International Journal of Entomology Research
ISSN: 2455-4758
Impact Factor: RJIF 5.24
www.entomologyjournals.com
Volume 2; Issue 6; November 2017; Page No. 88-93

Evaluation of repellent potentials of *Cichorium intybus, Inula racemosa, Tagetes minuta* and *Chrysanthemum cinerariaefolium* against pulse beetle, *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae)

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Abstract
In the present investigation, experiments were conducted in the Entomology Research plant product Laboratory, Department of Zoology, D.B.S. College, Kanpur, UP, India. Ten indigenous naturally occurring plants viz 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 used for their repellent effectiveness against early emerging adults of pulse beetle, *Callosobruchus chinensis* (L.) (Bruchidae:Coleoptera).Under storage condition the botanicals like seed extract of *Cichorium intybus* gave the highest repellency to early emerging adults of pulse beetle, *C. chinensis.* It shows 7.41 times more repellent to *C. chinensis* than *Acorus calamus* (1.00), which is taken as unit. The result reveals that relative repellent biopotential of remaining extractives are as - > *Inula racemosa* (6.39) > *Tagetes minuta* (5.88) > *Chrysanthemum cinerariaefolium* (5.42) > *Mantisalca duriaei* (2.77) > *Reichardia tingitana* (1.67) > *Scorzonera undulate* (1.61) > *Chromolaena odorata* (1.61) > *Chromolaena odorata* (1.35) > *Spilanthes paniculata* (1.00), respectively.

Keywords: *gynendropsis pentaphylla, callosobruchus chinensis* and repellent bioefficacy

1. Introduction

India is the largest producer, largest consumer and the largest importer of pulses in the world. In India Pulses are grown in around 24-26 million hectares of area producing 17-19 million tonnes of pulses annually. India accounts for over one third of the total world area and over 20 per cent of total world production. India primarily produces Bengal gram (chickpeas), red gram (tur), lentil (masur), green gram (mung) and black gram (urad) (Ramzan, 1994, Reddy and Singh, 1998, Sharm, 1984, Shaaya *et al.* 1997, Singh *et al.* 2001) [29, 30, 31, 32, 33]. Majority of vegetarian population in India, pulses are the major source of protein (Ahmed *et al* .1999) [3]. Pulses and pulse crop residues are also major sources of high quality livestock feed in India. Pulses continue to be a major source of protein in Indian diets and play a vital role in sustaining agricultural growth. Increasing pulse production is therefore important for improving food availability, soil health, diet quality and nutrition security (Sighamony, *et al.* 1984, Shaaya *et al.* 1997, Chandel and Singh 2017) [34, 31, 8, 9]. These provide a range of essential nutrients including protein, carbohydrates, dietary fibre, minerals and vitamins. They are: An economical dietary source of good quality protein and are higher in protein than most other plant foods. Among them, chickpea or chick pea (*Cicer arietinum* Linn.) is a legume of the family Fabaceae.

The differential rate of damage infected by *Callosobruchus chinensis* (Coleoptera: Bruchidae) in different pulses was reported to be 68, 56, 49 and 52 per cent in cowpea, bengal gram, red gram and green gram respectively over a storage period of 6 months (Al- Lavati *et al.*2002 Singh and Srivastava,1883, Srivastava *et al.*1998) [5, 36] and (Bhuiyan and Quiniones., 1990) [6]. The pulse grain damage was as high as 69.93% under storage condition (Chandel and Singh 2016) [7]. This pest is a serious problem at small farmer's level, village traders and average households where storage conditions are poor and inadequate. To control the pulse beetle in storage, a number of synthetic organic insecticides have been recommended. The admixture synthetic insecticide with food grains has more recently been banned in many countries (Singh, 2003, Zoubiri and Baaliouamer, 2015 and Chandel and Singh 2017) [8, 47, 35]. There are also reports that the pulse beetle is developing resistance to malathion (Tandon *et al.* 2004, Singh, 2003) [39, 40].

In contrast, naturally occurring indigenous plant products traditionally used against pulse beetle appear to be quite safe and promising. Several authors have reported the anti insect properties like repellent, insecticidal action and growth inhibiting effects of plant products on pulse beetle (Ketker, 1989, Lale, 1992, Pandey *et al,* 1976 and Pandey and Singh) [15, 16, 17, 23, 24, 25, 26], respectively. Botanicals extract can be used to keep the stored pulse free from pulse beetle attack (Subramanium *et al.* 1949, Sighamony, 1984, Won-Sik *et al.* 2002, Yalamanchilli *et al.* 2000, Yankanchi *et al.* 2010) [37, 34, 43, 44, 45]. Therefore, studies were undertaken to manage pulse beetle with plant products and to find the repellent biopotential of the naturally occurring plant product on treated chickpea seeds.

