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Original Article

Potential of plant oils against *Callosobruchus maculatus* (Coleoptera; Bruchidae)on stored mung bean (*Vigna radiata*)

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Abstract

In the present study four plant oils including neem oil (*Azadirachta indica*), castor oil (*Ricinus communis*), mustard oil (*Brassica rapa*) and almond oil (*Prunus amygdalus*) were evaluated against *Callosobruchus maculatus* (Coleoptera; Bruchidae) applied to mung bean (*Vigna radiata)* at a dosage of 10ml/kg. Free choice and no choice experiments were conducted to observe the oil effects on insect orientation, adult mortality, oviposition, adult emergence, seed weight loss and seed viability. All these oils showed 100% reduction in adult emergence and seed weight loss. Neem oil was proved to be most effective in oviposition deterrence as well as in deterring insects. These oils also did not affect seed viability.

Keywords: Plant oils, Mung bean, Seed viability, Leguminosae, *Callosobruchus maculatus*

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Introduction

Mung bean (*V. radiata*) is an important kharif crop in Pakistan and is easily digestible protein rich food containing 23.6% proteins (Albala, 2007; Morris and Brad, 2003). Mung bean is cultivated on about 18% of total cropped area with pulses in Pakistan. Eighty five percent of the total mung bean production of Pakistan is cultivated in Punjab province. In 2011-2012, total area cultivated with mung was 140.8 ha with the production of 93 tons (Pakistan Bureau of Statistics, 2012). *C. maculatus* (Coleoptera; Bruchidae) also known as bean bruchid is the most devastating stored grain pest of *Vigna spp*. (mung bean, black gram and cow pea). About 50% of damage in pulses is caused by this insect (Casewell, 1984). In some cases damage can increase up to 99% after 6 months of storage.The larvae of *C. maculatus* chew into the pulses which become useless for human feed as well as germination (Tapondjou et al., 2002). Apart from direct damage to grains, losses also occur due to contamination of grain with insect faecal material (Udo, 2011). As legumes provide the economical and richest source of plant

protein to man and animals, thus control of this pest is essential (Singh and Pandey, 2001).

In past practice of mixing plant ashes and inert dust or sand with pulses was common to prevent insect attack. Synthetic insecticides have been used to control such pests of stored products (Tapondjou et al., 2002).But recently the synthetic insecticide application such as DDT is reduced because they have environmental as well as health hazards as they are not biodegradable (Shukla et al., 2007; Bekele, 2002).For all these reasons attention has been diverted towards the botanicals with insecticidal properties (Obeng-ofori, 2007).

Essential oils are used as natural pesticides to protect and preserve crop, because they are less toxic to mammals and have less effect on environment as compared to some synthetic pesticides (Talukdar and Howse, 1994).Application of leaf powders and leaf extracts and oils of different plants to wheat to preserve it from grain weevil (*Sitophilus granaries*) evaluated that oils were more effective as compared to plant powders and extracts (Rahman et al., 2003; Rahman and Talukdar, 2006). Neem oil has been evaluated by many researchers against a number of insects who attack pulses (Ketkar 1987; Lale and Mustafa, 2000; Jilani et al., 2003; Lal et al., 2012). Castor oil proved to be effective against bean bruchids by causing mortality and reducing adult emergence as well (Khaireet al., 1992; Pacheco et al., 1995).

As bean bruchids are more susceptible to mung bean and cause considerable losses to mung bean, in this research neem oil, castor oil, almond oil and mustard oil were evaluated against infestation of *Callosobruchus maculatus*. The main objective of this research was to evaluate the effectiveness of these oils against adult mortality, oviposition, production of progeny and rate of infestation as well as effect on seed germination

Material and Methods

Culturing preparation of *Callosobruchus maculatus*

The insect pest studied in this research was *Callosobruchus maculatus* which is a serious pest of mung bean seeds. About 1kg mung bean heavily infested with *C.maculatus* was purchased from a local market of Sialkot. Fifty (25 pair) adults from this infested mung bean were separated. These adults were then introduced in 1kg of uninfested healthy mung bean in a plastic jar and the mouth of plastic jar was tied with muslin cloth and a rubber band. This jar was placed in the lab at ambient lab conditions. About 7 days after introduction of adults, oviposition was observed and all the insects either live or dead were removed again from bean. After 25-27 days of oviposition, new adults were emerged which were used for further study.

