Research Article

Insecticidal Efficacy of *Azadirachta indica* (A. Juss) and *Ricinus cummunis* (L.) Seed Oil Against *Callosobruchus maculatus* (F.) on Stored Mung Beans (*Vigna radiata* L. Wilczek)

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Abstract | Stored pulses suffer from great damage and weight loss due to attack of Cowpea seed beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), thus, it was important to handle the pest infestation on legume grains in order to reduce the economic losses. A laboratory experiment was conducted in August-November, 2019. The experiment was laid out at (30±2 °C and 65±5% relative humidity), to investigate the insecticidal efficacy of *Azadirachta indica* (A. Juss) (neem) and *Ricinus cummunis* (L.) (castor) seeds oil that effect the various biological parameters of *C. maculatus* fed on *Vigna radiata* (mung beans) including mortality, oviposition, and adult emergence using three replication for each treatment. Four concentrations *viz;* (0.25, 0.50, 0.75 and 1%) each of the oil were prepared to observe the contact toxicity and surface protectant effect. Results showed that as percentage of concentrations increased mortality of adult beetles also increased with increased exposure time. In addition, among the dissimilar concentrations of oils, it was found that 0.50% of both oils were lethal concentrations (LC_{50}) which killed almost (50%) population of beetles after 48 hrs of exposure. Furthermore, both oils were effective by reducing (100%) oviposition, population emergence and seeds infestation rate as compared to control in which 75% infested grains were found including the large number of eggs and adults. These two oils provided best protection at their higher concentrations to mung beans against *Callosobruchus maculatus*. In conclusion, it is strongly recommended that oil extracts from seeds of neem and castor plants can be used to manage the stored grain pest up to the tolerable limits in integrated pest management. Moreover, the present study suggests further work on the efficacy of some other local plant extractions as an alternative to chemical pesticides.

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Introduction

Vigna radiata (L. Wilczek) is also known as mung beans belong to family leguminosae. The mung bean crop is cultivated throughout the Indian subcontinent (Mondal *et al*., 2012). While in Pakistan *Vigna radiata* is cultivated on about 0.178 million hector area of the total agricultural land with the production of 0.13

million tons during the year 2016-2017 (Economic Survey of Pakistan, 2016-2017). Dry pulse seeds are used as a source of nutritional food for humans, they are also a favorite food for many people across the World (Vilakazi, 2016).

Callosobruchus species cause significant damage to almost all stored pulses and badly affected the quality of the seeds (Patil *et al*., 2003). Among the pulse beetles like *Callosobruchus chinensis* and *Callosobruchus analis*, *Callosobruchus maculatus* is one of notorious insect pest of legume seeds such as *Vigna radiata* (mung bean), *V. unguiculata* (cowpea) and *V. mungo* (mash bean) (Caswell, 1984). The larvae bore into the seeds for feeding and complete their development inside the grains; these infested grains become unsuitable for human consumption and planting. Also changed seeds have low market value. In this way, *C. maculatus* provided immense losses to the formers (Tapondjou *et al*., 2002). This polyphagous beetle causes heavy damage to the *Vigna* species within 2-3 months (Carlos, 2000).

Since *Vigna radiata* has economic value being the main food for infants and patients (Hozayn *et al*., 2007), it is important to protect the stored mung grains from infestation of *C. maculatus*.

Traditionally people protect their grains from infestation of insects during storage by different plant parts which have repellent efficacy against insects. This traditional method was applied by poor formers who had small landholding farms (Belmain *et al*., 2001). But now a day's several insecticides were recommended for storage management such as actellic (2%) or actellic super and phostoxin, however they are very expensive and require proper equipments and training for their use. They have lots of other disadvantages too like different kind of pollution and can kill the man and other useful organisms during their application (Ntoukam *et al*., 2000).

Due to these above reasons, agricultural researchers once again gave attention to plant extractions which have insecticidal efficacy as an alternative source of chemical insecticides for the control of stored grain insect pests.

Many plant species have insecticidal properties such as *Azadirachta indica*, *Murraya koenigii*, *Nicotiana tobacum* and liquorice and these were effective against two different strains (Lahore and Faisalabad) of *Callosobruchus chinensis* L. (Haidri *et al*., 2021). Neem, nishinda, bankalmi, safflower, eucalyptus, bablah and sesame plants derivates had insecticidal efficacy against *C. maculatus*, they can be used as a surface protectants on black gram (Rahman and Talukder, 2006). Some other plant oils like *R. communis*, *Brassica rapa*, *A. indica* and *prunus amygdalus* were also effective against Sialkot strain of *C. maculatus* on stored mung beans. These plant essential oils are less toxic to the other living organism in the ecosystem and are cheaply available to the formers (Sarwar *et al*., 2019).