2. Material and Methods

The present study was conducted in the post graduate Department of Zoology, Entomology, Biopesticides and Toxicological Laboratory, D.B.S. College, affiliated to CSJM University, Kanpur, India from May 2016 to February 2017.

2.1 Mass culturing of pulse beetle

Pulse beetle, *Callosobruchus chinensis* L. required for the study were mass reared on chickpea, *Cicer arietinum* L. (Fabaceae) (kabuli) in the laboratory. The moisture content of the grains was adjusted to 11.0 per cent by sun drying to have uniform moisture content. The mass culturing was initiated by confining 10-20 freshly emerged beetles in the plastic containers of 59 x 21 x 18 cm having 500 g of green gram which were then covered with kada cloth and secured tightly with rubber band. Such containers were stacked in iron shelves. Mass culturing of C. chinensis was done at room temperature in the plastic container and observed daily. Adult beetles were collected for the study.

2.2 Procurement of raw plant materials

In the present investigation ten indigenous botanical were collected 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 used for their repellent effectiveness against early emerging adults of pulse beetle, *Callosobruchus chinensis* Linn. In laboratory trials.

2.3 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 (Chandel and Singh, 2016)

2.4 Preparation of botanical extracts

For the extraction, 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 were 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.

2.5 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.

2.6 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.

2.7 The Insecticidal Formulations

Five concentrations (0.25, 0.5, 1.0, 1.5, 2.0 percent) were used for experiments on insecticidal and repellent tests in the laboratory conditions. However, only three concentrations (0.5, 1.0 and 2.0 percent) were used for insecticidal test in the laboratory and contact test in the field 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.

Table 1: List of selected asteraceous plants materials used for extraction

S.N	Scientific Name	Common names	Part Used	Family
1.	*Chromolaena odorata* Linn.	Siam weed	Leaves	Asteraceae
2.	*Chrysanthemum cinerariaefolium* (trev.) Vis.	Insecticide Daisy	Roots	Asteraceae
3.	*Cichorium intybus* (L.)	Chicory	Aerial parts	Asteraceae
4.	*Inula racemosa* Hook. f	Puskarmul	Roots	Asteraceae
5.	*Mantisalca duriaeri* Birq. Et Cavill.	Spach	Flowers	Asteraceae
6.	*Rechardia* tingitana (L.) Roth	False sowthistle	Flowers	Asteraceae
7.	*Rhaponticum acaule* (L.) DC.	Coffee plum	Flowers	Asteraceae
8.	*Scorzonera undulate* Vahl	Black Salsify	Aerial parts	Asteraceae
9.	*Spilanthes paniculata* Well ex DC	Toothache Plant	Leaves	Asteraceae
10.	*Tagetes minuta* Linn.	Wild Marigold	Flowers	Asteraceae

Table 2: Formulations of Extracts

Concentration (%)	Amount of Stock Solution (ml)	Amount of Benzene (ml)	Amount of Emulsifiable Water (ml)	Total Amount (ml)
0.25	2.50	22.50	475.00	500.00
0.50	5.00	20.00	475.00	500.00
1.00	10.00	15.00	475.00	500.00
1.50	15.00	10.00	475.00	500.00
2.00	20.00	5.00	475.00	500.00

2.8 Field Collection and culture of Pulse Beetle

Adults of *Callosobruchus chinensis* was drawn from laboratory mass cultures reared in glass jars at ambient laboratory temperature. The *Callosobruchus chinensis* used for experiment were early emerged adults used for experiment and fed on chickpea (channa).

3. Experimental protocol

For testing the repellent effect of plant extracts were used as food for emerging beetles, *C. chinensis* treated with different concentrations. The treated foods were kept in jar (23cm x 10 cm) on moist filter paper. Thirty (15 male and 15 female), 24 hours starved emerging beetles, *C. chinensis* were released in each jar along with control. The treated seeds were dipped in Benzene + emulsified water only. After four hours of the release emerging beetles, *C. chinensis.* Treated beetles either repelled and forced them to move from treated jars A to an empty jar B through the plastic pipe The ones found in plastic pipe were considered repelled individuals. The repellency data (in treated and untreated jars) and alive (in empty or untreated jars) were recorded for 14 days at an interval of 24 hours for each observation. The data was collected on the number of beetles, *C. chinensis* which reached the treated food and repellency over control was recorded and computed. (Abbott formul, 1925) [1].

4. Results

The data depicted in table 1 and figure 2 indicated that result based on their relative EC_{50} values the extracts of *G. pentaphylla* showed highest repellency (6.18) to the early emerged treated beetle followed by *W. somnifera* (5.67) and *A. galanga* (4.83) times more repellent than *A. calamus* (1.00), which is taken as unit.

