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Botanicals for Protection of Mungbean against *Callosobruchus maculatus* during Storage

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ABSTRACT

Background: The mungbean/green gram is one of the major grain legume crops native to the Indian subcontinent, which was found damaged extensively by the pulse beetle, *Callosobruchus maculatus* during storage. Moreover, insect damage also causes chemical constituents of seeds leading to alteration in flavor, nutritive value, marketability and acceptability of the stored seeds. Therefore, present investigation was focused on evaluating bioefficacy of botanicals in the protection of green gram grains from *C. maculatus* and also the changes on biochemical properties during six months of storage.

Methods: During the experimentation, green gram seeds were treated with selected botanicals in the form of oils, powders and their aqueous extracts and the effects on pulse beetle in terms of adult emergence and seed damage was recorded up to six months storage. Changes in biochemical constituents of the stored grains were also recorded .

Result: The study revealed significantly lesser number of exit holes/100 grains (1.33 to 13.33), adult emergence (4.33 to 15.00 no.), weight loss (0.92 to 3.10%) and grain damage (1.40 to 5.40%) in neem oil @ 3.0% after one month interval. The crude protein values did not vary significantly among the treatments, but the alcoholic acidity varied between 0.30-0.94%. The uric acid content in grains was increased with insect population. Significantly lower values of free fatty acids were recorded in the standard check which was at par with all the concentrations of neem oil, *Karanj* oil and 3.0% mustard oil.

Key words: Alcholic acidity, Biochemical constituents, Bioefficacy, Botanical, *Callosobruchus maculatus*, Free fatty acids, Protein, Seed damage, Uric acid.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the important and popular pulse crops cultivated throughout India since time immemorial (Dhage and Patil, 2022) and is consumed as a whole, dehulled and sprouted grain in a variety of dishes (Singh *et al.,* 2017). In Punjab, mungbean occupied 2.6 thousand hectares and the total production was 2.5 thousand tonnes during 2020-21 (Anonymous, 2022). The food grain losses caused due to various storage insect pests have been estimated to be 20-25% in India (Rajashekar and Shivanandappa, 2010). Most of these insect pests belong to the orders Coleoptera and Lepidoptera, accounting for about 60 and 10%, respectively (FAO, 2009). The continuous and indiscriminate use of pesticides has lead to several problems. There is no doubt of using botanical insecticides as an effective alternative to insect pest control and on the other side more than 2,50,000 plant species on our planet have been tested for this purpose (Khalequazzaman and Osmangoni, 2009).

Pulse beetle, *Callosobruchus maculatus* (Fabricius) (Bruchidae: Coleoptera) is a cosmopolitan, polyphagus and multivoltine species (Pajni,1987) causing significant damage to stored cowpea (68.2%) followed by moth bean (53.7%) and mungbean (50.3%) (Ramazan *et al.*, 1990). A significant change in chemical constituents of stored seed including alcoholic acidity, crude protein, uric acid, *etc*. with the increase in insect infestation has been confirmed in several researches (Singh, 2002; Modgil, 2003; Modgil and Mehta, 1995). Hence, an attempt has been made to investigate the effect of plant products against *C. maculaus* infesting mungbean but also the biochemical constituents of infested mungbean.

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MATERIALS AND METHODS

The experiment on bioefficacy of botanicals against *C. maculatus* was conducted at Post-harvest Technology Laboratory, Punjab Agricultural University, Ludhiana during 2017-18 under the ambient environment. The mother culture of pulse beetle, *C. maculatus* was obtained from this Laboratory, Department of Processing and Food Engineering, PAU, Ludhiana and mass cultured on mungbean seed (Variety: SML-668) collected from Instructional Farm, PAU, Ludhiana.

