AJAB

Asian J Agri & Biol. 2017;5(3):119-125.

Original Research Article

Bioefficacy of plant powders against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) in infested chickpea grains

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Received:
May 19, 2017
Accepted:
July 17, 2017
Published:
September 30, 2017

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Abstract

In this study we used dried leaf powders of five plants i.e., *Syzygium cumini*, *Citrus limon*, *Momordica charantia*, *Eucalyptus globulus* and *Piper nigrum* against *Callosobruchus chinensis* infesting chickpea seeds during storage. Free choice and no choice tests were conducted. Plant powders were applied at dose of 2% (w/w). Efficacy of all plant powders were compared by orientation, oviposition, adult mortality and adult emergence of the pulse beetle. Effect of plant powder on germination of seed was also observed by germination test. Leaf powder of *M. charantia* was found to be more effective while leaf powder of *S. cumini* was found to be least effective in adult mortality and oviposition deterrence. Furthermore, the results showed that leaf powders of all plants had no effects on germination of seeds.

Keywords: Leaf Powders, Chickpea seeds, Seed viability, Pulse beetle

Introduction

Pulses are among the major food sources in developing countries and vegetarian societies ((Aslam et al., 2006)). Chickpea (*Cicerarietinum* L.) contains 38-59% carbohydrates and 25.3-28.9% proteins (Khan et al. 1978 a; Khan 1981 b; Hulse 1991. Chickpea is widely cultivated crop in Pakistan, mostly after monsoon season.

Stored chickpea is heavily infested by pulse beetle (*Callosobruchus chinensis)*. Pulse beetles causes great economic losses as it reduces the dry weight and makes nutritional values poor. (Ahmed et al., 2003; Ahmed et al., 2009; Kumar et al., 2009; Righi-Assia et al., 2010). Pulse beetle, commonly known as Dhora, causes heavy loss about 90%, in stored grams (Qayyum et al., 1978; Kakepoter et al., 1985; Upadhyay et al., 2006). The use of commercially available insecticides and fumigants has adverse effects on environment as well as humans (Talukder (2006). Synthetic chemicals also induces the resistance in insects pests, toxic effects to grain handlers, and toxic residues affect the entire food chain after utilization (Schoonhoven (1982); Sharaby (1988); Shaaya et al. (1997); Upadhyay et al. (2011)). A number of plant products have been successfully tried as protectants against various stored grain insect pests (Gill et al., 1971; Pandey et al., 1986; Jilani et al., 1988; Ayyanger et al., 1989; Dixit et al., 1990; Kumari et al., 1998; Verma et al., 1999; Kim et al., 2003).These plant products included ash (Ajayi et al., 1987) vegetable oil (Schoovoven 1978;

Kazi et al., 1999) and plant powders and extracts (Abdullahi et al., 2004; Partil et al., 2006; Gupta et al., 2008; Shukla et al., 2009).The powders of certain plant parts i.e. leaves, bark, seeds as well as oil extracts suppressed the oviposition and adult emergence and increased mortality rate of bruchids and maintained the seed quality as well, when mixed with stored grains (Tapondjou et al., 2002; Adedire et al., 2004; Rahman et al., 2006; Bamaryi et al., 2007; Shukla et al.,2007). Botanical powders are safe and interesting alternatives of chemicals insecticides especially in developing tropical countries where plants are abundantly present throughout the year (Khalequzzaman et al., 2009).

Keeping in view the usefulness of plant products, the present research is performed to evaluate the repellent and deterrent effects of botanical powders from five angiospermic plants on adult mortality, oviposition and adult emergence of pulse beetle in stored chickpea. The weight loss and germination of treated seeds were also checked. In this research, leaf powders of *Syzygiumcumini* (Jaman), *Citrus limon* (Lemon), *Momordica charantia* (Bitter gourd), *Eucalyptus globulus* (Eucalyptus) and *Piper nigrum* (Black pepper) are used and according to best of my knowledge, leaf powders of bitter gourd, eucalyptus and black pepper are used for the first time against *Callosobruchus chinensis*.

