

EVALUATION OF BOTANICAL EXTRACTS AGAINST *Callosobruchus maculatus* F. (COLEOPTERA: CHRYSOMELIDAE) ON DIFFERENT HOST GRAINS

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ABSTRACT

As the damage of stored legume grains by *Callosobruchus maculatus* is severe in Sri Lanka, the present study was conducted to find the remedial solution for this existing problem. The experiment was designed to select the most effective botanical insecticides; *Azadirachta indica* 10 %, *Vitex negundo* 10 %, *Eucalyptus globulus* 10 %, *Annona squamosa* 10 % and *Allium sativum* 10 % against the insect pest, *Callosobruchus maculatus* (bruchid beetle) on stored grains; *Vigna unguiculata* var. *Waruni* (red cowpea), *Vigna unguiculata* var. *Dhawala* (cowpea with black eye), *Vigna radiata* (green gram) and *Cicer arietinum* (chickpea). Among these treatments, *A. indica* 10 % and *A. sativum* 10 % were found to be the significantly best along with 80 % of mortality in adult weevils and zero damaged seeds in all treated grains whereas *V. negundo* 10 % and *E. globulus* 10 % had average mortality effect (40-50 %) at the beginning of the study. Apart from this zero seed, damage was observed in chickpea treated with all treatments including untreated control. The study showed the non-preference of chickpea by *C. maculatus* as the seed coating was resistant to the beetle attack.

Keywords: *Allium sativum*, *Azadirachta indica*, Botanical insecticides, *Callosobruchus maculatus*, mortality, stored grains

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1.0 INTRODUCTION

Legume grains are the main food of one-third of the world's poorest people in Africa and Southeast Asia [1]. These grains play the major role in the income of farmers as well as the nutritional status and diet of people of these regions including Sri Lanka. These are high in protein contents and can be consumed directly or as flour, sprouts, etc. It can also be used during weaning food for young children thus ameliorating malnourishment [2]. Every year farmers for their future needs store a certain amount of the total grain production. It is important that the harvested seeds have to be stored for about 7-9 months until the next planting seasons [3]. However, post-harvest losses of legume grains are a serious problem due to damage caused by insect, *C. maculatus*, *Tribolium castaneum*, *Sitophilus oryzae*, *S. zeamais*, *Sototroga cerealella* etc. Huge losses between 20 and 50 % have been reported in stored grains due to attack by cowpea beetle, *Callosobruchus maculatus* and sometimes the loss may go up to 100 % [4]. It was reported that the grains such as cowpea, chickpea, Bambara groundnut, green gram, lentil, broad bean and green pea were the hosts of larvae of *C. maculatus* [5,6].

The damage is distinctive; larvae feed and develop inside the seed. Each larvae consumes approximately 25 % of the seed from which it develops and results in loss of quality and mould growth [7]. Further, as the larvae bore into the pulse grains, make the seeds unsuitable for human consumption and, replanting due to loss of viability and production of sprouts [8]. Thus, farmers are forced to sell their products early after harvest when prices are still low partly because of anticipated losses in storage [9].

A commonly used method of controlling insect pests of stored products is the application of synthetic contact insecticides and fumigants [10]. The indiscriminate use of broad-spectrum insecticides has created more problems than resolving them. The development of pesticide resistance by the pests, toxic residues in food and consequent health hazards, destruction of beneficial organisms, rapid resurgence of target pest populations and undesirable environmental pollution are critical problems that have arisen by the indiscriminate use of insecticides at storage [11].

It is well aware that the loss to stored products by the cowpea beetle is severe in worldwide including Sri Lanka and the hazardous effects of synthetic insecticide is much serious. Now there is an urgent need for the management practices, which are less toxic to human and environment. The past studies revealed that the pesticide plants have been in nature as its component for millions of years without any ill or adverse effect on the ecosystem [12, 13]. In addition, as the plant-based pesticides are renewable in nature, cheaper, very slow development of resistance in insect pest, etc. occupied a remarkable place in pest management [14]. Thus, now the era in insect pest management switched on towards pesticide plants, which are safer and recorded with no any ill effects to the ecosystem. As the plants, neem (*Azadirachta indica*), nochi (*Vitex negundo*), eucalyptus (*Eucalyptus globulus*) and annona (*Annona squamosa*) and the garlic bulbare easy to collect at the Eastern region of the Sri Lanka and their insecticidal properties are being tested against the management of various insect pests, the present study was undertaken to find the efficacy of these selected botanical insecticides against cowpea weevil, which is the most serious pest of stored products in Sri Lanka [15].

