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Biocidal Potential of Indigenous Flora of Soon Valley (Khushab, Pakistan) against *Helicoverpa armigera* Hübner and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) †

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Abstract: *Helicoverpa armigera* Hübner and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) are destructive pests of agricultural and horticultural crops. Excessive use of synthetic chemicals has created harmful impacts on non-target organisms and environment. This study was aimed to assess the insecticidal potential of acetone extracts of 40 indigenous plant species of Soon valley (Khushab, Punjab, Pakistan) against the 3rd instar larvae of *H. armigera* and *S. litura* using leaf-dip bioassay method. Results revealed that maximum mortality of *H. armigera* larvae was caused by the extracts of *Dodonaea viscosa* L. (88%), *Olea ferruginea* Wall. ex Aitch. (69%), *Mentha longifolia* (L.) Huds. (57%) and *Salvia officinalis* L. (52.22%), while the extracts of *S. officinalis*, *D. viscosa*, *O. ferruginea*, *Sonchus asper* (L.) Hill and *Nerium indicum* Mill. caused significant mortality (*i.e.* 70 to 90%) of *S. litura* larvae and exhibited minimum LC_{50} and LT_{50} values. These results propose the potential efficiency of indigenous flora against lepidopterous pests and there is a need of further biochemical characterization of these plant extracts.

Keywords: Lepidopterous pests; Botanical pesticides; *In-vitro* Toxicity; Salvia officinalis; *Salvia officinalis*; *Nerium indicum*; *Sonchus asper*; *Dodonaea viscosa*

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1. Introduction

Insect pests have always been an inevitable threat to global food production causing ubiquitously untold damage to different agricultural, horticultural and forest crops. *Helicoverpa armigera* Hübner and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) are destructive lepidopterous pests [1,2]. Farmers predominantly rely on the synthetic insecticides to combat the infestation of *H. armigera* and *S. litura*, which is manifesting many non-target effects such as the development of pesticide resistance, eradication of beneficial fauna including insect parasitoids and predators, and human health hazards [3–5]. These ecological drawbacks of synthetic insecticides necessitate looking for novel biorational insect pest management approaches which would be safer and environment-friendly than the synthetic chemical pesticides such as plant-based pesticides [6]. Furthermore, plant-based insecticides usually have low mammalian toxicity and reduced persistency in the environment as compared to conventional synthetic pesticides [7,8].

Native flora of any biogeographical region would contain specific phyto-constitutes potentially effective against indigenous pest species [9]. Soon valley is situated in district Khushab (Punjab, Pakistan) and is highly enriched in flora of ethnomedicinal value [10]. In this study, the insecticidal potential of this local flora (herbs, shrubs and trees) of Soon valley and surrounding salt range of Pakistan was explored against the above mentioned lepidopterous pests.

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

Larvae of *H. armigera* and *S. litura* were manually collected from the fields of berseem (*Trifolium alexandrinum* L.) and sunflower (*Helianthus annuus* L.), respectively, and were reared on respective plant leaves in plastic jars under controlled lab conditions at 27±3°C temperature, 60±5% relative humidity and 16h L: 8h D photoperiod. Healthy and active 3rd instar larvae of F_3 generation of both insect species were used in all toxicity bioassays.

3.3. Insecticidal Bioassays

Leaf-dip bioassay method was used in which leaf discs treated with different plant extract solutions were fixed on 2 mm layer of 1.5% agar in glass 90 mm Petri plates and after release of 10 3rd instar F_3 generation larvae on each leaf disc, these were incubated in a controlled chamber (at 26±2ºC, 60±5% RH and 16:8 L: D photoperiod). Three to six independent replicates were maintained for each treatment. Data regarding mortality of exposed larvae was recorded at 24, 48 and 72 h post-treatment. Moribund insects, which did not show any movement upon touching with camel hair brush, were considered as dead.

3.4. Statistical Analysis

Statistical interpretation of data was done using software Statistix 8.1® (Analytical Software, Tallahassee, Florida). Apart from graphical presentation, mortality data were analyzed by factorial analysis of variance (ANOVA) taking botanical solutions and time intervals as factors. Treatment means were compared using honestly significant different (HSD) test at 95% probability level ($p \leq 0.05$). Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were calculated by probit analysis using IBM SPSS® (Version 20) statistics regression software. Prior to probit analysis, mortality was corrected using Abbott's formula [11].

