Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 935-939

Received: 06-09-2017
Accepted: 07-10-2017

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Phagodeterrent bioefficacy of *Gynendropsis pentaphylla*, *Withamnia somnifera* and *Alpinia galanga* against chickpea bruchids, *Callosobruchus chinensis* (Linn.) (Coleoptera: Bruchidae)

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Abstract
Experiments were conducted to test phago-deterrent bio-potency of ten naturally occurring indigenous plant extracts in the Biopesticides and Toxicological research Laboratory, Department of Zoology, Entomology, D.B.S. College, Kanpur, U.P., India. Therefore, it becomes necessary to look for the suitable ten asteraceous plant extracts, which are known to be eco-friendly. Diferrent formulations of asteraceous plant extracts were tested for their repellent activity against the early emerged pulse beetle, *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae). Among them, only three extracts viz *Gynendropsis pentaphylla, Withamnia somnifera* and *Alpinia galanga* extracts was proved most effective repellent in compare to other. Based on relative EC_{50} values *G. pentaphylla* extract showed highest repellency (6.18) to the early emerged adult beetle, *C. chinensis* followed by W. *somnifera* (5.67) and *A. galanga* (4.83) times repellent than *A. calamus* against *C. chinensis*, which is taken as unit. Beside above the selected rest of the plants exhibited varying degree of lesser or moderate repellent effects. Thus, *Gynendropsis pentaphylla* and *Withamnia somnifera* formulations can be used as an effective option of commercial fumigants for the storage of chick pea seeds against pulse beetle, *Callosobruchus chinensis* Linn.

Keywords: *Callosobruchus chinensis*, *Gynendropsis pentaphylla,* repellent activity and plant extracts

Introduction
The chickpea beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) has been reported to cause serious damage to pulses in India and many countries of the world. (Pandey *et al,* 1992, 2010 [17, 18]. Ramzan, 1994 [21], and Shaaya *et al.* 1997 [23].) Qualitative and quantitative deterioration of pulses provoked by insects accounts for considerable loss of economic importance at international level. The *C. chinensis* are polyphagous nature and causing serious economic damage to several agricultural stored grains and pulses in India. (Ahmed *et al.*1999) [4] and many countries of the world Valsala and Gokuldas, 2015 [30],. *Callosobruchus chinensis* has a wide range of host, feeding on 112 species worldwide of which 40 species are known from India Chandel and Singh, 2016 [7], Ketker 1989 [10], Pandey and Singh, 1997 [19], Lale, 1992 [11], Reddy and Singh, 1998 [22], Lale, 2000 [12]. Broad spectrum insecticides used for control of *C. chinensis* has resulted in development of resistance to many of the registered pesticides for its control [4, 5]. The absence of resistance to *C. chinensis* in stored grains and the lack of adequate control measures make it difficult to manage this pest in the storage. In this scenario, new types of botanical products as insecticide originating from natural products, targeting *C. chinensis* could be a useful alternative for integrated pest management Singh, 2003 [25]. Plants are important natural sources of bioactive compounds and many such plant compounds have been included in commercial botanical pesticides Srivastava *et al.*1988 [26]. Many plant products are safer to non-target organisms and effective against phytophagous insects Yankanchi and Gadache [33]. Several plant products have been tested against *C. chinensis* and some promising plants have been reported Zoubiri and Baaliouamer [34]. However the screening of plant extracts against insects are still continuing throughout the world to find out different kinds of effects of botanicals to obtain an ecofriendly, naturally occurring and economic biopesticide [31, 32, 34]. Very few reports are present pertaining to the repellent activity of plant extracts against *C. chinensis* Tripathy *et al.*2001 [29], Al- Lavati *et al.* 2002 [5], Liu *et al.* 2006 [13], Govindan and Nelson (2008) [25, 26].

Therefore the present study deals with screening of various plant extracts for their repellent activity against *C. chinensis*. The use of botanical pesticides in pulse protection has a long account [7, 28] and there are a number of bibliographic records on the use of essential oils from different plants against stored product insect pests by researchers in developing countries were found [30, 31, 32]. So far, the effect of plant essential oils on *Callosobruchus* species has been investigated using fumigant toxicity [2], repellent activity [8, 9, 13, 20, 24, 28] as well as feeding deterrent activity. [2].

Materials and method

Present study was undertaken during the period from August 2016 to July 2017, at the Biopesticides and Toxicological research Laboratory, Post Graduate Department of Zoology, Entomology, D.B.S.College, Kanpur, U.P., India.

