

Biology and bio-intensive management of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) – a pest of kidney beans worldwide

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Abstract

Insects in the family Bruchidae are commonly called “pulse weevils” and are cosmopolitan in distribution. These beetles cause serious economic loss of legume commodities both in fields and every year. Pulses constitute the main source of protein for developing countries like India where per capita consumption of animal protein is very low. Due to their high protein quantity and quality, legumes are considered as “poor man’s meat”. A large number of non-native pulse beetles have crossed geographical boundaries and becoming cosmopolitan in distribution, thus posing major pest problem worldwide. A kidney bean pest, *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) native to Central and Southern America has recently infested stored kidney beans in the Indian subcontinent. The present investigations determined life cycle, behaviour, fecundity, pest status, host range and developmental compatibility on different legumes and different cultivars of kidney beans. Acetone and alcoholic extracts of some botanicals have been tested and proved effective to suppress fecundity, egg hatch and adult longevity of the pest population under laboratory conditions.

Keywords: *Acanthoscelides obtectus*, biology, resistance, developmental compatibility, botanical management

1. Introduction

Most pulses have 17-24% protein content which are 2.3 times higher than traditional cereals. Any stored materials of plant origin are vulnerable to attack by insect pests if the pulses are dried and stored improperly. The common bean, *Phaseolus vulgaris* can be infested by the common bean weevil, *Acanthoscelides obtectus* and Mexican bean weevil, *Zabrotes subfasciatus*, both in fields and stores and rendered unfit for consumption. The geographical distribution of both species is now almost cosmopolitan (Hill, 2002; Thakur, 2012). Common bean weevil is more common in temperate environments, while Mexican bean weevil prevails in tropical environments. The indiscriminate use of chemical pesticides and fumigants in storage have led to a number of problems including insect resistance, deleterious effects to non-target organism, toxic residues in food grains and environmental pollution. Synthetic insecticides can leave potentially toxic residues in food products and can affect non-target organisms in the environment (Isman, 2006). Hence, there is a need to implement safe and eco-friendly alternatives to protect stored grain products and to restrict the use of toxic chemicals.

Plant derived extracts and phytochemicals have been intensively investigated in an effort to develop alternatives to conventional insecticides with reduced health and environmental impacts. Several researchers have explored the utility of plant products as a potential source of managing agricultural pests. Therefore, in the present investigations two plants, *Juglans regia* Linnaeus and

Picrorhiza kurroa Royle ex Benth have been investigated to control the *A. obtectus* in stores. *J. regia* is a member of family Juglandaceae, commonly known as walnut, is an important species of deciduous trees found principally in temperate areas across the world. Leaves have been used in the cosmetic and pharmaceutical industries (Oliviera et al., 2008) and root bark contains naphthoquinones such as juglone and bisjuglone, used in dentistry and cosmetics (Hamayun et al., 2006). In the present study leaves, of walnut were used. *P. kurroa* belongs to family Scrophulariaceae, commonly known as kutki, is a small important alpine herb, growing at an altitude of 3,000-5,000 meters above mean sea level. The roots and rhizomes of *P. kurroa* are used in traditional and modern medicines for liver disorders, fever, asthma and jaundice. In Himachal Pradesh, kutki is found in the Thamsar and Dianasar areas of Bara and Chhota Bhangal in Kangra district, Pangi, Bharmaur of Chamba district, Lahaul, Kinnaur and Sirmaur. Most of the properties of this plant are attributed to an active constituent known as 'kutkin' which is a mixture of kutkoside and picroside. In the present study roots of kutki were used. Therefore, in an attempt to find natural and cheaper methods of control, bioefficacy of *J. regia* and *P. kurroa* have been tested on *A. obtectus* and methanol extract of both the plants were effective in increasing adult mortality, reducing the fecundity and F1 adult emergence.

