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

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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.) Pall., *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 LC_{50} values of 18.59, 20.27 and 20.73%, respectively. Similar trend of toxicity was observed in case of their LT_{50} 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

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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 (LC_{50}) and time (LT_{50}) 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 ($F = 44.82$; $P \leq 0.01$). 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 ($F = 181.30$; $P \leq 0.01$). 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 (LC_{50}) and median lethal time (LT_{50}) values were calculated for the botanical extracts. According to probit analysis, *S. officinalis* was the most effective at 48 h ($LC_{50} = 18.59\%$), followed by the extract of *N. indicum* ($LC_{50} = 20.27\%$) and *M. longifolia* ($LC_{50} = 20.73\%$), while the extracts of *S. officinalis*, *M. longifolia* and *N. indicum* showed minimum LC_{50} 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 (LT_{50}) values. The 40% extracts of *S. officinalis* and *O. ferruginea* showed minimum LT_{50} values (i.e. 17.73 and 20.05 %, respectively) (Table 3).

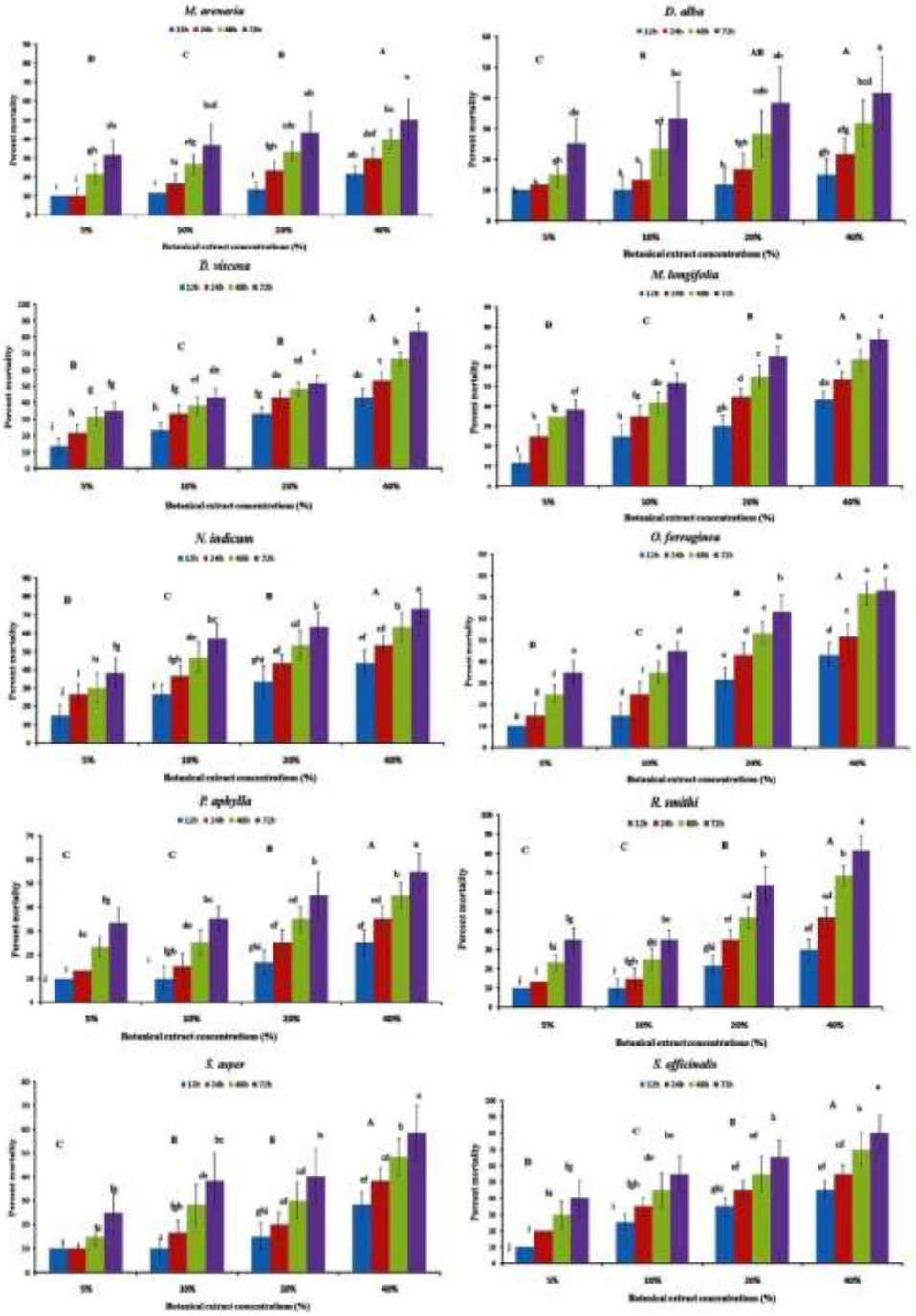

Figure 1. Percent mortality (mean ± SE; n = 10) of cotton aphid (*A. gossypii*) individuals exposed to different concentrations of botanical extracts observed at different post-exposure time intervals. For each botanical extract, small alphabets indicate statistical difference among time intervals for each concentration, while capital alphabets are indicating the statistical difference among different concentrations (one-way factorial ANOVA; HSD at $\alpha = 0.05$).

