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Research Article

Comparative Toxicity of Phyto-Extracts of Indigenous Flora of Soone Valley against some Insect Pests of Agricultural and Urban Importance

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Authors' Contributions
MZM conceived the idea and planned the experiment. MA provided technical assistance. MAR technically revised the manuscript. KSA prepared 1st draft of the manuscript. ML performed statistical analyses. MZS performed experiments on armyworm. MBT performed experiments on Asian citrus psyllid. MT performed experiments on house mosquito. SW performed experiments on subterranean termites.

Keywords
Ethnomedicinal plants, Botanical extracts, Soone valley, Toxicity bioassay, *Diaphorina citri*, *Spodoptera litura*, *Culex quinquefasciatus*, *Odontotermes obesus*

Abstract | This laboratory study encompasses comparative evaluation of insecticidal potential of indigenous ethnomedicinal flora of Soone Valley and surrounding Salt Range of Pakistan. Acetone extracts (10%) of forty plant species were evaluated against Asian citrus psyllid (*Diaphorina citri*), armyworm (*Spodoptera litura*), house mosquito (*Culex quinquefasciatus*) and subterranean termite (*Odontotermes obesus*) using twig-dip, leaf-dip, aqueous exposure and filter paper-dip bioassay methods, respectively. Results revealed that the extracts of *Mentha longifolia*, *Sonchus asper* and *Nerium indicum* were the most toxic to *D. citri* exhibiting 90% mortality. The extracts of *Dodonaea viscosa* and *Olea ferruginea* caused highest mortality of *S. litura* (*i.e.* 70 and 58%, respectively). Maximum mortality of *C. quinquefasciatus* larvae was observed by *Maerua arenaria* (87%), *N. indicum* (84%) and *Withania coagulans* (83%) extracts. While, the most toxic plant extracts against *O. obesus* termites were *Periploca aphylla*, *Rhamnus* spp. and *Buxus papillosa* causing 89, 62 and 52% mortality, respectively. These findings corroborate the effectiveness of indigenous plant extracts as safe and environment friendly alternates to hazardous synthetic insecticides and suggest the incorporation of these natural compounds in the pest management programs against agricultural and urban insect pests.

Novelty Statement | This study encompasses a first extensive evaluation of ethnomedicinal flora of Soone Valley and surrounding Salt Range for their toxicity potential against four major insect pests of economic importance. Results of this study demonstrate the relative insecticidal potential of indigenous plant extracts as biorational alternates to toxic synthetic insecticides and recommend the incorporation of these phyto-chemicals in the future insect pest management programs.

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Introduction

Apart from their great ecological impact, many species of insects pose a serious threat to humans. They are destructive pests of agricultural crops, notorious vectors of various plant and human diseases and cause many other direct and indirect losses. Insect pest problems have been an almost inevitable part of agriculture and urban sectors all over the world including Indo-Pak regions. For instance, armyworms and psyllids are among the major insect pests of agricultural and horticultural crops including fruits and vegetables. Similarly, mosquitos and termites are the most important urban and medical pests, respectively.

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Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is a sap feeding pest of citrus and other plants of Rutaceae family (Halbert and Núñez, 2004; Patt and Sétamou, 2010). Firstly, reported in Pakistan in 1927, it has become a major pest for all citrus growing regions of Pakistan (Husain and Nath, 1927). Both nymphs and adults desap plant foliage resulting in defoliation and curling of leaves, flowers and withering of branches and premature fruit drop (Mahmood *et al*., 2014). Moreover, this pest is also responsible for the transmission of citrus greening disease (Huánglóngbìng), a severe threat to citrus industry in Pakistan (Teixeira *et al*., 2005; Gottwald, 2010; Grafton-Cardwell *et al*., 2013; Hall *et al*., 2013; Razi *et al*., 2014; Canales *et al*., 2016).

Armyworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is polyphagous pest of cosmopolitan distribution causing severe losses to agricultural production worldwide (Sujatha *et al*., 2010). With a host range of more than 150 plants, *S. litura* infests and damage many fruit and vegetable crops of economic significance (Paulraj, 2001; Gallo *et al*., 2006; Ahmad and Gull, 2017). In Indo-Pak regions, considerable quantitative and qualitative losses are incurred by armyworm infestations in cotton, gram, potato, okra, tomato, chilies and many other horticultural crops.

With more than 3,500 described species, termites constitute an important part of all ecosystems and play a vital role in plant litter decomposition, turnover organic matter and soil acclimatization/reclamation (Jouquet *et al*., 2011; Brauman *et al*., 2015). However, many termite species particularly subterranean species are destructive pests of forest and orchard plantations, industrial crops and wooden infrastructures (Rouland-Lefevre, 2010). *Coptotermes heimi* Wasmann (Rhinotermitidae) and *Odontotermes obesus* Rambur (Termitidae) are most predominant and destructive termite species (Rasib *et al*., 2017). In Indo-Pak regions, these termites infest a wide range of agricultural crops including wheat, maize, gram, cotton, sugarcane and sesame (Rajagopal, 2002; Iqbal and Saeed, 2013). Moreover, they are serious threat to wooden infrastructures in urban and rural areas (Ahmed *et al*., 2005).

Mosquitoes are of the nature's most serious bioterrorists because they are responsible to transmit the world's most severe life-threatening diseases including malaria, filariasies, dengue, Zika and Chickungunya fevers (WHO, 2005). Among pest mosquito species, *Culex* mosquitoes, especially *C. quinquefasciatus*, are the principal vectors of nematode, *Wuchereria bancrofti* that cause a disease known as *Bancroftian filariasis*. *C. quinquefasciatus* is native to the West Africa from where it has been spread throughout the Asia (Belkin, 1962).

