Efficacy of few botanical extracts on the antifeedancy property against pink mealy bug 
Maconellicoccus hirsutus (green) in mulberry crop

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Abstract
Mulberry, Morus alba, (L.) leaves are the predominant source of food for silkworm, Bombyx mori L. rearing. Pink mealy bug Maconellicoccus hirsutus (Green) infests the mulberry plants and cause tukra diseases that leads to both quantitative and qualitative loss of leaves. Hence a study was carried out to evaluate the efficacy of commonly available botanical extracts for their antifeedancy property against pink mealy bug. The plant extracts obtained from Lantana, Garlic, Ginger, Neem and Vitex are viz. 2, 4, 8 and 10 percent respectively. After 24 hours, the highest antifeedancy of the nymphs was recorded with 10% methanolic (61%) and aqueous extract (50%) of lantana. The lowest antifeedancy percent was recorded with vitex (49%, 39%) respectively. In case of adult mealy bugs, the maximum antifeedancy recorded with 10% aqueous and methanol extract of lantana (58%, 47%). At 2% concentration, the range of antifeedancy recorded by the above plant extracts ranged from 9 to 27% and 15 to 36%. As the dose of the botanical extracts increases the effect of antifeedancy of the mealy bugs also increased.

Keywords: Plant extracts, Antifeedancy, Mealy bug Maconellicoccus hirsutus

1. Introduction
Mulberry Morus alba L. (Moraceae) is a very widespread and important fruit tree. It is also used as silkworm feed and timber in many parts of the world [1, 2]. It is attacked by about 300 insect and non insect species of pests in different parts of the world. Among the pests, sucking pests are considered as major pests causing considerable damage to mulberry in all growing stages of crop particularly in apical portion of the plant [3]. However, the pink mealy bug Maconellicoccus hirsutus Green (Pseudococcidae: Homoptera) is considered as an important cosmopolitan sucking pest and regular in occurrence. During infestation, the pests prefer tender portion of the plant because of succulence. It sucks the sap simultaneously releasing toxins which results in short internodes, curling, wrinkling and crumpling of apical leaves virtually stopping the growth of the plant by suppression of stem elongation affecting the yield of leaves. Further the affected region swells and turns into deep green color. Therefore, the symptoms of mealy bug infestation in mulberry collectively called as tukra [4]. Besides, reduction in leaf area, yellowing of leaves, premature leaf fall occurs due to impaired function of the petiole because of mealy bug infestation. The tukra affected mulberry plantations recorded 3 - 6 Tonnes leaf yield/ha/yr [5]. It was also reported that the mealy bug incidence caused an estimated loss in leaf yield of 4500 kg/ha/yr amounting to 34.24 per cent [6, 7], thus depriving the farmer from brushing about 450 dfls/ha/yr leading to decline in cocoon production by 150 kg/ha/yr (10-15%). The high incidence of tukra was noticed in March and reduced in August, the least was in December [8].

Generally insecticides are not advisable for mulberry ecosystem, because of the residual toxicity and also it directly influences the silkworm rearing [9]. Recently non chemical avenues like botanicals acted as an efficient alternative to the pesticides in mulberry garden [10]. Botanical pesticides are biodegradable [11] and their use of crop protection is a practical sustainable alternative. Therefore the present study attempts to evaluate the antifeedancy of few native botanicals against mealybug M. hirsutus of mulberry.
2. Materials and Methods

2.1 Culturing of pink mealy bug

The pure culture was released on well matured pumpkin which was cleaned using water and treated with 0.1% Bavistin 50 WP. The wounds present on pumpkins were plugged using wax. The culture was maintained throughout the research period without contamination. The nymphs and matured adult mealy bugs were collected from the tukra infected mulberry plants and placed on pumpkins for in vitro culture to study its antifeedant property.

2.2 Collection of botanicals:

The leaves of lantana (Lantana camara Linn.), neem (Azadirachta indica Juss.), vitex (Vitex negundo Linn.), cloves of garlic (Allium sativum Linn.) and rhizome of ginger (Zingiber officinale Rose.) were selected and used as study material.

2.3 Aqueous extraction of lantana, neem and vitex:

The extracts of the lantana, neem and vitex under study was prepared by homogenizing 10 g of plant material (leaf) in 100 ml of distilled water. The homogenate of each botanical extract was filtered through three layered muslin cloth. The resulting aqueous extract was used as a stock solution to prepare required dose [12].

2.4 Aqueous extraction of garlic and ginger:

50 g Garlic cloves were taken and ground well using mortar and pestle to obtain a paste form. Then 25 ml of distilled water is added to the paste and shaken well. The mixture was kept for three days; strained using a clean muslin cloth and the volume was made to 50 ml by adding further 25 ml of water, shaken well and stored in a dark bottle. Similar procedure was used to prepare ginger extract also.

