International Journal of Applied Research 2020; 6(4): 55-60

International Journal of Applied Research

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2020; 6(4): 55-60
www.allresearchjournal.com
Received: 21-02-2020
Accepted: 28-03-2020

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Antifeeding Potentials of *Cichorium intybus, Chromolaena odorata, Chrysanthemum cinerariaefolium* and *Inula racemosa* Hook against 3rd instars larvae of spotted bollworm, *Earias vittella* (Fabr.)

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DOI: https://doi.org/10.22271/allresearch.2020.v6.i4a.8643

Abstract
Experiment was conducted in the biorational pesticide Laboratory, Department of Zoology, Entomology, Dayanand Brijendra Swaroop Post-Graduate College, Kanpur Nagar. The tests insect spotted bollworm, *Earias vittella* Fabricius (Lepidoptera:Noctuidae) is the serious pest, causing enormous qualitative and quantitative losses to various malvaceous vegetables particularly okra. The ten naturally occurring asteraceous plants growing agricultural field and wild area namely: aerial parts of *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariaefolium* (trev.) Vis., *Inula racemosa* Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, *Acmella paniculata* Well ex DC and *Tagetes minuta* (Linn.) were tested for their antifeedant biopotency against 3rd instars larvae of spotted bollworm, *Earias vittella* Fabricius (Lepidoptera:Noctuidae) were used in the present investigation. Based on relative EC_{50} values, the *Chromolaena odorata* extract showed highest protectivity (EC_{50}=0.0180) to the larvae of *Earias vittella* followed by *Tagetes minuta* (EC_{50}=0.0185), *Cichorium intybus* (EC_{50}=0.0204), *Inula racemosa* (EC_{50}=0.0218), *Manisalca duriaeri* (EC_{50}=0.239), *Reichardia tingitana* (EC_{50} = 0.323), Rhaponticum acaule (EC_{50} = 0.389), *Scorzonera undulate*. (EC_{50} = 0.435), *Spilanthes paniculata* (EC_{50}=0.502) times less repellent than *C. cinerariaefolium*. (EC_{50}=1.370), respectively. Thus, *Chromolaena odorata*, *Tagetes minuta* and *Cichorium* intybus extractives may be a sound commercial herbal repellent alternatives to the more persistence synthetic pesticides for managing the against larvae of *Earias vittella* Fabricius.

Keywords: *Earias vittella, Chromolaena odorata, Tagetes minuta, Cichorium intybus* and antifeedant

Introduction
The crop Okra, *Abelmoschusm esculentus has* been called “a perfect villager’s vegetable” because of its robust nature, dietary fiber, and distinct seed protein balance of both lysine and tryptophan amino acids. Okra is a powerhouse of valuable nutrients, nearly half of which is soluble fibre in the form of gums and pectins which help to lower serum cholesterol, diuretic, reducing the risk of heart diseases. Bhindi (okra) is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds (Maramag, 2013) [1]. Okra mucilage has medicinal applications when used as a plasma replacement or blood volume expander. The mucilage of okra binds cholesterol and bile acid carrying toxins dumped into it by the liver. Okra seeds are a potential source of oil, with concentrations varying from 20% to 40%, which consists of linoleic acid up to 47.4%. Okra seed oil is also a rich source of linoleic acid, a polyunsaturated fatty acid essential for human nutrition (Adeboye and Oputa, 1996) [2].

Okra, *Abelmoschusm esculentus*, being an important vegetable, grown exclusively in and around Kanpur region for three quarters of a year. One of the important limiting factors in the cultivation of okra is insect pests. The major insect pests of okra in and around Kanpur region include aphid, (*Aphis gossypii* Glove.), jassid, (*Amrasca biguttula biguttula* Ishida), whitefly (*Bemisia tabaci* Gen.) and shoot and fruit borers, *Earias vittella* (Fabr.) and *Earias insulana* (Boisduval).

The studies were made for the management of major insect pests of okra (*Abelmoschus esculentus* (L.) Moench.) in agro-climatic condition of Kanpur, (Uttar Pradesh), India (Sumathi and Balasubramanian,2002) [3]. Among the above mentioned destructive insect pest the spotted bollworm, *Earias vittella* Fabricius (Lepidoptera: Noctuidae) is the most important pest causing direct damage to the leaves and marketable fruits (Panickar and Bharpoda 2003) [4]. *Earias vittella* is alone reported to cause 57.1% fruit infestation and 54.04% yield loss in okra [Chaudhary and Dadheech, 1989) [5].