It is seen from the table 1 and Fig.2 that all the plant extracts have proved to more or less repulsive against the emerging adults of *C. chinensis*. It is seen 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 minimum EC50 value (less than 0.50) and rest seven showed less repellent effect to *C. chinensis*. The seed extract of *Cichorium intybus* gave the highest repellency to early emerging adults of pulse beetle, *C. chinensis.* It shows 7.41 times more repellent to *C. chinensis* than *Acorus calamus* (1.00), which is taken as unit. The result reveals that relative repellent biopotential of remaining extractives are as *Inula racemosa* (6.39) > *Tagetes minuta* (5.88) > *Chrysanthemum cinerariaefolium* (5.42) > *Mantisalca duriaei* (2.77) > *Reichardia tingitana* (1.67) > *Scorzonera undulate* (1.61) > *Chromolaena odorata* (1.35) > *Spilanthes paniculata* (1.00), respectively.

Table 3: Calculation of log conc./Probit Repellency Regression graph using plant extracts on *C. chinensis*.

Plant Extracts	H*	X^2	Regression Equation	EC_{50}	Fiducial Limit	Relative EC_{50}
Chromolaena odorata	3	1.34	Y = 0.78x + 4.66	0.6622	M_1= 2.138738 M_2 = 1.771520	1.35
Chrysanthemum cinerariaefolium	3	1.16	Y =1.67x + 3.31	1645	M_1= 1.451225 M_2 = 0.216868	5.42
Cichorium intybus	3	1.05	Y = 1.90x + 2.77	0.1226	M_1= 2.3060579 M_2 = 1.871908	7.41
Inula racemosa	3	0.90	Y = 0.94x + 3.31	0.1422	M_1 = 2.386682 M_2 = 0.128688	6.39
Mantisalca duriaei	3	0.89	Y = 8.94x + 0.12	0.3277	M_1= 2.02679 M_2 = 1.263284	2.77
Reichardia tingitana	3	1.23	Y = 0.82x + 4.07	0.5412	M_1 = 2.026645 M_2 = 1.655775	1.67
Rhaponticum acaule	3	0.75	Y = 0.82x + 4.07	0.6244	M_1 = 2.136832 M_2 = 1.127868	1.45
Scorzonera undulate	3	1.64	Y = 0.79x + 4.08	0.5623	M_1 =1.948678 M_2 = 0.043214	1.61
Spilanthes paniculata	3	0.62	Y = 0.66x + 4.23	0.9088	M_1=2.186998 M_2 = 1.088144	1.00
Tagetes minuta	3	0.88	Y = 0.88x + 3.92	0.1544	M_1= 2.236882 M_2 = 1.128868	5.88

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, E.C.$_{50}$= Concentration Calculated at given 50% Repellency

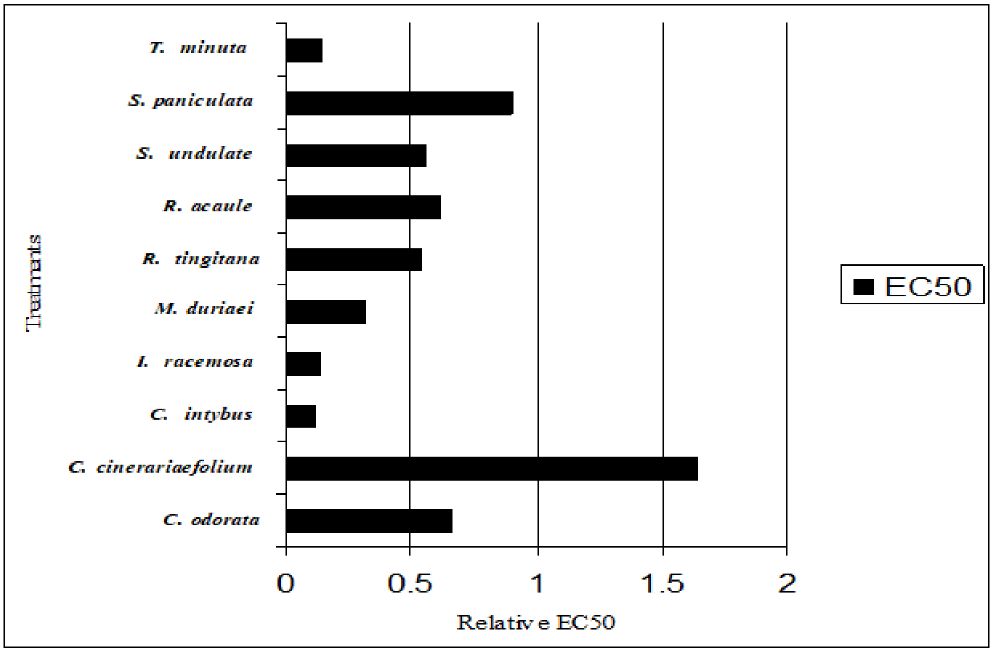

Fig 1: Calculation of log Conc./Probit repellency regression graph using plant extracts on C. chinensis.

