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USE OF BOTANICAL EXTRACTS AGAINST PULSE BEETLE (*Callosobruchus chinensis* L.) IN STORED CHICKPEA IN LABORATORY CONDITION

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ABSTRACT

Pulse beetle (*Callosobruchus chinensis* L.) is an economic pest of stored pulses. The chemicals used for seed protection create human health and environmental risks. Thus, an experiment was conducted to test the efficacy of locally available botanicals as an alternative against pulse beetle in chickpeas at the entomology laboratory of Lamjung Campus from April to June 2023. Eight treatments comprising untreated control and powdered botanicals like leaves of *Azadirachta indica* A. Juss*., Ocimum sanctum* L., and *Cinnamomum tamala* Buch.-Ham., the rhizome of *Acorus calamus* L. and *Curcuma longa* L., seed of *Zanthoxylum armatum* DC. and fruit of *Capsicum annum* L. were replicated three times in completely randomized design. Treatments were prepared by mixing botanicals at a rate of 0.5 grams per 50 grams of chickpea. Five pairs of newly emerged pulse beetle adults were used in each treatment. Weevil mortality was observed every two days. Progeny emergence was recorded from the 25th to 42 days. Weight loss and percentage of damaged seeds were recorded at the end of the experiment. All the botanicals differed significantly from the control in weevil mortality starting at 6 days of treatment application along with weight loss, seed damage and progeny deterrence which were assessed at the end of experiment. Among the botanicals, *A. calamus* and *Z. armatum* showed superior performance achieving 100% weevil mortality by the sixth day of treatment, while completely preventing seed damage and exhibiting 100% progeny deterrence and lowest weight loss. Hence, botanicals can manage beetles during pulse storage.

Keywords: Mortality, Progeny deterrence, Storage insect-pest

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a leading pulse crop in Nepal. It stands as the second most vital crop following lentils among winter legumes in the Terai and inner Terai of Nepal. It serves as a protein source for 1.8 million Nepalese individuals (Pande et al., 2005). Chickpea contains low digestible carbohydrates (40–60%), significant protein (15–22%), essential fats (4–8%), and a variety of minerals (phosphorus, calcium, magnesium, iron, zinc) and vitamins (Madurapperumage et al., 2021). The area under chickpea production was 10,793 ha with production of 12,196 metric tons (MoALD, 2023).

Stored grain pests cause both qualitative and quantitative losses resulting in food insecurity and reduced income. *C. chinensis* (Coleoptera: Bruchidae) also known as pulse beetle, is a destructive storage pest of legume seeds. Crop infestation may originate in matured pods in the field or in storage itself (Balmuchu et al., 2023). The pests' short life cycles and high reproductive capacity contribute to their status as significant stored grain threats (Hazarika & Patgiri, 2022). The developmental period (egg to adult) of *C. chinensis* is about 29 days (Hosamani et al., 2018). Larvae are the major destructive stage contributing to seed damage. They consume the entire internal content of the grain, leaving only the shell behind. They render the grains unsuitable for both human consumption and sowing purposes

(Mishra et al., 2022). The pulse beetles can cause a 30-40 % loss within six months, which could rise to 100% if not managed (Paikaray et al., 2022).

Nepal being a developing country with many farmers relying on traditional storage techniques (Kandel et al., 2021). Farmers often resort to pesticide use to mitigate losses due to easy availability. Pesticide use raises concerns about health risks, environmental harm, and the development of pesticide-resistant strains (Mehta et al., 2021). Additionally, pesticides bioaccumulate in target and non-target organisms and adversely impact soil, aquatic systems, plant physiology and human health (Pathak et al., 2022).The growing interest in plant-based pest control solutions has highlighted the importance of botanical compounds as alternatives to synthetic chemicals (Trivedi et al., 2018). Botanical products have pronounced insecticidal activity, pest repellency, oviposition deterrence, adult emergence inhibition, ovicidal, larvicidal activity, and feeding deterrence thereby, disrupting insect reproduction, growth and development (Kedia et al., 2015). Botanicals originate from easily accessible sources, are inexpensive and have varied modes of action. Their bio-degradability and low toxicity to non-target organisms make them favourable choices for sustainable agriculture crop production (Lengai et al., 2020). Nepal is rich in ethnobotanical plants, 324 species of 5345 flowering plant species have pesticidal properties (Budhathoki et al., 2020). The potential of botanicals to inhibit the reproductive cycle of the pulse beetle and prevent damage has not yet been explored in Lamjung. This study was conducted to evaluate the efficacy of locally available botanicals viz. *A. calamus* , *A. indica*, *C. annum*, *C. tamala*, *C. longa, O. sanctum* and *Z. armatum* for protection against pulse beetle with the purpose of promoting the use of locally available resources for seed protection during storage.

