



Efficacy of Fennel seed and Koseret Leaf Powders against Pulse Beetle (*Callosobruchus Chinensis* L.) in Stored Chickpea (*Cicer Arietinum* L.)

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Abstract

Pulse beetle (*Callosobruchus chinensis* L.) also commonly called bruchid, is one of the most destructive and cosmopolitan pests of stored legume. The present study was carried out to investigate the efficacy of botanical pesticides like fennel seeds and koseret leaf in management of pulse beetle (*C. chinensis*) in stored chickpea. An experimental design of A 1 x 1 x 3 [200grams of chickpea grains + 20 pulse beetles + (5gm, 10gm, and 15gms of fennel seed, koseret leaf, admixture of both fennel seed and koseret leaf powders)] factorial experiment was laid in a Complete Randomized Design (CRD) in two replications. The result of repellence test of pulse beetle against fennel seed and koseret leaf powders has indicated that, all the treatments have shown high repellence against pulse beetle at all amounts of treatments (5, 10, and 15grams) across the whole durations.

Mortality of pulse beetle, *C. chinensis* adult at different days after treatment with fennel seed and koseret leaf powders in solo and in their combined forms has shown significantly the highest pulse beetle mortality rate (60%) for koseret leaf powders followed by the admixture of koseret leaf and fennel seed powders during 20th days of treatment indicating toxicity effect of these powders on pulse beetle. The Effect of different concentrations of fennel seed and koseret leaf powders on chickpea seed damage as weight loss and germination loss caused by pulse beetle revealed that, there was no significance difference in weight loss and germination potential of chickpea seeds among treatments indicating that treatment with fennel seeds and koseret leaf powders did not affect the quality of chickpea seeds. The result of the present study demonstrated that both fennel seed and koseret seed can be used in control of pulse beetle. Further studies are required to test the effect of these botanical powders in the form of chemical extracts, or formulations with other ecofriendly pesticides in stored grains and foliage pests.

Keywords: Ecofriendly pesticides, Germination test, Mortality rate, Repellence test, Seed quality, Weight loss

Introduction

Pulses are important food crops as they nourish mankind with highly nutritive food being rich source of high protein and several essential amino acids. Apart from being an important source of dietary protein for human being, the pulse crops are also important for the management of soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture [1]. Chickpea, *Cicer arietinum* (L.) is one of the major pulse crops grown. Chickpea, besides a rich source of highly digestible dietary protein (17-20%) and 52-70 per cent carbohydrate. It is also a rich source of calcium, iron, niacin, vitamin 'C' and vitamin 'B'. Its leaves consist of mallic acid which is very useful for stomach ailments and blood purification. Its feed and straw are highly rich in nutrients and are mostly used as productive

ration for animals. Its protein is of high quality as compared to other pulse crops [2]. Apart from that it serves as a good source of energy (416 calories/100 gm), fat (4-10%), minerals (calcium, phosphorus, iron) and vitamins. It also helps in lowering the cholesterol level [3].

The production of chickpea is greatly hampered by both biotic and abiotic stresses and while addressing the biotic stresses, insect pests of chickpea play a significant role both in the field and in storage, limiting the chickpea production and market value. Many insect pests including lesser grain borer, red flour beetle, Grainary weevil damage chickpea in storages however, pulse beetle *Callosobruchus chinensis* L. is the most damaging one. Being cosmopolitan, it also damages lentil, cowpea, mung, sorghum and maize [4]. It enters

inside grains by making holes and start feeding until full damage. Normally infestation starts in the field because adult beetles can easily fly and lay eggs on the chickpea pods. Infestation is caused by grubs as well as adults [5]. Male and female pulse beetles can easily be distinguished on the basis of their antennae. Males have pectinate and females have serrate antennae [6]. Its female is larger than male (Howe and Currie, 1964). Normally 6-8 overlapping generations are observed in a year [7]. Mostly fumigants and synthetic insecticides are used in grain storages to manage insect pests that lead to issues like resistance, resurgence and poisoning of food [8].