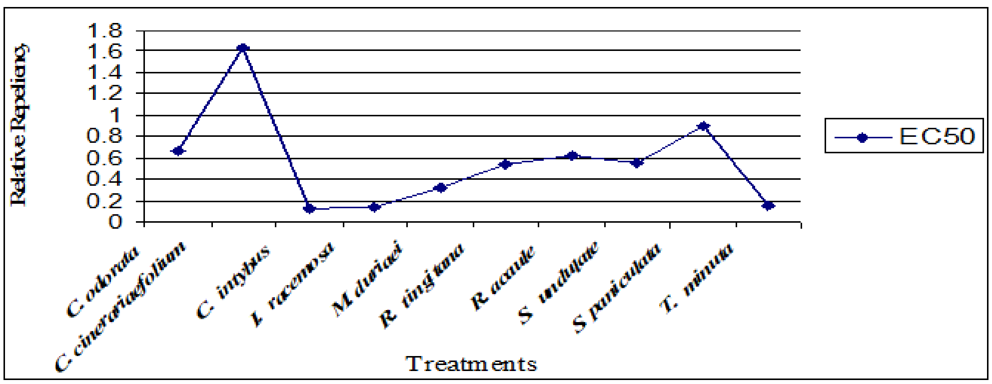

Fig 2: Calculation of log conc./Probit repellency regressing graph using botanicals on C. chinensis

5. Discussions

Various botanical products and their extractives works as repellent has been reported by several researchers against *C. chinensis* L., *C. maculatus* and *T. castaneum* etc. (Tripathi, *et al.* 2001, Tripathy *et al.*, 2001, Valsalan and Gokuldas, 2015) [41, 42, 43] tested effect of plant powders and extracts against *C. chinensis* L. attacking black gram. Certain vegetable oils i.e. turmeric, sweat flag, *neem* oil and Margosan-O extract 800 ug/ cm^2 applied to filter paper in choice chamber test, produced 59.00 and 67.00 per cent repellency against *T. castaneum* (Jilani *et al.* 1985, Mendes *et al.* 1997, Maredia *et al.* 1992, Pavela, 2011 and Safia and Aoumeur) [13, 20, 21, 27, 46]. *Neem* seed oil at different concentration against *Trichogramma chilonis*. *Neem* seed oil at 0.3 per cent showed high oviposition deterrent results (Ramamurthy *et al.* 2002, Manish Kumar *et al.* 2017) [28, 19].

In the support of present finding the many entomologist worked on therir sected botanical amomg them *Cleome gynandra* gave significant relent efficacy to spider mite (*Tetranychus urticae* Koch). *Ocimum suave* was found to repel the tick *Rhipicephalus appendiculatus* (Esther *et al.* 1995 and Abubakar *et al.* 2000) [11, 2]. Aqueous and methanolic plant extracts of *T. minuta*, which exhibited high repellency (IR = 0.04) againstrepellent effectiveness against the red flour beetle, Tribolium castaneum (Herbst) (Coleoptera Tenebrionidae). The application of these botanicals may be promising in protecting of stored grains against coleopteran pests. (Padin *et al.* 2013, Liu *et al.* 2006) [22, 18]. *Alpinia galanga* (L.) species of termites, *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren) (Isoptera: Rhinotermitidae). Repellent activity shows that 250 ppm of 1,8-cineol caused 50.00 ± 4.47% repellency for *C. gestroi* (Fauziah Abdullah *et al.*2015)[12]. The maximum repellent activity was observed at 500 ppm in methanol extracts of *N. nucifera*, ethyl acetate and methanol extract of *P. nigrum* and methanol extract of *T. ammi* and the mean complete protection time ranged from 30 to 150 min with the different extracts tested.(Chinnaperuma *et al.* 2011) [10].

6. Conclusion

Conclusively, the present investigation revealed that there appears prospects in selected botanicals *Cichorium intybus*, *Inula racemosa* and *Tagetes minuta* was registered promising repellency with minimum EC_{50} values to the early emerged *C. chinensis* adults, when compared to other extracts. The data depicted in table 1 and figure 1 indicated that result based on their relative EC_{50} values the extracts of *Cichorium intybus* showed highest relative repellency (7.41) to the early emerged treated beetle followed by *Inula racemosa* (6.39), *Tagetes minuta* (5.88) and *Chrysanthemum cinerariaefolium* (5.42) times more repellent than *A. calamus* (1.00), which is taken as unit. The above selected plant materials can be use in protection of pulses from *C.chinensis* infestations under storage conditions.

7. Acknowledgements
Authors would like thank to Council of Science and technology, Uttar Pradesh for providing the financial support in conducting this research work. Principal D.B.S. College, Kanpur, also thankful 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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