In Pakistan, synthetic insecticides have been the sole control measure being relied upon to suppress and control these agricultural and urban insect pests (Ahmed *et al*., 2006; Tiwari *et al*., 2011; Manzoor *et al*., 2012). Undoubtedly use of these insecticides increase farmers' production and improve their monetary benefits by their quick action against insect pests. However, their long-term negative effects on environment health and crop production sustainability cannot be overlooked. The frequent and indiscriminate application of these persistent synthetic insecticides have resulted into many non-target effects including environmental contamination (Edwards, 2013), pest resistance to insecticides (Kumar *et al*., 2012; Tong *et al*., 2013), resurgence of secondary pests (Hardin *et al*., 1995), eradiation of beneficial fauna including insect predator and parasitoid species (Armenta *et al*., 2003; El-Wakeil *et al*., 2013), and human health hazards (Isman, 2006; Shah and Devkota, 2009).

Due to above mentioned deleterious effects of synthetic insecticides being used in agricultural and urban environments; researchers have diverted their focus towards the development of biorational pesticides such as botanical pesticides. Many studies have demonstrated the efficacy of different phyto-extracts against *D. citri* (Khan *et al*., 2013; Ahmad *et al*., 2014; Shareef *et al*., 2016), *S. litura* (Nathan *et al*., 2005; Patil and Chavan, 2010; Gopalakrishnan *et al*., 2011; Arivoli and Tennyson, 2012; El-Wakeil *et al*., 2013; Ponsankar *et al*., 2016), *O. obesus* (Verma and Verma, 2006; Ahmed *et al*., 2007; Verma *et al*., 2011; Nisar *et al*., 2012; Verma *et al*., 2016) and *Culex* spp. (Dahchar *et al*., 2016; El-Bokl, 2016; Iqbal *et al*., 2018). Although lack quick knock-down effects as synthetic insecticides, plant-based pesticides can be effective alternatives to synthetic pesticides as most of these extracts are volatile in nature, target-specific and have reduced environmental risks (Elango *et al*., 2012).

As indigenous plants of a particular bio-geographical area may constitute effective and bioactive compounds against indigenous insect pest species (Isman, 2006; Yadav and Agarwala, 2011), the present study was aimed to determine the insecticidal potential of indigenous flora of Soone Valley situated in the North-West of district Khushab (Punjab, Pakistan). This valley and surrounding salt range harbor a rich diversity of medicinal plants including many herbs and shrubs (Ahmed *et al*., 2009; Shah and Rahim, 2017).

Materials and Methods

Sampling locations

Indigenous plant species were collected from Soone Valley and surrounding Salt Range. Sampling area was about 300 Km^2 located between longitudes 72°00' to 72°30' E and latitude 32°25' to 32°45' N (Ahmad *et al*., 2009). In the sampling area, six different sites, *i.e.* Khura, Khabikki, Kenhatti Garden, Daep Sharif, Angah and Uchhali, were selected for the collection of flora based on their vegetation

enrichment as detailed in Figure 1 and Table 1. Sampling was done during September to October, 2018 and then March to April 2019.

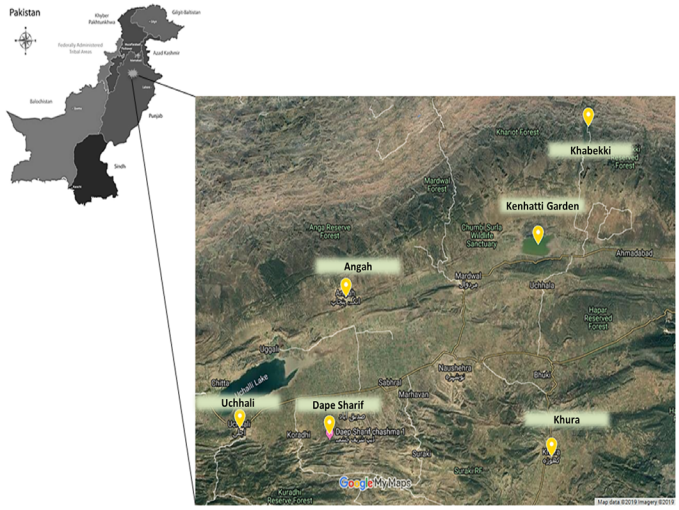

Figure 1: Main locations selected for the collection of indigenous flora of Soone Valley and surrounding Salt Range of Pakistan (cf: Table 1).

Table 1: Geographical coordinates of the plant sampling sites (cf: Figure 1).

Localities	Latitude N	Longitude E	Elevation (m)
Khura	32.23° N	72.11° E	866
Dape Sharif	32.30° N	72.04° E	890
Uchhali	32.56° N	72.02° E	794
Kenhatti Garden	32.40° N	72.14° E	783
Angah	32.35° N	72.05° E	821
Khabekki	32.35° N	72.12° E	774

Sampling and processing of plant samples

Samples of forty plant species were collected from above mentioned selected sites. Samples were consisted of leaves, stems, roots, fruits and flowers as mentioned in Table 2. Among this plant collection, 38 samples were identified with the help of their vernacular name told by local inhabitants and already published literature and verified by the Department of Botany, University of Sargodha, Sargodha. The collected plant samples were washed by clean tap water and shade-dried for about two weeks. After drying, plant materials were grinded to make fine powder using commercial electrical blender and were preserved separately in plastic zip bags for further processing.

Extraction of plant samples

The extraction of plant samples was carried out in the Laboratory of the Department of Entomology, College of Agriculture, University of Sargodha, Pakistan. Soxhlet apparatus (Daihan Scientific Co., Ltd. South Korea) was used to extract the phyto-constituents according to a previously described protocol (Mahmood *et al*., 2014). A known amount (50 g) of grounded material of each plant sample was loaded into the filter paper thimble in Soxhlet apparatus. A piece of cotton was plugged at the top of the thimble to stop the entry of crude extract into the siphoning tube. A known volume (500 mL) of organic solvent (acetone having polarity index of 5.1 and boiling point of 56 °C) was filled into the flask (1 L) fixed over the mantle of heating device. The extractions were performed for 6-8 hr at 60 °C. The crude extract obtained from Soxhlet apparatus was further concentrated by evaporating the excess of extraction solvent using rotary evaporator (Daihan Scientific Co., Ltd. South Korea) set at 60 °C. Prepared extracts were preserved in hermetic dark glass vials at 4 °C.