The use of synthetic insecticide is very effective in the controlling the insect pest of agricultural crops and vegetables in Indian conditions but their excessive and injudicious use of these synthetic insecticides led to many problems like development of resistance, induction of resurgence and environment pollutions. The risks to human health and environmental pollution (Gandhale *et al.* (1987, Konar and Rai,1990, Rao *et al.* 2002,) [6,7, 9]. side effects have forced to look for naturally occurring ecofriendly indigenous herbal alternatives to chemical pesticides especially for vegetables like okra where fruits are plucked at an interval of every 2-3 days. Moreover, as most aphid species have become resistant to many aphidicidal agents (Gajmer *et al.* 2003) [10], managing these pests in greenhouses and in the field is becoming problematic (Misra *et al.* 2002, Gupta and Misra, 2006,) [11, 12]. Therefore, efficient and environmentally friendly pest control alternatives must be developed to replace synthetic pesticides.

Researchers in different parts of world have been using plants products and derivates as antifeedants against number of crop, household insect pest for controlling insect pest infestatin (Arun Kumar *et al*.1979, Ambika Devi. and Mohandas 1982, Muralikrishna *et al*.1990, Rao *et al*.1990, Mishra and Singh *et al*.1992) [13, 14, 15, 16, 17]. Previous studies revealed that different plant compounds were used in controlling pest and they proved effective and eco-friendly (Huang and Okamura, Meshram and Kulkarni1996, Yasui and Kato 1998, Chiam *et al*.1999, Tripathi *et al*.1999, Abe and Matsuda *et al*.2000, Govindachari and Suresh, 2000) [18, 19, 20, 21, 22, 23, 24]. Many researchers investigated the compounds in plants that have a variety of properties including insecticidal activity, repellence to pests, antifeedant effects and in insect growth regulation (Juan and San *et al*.2000, Joshi and Lockwood, 2000, Ventura and Ito, 2000, Chandel *et al*.2001) [25, 26, 27, 28].

The research work on properties like insecticidal, antifeedant and repellent of various asteraceous plant species has been initiated on many insect species of economic importance. In the last two decades crude and refined extracts of different plant against insect-pest were tested and found effective against the insect pest infestation. Therefore, attempts were made to looking easily availability of asteraceous extractives and find out their antifeedant properties against okra spotted bollworm, *E. vittella* under the laboratory conditions.

2. Materials 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. The laboratory culture of spotted bollworm, *E. vittella* larvae collected from fields of farmers.. The insects were reared in the laboratory at 27 ± 2º C on a diet of okra leaves (Bajpai and Chandel, 2010) [29]. The collected eggs were placed in a well ventilated plastic container and okra leaves were provided to newly hatched larvae fom eggs also. The laboratory reared third instars larvae were used for the present investigation to evaluate the insecticidal efficacy of asteraceous extractives.

The larvae of *E. vittella* were obtained from the experimental farms of farmers field and maintained in the laboratory on natural diets. The collected larvae were kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments. *E. vittella* required for the study were mass reared on okra leaves in the laboratory. The mass culturing was initiated by confining 10-20 larvae of *E. vittella* in the plastic containers of 59 x 21 x 18 cm having green mustard leaves which were then covered with muslin cloth and secured tightly with rubber band. Mass culture of larvae of *E. vittella*. was done at 28 ± 2º C temperature in the plastic container and observed daily.

2.1: Procurement of Asteraceous plant materials: In the present investigation ten asteraceous plant materials were collected from wield and agricultural field and used for their antifeeding effectiveness against third instars larvae of *E. vittella* in laboratory trials Tabl-1.

