Potential of some botanical powders in reducing infestation of chickpea by *Callosobruchus chinensis* L. (Coleoptera:Bruchidae)

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The present study deals with the efficacy of some leaf powders via free choice and no choice assay against infestation of chick pea seeds by the pulse beetle *Callosobruchus chinensis* during storage. Among the plant powders tested, *Murraya koenigii* and *Eupatorium cannabinum* were found to be the most effective in reducing the orientation, oviposition and causing the mortality of bruchids at dose of 2% (w/w). The F_1 emergence from the infested chick pea was significantly reduced in treatments to which powders of *Murraya koenigii* (90.62%) and *Eupatorium cannabinum* (86.46%) had been added. Looking into the side effects of synthetic pesticides the study demonstrates that these plant powders can play an important role in protection of chickpea from insect invasion during storage.

Key words: *Callosobruchus chinensis***,** chickpea, F_1 emergence, mortality, oviposition, repellency

Introduction

Chickpea (*Cicer arietinum* L.) is a highly nutritious pulse cultivated throughout the world and is placed third in the importance list of the food legumes. India is the largest producer of this pulse contributing to around 63% of the world's total production (ICRISAT, 2007). It contains 38-59% carbohydrates and 25.3-28.9% proteins, which is the maximum provided by any pulse (Hulse, 1991) and does not contain any specific major anti - nutritional factor.

India has an annual production potential of 15.04 million tonnes of pulses recorded in year 2004-05 (India, 2006). However, nearly 8.5 % of total annual production is lost during post harvest handling and storage (Agrawal *et al.*, 1988). Chick pea seeds in developing countries suffer heavy qualitative and quantitative losses from the attack of pulse beetle, *Callosobruchus chinensis* L.

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(Alam, 1971). The insect invasion causes reduction in weight, market value and germination of chick pea seeds (IITA, 1989). The problem of residues resulting from mixing of synthetic organic pesticides with pulses beyond the permissible tolerance levels for control of beetle infestation has forced the researchers to look for some non-toxic pulse protectants. The integration of insecticidal natural products from locally available plants for use in storage by the farmers in developing countries appear to be quite safe and promising (Jillani *et al.*, 1988). Various products of plants have been tried recently with good degree of success as protectants against a number of stored grain insect pests (Gill and Lewis, 1971; Teotia and Tewari, 1971; Kothar, 1976; Ayyangar and Rao, 1989; Pandey *et al*., 1986; Yadav and Bhatnagar, 1987; Jilani *et al*., 1988; Dixit and Saxena, 1990; Varma and Dubey, 1999). The present study was performed to evaluate the repellent and deterrent effects of dried leaf powders from six angiospermic plants on oviposition and progeny production of *C. chinensis* in stored chickpea seeds.

Materials and methods

Rearing of insects

Insect rearing was carried out in department of Botany, Banaras Hindu University, India under the prevailing environmental conditions of 30 ± 2°C and 70 ± 5 RH (Talekar, 1988). To obtain newly emerged pulse beetles of same generation, 25 insects were released in a plastic container having 250 g of chickpea seeds covered by a muslin cloth. After 24 hours all the adults were removed and egg laid seeds were maintained at required temperature and humidity. The insects emerged after four weeks were used in the entire investigation. Insect eggs were counted by using hand lens.

Stored product

Chick pea seeds (variety -Radha) were obtained from the local market. Healthy and fresh seeds were used to avoid any pre storage infestation or egg laying of bruchids.

Plant products

Six plant powders used in this investigation were taken from the leaves of *Syzygium cumini* L. (fam.Myrtaceae), *Aegle marmelos* L. (fam. Rutaceae), *Eupatorium cannabinum* L. (fam.Asteraceae), *Murraya koenigii* L.

(fam.Rutaceae), *Ammomum subulatum* Roxb. (fam. Zingiberaceae), *Citrus medica* L. (fam. Rutaceae). Leaves were dried in Webcon's hot air oven at 40°C for 2 days and milled with electric grinder (Maharaja Whiteline) to powder. The resulting powder was passed through a 25-mesh sieve to obtain a fine dust and used at the dose of 2% (w/w).

Free choice chamber bioassay

The choice chamber is a circular device of seven transparent plastic boxes (300 ml) placed equidistantly to each other. The boxes were connected to a large transparent box (1L) placed in the centre of the chamber through glass tubes (1 cm in diameter and 8 cm long). The apparatus was placed in a plastic basin having a diameter of 42 cm and the height of 18 cm. The side walls were covered with black paper.

In each experiment 50 g. of chick pea seeds were dressed separately with six different botanical powders at the dose of 2% (w/w). The dressed seeds of chick pea were placed in six consecutive boxes leaving one box, which was occupied by control set of chick pea seeds without any treatment. Sixty pairs (120) adult bruchids (unsexed, 3 days old) were introduced to the central box and the chamber was placed in dark room. The number of adults oriented in each treated and control set was counted at 1, 2 and 3 days after release.

