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Rajani
Ph.D. Department of Zoology, S.R.K., P.G., College, Kurara, Hamirpur, Uttar Pradesh, India

BS Chandel
Ph.D., D.Sc., Biopesticides and Toxicological Lab, Department of Zoology, D.B.S. College, Kanpur, Uttar Pradesh, India

Correspondence
Rajani
Ph.D. Department of Zoology, S.R.K. P.G. College, Kurara, Hamirpur, Uttar Pradesh, India

Laboratory assessment and prospects of the Antifeedant potentiation of indigenous plant extractives against leaf webber, *Eucosma critica* Meyrick (Lepidoptera: Eucosmisdae)

Rajani and BS Chandel

Abstract
Screening test was done for antifeedant activity of some indigenous natural botanical extractives as discovery of new biorational agents for insect-pest management. The test insect leaf webber, *Eucosma critica* Meyrick (Lepidoptera: Eucosmidae) is a serious pest, causing enormous qualitative and quantitative losses to various leguminous crops and vegetables. The eleven edible and non-edible essential oil-containing plants growing wildly in India namely: *Ageratum conyzaides* Linn., *Argemone maxicana* Linn., *Arachis hypogea* Linn., *Azadirachta indica* A. juss., *Brassica juncea* Linn., *Chenopodium ambrosioides* Linn., *Mentha arvensis* Linn., *Mentha longifolia* Linn., *Ocimum basilicum* Linn., *Pongamia glabra* Vent. *Ricinus communis* Linn and Endosulphan were tested for their antifeedant biopotency against larvae of. *Eucosma critica* and one synthetic insecticide also used in the present investigation. Based on relative EC_{50} values *Chenopodium ambrosioides* seeds extract showed highest protectivity (EC_{50}=0.013) to the larvae of *Eucosma critica* followed by *Argemone maxicana* Linn. (EC_{50}=0.017), *Brassica juncea* Linn. (EC_{50}=0.037), *Azadirachta indica* A. Juss. (EC_{50}=0.197), *Ageratum conyzaides* Linn. (EC_{50} = 0.209), *Pongamia glabra* Vent (EC_{50} = 0.279), *Arachis hypogea* Linn. (EC_{50} = 0.317), *Ocimum basilicum* Linn. (EC_{50}=0.339), *Ricinus communis* Linn. (EC_{50}=0.413), *Mentha arvensis* Linn. (EC_{50}=0.447), *Mentha longifolia* Linn (EC_{50}=0.532), times repellent than Endosulfan (EC_{50}=0.516), respectively. Thus, *Chenopodium ambrosioides, Argemone maxicana* and *Azadirachta indica* seed oils showed a sound commercial herbal antifeedant alternatives to the more persistence synthetic pesticides for managing the against larvae of *Eucosma critica* Meyrick

Keywords: *Eucosma critica, Chenopodium ambrosioides, Azadirachta indica, Argemone maxica,* repellent activity and plant extracts

1. Introduction
Pulses are more important than cereals as they meet out protein requirements of the vegetarians in the developing countries, like India (Lefroy, 1906) [1]. India is vegetarian country and pulses play an important role in human diet. The minimum requirement of pulses is about 80g per day per capita (Brahmaprkash *et al*. 2004) [2]. Arhar. *Cajanus cajan* Millsp. crop contains 25.3 per cent protein, 57.2 per cent carbohydrate, 1.7 per cent fat and 12.2 per cent water (Meyrick, E. 1912) [3]. India is a major pulse growing country in the world sharing 35-36 and 27.28 per cent of the total area and production of these crops, respectively. Pigeonpea, *Cajanus cajan* occupies 6^{th} position in the total world production of grain legumes. It is very important pulse crop and ranks only next to grain in its consumption and availability. India accounts for about 85.0 per cent of its total supply in the world.