Table 1: List of selected indigenous naturally occurring plants materials for extraction

Sr. N.	Scientific Name	Vernacular name	Faimly	Plant parts used
1.	*Acmella paniculata* (Wall ex DC.) R.K.Jansen	Toothache Plant	Asteraceae	Aerial parts
2.	*Chromolaena odorata* Linn.	Siam weed	Asteraceae	Leaves
3.	*Chrysanthemum cinerariaefolium* (trev.) Vis.	Daisy	Asteraceae	Leaves
4.	*Cichorium intybus* (L.)	Chicory	Asteraceae	Roots
5.	*Inula racemosa* Hook. f	puskarmul	Asteraceae	Aerial parts
6.	*Mantisalca duriaeri* Birq. Et Cavill.	Spach	Asteraceae	Roots
7.	*Rechardia tingitana* (L.) Roth	False sowthistle	Asteraceae	Flowers
8.	*Rhaponticum acaule* (L.) DC.	coffee plum	Asteraceae	Flowers
9.	*Scorzonera undulate* Vahl	Black Salsify	Asteraceae	Flowers
10.	*Tagetes minuta* Linn.	Wild Marigold	Asteraceae	Leaves

2.2: Grinding of herbal collection in Powder Farm: Fresh collected asteraceous plant parts (leaves and seeds 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 (Bharti and Chandel, 2017) [30].

2.3: Soxhlet Extraction of Asteraceous plant materials
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 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.

2.4: Experimental tools applied: Small plastic jars (capacity 200 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.

2.5: Preparation of Stock Solution of Extractives: 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. The alcoholic extracts of asteraceous extractives were tested under laboratory against third instar starved larvae of *E. vittella*, which is noxious insect pest of malvaceous vegetables and crops. The details of which, are described as under:-

2.6: Preparation of various Concentrations of Extractives Five concentrations of asteraceous extractives (0.25,0.5,1.0,1.5,2.0 percent) and were used for experiments on antifeeding tests in the laboratory conditions. 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 To make five concentrations of extract the required quantity of the stock solution was calculated with the help of following formula.

$$\text{Amount of Stock Solution} = \frac{\text{Amount required x Concentration required}}{\text{Concentration of Stock Solution}}$$

The calculated amount of extracts to make different concentrations from the stock solution and amount taken are presented in the following table.2

Table 2: Preparation of various Concentrations of Extractives

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

2.7: Experimental Protocol
The ten naturally occurring asteraceous plants growing agricultural field, wildly are namely: aerial parts of *Acmella paniculata* Well ex DC, *Cichorium intybus* (L.)**,** *Chromolaena odorata* Linn., *Chrysanthemum cinerariaefolium* (trev.) Vis., *Inula racemosa* Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaul*e (L.) DC, *Scorzonera undulate* Vahl and *Tagetes minuta* (Linn.) were extracted and formulated in 0.25,0.5,1.0,1.5,2.0 percent concentrations. All selected extractives were tested for their antifeedant biopotency against 3rd instars larvae of spotted bollworm, *Earias vittella* Fabricius (Lepidoptera:Noctuidae) were used in the present investigation. *Earias vittella* is noxious insect pest of okra vegetables and crops. For testing the insecticidal effect the okra leaves were used as food against the third instar larvae of *Earias vittella* treated with different concentrations of ten asteraceous extracts used as biorational insecticide. The treated foods were kept in jar (23cm x 10cm) on moist filter paper. Then third instar, 24 hours starved *Earias vittella* larvae were released in each jar. In each set of extract and one control was introduced, where the leaves pieces were dipped in Benzene + emulsified water only. After four hours of the release of larvae the data was collected on the number of larvae reached at each treated food. Three replication of treatment were made. The antifeedant effect of all the extracts was judged by counting the number of larvae after 4 hours, present on the treated leaf in each treatment and the percentage of protectivity were adjudged over control. All the values were calculated as per Abbott formula (Abbott, 1925) [30] and statistical treatments of the sigmoid response by Probit analysis (Finney, 1952) [31].

Table 3: Calculation of log conc./Probit Protection Regression column.
(Summary of used plant extracts regarding Antifeeding test on *Earias vittella* Fabr.

