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Preference and progeny development of pulse beetle in response to black gram treated with botanicals and inert materials

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Abstract
Preference of pulse beetle towards black gram grain treated with inert materials such as sand as a surface layer of 3.0 cm thickness, cow dung ash @ 5% and red earth @ 5% and plant products including *Sesamum* oil @ 2%, neem seed powder @ 3%, dried neem leaves @ 5%, dried *Ocimum* leaves @ 5%, vasaka powder @ 3% and sweet flag rhizome bits @ 5% were assayed under free choice conditions by using ten arm olfactometer. The Excess Proportion Index for the sand layer treatment was -0.84 as the insects could not access the grains. Among the botanicals, sweet flag rhizome bits with an EPI of -0.82 showed strong repellency followed by vasaka plant powder (-0.78) and neem seed powder (-0.65). The sand layer and *Sesamum* oil treatments showed with no oviposition, adult emergence and per cent grain damage. The treatments; neem seed powder, sweet flag rhizome bits and vasaka powder recorded minimum oviposition (1.67, 2.0 and 6.0no.s respectively), less number of adult emergence (5.67, 7.67 and 10.0 respectively) and per cent grain damage (1.67, 3.0 and 12.0 respectively).

Keywords: Botanicals, inert materials, pulse beetle response, EPI

1. Introduction
Blackgram (*Vigna mungo* L.) is one of the important pulse crops cultivated in India and contributes 10 per cent towards the total pulses production. As grain legumes provide the cheapest and richest source of dietary protein to the most vegetarian population of India, there is an urgent need to enhance the production of pulses not only to meet the demand, but also for achieving food and nutritional security. Stored pulses suffer a great damage by the pulse bruchids, *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) (Coleoptera: Bruchidae) which are the most wide spread and destructive primary insect pests [1]. The bruchids cause an average of 10 – 15% loss across crops/regions in storage [2]. Generally, infestation starts in the field but population builds up in storage as the insect feed inside the grain and emerges as an adult and causes secondary infestation inflicting heavy losses. The infested pulses become unhygienic due to an increase of free fatty acids and the oxidative rancidity, reduction in weight, market value of grains and become unfit for human consumption [3].
Although synthetic pesticides are effective in controlling insect pests, several of them have shown adverse effects on environment and persist for longer period in the form of residues and entered in the food chain. It has also been reported that certain insect pests have acquired resistance against most of the insecticides [4]. Though the practice of using plant derivatives or botanical insecticides in agriculture is dates back, the problems associated with synthetic insecticides necessitated reconsideration of botanicals and inert materials for short term grain preservation at house hold level. Simple technologies like sun drying and repeated sieving, plant products which have insecticidal or repellent activity have been used for traditional storage by small-scale farmers [5]. Several plant derived products such as powders, oils, extracts from neem, castor, eucalyptus, *etc*. which are in use as grain protectants contain secondary metabolites that are effectively control various insect pests[6]. Keeping this in view, an experiment was conducted during 2016-17 to know the response of pulse beetle to blackgram treated with botanicals and inert materials under laboratory conditions at Post Harvest Technology Centre, Bapatla, Guntur district, Andhra Pradesh.

2. Material and Methods
Few selected indigenous techniques *viz.*, sand as a surface layer of 3.0 cm thickness, cow dung

ash @ 5% and red earth @ 5% and plant products including *Sesamum* oil smearing @ 2%, neem seed powder @ 3%, dried neem leaves @ 5%, dried *Ocimum* leaves @ 5%, vasaka powder @ 3% and sweet flag rhizome bits @ 5% were evaluated for the management of pulse beetle under free choice conditions. Thus, the experiments were conducted in completely randomized block design (CRBD) with ten treatments replicated thrice.

2.1 Rearing of the test insect: Fresh blackgram (LBG 645) produce was processed and disinfested by fumigating with aluminium phosphide tablets @ 9 g/tonne grain for seven days to ensure that they were free from field infestation. Later the grains were well aerated. About 250 g of blackgram grains were taken in plastic jars (500 ml capacity) and 50 freshly emerged adults of *C. maculatus* were released into each jar. After allowing mating and oviposition by females for a week the adults were removed. The containers were covered with muslin cloth and kept at room temperature (27±3°C) and 75% relative humidity throughout the period of study. After 35 to 40 days, adults emerged from the culture were utilized for the experiments.