Procurement of raw plant materials: For the experimental purposes asteraceous botanical parts were collected from wasteland and cultivated fields of the farmers. Among them, only ten asteraceous plants viz., aerial parts of *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariaefolium* (trev.) Vis., *Inula racem*osa Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, *Spilanthes paniculata* Well ex DC and *Tagetes minuta* Linn. were collected from different vicinity of from U.P. and India.

Preparation of powder: Fresh collected green plant parts (leaves, Flowers and seeds, rhizomes etc) were washed with distilled water and kept in the laboratory for 7 days for air drying followed by one day sun drying before making powder. Electric grinder was used to have coarse powder then these were passed through a 60-mesh sieve to get fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss as reported by earlier [14].

1. **Preparation of botanical extracts:** For the extraction, botanicals Soxhlet Apparatus was used; about 20g powder of each category of powder were extracted with 300 ml of different solvents (n-hexane, acetone, methanol, petroleum ether and distilled water). Extraction of each category of powder was done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.
2. **Apparatus used for experiment:** Small plastic jars (capacity 50 ml) were used for the experiment; there was one set of two jars joined by clear plastic pipe of 1cm diameter at an angle of 180 degree for each replication. One jar of each set was provided with 10 g of grains given the name ‘A’ while the other jar was kept empty and given the name ‘B’. In jar ‘A’, the grains treated with extracts were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.
3. **Preparation of Stock Solution:** For stock solution, 50ml. extract in each case was taken into reagent bottles and 50ml. benzene was added in it to dissolve the constituents of the materials. The mouth of the bottles were stopper with airtight corks after which, these bottles containing the solutions were kept in refrigerator.
4. **The Insecticidal Formulations:** Five concentrations (0.25, 0.5, 1.0, 1.5, 2.0 percent) were used for experiments on repellent tests in the laboratory experiment. The different concentrations of the herbal extracts were prepared from the stock solution using benzene as solvent and Triton X-100 as emulsifier. The level of solvent and emulsifier were kept constant.

Test insect and Host grain

In the present investigation, indigenous naturally occurring certain asteraceous plant parts extracts have been used for their phagodeterrent biological efficacy against *Callosobruchus chinensis* L. (pulse beetle) on stored gram, chickpea, *Cicer arietinum* L. (Fabales: Fabaceae) genotypes Kabuli variety Mexican white were used in preliminary trial under laboratory condition.

Establishment of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

The adult *Callosobruchus chinensis* L. (pulse beetle) were collected from Collector Gange Mandi, Kanpur Nagar and farmers godowns of farmers at Kanpur nagar Districts of Uttar Pradesh, India were cultured and reared on in the laboratory with uniparietal ad libitum population for various investigations. Rearing was done according to the method reported by Chandel and Singh, 2016 [7].

Table 1: Detailed List of plant materials used for extraction.

Sr. No	Scientific Name / Technical Name	Vernacular / Local Name	Family	Part used
1.	*Acorus calamus* Linn.	Sweet flag	Araceae	Rhizome
2.	*Adhatoda vasica* Nees.	Arusa	Acanthaceae	Aerial parts
3.	*Alpinia galanga* L.Willed.	Greater Galangal	Zingiberacea	Rhizome
4.	*Azadirachta indica* A.Juss	Neem	Meliaceae	Seeds kernel
5.	*Cassia tora* Linn.	Chakund	Caparadaceae	Areal parts
6.	*Gynendropsis pentaphylla* L.	Hulhul	Capridaceae	Seeds
7.	*Lantana camara* Linn.	Aripple	Verbenaceae	Unripe Fruits
8.	*Momordica charantia* Linn.	Bitter gourd	Cucurbitaceae	Unripe fruits
9.	*Vitex negundo* Linn.	Lagundi	Verbenaceae	Aerial parts
10.	*Withania somnifera* Dun	Winter Cherry	Solanaceae	Aerial parts

Experimental Protocol

Repellent activity were studied at 0.25, 0.5, 1.0, 1.5 and 2.0% concentration treated for two minutes for testing. The repellent effect stored gram, chickpea, *Cicer arietinum* L. (Fabales: Fabaceae) (channa) seeds ver. were used as food for the adults of *C. chinensis.* and chickpea seeds ver xxxx. The treated seed were left under electric fan for about half an hour, to make a dry film of the extracts on the seeds for each set of extract and one control. The treated seeds were kept in jar (23cm x 10cm) on moist filter paper. The untreated seeds were dipped in Benzene + emulsified water only. Five paired (male and female) *C. chinensis* beetle was introduced into a cage having treated stored chickpea seeds. Five paired (male and female), 24 hours starved adults were released in each jar along with control. Three replicates per treatments were maintained. The treated jars either repelled the insects or forced them to move from treated jars (T) to an empty jar(C) through the plastic pipe. The ones found in plastic pipe were considered repelled individuals. Total number of beetle reached to the food/repelled to untreated empty jars were recorded at an interval of 24 hours for each observation recorded and computed (Abbott W. S. 1925) [1].