MATERIALS AND METHODS

The experiment was conducted in the entomology laboratory of the Lamjung campus situated in Sundarbazar, Lamjung district, Gandaki province. Live insect specimens of *Callosobruchus* were collected from insect-infested black gram seeds. Mass rearing was performed on non-damaged seeds of pulse like chickpea, black gram, pea and rice bean. Maintenance and mass rearing of insects was performed under the room temperature recorded as 23 ±2 °C and a relative humidity of 65 ±5%.

Preparation of treatment materials

Leaves of *A. indica* , *O. sanctum*, and *C. tamala*, rhizomes of *A. calamus* and *C. longa* were collected from the premises of the Lamjung campus and shade-dried for 25 days to decrease the moisture without losing active ingredients (Neupane et al., 2016). Whereas, *Z. armatum* and *C. annum* were collected from nearby markets. Well-dried botanical materials were powdered using an electric mixture, mortar and pestle. The details of the different botanicals, their parts, and the amount used are presented in Table 1.

Table 1. Details of treatment and their doses used in the experiment

Common Name	Scientific Name	Concentration (Gram)	Parts used
Sweet flag	*A. calamus*	0.5	Rhizome
Neem	*A. indica*	0.5	Leaves
Basil	*O. sanctum*	0.5	Leaves
Turmeric	*C. longa*	0.5	Rhizome
Timur	*Z. armatum*	0.5	Seed

Indian bay leaf	*C. tamala*	0.5	Leaves
Red chili	*C. annum*	0.5	Fruit
Control	-	-	-

Experimental setup

The experimental design implemented in the research was a completely randomized design (CRD) consisting of eight treatments each replicated three times. Fifty grams undamaged chickpea of local variety was weighed and placed in each plastic jar and 0.5 gm of respective botanicals was applied and mixed properly to form 1 % concentration as used by Paneru and Shivakoti (1970). Following Seram et al., (2022) male and female were differentiated by their antennae as they are pectinate in male and serrate in female. Five pairs of newly emerged adults of weevils were placed in each plastic jar, covered with muslin cloth and kept at room condition. The treatment period lasted 42 days, beginning at the end of April and extending into June.

Data collection

To determine the number of dead weevils, data was collected at intervals of two days till complete mortality was obtained in each treatment. Data on weight loss and the number of damaged grains was collected at the end of the 42 days. Initial weights were measured prior to the experimental setup, while final weights were obtained after 42 days using weighing balance. Assessment of grain damage was done at the end of treatment period (42 days) by taking a random sample of 15 grams from each treatment. For progeny deterrence, newly emerged adults were counted starting from day 25 until no further emergence was detected. Different formulas were used for the calculation of the research data. Analysis of variance and Duncan's Multiple Range Test (DMRT) at 0.05 level of significance were done using R Studio software (version 4.3.1).

Per cent mortality = $\frac{\text{No. of dead weevil}}{\text{Total no. of weevil}} * 100$ (Kłys et al., 2020)

Total no. of weevil (number of live weevils + number of dead weevils)

Weight loss percentage = $\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} * 100$ (Zahir et al., 2014)

Per cent damaged seed = $\frac{\text{No. bored seed in the sample}}{\text{Total no. seed in the sample}} * 100$ (Singh et al., 2017)

F1 deterrence = $\frac{\text{No. progeny in control} - \text{No.of progeny in treatment}}{\text{No. of progeny in control}} * 100$ (Aldryhim, 1990)

RESULTS AND DISCUSSION

Effect of Treatments on Mortality of Pulse Beetle

A. calamus was the first to attain complete mortality of pulse beetle within 2 days (Table 2). Similar findings of complete mortality by *A. calamus* has been found by Paneru and Shivakoti (1970) within 6 days of treatment. It has antifeedant and repellent action (El-Nahal et al., 1989). Principal compounds like α-asarone, β-asarone, methyl isoeugenol, methyl eugenol, α-cedrene and camphor are responsible for its insecticidal action (Liu et al., 2013).

Z. armatum and *C. longa* also showed comparatively more effectiveness with respect to other treatments and caused complete mortality after 6 days of treatment (Table 2). The effect of *Z. armatum* is attributed to the exudation of non-human toxic and environmentally-friendly metabolites like thymol, myrtenol and citronellal (Okagu et al., 2023). The pesticidal activity of *C. longa* has also been previously identified because of bio-active compounds like alpha-pinene, beta-pinene, caryophyllene, eugenol and limonene (Chandra et al., 2014). Mortality in control might be due to ageing of beetles and experimental errors.