2. Materials and Methods

2.1. Sample collection

The infested and uninfested seeds of *P. vulgaris* were collected from different areas of the Himachal Pradesh. These seeds were cultured under controlled condition of temperature and relative humidity. Cultures were propagated in different Petri-dishes (90 mm diameter, Tarsons and 105 mm diameter, Borosil) and wire mesh cages (12×10×10 cm³) along with host seeds. Adults thus emerged were identified as *A. obtectus* according to dichotomous keys (Arora, 1977; Johnson, 1990; Kingsolver, 2004).

2.2. Maintenance of cultures

Reserve cultures of *A. obtectus* was maintained in the laboratory in 500 ml glass jars covered with muslin cloth. One pair of freshly emerged male and female were placed in each Petri-dish. A typical culture contains a pair of male and female insects. The batches of freshly laid eggs were separated regularly until the female died. Life cycle of the pests from the day of egg laying on host seeds and their development up to adult insects were carefully noted.

2.3. Collection and identification of the plant

The plants were collected and then identified by a taxonomic key developed by Eichler (1883). The plant materials were washed and air dried in the shade before use. Plant extracts was prepared in different solvents according to the method of Talukdar and Howse (1994) with a few modifications. Plant extracts were prepared in methanol. Twenty grams of ground leaves of plants were separately mixed with 100 ml of different solvents, stirred for 30 min using a magnetic stirrer and then left to stand for 24 hours. The mixture was then filtered through Whatman # 1 paper, and the solids were stirred again for 15 minutes with the same solvent and filtrates were combined. After complete evaporation of solvent, the final crude extracts were dissolved in solvent before use. Different concentration levels, 2, 4, 6, 8% were prepared and 1 ml of each concentration was applied to the filter papers placed in the Petri-dishes. The papers containing different concentration of botanicals were air dried until the complete evaporation of

solvents. Concentrations used were determined after conducting preliminary experiments to standardize the doses and then used for assessing their insecticidal and seed protective effects.

Methanol (1 ml each) treated filter paper placed in Petri-dishes were used as controls. Then 50 g seeds of *P. vulgaris* were placed on the extract treated filter papers inside the Petri-dishes. Five pairs of freshly emerged adults of *A. obtectus* were released in different Petri-dishes which were covered for the next few days for observation. The number of dead insects in each dish was counted after 24, 48, 72 and 96 h and also up to 100% mortality. All treatments were arranged in completely randomized design and all concentration levels were replicated three times.

2.4. Data analysis

Percentage insect mortality was calculated using Abbott's formula (Abbott, 1925) as follows:

$$\text{Correct \% mortality} = 1 - \frac{C_n - C_T}{C_T} \times 100$$

Where, C_n —number of insects in control and C_T —number of insects in treatment.

Duncan's Multiple Range Test (DMRT) was applied to all means using SPSS—16 software.

Bioefficacy of *J. regia* and *P. kurroa* as an insecticide against *A. obtectus* was studied in terms of mortality of adult pests, numbers of eggs laid and number of F_1 progeny emerged.

3. Results and Discussion

3.1. Biology

To study the biology of *Acanthoscelides obtectus* on *Phaseolus vulgaris*, all observations were recorded in the laboratory under natural temperature and relative humidity. This pest starts to infest beans in field and continues to develop during storage. Life cycle is holometabolous. These bean weevil species are cosmopolitan insect pests and feed on wild and cultivated common beans (Alvarez et al., 2005, Paul et al., 2009; Thakur, 2012). This pest is multi-voltine and completes 6-8 overlapping generation in a year. This pest shows sexual dimorphism, males were active, smaller in size with vertical pygidium, whereas females bulky with sub vertical pygidium. Like other bruchids, adults of *A. obtectus* do not feed, are weak fliers, and feign death when disturbed. Freshly emerged adults copulate at any time with in the 24 hours after their emergence. During copulation male normally raises its fore and middle legs to hold the female. Copulation lasted for 4-5 minutes.

