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RESEARCH ARTICLE

EFFICACY OF VARIOUS ESSENTIAL OILS AGAINST CHICKPEA PULSE BEETLE (*Callosobruchus chinensis* L.) IN STORAGE CONDITION AT RAMPUR, CHITWAN

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ABSTRACT

The research was carried out in the entomology laboratory of Agriculture and Forestry University, Rampur, Chitwan from March to June 2015. The experiment consisted of nine treatments and replicated thrice and laid out in Completely Randomized Design (CRD). The effects of nine treatments (Neem oil @ 3ml/kg, Clove oil @ 2ml/kg, Citronella oil @ 2.5ml/kg, Mentha oil @ 2ml/kg, Eucalyptus oil @ 3ml/kg, commercial Bojho oil @ 1ml/kg, French basil oil @ 2ml/kg, Malathion @ 1gm/kg, and Control) were evaluated against pulse beetle using ½ kg metal bins as the experimental unit. Among the treatments, the mortality of adult bruchids was significantly higher in chickpea seed treats with Citronell oil (27.67) followed by Malathion dust (27.33), Mentha oil (26.67) and Eucalyptus oil (24.33). Egg counts on 15 DAT, 45 DAT and 75 DAT were also recorded lowest in chickpea seed treated with Citronella oil (4.00, 5.00, 4.33) in all the dates of data recording followed by Mentha oil (4.33, 6.66, 6.00) and Eucalyptus oil (9.66, 13.00, 12.33), respectively. Interestingly, Malathion dust found effective for causing adult mortality (27.33) has reduced effect on next generation adult emergence 81.00 and 108.70 at 45 DAT and 75 DAT. Clove oil and Neem oil were also less effective with maximum egg counts and adult emergence. Thus, among all the oils used Citronella oil, Mentha oil and oils were found to be most promising in protecting grain damage by bruchids and maintaining optimum seed quality up to the 3 months of storage.

KEYWORDS

Essential oils, Chickpea pulse beetle, Chickpea seeds

1. INTRODUCTION

Globally chickpea is the third most important pulse crops mainly grown in the developing countries by resource-poor farmers in drought prone areas and on degraded soils (Joshi et al., 2001) and also one of the major legumes grown in Nepal. It is principally grown in the terai and inner terai in rainfed areas after rice and maize. It is traditionally grown either as a sole crop or as a mixed crop with mustard and/ or linseed on marginal lands with poor management (Pande et al., 2005). Among many other production constraints, insect pests are ranked third based on losses and the storage losses is estimated to be 9% in developed country to 20% or more in developing country (Philips and Throne, 2010). Feeding by the insect larva and adults accounts the major portion of the crop losses annually which causes the economic loss of millions of rupees. Chickpea pulse beetles are a major and growing problem in stored chickpea in all regions of Nepal. They breed rapidly in the storage condition and by the time they are detected, the infested grain lose its seed quality and is usually unmarketable.

The beetle infestation results in substantial reduction in the quality and quantity of the seed. When the infested seeds that harbored bruchid at varying stages of development are stored, adults emerge and lay eggs on the neighboring seeds (Akter et al., 2019). They are present in all the tropical and subtropical climates and attack a wide range of grain legume species (Southgate, 1978; Talekar, 1988). The pulse beetle, *Callosobruchus chinensis* is the major pests of stored pulse grains in

storage causing 15-20% losses in storage throughout the world (Kumar et al., 2003). The primary infestation occurs in the field, where the adults lay eggs on pod after which the larva hatch, penetrate the seed and feed on the cotyledonary and embryonic tissue (Biswas and Biswas, 2005). *Callosobruchus* causes seed weight loss, decreases germination potential, and reduces commercial value of the seeds (Casewell, 1981). Damage in the field is minor, but when such infected seeds are stored, the deploring stages of the beetle continue to feed on the seed and cause secondary infestation which is the major one. Secondary infestation causes loss in the seed quality as well as quantity.