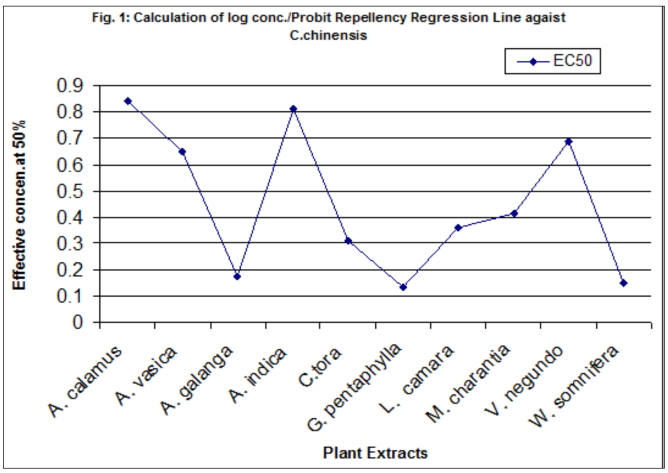
Table 1: Calculation of log conc. / Probit Repellency Regression histogram Summary of repellency test on emerging adults beetle *C. chinensis*.

Plant Extracts	H*	X^2	Regression Equation	EC_{50}	Fiducial Limit	Relative EC_{50}
A. calamus	3	0.52	Y=0.88X +4.42	0.8397	M1=1.8678 M2=0.1251	1.00
A. vasica	3	0.20	Y=0.84X +4.50	0.6470	M1=1.9418 M2=0.0432	1.29
A. galanga	3	1.93	Y=1.64X +3.26	0.1735	M1=1.4512 M2=0.2168	4.83
A. indica	3	0.52	Y=0.88X +4.42	0.8090	M1=1.8678 M2=0.1251	1.29
C.tora	3	0.83	Y=0.66X +2.98	0.3115	M1=2.9870 M2=1.1284	4.83
G. pentaphylla	3	4.02	Y=1.07X +2.70	0.1358	M1=2.3256 M2=1.9392	6.18
L. camara	3	0.96	Y=0.72X +3.19	0.3592	M1=2.9870 M2=1.1284	2.33
M. charantia	3	1.95	Y=0.88X +4.37	0.4122	M1=1.5188 M2=0.0148	2.03
V. negundo	3	0.28	Y=0.75X +4.1	0.6872	M1=2.1368 M2=1.278	1.22
W. somnifera	3	1.59	Y=1.08X +2.66	0.1480	M1=2.3362 M2=1.9781	5.67

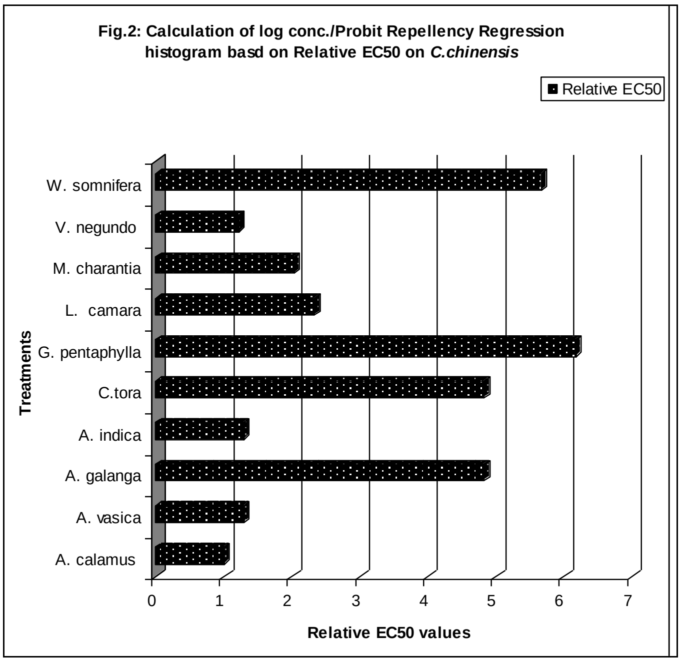
In case of X^2 was found non- significant heterogeneous at P=0.05, Y=Probit Repellency, X=Log Concentration X $10^{2,}$ D.F.=Degree of Freedom, EC_{50}= Conc. Calculated at given 50% Repellency

