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Full Length Research Paper

Repellence property of traditional plant leaf extracts against *Aphis gossypii* Glover and *Phenacoccus solenopsis* Tinsley

Anita Singh, Ruchika Kataria and Dolly Kumar*

[1*]Division of Entomology, Department of Zoology, Faculty of Science, The M.S.University of Baroda, Vadodara-390002, Gujarat, India.

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Aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) and mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) are major polyphagous pests in Vadodara agricultural fields. To control these sap sucking insect pests; farmers are using broad spectrum insecticides which are toxic to non target insects. Therefore, the objective of this study is to control these pests by using biorational control methods. Hence, laboratory assays were carried out to evaluate the repellent property of leaf extracts of three indigenous native botanicals namely; *Azadirachta indica* A. Juss; *Eucalyptus globules* L. and *Ocimum basilicum* L. against aphids and mealybugs. The methanol extract of leaf was isolated by using soxhlet method. The leaf extracts were applied against aphids and mealybugs at dose levels of 1, 2, 4, 8 and 10%. Observations were made at 12 and 24 h. After 24 h of release of aphids and mealybugs, the highest repellency was recorded in *A. indica* leaf extract which gives 99.0 and 97.0% followed by *E. globules* leaf extract giving 96.0 and 93.0%. While minimum repulsion was seen in *O. basilicum* leaf extract 91.0 and 88.0%, respectively. As the dose increases, the repellent effect also increased irrespective of the plant extracts. The use of such plant extracts can control the population of serious pests like aphids and mealybugs in an environmental friendly way.

Key words: Aphid, mealybug, biorational control, botanicals, *Azadirachta indica, Eucalyptus globules, Ocimum basilicum.*

INTRODUCTION

The present study showed that in Vadodara, the central part of West Gujarat has a number of agricultural fields surrounding it. Major crops of these fields are Cotton, Castor, Sugar cane, Pigeon pea, Ladies finger, Brinjal, Radish, Cauliflower, Wheat, and Maize etc. These agricultural areas are having rich diversity of insects. Approximately, 300 species were found. Out of these, 50 were serious insect pests (Naidu, 2008). 6 species of aphids *Aphis nerii* (Boyer de Fonscolombe), *Aphis gossypii* (Glover), *Aphis crassivora* (Koch), *Brevicoryne brassicae* (Linnaeus), *Aphis fabae* (Scopoli) and *Myzus persicae* (Sulzer) from the family; Aphididae and 3 species from Coccidae and Pseudococcidae families: *Ceroplastes ceriferus* (Fabricius), *Phenacoccus solenopsis* (Tinsley) and *Maconellicoccus hirsutus* (Green) were identified belonging to the order: Hemiptera in Vadodara. Among the major pests, Cotton aphid *A. gossypii* (Glover) and Mealybug *P. solenopsis* (Tinsley) were considered to be a serious pest to almost all agricultural crops and alternative host plants because of their polyphagous feeding habits (Minks and Harrewijn, 1987; Arif et al., 2009). The cotton aphid, *A. gossypii* Glover, is a serious pest of the cucumber, *Cucumis sativus* L., a greenhouse in Japan (Nozato, 1993). Aphid and mealybug species are economically important group of insects in all over the world as they are an important disease vectors and direct plant-sucking pests. They can cause serious problems on vegetable crops even at low densities, since they can transmit plant viruses among phylogenetically desperate host plants (Sharma and Joshi, 2010). The species of Aphididae, Coccidae and

*Corresponding author. E-mail: dollymsu@gmail.com. Tel: +91-265- 2343179.

