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Insectifuge Biopotency of *Chrysanthemum cinerariifolium*, *Cichorium intybus*, *Inula racemosa* and *Tagetes minuta* extractives against okra aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae)

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Abstract

In the present investigation alcoholic extract of ten indigenous naturally occurring asteraceous plants viz., aerial parts of *Acmella paniculata* (Wall ex DC.) R.K.Jansen, *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariifolium* (Trev.) Vis., *Inula racemosa* Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, and *Tagetes minuta* Linn. Extractives were prepared under the laboratory conditions. For testing the repellent effect on okra leaves were used as food against nymph and adults of *Aphis gossypii* Glover. After four hours nymph and adults of *A. gossypii* were release and the data was collected on the number of nymph and adults of *A. gossypii* reached and repelled at each treated food. All the comparisons were made with control. It is evident from the results of repellent test against nymph and adults of *Aphis gossypii* Glover that all the asteraceous extractives showed a good repellent property, when compared with the control. The repellent properties were observed based on their minimum EC₅₀ values as: *C. intybus* (0.1359) > *I. racemosa* (0.1531) > *T. minuta* (0.1833) > *M. duriaeri* (0.3208) > *S. undulate* (0.4123) > *A. paniculata*, (0.6365) > *R. acaule* (0.6469) > *R. tingitana* (0.7124) > *C. odorata* (0.9025), respectively. The relative EC₅₀ values of the above extracts are in descending order, 6.6409 > 5.8948 > 4.9236 > 2.8132 > 1.4179 > 1.3951 > 1.2668 > 1.2161 > 1.1668 > 1.0000 times as repellents, whereas *A. paniculata* was taken as a unit. *C. intybus* and *I. racemosa* extract significantly reduced the population of treated nymphs and adults of *Aphis gossypii* on okra vegetable compared to the untreated control.

Keywords: *Aphis gossypii*, *Cichorium intybus*, *Inula racemosa*, *Tagetes minuta*, okra

1. Introduction

Bhindi, *Abelmoschus esculentus* (Linn.) Moench commonly known as okra is an important vegetable crop grown all over Indian sub Zoogeographical region (Radake and Undirwade 1981) [1]. Okra contains large quantities of vitamin B, vitamin C, carbohydrate, potassium, protein, folic acid, and calcium (Adeboye and Oputa, 1996) [2]. It's low in calories and has high dietary fiber content. Okra is a nutritious vegetable which plays an important role to meet the demand of vegetables of the India when vegetable are scanty in the market. Okra mucilage is suitable for medicinal and industrial application (Kumar *et al.* 2013) [3].

The okra aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is a serious pest of vegetables ie; okra, brinjal, pepper melon, cucumber, pumpkin and cotton. The crop is vulnerable to attack by many serious insect pests among them, okra aphid, *A. gossypii* is the most important pest causing direct damage to the marketable fruits (Konar and Rai, 1990) [4]. *Aphis gossypii* and *Earias vittella* is alone reported to cause 57.1% fruit infestation and 54.04% yield loss in okra, *Abelmoschus esculentus* (Chaudhary and Dadheech, 1989) [5]. Initially, leaves will yellow and with an increasing number of okra aphids, they will begin to curl. Leaves can be damaged to such an extent that they wilt and fall off. This impairs photosynthesis, thereby weakening the okra plant even further, and may render fruits unmarketable and human consumption.

No doubt the synthetic chemical insecticides are very effective and used in insect pest management for a long time and to ensure higher vegetable and crop production (Chandel *et al.* 2018) [6].

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The injudicious and excessive use of these chemical pesticides led to many problems like development of resistance, environment pollutions and human health (Park *et al.* 2011) [7]. The side effects have forced to look for naturally occurring ecofriendly indigenous herbal alternatives aspect to chemical pesticides especially for vegetables like okra, where fruits are plucked at an interval of every 2-3 days (Chandel *et al.* 2018) [8]. Various plant extractives have been studied for insecticidal activity globally (Bajpai and Chandel, 2009, 2010 [9, 10] and majority of them are insect feeding deterrent (Antonius and Hagazy 1987, Chandel and Sengar 2018) [11, 12]. More than 140 compounds, which are chemically diverse and structurally complex, have been isolated from the seed oil, bark and leaves of neem (Daniel and Smith 1991) [13]. Phytochemicals are often distasteful and toxic to various insect pests. They can modify feeding deterrence behaviour of an insect (Schmidt and Strelake, 1994) [14]. Moreover, as most aphid species have become resistant to many aphidicidal agents (Rashid *et al.* 2013) [15] managing these pests in greenhouses and in the field is becoming problematic (Srivastava and Awasthi 1958, Dubey *et al.*, 2004, Dang *et al.* 2010, Kim *et al.* 2011) [16, 17, 18, 19]. Therefore, in the present investigation the selected naturally occurring asteraceous plant extractives are efficient and environment friendly pest control alternatives must be developed to replace toxic and hazardous synthetic pesticides.