Results and discussion

The results are summarized in Table-1 and Fig. which showed that all the plant extracts have proved to more or less repulsive against the emerging adults of *C. chinensis*. Among all selected plant extracts, only three plant extracts gave promising repellency with value less than 0.50 and rest seven showed less repellent effect to *C. chinensis*. On the basis of EC_{50} values order of merit can be arranged in following manner as: *G. pentaphylla* (0.1358) > *W. somnifera* (0.1480) > *A. galanga* (0.1735) > *C.tora* (0.3115) > *L. camara* (0.3192) > *M. charantia* (0.4122) > *A. vasica* (0.6470) > *V. negundo* (0.6872) > *A. indica* (0.8090) > *A. calamus* (0.8397), respectively.



The data depicted in table 2 indicated that maximum relative adults repellency was found in following order of effectiveness in descending order as: *G. pentaphylla* (6.18) > followed by W. *somnifera* (5.67) and *A. galanga* (4.83) times repellent than *A. calamus* against the emerging adults of *C. chinensis*. Beside above the remaining plant extract showed moderate repellency as *C. tora* (2.69) > *L. camara* (2.33) > *M. charantia* (2.03) > *A. vasica* (1.29) > *A. indica* (1.03)> *A. calamus* (1.00), respectively, whereas *A. calamus* taken as unit.



Various botanical products and their extractives works as repellent and has been reported by several researchers against pulse beetle. Different oils of neem, coconut, and castor was observed as surface protectants on green gram to check the pulse beetle and among them neem oil was the best surface protectant (Pandey *et al.* 1976 [18], Jilani *et al.* 1988 [9], Ketker 1989 [10]).

Cotton seed, sunflower, groundnut, soybean and mustard oils, when mixed with cowpea, completely suppressed adult emergence of *C. maculates* (Ramzan, 1994 [21]). The edible oils are potential control agents against *C. maculatus* and can play an important role in stored-grain protection (Shaaya *et al.*1997) [23]. Neem and sesame oils completely inhibited adult emergence and appeared to be most promising as a seed protectant against *C. chinensis* (Ahmed *et al.* 1999) [4]. It has been observed that the volatile oil from the leaves of *Curcuma domestica* could effectively protect the seeds, against *C. chinensis*, at a low concentration (Yalamanchilli and Punukollu 2000) [32]. Neem extractives and their isolates biobit and biolop tested against various crop pest and stored grain insect-pest and reported as promising repellency to the *C. chinensis*, (Sighamony *et al.* 1984, Maredia *et al.* 1992, Won-Sik *et al.* 2002) [24, 16, 31]. Several workers were tested a number of indigenous plants extractives, derivatives for their repellent activity to their test insect and found significant results when mixed with different varieties of gram seeds against pulse beetle. (Jilani *et al.* 1985, Jilani *et al.* 1988, Bhuiyan *et al.* 1990, Mendes *et al.* 1997, Abubakar *et al.* 2000, Tripathi *et al.* 2000, Pavela *et al.* 2011, Manish Kumar *et al.* 2017 [8, 9, 6, 15, 2, 28, 20, 7, 14].

Conclusion

The findings of the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use. Among ten plant extract, only *G. pentaphylla* (6.18) showed highest repellency followed by W. *somnifera* (5.67) and *A. galanga* (4.83) times repellent than *A. calamus* against the emerging adults of *C. chinensis*. All the extracts tested were effective to some degree of repellency reducing the feeding and destruction rates. More studies on major biochemical constituents responsible for repellent activity to the test insect on chickpea seeds against *C. chinensis* need to be investigated.

Acknowledgement

The authors are thankful to Council of Science and Technology, Uttar Pradesh, India funding the grant for conducting this research work and Dr. R.K.Singh, Principal, D.B.S.College, Kanpur for providing the necessary facilities. Prof. N.D.Pandey, Retd., Head, Division of Entomology, C S Azad University of Agriculture and Technology, Kanpur for rendering their support and help for the completion of this work.

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