Pseudococcidae families such as *A. gossypii* (Glover), *C. ceriferus* (Fabricius) and *P. solenopsis* (Tinsley) attack various plants, infesting leaves, stems, fruits and roots (Blackman and Eastop, 2000; Vinobaba and Prishanthini, 2009) and also causing damage indirectly by secreting honeydew which cause development of sooty mold as well as, attracts ants as transporting agents of the mealy bugs to different host plants (Prishanthini, 2009). According to Singh and Allen (1980), the pest causes up to 40% reduction of crop yields in Asia. Attle et al. (1987) reported as high as 100% yield reduction of different bean crops due to aphid infestation and during 2006, the mealybug caused economic damage, reducing yield by up to 40 to 50% in infested fields in several parts of Gujarat (Nagrare et al., 2009). Hodgson et al. (2008) in Pakistan and India described *P. solenopsis* (Tinsley) as a serious cum invasive pest of cotton.

To protect their crops from damages of aphid and mealybug infestation, growers often apply conventional synthetic chemical insecticides such as: Organophosphates, Carbonates, Synthetic pyrethroid and Nicotonoids. Although, conventional synthetic insecticides usually provide quick and adequate control for the time being, they are usually expensive (Hussain, 1989) and its continuous usage caused health hazards, development of pest genotypes resistant to insecticides, resurgence and upset of insect pests and environmental pollution (Nas, 2004). The use of these insecticides is hazardous to the environment and non target insects like pollinators and predators (Cork et al., 2003). Therefore, the use of pest control measures depends on the economic and ecological consequences. Recent past years showed the struggle of human being as against the control of harmful insects. Thus, the use of biorational approach is necessary for better environmental protection. In this, the use of plants, insecticidal allelochemicals appears to be promising. Aromatic plants, and their essential oils, are among the most efficient botanicals (Regnault-Roger, 1997). The plants showing fumigant and topical toxicity extracts like: *Lantana camara* L., *Catharanthus roseus* L. (Remia and Logaswamy, 2010) as well as, antifeedant or repellent effects like: *Cymbopogon nardus* L., *Pogostemon cablin* Benth, *Syzygium aromaticum* L., *Zanthoxylum limonella* L. and *Piper retrofractum* Trel. (Koul et al., 2008). Botanical products like tobacco extract, neem oil and extract, which can be easily and cheaply collected in rural Bangladesh, have been found promising and useful for pest control (Roy et al., 2005).

In this study, pure methanol leaf extract of *A. indica* A. Juss; *E. globules* L. and*. Ocimum basilicum* L. were screened under laboratory condition to know its repellent activity against aphids and mealybugs.

MATERIALS AND METHODS

Aphids and Mealy bugs were considered as one of the major pest of agriculture field in Vadodara used in the experiment. These appeared in the fields in the month of October and are polyphagous damaging almost all the crops.

Rearing of selected insects

The aphids and mealybugs were collected from farmer plots by using handpicking methods. These were reared at 24± 2℃ under a photoperiod of 16L: 8D at humidity of 70 to 75% RH in laboratory in big plastic containers. The adult females used for laboratory assay were separated and kept in large plastic containers having food. This was to ensure that the insects were well adjusted in laboratory condition before the experiment.

Selection of plant species and preparation of leaf extracts

Three plant species, *A. indica* A. Juss, *Ocimum sanctum* L., *E. globules* L. used in this study and the leaf parts from which extracts were obtained were selected because their repellent properties are already reported against other insects. These plants were abundantly available in the study area.

Methanol leaf extract was prepared using soxhlet extraction method. The freshly collected leaves were washed thoroughly under tap water. The washed leaves were rewashed with distilled water and excess water drained. The excess moisture of leaves was removed by using muslin cloth which was further allowed to air dry or shade dry. Due to this, the leaves were completely dried without any trace of moisture. These dried leaves were collected and made into fine powder by means of a blender or a mixer grinder. Each leaf sample was extracted separately at 50℃ for 8 h in 300 ml of the aforementioned solvent.

The crude extract was later evaporated to obtain concentrated slurry of about 10 ml. The concentrated extract thus, obtained was filtered through a sterilized Whatmann No.1 filter paper. The filtrate obtained was a pure leaf extract from which we can prepare different required dose levels (Reardon and Edwards, 1998).