Plant Extracts	Heter.	X^2	Regression Equation	LC_{50}	Fiducial Limit	Relative EC_{50}	Order of Merit
Acmella paniculata	3	0.40	Y=0.50X+3.17	0.5020	M_1=0.1280 M_2=1.3265	2.787	9
Chromolaena odorata	3	0.28	Y=0.61X+4.14	0.1801	M_1=1.6295 M_2=1.0877	1.000	1
C. cinerariaefolium	3	0.21	Y=2.94X+3.75	1.3702	M_1=0.5793 M_2=0.6608	7.607	10
Cichorium intybus	3	0.35	Y=0.45X+2.96	0.2045	M_1=1.7552 M_2=0.9345	1.135	3
Inula racemosa	3	0.42	Y=0.53X+1.41	0.2188	M_1=1.0234 M_2=0.0202	1.214	4
Manisalca duriaeri	3	0.88	Y=0.84X+3.84	0.2399	M_1=1.7533 M_2=1.0066	1.332	5
Reichardia tingitana	3	0.23	Y=1.9X+3.471	0.3238	M_1=1.8139 M_2=1.2065	1.797	6
Rhaponticum acaule	3	0.77	Y=0.89X+2.40	0.3894	M_1=1.5865 M_2=0.1390	2.162	7
Scorzonera undulate	3	1.29	Y=1.79X+2.09	0.4351	M_1 0.0955	2.415	8
Tagetes minuta	3	0.35	Y=1.8X+2.65	0.1850	M_1=0.0102 M_2=0.3015	1.027	2

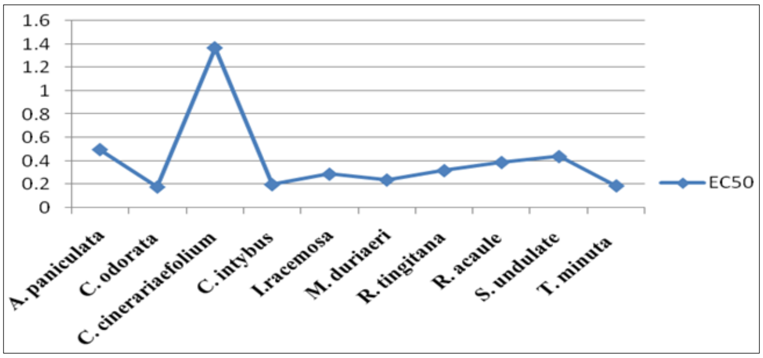

Fig 1: Calculation of Log Conc./Probit Protection Regression column against *E arias vittella* larvae based on ECso

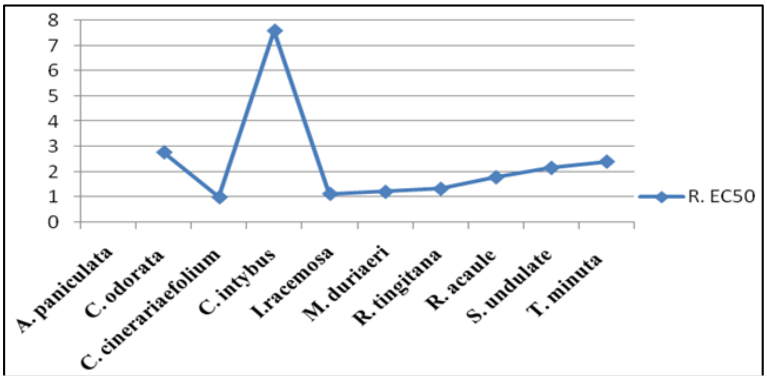

Fig 2: Calculation of Log Conc./Probit Protection Regression column a *gainst Earias vittella* larvae based on Relative EC50

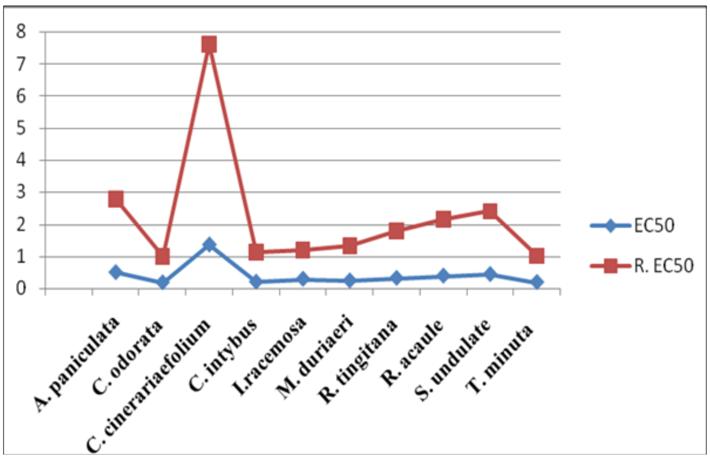

Fig 3: Calculation of Log Conc./Probit Protection Regression column against *Earias vittella* larvae EC50 & R. EC50