2.0 MATERIALS AND METHODS

The present study focused on the efficacy of fresh leaf extracts of different plants viz., neem 10 % (*Azadirachta indica*), nochi 10 % (*Vitex negundo*), eucalyptus 10 % (*Eucalyptus globulus*), annona 10 % (*Annona squamosa*) as well as extracts of bulbs of garlic 10 % (*Allium sativum*) against the insect pest, *Callosobruchus maculatus* of four stored grains; red cowpea (*Vigna unguiculata* var. Waruni), cowpea with black eye (*Vigna unguiculata* var. Dhawala), green gram (*Vigna radiata*) and chickpea (*Cicer arietinum*). The experiment was conducted in the laboratory of the Department of Agricultural Biology, Eastern University of Sri Lanka during the season Yala 2017 (June-August 2017).

2.1 Preparation of botanical insecticides

a. Leaf extracts

The method described by Gayatri and Rajani, (2017) [16] was slightly modified to prepare aqueous plant leaf extracts. Fresh and matured mixture of mid and lower leaves of neem, nochi, eucalyptus, and annona were separately collected from Agronomy farm, Faculty of Agriculture, Eastern University, Sri Lanka and brought immediately to the laboratory. One kilogram from each of the varieties' leaves was ground by using a domestic electric grinder and mixed them with one liter of water. Mud pots with the leaves solutions were buried in soil for 7 days. The properly soaked solution was strained and used as the treatment after diluting it in water for 1:10 ratio [17]. The methodology for leaf extraction was repeated prior to each application.

b. Garlic bulb extracts

The methodology by Suleria and co-authors (2012) [18] was slightly modified for the preparation of aqueous garlic bulb extract. Finely ground two hundred and fifty grams of dried garlic was mixed with one liter of water and the solution was poured in a mud pot and buried in soil for 7 days. Then the solution was filtered and diluted 1:10 [17] and used for experiments.

2.2 Mass rearing of *C. maculatus*

Adult insects of cowpea beetle were collected from the laboratory cultures and local market at the Batticaloa district and confirmed the species as *C. maculatus*. The collected insects were mass reared by adapting the methodology stated by Abdullah *et al.* (2017) [19]. Cleaned and well-sieved cowpea seeds were obtained from Agronomy Farm, Eastern University of Sri Lanka, Vantharumoolai. Five hundred grams cowpea seeds were taken separately into two sterilized polymate plastic insect cages with the capacity 20cmx6cmx15cm. About 100 male and female adults of cowpea beetles were added into each cage and covered by muslin cloths by tying them by rubber bands to prevent the weevils from getting out or in. Then the cages were placed at 30 °C ± 2.0 and 75 ± 5.0 % RH. After a week, the insects were sieved out and discarded, and the cowpea seeds with eggs of cowpea beetle were kept for about 4 weeks until the emergence of adult insects. Two to four days old, adult insects were used for the experiments.

Experimental setup

The efficacy of botanical insecticides viz., leaf extracts of neem, anona, nochchi, and eucalyptus and bulb extracts of garlic were evaluated against *C. maculatus* on different hosts viz., Red Cowpea, Cowpea with black eye, Green gram and Chick pea. All the treatments were arranged in Complete Randomized Design along with four replications at the laboratory of the Department of Agricultural Biology, Eastern University, Sri Lanka.

Application of treatments

Two thousand and four hundred grams of seeds from each variety namely, red cowpea, white cowpea, green gram and chickpea were taken and thoroughly cleaned and exposed in an oven dried under the condition of 60°C for about 6 hours to ensure the absence of insects, mites and disease causing microorganisms.