2. Materials and Methods

(A) Procurement of Raw Plant Materials: The plant parts used for extraction were surveyed, identified and collected mainly from wasteland, wild areas and some plants were collected from cultivated fields of the farmers. The investigations on the screening of various available indigenous naturally occurring ten plant extracts *viz.*, aerial parts of *Acemella paniculata* Well ex DC, *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariifolium* (trev.) Vis., *Inula racemosa* Hook. F., *Mantisalca duriaeri* Birq. Et Cavill., *Rechardia tingitana* (L.) Roth, *Rhaponticum acaule* (L.) DC, *Scorzonera undulate* Vahl, and *Tagetes minuta* Linn. were screened for their phagorepellent biopotency against nymph and adults of *Aphis gossypii* Glover in laboratory. For carrying out the present studies only ten selected asteraceous botanical Soxhlet extractives were assessed for their phagorepellent Bioefficacy under laboratory conditions.

(B) Preparation of Powder and their Extraction: Fresh collected ten green asteraceous plant parts (leaves, Flowers and roots, aerial parts etc) were washed with distilled water and kept in the laboratory for 7 days for shadow air 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.

For the extraction Soxhlet Apparatus was used; about 20g powder from each category of powder were extracted with 300 ml of solvents ie. Alcohol and distilled water. Extractions of each category powder were done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator (Trivedi and Chandel, 2009) [40]. 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.

- (C) Preparation of Stock Solution and their 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 contents of the botanical 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 and 2.0 per cent) were used for experiments on repellent 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.
- (D) 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 1.00 cm 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.
- (E) Experimental Protocol:** The repellent test was carried out under laboratory condition against nymph and adults of *A. gossypii*. The okra leaves and fruites were used as food for the nymph and adults of *A. gossypii*. Leaves were treated with different concentrations (0.25, 0.5, 1.0, 1.5 and 2.0 per cent) for two minutes. The treated leaves were left under electric fan for about half an hour, to make a dry film of the extracts on the leaves for each set of extract and one control. The treated foods were kept in jar (23cm x 10cm) on moist filter paper. The untreated leaves were dipped in Benzene + emulsified water only. Thirty starved nymphs and 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. Number of nymphs and adults of *A. gossypii*, reached to the food or repelled to untreated empty jar were recorded.

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

Sr. No.	Scientific Name	Vernacular name	Family	Plant parts used
1.	<i>Acmella paniculata</i> (Wall ex DC.) R.K. Jansen	Toothache Plant	Asteraceae	Aerial parts
2.	<i>Chrysanthemum cinerariifolium</i> (trev.) Vis.	Daisy	Asteraceae	Leaves
3.	<i>Chromolaena odorata</i> Linn.	Siam weed	Asteraceae	Leaves
4.	<i>Cichorium intybus</i> (L.)	Chicory	Asteraceae	Roots
5.	<i>Inula racemosa</i> Hook. f	Puskarmul	Asteraceae	Aerial parts
6.	<i>Mantisalca duriaeri</i> Birq. Et Cavill.	Spach	Asteraceae	Roots
7.	<i>Reichardia tingitana</i> (L.) Roth	False sowthistle	Asteraceae	Flowers
8.	<i>Rhaponticum acaule</i> (L.) DC.	Coffee plum	Asteraceae	Flowers
9.	<i>Scorzonera undulate</i> Vahl	Black Salsify	Asteraceae	Flowers
10.	<i>Tagetes minuta</i> Linn.	Wild Marigold	Asteraceae	Leaves

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

Repellent Bioassay: For testing the Phago-repellent effect of asteraceous extracts, okra leaves were used as food for nymph and adults of *Aphis gossypii* treated with 0.25, 0.5, 1.0, 1.5 and 2.0 per cent concentrations. The treated okra leaves and fruits were kept in jar (23cm x 10 cm) on moist filter paper. Thirty (15 male and 15 female), 24 hours starved nymph and adults of *Aphis gossypii* were released in each jar along with control. For control treated foods were dipped in Benzene + emulsified water only. After four hours of the release nymph and adults of *Aphis gossypii*. Treated nymph and adults of *Aphis gossypii* 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 6hr at an interval of 6 hours for each observation. The data was collected and calculated on the number of nymph and adults of *Aphis gossypii*, which reached the treated food and repellency over control was recorded and calculated (Finney,

1925)^[20].

