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Bioefficacy of Bidens pilosa L. against Acanthoscelides obtectus (Say) and Zabrotes subfasciatus (Boheman), stored pests of kidney beans, world wide

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ABSTRACT: A pioneer study has been conducted to test the bioefficacy of Bidens pilosa L. for the control of Acanthoscelides obtectus and Zabrotes subfasciatus reared on Phaseolus vulgaris L. seeds under laboratory conditions. Different concentrations of B. pilosa in methanol and acetone at 2, 4, 6 and 8% including control (0%) were tested against both the pest species. Extract prepared in acetone was found more effective than methanol at all concentration levels. Extract concentration of 2, 4, 6 and 8% in acetone gave 100% mortality after 2.66±0.66 days of the treatment among both the bruchids. On the other hand 8% methanol extract gave 100% mortality after 5.66±0.33 days, while 2, 4 and 6% gave 100% eradication of pest species mortality on 7.66±0.66 days after treatment. Mean oviposition decreased significantly ($p \leq 0.001$) with increase in treatment dosage from a high value of control (142.67±4.48 of A. obtectus and 134.33±2.33 of Z. subfasciatus) to a comparatively low value of total number of eggs at all concentration levels in both the solvents. Similarly there was a significant reduction in F_1 progenies with increase in treatment dosage as compared to the control. The bioefficacy of B. pilosa has been evaluated for the first time against the stored pests of kidney beans and the results thus obtained indicate that this is a wonderful botanical insecticide and utilized at large scale to suppress the pest populations of stored legumes.
Keywords: Acanthoscelides obtectus, Bidens pilosa, Biopesticides, Control, Phaseolus vulgaris, Zabrotes subfasciatus.

INTRODUCTION

Insect pests attack all crops and their products and minimum of 10% of cereals and legumes are lost after harvest globally (Boxall et al., 2002). But estimate from the developing countries shows that about one third of the potential yield of cultivated plants is destroyed by insect pests in fields and godowns every year. Loss of the plants produce through pests not only adversely affects the economy of a nation but also deprive the needy people of valuable food. Although insect pests cause heavy economic losses to stored grains world wide but their impacts are more devastating in developing and underdeveloped nations. Besides damaging the plants physically, many insect pests transmit viral, bacterial and fungal diseases while feeding on them thus adversely affect the total yield of the crops.

Acanthoscelides obtectus (Say) and Zabrotes subfasciatus (Boh.) are two universal and important pests of kidney beans, Phaseolus vulgaris and responsible for the heavy loss of bean cultivars both fields and stores (Thakur, 2012). Losses of stored bean due to these two bruchid pests, amount to 13-15% (Cardona and Posso, 1987). In Bako, 14% loss in haricot beans stored for 12 months by Z. subfasciatus was recorded by Adane and Abrham (1996). Insects multiply rapidly under favorable environmental conditions and it becomes difficult to reduce the loss caused by insects in tropical and subtropical regions of the world.

The indiscriminate use chemical pesticides and fumigants in the storage have led to a number of problems including insect resistance, deleterious to non target organism, toxic residues in food grains and environmental pollution. So there is an utmost need to implement safe and ecofriendly alternatives to protect stored grain products and to restrict the use of toxic chemicals globally.

The plant products because of their effective, eco-friendly and economically viable options have been intensively investigated in recent years in order to develop alternatives of chemical pesticides with lesser environmental impacts to control agricultural pests. To attain self-sufficiency in food, which is the goal of

agricultural practices of developing and underdeveloped countries, it is no doubt important to increase the crop yield, but it is more important to protect crops and their products from the attack of insect pests. To achieve this, it is necessary to have a thorough knowledge of the crop pests and the measures to control them. This knowledge not only benefits the farmers and horticulturist but also the nation as a whole.