To manage this serious pest, various physical and chemical pest management methods have been employed globally. Different synthetic fumigants developed and used to control this pest in storage are found to leave residues in/on grains and beetles have found to develop resistance against Ethylene-di-bromide (EDB) and aluminum phosphide during storage (Choubey, 2008). In Nepal, pesticides are most intensively applied on vegetables, high value crops, such as vegetables, mustards, and cotton (Pokhrel, 2015). Similarly, the farmers use chemical pesticides in stored product for easy and immediate control of storage pests. The most used pesticide in storage is aluminum phosphide. There are nearly 1500 pesticide poisoning related death annually in the country over the last five years (Pokhrel et al., 2015). Majority of cases are due to intentional poisoning and a significant number of accidental poisoning is with the use of storage pesticide.

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The plant products with insecticidal properties are becoming attractive alternatives to the synthetic, dangerous, and more expensive insecticide used in developing countries. On storage, most farmers do not treat crops with pesticides (Golob, 1994). Before the invent of the synthetic pesticides, local pesticidal plants were the major source of pest control against the pest of field crops as well as in storage. In the world, as many as 2400 plants species have been recorded that have pesticidal properties and biological activity against a wide range of pests (Grainage and Ahmed, 1988). Therefore, a research was conducted to seek plant derivatives as the alternatives of chemical pesticides (DARP, 2003). Few essential oils with insecticidal properties and derived from plant sources were tested in the laboratory.

2. MATERIALS AND METHODS

2.1 Experimental detail

An experiment was carried out at laboratory condition of department of entomology of Agriculture and Forestry University (AFU) during March to June 2005. The experiment was laid out in Completely Randomized Design (CRD) with three replications and nine treatments namely Neem oil @ 3ml/kg, Clove oil@ 2ml/kg, Citronella oil @ 2.5ml/kg, Mentha oil @ 2ml/kg, Eucalyptus oil@3ml/kg, commercial Bojho oil@1ml/kg, French basil oil@2ml/kg, Malathion @ 1gm/kg, and Control. Freshly emerged adult chickpea pulse beetles were used as test insect and metal bin of 2 kg capacity was used as storage structure with ½ kg normalized chickpea seeds of Kosheli variety were in each metal bin.

2.2 Insect rearing

Eggs laid by adult beetle were kept in plastic container covered with muslin cloth and allowed the adult to emerge from the seeds. The adults thus collected were introduced into chickenpea in plastic container and allowed to lay eggs for seven days (Jaiswal et al., 2019). Then adults were transferred into another sets of containers. The culture was maintained in laboratory at 27 ± 2 °C and relative humidity of 70 % to 80%.

2.3 Identification of male and female beetle

Male and female were identified in accordance to the type of antennae and the patch pattern in pygidium. Males were identified by the pectinate antennae and pygidium with dark patches. Females were identified by serrate antennae and pygidium with two dark patches, one on each side of the line.

2.4 Experimental trial setup

27 metal bins were used, and each metal bin was an experimental unit containing 0.5 Kg of chickpea seeds. Each of the metal bins with 0.5 Kg chickpea seeds were treated with assigned treatment of the essential oil and then covered with the muslin cloth from inside and capped tightly externally. In each experimental unit, 15 pairs of newly emerged adult bruchid (1:1 male : female ratio) were inoculated.

2.5 Observation from the experimental trial

The total number of dead adults bruchids after treatment setup were recorded at 15 days after treatment (DAT). In the same time number of eggs per fifty seeds from each experimental unit were also recorded. Fifty seeds were randomly selected from each experimental unit. Adults emerged (both live and dead) from each experimental unit were counted on 45 and 75 DAT. Adults counted on each observation were thrown out at each observation (Kumar et al., 2017). At the same time number of eggs per fifty seeds were also recorded. Initial moisture and germination percentage of the seeds were taken prior to the experimental setup. Moisture percentages were recorded on 45 and 75 DAT. And germination percentages were observed on 75 DAT of the experiment.

2.6 Measurement of the moisture and germination percentage

Wile moisture meter was used for the measurement of the seed moisture and germination percentage was analyzed by using blotting paper method (Park et al., 2016). For the percentage loss assessment, 100 grains were taken randomly at the end of the observation followed by counting of the damaged and undamaged grains. Weight of the damaged and the undamaged grains were recorded using electronic balance.