Experimental material

Stored product in this research was mung bean *(Vigna radiata).* Healthy mung bean about 5kg was purchased from local market. Before starting experiment it was placed in the freezer at 0^0C for about 24 hours to eliminate any insect infestation.

Four plant oils were evaluated in this study including:
neem oil (*Azadirachta indica)*
castor oil (*Ricinus communis)*
mustard oil (*Brassica rapa)*
almond oil (*Prunus amygdalus)*
All of these oils were purchased from local market.

The oils were mixed separately with mung bean in a bowl at rate of 10ml per 1kg of seed. The bowl was shaken well to ensure the uniform coating of oil on the surface of each seed. 1kg of untreated mung bean was used as control. Mung bean used in further research was taken from this stock.

Free choice experiment

Free choice test was conducted to observe orientation of adult insects toward different plant oil treatments. Free choice apparatus was self-constructed by connecting five jars with a capacity of 100g to a central one having a capacity of 500g. Each jar was equidistant to the central one. Holes were created in all jars at same level and then connected to central jar having five holes at same level through plastic pipes. About 50g of mung bean was placed in four different jars with 100g capacity each treated with specific oil. In the fifth jar (control) *i.e*. 50g of untreated mung bean was placed while in the central jar 60 (30 pairs) insects were released. These insects were allowed to move freely towards jars containing bean. The direction of insect movement was observed 1^{st}, 2^{nd} and 3^{rd} days after release of insects by counting number of insects in each jar. The experiment was replicated thrice.

No choice experiment

Fifty grams of seeds were weighed from each treatment as well as control and placed in 5 separate

jars. Each jar was labeled with the name of oil by which it was treated. Jar with untreated mung was labeled as control. Five pairs (0-2 days of bean beetles) were introduced into each jar and jars were placed in lab shelf at lab ambient conditions. Adult mortality, oviposition by adult females, adult emergence and weight loss in the seeds was observed. Germination test was performed at end of experiment. Each experiment was replicated thrice.

Adult mortality was observed on 2nd, 4th and 6th days after introduction of insects. All the live and dead insects were counted.

After six days of introduction adults either living or dead were removed. Number of eggs on all the bean seeds was noticed and calculated using hand lens or magnifying glass.

Adults were emerged about 21-27 days after oviposition. Number of live and dead adults was counted again in all treatments.

Percent weight loss was recorded by excluding weight of infested beans from the weight of healthy beans before infestation.

Effect of oils on seed viability was noticed by performing germination test. This test was performed after 35 days of starting experiment. Ten seeds from each treatment were picked and sown in soaked cotton in Petri dishes to see the germination.

Statistical analysis

All collected data regarding to insect orientation, adult mortality and oviposition were analyzed through a software package "Statistics" by using one way analysis of variance (ANOVA) method. Means of replicates for all treatments were separated by least significant difference (LSD) at 5% level of probability (Steel and Torrie, 1980). To check %age weight loss and %age reduction in adult emergence Abbots formula was applied (Abbot, 1925). %age deterrence in oviposition and %age germination were calculated by following formulae respectively:

%age deterrence = (no. of eggs in control – mean no. of eggs of each treatment)/control × 100

%age germination = no. of seeds germinated/ total seeds placed for germination×100

% reduction in adult emergence was corrected by Abbots formula (Abbot, 1925)

Results

All the plant oils tested were significantly effective in controlling *C.maculatus* in Mung bean (*V .radiata*). All the oils tested were found effective in resisting the orientation of insects as compared to control. Neem oil showed the higher potential in resisting orientation compared to other treatments including control. Castor, almond and mustard oils also resisted insect orientation but were not significantly different from control as revealed in Table 1.

