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. These polyphagous species have a cosmopolitan distribution worldwide. Synthetic insecticides are primarily used to control these lepidopterous pests. Excessive use of synthetic chemicals has created harmful impacts on non-target organisms and environment. Plant-based insecticides have been evidenced as important alternatives to conventional synthetic insecticides. 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 some botanical extracts exhibited significant toxicity against both lepidopterous larvae and their response was time and botanical concentration dependent. Initial screening bioassay revealed that the highest mortality of 3rd instar H. armigera larvae was caused by 10% extracts of Dodonaea viscosa L. (88%), followed by Olea ferruginea Wall. ex Aitch. (69%), Mentha longifolia (L.) Huds. (57%) and Salvia officinalis L. (52.22%). Further toxicity bioassay, conducted against 3rd instar larvae of S. litura with different concentrations of 10 most effective plant extracts, demonstrated that 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 LC50 and LT50 values. Overall study results demonstrated the insecticidal potential of these indigenous plant species of Soon valley against H. armigera and S. litura larvae, and suggest their further biochemical characterization and practical implication in the future management programs against these and other lepidopterous pests.

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. These are polyphagous pest species of cosmopolitan distribution, causing severe losses to agricultural production worldwide (Liu et al., 2004; Bragard et al., 2019). Both of these species are notorious pests of economic importance and have a wide range of host plants including fruits, vegetables, ornamental and agronomic crops (Armes et al., 1996; Bragard et al., 2019; Abbas et al., 2020).

 Farmers predominantly rely on the synthetic insecticides to combat the infestation of H. armigera and S. litura. Many non-target effects are being manifested by the extensive use of these synthetic chemicals such as the development of pesticide resistance, eradication of beneficial fauna including insect parasitoids and predators, and human health hazards (Isman, 2006; Desneux et al., 2007; Halstead et al., 2015; Naeem et al., 2016; Kim et al., 2017; Dhananjayan et al., 2020; Hadi et al., 2020). Moreover, many strains and field populations of H. armigera and S. litura have shown high resistance level
against insecticides of almost all major chemical groups (Shad et al., 2012; Tong et al., 2013; Walsh et al., 2018).

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. Plant-based pesticides for example appear as promising substitute of synthetic pesticides for insect pest management (Copping and Menn, 2000; Isman, 2006). Since last few decades, a number of studies have demonstrated the effectiveness of extracts and essential oils of many plant species against a wide range of sucking and chewing insect pests including *H. armigera* and *S. litura* (Rossetti et al., 2008; Regnault-Roger et al., 2012; Javed et al., 2018; Majeed et al., 2020). Plants belonging to families such as Meliaceae, Asteraceae, Piperaceae, Labiatae, Rutaceae and Annonaceae are found containing many effective phyto-constituents potentially capable of affecting insect behavior, development, molting and growth (Celis et al., 2008; de Cássia et al., 2010). Furthermore, plant-based insecticides usually have low mammalian toxicity and reduced persistency in the environment as compared to conventional synthetic pesticides (Turek and Stintzing, 2013; Isman et al., 2007; Isman, 2020).

Native flora of any biogeographical region would contain specific phyto-constituents potentially effective against indigenous pest species (Isman, 2006). Soon valley is situated in district Khushab (Punjab, Pakistan) and is highly enriched in flora of ethnomedicinal value (Ahmad et al., 2007). For instance, *Acacia modesta*, *A. farenesiana*, *A. nilotica*, *Achyanthus aspara*, *Albizia lebbeck*, *Amaranthus viridis*, *Buxus papilloosa*, *Calotropis procera*, *Capparis deciduas*, *Chenopodium album*, *Datura alba*, *Fumaria indica*, *Justice adhatoda*, *Mentha longifolia*, *M. royleana*, *Mellilotus alba*, *Olea ferruginea* and *Peganum hernala* are some of the important medicinal plant species found in Soon valley (Ahmad et al., 2009). 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.

**MATERIALS AND METHODS**

The study was conducted in the Department of Entomology, College of Agriculture, University of Sargodha to evaluate the insecticidal potential of indigenous flora of Soon valley and surrounding salt range of the Punjab province of Pakistan against two most destructive lepidopterous pests, *i.e.*, *H. armigera* and *S. litura*.