3. Result and Discussion

Data depicted from the results based on actual EC₅₀ values, the sequence of repellency of different plant extractives against nymph and adults of *A. gossypii* are as: *C. cinerariifolium* (0.0855) > *C. intybus* (0.1359) > *I. racemosa* (0.1531) > *T. minuta* (0.1833) > *M. duriaeri* (0.3208) > *S. undulate* (0.4123) > *A. paniculata*, (0.6365) > *R. acaule* (0.6469) > *R. tingitana* (0.7124) > *C. odorata* (0.9025), respectively., whereas *C. odorata* is taken as a unit. On the other hand data based on relative EC₅₀ values in the present phago-repellent test against nymph and adults of *A. gossypii*. The sequence of repellency from different plant extractives are as: *C. cinerariifolium* (10.550) > *C. intybus* (6.640) > *I. racemosa* (5.894) > *T. minuta* (4.965) > *M. duriaeri* (2.821) > *S. undulate* (2.188) > *A. paniculata*, (1.417) > *R. acaule* (1.395) > *R. tingitana* (1.267) > *C. odorata* (1.000), times as repellents, whereas *C. odorata* is taken as a unit.

Table 3: Calculation of log conc./Probit repellency Regression column of asteraceous extracts on *Aphis gossypii* Glover

Plant Extracts	Heterogeneous	X ²	Regression equation	EC ₅₀	Fiducial Limit	Relative EC ₅₀
<i>Acmella paniculata</i>	3	1.803	0.89X + 0.05	0.6365	M ₁ =2.8263 M ₂ =1.2632	1.417
<i>Chromolaena odorata</i>	3	1.492	1.08X + 2.65	0.9025	M ₁ =2.3362 M ₂ =1.9781	1.000
<i>C. cinerariifolium</i>	3	2.834	1.05X + 2.80	0.0855	M ₁ =2.3060 M ₂ =1.8719	10.550
<i>Cichorium intybus</i>	3	0.123	1.22X + 2.74	0.1359	M ₁ =2.0266 M ₂ =1.6557	6.640
<i>Inula racemosa</i>	3	1.93	1.41X + 3.26	0.1531	M ₁ =1.4512 M ₂ =0.2168	5.894
<i>Mantisalca duriaeri</i>	3	4.601	1.101X + 2.89	0.3208	M ₁ =2.1173 M ₂ =1.7484	2.821
<i>Reichardia tingitana</i>	3	1.080	1.25X + 2.42	0.7124	M ₁ =2.0267 M ₂ =1.9235	1.267
<i>Rhaponticum acaule</i>	3	1.95	0.92X + 4.37	0.6469	M ₁ =1.1588 M ₂ =0.0148	1.395
<i>Scorzonera undulate</i>	3	0.42	1.532X + 2.54	0.4123	M ₁ =0.0002 M ₂ =0.0005	2.188
<i>Tagetes minuta</i>	3	4.601	1.10x+2.89	0.1833	M ₁ =2.1173 M ₂ =1.7484	4.865

In case of X² was found non-significant heterogeneous at P=0.05, Y=Probit repellency, X=Log Concentration X 10⁻¹ D.F.=Degree of Freedom, E.C.₅₀= Concentration Calculated at given 50% repellency