Table 1: Effect of plant oils on isect orientation in free choice experiment

Treatments	No. of Insects With Respect to Days			Grand Mean
	1DAT*	2 DAT	3 DAT	
Azadirachta indica (neem oil)	3.667 ± 2.02^{d}	6.000 ± 1.52^{cd}	6.000 ± 1.52^{cd}	5.222 ± 0.93^{b}
Ricinus communis (castor oil)	9.000 ±0.57^{abc}	10.000 ±1.00^{abc}	9.333 ±0.88^{abc}	9.444 ± 0.44^{a}
Brassica rap (mustard oil)	7.000 ±2.30^{bcd}	10.667 ± 1.45^{ab}	9.667 ±0.88^{abc}	9.111 ± 0.99^{a}
Prunus amygdalus (almond oil)	9.000 ±2.30^{abc}	10.333 ±1.45^{abc}	9.667 ±0.88^{abc}	9.667 ± 0.84^{a}
Control	10.333 ±0.33^{abc}	13.333 ± 2.84^{a}	11.333 ± 0.88^{ab}	11.667 ±0.97^{a}

All data represent mean of three replicates ± standard error

Means with similar letters in same column are not significantly different according to LSD (5%)

Table 2, revealed the effect of four plant oils on adult mortality of *C. maculatus* after 2, 4 and 6 days of treatment. The highest mortality was observed in neem oil treated seeds having 6.667 dead insects at 2nd day after treatment. Mean number of insects for all the days in neem oil treatment was 3.333. Results for other oil treatments were also significantly similar to neem oil with 3.111, 3.000 and 3.111 insects for castor oil, mustard oil and almond oil, respectively, as shown in Table 2. Mean number of insects in control were significantly different from all other treatments.

Table 2: Effect of plant oils on adult mortality after 2nd, 4th, and 6th day after treatment

Treatments	No. of Dead Insects			Grand Mean
	2 DAT	4 DAT	6 DAT	
Azadirachta indica (neem oil)	6.667 ± 1.20^{a}	3.000 ±1.15^{ef}	0.333 ± 0.33^{h}	3.333 ± 1.04^{a}
Ricinus communis (castor oil)	3.333 ±0.33^{cbe}	4.666 ±0.33^{bcd}	1.333 ± 0.33^{f}	3.111 ± 0.51^{a}
Brassica rapa (mustard oil)	2.667 ±0.66^{efg}	5.333 ± 0.88^{ab}	1.000 ± 0.57^{gh}	3.000 ± 0.72^{a}
Prunus amygdalus (almond oil)	2.333 ±0.33^{efg}	5.000 ±0.57^{abc}	2.000 ±0.57^{efgh}	3.111 ±0.53^{a}
Control	0.333 ± 0.33^{h}	1.000 ± 1.00^{gh}	2.667 ± 0.33^{efg}	1.333 ± 0.47^{b}
Grand Mean	3.067 ± 0.60^{a}	3.800 ± 0.53^{a}	1.467 ± 0.27^{b}	2.778 ± 0.31

Data in all the columns show mean number of dead insects in three replicates ± standard error
Data shown by similar letters in same column is not significantly different according to LSD at 5%

Results for %age deterrence in oviposition and mean number of eggs laid on all treated bean seeds is demonstrated in Table 3. Maximum deterrence of oviposition (92.1%) was observed in neem oil treatment. Deterrence shown by other treatments was included 77.2, 57.8 and 57.3% for castor oil, mustard oil and almond oil respectively.
All oils showed 100% reduction in adult emergence as well as reduction in weight loss. These oils may have killed the eggs or larvae in the eggs thus inhibiting the adult emergence completely. As the eggs were not strongly bounded to mung bean seed therefore larvae could not feed on seed as well as could not penetrate inside the seed thus resulting mortality. These oils also did not affect the seed viability, 100% germination was observed with mustard oil (*Brassica rapa*), while castor (*Ricinus communis*) showed 80% and all others showed 90% germination (Table 4).