Insect cultures

Adults of Asian citrus psyllid (*D. citri*) were collected with an aspirator from the citrus (*Citrus reticulara cv.* kinnow mandarin) field situated near the College of Agriculture, University of Sargodha. These field collected psyllids were reared for 2-3 weeks on potted *Murraya paniculata* (orange jasmine) plants maintained in the insect rearing cages at optimum temperature (25±5°C), relative humidity (60±5%) and 16L:8D photoperiod. Healthy and active adult psyllids were used in toxicity bioassays.

Larvae of armyworm (*S. litura*) were collected from the field of sunflower (*Helianthus annuus*) and were maintained in the laboratory in plastic jars under controlled conditions (25±2°C, 60±5% RH and 16:8 (L: D) photoperiod). They were fed daily on un-contaminated fresh leaves of castor (*Ricinus cummunis*) plants. Adults upon emerging from pupa were transferred to separate plastic jars provided with 10% honey solution. Healthy and active larvae from F2 generation were used in bioassays.

Mosquito (*C. quinquefasciatus*) larvae were collected from different areas of Sargodha with the help of an aquatic net and dipper. Those collected larvae were identified on the basis of different distinguished morphological characteristics under microscope by using taxonomic keys available in literature (Azari-Hamidian and Harbach, 2009). It was ensured that collection site was never exposed to any insecticide at least two months before collection of mosquito larvae.

For subterranean termites, intact portions of termite nest were collected from the termite infested stubbles of sugarcane (*Saccharum officinarum*). Before collection, it was ensured that the sugarcane field was not treated with any pesticide for last three months. These termites were identified as *O. obesus* on the basis of their distinguished morphological characters (Shanbhag and Sundararaj, 2011). In order to acclimatize the termite individuals to lab conditions, collected termite nest portions were maintained in the lab in polystyrene glass cages for few weeks. Only healthy and active worker individuals were used in toxicity bioassays.

Table 2: Details of different plant samples collected from Soone Valley and surrounding Salt Range of Pakistan.

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)
1	*Chenopodium album*	Bathuwa	Khura	Leaves	Amaranthaceae	Alkaloids, Flavonoids, Saponin, Tannins (Mojab *et al.*, 2010; Pandey and Gupta, 2014)
2	*Buxus papillosa*	Shamshad	Khura	Leaves	Buxaceae	Alkaloids, Flavonoids, Phenols (Parveen *et al.*, 2001; Akhtar and Mirza, 2018)
3	*Cynodon dactylon*	Khabal	Khura	Leaves	Poaceae	Alkaloids, Anthroquinone, Flavonoids, Glycosides, Phenols, Saponins, Steroids, Tannins, Triterpenoids (Suresh, 2008; Kaleeswaran *et al.*, 2010)
4	*Petrophytum caespitosum*	Mat rock spiraea	Khura	Leaves and stem	Rosaceae	NI*
5	*Astragalus* Spp.	Koohni	Khura	Leaves and stem	Fabaceae	Flavonoids, polysaccharides, saponins, sterols (Huang *et al.*, 2019)
6	*Trichodesma indicum*	Juri/ Nil karaj, Doosi, Gao zaban	Khura	Leaves and stem	Boraginaceae	Alkaloids, Flavonoids, Phenols, Steroids, Terpenoids, Tannins, (Perianayagam *et al.*, 2012; Anusha *et al.*, 2014; Saboo *et al.*, 2014)
7	*Dicliptera bupleuroides*	Kaalu and Pipri	Daep Sharif	Leaves, flower and stem	Acanthaceae	Alkaloids, Carbohydrates, Flavonoids, Glycosides, Lipids, Proteins, Sterols, Saponin, Triterpenoids, Tannins (Riaz *et al.*, 2012)
8	*Marrubium vulgare*	Pahari gandana	Daep Sharif	Leaves	Lamiaceae	Alkaloids, Flavonoids, Saponin, Terpenoids, Tannins (Mojab *et al.*, 2010; Amessis-Ouchemoukh *et al.*, 2014)
9	*Fagonia indica*	Dhamasa	Daep Sharif	Leaves and stem	Zygophyllaceae	Alkaloids, Anthraquinons, Coumarins, Carbohydrates, Flavonoids, Glycosides, Phenol, Saponins, Steroids, Terpenoids, Tannins (Burm, 2011; Eman, 2011; Rashid *et al.*, 2013)
10	*S-16 (Unidentified)*	NI*	Daep Sharif	Leaves	NI*	NI*
11	*Mentha longifolia*	Desi podina	Daep Sharif	Leaves and stem	Lamiaceae	Essential oils, Flavonoids (Ghoulami *et al.*, 2001)
12	*Solanum surattense*	Kanda kari/ Choti Kateri	Daep Sharif	Leaves and fruit	Solanaceae	Alkaloids, Flavonoids, Glycosides, Sterols, Tannins, Triterpenoids (Muruhan *et al.*, 2013)
13	*Nerium indicum*	Kanera	Daep Sharif	Leaves	Apocynaceae	Alkaloids, Carbohydrates, Glycosides, Lipids, Proteins, Sterols, Saponins, Tannins, Triterpenoids (Bhuvaneshwari *et al.*, 2007)
14	*Nerium indicum*	Kanera	Daep Sharif	Fruit	Apocynaceae	Alkaloids, Carbohydrates, Glycosides, Lipids, Proteins, Sterols, Saponins, Tannins, Triterpenoids (Bhuvaneshwari *et al.*, 2007)
15	*Acacia melanoxylon*	Hickory	Daep Sharif	Leaves and stem	Fabaceae	Alkaloids, flavonoids, Phenols (Luis *et al.*, 2012)
16	*S-22 (Unidentified)*	NI*	Daep Sharif	Leaves	NI*	NI*
17	*Datura alba*	Dhatura	Uchhali	Leaves	Solanaceae	Flavonoids, Glycosides, Phenol, Reducing sugars, Steroids, Saponins, Terpenoids, Tannins (Uddin *et al.*, 2012)
18	*Suaeda fruticosa*	Lahnra	Uchhali	Leaves	Amaranthaceae	Anthraquinons, Alkaloids, Carbohydrates, Flavonoids, Phenol, Saponins, Steroids, Terpenoids, Tannins (Ullah *et al.*, 2012; Munir *et al.*, 2014)
19	*Alternanthera pungens*	Kandaa Booti/ Phakra	Uchhali	Leaves and stem	Amaranthaceae	Alkaloids, Anthocyanosides, Anthraquinons, Carbhydrates, Coumarins, Flavonoids, Lipids, Phenol, Saponins, Steroids, Triterpenoids, Tannins (Zongo *et al.*, 2011; Kalpana *et al.*, 2018)
20	*Opuntia dillenii*	Thor	Kanhati Garden	Leaves and roots	Cactaceae	Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Steroids, Terpeonids, Tannins (Pooja and Vidyasagar, 2016)
21	*Murraya koenigii*	Jangli curry Patta	Kanhati Garden	Leaves and stem	Rutaceae	Alkaloids, Anthraquinons, Carbhydrates, Flavonoids, Proteins, Phytosterols, Saponins, Tannin, Volatile oil (Handral and Prashanth, 2010)
22	*Periploca aphylla*	Bata	Kanhati Garden	Stem and leaves	Apocynaceae	Anthraquinons, Alkaloids, Carbhydrates, Flavonoids, Proteins, Phytosterols, Steroids, Saponins, Terpenoids (Khan *et al.*, 2012)