2.7 Analysis of germination percent

To determine the germination of seeds, a random sample of 50 seeds from each experimental unit were taken. Seeds were cleaned properly and

homogenously distributed in Petri dish(8.8cm) coated with over soaked blotting paper (Pokhrel, 2015). All the plates were kept in germination chamber under normal room temperature of 28°C. Finally, germination percentage was calculated using the following formula:

$$\text{Germination percent} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.8 Loss assessment

The activity was done simply using the count and weight method as adopted. For this, the number and weight of damaged and undamaged grains of composite sample of 100 grains were taken from each experimental unit at final observation (Pokhrel et al., 2015). The percent weight loss was calculated using the following formula:

$$\text{Percentage loss in weight} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u \times (N_d + N_u)} \times 100\%$$

$$\text{Percent loss in weight} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u \times (N_d + N_u)} \times 100$$

Where,

W_u = weight of undamaged seeds

N_u = number of undamaged seeds

W_d = weight of damaged seeds

N_d = number of damaged seeds

2.9 Weather records

Maximum and minimum temperature, rainfall and humidity were also recorded throughout the experimental period. The daily weather recorded is presented in Appendix 20.

2.10 Data management and analysis

Survey data were arranged and analyzed by using IBM SPSS Statistics 21 and biological parameters were arranged in Microsoft EXCEL program and statistically analyzed by using MSTATC statistical package (Sabbour, 2019). The data on insect population and egg count per fifty seeds were transferred square root. Similarly, data with higher CV were square root transferred. Abbott's correction was used in case of adult mortality in control treatment (Abbott, 1925).

$$\text{Corrected \%} = \frac{N \text{ in T after treatment}}{(1 - N \text{ in co}) \text{ after treatment}} \times 100\%$$

Where: N = Insect population, T = treated, Co = control

3. RESULT AND DISCUSSION

3.1 Effects of different treatment on adult mortality

There was significant difference (P< 0.05) on the adult mortality of *C. chinensis* among different treatments (Table 1).

Table 1: Effects of different treatments on adult mortality of <i>C. chinensis</i> at 15DAT in chickpea seeds at AFU, Rampur, Chitwan, 2015	
Treatments	Dead adults of <i>C. chinensis</i> at 15 DAT Number of dead adults (%)
Neem Oil @ 3ml/kg	16.00 ^b (44.00)
Clove Oil@ 2ml/kg	15.33 ^{bc} (41.32)
Citronella Oil @ 2.5ml/kg	27.67 ^a (90.68)
Mentha Oil@ 2ml/kg	26.67 ^a (86.68)
Eucalyptus Oil@3ml/kg	24.33 ^a (77.32)
Bojho Oil@1ml/kg	15.00 ^{bc} (40.00)
French basil Oil@2ml/kg	12.33 ^c (29.32)
Malathion@ 1gm/kg	27.33 ^a (89.32)
Control	5.00 ^d (0.00)
Mean	18.85
SEM±	3.22
LSD	3.07
CV%	9.52

Mean is the average of three replication of population, SEM is the standard deviation from mean value, LSD is the least significance difference and CV denotes the coefficient of variation. Figures in the parenthesis are abbots corrected percent mortality values.

The adult mortality was higher with the use of Citronella oil (27.33 adults/50 seeds), Eucalyptus oil (26.67 adults/50 seeds), Mentha oil (24.33 adults/50 seeds), which were comparable with Malathion (27.67 adults/50 seeds). In terms of adult mortality, Neem oil (16.00 adults/50 seeds), Clove oil (15.33 adults/50 seeds) and Bojho oil (15.00 adults/50 seeds) were of intermediate types, while French basil oil resulted in the lowest adult mortality (12.33 adults/50 seeds) (Sawant et al., 2019). However, among all the treatments, the lowest number of adult mortality was observed in control (5.00 adults/50 seeds).

3.2 Effects of different treatments on egg laying capacity

The treatments were differed significantly ($P < 0.05$) at each observation, i.e. 15DAT, 45 DAT and 75 DAT (Table 2).