In this experiment different plant oils (neem, karanj, mustard, castor, sesamum, soybean, groundnut); plant powders (neem kernel powder and turmeric powder) and

plant aqueous extracts (neem kernel extract and turmeric extract) against *C. maculatus* were taken. For start of experiment, total quantity of grains @ 500 g per treatment, of mungbean variety SML-668 was taken in a bin. The desired numbers (1 kg= 5 pairs) of freshly emerged pairs of *C. maculatus* were released in this bin and an exposure period of seven days was given for uniform multiplication of insect. Out of these grains, 500 g grains were taken from this lot for each treatment and these grains were treated with the botanicals. For each treatment, six sets were maintained with three replications. The jars were covered with muslin cloth under laboratory conditions along with untreated control. The duration of experiment was six months. At the end of each month, observations were taken and respective set was discarded after taking the observations at monthly interval. The following treatments were applied at different concentrations against *C. maculatus* in mungbean along with recommended practice (cover the pulses stored in bulk with 7 cm sand layer):

Treatments	Concentrations (%)
Neem oil	1.5, 2.0, 3.0
Karanj oil	2.0, 3.0
Mustard oil	2.0, 3.0
Castor oil	2.0, 3.0
Sesamum oil	3.0
Soybean oil	3.0
Groundnut oil	2.0, 3.0
Neem kernel extract (30%)	1.5, 2.5, 5.0, 10.0
Turmeric extract	10.0
Neem kernel powder	1.0, 2.0, 3.0, 4.0
Turmeric powder	4.0
Standard Check	Grains covered with 7 cm thick layer of sand
Untreated control	-

Different plant oils were purchased directly from the local market. The finely ground powder of these plant materials was obtained by grinding their required parts separately in an electronic blender and were sieved through a 90 mm mesh sieve to obtain a fine powder. The desired concentrations of powders were added to the grains in the jars. Each plant material was taken @ 300 g per 1 litre of water (30%) and boiled at 100°C in water bath for ten minutes to take its extract. The lid of the container during boiling was covered to avoid evaporation. The extract was then allowed to cool at room temperature, filtered through muslin cloth. The required concentrations (1.5, 2.5, 5.0 and 10.0%) were prepared from the stock solution. The following observations were taken:

Number of exit holes

A sample of 100 grains was taken and the number of exit holes were counted.

Adult emergence

The total number of adults emerged in 500 g sample were counted.

Weight loss (%)

It was calculated using count and weight method given by Adams and Schulten (1978).

Grain damage (%)

It was calculated by formula:

$$\text{Grain damage (\%)} = \frac{\text{Number of insect damaged grains}}{\text{Total number of grains}} \times 100$$

Seed germination

It was tested using 'paper towel method' (ISTA 1985).

Biochemical constituents

These were calculated by the following methods.
Uric acid: Venkatarao *et al* (1960).
Protein: Micro-Kjeldahl (1883).
Free Fatty acids: Mckillican (1966).
Alcoholic acidity: (AOAC 1960).

The data so generated were normalized through arc sine (√percentage) and square root transformations before subjecting for analysis of variance (ANOVA) under completely randomized design (CRD) using computer programme CPCS 1 (Computer Package for Computing Statistics1) (Cheema and Singh 1990).

RESULTS AND DISCUSSION

Exit holes (Nos.)

Neem oil @ 3.0% recorded significantly less number of exit holes/100 grains (1.33 and 4.33, respectively) (Fig 1) after one and second month of storage. After four months of storage period, neem oil @ 3.0% recorded significantly minimum number of exit holes followed by its concentrations of 1.5 and 2.0%. No significant increase in exit holes was recorded after five months of storage in mungbean grains treated with neem oil @ 3.0%. Similarly after six months of storage, the number of exit holes varied from 13.33 (neem oil @ 3.0%) to 29.33 (turmeric rhizome powder @ 4.0%).

Adult emergence (Nos.)

After two months of storage, significantly minimum adult emergence (6.00 adults/500 g) in neem oil @ 3.0% (Fig 2). After four months of storage, neem oil @ 3.0% was significantly better than all other treatments. After six months, significantly minimum adult emergence was recorded in neem oil @ 3.0%. Mulatu and Gebremedhin (2000) also showed that the plant oils of *Azadirachta indica* partially or completely inhibited *C. chinensis* emergence from the laid eggs. Jumbo *et al* (2014) reported reduced adult emergence of *C. maculatus* after treatment with plant based products.