Table 2. Effect of botanicals on mortality of Pulse beetle

Treatments	Weevil Mortality Percentage (%)					
	2 Days	4 Days	6 Days	8 Days	10 Days	12 Days
A. calamus	100 [a]	100 [a]	100 [a]	100 [a]	100 [a]	100 [a]
A. indica	33.33 [c]	53.33 [d]	70 [b]	90 [b]	100 [a]	100 [a]
O. sanctum	23.33 [d]	46.67 [de]	66.67 [b]	86.67 [b]	100 [a]	100 [a]
C. longa	66.67 [b]	86.67 [b]	100 [a]	100 [a]	100 [a]	100 [a]
Z. armatum	63.33 [b]	73.33 [c]	100 [a]	100 [a]	100 [a]	100 [a]
C. tamala	16.67 [d]	53.33 [d]	70 [b]	83.33 [bc]	100 [a]	100 [a]
C. annum	23.33 [d]	36.67 [ef]	53.33 [c]	76.67 [c]	86.67 [b]	100 [a]
Control	13.33 [d]	26.67 [f]	36.67 [d]	40 [d]	60 [c]	63.33 [b]
F-test	***	***	***	***	***	***
$LSD_{(0.05)}$	9.35	12.74	8.65	8.65	7.07	3.54
CV (%)	12.71	12.35	6.70	5.91	4.38	2.14
SEM	3.12	4.25	2.89	2.89	2.36	1.18
Grand Mean	42.5	59.58	74.58	84.58	93.33	95.42

Note: *, **, *** denote significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, CV = Coefficient of Variation, LSD = Least Significance Difference

Effect of Treatments on Weight Loss Percent

Notable differences were observed in the weight loss in stored chickpeas at $p<0.001$ after 42 days in laboratory conditions (Table 3). The lowest weight loss per cent was observed in *A. calamus* treated seeds (1.14%) while the highest weight loss was observed in control (6.18%). *Z. armatum* and *C. longa* followed the *A. calamus* in terms of preventing weight loss of chickpeas. Similar findings of lowered weight loss (1.5%) and minimized grain damage (1.43%) by *A. calamus* as reported by Tiwari et al (2018).

Table 3. Effect of botanicals on weight loss, F1 progeny deterrence, percent seed damage in chickpeas

Treatments	Weight Loss Percentage (%)	Progeny Deterrence (%)	Percent Seed Damage (%)
A. calamus	1.14 [f]	100 [a]	0 [f]
A. indica	3.55 [bc]	55.92 [f]	6.67 [b]
O. sanctum	3.95 [b]	60.72 [e]	6.67 [b]
C. longa	1.75 [e]	92.85 [b]	1.25 [e]
Z. armatum	1.67 [e]	100 [a]	0 [f]
C. tamala	3.26 [c]	75.02 [d]	3.33 [d]
C. annum	2.45 [d]	85.66 [c]	5.42 [c]

Control	6.18 [a]	0 [g]	10.83 [a]
F-test	***	***	***
$LSD_{(0.05)}$	0.52	3.95	0.99
CV (%)	10.1	3.20	13.37
SEM	0.18	1.32	0.33
Grand Mean	2.99	71.27	4.27

Note: *, **, *** denote significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, CV = Coefficient of Variation, LSD = Least Significance Difference

Effect of Treatments on F1 Progeny Deterrence

The progeny emergence in stored chickpeas was found to be significantly different among the treatments used at p<0.001 after 42 days of treatment in laboratory conditions (Table 3). The highest protection against progeny emergence observed in *A. calamus* (100%) and *Z. armatum* (100%). Botanicals like *A. calamus, O. sanctum*, *C. longa* and *C. annum* were found effective against progeny deterrence of *Callosobruchus* in stored green gram (Manju et al., 2019). Similar findings were reported where 100 % progeny deterrence was caused at a 2 % dose of *A. calamus*. Higher progeny deterrence is attributed to its ovicidal activity (Shukla et al., 2009).

Effect of Botanical Treatments on Percentage Seed Damage

There was a significant difference (p<0.001) among the treatments on the percentage of damaged seed of chickpea by *C. chinensis* (Table 3). After 42 days of treatments, the highest seed damage was seen in control (10.83%) while there was no seed damage in the case of *A. calamus* and *Z. armatum* treated seed. Reduction in egg laying and restriction of progeny development due to the high toxicity of beta-asarone content of *Acorus* lowered the number of exit holes or seed damage (Kaur et al., 2019).

CONCLUSION

All the botanicals used for the management of pulse beetle in stored chickpeas were found to be effective. However, *A. calamus* and *Z. armatum* were superior than other botanicals as they were effective in causing quicker weevil mortality and minimizing weight loss. *A. calamus* and *Z. armatum* treated seeds showed significantly greater progeny deterrence percentage and less seed damage percentage. The current research paves the way to provide awareness to the farmers to store the pulses by treating them with readily available, inexpensive and environment-friendly botanicals. This finding is beneficial for creating integrated pest management strategies in storage. This helps in extending the post-harvest life of pulses thereby positively affecting food security. Further research is needed to evaluate the effectiveness of botanicals under farmers' storage conditions, along with a detailed economic analysis to persuade farmers.

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