Table 2: Effects of different treatment in different days on fecundity of *C. chinensis* in chickpea at AFU, Rampur, Chitwan, 2015

Treatments	Eggs laid by <i>C. chinensis</i> in chickpea at indicated date		
	No of egg/50seeds		
	15DAT	45DAT	75DAT
Neem Oil @ 3ml/kg	16.00 ^{bc}	94.67 ^b	269.30 ^b
Clove Oil @ 2ml/kg	22.00 ^b	36.33 ^c	79.00 ^c
Citronella Oil @ 2.5 ml/kg	4.00 ^d	5.00 ^e	4.33 ^c
Mentha Oil @ 2ml/kg	4.33 ^d	6.66 ^e	6.00 ^c
Eucalyptus Oil @ 3ml/kg	9.66 ^{cd}	13.00 ^{de}	12.33 ^c
Bojho Oil @ 1ml/kg	14.00 ^c	20.00 ^{cde}	30.00 ^c
French basil Oil @ 2ml/kg	15.67 ^{bc}	22.33 ^{cde}	23.33 ^c
Malathion @ 1gm/kg	12.33 ^c	29.67 ^{cd}	52.67 ^c
Control	42.00 ^a	134.70 ^a	526.7 ^a
Mean	15.56	40.26	111.52
SEM±	2.28	6.95	26.78
LSD _{0.05}	6.79	20.66	79.56
CV%	25.48	29.91	41.59

Mean is the average of three replication of population, SEM is the standard deviation from mean value, LSD is the least significance difference and CV denotes the coefficient of variation.

At 15DAT, the lowest number of eggs were observed in Citronella oil treated chickpea seeds (4.00 eggs/50 seeds) followed by Mentha oil treated chickpea seeds (4.33 eggs/50 seeds), Eucalyptus oil treated seeds (9.66 eggs/50 seeds), Malathion dusted chickpea seeds (12.33 eggs/50 seeds), Bojho oil treated seeds (14.00 eggs/50 seeds), French basil oil treated chickpea seeds (15.67 eggs/50 seeds), Neem oil treated seeds (16.00 eggs/50 seeds), respectively and maximum no of egg were observed in control, i.e. without any treatments (42.00 eggs/50 seeds) (Shukla et al., 2016). The trend was similar at 45DAT, where the least number of eggs were observed in Citronella oil treated chickpea seeds (5.00 eggs/50 seeds) and then egg count gradually increased in Mentha oil treated chickpea seeds (6.66 eggs/50 seeds), Eucalyptus oil treated chickpea seeds (13.00 eggs/50 seeds) Bojho oil treated chickpea seeds (20.00 eggs/50 seeds), French basil oil treated chickpea seeds (23.33 eggs/50 seeds), Malathion dust treated chickpea seeds (29.00 eggs/50 seeds), respectively. Then maximum number of eggs were recorded on Clove oil treated chickpea seeds (36.33 eggs/50 seeds), Neem oil treated chickpea seeds (94.67 eggs/50 seeds) and Control (134.70 eggs/50 seeds), respectively. Even at 75DAT, the lowest number of eggs were observed in Citronella oil treated chickpea seeds (4.33 eggs/50 seeds), followed by Mentha oil (6.00 eggs/50 seeds), Eucalyptus oil (12.33 eggs/50 seeds), French basil (23.33 eggs/50 seeds), Bojho oil (30.00 eggs/50 seeds), Malathion dust (52.00 eggs/50 seeds) Neem oil (269.30 eggs/50 seeds), and the highest number of eggs were recorded in control (526.70 eggs/50 seeds).

