Insecticidal Potential of Indigenous Flora of Soon Valley against Asian Citrus Psyllid Diaphorina citri Kuwayama and Cotton Aphid Aphis gossypii Glover †

Muhammad Bilal Tayyab 1, Muhammad Zeeshan Majeed 1,* and Muhammad Asam Riaz 1

1 Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan; hmbt222@gmail.com (MBT); zeeshan.majeed@uos.edu.pk (MZM); asam.riaz@uos.edu.pk (MAR)
* Correspondence: zeeshan.majeed@uos.edu.pk
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Abstract: Diaphorina citri Kuwayama (Psyllidae: Hemiptera) and Aphis gossypii Glover (Aphididae; Hemiptera) are destructive sap-feeding pests of citrus and cotton, respectively. This study assessed the toxicity potential of indigenous flora of Soon valley and surrounding salt range (Punjab, Pakistan) against D. citri and A. gossypii. Among acetone extracts of 40 plant species, the extracts of Mentha longifolia L. Huds., Melilotus officinalis (L.) Huds., Nerium indicum Mill., Datura alba L. and Salvia officinalis L. showed highest psyllid mortality (i.e. 93, 91, 89, 88 and 81%, respectively). Bioassay with most effective extracts further revealed that the most toxic extracts were S. officinalis, N. indicum and M. longifolia with LCs50 values of 18.59, 20.27 and 20.73%, respectively. Similar trend of toxicity was observed in case of their LTs50 values. These results suggest the putative effectiveness and further biochemical characterization of these plant extracts for the management of D. citri and A. gossypii and other sap-feeding insect pests.

Keywords: Botanical pesticides; Phytoextracts; Sap-feeding pests; Diaphorina citri; Aphis gossypii; Toxicity evaluation

1. Introduction

Insect pests adversely affect the world food production. Among these, sap-feeding insect pests have been a serious threat to agricultural and horticultural crops all over the world including Pakistan. Although Pakistan ranks among the top citrus and cotton producing and exporting countries of the world, per unit area production of both these crops is far-behind than other top countries. Occurrence of insect pests and diseases are the major limiting factors for this decreased citrus and cotton production in Pakistan [1,2].

Asian citrus psyllid (ACP) Diaphorina citri Kuwayama (Psyllidae: Hemiptera) and cotton aphid Aphis gossypii Glover (Aphididae; Hemiptera) are the most destructive pests of citrus and cotton crops, respectively. For the control of both these pests, citrus and cotton growers rely exclusively on extensive applications of highly persistent synthetic insecticides which are also causing many environmental and health issues [3], insect pests resistance [4,5], eradication of non-target fauna including predators and parasitoids [6,7] and human health hazards [8].

This situation demands for searching novel biorational pest management strategies which would be more environment-friendly and safer than synthetic chemical insecticides such as plant-based pesticides which appear as promising alternative control measures [9]. This study was conducted to explore the indigenous flora (including herbs, shrubs and trees) of Soon valley and surrounding Salt Range (Khushab, Punjab, Pakistan) for their toxicity potential against D. citri and A. gossypii.

2. Methods
3.1. Collection and Extraction of Plant Materials

Samples of indigenous flora (including trees, shrubs and herbs) were collected from six selected sites (Angah, Dape Shareef, Kenhatti Garden, Khabeki, Khoora and Uchhali) of Soon valley and surrounding Salt Range of district Khushab (Punjab, Pakistan) as detailed in Table 1. Collected plants were identified up to species level and were extracted by the Soxhlet extractor (DH.WHM-12393, Daihan Scientific, South Korea) using pure acetone as extraction solvent using 1:10 plant material: acetone ratio. Pure botanical extract obtained from each plant sample was stored in 50 mL hermetic dark glass vial and was refrigerated until its downstream utilization in the toxicity bioassays.