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)
23	*Dryopteris filix-mas*	Male fern	Kanhati Garden	Leaves	Dryopteridaceae	Anthraquinons, Alkaloids, Flavonoid, Glycosides, Phenol, Reducing sugars, Saponins, Steroids, Tannins, Terpenoids (Erhirhie, 2018; Erhirhie *et al*., 2019)
24	*Ricinus communis*	Harnoli	Kanhati Garden	Leaves	Euphorbiaceae	Carbohydrates, Fatty acids, Flavonoids, Glycosides, Phenols, Proteins, Saponins, Steroids, Tannins (Yadav and Agarwala, 2011; Wafa *et al*., 2014)
25	*Cassia occidentalis*	Bana Chakunda	Kanhati Garden	Leaves	Fabaceae	Alkaloid, Flavonoid, Glycosides, Steroid, Saponin, Tannin (Saganuwan and Gulumbe, 2006; Yadav *et al*., 2010)
26	*Cassia occidentalis*	Bana Chakunda	Kanhati Garden	Fruit	Fabaceae	Anthraquinons, Flavonoids, Glycosides, Phenols, Steroid (Yadav *et al*., 2010)
27	*Adiantum capil-lus-veneris*	Venus hair fern/ Khatti booti	Kanhati Garden	Leaves	Pteridaceae	Alkaloids, Carbohydrates, Fiber, Fats and waxes, Flavonoids, Glycosides, Phenolics, Saponins, Steroids, Terpenoids, Tannins (Ibraheim *et al*., 2011; Rajurkar and Gaikwad, 2012; Ishaq *et al*., 2014)
28	*Justicia adhatoda*	Dhodhak Booti, Vaheakar/ Baikarr and Vasaka	Kanhati Garden	Leaves	Acanthaceae	Alkaloids, Anthraquinones, Flavonoids, Glycosides, Phenols, Polyphenols, Phytosterols, Saponins, Triterpenoids (Chanu and Sarangthem, 2014; Jayapriya and Shoba, 2015)
29	*Salvia virgata*	Meadow Sage	Khabikki	Flower	Lamiaceae	Amino acids, Alkaloids, Carbohydrates, Flavonoids, Glycosides, Phenolic compounds and Proteins, Saponins, Terpenoids (Koşar *et al*., 2008)
30	*Amaranthus viridis*	Jangli cholai/ Ghanyar	Kanhati Garden	Whole plant	Amaranthaceae	Amino acids, Alkaloids, Carbohydrates, Flavonoids, Glycosides, Phenolic compounds, Proteins, Saponins, Terpenoids (Kumar *et al*., 2012)
31	*Sonchus asper*	Bhattal	Kanhati Garden	Leaves	Asteraceae	Alkaloids, Flavonoids, Phenols, Saponins, Steroids, Tannins, Terpinoids (Hussain *et al*., 2010; Kumari *et al*., 2017)
32	*Melilotus officinalis*	Yellow sweet clover	Kanhati Garden	Leaves	Fabaceae	Flavonoids, Phenol, Saponins, Tannin, Terpenoids (Govindappa and Poojashri, 2011)
33	*Salvia officinalis*	Khalatra	Angah	Leaves	Lamiaceae	Alkaloids, Diterpenes, Flavonoids, Polyphenols, Saponins, Triterpenic acids (Kontogianni *et al*., 2013; Hernández-Saavedra *et al*., 2016)
34	*Solanum incanum*	Mahori	Angah	Fruit	Solanaceae	Alkaloids, Carbohydrates, Cardic glycosides, Cyanogenic glycosides, Flavonoids, Phenols, Resins Oxalates, Steroids, Saponins, Tannins (Auta *et al*., 2011; Indhumathi and Mohandass, 2014; Sambo *et al*., 2016)
35	*Portulaca oleracea*	Loonak	Angah	Leaves and stem	Portulacaceae	Fatty acids, Organic acids, Phenolic compounds (Oliveira *et al*., 2009)
36	*Dodonaea viscosa*	Santha/Pip-par	Angah	Leaves	Sapindaceae	Amino acids, Carbohydrates, Fatty acids Fixed oils, Flavonoids, Glycosides, Phenols, Proteins, Steroids, Saponins, Tannins, Triterpenoids (Venkatesh *et al*., 2008; Dimetry *et al*., 2015)
37	*Olea ferruginea*	Zatoon, Kao	Angah	Fruit	Oleaceae	β-amyrin, Ligstroside, Oleuropein, Quercetin (Hashmi *et al*., 2015)
38	*Rumex dentatus*	Toothed dock	Angah	Leaves and fruits	Polygonaceae	Alkaloids, Cardic glycosides, Cyanogenic glycosides, Carbohuydrates, Flavonoids, Phenols, Steroids, Saponins, Tannins (Nisa *et al*., 2013)
39	*Withania coagulans*	Paneer booti/ Khamjeera	Angah	Leaves, fruits	Solanaceae	Alkaloids, Amino acids, Carbohydrates, Organic acids, Phenolic compounds, Proteins, Steroids, Saponin, Tannins (Mathur *et al*., 2011)
40	*Eruca saiva*	arden rocket/ Jamahoon	Angah	Flower	Brassicaceae	Allyl isothiocyanate, 3-butenyl isothiocyanate, 4-methylsulfinybutyl isothiocyanate, sulforaphane), 2-phenylethyl isothiocyanate and bis (isothiocyanatobu-tyl) disulphide, fatty acids (Khoobchandani *et al*., 2010)