It is most abundant in the fields where tur is grown alone. It was described as a minor pest of regular occurrence on tur, *C. indicus* (Lefroy, 1907) [4]. Later on, it has been described as a destructive insect of tur. Fletcher (1914) [5] reported *E. critica* was found throughout the India on red gram as a minor pest. He again in 1916 stated in his report in proceedings of the Second Entomological Meeting (SEM) that *E. critica* either to called *Eucelis critica* in Indian. Entomological literature, occurred commonly in most parts of India and causes bunching of the shoots and leaves of arhar, *C. indicus* [6]. As a pest on young arhar plants, it has been reported only by Fletcher (1917) [7]. Fletcher (1920) [8]. Found that the larvae of this pest at Pusa boring the pods and eating the seeds of arhar, *C. indicus* in April and May and also on the flower-buds and to leaves at Coimbatore. Ayyar (1963) [9] recorded as arhar leaf and shoot folder. Srivastava (1964) [10] found it causing damage to the red gram (*Cajanus cajan*).

Pigeonpea, *Cajanus cajan* pulse crop is subjected to the attack of a large number of insect-pests, namely, leaf folder or leaf webber, *Eucosma critica* Meyrick; pod fly, *Melanagromyza obtusa* Malloch, plume-moth, *Exelastis atomosa* W.; bud butter fly, *Euchrysops cnejus* F.; gram pod borer, *Heliothis armigera* (*Helicoverpa armigera*) Hubner, spotted pod borer, *Maruca testululis* Geyer and pod bug, *Clavigralla gibbosa* Spin as reported by Meyrick, E. 1912 [11]. Out of these insects, *Eucosma critica* has been found causing enormous damage to red gram in various parts of U.P. and its adjoining States. Parshad and Rao (1964) [12] have noted varied degree of its incidence in different parts of India. At times, it may cause 40-50 per cent infestation (Khare and Ujagir, 1977) [13]. It was once considered to be a minor pest of pigeon pea, but now, has assumed the status of a major pest (Singh and Singh, 1978 a and b, Thobbi and Singh1978.) [14, 15, 16]. According to the annual report of ICRISAT, 100.0 per cent infestation to this crop has been done by *Eucosma critica* (Anonymous, 1978) [17].

Eucosma critica Meyrick has been recorded from Gujrat, Bihar, Nagpur and Surat (Lefroy, 1909) [18]. throughout the plains of Southern India all over Madras, Bombay, Bihar, Central Provinces, Bengal, United Provinces, Assam and Punjab (Fletcher, 1917) [19]. Surat, Poona and Coimbatore (Fletcher, 1920) [20]. South India (Ayyar, 1963) [21]. Sitapur, Kanpur and Unnao in U.P. (Parshad and Rao, 1964) [12]. Recorded it in a serious form and observed that this type of attack did not permit further growth of the arhar plant resulting the poor pod formation. Lal *et al*. (1980) [22]. revealed that it is a serious and important pest of arhar or tur. (*C. cajan* Millsp.) crop, previously it was considered as a minor pest of arhar, but for the last few years it has assumed a serious status of a pest and causing severe damage to this crop. Now, it has attained a 'major' status of arhar pest (Koul *et al*. 2004) [23]. The larvae of this pest damage upper leaves of pigeon pea, *C. cajan*. (Nair, 1975, Sharma and Singh, 1980 and Koul *et al*. 2004) [24, 25, 26].

These natural insecticides are so called bio-insecticides, present several advantages in relation to synthetic hazaourdous toxic compounds as their rapid biodegradation, cheap, easy to handling, reducing the risks of environment and food contamination. These botanical insecticides have two basic objectives: First direct application in pest control by using powders, aqueous extracts and oils in small areas or in organic crops and other as source for novel product aiming for the development of novel products more selective and less toxic than currently used synthetic insecticides (Chandel and Singh, 2016) [27].

Practically no systematic approach has so far been adopted to work out the economical effective control measures of *Eucosma critica*. The complicated problem of pest management has oriented the attention of agricultural experts, researchers and administrators for rethinking of its solution. It is realized now, that for satisfactory solution of many of the major problems in the pest management researches, study of insect life history and its extent of crop damage is of most importance. It is accepted that knowledge of the factors that influence the distribution and abundance of insect-pests will be more essential arms in wagging effective warfare against these injurious enemies.