3. Results and Discussions

The data given in table 3 and figure 1, 2, 3 indicates that the tested all extracts had significant antifeedant effect against larvae of *Earias vittella*. Based on relative EC_{50} values, the *Chromolaena odorata* extract showed highest protectivity (EC_{50}=0.0180) to the larvae of *Earias vittella* followed by *Tagetes minuta* EC_{50}=0.0185), *Cichorium intybus* (EC_{50}=0.0204), *Inula racemosa* (EC_{50}=0.0218), *Manisalca duriaeri* (EC_{50}=0.239), *Reichardia tingitana* (EC_{50}=0.323), *Rhaponticum acaule* (EC_{50}=0.389), *Scorzonera undulate*. (EC_{50} = 0.435), *Spilanthes paniculata* (EC_{50}=0.502) times less repellent than *C. cinerariaefolium*. (EC_{50}=1.370), respectively. Thus, *Chromolaena odorata*, Tagetes minuta and *Cichorium* intybus extractives may be a sound commercial herbal antifeedant alternatives to the more persistence synthetic pesticides for managing the against larvae of *Earias vittella* Fabricius.

For the conformity of the above findings those workers on antifeeding bioefficacy of plant products against number of agricultural insect pest. (Sandhu and Singh, 1975, Muralikrishna *et al.* 1990, Mishra and Singh, 1996, Meshramand Kulkarni, 1996, Murugan *et al.*1998, Sharma *et al.* 2012).

These results are in agreement with Sardana and Kumar (1989) reported weekly applications of various plant oils, especially neem (*Azadirachta indica*) oil at 2%, were shown to be effective in controlling *Earias vittella* on okra, treated plots having lower fruit damage and increased yields in comparison with untreated ones. Neem oil was as effective as monocrotophos at 0.05%, and can therefore be recommended for use in an integrated control scheme for the pest. Mallik and Lal (1989) reported application of neem oil cake [neem seed cake] and fertilizer (2.5 kg of each on 200 square metre plots) or of neem seed cake on its own (5 kg per plot) reduced *Earias fabia E. vittella* infestation of okra and increased yield. Murthy *et al.* (1996) conducted a field experiment to investigate the best insecticide management strategy to control bhendi fruit borer (*Earias vittella* and *E. insulana*) incidence. Fruit borer incidence gradually increased and reached its peak (34.5%) in the last picking (78 days after sowing (DAS). This treatment was concluded to be the best (witha cost benefit ratio of 1:4.6 and a net yield of 5.9 t/ha).

Thara and Kingsly (2001) tested the neem tree, *Azadirachta indica*, is one of the most promising carries of antifeedent factors. In this study, the antifeedent effect of neem oil and neem cake extract in different concentrations along with low dose of endosulfan on third instar larvae of Bhindi (okra) pest *Earias vittella*. This study confirms the antifeedent effect of neem oil and neem cake extract on *Earias vittella*. The test was conducted on laboratory reared *Earias vittella*. High level of feeding deterrence was exhibited at all concentrations of neem cake extract. While in neem oil 2.0, 1.0 and 0.5 percentages, the deterrent effects declined considerably on their decreased concentrations. Endosulfan 0.05% showed the least antifeedant effect. It was concluded that neem cake extract was found to be highly effective followed by neem oil and endosulfan against *Earias vittella*.

Conclusively, our findings indicated that selected certain plants contains significant Antifeedant activity to the larvae of *Earias vittella*. The *Chromolaena odorata* extract showed highest protectivity to the larvae of *Earias vittella* followed by *Tagetes minuta*, *Cichorium intybus*, *Inula racemosa* and *Manisalca duriaeri*, respectively. Thus, *Chromolaena odorata*, *Tagetes minuta* and *Cichorium* intybus extractives may be a sound commercial herbal antifeedant alternatives to the more persistence synthetic pesticides for managing theagainst larvae of *Earias vittella* Fabricius.

4. Acknowledgement: The authors are thankful to Principal, Dr. C.S. Prasad, and Dr. Indrani Dubey, Head, Department of Zoology, D.B.S.College, Kanpur for providing the necessary facilities. Prof. N.D.Pandey, farmer Head, Division of Entomology, C. S. Azad University of Agriculture and Technology, Kanpur for rendering their encouragement, support and help for the completion of this work and manuscript.

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