Oviposition starts within few hours after mating. The number of eggs laid per female per day was observed and the freshly emerged female laid the maximum number of eggs on the first and second day of oviposition and then oviposition decreased subsequently. The female glued only a small number of eggs to the seeds, while the majority of the eggs were released freely among the seeds. Freshly laid eggs were milky white and ellipsoidal in shape. Oviposition lasted for 7-10 days and the incubation period was 8-10 days. Since most of the eggs were not glued onto the seeds it is essential for the freshly hatched first instar larva to find and select the host seeds for the remaining stages of development and food requirements. Females of *A. obtectus* lay eggs loosely among the seeds the 1st instar larva burrows into the bean (Parsons and Credland, 2003; Paul et al., 2009; Thakur, 2012).

Further development of successive larval instars was completed inside the host seeds. All the larval instars were voracious feeders. The last larval instar prepares an emergence window before molting to pupal stage. Larval development was completed in 14-20 days. The pupal stage also

completes development inside the host seeds and pupal development took 14-17 days. The total life cycle required 44-54 days. Similar observations on biology, oviposition and larval - pupal development of bruchids have been observed by Southgare (1979) Thakur and Banyal (2007).

3.2. Biointensive management

The use of natural products is an important alternative for the control of stored product pests. In the present investigations leaves of *Juglans regia* and root extracts of *Picrorhiza kurroa* were toxic to the adults of *A. obtectus*. All application rates produced 100% mortality of both males and females compared to the controls where 100% mortality was recorded on the 17th day. Methanol extracts of *J. regia* and *P. kurroa* at 8% concentration were more effective, resulting in 100% mortality after the 6th day and 5th day respectively (Fig. 1, 2 and Table 1, 2). A 6% concentration of *J. regia* gave 100% mortality after the 7th day while same concentration of *P. kurroa* extract in methanol, resulted in 100% mortality on the 6th day. Concentration of 2 and 4% of *J. regia* gave 100% mortality after the 11th and 9th day respectively whereas the same concentration of *P. kurroa* gave 100% mortality after the 8th and 7th day respectively (Fig. 1, 2 and Table 1, 2). Essential oils and natural products affect insects as contact insecticides and also disrupt metabolic pathways (Saxena et al., 1992), act as repellents (Plarre et al., 1997), and can deter or modify oviposition (Abd-Elhady, 2012). *P. kurroa* extract was more effective than *J. regia* at all concentration levels. An 8% concentration of *P. kurroa* gave 100% mortality on the 5th day whereas *J. regia* resulted in 100% mortality on the 6th day after treatment. Renuka et al. (2014) recorded 100% mortality of *A. obtectus* after 5.66 ± 0.33 days at 8% concentration of *Bidens pilosa* extract in methanol. Koon and Bouda (2006) prepared extracts of *Pachypodanthium staudtii* and showed that 0.16% (w/w) killed 100% adults of *A. obtectus* 96 h after treatment.

The methanol extract of *J. regia* and *P. kurroa* at all concentration levels significantly reduced the number of eggs on the seeds as compared to the control where a maximum of 142.67 ± 4.48 eggs were recorded. An 8% methanol extract of *J. regia* and *P. kurroa* resulted in 52.33 ± 1.45 and 50.33 ± 2.60 eggs respectively (Table 3). In the case of a 6% concentration of *J. regia* and *P. kurroa*, total numbers of 60.33 ± 1.45 and 71.66 ± 1.76 eggs were observed respectively. Total numbers of 78.66 ± 0.88 and 88.33 ± 1.45 eggs were observed under 4% concentrations of *J. regia* and *P. kurroa* respectively and under 2% concentrations of *J. regia* and *P. kurroa* each 95.33 ± 1.45 and 100.67 ± 1.20 eggs were recorded respectively. However, results are contrary to the findings of Jovanovic et al. (2007) who reported ineffectiveness of *Sambucus nigra* L. and *J. regia* extracts against *A. obtectus*. Significant decrease in fecundity, fertility and F₁ emergence of *A. obtectus* and *Z. subfasciatus* observed when crushed leaves of *Tetradentia riparia* at 4% application rate were applied (David et al., 1998). Vanmathi et al. (2010) recorded maximum oviposition deterrent of 84.32% of *C. maculatus* against aqueous extract of *Ocimum tenuiflorum* at higher concentrations.