2. Materials and Methods

2.1. Experimental Site

Experiments were conducted in the Department of Zoology, Dayanand Brijendra Swaroop Post-Graduate College, Kanpur U.P. India., under 25 ± 2 ºC, 60 ± 10 % Rh. and photophase of 12 h. The districts Kanpur Nagar is located in Indian sub region of Oriental Zoogeographical region and in between latitudes 25.26º and 26.58º North and longitudes 19.31º and 84.34º East, Kanpur is situated at an elevation of about 127.117º metres above the mean sea level and has a semi-arid subtropical climatic conditions.

2.2. Test Insect

For the experimental study, the leaf webber, *Eucosma critica* Meyrick (Lepidoptera: Eucosmidae) have been used as test insects for the anti-feeding activities of selected plat extracts. Last instar larvae of *E. critica* were used as test insect. Insects used in the bioassays were obtained from a stock culture maintained on arhar, *Cajanus cajan* leaves inside 300 ml glass petri-dishes covered with fine muslin cloth under laboratory conditions.

Trials were carried out at the Plants Product Laboratory of the Department of Zoology, D.B.S. College, Kanpur, U.P., India., under 25 ± 2 ºC, 60 ± 10 % Rh. and photo phase of 12 h. Insects used in the bioassays were obtained from a stock culture maintained on arhar leaves inside 300 ml glass petri-dishes covered with fine muslin cloth under laboratory conditions.

2.3. Procurement of raw plant materials

After preliminary experiment of aqueous extract on different aspects mention already in first report, the regular experiments of selected eleven botanical soxhlet extractives regarding phago-deterrent bio-efficacy were conducted under laboratory conditions. The plants parts used for extracts were collected mainly from wasteland and wild areas and some plants were collected from cultivated fields of the farmers. The investigations on the screening of eleven s available indigenous naturally occurring plant extracts on one hand *viz*., aerial parts and seeds, rhizomes etc of *Ageratum conyzaides* Linn., *Argemone maxicana* Linn., *Arachis hypogea* Linn., *Azadirachta indica* A. juss, *Brassica juncea* Linn., *Chenopodium ambrosioides* Linn., *Mentha arvensis* Linn., *Mentha longifolia* Linn., *Ocimum basilicum* Linn., *Pongamia glabra* Vent. *Ricinus communis* Lin were obtained from plants grown in the vicinity of Kanpur region, U.P. were screened for their antifeedant bioefficacy against Eucosma critica in laboratory.

2.4. Preparation of powder: Fresh collected green plant parts (aerial parts 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.

2.5. 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 and distilled water. Extraction of each category of powders 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 (Bajpai and Chandel, 2009) [26].

2.6 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 leaves given the name 'A' while the other jar was kept empty and given the name 'B'. In jar 'A', the leaves treated with extracts were placed, while the jar B remained empty. The jars used for experiment were disinfected with alcohol.

2.7. Preparation of Stock Solution and Insecticidal formulations: 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. 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 (0.25, 0.5, 1.0, 1.5, 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 (Trivedi and Chandel, 2010) [27]. The formula used to find out actual amount of stock solution required for making various concentrations of an insecticide is given below:

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

3. Experimental Protocol

Pegionpea *C. cajan* leaves of five square centimeter were cut and dipped in the different concentrations two minutes of the extracts. They were dried under clip and left under electric fan an about 1.0 hrs, to make a dry film of the extract on the leaves for each set of extract, one control was kept in which the leaf pieces were dipped in Benzene + emulsified water only. The treated pieces were kept in petri-dishes on moist filter papers and two 24 hr. starved third instar larvae of *Eucosma critica* were released in each petri-dish to feed for 4 hours. Three replicates per treatments were maintained. The area of leaf consumed by two grubs in each replication and concentration was measured. The results were compared with control (Dubey *et al*., 2004) [28].