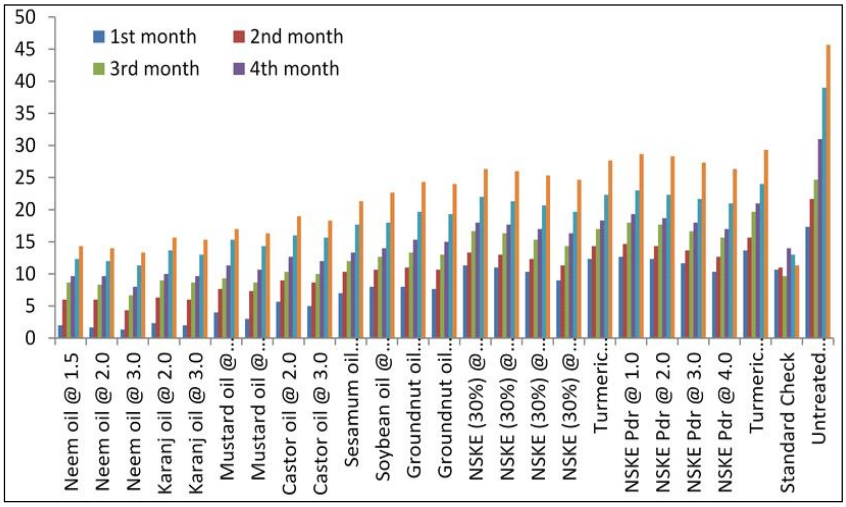

Fig 1: Effect of different botanicals on exit holes by *C.maculatus*.

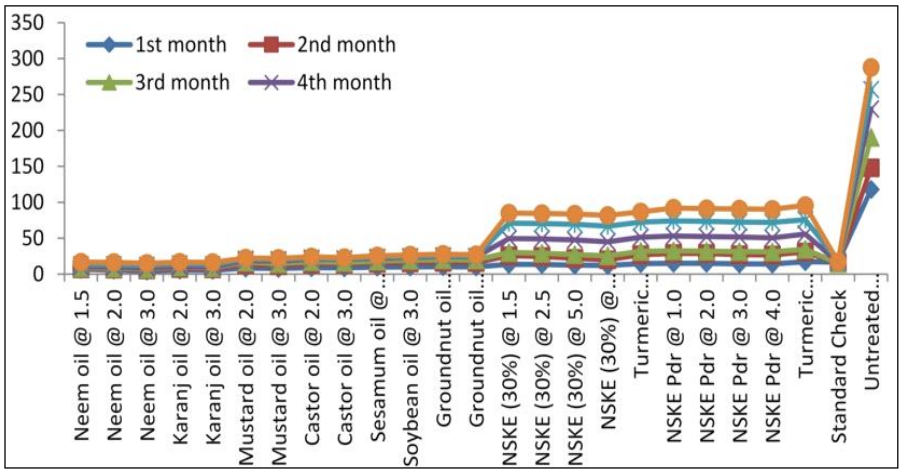

Fig 2: Effect of different botanicals on adult emergence of *C. maculatus*.

Weight loss (%)

Significantly minimum weight loss was recorded in neem oil @ 3.0% (Fig 3). After two and four months of storage, significantly minimum weight loss was recorded in neem oil @ 3.0%. After five months, no significant increase in weight loss was noticed in the standard check and neem oil @ 3.0%. After six months, significantly minimum weight loss was recorded in neem oil @ 3.0%.

Grain damage (%)

The data on % grain damage given in Fig 3 revealed that Neem oil @3.0% recorded significantly less grain damage (1.40%) grain damage after one month. After two months, neem oil @ 3.0% recorded significantly lower value for % grain damage. After three months and neem oil @ 3.0% was significantly better than all other treatments. After four months of storage, neem oil @ 3.0% recorded significantly minimum grain damage followed by its concentrations of 1.5 and 2.0%. Chaudhary (1990) reported no grain damage in chickpea grains upto 6 months of storage, when grains were treated with neem, groundnut and castor oil @ 0.5 and 1.0% against *C. chinenesis*. Similarly after six months of storage, grain damage was significantly minimum (5.40%) in neem oil @ 3.0%. Significantly less % seed content loss was reported with neem oil (@ 2.40 and 1.20%, respectively) (Kumar *et al.*, 2017; Rahman and Talukder, 2006).