The methanol extract significantly reduced the F₁ progeny at all concentration levels as compared to control where a maximum 114.67 ± 6.07 F₁ adults emerged. The minimum number of 30.33 ± 1.45 and 33.66 ± 2.02 adults emerged under 8% concentration of *J. regia* and *P. kurroa* respectively. Under 6% concentration of *J. regia* and *P. kurroa* extracts 42.35 ± 1.76 and 50.33 ± 2.02 adults emerged respectively. Whereas in 4% concentration of *J. regia* and *P. kurroa* 88.33 ± 1.45 and 62.66 ± 2.02 and under 2% concentration 100.67 ± 1.20 and 33.66 ± 1.76 adults emerged (Table 3). The extracts of *Urtica dioica* L. and *Taraxacum officinale* L., were tested against the bean weevil, *A. obtectus* for insecticidal potential to protect stored legume seeds in terms of their repellency, toxicity and reduction of F₁ progeny and significant insecticidal

activities, effectiveness in repellency and reduction in F1 progeny exhibited by 100% concentrated extract was recorded (Jovanovic et al., 2007). High mortality rates and inhibition of F1 progeny production of *C. maculatus* were recorded by contact with seeds treated with essential oil of *Artemisia judaica* (Abd-Elhady, 2012). The powder of orange peel applied at 15 gm against *Z. subfasciatus* significantly reduced the F1 progeny (Dawit and Bekelle, 2010).

The present study thus showed that the methanol extracts of *J. regia* and *P. kurroa* were effective against *A. obtectus* at all application rates in increasing mortality, decreasing fecundity and decreasing progeny emergence. The plant extracts were significantly different from the controls at all concentrations applied and best results were obtained at 8% application of plant extracts. Both plants could be used as potential botanical insecticides for stored grain pests but more research is required for commercial development on an enlarged scale.

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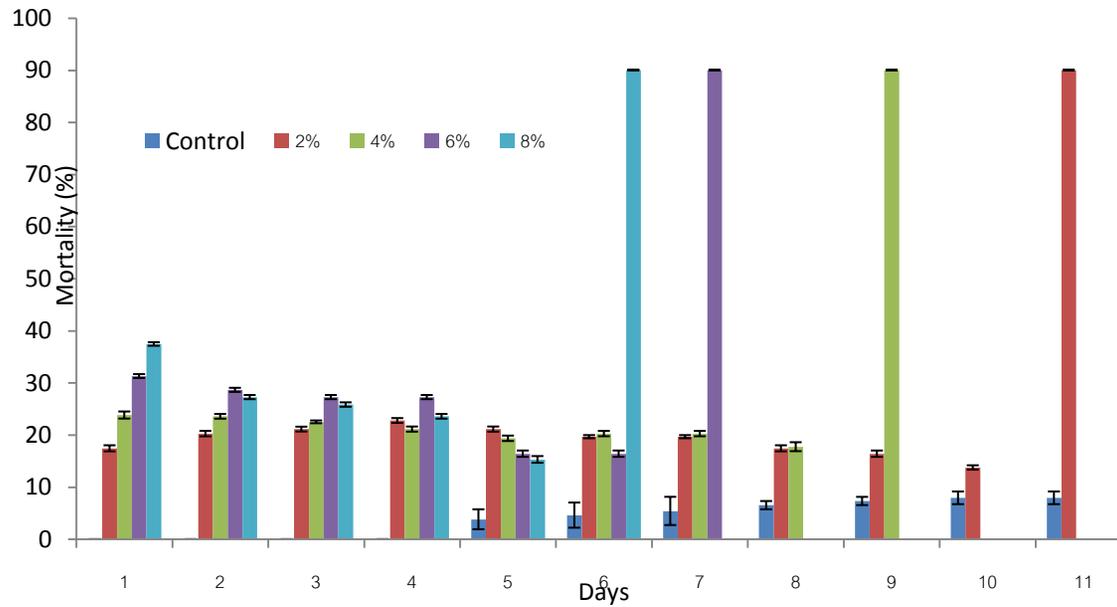


Figure 1 Percent mortality of adult *A. obtectus* treated with different concentrations of *J. regia* extract in methanol.