As the beetle *C. maculatus* is a major pest of stored mung bean pulse and needs some less toxic or biorational methods to control its infestation.

Keeping in view, the objectives of present study is to assess the insecticidal efficacy of *A. indica* (neem) and *R. communis* (castor) oil against the Hyderabad strain of *C. maculatus* in order to protect the grains during storage.

Materials and Methods

The present experiments were conducted in the laboratory of the Department of Zoology University of Sindh, Jamshoro, Pakistan, from August-November, 2019, under constant environmental conditions (temperature 30±2 °C and 65±5% relative humidity) by performing three replications of each treatment, the method described by (Hanif *et al*., 2015).

Plant materials used for experiments

Dry *V. radiata* seeds about 5 kg were brought from grain market Hyderabad. All the grains were sterilized at 0 °C in the freezer for about 1 day to kill the developmental stages of insect, if already present on them following (Sarwar *et al*., 2019). *A. indica* (pure neem oil) and *R. communis* (pure castor oil) were also purchased from Malkani oil products of Pakistan.

Collection and rearing of C. maculatus

The adults of Hyderabad strain of *C. maculatus* were obtained from highly infested mung beans bought from grain storage houses in Hyderabad market. One pair from infested grains was separated; this pair was then introduced in screened 500 g of mung grains to get homogenous population. The plastic jars used for rearing the beetles having 2 kg capacity. Each jar was covered with net sieve to allow free air for respiration and to prevent adult insects from escape. After 25-27 days of ovi position new adults emerged which were used for further study following (Sarwar *et al*., 2019).

Sample preparation of plants oil

To observe the *in vitro* insecticidal potential of neem and castor oil four different concentrations *i.e*., 0.25, 0.50, 0.75 and 1% of both oils were prepared simultaneously by the method described by Raham and Talukder (2006). The oil is diluted in acetone (Hanif *et al*., 2015). For the preparation of (0.25%) concentration of tested plant oils, 0.25 mL of oil was added in 99.75 mL of acetone. In the same way 0.50, 0.75 and 1% concentrations were also prepared.

Experimental protocol

After sample preparation, 15mL of oil concentration was sprayed on forty grams (40 g) of mung beans by the help of syringe and then mixed with the help of fine brush until the seeds were uniformly and equally coated with the oil, in this way four different treatments of both oils were prepared.

After that all the oil treated grains were left for an hour to evaporate the acetone and oil dried. Ten grams (10 g) of treated seeds were separated from 40 g of each treatment for seed germination test. While the rest of 30 g seeds were put into the plastic jars having half kg capacity, for control 30 g of untreated mung beans were put into the jar for comparison purpose, Raham and Talukder (2006). After that thirty-five (35) one day old adults (male and female) were released on each experimental jar and then covered with thin cloth for aeration. Mortality of adult beetles was observed after 24, 48 and 72 hrs of exposure to contact toxicity.

For mortality tests original data were corrected by Abbott's (1925) formula and calculates as follow:

$$CM\ (\%) = [(T - C) / (100 - C)] \times 100$$

Where; CM is Corrected mortality; T is Mortality in treated seed; C is Mortality in untreated seeds.

While surface protectants effect of seeds were observed by estimating oviposition, adult emergence and weight loss of grains after 7, 30 and 42 days of storage.

Ten seeds were randomly picked and sown in pots from separated 10 g treated seeds of each treatment, to observe the seed germination after 120 days of treatment. The data regarding surface protection efficacy and seed germination was calculated as follow (Rahman and Talukder, 2006; Gereziher *et al*., 2016).

$$Eggs\ hatching\ (\%) = Total\ eggs\ hatch / Total\ eggs\ in\ each\ jar \times 100$$

$$Weight\ loss\ (\%) = Initial\ weight - Final\ weight / Initial\ weight \times 100$$

$$Seed\ germination\ (\%) = NG / TG \times 100$$

Where; NG is number of seeds germinated; TG is total number of seeds tested in pots.

$$Protection\ (\%) = [TF1PC - TF1PT / TF1PC] \times 100$$

Where; TF1PC is total first filial progeny in control; TF1PT is total First filial progeny in treatment.

Statistical analysis

Means of three replications were taken from each treatment and statistical significance was calculated by (ANOVA) using statistics 8.0 software (Haidri *et al*., 2021).

Results and Discussion

Contact toxicity

Efficacy of tested essential oils as a contact toxicant was observed by mortality of adult beetles by direct contact with oils after 24, 48 and 72 hrs of exposure. Mortality of test insect was considerably increased with in exposure time and percent concentration of oils. In control group (untreated grains) no death of adults was observed ($P \leq 0.05$) after 72 hrs of infestation, because introduced beetles could not find any difficulty in respiration due to oil free contact with host.