Repellency bioassays

The laboratory assays were carried out to evaluate the repellent property of leaf extracts of indigenous native botanical such as *A. indica* A. Juss; *E. globules* L. and *O. basilicum* L. against aphids. The native botanicals leaf extracts were applied against aphids and mealybugs at different dose levels namely; 1, 2, 4, 8 and 10%.

Bioassays were conducted under ambient laboratory condition of 28 + 2℃ and relative humidity of 80 + 20%. The repel lent action of the extracts against the aphids and mealybugs adult females was evaluated by the following method as subsequently shown:

In this study, large petri plates were used. The test areas consisted of No. 1 Whatman filter papers which were cut in triangular shape. Different test extracts and concentrations were applied to these triangular strips as uniformly as possible with pipette. The treated strips were air dried. On the other side of Petri plates the water dip strip was used as control. These were placed in a petri plate and 15 insects (aphids and mealybugs) were placed at the centre of the petri plate and covered with lids. Each treatment was replicated three times. The number of insects (aphids and mealybugs) on control and extract treated strip was recorded after 12 and 24 h. Percentage repellency (PR) values were computed using the formula:

$$PR = [(NC-NT)/ (NC+NT)] \times 100$$

Where; PR = percentage repellency

Table 1. Mean percentage repellency values for five concentrations (1, 2, 4, 8 and 10%) of methanol extracts of three plants obtained on 12 h time in a filter paper bioassay on adult mealybugs.

Plant extract	Concentration				
	1%	2%	4%	8%	10%
A. indica	16.67±0.8819^{j}	34.33±1.202^{g}	44±0.5774^{e}	61.33±0.333^{c}	70.33±0.8819^{a}
E. globules	19.67±0.8819^{i}	27±0.5774^{h}	40.3± 0.8819^{f}	54.33±0.333^{d}	64.67±0.8819^{b}
O. basilicum	11.33±0.6667^{k}	22.33±1.202^{i}	34±1.155^{g}	45±1.000^{e}	54±1.155^{d}
Control	0	0	0	0	0

*Value of mean of three replicates ±SE. Column Means followed by different letters are significantly different at 5% level of Duncan's Multiple Range Test.

Table 2. Mean percentage repellency values for five concentrations (1, 2, 4, 8 and 10%) of methanol extracts of three plants obtained on 12 h time in a filter paper bioassay on adult aphids.

Plant extract	Concentration				
	1%	2%	4%	8%	10%
A. indica	27.0±0.5774^{gh}	34±1.115^{f}	50.3±0.8819^{d}	64.3±1.528^{a}	78± 1.155^{a}
E. globules	25.33±0.8819^{h}	33.6±1.453^{f}	47±0.5774^{d}	59.6±0.8819^{c}	76±0.5774^{a}
O. basilicum	16.67±0.8819^{i}	30±1.155^{g}	39.3±1.453^{e}	47.6±1.453^{d}	60.6±1.202^{c}
Control	0	0	0	0	0

*Value of mean of three replicates ±SE. Column Means followed by different letters are significantly different at 5% level of Duncan's Multiple Range Test.

Table 3. Mean percentage repellency values for five concentrations (1, 2, 4, 8 and 10%) of methanol extracts of three plants obtained on 24 h time in a filter paper bioassay on adult mealybugs.

Plant extract	Concentration				
	1%	2%	4%	8%	10%
A. indica	55±0.5774^{g}	62.33±0.8819^{f}	79.67±0.8819^{d}	86±0.8819^{c}	97±0.5774^{a}
E. globules	44±1.155^{h}	55±0.577^{g}	71±0.5774^{e}	77.3± 0.6667^{d}	93±1.155^{b}
O. basilicum	34.33±0.6667^{i}	41.67±1.202^{h}	55.67±0.881^{g}	70±0.5774^{e}	88±1.155^{c}
Control	0	0	0	0	0

*Value of mean of three replicates ±SE. Column Means followed by different letters are significantly different at 5% level of Duncan's Multiple Range Test.