3. Results

Results of preliminary screening bioassay revealed that some botanical extracts caused significant mortality of 3rd instar larvae of *H. armigera* ($p \leq 0.05$). Maximum larval mortality was observed in case of *D. viscosa* (88%), *O. ferruginea* (69%), *M. longifolia* (58%) and *S. officinalis* (52%). Second bioassay conducted with 10 most effective botanical extracts recorded from the first bioassay revealed a differential response of *S. litura* larvae against different plant extracts. According to results, all plant extracts exhibited considerable mortality of S. litura larvae and this mortality response was concentration and exposure time dependent as it increased along with the increase of concentration of botanicals and exposure time (Figure 1). The extracts of *S. officinalis*, *D. viscosa*, *O. ferruginea*, *S. asper* and *N. indicum* caused significant mortality (i.e. 70 to 90%) of *S. litura* larvae.

Probit analysis demonstrated the similar trend of toxicity of botanical extracts against S. litura larvae. The most effective extract was of S. officinalis (LC_{50} = 0.11 and 1.04 % at 48 and 72 h post-treatment, respectively), followed by D. viscosa (LC_{50} = 12.25%) and *O. ferruginea* (LC_{50} = 14.79%) at 72 h of application (Table 2). Least significant effect was observed in the case of M. arenaria. In case of medial lethal time (LT_{50}) values, N. indicum (LT_{50} = 22.82 h) and O. ferruginea (LT_{50} = 25.44 h) were the most toxic at 40% concentration. Least significant effect was observed in the case of M. arenaria (Table 3).

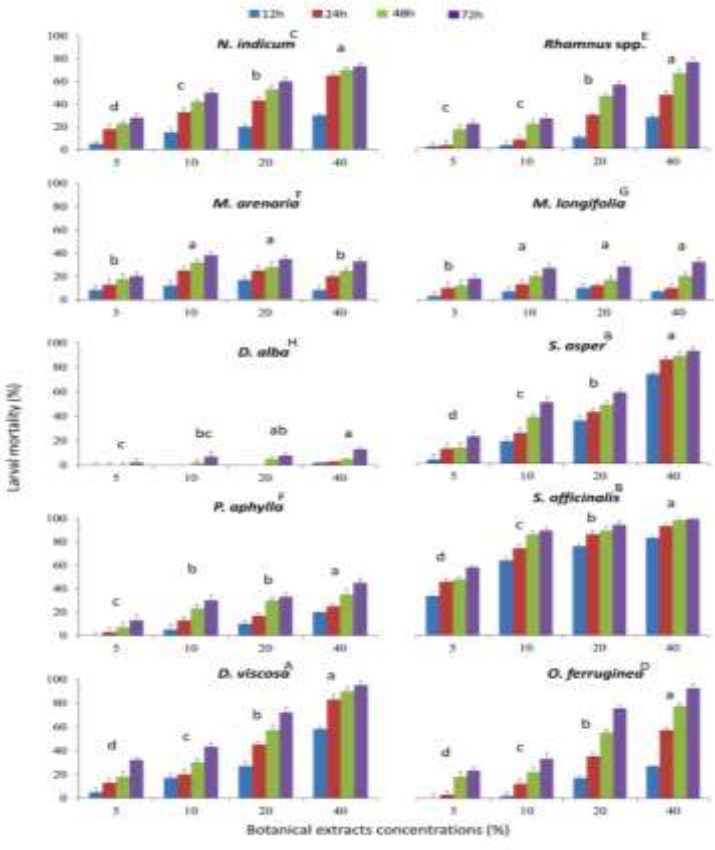

Figure 1. Percent mortality (mean ± SE; n = 10) of 3rd instar larvae of *Spodoptera litura* exposed to different concentrations of botanical extracts observed at different post-exposure time intervals. Capital alphabets indicate the overall statistical difference among the plant extracts (two-factor factorial ANOVA; HSD at α = 0.05), while small alphabets indicate the statistical difference among different concentrations of each botanical extract (one-way ANOVA; HSD at α = 0.05).

Table 2. Median lethal concentration (LC_{50}) values of different acetone extracts of Soon valley flora bioassayed against 3rd instar larvae of Spodoptera litura.