Neem oil exhibits maximum percent mortality to adults with 65.71, 88.57 and 100% at 1% followed by 45.71, 82.86 and 100% at 0.75%, while the same oil gave minimum percent mortality with 0, 11.43 and 31.43% at 0.25% oil concentration after 24, 48 and 72 hrs of exposure, respectively (Figure 1).

Whereas castor oil also showed maximum percent mortality with 64.70, 87.56 and 100% also at 1% followed by with 44.70, 81.85 and 99% at 0.75% oil concentration while minimum percent mortality with 0, 10.40 and 30.40% at 0.25% after 24, 48 and 72 hrs of treatment, respectively (Figure 2).

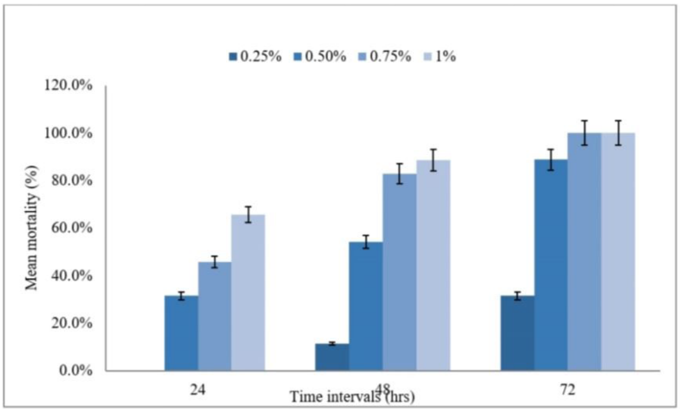

Figure 1: *Percent mean mortality of Callosobruchus maculatus adults against four different concentrations of Azadirachta indica (neem) oil.*

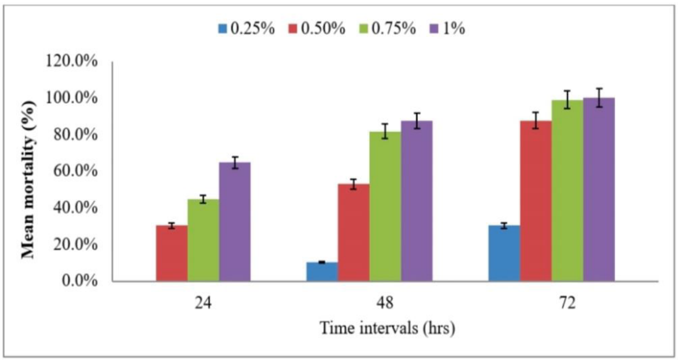

Figure 2: *Mortality of Callosobruchus maculatus adults against four different concentrations of Ricinus communis (castor) oil.*

At the same time neem and castor oil provided with 54.29 and 53% mortality to beetles respectively with their 50% concentrations after 48 hrs of treatment. Therefore, based on above data regarding percent mortality the lethal concentration of two tested oils for *C. maculatus* was estimated and it was 50% concentration at that almost half pest population was found dead.

Surface protectant effect of oils

The efficacy of neem and castor oil as surface protectants of grains was investigated by comparing the total number of mean eggs laid; total number of mean adults emerged, hatching percentage of adult beetles and weight loss of treated grains. Four dissimilar concentrations of two different oils were tested *i.e.*, 0.25, 0.50, 0.75 and 1.0%. The data was significantly different when we compared with the control (P≤ 0.05). Table 1 revealed that more mean numbers of eggs laid, adults emerged and eggs hatching percent of Cowpea weevil and weight loss of seeds found in control group, while in treatments the mean maximum number of eggs was laid at 0.25% whereas mean minimum number of eggs were laid at 0.50, 0.75 and 1.0% oil treatments.

The lowest mean eggs hatching percent as well lowest mean adult emergence was observed at 0.50, 0.75 and 1.0% treatments of both oils, on the other hands highest mean eggs hatching percent and mean maximum adults emergence were found at 0.25% treatment of castor oil followed by neem oil. The highest percent surface protection of treated mung grains was recorded at higher concentrations of tested oils.

Table 1: *Effect of neem and castor oil on eggs laid, eggs hatching percent, adult emergence of Callosobruchus maculatus, percent protection and weight loss of seeds.*

Name of oil and extract concentration (%)	Total number of mean eggs laid after 7 days	Eggs hatching (%)	Number of mean adult emerged after 30 days	Protection (%)	Weight loss (%) after 42 days
Neem					
0.25	84b	1.1c	1c	99.7c	00c
0.50	38bc	0c	0c	100c	00c
0.75	9c	0c	0c	100c	00c
1	4c	0c	0c	100c	00c
Castor					
0.25	115b	10.43bc	12bc	96.70bc	1bc
0.50	64bc	0c	0c	100c	00c
0.75	32c	0c	0c	100c	00c
1	18c	0c	0c	100c	00c
Control	348a	82.7a	288a		75a

Means in the same columns followed by the variable letters are significantly different (P≤0.05).