The sterilized seeds of one variety were divided into six seed lots each containing four hundred grams of seeds. Each seed lot was separately treated with each of the diluted botanical solution viz., leaf extracts of neem 10 %, anona 10 %, nochchi 10 %, and eucalyptus 10 % and bulb extracts of garlic 10 % and untreated control. 100 ml of each of the botanical pesticide was taken in a hand sprayer and sprayed evenly over the respective seed lot until all the seeds were wetted. Similarly, water was sprayed to the untreated control treatment. The treated seed lots were air-dried under shaded condition. Likewise, other seed varieties were treated.

In order to set the replications, each seed lot consisting four hundred grams of seeds of one variety was further grouped into four as one contained one hundred grams of grains. Each 100 g seed lot was kept in a gunny bag and five pairs of male and female cowpea beetle were introduced into each bag. After the introduction of stored pest, the gunny bags containing treated and untreated seeds were tightly tied to prevent the movement of insects from inside to outside or vice versa.

Similar manner all treatments and replications were designed and kept in the laboratory. In order to increase the efficacy of botanical insecticides the second application was made at 2 weeks after 1st application. At the time of second application of botanical insecticides to the respective seed lots, the live beetles were removed carefully and after drying of insecticides the beetles were replaced in the seed lot and gunny bags were tied. The dead insects and damaged grains, with exiting holes of adult bruchid beetles were counted in weekly interval after the first and second application of botanical pesticides to the respective seed lots.

3.0 Data Analysis

The data collected were subjected to PROC MIXED in SAS 9.1 (two-factor ANOVA). Multiple comparisons were done on least square means using DMRT. All the comparisons were considered significant when $p < 0.05$.

4.0 Results and Discussion

Five indigenous plants leaf extracts and untreated control were evaluated against cowpea beetle on stored grains; red cowpea (*Vigna unguiculata* var. Waruni), cowpea with black eye (*Vigna unguiculata* var. Dhawala), green gram (*Vigna radiate*) and chickpea (*Cicer arietinum*). The results of this experiment conducted during the period Yala 2017 (June-August 2017) is presented and discussed below.

Mortality Rate of adult *Callosobruchus maculatus*

The percentage mortalities of *C. maculatus* exposed to different treatments are given in Table 1. Results revealed that 100 % of mortality in almost all the treatments in chickpea except un-treated control at 7 days after 1st spray whereas since 14 DAS 100 % of mortality was noted in all treatments of chickpea including un-treated control. The findings of present study showed that the mortality of cowpea beetles at the treated and non-treated chickpea due to the effect of insecticide as well as seed characteristics of chickpea, which was evidenced with the statement of Muhammad Sarwar (2012) that the tolerant varieties of chickpea that exhibited hard and wrinkled seed coat lead to harmful relation with pest appearance and grain damage, and these characteristics of chickpea had a negative relation with pest manifestation [20]. Further, it was noted that, the chickpea genotypes with wrinkled seed coat and black colour had affected beetle development and seemed to be less preferred than smooth, plumpy and white colour seeds of chickpea cultivars [21]. In addition, a study stated that the female bruchids usually oviposits on the smooth side of a bean rather than the rough top and it avoids legumes without any smooth surface [22]. The bruchid weevil would choose its host depending on the variety and size of the bean as well as texture of the seeds [23]. In contrast, in few studies it was pointed out that the chickpea (*Cicer* spp.) was the host of cowpea bruchids (*C. maculatus*) [5,20]. Based on these discussions and results of the present study, it can be concluded that chickpea was not the host of cowpea bruchids.

Apart from this finding, almost all the tested botanical pesticides reduced the survival of *C. maculatus* in all tested hosts. However, the study proved the superiority of garlic 10 % in controlling the cowpea weevil especially in green gram (88.9 %) followed by cowpea with black eye (80.9 %) and red cowpea (80 %) at 7 day after first spray. At the same time neem 10 % also exhibited 80 % of bruchid mortality in green gram, cowpea with black eye and red cowpea. However, 100 % of bruchid mortality was reached in garlic 10 % and neem 10 % treated grains after 14 day of first treatment. Meanwhile, significantly very low mortality (10 %) revealed in green gram, cowpea with black eye and red cowpea treated with annona. The garlic 10 % and neem 10 % were considered as better treatment than other botanicals according to the results of the present study. Though annona 10 %, nochi 10 % and eucalyptus 10 % were recorded with significantly low mortality than garlic 10 % and neem 10 % at the beginning of experiment, they destroyed almost all the insects after second treatment in all tested hosts.

