level. According to the statistical analysis, mung beans treated with 100% *A. calamus* showed no survival of *C. maculatus* two days after treatment, whereas those treated with 90% *A. calamus* powdery pesticide showed 3.3% survival. Though minimum cumulative number of eggs was detected in 90% and 100% (11.0, 9.7) of *A. calamus* treated mung bean seeds, zero progeny development was observed until 60 days; therefore, no weight loss was detected on mung bean seeds. The study concluded that 90 and 100% of the powdery insecticides from *A. calamus* were efficacious in safeguarding the mung bean from *C. maculatus*.

Key words

Adjuvant, Botanicals, Formulations, Infestation, Survival

Introduction

Mung bean (*Vigna radiata* L.) production is seasonal; however, the harvest can be utilized the entire year for various purposes. Subsequently, the seeds have to be stored in storage until their next cultivation, sale and consumption. During storage, *C. maculatus* (cowpea beetle) is one of the most destructive storage pests of mung beans (Pandiselvam *et al.*, 2019). The beetle has the ability to reproduce at both store and field conditions and the larvae bore into the mung bean seeds and feed on the whole content of the grain. Therefore, grains lost their weight, nutrient composition, odor, color, germination rate and ended up

being rejected by traders. Thus, farmers lose their authority in deciding the price and try to sell their products immediately after harvesting at a low price (Gupta and Apte, 2016). Thus, control of *C. maculatus* is essential to getting profit from mung bean cultivation. Botanical insecticides are one of the most eco-friendly and sustainable methods of controlling *C. maculatus* due to their easy formulation, lack of toxic levels to humans or animals, lack of biological eradication, cost-effectiveness for local farmers, and abundance of active ingredients that collectively impede pests from developing resistance (Kareru *et al.*, 2013). Two botanicals, *A. calamus* and *P. longum*, have proven to be effective against various kinds of insects (Kumar *et al.*, 2015; Sharmah *et al.*, 2018). Considering these details, the current study has aimed to formulate a botanical insecticide employing *P. longum* and *A. calamus* botanicals to control the cowpea beetle. This formulation may improve the insecticides' effectiveness and ease of handling.

Materials and Methods

Experimental location

This study was carried out from September to November 2022 in the laboratory of the Department of Agricultural Biology at Eastern University, Sri Lanka.

***C. maculatus* collection and mass culture**

Cowpea beetles were collected from the culture at the laboratory of the Department of Agricultural Biology. Ten unsexed adult beetles screened from laboratory insect stock were placed into a beaker containing uninfected, sterilized mung bean seeds and kept in the laboratory at room temperature. On day seven, all the parent stock mortality was observed and they were sieved out. The mung bean grains containing eggs were kept in an undisturbed place until new emergence appeared and were then utilized as bioassays for the experiment. All the experiments were done under laboratory conditions, $30 \pm 5^\circ\text{C}$ temperature and $70 \pm 5\%$ RH.

Pesticide keeping pack preparation for storage studies

Twenty-four gunny bags were prepared with an appropriate size of 15 cm in width and 25 cm in length for keeping all the treatments. For retaining the formulated pesticide, 6×6 cm² sized small bags were prepared using a minutely perforated cloth to release the fumes of the botanical pesticide throughout the storage studies.

Preparation of botanical pesticides

The inert materials mixed with the plant components were removed manually, then washed with distilled water. The rhizome of *A. calamus* and the fruit of *P. longum* were chopped and allowed to dry in the shade for seven days. Subsequently, each plant material was ground separately using an electrical grinder. Afterward, powder was sieved through a 500 µm screen mesh to obtain a fine powder with uniform particle size.

The extraction and purification of plant gum utilized for the development of botanical insecticide.

Neem plant gum (*Azadirachta indica* A.) was chosen to serve as the adjuvant for the current investigation based on its binding capacity with substances and the fact that it is released in order to protect plants from biotic and abiotic stresses. Plant gum exudates were dissolved in water at room temperature (25–30°C) and the contaminants were filtered out using muslin cloth. After that, the sample was centrifuged for 15 minutes at 10°C at 4500 rpm (Samrot et al., 2020). Ultimately, a transparent solution was acquired. After that, 50 ml of the obtained solution and 100 ml of methanol were combined, and gum was allowed to precipitate. Thereafter, the precipitate was rinsed, sun-dried and milled to produce a powdered adjuvant (Hamdani et al., 2019).