Table 1. Geographical coordinates of selected flora collection sites in Soon Valley and surrounding Salt Range of Pakistan.

| Sr. No. | Localities     | Latitude N  | Longitude E | Elevation (m) |
|---------|----------------|-------------|-------------|---------------|
| 1.      | Angah          | 32.35° N    | 72.05° E    | 821           |
| 2.      | Dape Sharif    | 32.30° N    | 72.04° E    | 890           |
| 3.      | Kenhatti Garden| 32.40° N    | 72.14° E    | 783           |
| 4.      | Khabeki        | 32.35° N    | 72.12° E    | 774           |
| 5.      | Khoora         | 32.23° N    | 72.11° E    | 866           |
| 6.      | Uchhali        | 32.56° N    | 72.02° E    | 794           |

3.2. Insect Culture

Active adults of ACP (D. citri) were collected from the citrus field (Citrus reticulata Blanco cv. kinnow mandarin) by means of manual aspirator and were reared on potted citrus jasmine (Murraya paniculata (L.) Jack) plants. Aphid (A. gossypii) colonies were collected cotton field and were reared on potted cotton (Gossypium hirsutum L.) plants. Insects were reared at 27±3°C temperature, 60±5% relative humidity and 16h L: 8h D photoperiod.

3.3. Insecticidal Bioassays

First bioassay was conducted in order to screen out the most effective botanical extracts against ACP (C. citri) individuals using twig-dip bioassay method. Later on, based on the results of this preliminary toxicity experiment, we conducted our 2nd series of toxicity bioassays with detailed experimental parameters using laboratory reared aphid (A. gossypii) individuals using leaf-dip bioassay method. After exposure of 5 to 10 freshly molted lab reared insect individuals to different concentrations, they were incubated in an environmental chamber under controlled conditions i.e. at 27±2°C and 60±5% relative humidity. Experimental design was completely randomized with three to five replicates maintained for each treatment. Data regarding the mortality of exposed insect individuals was recorded at 12, 24, 48 and 72 h post-treatment.

3.4. Statistical Analysis

Statistical interpretation of data was performed using analytical software Statistix V. 8.1®. Apart from graphical presentation, percent mortality data were analyzed by factorial analysis of variance (ANOVA) taking botanical solutions, concentrations and time intervals as factors. Treatment means were further compared using HSD test at 95%
probability level (P < 0.05). Median lethal concentration (LC50) and time (LT50) values were calculated by probit analysis using IBM SPSS® statistics regression software. Prior to statistical analysis, mortality data was corrected using Abbott’s formula [10].

3. Results

Results of preliminary screening experiment performed with 10% extracts showed that some botanical extracts caused significant mortality of adult psyllid individuals (F = 44.82; P ≤ 0.01). The extracts of M. longifolia (93%), followed by M. officinalis (91%) and N. indicum (89%), while the extracts of D. alba and S. officinalis showed 88 and 81% mortality, respectively. Whereas about 57% mortality was caused by R. smithi and remaining all botanicals caused less than 50% psyllid mortality (data not shown).

Results of detailed toxicological bioassays carried out against A. gossypii revealed a differential response of aphid individuals against different plant extracts. There was a significant effect of all botanical extracts on aphid mortality (F = 181.30; P ≤ 0.01). Overall, the highest average mortality (63%) of aphid individuals was observed in case of S. officinalis, followed by D. viscosa, and O. ferruginea exhibiting 62 and 60% average aphid mortality, respectively, while M. longifolia and N. indicum both exhibited about 58% aphid mortality for 40% concentrations (Figure 1).

Similar trend of toxicity was observed for median lethal concentration (LC50) and median lethal time (LT50) values were calculated for the botanical extracts. According to probit analysis, S. officinalis was the most effective at 48 h (LC50 = 18.59%), followed by the extract of N. indicum (LC50 = 20.27%) and M. longifolia (LC50 = 20.73%), while the extracts of S. officinalis, M. longifolia and N. indicum showed minimum LC50 values (i.e. 9.24, 9.51 and 10.98%, respectively) at 72 h (Table 2). Similar trend was found in case of median lethal time (LT50) values. The 40% extracts of S. officinalis and O. ferruginea showed minimum LT50 values (i.e. 17.73 and 20.05 %, respectively) (Table 3).
Figure 1. Percent mortality (mean ± SE; n = 10) of cotton aphid (A. gossypii) individuals exposed to different concentrations of botanical extracts observed at different post-exposure time intervals. For each botanical extract, small alphabets indicate statistical difference among time intervals for each concentration, while capital alphabets are indicating the statistical difference among different concentrations (one-way factorial ANOVA; HSD at α = 0.05).
Table 2. Median lethal concentration (LC₅₀) values of different acetone extracts of Soon valley flora bioassayed against freshly molted adults of cotton aphid (Aphis gossypii)