Materials and Methods

Rearing of *Callosobruchus chinensis*

Chickpea seeds were taken from a local market in Gujranwala and naturally infested adult pulse beetles (*Callosobruchus chinensis*) were obtained. From these infested seeds hundred adult beetles were separated in order to obtain newly emerged pulse beetles (Halstead, 1963). Newly emerged pulse beetles were released in 1 kg clean and un-infested chickpea seeds in a plastic jar covered with cloth and capped with muslin cloth. The jar was kept in lab at temperature 25-35 C. Adult beetles were left for about seven days for the purpose of mating and oviposition. After seven days, the parent beetles were separated from chickpea seeds and eggs. Chickpea seeds with eggs were left in jar for about 25 days in order to obtain adult beetles which were used in the entire study.

Stored product

Five kilogram of chickpea seeds was bought and placed in refrigerator for 4 days in order to prevent prestorage infestation.

Preparation of botanical powders

i. *Piper nigrum* (Black pepper)
ii. *Citrus limon* (Lemon)
iii. *Eucalyptus globules* (Eucalyptus)
iv. *Momordica charantia* (Bitter gourd)
v. *Syzygium cumini* (Jaman)

Cleaned leaves of these plants were dried in shade. Then crush into powdered form by using electric grinder (Nisar et al., 2012; Udoh et al., 2010). Finely ground powder was collected by passing through 25-mesh sieve.

Free choice test

For this test, free choice chamber used which was circular in shape and having 6 transparent plastic jars. Side walls of jars were wrapped with black paper. Then jars were positioned at equal distance from each other and a transparent jar was placed in center. All jars connected with central jar through plastic tube. The orientation of adult pulse beetles i.e. treated chickpeas and untreated chickpeas were studied by performing free choice test. Fifty grams of chickpea seeds were treated separately with 5 botanical powders at dose of 2% (w/w). The treated seeds were placed in 5 boxes of the device. The control set of chickpea seeds were also placed in the remaining box without any treatment. In the central box of chamber 20 adult pulse beetles were placed and the chamber was placed in a dark room. The number of adults oriented in each treated and control set of chickpea seeds were counted daily for 3 days. The experiment was repeated 3 times.

No choice test

Fifty grams of chickpea seeds were treated with each botanical powder separately at dose of 2% (w/w) and then placed in 5 plastic jars separately. Control set was also placed in one container without any treatment. About 0-2 days old five pairs of pulse beetles were introduced into each jar.

Jars were placed in lab shelf after covering with muslin cloth. Weight loss in the seeds, mortality of adult beetles, adult emergence and oviposition was observed. Treated seeds were then subjected to germination test at end of experiment. The experiment was repeated three times.

Statistical analysis

Data was analyzed statistically by using one way ANOVA and for significant means; LSD was applied (Steel et al., 1980). For statistical analysis a software package Statistics was used. To check % oviposition deterrence, % adult emergence deterrence and % feeding deterrent index (FDI), Abbott's formula was applied (Abbott (1925) as follows.

$$\textit{Per cent control} = X - Y / X \times 100$$

Results and Discussion

In order to observe effects of botanical powders free choice and no choice tests were performed. Weight loss and germination of chickpea seeds, Adult orientation, adult mortality, oviposition and adult emergence of *Callosobruchus chinensis*, were observed. The obtained results are as follows:

Effect of powders on orientation of *C. chinensis*

In this study the plant powders of *Syzygium cumini*, Citrus limon, *Momordica charantia*, Eucalyptus globulus and *Piper nigrum* were used. Powders found effective over control in repelling the *C. chinensis* adults, when their orientation was observed through free choice test (Table 1). Leaf powders of *Momordica charantia* and *Eucalyptus globulus* significantly ($P<0.05$) hindered the orientation of adult beetles as compared to other three. Among the treatments, *Citrus limon* leaf powder showed the highest mean orientation of beetles followed by *Syzygium cumini* and *Piper nigrum* and these three powders were not significantly different from each other. Maximum orientation of bruchids was recorded in control set of chickpeas.