A study by Bamphitlhi Tiroesele (2015) [24] stated 87.5 % of mortality in *C. maculatus* on in *Vigna subterranea* treated with 0.55gm of chopped garlic per 50gm of grains, and this finding supports the present study results. Certain studies revealed a positive response against brichid beetles by the application of African nutmeg, clove and garlic [25], and chili pepper and black pepper [26].

Table 1: Percentage mortality of adult cowpea beetles (*Callosobruchus maculatus*) by the application of botanical insecticides on different host grains

Hosts	Treatments	Percentage of Mortality*			
		1 st Spray		2 nd Spray	
		7 Days after spray	14 days after spray	7 Days after spray	14 days after spray
Red Cowpea (<i>Vigna unguiculata</i> Var: Waruni)	Neem	80.0 (63.4) ^c	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Eucalyptus	30.0 (33.2) ^g	80.0 (63.4) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a
	Nochi	50.0 (45.0) ^e	80.0 (63.4) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a
	Annona	10.0 (18.4) ^h	80.7 (63.4) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a
	Garlic	80.0 (63.4) ^c	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Control	0.0 (2.03) ⁱ	40.0 (39.2) ^e	60.0 (50.8) ^c	100.0 (90.0) ^a
Cowpea with black eye (<i>Vigna unguiculata</i> Var:Dhawala)	Neem	80.0 (63.4) ^c	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Eucalyptus	40.0 (39.2) ^f	80.0 (63.4) ^b	88.9 (70.5) ^b	100.0 (90.0) ^a
	Nochi	30.0 (33.2) ^g	66.7 (54.76) ^d	100.0 (90.0) ^a	100.0 (90.0) ^a
	Annona	10.0 (18.4) ^h	71.4 (57.67) ^c	100.0 (90.0) ^a	100.0 (90.0) ^a
	Garlic	80.7 (63.4) ^c	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Control	0.0 (2.03) ⁱ	20.0 (26.6) ^f	40.0 (39.2) ^d	100.0 (90.0) ^a
Green gram (<i>Vigna radiata</i>)	Neem	80.0 (63.4) ^c	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Eucalyptus	60.0 (50.8) ^d	60.0 (50.8) ^d	90.0 (71.6) ^b	100.0 (90.0) ^a
	Nochi	40.0 (39.2) ^f	80.0 (63.4) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a
	Annona	10.0 (18.4) ^h	80.0 (63.4) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a
	Garlic	88.9 (70.5) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Control	10.0 (18.4) ^h	60.0 (50.8) ^e	80.0 (63.4) ^b	100.0 (90.0) ^a
Chick pea (<i>Cicer arietinum</i>)	Neem	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Eucalyptus	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Nochi	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Annona	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Garlic	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a
	Control	90.0 (71.6) ^b	100.0 (90.0) ^a	100.0 (90.0) ^a	100.0 (90.0) ^a

CV %	5.15	21.76	2.64
CD (0.05)			
Hosts	2.25	7.01	0.51
Treatments	2.76	8.59	0.63
Interaction	5.51	17.18	1.26

*Values are mean of three replications.

Values in parentheses are arcsine and square root ($\sqrt{X+0.5}$) transformations.

In each column, means with similar alphabets do not vary significantly at P=0.05 and P=0.01 by DMRT.

CV – Coefficient of Variation

CD – Critical Difference

A study on certain selected botanicals as protectants of cowpea bruchids evidenced the superiority of garlic with 81.2 % mortality among the treated botanicals viz., *Curcuma longa*, *Ficus exasperate*, *Garcinia kola*, *Zingiber officinale* and *Z. officinale* oil [7]. In addition, the bruchid development had been suppressed by garlic oil applied on cowpea grains [27] whereas oviposition of *C. maculatus* had significantly reduced in chickpea seeds when it was treated with garlic clove powder [28]. Further, a study reported that garlic was second best botanical due to the presence of diallyl sulphate, which has high fumigant action [29].

Study has proved that azadirachtin from neem plant has a deterrent, antifeedant, growth disrupting, ant ovipositional and fecundity reducing properties on a range of insects [30]. The potential of botanicals such as leaf powder of *Tephrosia vogelii*, the combination of neem and *T. vogelii* and tobacco snuff as significantly excellent measures in controlling the beetles and thus recommended as protectant of grains used for both food and seed purposes [9].