| Treatments                        | Observation time (h) | LC₅₀ (%) | Lower and Upper 95% Fiducial Limits (%) | X²(DF =10)* | P-value |
|-----------------------------------|----------------------|----------|----------------------------------------|-------------|---------|
| Maerua arenaria H & Thom.         | 48                   | 63.76    | 50.29-110.45                           | 52.28       | ≤ 0.001 |
|                                   | 72                   | 58.80    | 46.71-104.04                           | 47.09       | 0.001   |
| Mentha longifolia (L.) Huds.      | 48                   | 20.27    | -38.57-42.65                           | 31.59       | 0.06    |
|                                   | 72                   | 10.98    | -53.43-34.84                           | 42.51       | ≤ 0.001 |
| Nerium indicum Mill.              | 48                   | 20.73    | -233.28-52.07                          | 76.59       | ≤ 0.001 |
|                                   | 72                   | 9.51     | -192.30-43.54                          | 77.35       | ≤ 0.001 |
| Rhamnus smithi Greene             | 48                   | 25.63    | -5.3-36.68                             | 53.36       | ≤ 0.001 |
|                                   | 72                   | 15.67    | -21.99-29.56                           | 60.05       | ≤ 0.001 |
| Datura alba L.                    | 48                   | 66.67    | NC                                     | 83.58       | ≤ 0.001 |
|                                   | 72                   | 72.40    | NC                                     | 67.28       | ≤ 0.001 |
| Periploca aphylla Decne.          | 48                   | 54.17    | 40.16-91.96                            | 67.92       | ≤ 0.001 |
|                                   | 72                   | 50.03    | 38.06-75.57                            | 63.27       | ≤ 0.001 |
| Sonchus asper (L.) Hill           | 48                   | 41.13    | NC                                     | 83.23       | ≤ 0.001 |
|                                   | 72                   | 30.11    | NC                                     | 69.70       | ≤ 0.001 |
| Salvia officinalis L.             | 48                   | 18.59    | -37.88-37.87                           | 48.74       | 0.001   |
|                                   | 72                   | 9.24     | -64.61-32.39                           | 52.07       | ≤ 0.001 |
| Dodonaea viscosa (L.) Jacq.       | 48                   | 22.51    | -55.11-41.97                           | 17.49       | 0.012   |
| s Olea ferruginea Wall. ex Aitch. | 48                   | 21.30    | -2.12-32.91                            | 46.10       | 0.001   |
| v                                    | 72                   | 14.62    | -29.37-33.02                           | 45.71       | 0.001   |

*ince the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits. DF = degree of freedom; NC = not calculable.
Table 3. Median lethal time (LT₅₀) values of different acetone extracts of Soon valley flora bioassayed against freshly molted adults of cotton aphid (Aphis gossypii)