2.5 Soxhlet extraction

Similarly the extracts of the botanicals under study were obtained using the soxhlet extraction method. This was carried out by using the following procedure; 20 grams of washed, dried and powdered plant part were taken and placed in a thimble made up from the thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a round bottom flask containing the extraction solvent i.e. distilled water. The Soxhlet was then equipped with a condenser. The solvent was heated at 90 °C to reflux: As the solvent vapour travelled up a distillation arm, into a condenser, the condensed vapours dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled up with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. After extraction the solvent was removed, by means of a rotary evaporator. The extracted compound was collected. The non-soluble portion of the extracted solid in the thimble was discarded [12]. Similarly soxhlet extraction of garlic and ginger was also done and obtained the stock solution. The aqueous and methanolic extract prepared from the botanicals were utilized to prepare the serial dilutions viz. 2, 4, 8 and 10% for evaluation of antifeedancy of mealy bugs.

2.6 Evaluation of Antifeedancy

The nymph and adult mealy bugs were collected from the in vitro culture and kept in the plastic bag separately. The adult mealy bugs (20 mealy bugs/treatment) were released on the mulberry leaves treated with different concentrations of the aqueous and methanolic extracts plant materials viz. 2, 4, 8 and 10% kept inside the petri dishes under controlled condition (Temp. 25± 2 °C; RH 54-65%) along with untreated check. The antifeedant effect of the plant extracts was calculated based on the repellency of the mealy bugs from the treated mulberry leaves.

Similarly the study was carried out for nymph mealy bugs also with aqueous and methanolic botanicals extract treated mulberry leaves. The treatments were replicated thrice and the data collected after 24 hours was subjected to the statistical analysis for its significance among various plants extract treatments.

2.7 Statistical Analysis of Data

The data obtained from the experiment were subjected to “t” test in order to assess the antifeedant property of the plant extracts against nymphs and adults of the pink mealy bug M. hirsutus after 24 hrs.

3. Results

The results revealed that the methanolic botanical extracts were comparatively more effective than aqueous extracts. The antifeedant response in all the extracts was correspondingly increased with increase in concentration of the extracts. In case of untreated check, there was no antifeedent effect.

3.1 Evaluation of the antifeedancy of nymph mealy bugs:

Among the extracts, maximum antifeedancy of the nymphs recorded in aqueous and methanolic extracts of lantana at 10% conc. was 50% and 61% respectively. In case of 10% aqueous and methanolic extracts of garlic, ginger and neem, it ranged from 47% and 57%, 46% and 57% and 42% and 52% respectively. The least antifeedancy was recorded with aqueous and methanolic extracts of vitex are 39% and 49%. Similar observations were also made with other concentrations of the botanical extracts which were depicted in Table-1.

3.2 Evaluation of the antifeedancy of adult mealy bugs:

In case of adult mealy bugs, maximum antifeedancy was recorded at 10% aqueous and methanol extract of lantana (58% and 47%). Whereas the range of antifeedancy recorded with 10% aqueous and methanolic extracts of garlic, ginger and neem was 46% and 54%, 45% and 53% and 41% and 47% respectively. The least antifeedancy was recorded with aqueous and methanolic extracts of vitex was 37% and 45%. At 2% conc. the range of antifeedant recorded by the above botanical extracts ranged from 9 to 19% and 15 to 29% respectively. The antifeedant data recorded with other concentrations of the botanical extracts are showed in the Tables-2.
Table 1: Efficacy of botanical extracts on the antifeedancy property against the nymphs of pink mealy bug *Macrollicoccus hirsutus* (after 24 hrs.)

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
<th>10%</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
<tr>
<td>Lantana</td>
<td>5.4±0.1</td>
<td>7.2±0.1</td>
<td>6.2±0.1</td>
<td>8.4±0.4</td>
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<tr>
<td>Garlic</td>
<td>4.4±0.1</td>
<td>6.2±0.1</td>
<td>4.4±0.09</td>
<td>6.6±0.09</td>
</tr>
<tr>
<td>Ginger</td>
<td>3.4±0.14</td>
<td>5.2±0.14</td>
<td>4.6±0.09</td>
<td>6.4±0.08</td>
</tr>
<tr>
<td>Neem</td>
<td>2.8±0.08</td>
<td>5.2±0.08</td>
<td>4.4±0.08</td>
<td>6.4±0.09</td>
</tr>
<tr>
<td>Vitex</td>
<td>1.8±0.11</td>
<td>3.2±0.11</td>
<td>3.8±0.09</td>
<td>6±0.10</td>
</tr>
<tr>
<td>Untreated check</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

Table 2: Efficacy of botanical extracts on the antifeedancy property against the adult pink mealy bug *Macrollicoccus hirsutus* (after 24 hrs.)