B. pilosa is a plant species belonging to the family Asteraceae. It is considered a weed in some tropical habitats but in some part of the world it is source of the food (Grubben and Denten, 2004). It is a small, erect annual herb having bright green leaves with serrated, prickly edges and produces small, yellow flowers and black fruit. This plant is commonly used to reduce inflammation, increase urination, support and protect liver, hepatitis, conjunctivitis, abscesses, fungal infections, urinary infections, as a weight loss aid and to stimulate child birth, to clot blood in fresh wounds, headaches, ear infection, kidney problems and for the treatment of HIV/AIDS infections also (Tadesse, 1994; Duke, 1997; Taylor, 2005; Theta, 2005). Preliminary phytochemical investigation reveals that this plant is rich in tannins, phlobatannins, flavonoids, terpenoids, cardiac glycosides, phenylpropanoids, lipids and benzenoids (Ezeonwumelu et al., 2011; Chang et al., 2001; Abajo et al., 2004; Wu et al., 2004; Sundararajan et al., 2006). Due to the presence of polyacetylenes, flavonoids, terpenoids, phenylpropanoids, B. pilosa is used in folk medicine for various applications and Kumari et al. (2009) investigated that the leaves of this plant contains anticancerous and antimalarial compounds.

The present study was carried out to investigate the insecticidal property of B. pilosa extract prepared in methanol and acetone against A. obtectus and Z. subfasciatus and also to evaluate the effect of different concentrations on total number of eggs and F_1 progeny of both the pests. B. pilosa is known to possess insecticidal properties against tea pests (Mamun and Ahmed, 2011) but used for the first time to control the bruchid pests of common beans in storage.

MATERIALS AND METHODS

Collection and identification of pests

The infested and uninfested seeds of P. vulgaris were collected from the different pars of Himachal Pradesh viz. Kullu Distt., Chaudhry Sarvan Kumar Krishi Vishav Vidyalaya Palampur Research Sub-station, Katrain (Kullu) and National Bureau of Plant Genetic Resources (NBPGR) Research Station Phagli (Shimla). After emergence of the adult bruchids from the samples thus collected, cultures were initiated under controlled condition of temperature and relative humidity. Adults thus emerged were identified as Z. subfasciatus and A. obtecuts by running in dichotomous key developed by Arora (1977); Johnson (1990) and Kingsolver (2004). Cultures were propagated in different petri-dishes 90 and 105 mm diameter of Tarsons and Borosil and in wire mesh cages (12×10×10 cm^3) along with host seeds. Insect culture was subletted and maintained in BOD (Biological oxygen demand) Decibel made at 25.3±0.57°C and 70.6±1.15% relative humidity and reserve culture of both the insect pests were maintained in 500 ml glass jars each with the open mouth covered with muslin cloth tightly.

Collection and identification of the plant samples

B. pilosa was collected from the campus of Himachal Pradesh University Summer Hill, Himachal Pradesh and then identified through taxonomic key developed by Eichler (1883) and also with the help of Plant taxonomist, Prof. M. K. Seth, Department of Biosciences, Himachal Pradesh University, Shimla H. P.

The plant materials were washed and air dried in the shade before use. Aerial parts of the plant were used and their extract prepared in methanol and acetone according to the method of Talukdar and Howse (1993) with a slight modifications. Twenty grams of ground leaf powder of plant was mixed with 100 ml of solvents, stirred for 30 minutes by using a magnetic stirrer and then left undisturbed for 24 hrs. 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. The solvent from the pooled filtered solution was evaporated in a water bath by setting the temperature of water bath according to the boiling point of the solvent used. After complete evaporation of solvents, the final crude extracts were dissolved in solvents before use. Different concentration levels of 0, 2, 4, 6, 8% were prepared and 1ml of each concentration applied to the filter papers placed in the petri-dishes and 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 insecticidal properties and seed protective effects.

Methanol and acetone treated filter paper placed in petri-dishes were taken as control and both of them non toxic to the pests. 50 seeds of P. vulgaris were placed on the extract treated filter papers inside the petri-dishes. Five pairs of A. obtectus and Z. subfasciatus were released in different petri-dishes and covered for the next 7 days for observation. The number of dead insects in each petri-dish was counted after 24, 48, 72 and 96 hrs and also up to 100% mortality of pests. Percentage insect mortality was calculated using Abbott's formula (Abbott, 1925) as follows:

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

Where, C_n- number of insects in control and C_T-number of insects in treatment.
The experiment was designed in a completely randomized design in three replications.
Efficacy of B. pilosa as an insecticide against bruchids was studied in terms of mortality of adult pests, numbers of eggs laid and numbers of F_1 progeny emerged.