Formulation of pesticides

Ten grams of pure adjuvant powder and ninety grams of *P. longum* fruit powder were weighed and combined to make 90% of *P. longum*. In a similar way, 90% of *A. calamus* was formed. Each combination was given the adequate amount of water and thoroughly combined to generate a well – formed mixture. The mixes

were subjected to a 48 hour shade drying process to reduce the moisture level, milled and screened using a 500 µm mesh in order to produce an insecticide that was consistently formulated. Similarly, 80 g of *A. calamus* and *P. longum* were combined with 20 g of pure adjuvant powder to formulate 80% of the insecticides. Furthermore, no adjuvants were added to the preparation of 100% of *A. calamus* and *P. longum*.

The experimental design to evaluate the performance of insecticides

Eight hundred grams of sterilized mung beans were equally divided into eight sterilized gunny bags. Five grams of each of the formed insecticides 80%, 90% and 100% of *P. longum* and 80%, 90% and 100% of *A. calamus*, were taken. Among them, each 1 g was inserted into minutely perforated 6 cm x 6 cm² sized bags. Similarly, five grams of adjuvant powder were also split into five perforated bags of 1 g each. Five perforated bags, each comprising similarly formulated pesticides or adjuvants, were inserted separately into each of the gunny bags, which contained 100 grams of mung bean and carefully shaken to achieve uniform distribution. Following that, control was maintained without the addition of insecticides or adjuvants. Then ten unsexed, newly emerged and vigorous *C. maculatus* were taken from mass culture and introduced to each gunny bag and tied with an elastic band. Likewise, three replicates were setup for each treatment and arranged according to Completely Randomized Design.

Phytochemical analysis

To confirm the presence of alkaloids, flavonoids, phenol, tannin, steroids, cardiac glycosides, saponin, and anthraquinone, phytochemical analysis was performed on the methanol extraction of *A. calamus* and *P. longum* based on the following references (Banu and Cathrine, 2015; Moses *et al.*, 2014; Parab and Mengi, 2002; Shaheen *et al.*, 2015; Trivedi *et al.*, 2011).

Data collection

The cowpea beetle's survival percentage, fecundity, number of newly emerged adult beetles of the F1 and F2 generations, time duration from egg to adult

development, and weight loss in the mung bean seeds were measured and observations were taken every day after treatment.

Following that, mung bean seeds with laid eggs were kept under laboratory conditions at $30 \pm 5^{\circ}\text{C}$ temperature and $70 \pm 5\%$ RH to determine the emergence of the F1 generation and the cumulative number of eggs laid by the F1 generation and they were further maintained to determine the emergence of the F2 generation.

Statistical Analysis

Data were subjected to the analysis of variance aided by SAS 9.4 version and means were separated by using the Duncan Multiple Range Test at the 5% significance level.

Results and Discussion

A) Phytochemical Analysis

Table 1 shows the results of the phytochemical analysis of *A. calamus* and *P. longum* botanicals. The qualitative analysis revealed that the methanol extract of *A. calamus* contained phytochemicals such as alkaloids, flavonoids, phenol, tannin, steroids, cardiac glycosides, saponin, and anthraquinone, while the methanol extract of *P. longum* contained flavonoids, phenol, tannin, and steroids. It was also noted that, in comparison to *P. longum*, *A. calamus* had certain components such as alkaloids, cardiac glycosides, saponins, and anthraquinones. Perhaps due to the fact that *A. calamus* is more toxic to *C. maculatus* than *P. longum*. These findings were in par with the study of Mamta and Jyoti (2012) who reported the same result.

Table 1. Results of phytochemical analysis of *Acorus calamus* and *Piper longum* botanicals

Phytochemical	Methanol extract of <i>A. calamus</i>	Methanol extract of <i>P. longum</i>
Alkaloid	+	-
Flavonoids	+	+
Phenol	+	+
Tannin	+	+
Steroids	+	+
Cardiac glycosides	+	-
Terpenoids	-	-
Saponin	+	-
Anthraquinone	+	-

+ Indicates the presence of the substance

- Indicate the absence of the substance

B) Efficacy of botanical pesticide against *C. maculatus***a) Survival percentage**

Table 2 shows the least survival percentage of *C. maculatus* in mung beans treated with 100% *A. calamus*, which was statistically ($p < 0.05$) equivalent to 90% *A. calamus* in two days after treatment.