NC= insect no. present on control
NT= insect number present on strip treated with extract

RESULTS

The result was shown at 12 and 24 h. At 1% concentration, in both aphids and mealybugs among the treatments the botanical extracts of *A. indica* A. Juss (27.0, 16.67%) showed higher repellency. *E. globules* L. extract stands the next position (25.33, 19.67%). The least repellency was recorded from *O. basilicum* L. leaf extract (16.67, 11.33%) as against aphids and mealybugs respectively, during 12 h after release (Tables 1 and 2). At the same level of concentration after 24 h of insect release, the highest repellency was noticed from *A. indica* A. Juss leaf extract (59.33, 55.0%), followed by *E. globules* L. leaf extract (50.0, 44.0%) and *O. basilicum* L. (38.0, 33.0%) as against aphids and mealybugs, respectively (Tables 3 and 4).

Among the botanical extracts, there was not a significant difference between 12 and 24 h of release. Similar trend, as like 1% concentration, was noticed in case of 2, 4, and 8% concentrations both at 12 and at 24 h of release (Tables 1, 2, 3 and 4). Repellency was recorded by methanol leaf extract in the following order *A. indica* A. Juss > *E. globules* L. > *O. basilicum* L. as against aphids and mealybugs.

After 24 h of release of aphids and mealybugs, the highest repellency was recorded in the case of *A. indica* A. Juss leaf extract (99.0 and 97.0%) followed by *E. globules* L. leaf extract (96.0 and 93.0%). Minimum repulsion was seen in *O. basilicum* L. leaf extract (91.0 and 88.0%) at 10% concentration (Tables 3 and 4). After 12 h

Table 4. Mean percentage repellency values for five concentrations (1, 2, 4, 8 and 10%) of methanol extracts of three plants obtained on 24 h time in a filter paper bioassay on adult aphids.

Plant extract	Concentration				
	1%	2%	4%	8%	10%
A. indica	59.33±0.6667^{f}	70.3±0.8819^{e}	81±1.528^{c}	90±0.5774^{b}	99±0.5774^{a}
E. globules	50±1.155^{g}	59.6±0.8819^{f}	77.6±0.8819^{cd}	80.3±0.8819^{c}	96±1.5774^{a}
O. basilicum	38±1.155^{h}	49±0.5774^{g}	60±1.155^{f}	74.3±2.333^{d}	91±0.577^{b}
Control	0	0	0	0	0

*Value of mean of three replicates ±SE. Column Means followed by different letters are significantly different at 5% level of Duncan's Multiple Range Test.

of release, there was no marked difference in repellent effect between these three native botanicals. No significant effect was noticed in case of time duration between treatments that is, 12 and 24 h of release. After 24 h of release all the native botanical extracts reported highest repellency effect without any deviation from the earlier dose levels. Thus, as the time increased, the repellency property also increased. Irrespective of all the three botanical extract treatments, as the concentration level was increased the repellency property was also increased.

DISCUSSION

All plants extracts selected for the studies were reported to inhibit the bioactivity of several insects. The three plant leaf extracts caused repulsion of aphids and mealybugs under laboratory condition.

The repellent property of the plant extracts under discussion resulted to the effect of active compound of these plants. The plant extract of *A. indica* A. Juss which is globally accepted as good green insecticides having bio-active alkaloid, Azadirachtin and other tetranor-triterpenoids compounds responsible for the repellency (Jeyasanker et. al., 2005). Neem products were reported to reduce the infestation of various insect pests in tea (Selvasundaram and Muraleedharan, 1999), okra (Anaso and Lale, 2001a, b) and cowpea (Lale and Kabeh, 2004). Saikia et al. (2000) reported that leaf (10 to 50%) and seed kernel (5%) extracts of neem caused significant mortality of the bean aphid. The treatment of neem leaf and kernel extract along with cow urine against *Lipaphis erysimi* (Kalt) the mustard aphid leads to the reduction in incidence of this aphids and increase in the yield of mustard Gupta, 2010.