2.2 Relative preference of pulse bruchid to the treated blackgram grain: The relative preference of *C. maculatus* to the blackgram grain treated with the indigenous and natural products were evaluated under free choice condition, where the insects were allowed to choose their preferred host/treated grain. Many insects use olfactory cues to orient towards host habitat as well as to a host. Hence an olfactometer with 10 arms was designed (Figure 1) in which the bruchids can choose among the different odours emanating from the head space of different treatments. The olfactometer was consisted of around shaped response chamber (16 cm diameter and 8 cm height) made of transparent polypropylene and the response chamber was connected by ten test chambers (6 cm in diameter and 200 ml capacity) through PVC tubes (12 cm in length). Accordingly, 100 g of treated blackgram grains along with untreated grain was taken in the respective labeled test chambers connecting the response chamber and closed tightly with lids. Later one hundred newly emerged unsexed adults were released into the response chamber of the olfactometer and were allowed for 24 h to move into the arms of the olfactometer so as to reach the test material (treated or untreated blackgram grains). The principle involved is to attract the insects toward the entrances of the test chambers as influenced by the head space odours drawn through the arms where nine arms serving as an attractive or repellent side and the other arm as the control side. Thus, three sets were maintained to have three replications of the experiment. Data on the number of insects moved in to each treatment, oviposition preference, adult emergence and grain damage were collected and analysed for comparision. Excess Proportion Index (EPI) gives an idea about the repellency and attractancy of chemical substance against a test insect [7]. A negative value of EPI indicates a repellency effect where as a positive value indicates an attraction and zero values, a neutral response [8]. Itwas calculated by using the following formula:

$$EPI = (N_T - N_C) / (N_T + N_C)$$

Where
N_C = No. of insects moved in to the control test chamber
N_T = No. of insects moved in to the treated test chamber

Preference for oviposition was assessed by counting the numbers of eggs laid by the insects on blackgram grains in each treatment at 10 days after release by observing the grains under the illuminated magnifying lens. Later the jars were kept for observation under laboratory conditions till the emergence of adults. To estimate the progeny build up, the number of adults of *C. maculatus* that emerged from different treatments was counted at 40 and 80 days after release. Final data was pooled to get the total number of adults emerged from each treatment. Similarly, the damaged grains were separated from the sample of blackgram grains and counted and expressed as per cent grain damage.

Fig 1: The 10-armed olfactometer used for the free choice tests

3. Results and Discussion

The insects responded differently to the head space volatiles of the treated grain in the each test chamber by the cues emanated through arms connected to the response chamber.

3.1 Number of insects moved into test chamber: The mean number of beetles (28.0) moved into the test chamber containing untreated grain was the highest (Table 1). The mean numbers of insects responded to sand layer (2.33) and sweet flag rhizome bits (2.67) was significantly lower compared to other treatments, but at par with vasaka plant powder (3.33) and cow dung ash (3.67) treatments. Neem seed powder (6.0), red earth (7.33) and *Ocimum* leaves (8.0) treatments were found at par with each other in attracting the insects. *Sesamum* oil and neem leaves treatments had 11.67 and 14.0 adults respectively moved into them.

3.2 Excess Proportion Index: All the treatments showed some degree of repellency to *C. maculatus* as indicated by the negative values of EPI. For the sand layer treatment the EPI was -0.84 indicating that the sand layer acted as physical barrier and the insects could not access the grains. Among the botanicals, sweet flag rhizome bits with an EPI of -0.82 showed strong repellency followed by vasaka plant powder (-0.78) and neem seed powder (-0.65). Surprisingly cow dung ash (-0.76) treatment also recorded better repellency. Whereas for the neem leaves (-0.32), *Ocimum* leaves (-0.54) and *Sesamum* oil (-0.41) had lower EPI (Table 1).

The repellent activity of various plant powders against *T. castaneum* using the olfactometer found maximum repellent activity with *Tephrotia purpurea* (L.) and also showed the highest negative value of the Excess Proportion Index (EPI)[9, 10]. The repellence of crude solvent (hexane, chloroform and ethyl acetate) extracts of plant *viz.*, *Argemone mexicana* (L.), *Artemesia vulgaris* (Wall.), *Sphaeranthus indicus* (L.) and *T. pupurea* against *S. oryzae* adults using a multi arm glass olfactometer and found that ethyl acetate extracts of *A.*

vulgaris and *S. indicus* at 5% concentration exhibited repellent activity (66.23 and 54.55% respectively) on exposure for one hour. Similarly, repellent activities of medicinal plant extracts against *T. castaneum* were also assessed based on EPI [11] who concluded that repellent activity was maximum with hexane extract of *Prosopis juliflora* (Sw.). The repellent activity of few plant oils against *S. oryzae* at 10µl based on EPI values on 6 h exposure and found maximum repellency in camphor (-0.90) followed by winter green (-0.88), lavender (-0.70) and citronella (-0.70)[8].