3.3 Effects of different treatments on adult emergence

There existed significant differences ($P < 0.05$) on adult emergence among the treatments both at 45 DAT and 75 DAT (Table 3). At 45DAT, the least number of adult emergence were observed in Citronella oil treated chickpea seeds (11.67), which slightly higher in Mentha oil treated chickpea seeds (14.00) and Eucalyptus treated chickpea seeds (29.67), respectively followed by French basil oil treated chickpea seeds (60.67), Bojho oil treated chickpea seeds (68.00), Malathion dust treated chickpea seeds (81.00). Adult emergence was found to be higher in Clove oil treated chickpea seeds (235.00) and maximum number of adult emergence were observed in Neem oil treated chickpea seeds (311.70) and in Control

(345.00). The trend was similar at 75DAT, the lowest numbers of adult emergence were observed in Citronella oil treated chickpea seeds (7.00), which slightly higher in Mentha oil treated chickpea seeds (10.33), Eucalyptus oil treated chickpea seeds (11.67) followed by Bojho oil treated chickpea seeds (52.00), French basil oil treated chickpea seeds (52.33), Clove oil treated chickpea seeds (202.00). The maximum number of adult emergence were observed in Neem oil treated chickpea seeds (1800.00), were observed slightly higher in Control (2183.00). Similar finding was reported by Biswas and Biswas (2005) conducted a laboratory experiment on pre-storage seed treatment of gram against *Callosobruchus chinensis* L. and reported that citronella oil at 2.5ml/kg of seed effectively controlled *C. chinensis* L. population by reducing oviposition rate and the treatments also recorded the least seed damage and weight loss due to pulse beetle infestation, as well as the highest percentage of gram seed germination.

Table 3: Effect of different treatments on adult emergence in chickpea at AFU, Rampur, Chitwan, 2015

Treatments	Number of adults emerged at different date	
	45DAT	75DAT
Neem Oil @ 3ml/kg	311.70 ^a	1800.00 ^b
Clove Oil @ 2ml/kg	235.00 ^b	202.00 ^c
Citronella Oil @ 2.5ml/kg	11.67 ^e	7.00 ^d
Mentha Oil @ 2ml/kg	14.00 ^e	10.33 ^d
Eucalyptus Oil @ 3ml/kg	29.67 ^{de}	11.67 ^d
Bojho Oil @ 1ml/kg	68.00 ^{cd}	52.00 ^{cd}
French basil Oil @ 2ml/kg	60.67 ^{cd}	52.33 ^{cd}
Malathion @ 1gm/kg	81.00 ^c	108.70 ^{cd}
Control	345.00 ^a	2183.00 ^a
Mean	128.51	491.92
SEM±	13.21	56.50
LSD	39.24	167.90
CV%	17.80	19.90

Mean is the average of three replication of population, SEM is the standard deviation from mean value, LSD is the least significance difference and CV denotes the coefficient of variation

3.4 Effects of different treatment in percent weight loss

The treatments showed significant difference ($P < 0.05$) on percent weight loss on chickpea seed at 75 DAT (Table 4). There were no significant difference on percent weight loss of chickpea seeds among the treatments, i.e. Citronella oil, Mentha oil, Eucalyptus oil, Bojho oil and French basil oil, minimum percent of weight loss were observed in Citronella oil treated chickpea seeds (0.28), but slightly higher in Mentha oil treated chickpea seeds (0.39), French basil oil treated chickpea seeds (0.56), Eucalyptus oil treated chickpea seeds (0.73), Bojho oil treated chickpea seeds (0.84), respectively. Whereas Clove oil treated chickpea seeds (2.06) and Malathion dust treated chickpea seeds (2.45), Neem oil treated chickpea seeds (5.62) showed intermediate effects in percent weight loss in chickpea seeds. The maximum percent of weight loss in chickpea seeds were observed in Control treatment (15.76).

Table 4: Effects of different treatments in percent weight loss of chickpea seeds at 75DAT at AFU, Rampur, Chitwan, 2015

Treatments	Percent weight loss of chickpea at 75 DAT	
	% loss in weight	
Neem Oil @ 3ml/kg	5.62 ^b	
Clove Oil @ 2ml/kg	2.06 ^{cd}	
Citronella Oil @ 2.5 ml/kg	0.28 ^e	
Mentha Oil @ 2ml/kg	0.39 ^e	
Eucalyptus Oil @ 3ml/kg	0.73 ^{de}	
Bojho Oil @ 1ml/kg	0.84 ^{de}	
French basil Oil @ 2ml/kg	0.56 ^{de}	
Malathion @ 1gm/kg	2.45 ^c	
Control	15.76 ^a	
Mean	3.19	
SEM±	0.49	
LSD	1.46	
CV%	26.71	