E. globulus Labill. consisting of 1.8-cineole, á-pinene, and *p*-cymene (Koul et al., 2008) acted as a good repellence as against insects. In Zambia, Mukanga et al. (2010) tested leaf powder of *E. globules* L. and *A. indica* A. Juss. for insecticidal activity as against larva and adult of *Prostephanus truncatus* horn found neem as a good anti-feedent and repellent than eucalyptus.

Oladimeji and Kannik (2010) found that *A. indica* A. Juss and *O. basilicum* L. leaf extract were not phytotoxic but increase in concentrations increased its effectiveness and its treated field yield was higher when compared to the treated synthetic pesticides as against *Podagrica spp.* Jac. in okra fields of the University of Ilorin Teaching and Research Farm situated in the Southern Guinea Savannah of Nigeria.

Okigbo et al. (2010) found that *A. indica* A. Juss leaf extract caused 100% mortality to culex larva species after 24 h at a concentration of 40% whereas, 100% mortality was seen at 50% concentration of *Ocimum gratissimum* L. after 24 h. Oparaeke et al. (2005) found that the treatment of mixtures of neem and eucalyptus leaf extract with extracts of lemongrass, African curry, tomato, bitter leaf and African bush tea on *Maruca vitrata* Fab. pod borers *and Clavigralla tomentosicollis* Stal caused great reduction in pod damage/plant and ensured higher yield as compared to untreated plants in the Research Farm of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

Inyang and Emosairue (2005) found good repulsion and antifeedant activity of aqueous solution of *A. indica* A. Juss and *O. gratissimum* L. leaves against banana weevil *Cosmopolites sordidus* Germar in Nigeria. Tandon and Sirohi (2009) found that ethanol extract of *A. indica* A. Juss was showing good repellent activity against *Raphidopalpa foveicollis lucas,* major pest of cucurbits crops and under repellency of Class IV (60.1 to 80%). Asogwa et al. (2010) emphasized on the use of *A. indica* A. Juss which is a biodegradable pesticide for the control of brown coca mirids, *Sahlbergella singularis* Haglund which caused major damage to coca in Nigeria.

According to the study of Regnault-Roger (1997), *O. basilicum* L. has linalool, limonene, eugenol and estragole. In Tamil Naidu agricultural university, Sathyaseelan and Bhaskaran (2010) found the highest repellency by *A. indica* A. Jus (99%) kernel extracts (99%) then *O. basilicum* L. (90.1%) leaf extract after 48 h of release of against *Maconellicoccus hirsutus* Green which was a major pest of mulberry crop. Gorski and Tomczak (2010) at Poznan university of Life Sciences, Poland reported the usefulness of natural essential oils in the control of foxglove aphid (*Aulacorthum solanis* Kalt.) occurring on eggplant (*Solanum melanogena* L.). Natural

essential oils not only exhibit toxic action against aphids, but may also have a repellent activity. The efficacy of natural essential oils in control of foxglove aphid occurring on eggplant was conducted in 2009. The following essential oils were tested: basil (*O. basilicum* L.), citronella (*Cymbopogon winterianus* Jowitt), eucalyptus (*E. globules* L.), juniper (*Juniperus communis* L.) and patchouli (*Pogostemon patchouli* Benth.) oils, which were applied at a concentration of 0.02, 0.05 and 0.10% respectively

The mealybug vector of cocoa swollen shoot disease, *Planococcoides njalensis* (Dist.), and its attendant ants, *Pheidole megcephala* and *Camponotus* spp. and some minor pests, including the mirid *Helopeltis* sp. and the psyllid *Tyora tessmanni* (Aulm.), were also adversely affected by the crude neem extract and the commercial neem formulations, especially, neem Azal (Padi et al., 2000). Much work on the selected plants was done on different agricultural as well as stored insect pests. Results obtained from the laboratory bioassay study demonstrated the good potential of these plants in field trials. These plants were consumed or used by human being. Thus, it is safe for environment and non target organisms like humans. It is abundant in the study area and easily available to farmers. Therefore, the plant product can be utilized for preparing phytochemical product which is a good alternative to conventional synthetic insecticides as they are safe, economical and readily available in many areas of the world.

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