In this study it was found that leaf powders of these plants contains repellent properties against *C. chinensis*. In case of each treatment diverse results found that pest repelling factors are not evenly present in the plants. The leaf powder of *Momordica charantia* was most effective then *Eucalyptus globulus* in repellency and these results are in accordance with the previous studies. Oil of *E. globulus* exposed to contain highest insecticidal potential among other treatments i.e. neem oil, castor oil and sunflower oil (Lal et al., 2012). From different studies it was found that fruit powder of P. nigrum at different concentration is proved effective post-harvest grain protectant (Aslam et al., 2002; Mahdi et al., 2008). In this study P. nigrum was also found effective against *C. chinensis* which confirmed the previous findings. But here for the first time, powder of leaves of *P. nigrum* was used.

Table 1: Effects of leaf powders on orientation of *C. chinensis*

Treatments 2% (w/w)	Insects with respect to days			Grand Mean
	1DAT	2DAT	3DAT	
Syzygiumcumini	3.00 ± 1.00	2.66 ± 1.2	3.00 ± 0.00	2.89 [b] ± 0.45
Citrus limon	3.33 ± 0.33	3.33 ± 0.33	3.00 ± 0.58	3.22 [b] ± 0.22
Momordica charantia	1.00 ± 0.58	1.00 ± 1.00	1.00 ± 0.58	1.00 [c] ± 0.37
Eucalyptus globules	1.00 ± 0.58	1.33 ± 0.66	1.33 ± 0.33	1.22 [c] ± 0.28
Piper nigrum	3.33 ± 0.66	2.66 ± 0.33	2.33 ± 0.33	2.78 [b] ± 0.28
Control	6.00 ± 0.58	*7.33* ± 0.33	7.00 ± 1.15	6.78 [a] ± 0.43
Grand Mean	2.94 ± 0.47	*3.06* ± *0.56*	2.94 ± 0.52	2.98 ± 0.29

DAT= Days after treatment

Each value represents the mean of number of insects oriented in each treatment. The mean followed by same letter are not different significantly according to LSD.

The mean difference is significant at the level of 0.05.

Effect of powders on adult mortality of *C. chinensis*

In table 2 mortality of adult *C. chinensis* described which were recorded after 2nd, 4th and 6th day of treatment and revealed the effects of plant powders. Adult mortality was observed during 6 days of exposure to treatments. The greatest mortality was presented by *Momordica charantia* and *Citrus limon* followed by Eucalyptus globulus and *Piper nigrum* while slightest mortality was observed in *Syzygium cumini*, still higher than control set of chickpeas.

The powders play important role in killing insects by occlusion of spiracles, by causing tracheal resistance in respiration and by obstructing movement of insects, eventually causing death (Shukla et al., 2007). To the best of our knowledge, the dried leaf powders of *M. charantia, E. globulus and P. nigrum* are tested for the

first time for the control of *C. chinensis* in chickpea seeds. It was hypothesized that powder leaves of *M. charantia* would be efficient in grain protection after harvesting and results of this study supported our hypothesis. *M. charantia* leaf powder caused greatest mortality, followed by *C. limon*. All the Citrus spp. possesses insecticidal factors. A high mortality rate was reported in *C. chinensis* and other insect pests from vital oils of Citrus spp. such as *C. reticulata, C. sinensis, C. paradise* and *C. grandis* (Zia et al., 2013). *C. medica* reported to be highly active as compared to *Syzygium cumini*, *Aegle marmelos* and *Ammomum subulatum* in controlling of pulse beetle (Shukla et al., 2007).