| Treatments            | Botanical conc. (%) | LT₅₀ (h) | Lower and Upper 95% Fiducial Limits (h) | X² (DF = 10)* | P-value |
|-----------------------|---------------------|----------|----------------------------------------|---------------|---------|
| Maerua arenaria H & Thom. | 20                  | 80.88    | 72.35-93.52                            | 35.85         | 0.02    |
|                       | 40                  | 70.16    | 58.43-93.37                            | 96.97         | ≤ 0.001 |
| Mentha longifolia (L.) Huds. | 20                  | 41.29    | 37.63-45.06                            | 29.76         | 0.012   |
|                       | 40                  | 21.63    | 16.03-26.18                            | 25.33         | 0.028   |
| Nerium indicum Mill.  | 20                  | 42.47    | 35.19-50.49                            | 58.94         | ≤ 0.001 |
|                       | 40                  | 21.63    | 10.56-29.21                            | 60.37         | ≤ 0.001 |
| Rhamnus smithi Greene | 20                  | 51.90    | 48.71-55.49                            | 28.81         | 0.15    |
|                       | 40                  | 30.55    | 26.00-34.65                            | 64.08         | ≤ 0.001 |
| Datura alba L.        | 20                  | 90.19    | 78.09-110.94                           | 59.75         | ≤ 0.001 |
|                       | 40                  | 86.16    | 69.68-126.09                           | 129.43        | ≤ 0.001 |
| Periploca aphylla Decne. | 20                  | 79.35    | 65.92-107.27                           | 102.96        | ≤ 0.001 |
|                       | 40                  | 59.87    | 49.81-77.61                            | 101.16        | ≤ 0.001 |
| Sonchus asper (L.) Hill | 20                  | 90.51    | 73.84-128.55                           | 103.87        | ≤ 0.001 |
|                       | 40                  | 53.02    | 46.06-62.48                            | 52.03         | ≤ 0.001 |
| Salvia officinalis L. | 20                  | 38.93    | 34.72-43.14                            | 27.85         | 0.018   |
|                       | 40                  | 17.73    | 8.81-24.14                             | 57.99         | ≤ 0.01  |
| Dodonaea viscosa (L.) Jacq. | 20                  | 60       | 51.87-72.54                            | 25.22         | 0.028   |
|                       | 40                  | 21.09    | 17.28-24.37                            | 28.22         | 0.016   |
| Olea ferruginea Wall. ex Aitch. | 20                  | 43.17    | 39.21-47.39                            | 25.28         | 0.028   |
|                       | 40                  | 20.05    | 11.69-26.22                            | 44.63         | ≤ 0.001 |

*Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits. DF = degree of freedom; NC = not calculable.
4. Discussion

This study determined the bioactivity of acetonic extracts of 40 indigenous plant species of Soon valley and surrounding Salt Range of Pakistan against D. citri and A. gossypii. Among all 40 botanical extracts, overall the extracts of M. longifolia, M. officinalis and N. indicum and extracts of S. officinalis, D. viscosa and O. ferruginea proved to be most toxic against D. citri and A. gossypii, respectively, exhibiting minimum LC50 and LT50 values. The observed mortality of citrus psyllid by M. longifolia, M. officinalis and N. indicum and extracts of S. officinalis, D. viscosa and O. ferruginea would be due to diverse terpenoids and phenolic compounds present in these plant extracts [11–13]. In our study, M. longifolia exhibited about 90% mortality of D. citri and 58% mortality of A. gossypii. The extracts of this plant species have been demonstrated to constitute of such bioactive compounds as flavonoids, phenol, saponins, tannin and terpenoids [14,15] that might be responsible for the observed psyllid and aphid mortality in this study. Similarly, D. alba, S. officinalis and N. indicum extracts is reported to have chemicals such as flavonoids, phenol, saponins, tannin and terpenoids that might be responsible for significant psyllid mortality recorded in this study [15–18]. Similarly, D. viscosa and O. ferruginea plant extracts constitute such phytochemicals as lupeol, stimgasterols, diterpenoids, flavonol-3-methyl ethers and certain fatty acids which have been demonstrated to show bioactivity against different insect pests including lepidopterous [19–20], coleopterous [21] and homopterous pests [22].

5. Conclusions

In brief, the extracts of M. longifolia, M. officinalis, N. indicum, D. alba and S. officinalis are found relatively toxic to D. citri, while the extracts of S. officinalis, D. viscosa, O. ferruginea and M. longifolia appeared effective against A. gossypii, suggesting their incorporation in future integrated pest management for sucking insect pests. Moreover, the biochemical characterization of these plant extracts in order to find out their bioactive constituents responsible for the observed psyllid and aphid mortality and the laboratory and field evaluation of these plant extracts against natural enemies (insect predators and parasitoids) constitute important future perspective of this research work.

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