Table 3: Mean number of eggs laid by females in all treatments and control

Treatments	No. of eggs	Percentage deterrence
Azadirachta indica (neem oil)	16.33 ± 4.05^{c}	92.1
Ricinus communis (castor oil)	47.33 ± 12.19^{c}	77.2
Brassica rapa (mustard oil)	87.67 ± 12.00^{b}	57.8
Prunus mygdalus (almond oil)	88.66 ± 6.98^{b}	57.3
Control	208.0 ± 17.09^{a}	

% deterrence = (no. of eggs in control – mean no. of eggs of each treatment)/control × 100
Data shown by similar letters in same column is not significantly different according to LSD at 5%

Discussion

Quite similar results for castor and mustard oils were observed in a study evaluated by Fouad (2013) in cowpea seeds. He reported 20% repellency for both castor oil and mustard oil. Maximum repellency as well as best surface protectant property was also observed for neem oil by Ketkar (1989) in cowpea seeds.

Table 4: Total no. of adults emerged, percentage adult emergence, percentage weight loss and percentage seed germination

Treatments	No. of Adults Emerged	Percentage Reduction in Adult Emergence	Percentage Weight Loss	Percentage of Seed Germination
Neem oil	0.00	100%	00	90%
Castor oil	0.00	100%	00	80%
Mustard oil	0.00	100%	00	100%
Almond oil	0.00	100%	00	90%
Control	111.66		5.4%	100%

The effect of four plant oils on adult mortality of *C. maculatus* after 2, 4 and 6 days of treatment is shown in Table 2. Results were in confirmation with findings of Ahmed *et al.,* (1999). They reported 100% adult mortality of *C. chinensis* infested on azuki bean with the treatment of *Azadirachta indica* just in three days. Our findings were in consonance with Lal and Deepshikha (2003) who reported mean number of eggs laid on pigeon pea by *C. maculatus* as 14.52 and 15.23 with neem and castor treatment respectively. Size of eggs laid on treated seeds was same as on untreated seeds. But eggs were loosely attached to treated seeds as compared to untreated seeds. Jagjeet et al.(2005) reported that the oviposition of insects is reduced on the grains by the application of oils.

Complete reduction in weight loss was observed because no infestation took place. Our results were in accordance with findings of Lal and Deepshika (2012) who reported that neem oil and castor oil completely inhibited the adult emergence of *C. maculatus* when applied at a dosage of 3ml/kg of the pigeon pea and weight loss observed was zero due to the application of these oils. Percentage of seed germination observed by Lal and Deepshikha (2012) for neem and castor oil were 82.96 and 78.23 which are very close to present results. Moreover results of experiments of Raghvani and Kapadia (2003) were also in confirmation that plant oils have no effect on seed viability.

Conclusion

Protection of stored pulses has been under consideration for many years. Many insecticides were tested against insect pests of stored pulses. But these were not safe. Then different botanicals were evaluated to check their efficacy against different insect pest of pulses. In the present research mung bean was treated with different oils against *Callosobruchus maculatus*. All the oils tested in present research including *Azadirachta indica, Ricinus communis, Prunus amygdalus* and *Brassica rapa* were found very effective and quite safe to use as they have no effect on stored bean. All oils completely inhibited adult emergence. *Azadirachta indica* was found effective in all parameters including resistance of insects, adult mortality as well as seed germination. *Ricinus communis* was also found effective in adult mortality as well as in oviposition deterrence after Neem oil. *Prunus amygdalus* and *Brassica rapa* also showed quite safe to use. Further studies could be done to explore the potential of different other oils occurring naturally against insect pests. Use of oils in combination may be recommended as cheap, easily available, eco-friendly and non-toxic in management of *C. maculatus* and other insect pests and thus are efficacious in protecting mung bean and other pulses from insect invasion at farmer level. Isolation of active ingredients from these oils and preparation of their formulations could be done and could be evaluated against pests.

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Contribution of Authors

Sarwar A: Literature Search, Data Collection, Manuscript Writing
Afsheen S: Conceived Idea, Designed Research Methodology, Data Interpretation, Manuscript final reading and approval
Shah SS: Data Interpretation, Manuscript Writing
Afzal S: Data Interpretation, Manuscript Writing
Zia A: Data Interpretation, Statistical Analysis
Khan I: Data Interpretation, Statistical Analysis
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