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

Treatments 2% (w/w)	Insects with respect to days			Grand Mean
	1DAT	2DAT	3DAT	
Syzygium cumini	1.67 ± 0.67	2.67 ± 0.33	2.67 ± 0.67	2.33 [a] ± 0.33
Citrus limon	1.67 ± 1.2	4.00 ± 0.58	3.33 ± 1.20	3.00 [a] ± 0.62
Momordica charantia	3.33 ± 0.33	4.33 ± 0.33	1.67 ± 1.20	3.11 [a] ± 0.54
Eucalyptus globulus	2.67 ± 0.67	3.33 ± 0.88	2.33 ± 1.86	2.78 [a] ± 0.64
Piper nigrum	4.33 ± 1.2	2.33 ± 0.33	1.67 ± 0.33	2.78 [a] ± 0.6
Control	1.33 ± 0.88	*1.67 ± 0.33*	0.33 ± 0.33	1.11 [b] ± 0.35
Grand Mean	2.5 ± 0.4	*3.06 ± 0.31*	2.00 ± 0.44	2.52 ± 0.23

DAT= Days after treatment

Each value represents the mean of number of insects died in each treatment. The mean followed by same letter are not different significantly according to LSD. The mean difference is significant at the level of 0.05.

Effect of powders on oviposition of *C. chinensis*

In this experiment all the plant powders used found to be effective in reducing the oviposition of *C. chinensis*. The results in treated and untreated chickpea seeds regarding oviposition are shown in Table 3. Chickpea seeds treated with *Momordica charantia* shows maximum prevention in oviposition i.e. 93.86% followed by *Eucalyptus globulus* and *Citrus limon* causing 63.60% and 61.84 % prevention in the oviposition of bruchids on chickpea seeds respectively. *Citrus limon* powder effective at very small rate on oviposition of bruchids. Powder of *Syzygium cumini* found least effective in oviposition deterrence as shown in adult mortality. *C. limon* leaf powder causes high mortality of bruchids as compared to *E. globulus* and *P. nigrum* while *E. globulus* and *P. nigrum* reduced oviposition as compared to *C. limon*. The reason behind this may be due to semi chemical nature of *E. globulus* and *P. nigrum* which affects behaviour and interrupt physiology of the insects' thus disturbing oviposition and adult emergence. *S. cumini* found least effective in oviposition deterrence and adult mortality as found in another study in which it was used along with other treatments i.e. *Aeglemarmelos, Eupatorium cannabinum, Murraya koenigii, Ammomum subulatum* and *Citrus medica against C. chinensis* in stored chickpea (Shukla et al., 2007).

Table 3: Effects of leaf powders on oviposition of *C. chinensis*

Treatments 2% (w/w)	Mean number of eggs laid	% deterrency
Syzygium cumini	121.67 [ab] ± 23.6	19.95%
Citrus limon	112.00 [ab] ± 32.6	26.32%
Momordica charantia	9.33 [c] ± 6.01	93.86%
Eucalyptus globules	55.33 [bc] ± 31.54	63.60%
Piper nigrum	58.00 [bc] ± 16.92	61.84%
Control	152.00 [a] ± 27.57	
Grand Mean	84.72 ± 14.45	

The mean followed by the same letter are not significantly different according to LSD. The mean difference is significant at the 0.05 level.

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

Effect of powders on adult emergence of *C. chinensis*

The adult emergence of *C. chinensis* was found to be reduced in treatment sets which are given in Table 4.

Reduction in emergence of adults from eggs was found 47.24%-97.12% in all treatments. In case of adult emergence *Momordica charantia* showed highest i.e. 97.12% reduction and the lowest of *Citrus limon* i.e., 47.24% was observed in treated chickpea seeds. *Syzygium cumini* powder found more effective giving 64.51% reduction in emergence of adults as compared to its results shown in adult mortality and oviposition of beetles.