There are a considerable quantitative and qualitative losses due to insect pests especially in stocks of chickpeas. The losses are primarily due to the attacks of insect pests, particularly the Chinese beetle (*Callosobruchus chinensis* L.). This pest is a potentially ubiquitous cosmopolitan beetle which can infest its host plant *Cicer arietinum* L. both in the field and in stocks. Within the framework of plant health protection, the use of insecticides had always been the solution [9]. But use of insecticides has had bad consequences, such as increased resistance (where increasingly insatiable species have appeared), an imbalance of the ecosystem (the massive and random destruction of the harmful and useful insects), and disturbances of the environment as there is a risk of toxicity due to the problems of residues [10,11]. With this view, the present was carried out to investigate the efficacy of botanical pesticides like funnel seeds and koseret leaf in management of pulse beetle (*Callosobruchus chinensis*) in stored chickpea.

Materials and Methods

The experiment was conducted in Biotechnology Laboratory, School of Biological Sciences and Biotechnology, Haramaya University. Four-kilogram chickpea seeds were obtained from Haramaya University Rare Research Station and pulse beetle infested chickpea grain was bought from Shewaber market, Harar city. The grain was sieved to remove dead seed, dirty and broken particles. Then, 2kg grains were randomly sampled and stored in a refrigerator for 2 weeks to kill any prior sources of the pulse beetle inoculum and eggs that might be already pre-existing in the grain as procedure followed by Parugrug & Roxas [12]. After 2 weeks in the freezer, subsamples of 200g grains were placed in 375ml bottles with perforated lids to prevent weevils from escaping and for aeration. one-kilogram Funnel seeds and koseret leaf powders will be bought from local market at Haramaya town. The funnel seeds and koseret leaves samples will be air-dried under shade at an ambient temperature to avoid photo degradation of active ingredient by ultra-violet ray and koseret leaf sample was chopped and sun dried. The dried materials were then ground into fine powder using grinding machine and sieved with a 10mm sieve. The fine powders were then kept in air-tight containers until required.

Treatment and experimental Design

A 1 x 1 x 3 [200grams of chickpea grains + 20 pulse beetles + (5gm, 10gm, and 15gms of funnel seed, koseret leaf, admixture of both funnel seed and koseret leaf powders)] factorial experiment was laid in a Complete Randomized Design (CRD) in two replications.

Preparation of Insect Culture

The parent stock of *Callosobruchus chinensis* was obtained from infested chickpea grains. The insects were cultured under room temperature. The food media for the insect culture was 1kg chickpea grains for *C. chinensis*. 500gm of chickpea food medium was weighed into two different glass jars. Two hundred adult insect pests were introduced into each culturing medium. The culturing spanned for 50 days; at the end about eighty (540) adult insect pests will be randomly selected for the study.

Test for Repellence

The method employed by Garcia [13] with some modifications was followed. Briefly, transparent plastic tubing, 13cm long x 1.3cm diameter as test cylinders were used in the experiment. Each test cylinder was plugged at one end with fine mesh tulle containing 5, 10 and 15grams of funnel seed, koseret leaf, and admixture of both powders, while the other end was plugged with clean cotton ball which served as a control. About 20 pulse beetles were introduced at the middle of each test cylinder through a hole at the middle portion of the cylinder. The hole was covered with nylon tulle mesh to keep the insects inside the cylinder. The cylinders were grouped accordingly to represent the treatments and replications. Each treatment consisted of three cylinders and replicated twice.

The cylinders were left undisturbed and the number of weevils that moved towards the untreated halves of the cylinders were counted and rated every hour for the first five hours and at 24, 48, 96 and 144 hours thereafter. Repellency rating was calculated following the formula:

$$\text{Repellency rating} = \frac{n(1) + n(3) + n(5) + n(7)}{N}$$

Table 1: Scale for the determination of the degree of repellency of the test materials.