Further, the neem leaf powder is identified as effective protects of black gram, pigeon pea and green gram seeds, against *C. chinensis* with maximum adult mortality [31,32,33,34]. Meanwhile a study in India on the insecticidal action of nine plant species such as neem, garlic, eucalyptus, pongam, tulsi, custard apple, mint, arduci and kalmegh against *C. chinensis* in black gram showed the significant role of neem leaf powder in controlling the *C. chinensis* followed by garlic bulb powder where acceptable efficacy has been exhibited by eucalyptus, pongam, tulsi and custard apple leaf powder [31]. However, the botanicals, mint, arduci and kalmegh showed less effective against infesting black gram [31].

Number of damaged seeds

A significant difference ($p < 0.05$) was found between the numbers of damaged seeds in different treatments when compared with the control (Table 2). The zero seed damage was observed in all hosts sprayed with neem and garlic especially after 14 days of 1st spray except chickpea, which was not selected as host by bruchid beetles during the study period. The seed damage was visible in grains treated with other treatments viz., eucalyptus, nochi, annona and untreated control from 14th day onwards.

The reference studies revealed that plant products significantly reduced the number of eggs laid by cowpea weevils on the grains and a significant reduction in the exit holes was exhibited on the garlic and chilies treated grains compared with the peppermint and untreated control treatments [24]. However, the authors stated a comparable reduction by the peppermint than the untreated control in the number of exit holes. Neem leaf powder was the most effective grain protectant in suppressing the population of pulse beetle in grains [35]. The same botanical was also registered earlier as superior in soybean and cowpea [36].

Table 2: Number of damaged grains in different host grains treated with different botanical pesticides by the infestation of *Callosobruchus maculatus*

Treatments	Red Cowpea (<i>Vigna unguiculata</i> Var: Waruni)				Cowpea with black eye (<i>Vigna unguiculata</i> Var:Dhawala)				Green gram (<i>Vigna radiata</i>)				Chick pea (<i>Cicer arietinum</i>)			
	1 st Spray		2 nd Spray		1 st Spray		2 nd Spray		1 st Spray		2 nd Spray		1 st Spray		2 nd Spray	
	After7 th day	after14 th day	after7 th day	After14 th day	after7 th day	after14 th day	after7 th day	after14 th day	after7 th day	after14 th day	after7 th day	after14 th day	after7 th day	after14 th day	after7 th day	after14 th day
Neem	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Eucalyptus	0.00 ^a	10.00 ^c	12.00 ^c	0.00 ^a	0.00 ^a	8.00 ^c	18.00 ^d	46.00 ^d	0.00 ^a	9.00 ^b	12.00 ^b	12.00 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Nochi	0.00 ^a	2.00 ^b	2.00 ^b	2.00 ^b	0.00 ^a	8.00 ^c	12.00 ^c	12.00 ^c	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Annona	0.00 ^a	12.00 ^{cd}	22.00 ^d	22.00 ^c	0.00 ^a	5.00 ^b	7.00 ^b	7.00 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Garlic	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Control	0.00 ^a	20.00 ^d	41.00 ^e	68.00 ^d	0.00 ^a	10.00 ^d	38.00 ^e	80.00 ^e	0.00 ^a	13.00 ^c	43.00 ^c	74.00 ^c	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a

5.0 Conclusion

The study stated that chickpea (*Cicer arietinum*) was a non-host of Cowpea bruchid, *Callosobruchus maculatus* as the grain was not preferred by the bruchids.

The application of neem 10 % (*Azadirachta indica*) and garlic 10 % (*Allium sativum*) would be effective treatments with respect to the mortality of *Callosobruchus maculatus* on stored grains; Red Cowpea (*Vigna unguiculata* var. Waruni), Cowpea with black eye (*Vigna unguiculata* var. Dhawala) and Green gram (*Vigna radiate*). Nochi 10 % (*Vitex negundo*), eucalyptus 10 % (*Eucalyptus globulus*) and annona 10 % (*Annona squamosa*) also could be considered as potential plant extracts against Cowpea weevil, *Callosobruchus maculatus*.

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