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

Anju Bharti and BS Chandel

**Abstract**

In the present investigation alcoholic extract of ten indigenous naturally occurring asteraceous plants viz., aerial parts of *Acnella paniculata* (Wall ex DC.) R.K.Jansen, *Cichorium intybus* (L.), *Chromolaena odorata* Linn., *Chrysanthemum cinerariifolium* (trev.) Vis., *Inula racemosa* Hook. F., *Mantisalca duriaeeri* Birq. Et Cavill., *Reichardia 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. duriaeeri* (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 in meeting 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]. 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 (Antionius 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 Strelke, 1994) [14]. Moreover, as most aphid species have become resistant to many aphidical 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 cinerarifolium (trev.) Vis., Inula racemosa Hook. F., Mantisalca duriaeiri Birq. Et Cavill., Reichardia tingitana (L.) Roth, Rhaponticum aculea (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 fruits 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 starred 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.
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 (23 cm 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 6 hr 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].

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

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

### Table 2: Formulations of Extracts

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Amount of Stock Solution (ml)</th>
<th>Amount of Benzene (ml)</th>
<th>Amount of Emulsifiable Water (ml)</th>
<th>Total Amount (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>2.50</td>
<td>22.50</td>
<td>475.00</td>
<td>500.00</td>
</tr>
<tr>
<td>0.50</td>
<td>5.00</td>
<td>20.00</td>
<td>475.00</td>
<td>500.00</td>
</tr>
<tr>
<td>1.00</td>
<td>10.00</td>
<td>15.00</td>
<td>475.00</td>
<td>500.00</td>
</tr>
<tr>
<td>1.50</td>
<td>15.00</td>
<td>10.00</td>
<td>475.00</td>
<td>500.00</td>
</tr>
<tr>
<td>2.00</td>
<td>20.00</td>
<td>5.00</td>
<td>475.00</td>
<td>500.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Heterogeneous</th>
<th>$X^2$</th>
<th>Regression equation</th>
<th>EC$_{50}$</th>
<th>Fiducial Limit</th>
<th>Relative EC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acmella paniculata</td>
<td>3</td>
<td>1.803</td>
<td>0.89X + 0.05</td>
<td>0.6365</td>
<td>M1=2.8263 M2=1.2632</td>
<td>1.417</td>
</tr>
<tr>
<td>Chromolaena odorata</td>
<td>3</td>
<td>1.492</td>
<td>1.08X + 2.65</td>
<td>0.9025</td>
<td>M1=2.3362 M2=1.9781</td>
<td>1.000</td>
</tr>
<tr>
<td>C. cinerarifolium</td>
<td>3</td>
<td>2.834</td>
<td>1.05X + 2.80</td>
<td>0.0855</td>
<td>M1=2.3060 M2=1.8719</td>
<td>10.550</td>
</tr>
<tr>
<td>Cichorium intybus</td>
<td>3</td>
<td>0.123</td>
<td>1.22X + 2.74</td>
<td>0.1359</td>
<td>M1=2.0266 M2=1.6575</td>
<td>6.640</td>
</tr>
<tr>
<td>Inula racemosa</td>
<td>3</td>
<td>1.93</td>
<td>1.41X + 3.26</td>
<td>0.1531</td>
<td>M1=1.4512 M2=0.2168</td>
<td>5.894</td>
</tr>
<tr>
<td>Manitasca duriaeri</td>
<td>3</td>
<td>3.601</td>
<td>1.101X + 2.89</td>
<td>0.3208</td>
<td>M1=2.1173 M2=1.7484</td>
<td>2.821</td>
</tr>
<tr>
<td>Reichardia tingitana</td>
<td>3</td>
<td>1.080</td>
<td>1.25X + 2.42</td>
<td>0.7124</td>
<td>M1=2.0267 M2=1.9235</td>
<td>1.267</td>
</tr>
<tr>
<td>Rhoponticum acaule</td>
<td>3</td>
<td>1.95</td>
<td>0.92X + 4.37</td>
<td>0.6469</td>
<td>M1=1.1588 M2=0.0148</td>
<td>1.395</td>
</tr>
<tr>
<td>Scorzonera undulate</td>
<td>3</td>
<td>0.42</td>
<td>1.532X + 2.54</td>
<td>0.4123</td>
<td>M1=0.0002 M2=0.0005</td>
<td>2.188</td>
</tr>
<tr>
<td>Tagetes minuta</td>
<td>3</td>
<td>4.601</td>
<td>1.10X + 2.89</td>
<td>0.1833</td>
<td>M1=2.1173 M2=1.7484</td>
<td>4.865</td>
</tr>
</tbody>
</table>

In case of $X^2$ was found non-significant heterogeneous at P=0.05, Y=Probit repellency, X=Log Concentration X 10, D.F.=Degree of Freedom, E.C.$_{50}$= Concentration Calculated at given 50% repellency.
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: Rhinotermidae). 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. cinerarifolium, 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. cinerarifolium 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. cinerarifolium, C. intybus, I. racemosa and Tagetes minuta extractives can be applied effectively by
farmers as a component of integrated management of insects of solanaceous vegetable and crop in India.

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7. References
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