Rating	Distance (cm) from center of the cylinder towards the untreated plug	Description
1	0	Ineffective
3	1-2	Slightly repellent (SR)
5	3-4	Moderately repellent (MR)
7	5-6	Highly repellent (HR)

Where: n = number of insects stayed 0, 1-2, 3-4 and 5-6 cm from the center of the cylinder towards the untreated cotton plug, respectively. 1, 3, 5 and 7 = rating scale on the reaction of the insects on different test materials. N= Total number of insects introduced per cylinder. The degree of repellency of each test material was based on the following scale (Table 1).

Test for Weevil Mortality

Two hundred grams of chickpea grains adjusted to 10% moisture content (MC) was treated with 5, 10, & 15g of each of the test treatment in 12 cm high x 6.5 cm diameter glass jars. In the first treatment funnel seed powder; in the second experimental unit koseret leaf powder; and in the third experimental unit admixture of both funnel seed and koseret leaf powders were used. The admixtures were shaken manually for 5 minutes and then tumbled for 15 minutes in a mechanical tumbler. The treated grains were left undisturbed for an hour. Thereafter, mixture 20 adult pulse beetles were introduced per treatment. The glass jars were covered with filter paper and sealed with molten wax to keep the insects inside. Untreated chickpea grains were served as control. Each treatment was replicated twice. Pulse beetle mortality rate was measured by physically counting dead weevils at 10 and 30 days after exposure to the treatment.

The mortality counts were done during the day when the weevils are highly active due to high temperatures and relative humidity. Percent adult mortality was determined by counting

the number of dead insects divided by the total number of insects introduced multiplied by 100.

Grain loss and Germination test

Grain loss assessment was determined by using hundred Grain Method (HGM) as follow:

$$HGM = \frac{\text{initial HGM} - \text{final HGM}}{\text{initial HGM}} * 100$$

Mass of 100 grains at the beginning of the storage period will be compared with mass of 100 grains after 48 days intervals during the experiment.

Data Analysis

Percentages and mean mortality and migration rate of adult insect pest which occurred was calculated. Mean separation based on least significance difference (LSD) test and ANOVA were conducted using SAS software version 9.2.

Result and Discussions

Repellence test of pulse beetle against fennel seed and koseret leaf powders is indicated in Table 2. All the treatments have shown high repellence against pulse beetle at all amounts of treatments (5, 10, and 15grams) in the whole durations. However, there was no significant difference among treatments. Various botanical products and their extractives works as repellent has been reported by several researchers against *C. chinensis* L. [14-16] tested effect of plant powders and extracts against *C. chinensis*.

Table 2: Mean values for repellence test against pulse beetle (*Callosobruchus chinensis* L.) with different amounts of fennel seeds and koseret leaf powders

Treatment	24hrs			48hrs		
	FE	KL	FK	FE	KL	FK
0gm	2.40±0.14b	2.40±0.14b	2.40±0.14b	2.40±0.14b	2.40±0.14c	2.40±0.14b
5gm	6.22±0.12a	6.00±0.28a	6.10±0.05a	6.00±0.38a	5.73±0.19b	6.07±0.28a
10gm	6.30±0.04a	6.02±0.45a	6.17±0.05a	6.03±0.05a	6.07±0.09ab	6.13±0.10a
15gm	6.30±0.24a	6.32±0.50a	6.47±0.28a	6.20±0.09a	6.13±0.01a	6.28±0.07a
	96hrs			144hrs		
5gm	6.27±0.02a	6.26±0.19a	6.17±0.24a	6.23±0.14a	6.20±0.09a	6.42±0.12a
10gm	6.10±0.24a	6.27±0.19a	6.43±0.05a	6.43±0.05a	6.10±0.14a	6.30±0.04a
15gm	6.13±0.09a	6.23±0.33a	6.23±0.14a	6.30±0.05a	6.40±0.09a	6.30±0.14a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. FS: Fennel Seed Powder; KL: Koseret Leaf Powder; FS+KL: Combinations of Fennel Seeds and Koseret Leaf Powders.