RESULTS

Mortality response of adult bruchids

B. pilosa extract prepared in methanol and acetone found toxic to adults of both A. obtectus and Z. subfasciatus at all application rates and significantly resulted in 100% mortality of both the pests as compared to the control. Eight percent methanol extract against both bruchid pests proved most effective than other concentrations of 2, 4 and 6% and resulted in 100% mortality after 5.66±0.33 days of treatment. But acetone extract of 2, 4, 6 and 8% concentration had given 100% mortality of the both bruchids after 2.66±0.66 days of treatment. Thus acetone extract proved more effective than methanolic extract against both the insect pests. 2, 4 and 6% concentrations of methanolic extract against both the pests had given 100% mortality 7.66±0.66 days after treatment. Methanol and acetone extract at all concentration levels against both the pests were statistically different to the control (Fig. 1, 2, 3 &4 and Table 1 & 2).

Eggs deterrence

The plant extract of all concentration significantly ($p \leq 0.001$) reduced the fecundity of gravid females as compared to control where 142.67±4.48 and 134.33±2.33 eggs were laid by A. obtectus and Z. subfasciatus respectively. Eight percent methanol and acetone concentration of B. pilosa has proved effective and resulted in 51.66±1.45 & 47.66±1.45 eggs production by A. obtectus and 40.33±1.45 & 44.66±1.45 by Z. subfasciatus respectively (Table 3).

F_1 progeny reduction

Eight percent methanolic concentration of B. pilosa resulted in 37.33±1.20 and 36.33±0.88 F_1 adult's emergence of A. obtectus and Z. subfasciatus and it was statistically almost similar to 6% methanol extract which resulted in 41.33± 2.33 of A. obtectus and 40.33±1.20 of Z. subfasciatus F_1 adult's emergence respectively. The acetone extract resulted in 31.66±2.08 and 24.66±2.02 F_1 adult emergence at 8% for A. obtectus and Z. subfasciatus respectively and was statistically almost similar to 6% concentration which resulted in 37.66 ± 1.45 F_1 adult emergence in A. obtectus but statistically different in Z. subfasciatus who resulted in 35.66 ± 1.20 F_1 adult emergence. All other concentrations viz. 2 and 6% of methanol and acetone extract of B. pilosa significantly reduced the emergence of F_1 adults as compared to control where 114.67±2.60 and 107.67±4.05 adults of A. obtectus and Z. subfasciatus were emerged successfully (Table 3).