Seed germination

The data on seed germination in Table 1 revealed that it was not significantly affected in treated and control samples after three and six months of storage. This indicated that there was no ill effect of application of botanicals on the mungbean grains. The findings of Meghwal *et al.* (2007) reported that germination of mothbean seeds was not effected at all by oils of neem, castor, mustard and groundnut oil each @ 0.4, 0.8 and 1.2 ml/100 g grains.

Biochemical constituents

Crude protein

All the botanical treatments and the standard check were statistically at par with each other and crude protein values were non-significant in these treatments except untreated

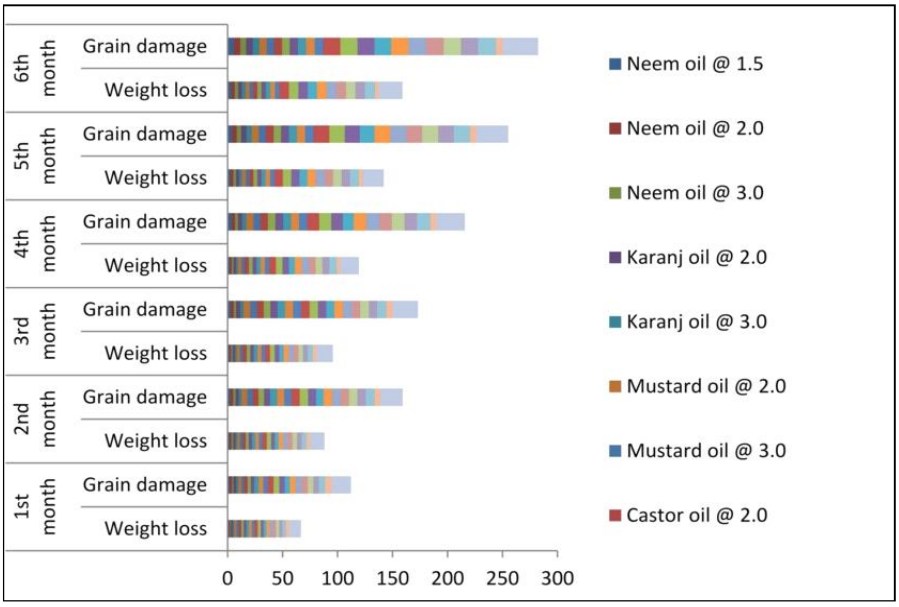

Fig 3: Effect of different botanicals on weight loss and grain damage of green gram grains.

Table 1: Efficacy of different plant oils, powders and aqueous extracts on seed germination of mungbean.

Treatment	Concentration (%)	%seed germination after#	
		3rd month	6th month
Neem oil	1.5	88.67±0.53	90.00±0.45
Neem oil	2.0	89.00±0.53	89.00±0.52
Neem oil	3.0	89.00±0.29	88.00±0.27
Karanj oil	2.0	88.67±0.43	89.00±0.52
Karanj oil	3.0	88.00± 0.25	88.00±0.48
Mustard oil	2.0	89.00±0.50	89.00±0.39
Mustard oil	3.0	87.33±0.42	89.67±0.50
Castor oil	2.0	87.67±0.55	89.00±0.42
Castor oil	3.0	89.33±0.50	88.67±0.41
Sesamum oil	3.0	88.67±0.12	88.67±0.39
Soybean oil	3.0	89.00±0.32	88.67±0.65
Groundnut oil	2.0	88.67±0.63	89.00±0.79
Groundnut oil	3.0	89.00±0.69	89.33±0.44
Neem kernel extract (30%)	1.5	88.67±0.78	88.67±0.58
Neem kernel extract (30%)	2.5	87.33±0.74	88.00±0.89
Neem kernel extract (30%)	5.0	88.00±0.40	89.00±0.40
Neem kernel extract (30%)	10.0	88.00±0.35	87.33±0.52
Turmeric rhizome extract	10.0	88.00±0.29	87.67±0.29
Neem kernel powder	1.0	88.00±0.48	89.33±0.17
Neem kernel powder	2.0	89.00±0.57	88.67±0.38
Neem kernel powder	3.0	88.67±0.21	89.00±0.60
Neem kernel powder	4.0	88.67±0.15	88.67±0.25
Turmeric rhizome powder	4.0	88.67±0.41	89.00±0.51
Standard check	Grains covered with 7 cm thick layer of sand	89.00±0.35	88.67±0.79
Untreated control	-	89.33±0.59	88.00±0.51
CD (p=0.05)	NS	NS	