The data of leaf area of mustard consumed by fifteen larvae of *Eucosma critica* in each replication was bulked in these values and the percentage of leaf area protected over control. The protection was estimated over damage. The concentrations were converted into log concentrations (100X). The data were subjected to the Probit analysis. The EC50 value in respect of each extract was calculated. The fitness of test was obtained by comparing X2 table at respective degree of freedom (D F). The variance rate was calculated and the fiducial limits were worked out (Finney, 1952) [29]. Finally, the regression column was drawn with the regression equation. The details of the calculation in respect of all selected indigenous plant extracts have been shown in table -3 and figure 1 to 2 and 3.

Table 1: Details of the selected plant parts and their common name.

Botanical Name	Part Used	Common Name	Part Used
Ageratum conyzoides Linn.	Asteraceae	Goat weed	Arial part
Argemone maxicana Linn.	Papavaraceae	Satyanashi	Seed oil
Azadirachta indica A. Juss.	Meliaceae	Neem	Seed oil
Arachis hypogea Linn.	Fabaceae	Groundnut	Seed oil
Brassica juncea Linn.	Brassicaceae	Brown mustard	Seed oil
Chenopodium ambrosioides L.	Chenopodiceae	Indian warm seed	Seed oil
Mentha arvensis Linn.	Lamiaceae	Cornmint	Leaves
Mentha longifolia Linn.	Lamiaceae	Horsemint	Leaves
Ocimum basilicum Linn.	Lamiaceae	Sweetbasil	Leaves
Pongamia glabra Vent.	Fabaceae	Karanja	Seed oil
Ricinus communis Linn.	Euphorbiaceae	Castorbean	Leaves
Endosulfan.	M/s.Hoechst pharmaceuticals Ltd, Mumbai	Trade name-Thiodon	

- Table No.2: Details of the selected plant parts and their common name.

Table 2: Formulations of Plant 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

4. Results

The protectivity on the basis of relative EC_{50} values of selected eight indigenous plant extracts against *Eucosma critica* is summarized in table 3 and figure 1 to 3. Based on relative EC_{50} values it is evident from the table 3 that *Chenopodium ambrosioides* seeds extract showed highest protectivity (EC_{50}=0.013) which is followed by *Argemone maxicana* Linn. (16.0769) >, *Brassica juncea* Linn. (EC_{50}= 0.037), *Azadirachta indica* A. Juss. (EC_{50}=0.197), to the larvae of *Eucosma critica*, the least.

On the basis of their order of merit the antifeeding result the relative EC_{50} values remaining botanicals are summarized as under *viz*., *Argemone maxicana* Linn.(16.0769)>, *Brassica juncea* Linn. (EC_{50}=0.037), *Azadirachta indica* A. Juss. (EC_{50}=0.197), *Ageratum conyzaides* Linn. (EC_{50} =0.209), *Pongamia glabra* Vent (EC_{50} = 0.279), *Arachis hypogea* Linn. (EC_{50}=0.317), *Ocimum basilicum* Linn. (EC_{50}=0.339), *Ricinus communis* Linn. (EC_{50}=0.413), *Mentha arvensis* Linn. (EC_{50}=0.447), *Mentha longifolia* Linn. (EC_{50}=0.532), times feeding deterrent than Endosulfan (EC_{50}= 0.516), respectively.

The summary of result of antifeeding test and relative protectivity on the basis of EC_{50} values of selected ten plant extract against third instar larvae of *Eucosma critica*. Is shown in table 3. Based on relative EC_{50} the antifeeding biopotency are summarized as:-*Chenopodium ambrosioides* > *Argemone maxicana* > *Brassica juncea* > *Azadirachta indica* > *Ageratum conyzaides*.> *Pongamia glabra*.> *Mentha longifolia* > *Arachis hypogea* > *Mentha arvensis* > *Ricinus communis* > Endosulfan, respectively. Their relative order of antifeedant protectivity merit being: 39.692 > 30.352 > 13.940 > 2.619 > 2.468 > 1.849 > 1.155 times protective, respectively as Endosulfan taken as unit.