DISCUSSION

Eight percent methanol extract of B. pilosa gave 100% mortality after 5.66±0.33 days in both the bruchids species and 2, 4 and 6% concentration of same extract also gave 100% mortality but 7.66±0.66 days after treatment, whereas, no mortality was observed until 6th day in A. obtectus and Z. subfasciatus both at control. Acetone extract of B. pilosa was most effective resulting 100% mortality after 2.66±0.66 days and mortality was directly proportional to extract concentrations. Kim et al. (2003) tested methanol extracts of Acorus sp., rhizomes and fruits of Illicium vervum and Foeniculum vulgare against Sitophilus oryzae and Callosobruchus chinensis and found 90% mortality on 3-4 days after treatment but root bark extract of Clematis sieboldii gave 100% mortality on 2 days after treatment. Rahman and Talukder (2006) showed that acetone extract of Vitex negundo was most toxic to C. maculatus and found that number of eggs laid was lowest and ovipostion inhibition highest in the food treated with above plant materials. Mean oviposition decreased significantly ($P \leq 0.001$) with increase in treatment dosage from 142.67±4.48 and 134.33±2.33 eggs per female of A. obtectus and Z. subfasciatus respectively at control to a comparatively low value of 51.66±1.45 and 47.66±1.45 eggs per female of A. obtectus and 40.33±1.45 and 44.66±1.45 eggs per female of Z. subfasciatus at 8% methanol and acetone concentration respectively. Similarly F_1 adults decreased significantly from a high value of control, 114.67±2.60 and 107.67±4.05 of A. obtectus and Z. subfasciatus respectively to a comparatively low value of 37.33±1.20 and 31.66±2.08 of A. obtectus and 36.33±0.88 and 24.66±2.02 of Z. subfasciatus at 8% concentration respectively. Mamun and Ahmed (2011) used B. pilosa for the control of tea pests and observed significant results of adult mortality. Koona and Bouda (2006) prepared different doses of extract of Pachypodanthium staudtii and showed that 0.16% (w/w) killed 100% adults of A. obtectus after 96 hrs. Jovanovic et al. (2007) showed that 100% Utrica dioica and Taraxacum officinale efficiently killed adults of A. obtectus and significant level of reduction in F_1 progeny was also achieved. Vanmathi et al. (2010) found that the adult emergence of C. maculatus was reduced to 5.66±0.66 at 1%, 2.66±0.33 at 3% and 1.66±0.33 at 5% concentration in the seeds treated with Azadirachta

indica. Mortality of adult bruchids with the treatment of plant extract was directly proportional to the concentration levels and the exposure time of the pest. It was observed that higher doses and longer exposure periods were needed to achieve appreciable management of bruchids in methanolic extract of B. pilosa and this observation is in accordance with the study of Ziaee and Moharramipour (2013), who found the effectiveness of Carum copticum and Cuminum cyminum powders on Sitophilus granaries and Tribolium confusum.

CONCLUSIONS

The present study therefore, revealed that B. pilosa possesses insecticidal properties against A. obtectus and Z. subfasciatus and methanol and acetone extracts were found effective against both the pest species. However, acetone extract was more effective resulted in 100% mortality of pests after 2.66±0.66 days irrespective of the concentration levels. Plant extracts in methanol and acetone at all concentration levels significantly reduced the fecundity and F_1 progeny. This plant is easily available and could be used as a potential botanical insecticide for stored grain. Insecticidal property of this plant is due to its richness in phytochemical compounds which released in solvents during extraction and produced desired effects when applied in insectary. Since synthetic pesticides cause environmental hazards and this has lead to a demands of botanical pesticide and B. pilosa may prove a wonderful substitute of synthetic chemicals may exploited at the large scale for the management of stored grain pests in general and bruchids in particular.

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Table 1. Adult mortality of A. obtectus treated with different concentrations of methanol and acetone extracts of B. pilosa.

Extracts	Dose (%)	A. obtectus mortality (%)							
		1st D†	2nd D	3rd D	4th D	5th D	6th D	7th D	8th D
Methanol	0	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}	3.82±1.91^{d}	6.53±0.79^{d}	7.94±1.22^{b}
	2	15.31±0.64^{c}	17.44±0.58^{d}	18.42±0.55^{c}	21.97±0.47^{b}	24.35±0.43^{a}	27.97±0.41^{b}	27.27±0.40^{a}	90.04±0.00^{a}
	4	26.08±0.63^{b}	22.78±0.46^{c}	22.50±0.72^{b}	22.23±0.71^{b}	21.13±0.49^{b}	20.23±1.02^{c}	19.95±0.79^{b}	90.04±0.00^{a}
	6	27.27±0.40^{b}	25.84±0.42^{b}	23.58±0.45^{b}	22.78±0.46^{b}	21.13±0.49^{b}	20.26±0.51^{c}	11.47±0.85^{c}	90.04±0.00^{a}
	8	30.00±0.38^{a}	30.00±0.38^{a}	27.27±0.40^{a}	25.84±0.42^{a}	18.42±0.55^{c}	90.04±0.00^{a}		
Acetone	0	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{b}					
	2	43.87±0.32^{d}	46.16±0.33^{a}	90.04±0.00^{a}‡					
	4	46.73±0.33^{c}	43.29±0.33^{b}	90.04±0.00^{a}					
	6	52.55±0.34^{b}	37.47±0.34^{c}	90.04±0.00^{a}					
	8	66.45±0.45^{a}	23.58±0.45^{d}	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 √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 2. Adult mortality of Z. subfasciatus treated with different doses of methanol and acetone extracts of B. pilosa.