Table 2. Survival percentage of *Callosobruchus maculatus* in different treatments

Treatments	*Survival percentage of <i>Callosobruchus maculatus</i> (Coleoptera; Chrysomelidae) in 2 DAT
Mung bean treated with 90% <i>Acorus calamus</i>	03.3 ± 5.8 ^c
Mung bean treated with 80% <i>Acorus calamus</i>	73.3 ± 5.8 ^a
Mung bean treated with 90% <i>Piperlongum</i>	46.7 ± 5.8 ^b
Mung bean treated with 80% <i>Piperlongum</i>	73.3 ± 5.8 ^a
Mung bean treated with 100% adjuvant	80.0 ± 0.0 ^a
Mung bean treated with 100% <i>Acorus calamus</i>	00.0 ± 0.0 ^c
Mung bean treated with 100% <i>Piperlongum</i>	43.3 ± 5.8 ^b
Untreated mung bean (control)	80.0 ± 0.0 ^a

*The values are means of triplicates ± standard deviation. Means followed by same alphabet don't vary significantly at $p < 0.05$. 2

DAT- Two days after treatment.

It might be explained by the fact that the rhizome powder of *A. calamus* acts as a pesticide and has pesticide properties that kill insects. The present study's findings align with Anvesh et al. (2022) who confirmed that *A. calamus* has insecticidal compounds viz. β -asarone and unidentified insecticidal compounds which are responsible for causing mortality in *C. maculatus*.

b) Ovipositional capacity

Figure 1 illustrates the total number of eggs laid on grains for each treatment. On the seventh day after the introduction, it was evaluated. The highest number of eggs were discovered in mung beans treated with 100% adjuvant, followed by mung beans treated with 80% *P. longum* and the untreated control with the mean values of 44.7, 36.7 and 37.0, respectively. Notably, mung beans treated with 90% and 100% *A. calamus* had the lowest mean values of 11.0 and 09.7 for the number of eggs.

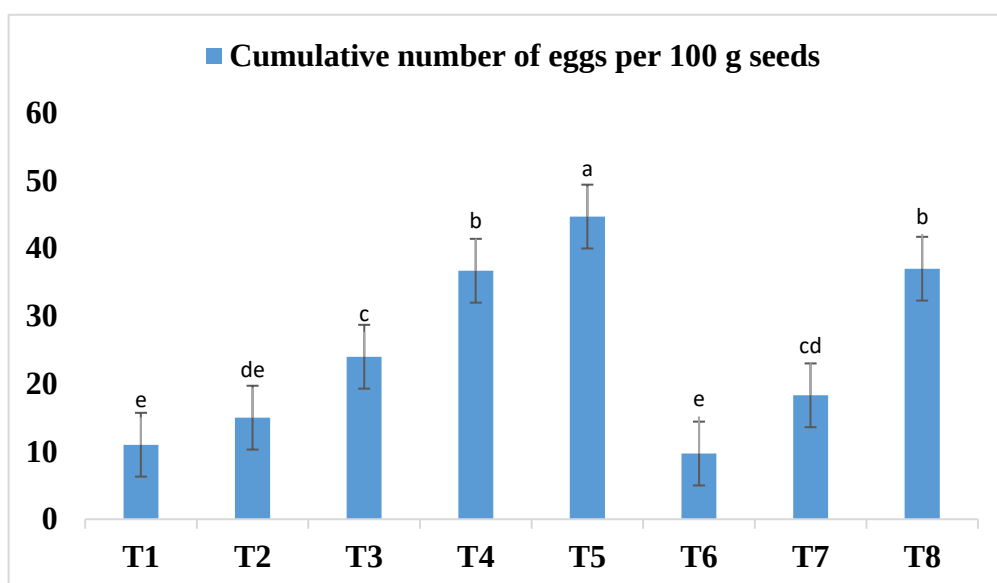


Figure 1: Cumulative number of eggs

The bar indicates the mean value of three replicates. Means followed by same alphabet don't vary significantly at $p < 0.05$.

T1 (mung bean treated with 90% *A. calamus*), T2 (mung bean treated with 80% *A. calamus*), T3 (mung bean treated with 90% *P. longum*), T4 (mung bean treated with 80% *P. longum*), T5 (mung bean treated with 100% adjuvant), T6

(mung bean treated with 100% *A. calamus*), T7 (mung bean treated with 100% *P. longum*), T8 (untreated mung bean - control).