Table 3: List of plant extract Log concentration Probit Protection regression column to *Eucosma critica* Meyrick

Plant Extracts	Het.	X^2	Regression Equation	EC_{50}	Fiducial Limit	Relative EC_{50}
Ageratum conyzaides Linn.	3	0.88	Y=0.84x+ 3.84	0.209	M_1 = 1.753 M_2= 1.006	2.468
Argemone maxicana Linn.	3	0.42	Y = 0.48x +1.11	0.017	M_1 = 1.017 M_2 = 1.760	30.342
Arachis hypogea Linn.	3	0.54	Y = 5.36x +2.65	0.317	M_1= 0.109 M_2 = 0.856	1.627
Azadirachta indica A. Juss.	3	0.75	Y = 0.77x + 3.96	0.197	M_1= 1.744 M_2 = 0.935	2.619
Brassica juncea Linn.	3	1.34	Y = 0.73x + 4.56	0.037	M_1= 1.167 M_2 = 0.032	13.945
Chenopodium ambrosioides Linn.	3	0.51	Y = 0.47x + 4.44	0.013	M_1 = 1.529 M_2 = 1.087	39.692
Mentha arvensis Linn.	3	1.52	Y = 2.10x + 1.36	0.447	M_1= 1.514 M_2 = 0.406	1.155
Mentha longifolia Linn.	3	0.38	Y=0.50x+ 3.8711	0.532	M_1 =0.119 M_2 =1.3165	1.821
Ocimum basilicum Linn.	3	1.48	Y = 0.58x + 0.32	0.339	M_1 = 1.726 M_2 = 1.427	1.522
Pongamia glabra Vent.	3	0.17	Y = 1.00x + 3.49	0.279	M_1 = 1.813 M_2 = 1.206	1.849
Ricinus communis Linn.	3	0.78	Y = 0.69x + 2.38	0.413	M_1= 1.586 M_2 = 1.138	1.249
Endosulfan	3	1.17	Y = 2.72x + 0.58	0.516	M_1= 1.600 M_2 = 0.033	1.000

Data found significantly heterogeneous at P= 0.05, Y =Probit repellency, X =Log Concentration X^{10}, D.F.= Degree of Freedom and EC_{50}= Concentration calculated to give 50% repellency