No choice ovipositional test

50 g chickpea seeds treated separately with each plant material (2 % w/w) were kept separately in small plastic containers of 150 ml covered with muslin cloth. Five pairs of adult female bruchids (female bruchids possess larger abdomen than the males) were released in each container. The adult mortality was recorded at 2, 3 and 5 days after the release and the per cent mortality was calculated by Abott's formula (Abbott, 1925). All the remaining adults were removed after 5 days of release and number of eggs laid were recorded. Total number of adults emerged in each treatment was counted after 25 days of their release. A control set was also maintained without any treatment of powder. Per cent weight loss of treated and control chick pea seeds were recorded. Feeding deterrent efficacy of the powders was calculated following Isman *et al*. (1990).

Statistical analysis

All the treatments were done in triplicate. The data obtained during free choice and no-choice tests were analysed statistically using one-way ANOVA and the means were compared using Tukey's multiple comparison tests (SPSS, 1999). Standard deviation of the differences were computed for mean comparisons.

Results

The most of the plant powders were significantly effective over control with regards to orientation and repellency (Table 1). It was estimated that per cent repellency decreased with the increase in days of treatment. *M. koenigii* and *E. cannabinum* leaf powders hindered the orientation of adult significantly than rest of the plant powders. Poor effects were recorded with the leaf powders of *A. subulatum*. Control and *A. subulatum* treatment showed the higher mean orientation of beetles and they were not significantly different from each other.

The effect of different plant powders on mortality of adult *C. chinensis* after 2, 3 and 5 days of treatment is showed in Table 2. All treatments showed 25.07 to 80.03% mortality during 5 days of exposure. *E. cannabinum* offered maximum mortality (80.03%) followed by *M. koenigii* (75.07%) and *C. medica* (65.01%). *S. cumini* and *A. marmelos* showed 34.98% and 45.04% mortality respectively.

Mean number of eggs laid on chickpea seeds treated with different leaf powders are shown in Table 3. *M. koenigii* powder was significantly more effective causing 86.15% oviposition deterrence of bruchids on chickpea followed by *E. cannabinum* (82.50%), *C. medica* (72.58%) and *A. marmelos* (71.27%). It was visually observed that the eggs laid on seeds in treated sets were smaller in size than on untreated seeds. In addition, the eggs of treated sets were not firmly attached with the seeds. The F_1 emergence of adult beetles was also found to be reduced in treatment sets. The highest per cent reduction in adult emergence was observed in case of *M. koenigii* treated chickpea seeds (90.62%) and the lowest in case of *A. subulatum* (26.03%). Feeding deterrent index (FDI) was recorded maximum in case of *M. koenigii* (96.49%) followed by *E. cannabinum* (92.31%) and minimum in *A. subulatum* (13.42%).

Discussion

The findings of the present study indicate the repellent and deterrent effects of some of the leaf powders on oviposition and adult emergence of *C. chinensis.* Varying activity by different powders indicate that the pest controlling factors are not uniformly present in every aromatic plant. The leaf powder of *A. subulatum* showed poor repellent activity whereas that of *E. cannabinum* and *M. koenigii* exhibited strong activity against orientation, longevity, oviposition and adult emergence of bruchids. The findings of the present investigation are in accordance with those of other workers who have previously reported that plant powders reduce life span and oviposition of bruchids, which include neem kernel powder (Sowunmi and Akinnsi, 1983; Maredia *et al*., 1992), *Tridax procumbens* (Bhaduri *et al*, 1985), *Lantana camara* (Koona and Njoya, 2004) and seed powder of custard apple (Ali *et al.*, 1983). Powdered materials of orange, lemon, lime, lemon grass, cinnamon, derris, nutmeg, cactus, ginger have been evaluated by Rajpakse and Vaneben (1997) against bruchids upto higher doses of 300g/kg seeds. Dried powders of clove, red and black pepper have also been reported to prevent the infestation of bruchids at dose of 25g/kg (Aslam *et al*., 2002). However, in the present investigation an attempt has been made to find out the effect of the leaf powders as insecticidal at comparatively lower dose of only 2% (20g/kg seeds).

E. cannabinum causes more mortality than *M. koenigii* but the later prevent the bruchids more to oviposit. It could be due to semiochemical nature of *M. koenigii* powder which alters the behaviour and physiology of the insects affecting adversely the egg laying and F_1 emergence. The dried leaf powders investigated were taken from the plants used as pharmaceuticals, spice or flavour yielding and could be, therefore, considered less harmful to humans than most of the conventional pesticides. Leaves of *M. koenigii* (known as Curry leaf) is used in Indian dishes as flavouring agent and the leaves of *E. cannabinum* (known as Agrimony Hemp) used medicinally as diuretic and blood purifier (Grieve, 2007). Insecticidal and antifeedant activity by these two powders can be ascribed to its sesquiterpenoid contents (Semnani *et al*., 2006; Onayade, 2000; Raina *et al*., 2002) as this compound is one of the most associated with deterrence against insects (Gonzalez *et al*., 1997). Such deterrence could be attributed to the diffusing of persistant odors capable of suffocating bruchids in boxes as observed by other workers (Koona and Njoya, 2004). These powders can reduce insect movement and also cause death through occlusion of their spiracles, thereby, preventing respiration via trachea.