Extracts	Dose (%)	Z. subfasciatus mortality (%)							
		1st D†	2nd D	3rd D	4th D	5th D	6th D	7th D	8th D
Methanol	0	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}	1.91±1.91^{d}	4.62±2.41^{d}	6.53±0.79^{d}	7.94±1.22^{b}
	2	16.41±0.61^{d}	17.44±0.58^{d}	18.42±0.55^{c}	21.97±0.47^{bc}	24.35±0.43^{a}	27.97±0.40^{b}	26.56±0.41^{a}	90.04±0.00^{a}
	4	26.56±0.41^{c}	23.58±0.45^{c}	22.78±0.46^{b}	21.13±0.49^{c}	21.13±0.49^{b}	21.13±0.49^{c}	18.42±0.55^{b}	90.04±0.00^{a}
	6	27.97±0.40^{b}	25.84±0.45^{b}	23.58±0.45^{b}	22.78±0.46^{b}	20.26±0.51^{bc}	19.36±0.52^{c}	12.88±0.76^{c}	90.04±0.00^{a}
	8	30.66±0.37^{a}	30.00±0.38^{a}	27.27±0.40^{a}	25.84±0.42^{a}	17.44±0.58^{c}	90.04±0.00^{a}		
Acetone	0	0.00±000^{e}	0.00±0.00^{e}	0.00±0.00^{e}					
	2	46.73±0.33^{d}	43.29±0.33^{a}	90.04±0.00^{a}‡					
	4	51.37±0.33^{c}	38.66±0.34^{b}	90.04±0.00^{a}					
	6	58.08±0.36^{b}	31.94±0.36^{c}	90.04±0.00^{a}					
	8	66.84±0.39^{a}	23.19±0.40^{d}	90.04±0.00^{a}					

All values are mean ±SE of three replicates. The data on original mortality of Z. subfasciatus were corrected by Abbott's formula and then transformed into arcsin √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. Numbers of eggs and adults of A. obtectus and Z. subfasciatus both treated with methanol and acetone extracts of B. pilosa at different dose. Values are mean ± SE of three replicates. Values followed by different letters within a column are significantly different at p≤0.05 (Duncan's Multiple Range Tests). F values

Plant extracts	Dose (%)	A. obtectus		Z. subfasciatus	
		No of eggs	No of adults	No of eggs	No of adults
Methanol	0	142.67±4.48[a]	114.67±2.60[a]	134.33±2.33[a]	107.67±4.05[a]
	2	95.33±1.45[b]	74.66±2.60[b]	94.33±3.48[b]	71.66±1.76[b]
	4	74.66±2.02[c]	54.33±2.02[c]	72.33±1.45[c]	53.33±1.76[c]
	6	63.33±4.50[d]	41.33±2.33[d]	60.66±1.20[d]	40.33±1.20[d]
	8	51.66±1.45[e]	37.33±1.20[d]	40.33±1.45[e]	36.33±0.88[d]
	F	181.63[***]	203.52[***]	277.90[***]	169.86[***]
Acetone	0	142.67±4.48[a]	114.67±2.60[a]	134.33±2.33[a]	107.67±4.05[a]
	2	90.66±1.20[b]	65.66±3.48[b]	89.66±2.02[b]	63.66±2.33[b]
	4	72.33±1.76[c]	49.66±1.45[c]	67.66±1.45[c]	49.66±2.02[c]
	6	58.33±1.76[d]	37.66±1.45[d]	58.66±0.88[d]	35.66±1.20[d]
	8	47.66±1.45[e]	31.66±2.02[d]	44.66±1.45[e]	24.66±2.02[e]
	F	233.67[***]	203.36[***]	420.47[***]	164.91[***]

followed by asterisks are significant at p≤0.001 (One-way ANNOVA test).