*NI, not informed.

Toxicity bioassays

For screening toxicity potential of forty plant extracts, 10% solutions of these extracts were made using acetone and the same was used in control treatments. Bioassays were performed using completely randomized design (CRD) with five replications for each treatment.

For *D. citri*, twig-dip method was used. Freshly cut twigs (5 cm long) of orange jasmine (*C. reticulata*) were dipped into 10% solutions of botanical extracts for 30 sec and were placed at towel paper to soak up the excess solution from leaves. These treated twigs were then fixed in 2% agar solution in sterile Eppendorf tubes (1.5 mL) and these Eppendorf tubes were placed into sterile falcon tubes (50 mL). Laboratory maintained adult psyllids were collected with the help of aspirator and were kept into freezer for 5 min at 0 ºC to inactivate psyllids. Ten inactive psyllids were released into each falcon tubes with the help of a soft camel hair brush. Each falcon tube was covered with a piece of muslin cloth and tied with rubber band and all tubes were incubated in the rearing lab at controlled conditions (25 ± 2 ºC, 60 ± 5% RH and 16:8 (L: D) photoperiod). Data regarding mortality of psyllids was recorded at 24, 48 and 72 h post-exposure.

For *S. litura*, leaf-disc method was used. Uncontaminated fresh leaves of *R. cummunis* were washed and air-dried at room temperature (24 ˚C) for 5 min. Leaf discs (60 mm) were prepared and treated with treatment solutions and put to dry on towel paper for 15 min at room temperature. Treated and control leaf discs were placed in Petri plates (60 mm) over a thin layer of 2% agar to maintain the moisture within the Petri plates. Ten 2nd instar starved larvae of lab reared *S. litura* were released into each Petri plate and these plates were incubated in the rearing lab at controlled conditions (25 ± 2 ºC, 60 ± 5% RH and 16:8 (L: D) photoperiod). Data regarding the mortality of exposed larvae was recorded at 24, 48 and 72 h post-exposure.

Aqueous solution bioassay method was used for *C. quinquefasciatus*. Ten early 4th instar larvae of *C. quinquefasciatus* mosquito were dropped into disposable glasses (200 mL) having 100 mL of 0.5% aqueous solution of each botanical. Whole experimentation was performed in controlled conditions (25 ± 2 ºC, 60 ± 5% RH and 16:8 (L: D) photoperiod). Data regarding the mortality of exposed mosquito larvae was recorded at 24, 48 and 72 h post-exposure.

For *O. obesus*, filter paper disc method was used. Filter paper (Whatman No. 1) discs were dipped in 10% solution of each botanical extract for 30 sec and allowed to dry for 30 min at room temperature (24 ˚C). Treated and control leaf discs were placed in Petri plates (60 mm) over a thin layer of 2% agar to maintain the moisture within the Petri plates. Ten healthy worker termites were released in each Petri plate and these plates were incubated in the laboratory at 25 ± 2 ºC, 60 ± 5% RH and 16:8 (L: D) photoperiod). Data regarding the mortality of exposed termite individuals was recorded at 24, 48 and 72 h post-exposure.

Statistical analysis

Statistical analysis of data was performed using Statistix V. 8.1. analytical software (Tallahassee, FL, USA). In addition to graphical presentation of percent mortality of the exposed insect individuals, one-way factorial ANOVA was run using botanical extracts and time intervals as factors. Treatment means were compared using Tukey's honest significant difference (HSD) at standard level of significance (α = 0.05).

Results and Discussion

Insecticidal potential of forty indigenous plant species (including trees, herbs and shrubs) was evaluated in this laboratory study against four insect pests of economic importance. Most of the plant species collected belongs to Apocynaceae, Amaranthacea, Fabaceae, Lamiaceae and Solanaceae families and are usually enriched in such phyto-constitutes as alkaloids, carbohydrates, cardiac glycosides, cyanogenic glycosides, flavonoids, phenols, resins oxalates, steroids, saponins and tannins (Table 2).