Leaf powder of P. nigrum had shown 88.73% deterrence in adult emergence. In order to control pulse beetle in stored green and black gram insecticidal potency of some spices i.e. clove,

cinnamon, black cardamom, black cumin, nutmeg, turmeric, black pepper, red pepper, cumin, green cardamom, bay leaf, aonla and ginger was tested. Black pepper and clove were observed highly effective as compared to all other spices (Gautam et al., 2000; Aslam et al., 2002; Mahdi et al., 2008).

Table 4: Effects of leaf powders on adult emergence of *C. chinensis*

Treatments 2% (w/w)	Mean number of adults emerged	% Deterrency
Syzygiumcumini	49.33 ^{bc} ± 24.99	64.51%
Citrus limon	73.33 ^{b} ± 20.61	47.24%
Momordica charantia	4.00 ^{c} ± 3.06	97.12%
Eucalyptus globules	23.00 ^{bc} ± 21.50	83.45%
Piper nigrum	15.67 ^{bc} ± 10.73	88.73%
Control	139.00 ^{a} ± 24.70	
Grand Mean	50.72 ± 12.90	

The mean followed by the same letter are not significantly different according to LSD. The mean difference is significant at the 0.05 level.
Percent deterrency = (control – mean of each treatment / control x 100).

Effect of powders on weight loss and germination of chickpea seeds

The weight loss of chickpea seeds is due to the feeding activities of larvae of pulse beetle. So, more the emerged beetles, more the weight of seed will be lost as depicted in our findings as well. Weight loss in high amount recorded in the seeds treated with *Citrus limon* and seeds of control set as well and the same results were obtained in case of adult emergence. Least weight loss observed in *Momordica charantia* and *Eucalyptus globulus* treated seeds. In case of each treatment feeding deterrent index (FDI) was calculated by using Abotts formula. *Momordica charantia* (98.03%) was observed having maximum feeding deterrent index followed by *Piper nigrum* (88.24%) and minimum in *Citrus limon* (56.85%) shown in (Table5). The seeds, attained from each treatment, were germinated envisaged no effects of plant powders on the germination of seeds i.e., 100% germination result was obtained in case of each treatment.

Table 5: Percentage seed germination and weight loss in treated and untreated seeds

Treatments 2% (w/w)	% Seed Germination	Mean Weight loss	% FDI
Syzygiumcumini	100%	50.03 ^{ab} ± 0.52	71.56%
Citrus limon	100%	49.53 ^{b} ± 0.47	56.85%
Momordica charantia	100%	50.93 ^{a} ± 0.07	98.03%
Eucalyptus globulus	100%	50.60 ^{ab} ± 0.40	88.24%
Piper nigrum	100%	50.57 ^{ab} ± 0.28	87.26%
Control	100%	47.60 ^{c} ± 0.47	
Grand Mean		49.88	

The mean followed by the same letter are not significantly different according to LSD. The mean difference is significant at the level of 0.05.
FDI= Feeding Deterrent Index
% FDI = (weight loss in control – weight loss in treatment / weight loss in control x 100)

Conclusion

All the results proved that *Momordica charantia* (Bitter gourd) is best protectant among all the plants tested in this study. The leaf powders of this plant gave maximum mortality and ovipositional and adult emergence detergency against *C. chinensis*.
Leaf powder of *C. limon* when compared with the leaf powder of *E. globulus* was found more effective in reduction of life span than egg laying ability and adult emergence, whereas the leaf powder of *E. globulus* was found highly effective in reduction of egg laying abilities and adult emergence. So, mixtures of both powders can be applied for effective control in already infested chickpeas.
It is concluded that botanical powders used in this study can be suggested as economical and easily available post-harvest grain protectants due to their non-toxic and eco-friendly nature. Insecticidal potential of other plants can be find by further studies on them. Moreover, different concentrations of these plants powders can also be tested.

Acknowledgements

The authors are thankful to the Competent Authority at University of Gujrat, Gujrat, Pakistan and all others for all the possible help extended for the accomplishment of this project.

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