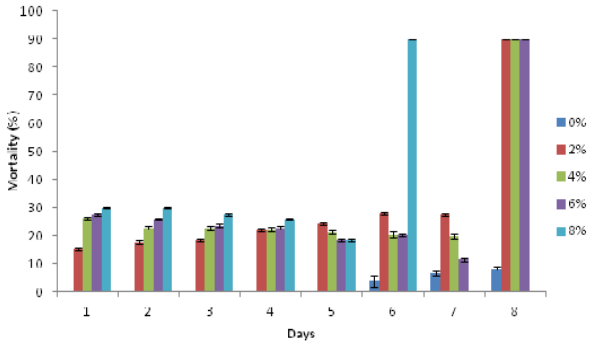

Fig. 1. Daywise percent mortality of adult *A. obtectus* due to different concentrations of methanolic extract of *B. pilosa*.

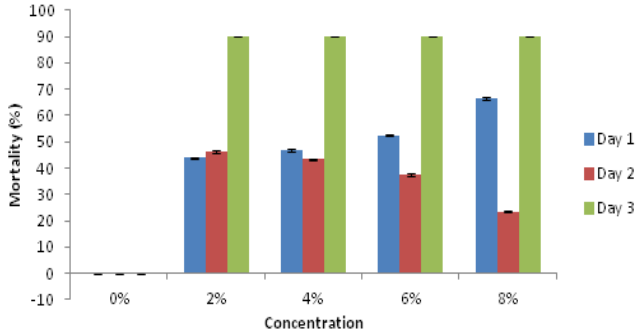

Fig. 2. Daywise percent mortality of *A. obtectus* due to different concentrations of acetone extract of *B. pilosa*.

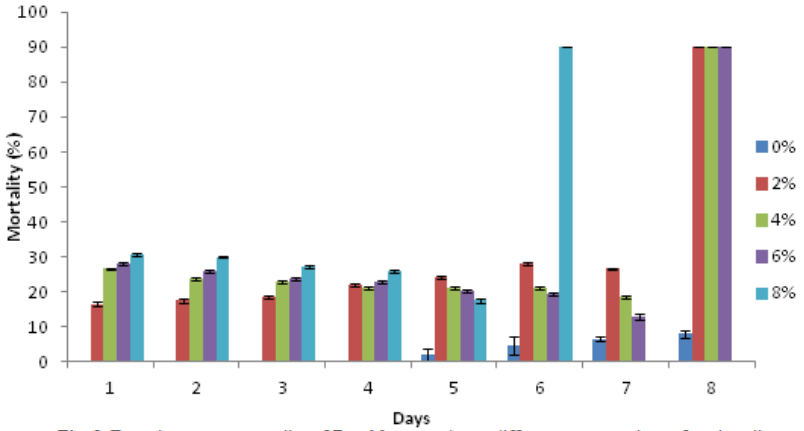

Fig. 3. Daywise percent mortality of *Z. subfasciatus* due to different concentrations of methanolic extract of *B. pilosa*.

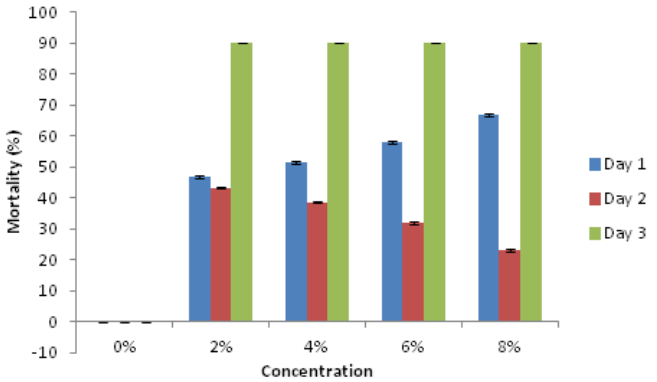

Fig. 4. Day wise percent mortality of *Z. subfasciatus* due to different concentrations of acetone extract of *B. pilosa*.