3.3 Oviposition preference: The results indicated that there was no oviposition in the treatments, sand layer and *Sesamum* oil. The number of eggs laid by *C. maculatus* in other treatments varied from 1.67 to 61.0 per 100 g (Table 1). Among the other treatments, neem seed powder recorded a less number of eggs (1.67) which was at par with sweet flag rhizome bits (2.0) and also followed by vasaka plant powder (6.0) treatments. *Ocimum* leaves recorded less oviposition (29.0) and was at par with neem leaves (31.67) treatment. Significantly lower number of eggs recorded in cow dung ash (18.0) compared to red earth treatment (30.0).

3.4 Adult emergence: The results showed that was no adult emergence even at 80 days after release of insects from blackgram in sand layer and *Sesamum* oil treatments (Table 2). The number of adults emerged from neem seed powder (1.33) was at par with sweet flag rhizome bits (2.0) and followed by vasaka plant powder with 6.0 per cent grain damage. Parmar *et al* evaluated the botanicals on mung bean against the pulse beetle, *Callosobruchus chinensis* (L.) recorded higher adult mortality, half-life value (58.84 days), gross persistency (2495.0) and less adult emergence (13.21) with the neem leaf powder @ 2% (w/w) during six months of storage. *Ocimum* leaves recorded less (29.0) adult emergence which was on par with neem leaves treatment (31.67). Cow dung ash (16.67) recorded significantly less adult emergence compared to red earth treatment (80.33).

At 80 DAT, the number of adults emerged ranged from 4.33 to 373.67. Neem seed powder (4.0) recorded the lowest adult emergence which remained at par with sweet flag rhizome bits (4.33) and vasaka plant powder (5.67). *Ocimum* leaves resulted in emergence of 167.33 adults, while in the neem leaves treatment the adult emergence increased to 184.0. Cow dung ash recorded the emergence of 139.67 adults, however found significantly lower than the red earth treatment from which as many as 259.33 adults were emerged.

Overall, there was no adult emergence from sand layer and *Sesamum* oil treatments. Among other treatments, neem seed powder (5.67) was on par with both the treatments sweet flag rhizome bits (7.67) and vasaka plant powder (10.0) in total number of adults emerged from blackgram. *Ocimum* leaves recorded lower number of adults (196.33) which was on par with neem leaves (215.67). Cow dung ash resulted in 156.33 adults though significantly superior to red earth with emergence of 339.67 adults.

Table 1: Response of pulse bruchid to black gram in different treatments

Treatments	Insects (No.) moved into test chamber*	Excess Proportion Index	No. of eggs/ 100 g*
Sand layer (3 cm)	2.33 (1.52)[a]	-0.84	0.0 (0.70)[a]
Cow dung ash @ 5%	3.67 (1.96)[ab]	-0.76	18.0 (4.29)[d]
Red earth @ 5%	7.33 (2.68)[c]	-0.59	30.0 (5.52)[e]
Sesamum oil @ 2%	11.67 (3.39)[de]	-0.41	0.0 (0.70)[a]
Neem seed powder @ 3%	6.0 (2.44)[bc]	-0.65	1.67 (1.46)[b]
Neem leaves @ 5%,	14.0 (3.71)[e]	-0.32	31.67 (5.66)[e]
Ocimum leaves @ 5%	8.0 (2.81)[cd]	-0.54	29.0 (5.39)[e]
Vasaka plant powder @ 3%	3.33 (1.82)[ab]	-0.78	6.0 (2.54)[c]
Sweet flag rhizome bits @ 5%	2.67 (1.60)[a]	-0.82	2.0 (1.56)[b]
Untreated control	28.0 (5.26)[f]	0.0	61.0 (7.83)[f]
CD (P=0.05)	0.70	-	0.72
SEm±	0.23	-	0.24

* Figures in parentheses are square root transformed values;

3.5 Grain damage (%): No grain damage was observed in sand layer and *Sesamum* oil treatments even after 80 DAT (Table 2). At 40 DAT, neem seed powder recorded negligible grain damage (0.67%) which was at par with sweet flag rhizome bits (1.33%) followed by vasaka plant powder (3.67%). *Ocimum* leaves (13.33%) recorded lower damage by bruchids compared to neem leaves (20.67%). Significantly lower grain damage was observed in cow dung ash (6.33%) compared to red earth (22.0%) and untreated control (28.67%) treatments.