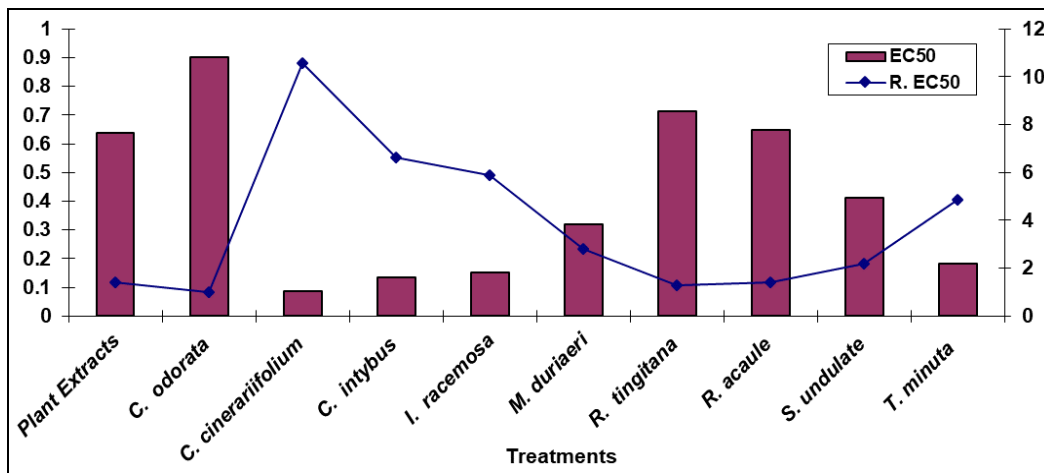


Fig 1: Log conc./Probit repellency Regression column of *Aphis gossypii* Glover

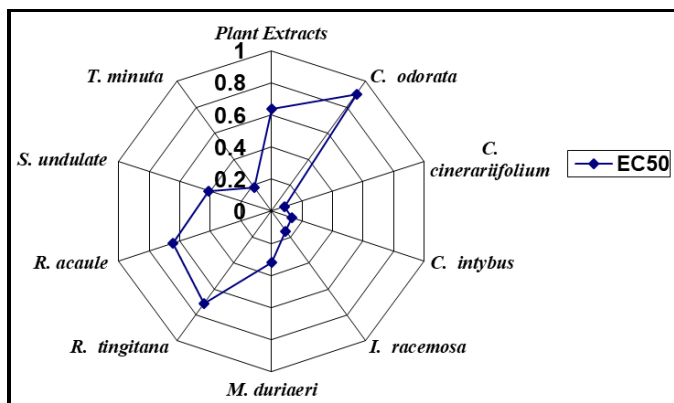


Fig 2: Log conc./ Probit repellency Regression radar based on EC50 of *Aphis gossypii* Glover.

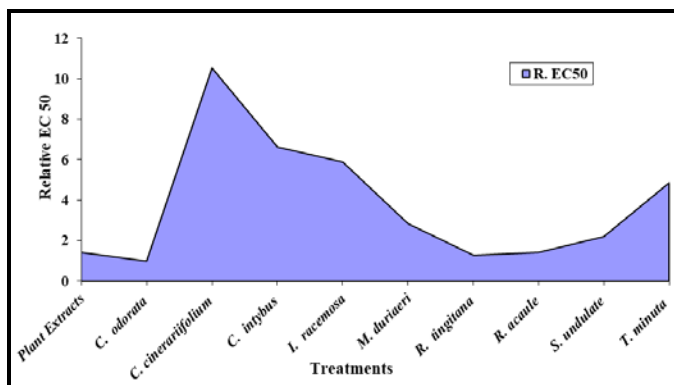


Fig 3: Log conc./ Probit repellency Regression area based on relative EC50 of *Aphis gossypii* Glover.

4. 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. 2000, Valsalan and Gokuldas, 2015) [21, 22] 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/ cm² applied to filter paper in choice chamber test, produced 59.00 and 67.00 per cent repellency against *T. castaneum* (Jilani et al. 1985, 1988 Mendes et al. 1997, Maredia et al.1992, Pavela, 2006, 2011 and Zoubiri and Aoumeur) [25, 26, 27, 28, 29] *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) [30]

In the support of present finding the many entomologist worked on their selected botanicals, among them *Cleome gynandra* gave significant repellent 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) [31, 32]. Aqueous and methanolic plant extracts of *T. minuta*, which exhibited high repellency (IR = 0.04) against repellent 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) [33, 34]. *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) [35]. 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. 2012) [36]. The repellent properties of certain asteraceous plant extractives as stored grain pest (Chandel and Singh,2016, 2017) [37, 38] and crop pests were earlier reported by (Singhamony 1984, Won- Sik, et al. 2002, Trivedi and Chandel, 2009, 2010, Manish Kumar et al. 2017) [39, 40, 41, 42, 43].

5. Conclusion

Conclusively, the present investigation revealed that the appear prospects in selected botanicals *C. cinerariifolium*, *C. intybus*, *I. racemosa* and *Tagetes minuta* were registered promising repellency with minimum EC₅₀ values to the nymph and adults of *Aphis gossypii*, when compared to other extracts. The data depicted in table 1 and figure 1 indicated that result based on their relative EC₅₀ values the extracts of *C. cinerariifolium* showed highest relative repellency with minimum relative EC₅₀ Values (19.550) to the treated nymph and adults of *Aphis gossypii* followed by *C. intybus* (6.39), *I. racemosa* (5.88) and *T. minuta* (5.42) times more repellent than *C. odorata* (1.00), which is taken as unit. The above selected plant materials can be use in protection of okra, *Abelmoschus esculentus* crop from *Aphis gossypii* infestations. The *C. cinerariifolium*, *C. intybus*, *I. racemosa* and *Tagetes minuta* extractives can be applied effectively by

farmers as a component of integrated management of insect-pests of solanaceous vegetable and crop in India.

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