Table 2. Median lethal concentration (LC_{50}) values of different acetone extracts of Soon valley flora bioassayed against freshly molted adults of cotton aphid (Aphis gossypii)

Treatments	Observation time (h)	LC_{50} (%)	Lower and Upper 95% Fiducial Limits (%)	X^2 (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
	72	27.61	19.66-32.07	27.63	0.015
* *Olea ferruginea* Wall. ex Aitch.	48	21.50	-2.12-32.91	46.10	0.001
S	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 (LT_{50}) values of different acetone extracts of Soon valley flora bioassayed against freshly molted adults of cotton aphid (Aphis gossypii)

Treatments	**Botanical conc. (%)**	**LT_{50} (h)**	**Lower and Upper 95% Fiducial Limits (h)**	**X^2 (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 LC_{50} and LT_{50} values. The observed mortality of citrus psyllid by *M. longifolia*, *M. officinalis* and *N. indicum* 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. indicuum* 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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References

1. Ashfaq, S.; Khan, I.A.; Saeed, M.; Saljoqi, A.U.R.; Manzoor, F., Sohail, K., Sadozai, A. Population dynamics of insect pests of cotton and their natural enemies. *SJA* **2011** 27 2, 251-253.
2. Mahmood, R.; Rehman, A.; Ahmad, M. Prospects of biological control of citrus insect pests in Pakistan. *Pakistan J Agri. Res* **2014** 52 2, 229-244.
3. Deng, F.; Sun, J.; Dou, R.; Yu, X.; Wei, Z.; Yang, C.; Zhu, L. Contamination of pyrethroids in agricultural soils from the Yangtze River Delta, China. *Sci. Total Environ* **2020** 731, 139-181.
4. Tiwari, S.; Mann, R.S.; Rogers, M.E.; Stelinski, L.L. Insecticide resistance in field populations of Asian citrus psyllid in Florida. *Pest Manage Sci* **2011** 67 10, 1258-1268.
5. Naeem, A.; Freed, S.; Jin, F.L.; Akmal, M.; Mehmood, M. Monitoring of insecticide resistance in *Diaphoria citri* Kuwayama (Hemiptera: Psyllidae) from citrus groves of Punjab, Pakistan. *Crop Protec* **2016** 86, 62-68.

6. Desneux, N.; Decourtye, A.; Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol* **2007** 52, 81-106.
7. Haddi, K.; Turchen, L.M.; Viteri Jumbo, L.O.; Guedes, R.N.; Pereira, E.J.; Aguiar, R.W.; Oliveira, E.E. Rethinking biorational insecticides for pest management: unintended effects and consequences. *Pest Manage Sci* **2020** 76 7, 2286-2293.
8. Kim, S.I.; Roh, J.Y.; Kim, D.H.; Lee, H.S.; Ahn, Y.J. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *J. Stored Prod. Res* **2003** 39 3, 293-303.
9. Isman, M.B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol* **2006** 51, 45-66.
10. Abbot, W.S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol* **1925** 18 2, 265–267.
11. Odeyemi, O.O.; Masika, P.; Afolayan, A.J. Insecticidal activities of essential oil from the leaves of *Mentha longifolia* L. against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Afr Entomol* **2008** 16 2, 220-225.
12. EL-Kamali, H.H. Effect of certain medicinal plants extracts against storage pest, *Tribolium castaneum* Herbst. *AEJSA* **2009** 3 2, 139-142.
13. Hussain, J.; Muhammad, Z.; Ullah, R.; Khan, F.U.; Khan, I.U.; Khan, N.; Jan, S. Evaluation of the chemical composition of *Sonchus eruca* and *Sonchus asper*. *Am. J. Sci* **2010** 6 9, 231-235.
14. Lee, S.E.; Lee, B.H.; Choi, W.S.; Park, B.S.; Kim, J.G.; Campbell, B.C. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L). *Pest Manage Sci* **2001** 57 6, 548-553.
15. Govindappa, M.; Poojashri, M.N. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. *J. Pharmacogn Phytotherapy* **2011** 3 3, 43-51.
16. Bhuvaneshwari, L.; Arthy, E.; Anitha, C.; Dhanabalan, K.; Meena, M. Phytochemical analysis and antibacterial activity of *Nerium oleander*. *Anc. Sci. Life* **2007** 26 4, 24-29.
17. Tomczyk, A.; Suszko, M. The role of phenols in the influence of herbal extracts from *Salvia officinalis* L. and *Matricaria chamomilla* L. on two-spotted spider mite *Tetranychus urticae* Koch. *Biol. Lett* **2011** 48 2, 193-205.
18. Uddin, G.; Rauf, A.; Akhtar, S. Studies on chemical constituents, phytochemical profile and pharmacological action of *Datura alba*. *MEJMPR* **2012** 1 1, 14-18.
19. Malarvannan, S.; Giridharan, R.; Sekar, S.; Prabavathy, V.R.; Nair, S. Ovicidal activity of crude extracts of few traditional plants against *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). *J. Biopestic* **2009** 2 1, 64-71.
20. Mohammed, A.K.; Nawar, M.H. Study of the effect of alcoholic extract of *Dodonaea viscosa*. leaves on the life performance of the greater wax worm *Galleria mellonella* L. (lepidoptera: pyralidae). *Plant Arc* **2020** 20, 3449-3454.
21. Dimetry, N.Z.; El-Gengaihi, S.; Hafez, M.; Abbass, M.H. Pesticidal activity of certain plant extracts and their isolates against the cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae). *Herba Pol* **2015** 61 3, 77-92.
22. Díaz, M.; Díaz, C.E.; Álvarez, R.G.; González, A.; Castillo, L.; González-Coloma, A.; Rossini, C. Differential anti-insect activity of natural products isolated from *Dodonaea viscosa* Jacq. (Sapindaceae). *J Plant Prot Res* **2015** 55 2, 172-178.