Mortality of pulse beetle, *C. chinensis* adult at different days after treatment with 5gm, 10gm and 15gms of fennel seed and koseret leaf powders and their combined effects is presented in Table 3. Significance difference between control and treatment groups were observed throughout duration of exposure (from 10th to 20th days of treatment) indicating that both koseret leaf and fennel seed powders can be used as biological control of pulse

beetle. Significantly the highest pulse beetle mortality rate (60%) was observed for koseret leaf powders followed by the admixture of koseret leaf and fennel seed powders during 20th days of treatment indicating toxicity effect of these powders on pulse beetle. It was found that the percent mortality was increasing proportionately with the increase of amount of powders and exposure time.

Table 3: Weevil mortality rate (%) after 10 and 20 days as pulse beetle (*Callosobruchus chinensis* L.) treated with different amounts of fennel seed and koseret leaf powders in stored chickpea.

Treatment	10days		
	FE	KL	FK
0gm	0.00c	0.00d	0.00d
5gm	2.5±3.54aB	12.5±2.43bA	10.00±4.21bA
10gm	12.50±3.5aB	25.00±7.07abA	20.00±1.23abAB
15gm	20.00±6.7aB	45.00±5.42aA	45.00±1.81aA
	20days		
0gm	0.00c	0.00d	0.00d
5gm	7.50±7.1cB	17.50±2.3cA	22.50±3.53cA
10gm	37.50±6.1bB	47.50±4.8bA	42.50±3.54bAB
15gm	55.00±2.04aA	60.00±7.5aA	57.50±2.56aA

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance within row. FS: Fennel Seed Powder; KL: Koseret Leaf Powder; FS+KL: Combinations of Fennel seeds and Koseret Leaf Powders.

The result of this study is in accordance with the findings of many authors Singh, Kumari and Singh, Aslam, Umrao and Verma, Singh & Boeke [17-22] who concluded that plant dusts were proved to be equally effective against bruchids in respect of control of number of eggs laid, number of adults emergence, reduction in damage to grain by the pest, weight loss, moisture content and even in germination of the seed. DARP [23] also reported that malathion resistance in stored product insect pests was found from all over the world and currently, there are 122 insect-pest species, which are found as resistant to this insecticide.

The Effect of different concentrations of fennel seed and koseret leaf powders on chickpea seed damage as weight loss and germination loss caused by pulse beetle was presented in Table 4. Significance difference between control or untreated group and treatment groups for both weight loss and germination potential of

chickpea seeds. The highest percentage weight loss was recorded in control (33.79%) followed by 5gm fennel seed powder (16.04%). The lowest percentage weight loss (2.92% and 3.45%) was found in 15gm koseret leaf and fennel seed powders respectively. It was observed that there was no significance difference in weight loss and germination potential of chickpea seeds among 5gm, 10 gm and 15 gm treatments indicating that treatment with fennel seeds and koseret leaf powders did not affect the quality of chickpea seeds. This result was in agreement with findings of Chowdhury, Regmi & Dhoj [24,25] who reported seeds treated with botanical powders reduced the number of damaged seeds. Baral [26] reported more number of populations and more weight loss (21.7 %) even morethan control (16.55 %) in *Azadirachta indica* leaf powder treated grains, where as in *Acorus calamus*, it was nil. *A. calamus* was found even more effective than malathion in controlling *S. oryzae* in wheat seed storage.

Table 4: Grain loss and germination test due to damage by pulse beetle (*Callosobruchus chinensis* L.) on chickpea (*Cicer arietinum* L.) seeds after 30 days of treatment with fennel seed and koseret leaf powders.