Treatments	**Observation time (h)**	**LC_{50} (%)**	**X^2 (DF = 10)***	**P-value**
Maerua arenaria H & Thom.	48	NC	113.622	< 0.05
	72	NC	120.401	< 0.05
Mentha longifolia (L.) Huds.	48	NC	126.497	< 0.05
	72	90.405	85.521	< 0.05
Nerium indicum Mill.	48	21.059	72.056	< 0.05
	72	15.936	104.924	< 0.05
Rhamnus smithi Greene	48	27.407	119.073	< 0.05
	72	21.421	125.277	< 0.05
Datura alba L.	48	NC	158.925	< 0.05
	72	93.812	169.316	< 0.05
Periploca aphylla Decne.	48	53.178	173.349	< 0.05
	72	43.241	137.701	< 0.05
Sonchus asper (L.) Hill	48	19.533	137.485	< 0.05
	72	14.903	114.149	< 0.05
Salvia officinalis L.	48	0.108	173.008	< 0.05
	72	0.104	178.643	< 0.05
Dodonaea viscosa (L.) Jacq.	48	18.488	137.104	< 0.05
	72	12.248	89.681	< 0.05
Olea ferruginea Wall. ex Aitch.	48	22.713	126.258	< 0.05
s	72	14.799	162.426	< 0.05

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 3rd instar larvae of Spodoptera litura.

Treatments	**Botanical conc. (%)**	**LT_{50} (h)**	**X^2 (DF = 10)***	**P-value**
Maerua arenaria H & Thom.	20	115.58	89.83	< 0.05
	40	101.84	162.75	< 0.05
Mentha longifolia (L.) Huds.	20	106.13	129.51	< 0.05
	40	99.22	170.28	< 0.05
Nerium indicum Mill.	20	48.99	112.94	< 0.05
	40	22.82	163.86	< 0.05
Rhamnus smithi Greene	20	58.41	123.13	< 0.05
	40	32.12	96.84	< 0.05
Datura alba L.	20	122.74	126.64	< 0.05
	40	122.52	153.38	< 0.05
Periploca aphylla Decne.	20	97.28	138.15	< 0.05
	40	81.77	77.87	< 0.05
Sonchus asper (L.) Hill	20	50.27	67.43	< 0.05
	40	39.60	-	< 0.05
Salvia officinalis L.	20	38.86	-	< 0.05
	40	NC	-	-
Dodonaea viscosa (L.) Jacq.	20	38.86	-	< 0.05
	40	NC	-	-
Olea ferruginea Wall. ex Aitch.	20	44.00	111.60	< 0.05
	40	25.44	133.71	< 0.05

*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

In preliminary screening of 40 indigenous plants, some extracts, particularly of *D. viscosa*, *O. ferruginea*, *M. longifolia* and *S. officinalis* expressed significant toxicity against H. *armigera*, while in second bioassay the extracts of *S. officinalis*, *D. viscosa*, *S. asper* and *O. ferruginea* exhibited maximum mortality of *S. litura*. These findings are consistent with many previous studies documenting the contact and oral toxicity, anti-feedant, larvicidal and ovicidal effects of different plant extracts and essential oils against lepidopterous pests including *H. armigera* and *S. litura* [12–21].

Extracts of *D. viscosa* usually constitute such chemicals as flavonoids, certain fatty acids, diterpenoids, lupeol and stimgasterols which have been evidenced to exhibit bio-activity against a number of insect pest species including homopterous **[**22], coleopterous 23] and lepidopterous pests [24,25]. Likewise, plant species of Oleaceae family contain many toxic phytochemicals putatively effective against different insect pests. Extracts of *O. europaea* for example contain higher triterpene (maslinic acid) and phenolic contents showing considerable toxicity against stored grain insect pests (*Tribolium confusum* and *Sitophilus granaries*) and aphids (*Brevicoryne brassicae* and *Schizaphis graminum*) [26,27]. Moreover, our results corroborate the findings of Zavala-Sánchez et al. [28], Polatoğlu et al. [29] and Sharaby and Nujiban [30] showing the insecticidal potential of different Salvia species including *S. officinalis* against armyworms *S. frugiperda*, *S. exigua* and *Agrotis ipsilon*, respectively.

5. Conclusions

Based on overall study results, it is concluded that the extracts of *S. officinalis*, *D. viscosa*, *O. ferruginea*, *S. asper* and *N. indicum* exhibited significant toxicity potential against both lepidopterous pests. These findings suggest the incorporation of these plants extracts in future management of *H. armigera*, S. litura and other lepidopterous pests. Nevertheless, the biochemical characterization of these plant extracts in order to find out their bioactive constituents responsible for the observed larval mortality and the laboratory and field evaluation of these plant extracts against natural enemies (insect predators and parasitoids) constitute important future perspectives of this research work.

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