**Rearing of H. armigera and S. litura**

Larvae of *H. armigera* and *S. litura* were collected from the fields of berseem (*Trifolium alexandrinum* L.) and sunflower (*Helianthus annuus* L.), respectively, and were brought to the Laboratory of Entomology, College of Agriculture University of Sargodha. Cultures were kept under controlled conditions at 25±2°C, 60±5% RH and 16:8 (L: D) photoperiod. Plastic jars of different sizes were used for rearing purposes. Castor (*Ricinus communis*) leaves were fed to the larvae of *S. litura*, while *H. armigera* larvae were reared on the flowering parts of *T. alexandrinum*. Pupae were kept individually in Petri plates (diameter 60 mm) over moist filter paper discs and then after emergence, adults were fed on 10% honey solution and were kept in separate plastic cages. Eggs of *H. armigera* and *S. litura* were collected from cages and kept in Petri plates on moist filter paper discs till hatching and newly hatched larvae were shifted to fresh leaf discs. Healthy and active 3rd instar larvae of *F.* generation of both insect species were used in all toxicity bioassays.

**Plant collection**

Samples of indigenous plants (including herbs, shrubs and trees) were randomly collected from six different ecological sites of Soon valley situated in between 71°55'0.9'' E longitude and 32°33'2.5'' N latitude in the north-west of district Khushab (Punjab, Pakistan). These sampling sites were Angah, Dape Sharif, Kenhatti Garden, Khabeki, Khoora and Uchhali Garden (Table I; Supplementary Fig. 1). Plant samples were brought to the laboratory and were identified up to species level by experts from the Department of Botany, University of Sargodha. Collected plant samples were washed twice with tap-water and shade-dried at ambient room temperature (28°C) for a week and then were ground using electrical blender (750W; TCB-318). These powdered plant materials were stored in zip-lock bags, labeled and kept in refrigerator at 4°C until their extraction.

**Table I. 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           |
**Plant extraction**

Extraction of powdered plant samples was carried out using Soxhlet apparatus (DH.WHM-12393, Daihan Scientific, South Korea). Acetone was used as a solvent for the extraction. Fifty gram of each powdered sample was placed in a thimble and plugged with cotton. Heating flask was filled with 500 mL of extraction solvent (acetone). Extraction of each sample was done for about 4 to 6 h. Soxhlet apparatus extracted samples were further purified individually using the rotary evaporator (WEV-1001L, Daihan Scientific, South Korea) fitted with a vacuum pump and chiller. 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.

**Toxicity bioassays**

Leaf-dip bioassay method was used in the preliminary screening of insecticidal potential of 40 local plant extracts against the larvae of *H. armigera*. Discs made from the freshly clipped and rinsed leaves of *R. communis* were dipped in 10% acetone extracts for 3 min and then were fixed on 2 mm layer of 1.5% agar in glass Petri plates (diameter 90 mm) and were left to dry for 30 min. Ten healthy and active 3rd instar *F*₃ generation larvae were released on each leaf disc and these Petri plates were incubated in a controlled chamber (at 26±2ºC, 60±5% RH and 16:8 L: D photoperiod). Three independent replicates were maintained for each treatment. Data regarding mortality of exposed *H. armigera* 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. Ten most effective botanical extracts recorded from the previous screening trial were further bioassayed against the larvae of *S. litura*. Four different concentrations (*i.e.*, 5, 10, 20 and 40%) were used in this bioassay following the same protocol as described above. However, six replicates per treatment were maintained in this bioassay.

**Data 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 ≤ 0.05). Median lethal concentration (LC₅₀) and median lethal time (LT₅₀) were calculated by probit analysis using IBM SPSS® (Version 20) statistics regression software. Prior to probit analysis, mortality was corrected using Abbott’s formula (Abbott, 1925).

**RESULTS**

Results of preliminary screening bioassay revealed that some botanical extracts caused significant mortality of 3rd instar larvae of *H. armigera* (*P* ≤ 0.05). Maximum larval mortality was observed in case of *D. viscosa* (88%), followed by *O. ferruginea* (69%), *M. longifolia* (58%) and *S. officinalis* (52%). All other plant extracts did not show any significant mortality during this preliminary screening (Supplementary Table I).

![Fig. 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).](image)
this mortality response was concentration and exposure time dependent as it increased along with the increase of concentration of botanicals and exposure time (Fig. 1).