Table 1 Percent mortality of adult *A. obtectus* treated with different concentrations of *J. regia* extract in methanol.

Dose (%)	Day wise insect mortality (%)										
	1	2	3	4	5	6	7	8	9	10	11
Control	0.00±0.00 ^e	0.00±0.00 ^d	0.00±0.00 ^e	0.00±0.00 ^d	3.82±1.91 ^d	4.62±2.41 ^d	5.42±2.71 ^c	6.53±0.79 ^c	7.33±0.79 ^c	7.94±1.22 ^c	7.94±1.22 ^b
2	17.44±0.58 ^d	20.26±0.51 ^c	21.13±0.46 ^d	22.78±0.46 ^b	21.13±0.49 ^a	19.67±0.30 ^b	19.67±0.30 ^b	17.44±0.58 ^b	16.41±0.61 ^b	13.76±0.42 ^b	90.04±0.00 ^a
4	23.83±0.68 ^c	23.58±0.45 ^b	22.52±0.27 ^c	21.13±0.49 ^c	19.36±0.52 ^{ab}	20.26±0.51 ^b	20.26±0.51 ^b	17.75±0.86 ^b	90.04±0.00 ^a	90.04±0.00 ^a	90.04±0.00 ^a
6	31.31±0.37 ^b	28.66±0.39 ^a	27.27±0.40 ^a	27.27±0.40 ^a	16.41±0.61 ^{bc}	16.41±0.61 ^c	90.04±0.00 ^a				
8	37.47±0.34 ^a	27.27±0.40 ^a	25.84±0.42 ^b	23.58±0.45 ^b	15.31±0.64 ^c	90.04±0.00 ^a					

Values are mean ±SE of three replicates. The data original mortality of *A. obtectus* were corrected by Abbott's formula and then transformed into arcsin $\sqrt{\text{percentage}}$ values before statistical analysis. Values followed by different letters within a column are significantly different at the 5% level of probability (Duncan's Multiple Range Tests). *D (day). **90.04 represent 100% mortality of pests.