The per cent grain damage by bruchid infestation ranged from 1.67 to 57.33 in other treatments at 80 DAT. Neem seed powder recorded 1.67 per cent grain damage which was at par with sweet flag rhizome bits (3.0%) and followed by vasaka plant powder (12.0%) and cow dung ash (18.67%) treatments. *Ocimum* leaves showed 30.67 per cent grain damage which was at par with neem leaves (38.0%) which was again at par with red earth treatment (43.0%).

The results were in agreement with the findings of Swamy *et al.* [12] who also observed no adult emergence of pulse beetles from stored blackgram under the sand layer even after 150 days of storage, as the adults could not enter through the sand layer and lay eggs over the grain, and neem seed powder @ 5% was also found effective in reducing adult emergence (30.67 no.s), weight loss (4.25%) and grain damage (1.0%) while there was 15.31% weight loss and 44.0% grain damage due to emergence of 4688 adults in untreated control. Significantly less adult emergence of pulse beetle was recorded during six months of storage in neem leaf powder @ 2% (w/w) treated mung bean [13].

Thus free choice test confirmed that sand layer and *Sesamum* oil treatments were found superior recording no oviposition, adult emergence and grain damage while the treatments neem seed powder, sweet flag rhizome bits and vasaka plant powder with significantly less oviposition, adult emergence and grain damage were also proved better for the management of pulse bruchid. Cow dung ash powder at present rate of application was though found superior to the treatments of red earth, neem leaves and *Ocimum* leaves, further indicating that it may be effective at higher rate of application. The efficacy of inert dusts applied at two rates 0.5% and 1.0% w/w of grains against Angoumois grain moth in stored maize and found that higher rate cow dung cake ash gave better control, while fly ash at all its application rates [14].

Table 2: Pulse bruchid emergence and grain damage of blackgram in different treatments under free choice condition

Treatments	Adult emergence (No.) *			Grain damage (%) **	
	40 DAT	80 DAT	Total	40 DAT	80 DAT
Sand layer (3 cm)	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.52)[a]	0.0 (0.52)[a]
Cow dung ash @ 5%	16.67 (4.14)[d]	139.67 (11.81)[c]	156.33 (12.51)[c]	6.33 (14.54)[d]	18.67 (25.50)[d]
Red earth @ 5%	80.33 (8.96)[f]	259.33 (16.12)[e]	339.67 (18.45)[e]	22.0 (27.95)[f]	43.0 (40.97)[f]
Sesamum oil @ 2%	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.70)[a]	0.0 (0.52)[a]	0.0 (0.52)[a]
Neem seed powder @ 3%	1.33 (1.35)[ab]	4.0 (2.18)[b]	5.67 (2.48)[b]	0.67 (4.0)[b]	1.67 (7.33)[b]
Neem leaves @ 5%,	31.67 (5.66)[e]	184.0 (13.54)[d]	215.67 (14.67)[d]	20.67 (27.03)[f]	38.0 (38.0)[ef]
Ocimum leaves @ 5%	29.0 (5.39)[e]	167.33 (12.94)[d]	196.33 (14.03)[d]	13.33 (21.40)[e]	30.67 (33.60)[e]
Vasaka plant powder @ 3%	6.0 (2.54)[c]	5.67 (2.47)[b]	10.0 (3.25)[b]	3.67 (10.95)[c]	12.0 (19.99)[c]
Sweet flag rhizome bits @ 5%	2.0 (1.56)[b]	4.33 (2.11)[b]	7.67 (2.85)[b]	1.33 (6.53)[b]	3.0 (9.88)[b]
Untreated control	96.67 (9.85)[g]	373.67 (19.35)[f]	470.33 (21.70)[f]	28.67 (0.52)[a]	57.33 (49.23)[g]
CD (P=0.05)	0.81	0.92	0.96	2.55	4.46
SEm±	0.28	0.32	0.32	0.86	1.51

* Figures in parentheses are square root transformed values;
******Figures in parentheses are angular transformed values

4. Conclusion

The treatments *viz*; sand layer of 3 cm over grain surface, *Sesamum* oil @ 2%, sweet flag rhizome bits @ 5% were found effective for the management of pulse bruchid as they recorded nil or minimum oviposition, adult emergence, grain damage and weight loss. It is also evident that traditional method of placing dried neem leaves and *Ocimum* leaves could not offer much control of bruchid infestation. However, the plant powders such as neem seed and vasaka can be considered only for very short term storage of pulses as they could suppress the insect populations although not eliminated. Adoption of these effective indigenous techniques and botanicals can be considered especially in rural households for protecting the food grains and seeds from insect pest attack as they are eco-friendly, cheaper and locally available materials.

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