After 12 h of exposure, maximum mortality of armyworm larvae was depicted by 40% concentration of *S. officinalis* (82%), followed by *S. asper* (73%) and *D. viscosa* (58%), while the minimum *S. litura* mortality was observed by the extracts of *D. alba*, *P. aphylla* and *M. arenaria* (Fig. 1).

At 24 h post-treatment, the extract of *S. officinalis* was more effective with maximum mortality (89%) of *S. litura* larvae, followed by *S. asper* (85%) and *O. ferruginea* (57%). At 48 h post-treatment, the extract of *S. officinalis* was more effective with maximum mortality (89%) of *S. litura* larvae, followed by *S. asper* (85%) and *O. ferruginea* (57%). At 72 h post-treatment, maximum mortality was recorded in case of *S. officinalis* (92%), followed by *D. viscosa* (90%), *S. asper* (88%) and *O. ferruginea* (77%). Similar trend was recorded at 72 h post-treatment against 3rd instar larvae of *S. litura*. Moreover, minimum mortality was examined by 5% extract of *D. alba* (8%) and maximum mortality was recorded by 40% extract of *S. officinalis* (98%).

Probit analysis demonstrated similar trend of toxicity of botanical extracts against *S. litura* larvae. The most effective extract was of *S. officinalis* (LC50 = 0.11 and 1.04% at 48 and 72 h post-treatment, respectively), followed by *D. viscosa* (LC50 = 12.25%) and *O. ferruginea* (LC50 = 14.79%) at 72 h of application (Table II). Least significant effect was observed in case of *M. arenaria*. In case of median lethal time (LT50) values, *N. indicum* (LT50 = 22.82 h) and *O. ferruginea* (LT50 = 25.44 h) were the most toxic at 40% concentration. Least significant effect was observed in the case of *M. arenaria* (Table II).

**DISCUSSION**

Naturally occurring plant compounds are abundant having certain type of defensive properties. Different plant parts such as leaves, roots, flowers, fruits and bark have been used in crude form as conventional insecticidal tools for centuries (Isman, 2006, 2020). Anti-insect activities of these phytoextracts are usually due to complex combinations of their different bioactive constituents. For the determination of potential plants with such insecticidal properties, preliminary screening is a good source of evaluation (Copping and Menn, 2000).

**Table II. Median lethal concentration (LC50) and median lethal time (LT50) values of different acetone extracts of Soon valley flora bioassayed against 3rd instar larvae of *Spodoptera litura*.

| Treatments                  | Observation time (h) | LC50 (%) | X2 (DF = 10)* | P value | Observation time (h) | LT50 (h) | X2 (DF = 10)* | P value |
|-----------------------------|----------------------|----------|---------------|---------|----------------------|-----------|---------------|---------|
| *Maerua arenaria* Hook & Thomson | 48                   | NC       | 113.622       | < 0.05  | 20                   | 115.58    | 89.83         | < 0.05  |
|                            | 72                   | NC       | 120.401       | < 0.05  | 40                   | 162.75    | < 0.05        |         |
| *Mentha longifolia* (L.) Huds. | 48                   | NC       | 126.497       | < 0.05  | 20                   | 129.51    | < 0.05        |         |
|                            | 72                   | 90.405   | 85.521        | < 0.05  | 40                   | 170.28    | < 0.05        |         |
| *Nerium indicum* Mill.      | 48                   | 21.059   | 72.056        | < 0.05  | 20                   | 112.94    | < 0.05        |         |
|                            | 72                   | 15.936   | 104.924       | < 0.05  | 40                   | 163.86    | < 0.05        |         |
| *Rhamnus smithi* Greene     | 48                   | 27.407   | 119.073       | < 0.05  | 20                   | 123.13    | < 0.05        |         |
|                            | 72                   | 21.421   | 125.277       | < 0.05  | 40                   | 96.84     | < 0.05        |         |
| *Datura alba* L.            | 48                   | NC       | 158.925       | < 0.05  | 20                   | 126.64    | < 0.05        |         |
|                            | 72                   | 93.812   | 169.316       | < 0.05  | 40                   | 153.38    | < 0.05        |         |
| *Periploca aphylla* Decne.  | 48                   | 53.178   | 173.349       | < 0.05  | 20                   | 138.15    | < 0.05        |         |
|                            | 72                   | 43.241   | 137.701       | < 0.05  | 40                   | 77.87     | < 0.05        |         |
| *Sonchus asper* (L.) Hill   | 48                   | 19.533   | 137.485       | < 0.05  | 20                   | 67.43     | < 0.05        |         |
|                            | 72                   | 14.903   | 114.149       | < 0.05  | 40                   | 39.60     | < 0.05        |         |
| *Salvia officinalis* L.     | 48                   | 0.108    | 173.008       | < 0.05  | 20                   | 38.86     | < 0.05        |         |
|                            | 72                   | 0.104    | 178.643       | < 0.05  | 40                   | NC        | -             |         |
| *Dodonaea viscosa* (L.) Jacq. | 48                   | 18.488   | 137.104       | < 0.05  | 20                   | 38.86     | < 0.05        |         |
|                            | 72                   | 12.248   | 89.681        | < 0.05  | 40                   | NC        | -             |         |
| *Olea ferruginea* Wall. ex Aitch. | 48                   | 22.713   | 126.258       | < 0.05  | 20                   | 111.60    | < 0.05        |         |
|                            | 72                   | 14.799   | 162.426       | < 0.05  | 40                   | 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.
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* larvae. These findings are consistent with many previous studies documenting the contact and oral toxicity, anti-feedant, larvicidal and oviducal effects of different plant extracts and essential oils against lepidopterous pests including *H. armigera* and *S. litura* (Kamaraj et al., 2008; Krishna et al., 2008; Sujatha et al., 2010; Tomczyk and Suszko, 2011; Mir, 2015; Chauhan and Mishra, 2016; Chennaiyan et al., 2016; Kaleeswaran et al., 2017; Benelli et al., 2018; Nobsathian et al., 2019).