Table 2: Efficacy of different plant oils, powders and aqueous extracts on different biochemical constituents in mungbean.

Treatment	Concentration (%)	Biochemical constituents after six months			
		Crude protein (%)#	Alcoholic acidity (%)#	Uric acid (mg/100 g)#	Free fatty acids (%)#
Neem oil	1.5	24.50±0.14 (29.66)	0.38±0.12 (3.54)	34.25±0.66 (35.80)	0.23±0.09 (2.74)
Neem oil	2.0	24.50±0.08 (29.66)	0.35±0.05 (3.39)	33.81±0.63 (35.53)	0.23±0.16 (2.74)
Neem oil	3.0	24.42± 0.28 (29.60)	0.36±0.03 (3.24)	33.53±0.35 (35.37)	0.20±0.10 (2.56)
Karanj oil	2.0	24.50±0.24 (29.66)	0.43±0.10 (3.76)	44.87±0.62 (42.04)	0.240.12 (2.80)
Karanj oil	3.0	24.40± 0.49 (29.58)	0.40±0.50 (3.56)	44.00±0.50 (41.54)	0.23±0.06 (2.75)
Mustard oil	2.0	24.55± 0.56 (29.68)	0.51±0.06 (4.09)	55.56±0.24 (48.17)	0.26±0.10 (2.92)
Mustard oil	3.0	24.52±0.45 (29.67)	0.47±0.04 (3.93)	55.10±0.36 (47.91)	0.24±0.06 (2.81)
Castor oil	2.0	24.67±0.48 (29.76)	0.57±0.04 (4.33)	56.60±0.26 (48.77)	0.28±0.11 (3.03)
Castor oil	3.0	25.62±0.52 (30.39)	0.53±0.02 (4.17)	56.21±0.45 (48.55)	0.26±0.07 (2.92)
Sesamum oil	3.0	24.66±0.31 (29.76)	0.59±0.04 (4.40)	57.80±0.36 (49.47)	0.28±0.06 (3.03)
Soybean oil	3.0	24.70±0.19 (29.79)	0.61±0.06 (4.48)	58.92±0.71 (50.12)	0.30±0.05 (3.14)
Groundnut oil	2.0	27.78±0.41 (31.79)	0.67±0.04 (4.69)	59.84±0.35 (50.66)	0.32±0.11 (3.24)
Groundnut oil	3.0	24.72±0.20 (29.80)	0.650.08 (4.62)	59.19±0.31 (50.28)	0.30±0.14 (3.13)
Neem kernel extract (30%)	1.5	24.80±0.33 (29.85)	0.79±0.09 (5.09)	60.54±0.26 (51.06)	0.38±0.07 (3.53)
Neem kernel extract (30%)	2.5	24.75±0.23 29.82)	0.76±0.06 (5.00)	60.40±0.69 (50.99)	0.37±0.08 (3.48)
Neem kernel extract (30%)	5.0	24.64±0.19 (29.75)	0.70±0.10 (4.80)	60.28±0.96 (50.92)	0.33±0.09 (3.29)
Neem kernel extract (30%)	10.0	24.50±0.28 (29.65)	0.65±0.07 (4.62)	60.12±0.19 (50.82)	0.30±0.05 (3.14)
Turmeric rhizome extract	10.0	24.85±0.46 (29.88)	0.82±0.07 (5.19)	61.14±0.47 (51.42)	0.42±0.10 (3.71)
Neem kernel powder	1.0	24.85±0.23 (29.88)	0.88±0.06 (5.38)	61.87±0.52 (51.85)	0.48±0.05 (3.97)
Neem kernel powder	2.0	24.80±0.19 29.85)	0.85±0.07 (5.29)	61.80±0.13 (51.80)	0.45±0.07 (3.84)
Neem kernel powder	3.0	24.78±0.20 (29.84)	0.81±0.09 (5.16)	61.71±0.19 (51.75)	0.43±0.07 (3.76)
Neem kernel powder	4.0	24.76±0.31 (29.83)	0.78±0.07 (5.06)	61.64±0.42 (51.71)	0.38±0.07 (3.53)
Turmeric rhizome powder	4.0	24.90±0.24 (29.92)	0.90±0.35 (5.42)	62.05±0.33 (51.95)	0.50±0.11 (4.05)
Standard check	Grains covered with cm 7 thick layer of sand	24.32±0.10 (29.54)	0.30±0.31 (3.11)	24.67±0.32 (29.76)	0.20±0.14 (2.56)
Untreated control	-	29.05±0.33 (32.60)	0.94±0.05 (5.56)	96.00±0.50 (78.45)	0.58±0.10 (4.36)
CD (p=0.05)		0.92	0.43	1.33	0.27