The findings suggested that *A. calamus* has the ability to resist ovipositional, or in other words, it prevents or reduces *C. maculatus* from producing eggs. The findings of this investigation appeared to be in line with Anvesh et al (2022).

c) F1 generation of newly emerged *C. maculatus* and cumulative number of eggs laid by F1 generation of *C. maculatus*

The percent emergence of adult *C. maculatus* at F1 generation and the cumulative number of eggs at F1 generation are given in Table 3. Newly emerged insects were observed following three weeks of egg-laying. The untreated mung bean had the highest percentage of the new generation, with a mean value of 84.8. It was followed by mung beans treated with a 100% adjuvant and 80% *P. longum*. After all the F1 progeny of *C. maculatus* died on the 34th day of the experiment, the cumulative number of eggs was quantified. The highest number of eggs was recorded in mung bean treated with 100% adjuvant, followed by untreated mung bean with mean values of 195.7 and 114.0, respectively.

Table 3.Percent emergence of adult *Callosobruchus maculatus* F1 generation and cumulative number of eggs of F1 generation of *Callosobruchus maculatus*

Treatments	*Percent emergence of adult <i>Callosobruchus maculatus</i> (Coleoptera; Chrysomelidae) F1 generation	*Cumulative number of eggs laid by F1 generation of <i>Callosobruchus maculatus</i> (Coleoptera; Chrysomelidae)
Mung bean treated with 90% <i>Acoruscalamus</i>	00.0 ± 00.0 ^d	000.0 ± 00.0 ^f
Mung bean treated with 80% <i>Acoruscalamus</i>	00.0 ± 00.0 ^d	000.0 ± 00.0 ^f
Mung bean treated with 90% <i>Piperlongum</i>	55.4 ± 13.2 ^c	041.7 ± 05.5 ^d
Mung bean treated with 80% <i>Piperlongum</i>	62.1 ± 06.7 ^{bc}	058.7 ± 10.0 ^c
Mung bean treated with 100% adjuvant	71.3 ± 14.7 ^b	195.7 ± 11.6 ^a
Mung bean treated with 100% <i>Acoruscalamus</i>	00.0 ± 00.0 ^d	000.0 ± 00.0 ^f
Mung bean treated with 100% <i>Piper longum</i>	52.9 ± 18.5 ^c	024.7 ± 03.3 ^e
Untreated mung bean (control)	84.8 ± 01.9 ^a	114.0 ± 19.7 ^b

*The values are means of triplicates± standard deviation. Means followed by same alphabet don't vary significantly at $p < 0.05$. 2

DAT- Two days after treatment.

The study revealed that no progeny development was recorded in mung bean treated with 80%, 90% and 100% *A. calamus*. It confirmed its efficacy against *C. maculatus*. The reason might be due to reduced egg hatchability or the ovicidal effect of *A. calamus*, which was also supported by Hafez *et al.* (2014). There was no egg development in mung bean treated with *A. calamus* powdery pesticides. This result may be explained by the fact that *A. calamus* has the

ability to prevent *C. maculatus* from developing progeny, which impedes egg development.

To formulate the botanical pesticide, the resins from *Azadirachta indica* (neem plant) were used as an adjuvant. Surprisingly, the resins attracted more *C. maculatus*, thus in mung bean treated with 100% adjuvant, it received a higher number of eggs than the control.

d) Newly emerged *C. maculatus* in F2 generation

Table 4. The percentage of newly emerged *Callosobruchus maculatus* in F2 generation of *Acorus calamus* and *Piper longum* treated mung beans

Treatments	*Percent emergence of adult <i>Callosobruchus maculatus</i> (Coleoptera; Chrysomelidae) F2 generation
Mung bean treated with 90% <i>Acorus calamus</i>	00.0 ± 0.0 ^c
Mung bean treated with 80% <i>Acorus calamus</i>	00.0 ± 0.0 ^c
Mung bean treated with 90% <i>Piper longum</i>	56.3 ± 9.9 ^b
Mung bean treated with 80% <i>Piper longum</i>	58.3 ± 5.6 ^b
Mung bean treated with 100% adjuvant	85.6 ± 5.6 ^a
Mung bean treated with 100% <i>Acorus calamus</i>	00.0 ± 0.0 ^c
Mung bean treated with 100% <i>Piper longum</i>	48.2 ± 8.5 ^b
Untreated mung bean (control)	83.6 ± 7.8 ^a

*The values are means of triplicates ± standard deviation. Means followed by same alphabet don't vary significantly at $p < 0.05$. 2

DAT- Two days after treatment.

The findings of this investigation indicated that *A. calamus* has the ability to completely suppress the progeny of *C. maculatus*. Govindan et al.