Toxicity of indigenous flora of Soone Valley against D. citri

Toxicity bioassays revealed that the 10% acetone extracts of *M. longifolia*, *S. asper, N. indicum*, *D. alba* and *S. officinalis* exhibited highest average mortality of *D. citri i.e.* 93, 91, 89, 88, and 81%, respectively, whereas the other plant extracts caused less than 50% mortality as observed at 72 h post-exposure (Figure 2). Least toxic plant extracts were of *Astragalus* spp., *W. coagulans*, *O. dillenii*, *T. indicum* and *A. viridis*.

This observed mortality of *D. citri* by *M. longifolia*, *S. asper* and *N. indicum* would be due to diverse terpenoids and phenolic compounds present in these plant extracts (Hiremath *et al.*, 1997; Lee *et al.*, 2001; Odeyemi *et al.*, 2008; El-Kamali, 2009; Hussain *et al.*, 2010). Our results are in line with the findings of Kuganathan *et al.* (2008) demonstrating significant mortality of aphids by the extracts of *D. alba*, probably due to the alkaloids present in the leaves of this plant. Khan *et al.* (2013) demonstrated significant toxicity of *D. alba* extract against citrus psyllids (*D. citri*) causing 60±9.7% nymphal mortality. Similarly, the toxic effect of essential oil of *S. officinalis* was revealed by Tomczyk and Suszko (2011) against two spotted spider mites and reported 56% mite mortality in 4 days of treatment. Govindappa and Poojashri (2011) examined the presence of chemicals such as flavonoids, phenol, saponins, tannin and terpenoids in *M. officinalis* that might be responsible for psyllid mortality in this study.

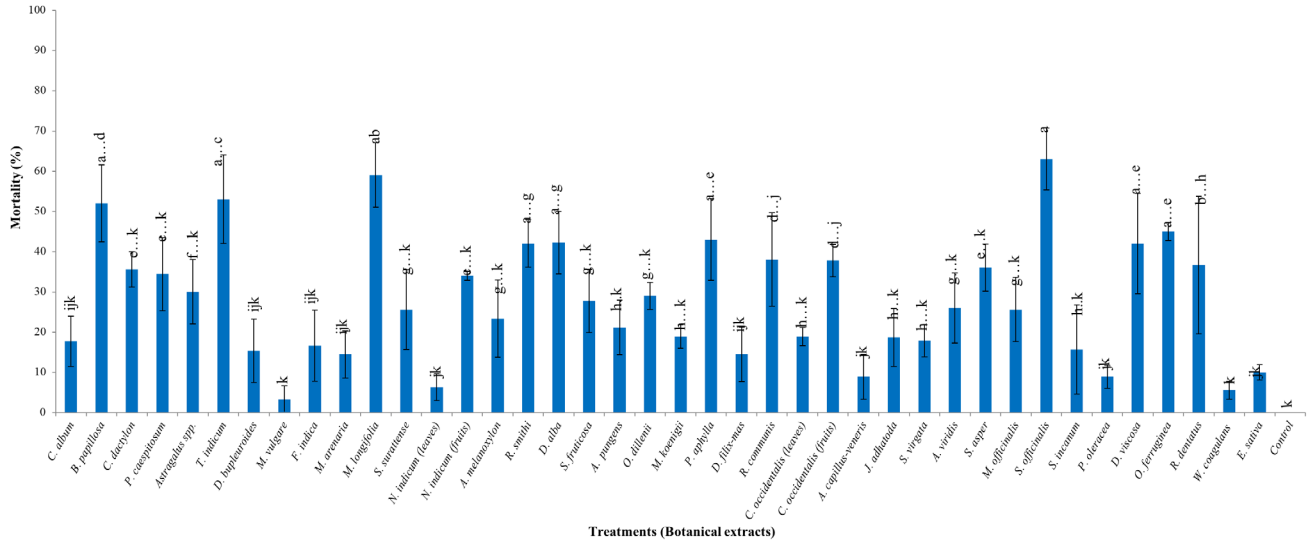

Figure 2: Percent mortality (mean ± SE) of citrus psyllid (*D. citri*) adults bioassayed with 10% acetone solutions of different indigenous plant species collected from Soone Valley and surrounding Salt Range.

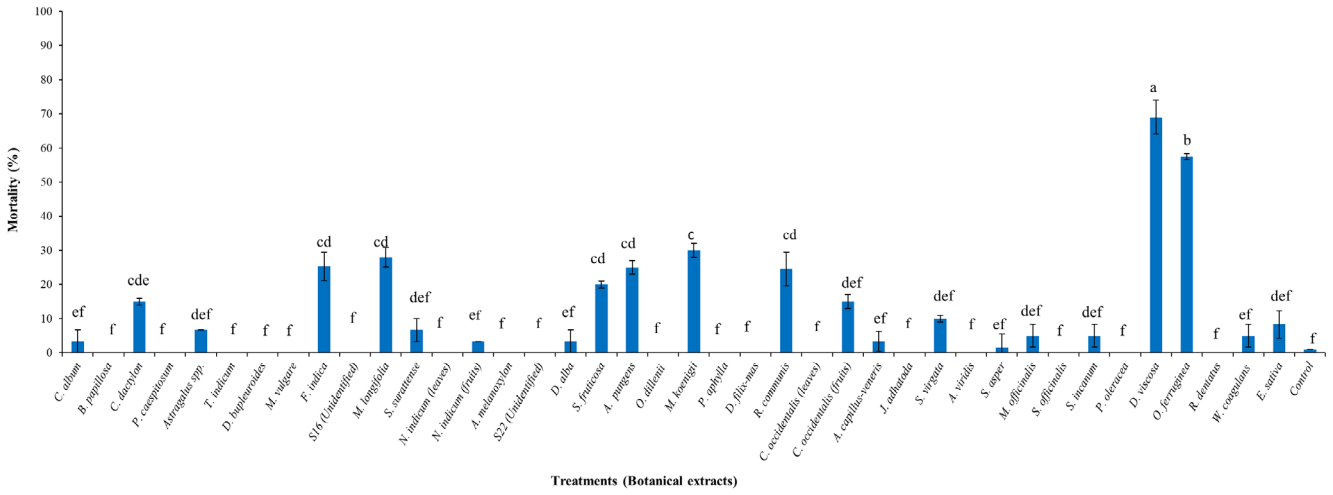

Figure 3: Percent mortality (mean ± SE) of armyworm (*S. litura*) larvae bioassayed with 10% acetone solutions of different indigenous plant species collected from Soone Valley and surrounding Salt Range.