Mean is the average of three replication of population, SEM is the standard deviation from mean value, LSD is the least significance difference and CV denotes the coefficient of variation

3.5 Effects of different treatments on moisture percent and germination

The initial moisture recorded was 12.8% and initial germination percent recorded was 93.5%. At 45DAT, Minimum moisture percent were observed on Mentha oil treated chickpea seeds (13.2) which slightly higher in Citronella oil chickpea seeds treated (13.37) followed by Eucalyptus oil treated chickpea seeds (13.63), Neem oil treated chickpea seeds (13.6). Clove oil treated chickpea seeds (13.80), French basil treated chickpea seeds (13.97), and Malathion dust treated chickpea seeds showed intermediate effects in maintain moisture in chickpea seeds. Maximum moisture was observed in control treatment (15.5). Similar trend was recorded at 75DAT, where moisture percent were properly maintained in Citronella oil treated chickpea seeds (13.77), then after moisture percent were increased slightly and were observed in Mentha oil treated chickpea seeds (13.83). Moisture percent were observed intermediate in Clove oil treated chickpea seeds (14.97), Eucalyptus oil treated chickpea seeds (15.23), Bojho oil treated chickpea seeds (15.97) while moisture percent were observed higher in chickpea seeds treated with French basil oil (16.68) and Malathion dust (17.33). Maximum percent of moisture were observed in Control treatment (18.90).

At the 75DAT, Maximum germination percent were maintained in Citronella oil treated chickpea seeds (90.33) which slightly lower in Mentha oil treated chickpea seeds (87.00) followed by Mentha oil (87.00), Eucalyptus oil treated chickpea seeds (86.33). Germination percent were observed lower in Bojho oil treated chickpea seeds (77.67), Malathion dust treated chickpea seeds (72.67), French basil oil treated chickpea seeds (66.00), Neem oil treated chickpea seeds (64.67), and Clove oil treated chickpea seeds (64.33). Minimum percent of germination was observed in Control treatment (48.67). A study reported that repellent activity of Citronella (*Cymbopogon nardus* L.) oil vapor was evaluated against *Callosobruchus chinensis* L in bulk stored green gram (Nayantara and Ratnasekera, 2010). Citronella oil used at 2ml/kg of seed effectively controlled the population of *Chinensis*, and this treatment has no harmful effects on germination of seed.

Figure 1: Effects of treatments on moisture and germination

4. CONCLUSION

Various essential used in the experiment have significant effects on adult mortality, fecundity, adult emergence, moisture of chickpea seeds and germination of chickpea seeds. In laboratory experiment, among eight pesticidal materials tested, mortality of adult bruchids was obtained significantly higher in chickpea seed treated with Citronella oil@ 2.5ml/kg (27.67) followed by Malathion dust@1gm/kg (27.33), Mentha oil@2ml/kg (26.67) and Eucalyptus oil@3ml/kg (24.33). Egg count was also low in chickpea seeds treated with Citronella oil(4.00, 5.00, 4.33) in all the dates of data recording followed by Mentha oil(4.33, 6.66, 6.00) and Eucalyptus oil(9.66, 13.00, 12.33), respectively. Among the essential oils used Citronella oil, Mentha oil and Eucalyptus oil was found to effective in controlling the adult emergence. Malathion, initially found to be effective in causing adult mortality, however, is moderately effective form F₂ generation causing higher adult emergence (81.00 and 108.70).

Clove oil and Neem oil becomes in effective form F₂ generation causing maximum egg count and adult emergence. Among the essential oils used, Citronella oil, Mentha oil and Eucalyptus oil found to be excellent in protecting grain damage by bruchids and maintain the optimum seed quality. Thus, these three essential oils can be promoted as ecofriendly measures for the management of bruchids in chickpea seeds. It is hoped that they will reduce the overuse of chemical pesticide and their negative impacts like health hazard, pesticide pollution in environment and pest resistance against chemical pesticide. As Citronella oil, Mentha oil and

Eucalyptus oil may not be available in all places so more plant materials having pesticidal properties are to be explored.

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