(2020) reported that *A. calamus* acted as a strong anti-emergence substance against *C. maculatus*. It prevents the adult emergence of the F1 generation; thus, no eggs or F2 progeny development were observed in these treatments. One more interesting finding from the study was that *A. calamus* could be used to store mung beans for more than 60 days without the development of *C. maculatus*.

e) Percentage of weight loss on the mung bean

Table 5 displays the percentage of weight loss on mung beans by *C. maculatus* in each treatment. Mung beans treated with 90% *P. longum*, 100% adjuvant, and untreated (control) mung beans had the highest percentages of seed weight loss. In mung beans treated with 100%, 90%, and 80% *A. calamus* pesticides, a minimal percentage of seed weight loss was recorded with mean values of 0.4, 0.6, and 0.8, respectively, which were statistically similar to one another at the 5 % significant level.

Table 5. Percentage of weight loss in mung beans by *Callosobruchus maculatus* population

Treatments	*Percentage of weight loss
Mung bean treated with 90% <i>Acoruscalamus</i>	00.6 ± 0.8 ^c
Mung bean treated with 80% <i>Acoruscalamus</i>	00.8 ± 0.9 ^c
Mung bean treated with 90% <i>Piperlongum</i>	09.9 ± 1.6 ^b
Mung bean treated with 80% <i>Piperlongum</i>	17.7 ± 2.9 ^a
Mung bean treated with 100% adjuvant	24.8 ± 3.6 ^a
Mung bean treated with 100% <i>Acoruscalamus</i>	00.4 ± 0.5 ^c
Mung bean treated with 100% <i>Piperlongum</i>	07.7 ± 2.3 ^b
Untreated mung bean (control)	24.9 ± 4.1 ^a

*The values are means of triplicates $\pm$ standard deviation. Means followed by same alphabet don't vary significantly at $p < 0.05$. 2
DAT- Two days after treatment.

The reason for this is that *A. calamus* has the ability to minimize the infestation of *C. maculatus*, which in turn protects the mung bean seeds from the feeding by *C. maculatus* and prevents weight loss of grains.

f) *C. maculatus* developmental period

The study discovered that almost all the treatments except mung bean treated with *A. calamus* have taken a statistically similar duration in F1 (21 to 22 days) and F2 (20 to 21 days) generations to complete the *C. maculatus* lifecycle. There were no progenies developed in the F1 and F2 generations of mung beans treated with *A. calamus* pesticides.

Table 6. Total developmental periods of *Callosobruchus maculatus* under *Acorus calamus* and *Piper longum* treated mung bean

Treatments	Developmental period	
	*F1 (days)	*F2 (days)
Mung bean treated with 90% <i>Acorus calamus</i>	00.0 $\pm$ 0.0 ^b	00.0 $\pm$ 0.0 ^b
Mung bean treated with 80% <i>Acorus calamus</i>	00.0 $\pm$ 0.0 ^b	00.0 $\pm$ 0.0 ^b
Mung bean treated with 90% <i>Piperlongum</i>	21.3 $\pm$ 0.6 ^a	20.3 $\pm$ 0.6 ^a
Mung bean treated with 80% <i>Piperlongum</i>	22.0 $\pm$ 1.0 ^a	21.0 $\pm$ 0.0 ^a
Mung bean treated with 100% adjuvant	21.7 $\pm$ 0.6 ^a	20.0 $\pm$ 0.0 ^a
Mung bean treated with 100% <i>Acorus calamus</i>	00.0 $\pm$ 0.0 ^a	00.0 $\pm$ 0.0 ^b
Mung bean treated with 100% <i>Piper longum</i>	21.3 $\pm$ 0.6 ^a	20.7 $\pm$ 0.6 ^a
Untreated mung bean (control)	21.7 $\pm$ 0.6 ^a	21.0 $\pm$ 2.0 ^a

*The values are means of triplicates $\pm$ standard deviation. Means followed by same alphabet don't vary significantly at $p < 0.05$. 2
DAT- Two days after treatment.

The study proved the ability of *A. calamus* to completely prevent *C. maculatus* from developing. Similar results of the current investigation were also noted by Saranya et al. (2019, 2020).

Conclusions

The study confirmed that 90% and 100% of *A. calamus* powdered insecticides were effective against *C. maculatus* with minimum survival percentage, lowest cumulative number of eggs, zero F1 generation and minimum reduction of weight on mung bean. Thus, they could be recommended as an effective insecticide for protecting mung bean, as they are an environmentally friendly alternative to synthetic pesticides.

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Declaration of Conflict of Interest

Authors have no conflict of interest to declare.

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