Treatment	Weight loss (%)		
	FS	KL	FK
0gm	33.79±1.40a	33.79±1.40a	33.79±1.40a
5gm	16.04±0.89b	12.62±8.42a	14.69±8.99b
10gm	8.89±0.28c	1.14±1.61b	8.02±1.51b
15gm	3.92±1.31d	2.96±1.47b	8.65±1.35b
	Germination (%)		
Treatment	FS	KL	FK
0gm	87.50±2.12c	87.50±2.12c	87.50±2.12c
5gm	91.50±2.12bc	93.75±0.35b	93.50±2.11b
10gm	95.00±1.41ab	97.00±1.45ab	98.50±0.71a
15gm	99.50±0.50a	98.50±0.72a	97.00±1.42ab

Means followed by same letter within a column were not significantly different at 0.05 probability level based on Tukey HSD (Honestly Significantly Different) test. FS: Fennel Seed Powder; KL: Koseret Leaf Powder; FS+KL: Combinations of Fennel Seeds and Koseret Leaf Powders.

Shivanna [27] studied *A. calamus* at 0.5, 1.5 and 2.5 g-50g of seeds as prestorage treatments against *C. chinensis* on red gram (*Cajanus cajan*). They measured fecundity; adult emergence and percent grain weight loss and found that the *A. calamus* powder applied at all 3 rates gave maximum protection against all 3 generations of the pest. Similarly moisture percent and germination percent were highly maintained in chickpea treated by *Sesamum* oil (13.10; 95.00), *C. camphora* balls (13.57; 93.67) and *A. calamus* rhizome dust (13.15; 93.33) till the end of the experiment as initial moisture percent and germination percent of chickpea was 12.30 and 96.00 respectively. *A. indica* leaf dust was poor for maintaining moisture percent (18.03) and germination percent (31.67). Malathion, too, could not show better performance in maintaining moisture percent (17.08) and germination percent (33.67). *X. armatum* fruit dust was intermediate among them. Pandey & Singh confirmed that the Neem powder reduced the damage of the beetle. Al Lawati [28] proved the great inhibiting capacity of the powder extract of *Annona squamosa* L. on *C. chinensis*. Several manioc and bean plants tested on the Coleoptera of the Bruchidae type, showed an insecticidal and ovicide effect [29].

Mihret Alemayehu & Emanu Getu [30] study on germination of chickpea seeds treated with botanicals tested after 90 days of the experiment reported that all the botanical treated seeds showed significantly higher germination that ranged from 80.39% to 100% compared to the untreated check (66.67%). Noug oil and primiphos-methyl treated grains gave 100% germination followed by Lemon oil (98%) and neem seed (96.08%). Thus, the result indicated that chickpea seeds treated with botanicals after 90 days of application germinated well [31].

Conclusion

The use of natural substances for pest control in agriculture is, economically, a viable option and has benefits for both the human being and the environment, due to its low persistence and toxicity. All the treatments tested were statistically superior and provided a better protection against *C. chinensis* compared to the untreated chickpea. However, the results revealed that powdered form of fennel seed and koseret leaf were effective against the Bruchids. Botanical materials resulted significant difference over other treatments in terms of adult mortality, adult emergence, percent grain damage, percent weight loss, moisture content and germination percent. Both fennel seed and koseret leaf powders can be used as an alternative control option in integrated storage pest management strategies. Further studies are required to check the effectiveness of repellence and pesticidal activity of fennel seed and koseret leaf powders as repellence of foliar insects; further studies to be conducted to select the most effective botanical pesticides; Studies are also required to identify biopesticides with minimum effect on seed on germination; biopesticides have great potential application in integrated pest management, due

to customer preferences, health and environmental issues. Thus, studies are required to optimize biopesticide application and their control efficiency, and various form of botanicals either in the form of crude extracts, oils, and various formulations with other ecofriendly pesticides.

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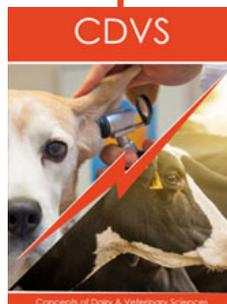
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