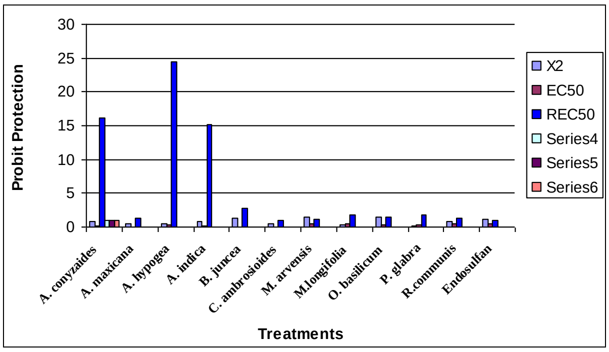

Fig 1: Log concentration/Protection Regression Column to *Ecosma critica*

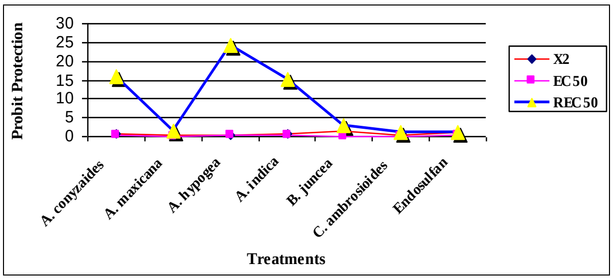

Fig 2: Log concentration/Probit Protection Regresson Column of *E. critica*

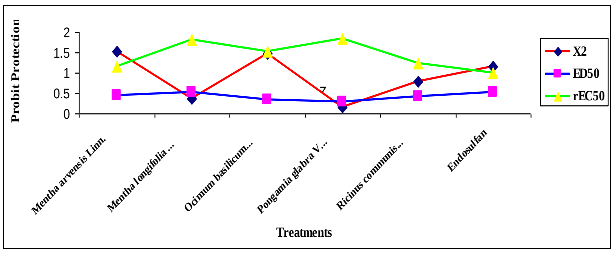

Fig 3: Log concentration/Probit Protection Regresson Column of *E. critica*

5. Discussion

The finding of the present investigation revealed that, the leaves extract of *Argemone mexicana* possesses remarkable antifeedant activity (EC_{50}=0.013 mg/ml) against *E. critia* larvae. Based on relative EC_{50} the antifeeding biopotency are summarized as:-*Chenopodium ambrosioides* L. (rEC_{50} = 39.692) > *Argemone maxicana* Linn. (rEC_{50} = 30.342) > *Brassica juncea* Linn. (rEC_{50}= 13.945)> *Azadirachta indica* A. Juss. (rEC_{50}= 2.619) > *Ageratum conyzaides* Linn. (rEC_{50}= 2.468) > *Pongamia glabra* Vent. (rEC_{50}= 1.849) > *Mentha longifolia* Linn. (rEC_{50} = 1.821 > *Arachis hypogea* Linn. (rEC_{50} = 1.627) > *Mentha arvensis* Linn. (rEC_{50}= 1.155) > *Ricinus communis* Linn. (rEC_{50} = 1.249) > Endosulfan (rEC_{50}= 1.00), respectively times protective, respectively as Endosulfan taken as unit.

Alcoholic extracts of all selected plant materials of the eleven botanicals the *Chenopodium ambrosioides* Linn. 0 per cent extract found highest antifeedant effect in relation to *Eucosma critica* larvae followed by *Argemone maxicana* Linn. *Brassica juncea* Linn and *Azadirachta indica* A. Juss which did not differ significant one and other.

The study needs further investigation to find out active ingredients responsible for antifeedant biopotentials of *C. ambrosioides*, *A. mexicana*, *B. juncea* and *A. indica* to reach any final recommendations. In many countries, plant derived products are being used by the farmers from ancient times and it triggered the scientists to search for ecofriendly insecticides from plant kingdom. In the support of above finfings several hundred plants have been reported as insect antifeedants, attractants, insecticides, ovicides and oviposition deterrents (Chander *et al*. 1991, Gbolade, *et al*. 1991, *Viji* and Bhagat, 2001) [31, 32, 33]. Facknath and Kawol (1993) tested *Argemone mexicana* extract for their antifeedant and insecticidal effects on third-instar larvae of the cabbage webworm, *Crocidolomia binotalis* on cruciferous crops. Among them *A. mexicana* showing the highest control potential against *C. binotalis* [34].

Similarly, many workers reported antifeedant responses of neem, *Azadirachta indica* towards their test insect. Sandhu and Singh, 1975 (*Pieris brassicae*) [35], Muralikrishna *et al*.1990 (*H. vigintioctopunctata*) [36], Misra and Singh, 1992 (*Schistocerca gragaria*) [37], Meshram and Kulkarni,1996 (*Papelio demaleus* [38], Murugan *et al*., 1998 (*Helicoverpa armigera*) [39], Park *et al*. 2000 (*Pieris brassicae*) [40] Joshi, and Lockwood, 2000 (*Oxya velox*) [41] Bosch, (2007) studied the effect of crude extract from leaves of of *Argemone mexicana* having antifeedant effect on the larvae of *Crocidolomia binotalis* Zeller (Lepidoptera: Crambidae) and *S. litura* [42].

Practically no systematic approach has so far been adopted to work out the antifeedant biopotentials of selected plant extractives to control of *Eucosma critica*. The complicated problem of pest management has oriented the attention of agricultural experts and administrators for rethinking of its solution. It is realized now, that for satisfactory solution of many of the major problems in the pest management researches, study its extent of damage is of rime importance. It is accepted that knowledge of the factors that influence the distribution and abundance of insect-pests will be more essential arms in wagging effective warfare against these injurious enemies.

6. Conclusion

In the present investigation has confirmed that the *Chenopodium ambrosioides* Linn. *Argemone maxicana* Linn., *Brassica juncea* Linn and *Azadirachta indica* A. Juss. has been explored the potential antifeedant biopesticide and plant protecting activity against 3rd instar larvae of *Ecosma critica.* In our study leaf area protected over control assessed and observed that feeding deterrence potential increased with increase in concentration at all the concentration up to 2.0 per cent concentration of exposure.

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