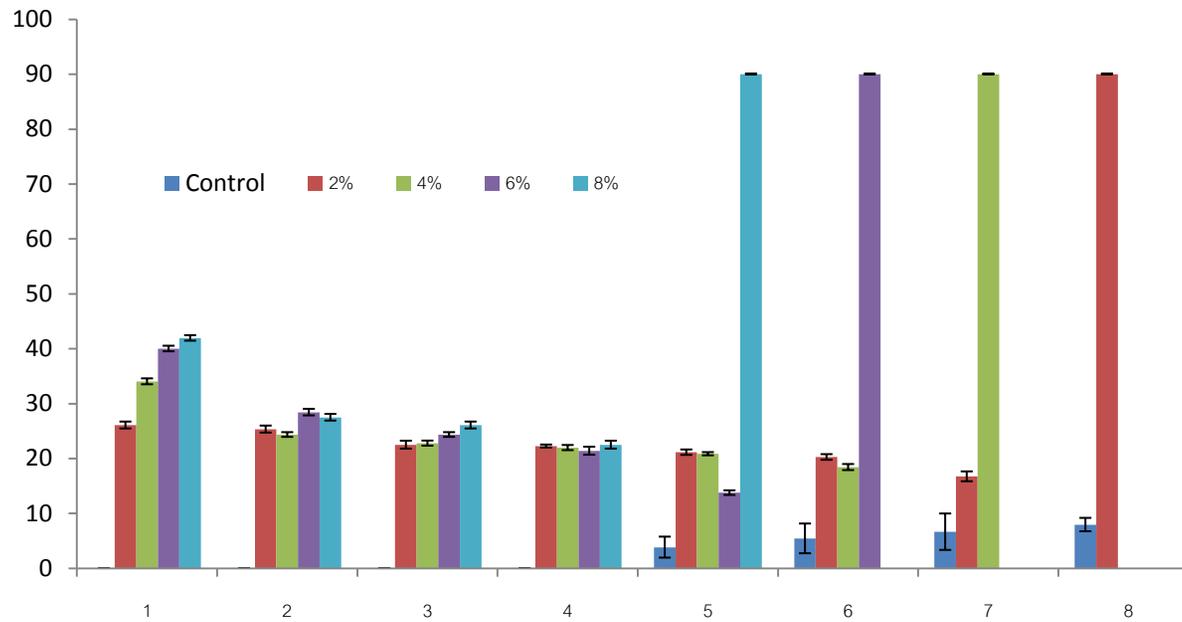


Figure 2 Day wise percent mortality of adult *A. obtectus* treated with different concentrations of *P. kurroa* extract in methanol.

Table 2 Day wise mortality of adult *A. obtectus* treated with different concentrations of *P. kurroa* extracts in methanol.

Dose (%)	Day wise insect mortality (%)							
	1	2	3	4	5	6	7	8
Control	0.00±0.00 ^e	0.00±0.00 ^c	0.00±0.00 ^d	0.00±0.00 ^c	3.82±1.91 ^d	5.42±2.71 ^c	6.64±3.32 ^c	7.94±1.22 ^b
2	26.08±0.63 ^d	25.34±0.64 ^b	22.50±0.72 ^c	22.25±0.27 ^a	21.13±0.49 ^b	20.26±0.51 ^b	16.73±0.90 ^b	90.04±0.00 ^a
4	34.04±0.54 ^c	24.35±0.43 ^b	22.78±0.46 ^{bc}	21.97±0.47 ^a	20.85±0.29 ^b	18.42±0.55 ^b	90.04±0.00 ^a	90.04±0.00 ^a
6	40.02±0.51 ^b	28.43±0.60 ^a	24.35±0.43 ^b	21.40±0.73 ^a	13.76±0.42 ^c	90.04±0.00 ^a	90.04±0.00 ^a	90.04±0.00 ^a
8	41.95±0.50 ^a	27.50±0.61 ^a	26.08±0.63 ^a	22.50±0.72 ^a	90.04±0.00 ^a	90.04±0.00 ^a	90.04±0.00 ^a	90.04±0.00 ^a

Values are mean ±SE of three replicates. The data original mortality of *A. obtectus* were corrected by Abbott's formula and then transformed into arcsin $\sqrt{\text{percentage}}$ values before statistical analysis. Values followed by different letters within a column are significantly different at the 5% level of probability (Duncan's Multiple Range Tests). *D (day). **90.04 represent 100% mortality of pests.

Table 3 Number of eggs laid and F₁ adults emerged of *A. obtectus* treated with different doses of *J. regia* and *P. kurroa* extracts in methanol.

Plant	Dose (%)	Number of eggs laid	Number of F ₁ adults emerged
<i>J. regia</i>	Control	142.67±4.48 ^a	114.67±2.60 ^a
	2	95.33±1.45 ^b	71.66±1.76 ^b
	4	78.66±0.88 ^c	50.33±1.45 ^c
	6	60.33±1.45 ^d	42.33±1.76 ^d
	8	52.33±1.45 ^e	30.33±1.45 ^e
	F	236.21***	318.83***
<i>P. kurroa</i>	Control	142.67±4.48 ^a	114.67±2.60 ^a
	2	100.67±1.20 ^b	81.33±1.76 ^b
	4	88.33±1.45 ^c	62.66±2.02 ^c
	6	71.66±1.76 ^d	50.33±2.02 ^d
	8	50.33±2.60 ^e	33.66±2.02 ^e
	F	178.70***	270.88***