#Figures in parentheses are the means of arc sine√percentage transformations.

control (Table 2), where a significant increase in crude protein value to 29.05% was observed which might be due to addition of body fragments and uric acid, the main excretory product of insects. The results are in corroboration with Ekeh *et al.* (2013) and Modgil and Mehta (1995) who reported increase in values of crude protein with increase in infestation by *C. chinensis*.

Alcoholic acidity

The data given in Table 2 showed hidden infestation of insects and other metabolic changes that might have led to the increase in alcoholic acidity. Significantly lower value of 0.30% was recorded in standard check followed by neem oil @ 1.5, 2.0 and 3.0%, where the values for alcoholic acidity were 0.38, 0.35 and 0.36%, respectively and all these treatments were statistically at par with each other and also with *Karanj* oil @ 3.0% (0.40%). Similarly, Singh (2002) reported alcoholic acidity in mungbean samples increased from 0.30 to 0.57% in control samples during six months storage of grains.

Uric acid

Data presented in Table 2 inferred that the uric acid content in mungbean grains increased with increase in insect population and it ranged from 24.67 in the standard check to 96.00 mg/100 g in untreated control. The standard check was significantly better than all other treatments. Treatments of mungbean grains treated with neem oil @ 1.5, 2.0 and 3.0% were at par with each other. Modgil and Mehta (1995) reported increase in values of uric acid content, crude protein with increase in infestation by *C. chinensis*. Singh (2002) observed increase in uric acid values in mungbean grains during six months storage period.

Free fatty acids

Analysis of data presented in Table 2 revealed that fat acidity increased with increase in insect population and ranged from 0.20 to 0.58%. Singh (2002) observed 0.10 to 0.50% increase in fat acidity due to *C. maculatus* infestation and 0.10 to 0.24% increase in treated mungbean samples during six months of storage.

Throughout the storage studies, neem oil @ 3.0% remaines as the best treatment. Neem and sesame oils completely inhibited adult emergence of *C. chinensis* and protected the seeds from damage by this pulse beetle as confirmed by Ahmed *et al.* (1999). Rahman and Talukder (2006) also reported minimum adult emergence of *C.maculatus* when blackgram seeds were treated with plant oils of nishinda, eucalyptus and bankalmi @ 3.0%. The present findings are in close conformity with Shaaya *et al* (1997) who reported that edible oils act as good seed protecting agents against *C. chinensis*.

CONCLUSION

Storage studies on efficacy of botanicals against *C. maculatus* revealed that neem oil @ 3.0% is very effective against *C. maculatus* recording significantly lesser number of exit holes, adult emergence, weight loss and grain damage. The treatments with botanicals did not show any ill effect on seed germination. So the botanicals can be taken as an integral part of integrated programmes for stored grain insect pest management.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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