Local plant species of the study area (i.e., Soon valley and surrounding salt range of Punjab, Pakistan) including *N. indicum*, *O. ferruginea* and *D. viscosa* are known for their ethnomedical value and for their herbal remedial potential (Simonsen et al., 2001; Hashmi et al., 2015; Shah and Rahim, 2017). Extracts of *D. viscosa* usually constitute such chemicals as flavonoids, certain fatty acids, diterpenoids, lupeol and stigmasterylols which have been evidenced to exhibit bioactivity against a number of insect pest species including homopterous (Díaz et al., 2015), coleopterous (Dimetry et al., 2015) and lepidopterous pests (Malarvannan et al., 2009; Mohammed and Nawar, 2020). 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*) (Yali and Anaya, 2017; Kisa et al., 2018).

Moreover, our results corroborate the findings of Zavala-Sánchez et al. (2013) and Polatoğlu et al. (2017) showing the insecticidal potential of different *Salvia* species including *S. officinalis* against armyworms *S. frugiperda* and *S. exigua*, respectively. Extracts of aerial parts of sage (*S. officinalis*) and thyme (*Thymus vulgaris*) plants showed significant anti-insect effects including larvicidal and antioxidant activities against 4th instar larvae of *S. littoralis*. Similarly, Sharaby and Nujiban (2019) demonstrated that the leave extracts of *S. officinalis* caused significant mortality and feeding deterrence effects on lepidopterous larvae including *Agrotis ipsilon*, *H. armigera* and *S. litura*.

**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 extracts of indigenous plant species in future integrated 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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**Supplementary material**

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210411140405

**Statement of Conflict of Interest**

The authors have declared no conflict of interest.

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Supplementary Material

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

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Supplementary Fig. 1. Sampling sites regarding the collection of indigenous flora of Soon Valley and surrounding Salt Range of Pakistan.

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Supplementary Table I. Percent mortality of 3rd instar larvae of *Helicoverpa armigera* by 10% acetone extracts of different plant species collected from Soon Valley and surrounding salt range of Pakistan.