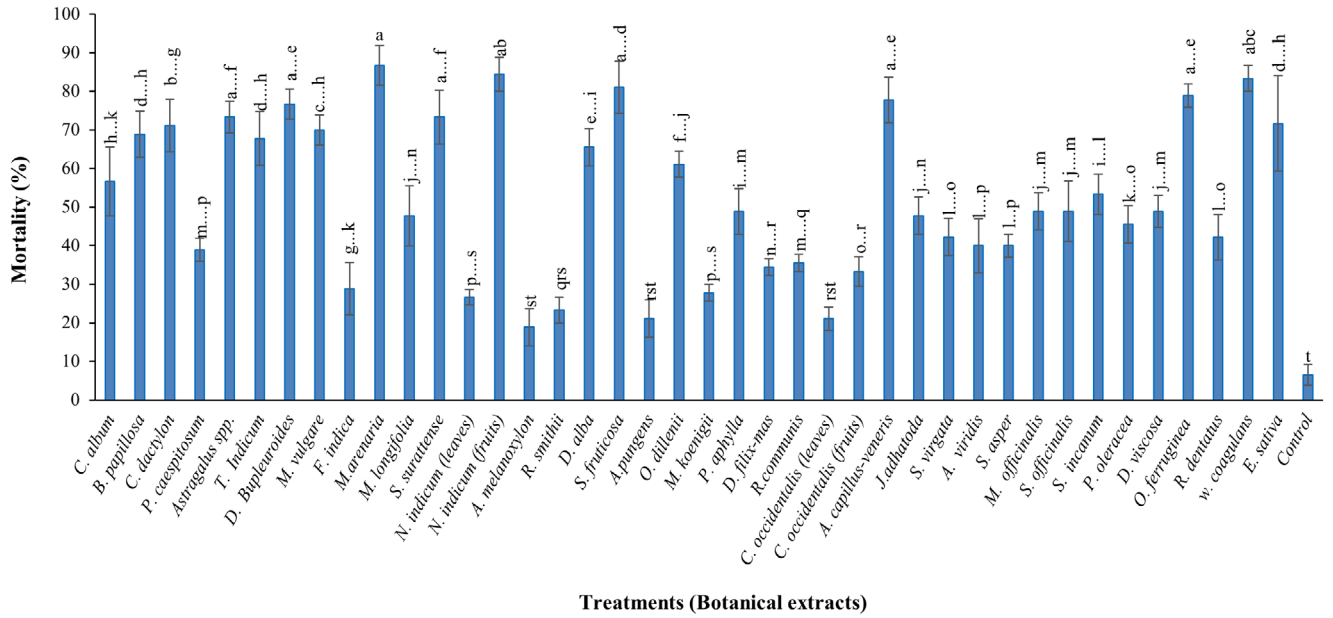

Figure 4: Percent mortality (mean ± SE) of mosquito (*C. quinquefasciatus*) larvae bioassayed with 10% acetone solutions of different indigenous plant species collected from Soone Valley and surrounding Salt Range.

Toxicity of indigenous flora of Soone Valley against S. litura

In case of *S. litura*, extracts of *D. viscosa* and *O. ferruginea* caused highest average mortality of *S. litura*, *i.e.* 70 and 58%, respectively. The extracts of *M. koeingii*, *M. longifolia*, *F. indica*, *A. pungens* and *R. cummunis* exhibited moderate toxicity causing 20 to 40% mortality of the exposed 2nd instar larvae of *S. litura*, whereas other plant extracts caused minimum or negligible mortality (Figure 3).

Ethnomedicinal plant species of Soone Valley and surrounding Salt Range such as *D. viscosa* and *O. ferruginea* have been known as excellent herbal remedies against many diseases including diarrhea and malaria (Shah and Rahim, 2017). *D. viscosa* 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 (Malarvannan *et al.*, 2009; Mohammed and Nawar, 2020), coleopterous (Dimetry *et al.*, 2015) and homopterous pests (Díaz *et al.*, 2015). Similarly, many species of Oleaceae family contain toxic compounds potentially effective against different insect pests. For instance, *O. europaea* constitute higher phenolic contents and a triterpene compound (maslinic acid) exhibiting significant toxicity against aphids (*Myzus persicae*) and stored grain insect pests (*Sitophilus granaries* and *Tribolium confusum*) (Hamouda *et al.*, 2015; Kisa *et al.*, 2018).

Toxicity of indigenous flora of Soone Valley against C. quinquefasciatus

Figure 4 presents the average percent mortality of *C. quinquefasciatus* larvae by 0.5% botanical extracts. Maximum mortality of mosquito larvae was observed by the *M. arenaria* extract (87%), followed by the extracts *N. indicum* (84%), *W. coagulans* (83%), *S. fruticosa* (81%), *O. ferruginea* (79%), *A. capillus-veneris* (78%), *D. bupleuroides* (77%), *Astragalus spp.* (73%), *S. surattense* (73%), *E. Sativa* (72%), *C. dactylon* (71%), *M. vulgare* (70%), *B. papillosa* (69%), *T. indicum* (68%), *D. alba* (66%), *O. dillenii* (61%), *S. incanum* (53%). Other plant extracts showed less than 50% mortality. *A. melanoxylon*, *C. occidentalis* and *A. pungens* were least toxic extracts showing 20-25% mortality (Figure 4).