The weight loss percent of all the treated and control grains were calculated after 42 days of treatments. It was observed that no weight loss of treated grains occurred in all treatments except at 0.25% treatment of castor oil, although it had very low percent weight loss in comparison with control group that had an extensive percentage of weight loss of seeds.

The above results showed that both tested oils showed strong insecticidal efficacy against adult and developmental stages by inhibiting the mating and physiological activities of *C. maculatus.*

Seeds germination test

Seeds treated with different concentrations of *A. indica* **(**neem) and *R. communis* (castor) oil showed 80 and 100% germination, respectively while control grains showed 100% germination after 120 days of storage.

The findings of present bioassay tests revealed that the application of neem and castor oils acted as contact toxicant against *C. maculatus* on stored seeds of *V. radiata*. It was observed that the 100% mortality of adults has occurred after 72 hrs of treatments by direct contact to higher concentrations of both oils neem and castor due to the blockage in respiratory passage. Our results are related to (Ahmed *et al*., 1999) who found that 100% dead adults of *C. chinenesis* on neem oil treated Azuki grains after three days of exposure. Hanif *et al*. (2015) also revealed that the percent mortality of *Trogoderma granarium* against essential oils of *A. indica*, *Melia azadarach* and *Datura stamonium* was time and concentration dependent.

It was also found that both different tested oils reduced the population growth and weight loss of grains even at their lower 0.25% concentration (Table 1) and protects the grains from infestation of *C. maculatus*. Our outcomes that neem and castor oils worked as a surface protectants are close to Rahman and Talukder (2006) they used different plants oil of neem, safflower and sesame to observe their surface protectants efficacy on black grams against *C. maculatus* and revealed that among different oils neem oil was the best surface protectant.

Nisar *et al*. (2022) also assessed the effectiveness of some botanical essential oils against *C. maculatus i.e.,* Neem, mustard, pumpkin and popy. They prepared o.5, 1.0, 1.5 and 2.0 mL concentrations of the oils. They observed the highest mortality of beetles on neem oil treated grains while all the oils concentrations 100% inhibited population emergence, damage and weight loss of the treated grains, finally they concluded that the tested oils could be utilized to control *C. maculatus* during storage.

In addition to this neem, mustard and almond oil at a rate of 10 mL/ kg significantly reduced weight loss of *V. radiata* against *C. maculatus* (Sarwar *et al*., 2019). Furthermore, according to Haidri *et al*. (2021) plants derivatives of *A. indica*, *M. koenigii*, liquorica and *N. tobacum* were effective against two strains of *C. chinensis,* among them neem exhibited the best repellent and growth inhibitor as compared to other plant extractions, its higher concentration 20% showed 93.32% repellency.

Sarwar *et al*. (2019) observed that plants extracted essential oil treated mung grains did not lose their viability.

The Present study has clearly shown that different concentrations of neem and castor oil were effective against *C. maculatus* and did not affect the quality and seed germination rate of mung seeds. Therefore, botanical parts could be used for controlling insect pests under environmental friendly management.

Conclusions and Recommendations

The findings of this research work confirmed that plant seeds oils might have useful as insect control agents for marketable use. *A. indica* (neem) *and R. communis* (castor) oil were very effective when as contact toxicant they provide 100% mortality to adult beetles as their concentration and exposure time increased. In addition, lethal concentration of both oils was 50% concentration and this could be used to develop bio insecticides against *C. maculatus*. While like a surface protectant both oil reduced oviposition, adult emergence and to minimized the sever weight loss of mung beans even at their lower concentrations. Finally, it was recommended that the application of plant oils to stored grains of pulses is an inexpensive and effective technique and has less environmental hazard. They can be use as an alternative to chemical pesticides in integrated pest control program.

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Novelty Statement

The current research work gives data that neem and castor plants provide oils and could be used to treat the mung beans even at their lower concentration to protect the grains from infestation of Hyderabad strain of *C. maculatus* during storage. It also gives baseline information to keep the seeds protected during export.

Author's Contribution

Nosheen Jehajo and Nasreen Memon: Conceived the idea and supervised the work.
Nosheen Jehajo: Executed the laboratory research.
Mansoor Ali Shah: Helped in statistical analysis.
Naheed Shah: Helped in paper write up.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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