| Botanicals               | Vernacular name             | Plant part extracted | Mortality*±S.E (%) | Homogenous groups |
|--------------------------|-----------------------------|----------------------|--------------------|-------------------|
| *Acacia melanoxylon* R.Br. | Hickory                     | Leaves               | 0.00±0             | F                 |
| *Adiantum capillus-veneris* L. | Khatti booti                | Leaves               | 0.00±0             | F                 |
| *Alternanthera pungens* Kunth | Phakra                      | Leaves               | 0.00±0             | F                 |
| *Amaranthus viridis* L.  | Jangli cholai              | Leaves               | 0.56±0             | F                 |
| *Astragalus* spp. L.     | Koohni                      | Leaves               | 6.67±0             | EF                |
| *Buxus papillosa* Schneid. | Shamshad                    | Leaves               | 0.00±0             | F                 |
| *Cassia occidentalis* L. | Bana Chakunda               | Leaves               | 5.56±0             | EF                |
| *Cassia occidentalis* L. | Bana Chakunda               | Fruit                | 5.56±2             | EF                |
| *Chenopodium album* L.   | Bathua                      | Leaves               | 3.33±3             | F                 |
| *Cynodon dactylon* (L.) Pers. | Khabal                      | Leaves               | 12.22±1            | DEF               |
| *Datura alba* L.         | Dhatura                     | Leaves/flower        | 3.33±3             | F                 |
| *Dicliptera bupleuroides* Nees | Kaalu                      | Leaves               | 0.00±0             | F                 |
| *Dodonaea viscosa* (L.) Jacq. | Santha                     | Leaves               | 87.78±4            | A                 |
| *Dryopteris filix-mas* (L.) Schott | Male fern                | Leaves               | 1.56±0             | F                 |
| *Eruca sativa* Mill.     | Jamahoon                    | Leaves               | 8.89±4             | EF                |
| *Fagonia indica* Burn.f. | Dhamasa                     | Leaves               | 17.78±4            | DE                |
| *Justicia adhatoda* L.   | Dhodhak booti               | Leaves               | 0.00±0             | F                 |
| *Maerua arenaria* Hook & Thomson | Hemkand                  | Leaves/Stem          | 17.20±4            | F                 |
| *Marrubium vulgare* L.   | Pahari gandana              | Leaves               | 0.00±0             | F                 |
| *Meliotus officinalis* (L.) Pall. | Sweet clove               | Leaves               | 0.00±0             | F                 |
| *Mentha longifolia* (L.) Huds. | Desi podina              | Leaves               | 57.78±3            | BC                |
| *Murraya koenigii* (L.) Spreng. | Jangli curry patta       | Leaves               | 0.00±0             | F                 |
| *Nerium indicum* Mill.   | Kanera                      | Leaves               | 0.00±0             | F                 |
| *Nerium indicum* Mill.   | Kanera                      | Fruit                | 3.33±0             | F                 |
| *Olea ferruginea* Wall. ex Aitch. | Kao                      | Leaves               | 68.89±1            | B                 |
| *Opuntia dillenii* (Kar Gawli) Haw. | Thor                    | Leaves               | 0.00±0             | F                 |
| *Periploca aphylla* Decne.| Bata                        | Stem                 | 0.00±0             | F                 |
| *Petrophytum caespitosum* Rydb. | Mat rock spiraea         | Leaves               | 0.00±0             | F                 |
| *Portulaca oleracea* L.  | Loonak                      | Leaves               | 5.78±0             | EF                |
| *Rhamnus smithii* Greene | Buckthorn                  | Leaves/stem          | 0.00±0             | F                 |
| *Ricinus communis* L.    | Harnoli                     | Leaves               | 0.00±0             | F                 |
| *Rumex dentatus* L.      | Toothed dock               | Leaves               | 0.00±0             | F                 |
| *Salvia officinalis* L.  | Sage                        | Leaves               | 52.22±0            | C                 |
| *Salvia virginata* Jacq. | Meadow sage                | Leaves               | 6.67±1             | EF                |
| *Solanum incanum* L.     | Mahori                      | Leaves               | 0.00±0             | F                 |
| *Solanum surattense* Burm. f. | Kanda kari               | Leaves               | 6.67±3             | EF                |
| *Sonnchus asper* (L.) Hill | Bhattal                    | Leaves               | 11.11±4            | DEF               |
| *Suaeda fruticosa* (L.) Delile | Lahhra                | Leaves               | 23.3±1             | D                 |
| *Trichodesma indicum* (L.) Lehms. | Juri                  | Leaves               | 0.00±0             | F                 |
| *Withania coagulans* (Stocks) Dunal | Khamjeera             | Leaves               | 6.67±3             | EF                |

*a*, mean of three to five independent replications.