Extracts of *N. indicum* constitute different alkaloids and triterpenoids which show anti-feedant, ovicidal, larvicidal and repellant activities against a wide range of insect pests including mosquitoes (Hiremath *et al.*, 1997; Sharma *et al.*, 2005; Rahuman and Venkatesan, 2008; Dey *et al.*, 2017; Kumar *et al.*, 2019). Acetone and methanolic extracts of *N. indicum* at 0.02 to 0.03% concentrations showed significant mortality (more than 50%) of *C. quinquefasciatus* larvae (Sharma *et al.*, 2005; Bhuvaneshwari *et al.*, 2007; Rahuman and Venkatesan, 2008). Similarly, *W. coagulans* and *S. fruticosa* constitute different alkaloids and phenols, and α-pinene and borneol, respectively (Koliopoulos *et al.*, 2010; Mathur *et al.*, 2011) and these plant extracts (10%) have shown to cause significant mortality (up to 63%) in *Callosobruchus chinensis* (Gupta and Srivastava, 2008) and up to 50% mortality in larvae of *Culex pipiens* (Koliopoulos *et al.*, 2010). Our results are in line with the findings of Teressa *et al.* (2019) showing 60% mortality in *Anopheles* mosquito larvae by the extract of *O. europea* plant. Similarly, 0.03% hexane extract of *A. capillus-veneris* has been found determinant to *Plutella xylostella* (causing 80% mortality) and to *Aphis craccivora* (causing up to 70% mortality) (Sharma and Sood, 2012).

Toxicity of indigenous flora of Soone Valley against O. obesus

In case of subterranean termites, the most toxic plant extracts were *P. aphylla*, *Rhamnus* spp., *B. papillosa* and *T. indicum* causing 89, 62, 52 and 50% termite mortality, respectively. Minimum average termite mortality was exhibited by the 10% extracts of *M. vulgare*, *W. coagulans*, *P. oleracea* and *A. capillus-veneris* (Figure 5).

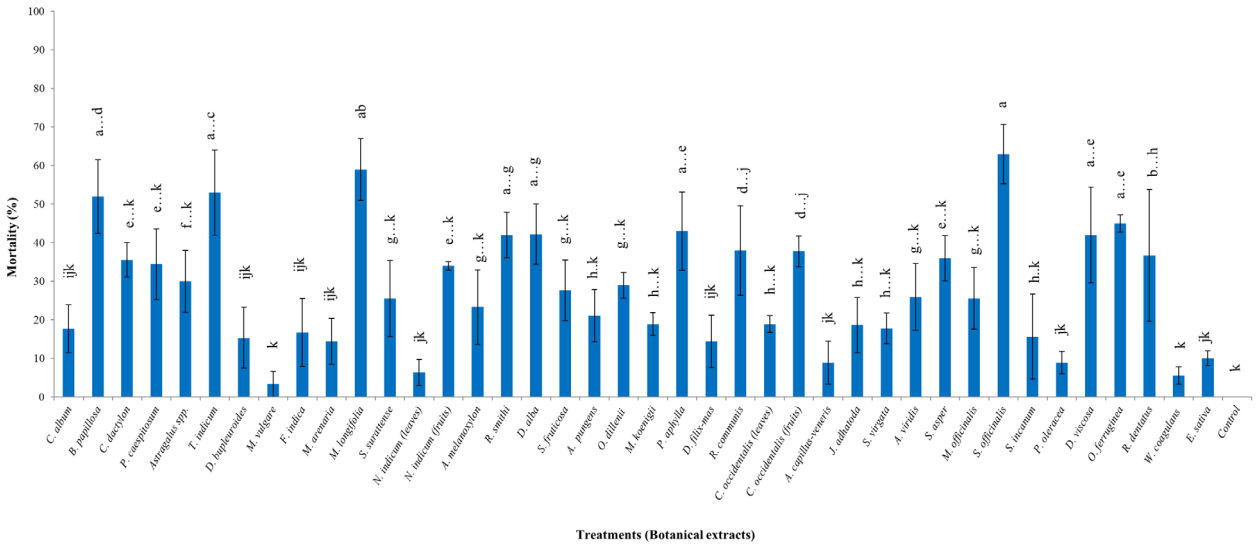

Figure 5: Percent mortality (mean ± SE) of subterranean termite (*O. obesus*) worker termites bioassayed with 10% acetone solutions of different indigenous plant species collected from Soone Valley and surrounding Salt Range.

The triterpenes isolated from the stems of *P. aphylla* showed antibacterial activity (Iqbal *et al*., 2012) but insecticidal activity of this plant species has not tested against any insect pest. Acetone and ethanol extracts of *Rhamnus dispermus* caused significant mortality of peach trunk aphid (*Pterochloroides persicae*) (Ateyyat and Darwish, 2009; Elango *et al*., 2012). The methanolic extract of *B. papillosa* showed acaricidal activity against *Rhipicephalus microplus* (Jonsson and Iqbal, 2012). Similarly, different organic solvent derived and aqueous extracts of *T. indicum* have been shown significant effectiveness against armyworms (*Mythimna separate*), dengue vector mosquitos (*Aedes aegypti*) and many stored grain pests (Buhroo *et al*., 2017; Kazmi *et al*., 2017; Chellappandian *et al*., 2019).

Conclusions and Recommendations

Toxicity bioassays conducted with methanolic extracts of forty indigenous plant species of Soone Valley revealed that *M. longifolia* caused highest mortality in *D. citri*, *D. viscosa* caused 70% mortality in *S. litura*, *M. arenaria* caused 87% mortality in *C. quinquefasciatus* and *P. aphylla* caused 89% mortality in *O. obesus*. So, for the further studies' chemical characterization of these most effective plant extracts will be analyzed for their chemical constituents.

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Conflict of interest

The authors have declared no conflict of interest.

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