The reduction in adult emergence could either be due to egg mortality or larval mortality or even reduction in the hatching of the eggs. It has been reported that the larvae which hatch from the eggs of *Callosobruchus* species must penetrate the seeds to survive (FAO, 1999). The larvae are unable to do so unless the eggs are firmly attached to the seed surface. In the present study the eggs were found to be loosely attached to the chick pea seed surface in the treated sets of *M. koenigii* and *E. cannabinum.* The leaf powders might thus have inhibited the larval penetration into the seed and thus showed maximum FDI. The bitter taste, pungent smell and semiochemical nature of these two leaf powders causing quick mortality within five days would not allow the formation of resistant races of the insect which is quite prevalent with most of the synthetic pesticides.

In conclusion, admixing the powders of *E. cannabinum* and *M. koenigii* may be recommended as cheap, easily available, eco friendly and non-toxic in management of *C. chinensis* and thus are efficacious in protecting chick pea seeds from insect invasion at farmer level.

Table 3. Oviposition deterrence and progeny production of *C. chinensis* on chickpea seeds treated with different leaf powders.

Treatment 2% (w/w)	**Mean number of eggs laid**	**% deterrency**	**Mean number of F_1 emerged**	**% ddeterrency**	**% FDI**
S. cumini	46.33 ± 5.50c	63.70	14.67 ± 2.08c	54.15	43.20 ± 1.00c
A. marmelos	36.67 ± 6.65cd	71.27	10.34 ± 2.88cd	67.68	61.33 ± 1.44bc
E. cannabinum	22.34 ± 4.50d	82.50	4.33 ± 1.15d	86.46	92.31 ± 0.50a
M. koenigii	17.67 ± 5.50d	86.15	3.00 ± 2.00d	90.62	96.49 ± 0.64a
A .subulatum	70.00 ± 7.54b	45.17	23.67 ± 4.72b	26.03	13.42 ± 1.40d
C. medica	35.00 ± 9.84cd	72.58	9.67 ± 2.30cd	69.78	79.25 ± 0.96ab
Control	127.67 ± 8.05a		32.00 ± 3.60a		

Each data point represents mean of three replicates ± SD.

The mean followed by the same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison test.

The mean difference is significant at the 0.05 level.

Percent deterrency = (control – mean of each treatment / control x 100).

% FDI = (weight loss in control – weight loss in treatment / weight loss in control x 100)

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Table 1. Effect of leaf powders on orientation and repellency of *C. chinensis*

Treatment	1 DAT*	% repellency	2 DAT*	% repellency	3 DAT*	% repellency
S. cumini	12.33 ± 2.51b	6.00	13.33 ± 1.52bc	4.00	13.00 ± 1.73b	3.66
A. marmelos	12.33 ± 2.51b	6.00	11.66 ± 1.52cd	5.67	9.66 ± 0.57c	5.00
E .cannabinum	5.00 ± 1.00c	13.33	5.00 ± 1.00ef	12.33	4.66 ± 1.15d	12.00
M. koenigii	4.33 ± 0.57c	14.00	4.33 ± 1.15f	13.00	4.00 ± 1.00d	12.66
A .subulatum	19.33 ± 0.57a	-1.00	17.66 ± 1.15a	-0.33	16.33 ± 1.15a	0.00
C. medica	9.66 ± 2.08b	8.67	9.00 ± 2.00de	8.33	9.66 ± 1.15c	7.00
Control	18.33 ± 0.57a		17.33 ± 2.08ab		16.66 ± 1.15a	

DAT= Days after treatment

*Each data point represents the number of insects oriented (mean of three replicates ± SD) The mean followed by the same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison test.

The mean difference is significant at the 0.05 level.

Table 2. Effect of leaf powders on life span of *C. chinensis.*

Treatment	2 DAT*	% Mortality	3 DAT*	% Mortality	5 DAT*	% Mortality
S. cumini	4.66 ± 0.57bc	42.87	5.66 ± 0.57abc	51.77	5.66±0.57bc	34.98
A. marmelos	4.00 ± 1.73c	35.76	5.00 ± 0.00bc	44.44	6.33±0.57abc	45.04
E .cannabinum	7.66 ± 0.57a	75.00	8.33 ± 0.57a	81.44	8.66±0.57a	80.03
M. koenigii	5.33 ± 0.57abc	53.59	7.66 ± 1.52ab	74.00	8.33±0.57a	75.07
A .subulatum	4.33 ± 1.15bc	39.33	4.66 ± 1.52c	40.66	5.00±1.73cd	25.07
C. medica	7.00 ± 1.00ab	67.88	7.33 ± 0.57a	70.33	7.66±0.57ab	65.01
Control	0.66 ± 0.57d		1.00 ± 1.00d		3.33±0.57d	

DAT= Days after treatment

*Each data point represents the number of insects died (mean of three replicates ± SD) The mean followed by the same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison test.

The mean difference is significant at the 0.05 level.