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>2%</th>
<th>4%</th>
<th>8%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
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<td>Lantana</td>
<td>3.8±0.7</td>
<td>5.8±0.7</td>
<td>6.4±0.5</td>
<td>6.4±0.5</td>
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<tr>
<td>Garlic</td>
<td>2.8±0.7</td>
<td>4.8±0.7</td>
<td>4±0.5</td>
<td>6±0.5</td>
</tr>
<tr>
<td>Ginger</td>
<td>2.6±0.65</td>
<td>4±0.65</td>
<td>5.2±0.7</td>
<td>6±0.75</td>
</tr>
<tr>
<td>Neem</td>
<td>1.8±0.7</td>
<td>3.2±0.7</td>
<td>4.2±0.6</td>
<td>5.2±0.6</td>
</tr>
<tr>
<td>Vitex</td>
<td>1.8±0.6</td>
<td>3±0.6</td>
<td>4±0.6</td>
<td>5±0.6</td>
</tr>
<tr>
<td>Untreated check</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

Note: values in the table 1 & 2 are mean±SD; values in parenthesis are in terms of%.

4. Discussion

Natural pesticides, especially those derived from plants are promising elements for pest control and are considered as an alternative to synthetic pesticides as it reduces the negative impacts on the human health and the environment [13]. Essential oils are very important botanicals that can act as a fumigants, insecticides, repellents, and antifeedants [14]. Antifeedant is a chemical that inhibits the feeding without killing the insect pests directly, while it remains near to the treated crops and dies through starvation [15]. Higher antifeedant index normally indicate decreased rate of feeding. Botanicals are reported to be safer than synthetic insecticides, easily biodegradable, environmentally safe, non-persistent and easily available and processed [16].

In the present study, the plant extracts tested against mealy bugs revealed the higher percent of antifeedancy of nymph and adult mealy bugs with 10% lantana (aqueous and methanol) extract compare to other botanical extracts. The above discussed effects of crude aqueous extract of *L. camara* leaves may be due to the active toxic group like Lantadene present in the leaves [17]. The effect of neem extract on mealy bugs in the experiment also recorded moderate percent of antifeedancy. As the dose of the plant extracts increases, the percent of antifeedancy of the nymph and adult mealy bugs also increases. The results are in agreement with the other research findings that the use of aqueous plant extracts on *M. hirsutus* prepared from Azadirachta indica, Rhizophora apiculata, Adalibah vasica, Parthenium hysterophorus, Lantana camara and Prosopis juliflora directly used as a foliar spray on six mulberry varieties viz., M5, S13, MR2, Kosen, BC2-59 and Tr4 revealed prevention of spread of *M. hirsutus*. Application of Azadirachta indica and Adalibah vasica sprayed directly on mulberry have controlled the tukra and did not affect nutritional status of mulberry and silkworm rearing parameters. This decrease in incidence may be due to the presence of biochemical constituents in the botanicals with property of repellency, antifeedent, insecticidal, sterilant effect, oviposition deterrent effect, insect growth regulatory effect, toxic effect etc., and also their availability during the infestation of mealy bug [18].

Garlic extracts have shown a considerable toxicity to a number of species of different insect orders and to different developmental stages [19, 20]. In garlic, there are many secondary metabolites such as saponins, tannins, alkaloid steroids and glycosides that may affect the antifeedant [21]. Similarly, the garlic extracts were found to act as antifeedants against different insect orders, for example, Coleoptera [22], Lepidoptera and Hemiptera [23, 24]. The results recorded from the experiment also showed significant antifeedancy percent in mealy bugs with garlic and ginger extracts. Though these extracts were found effective in controlling leaf roller, thrips, mealy bugs, fruit, stem and bark borers, hairy caterpillar and aphids also [25], due to its persistent odour in mulberry after treatment, the silkworm *Bombyx mori* consumption was less. Further the treatment of mulberry with *Vitex negundo* also recorded the antifeedancy in mealy bugs at significant level. This is in agreement with the findings that the leaf extract of *V. negundo* was found to be toxic against *Spodoptera littoralis* [26], *Platella xylostella* [27]. The active biomolecule sabeniene present in *V. negundo* exhibited a maximum repellency against mosquito *Culex quinquefasciatus* [28]. The presence of toxic compounds like terpenes, cinol, sabeniene, sesquiterpenes in *V.negundo* extract might be the reason for its higher repellency property [29]. The above findings revealed...
that the decrease in incidence may be due to the presence of biochemical constituents in the botanicals with property of repellency, antifeedant, insecticidal, sterilant effect,

5. Conclusion
The outcome of the study revealed that the aqueous extracts of the botanicals at lowest concentration i.e. 2% may be used as a substitute for chemical pesticides in controlling the mealy bugs in the mulberry field which is safe for silkworm and also it is ecofriendly. This inturn increases the leaf yield of the mulberry and enhances the cocoon production of silkworms.

6. Acknowledgement
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7. References
22. Chiam WY, Huang Y, Chen SX, Ho SH. Toxic and antifeedant effects of allyl disulfide on Tribolium castaneum (Coleoptera: Tenebrionidae) and Sitophilus zeamais(Coleoptera: Curculionidae). Journal of Economic Entomology. 1999; 92:239-245.