Pakistan J. Zool., pp 1-9, 2021. DOI: https://dx.doi.org/10.17582/journal.pjz/20210705100711

Comparative Efficacy of Selected Biorational Insecticides against Larvae of Southern House Mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae)

Mujahid Tanvir[1], Muhammad Asam Riaz[1]*, Muhammad Zeeshan Majeed[1], Mazhar Iqbal Zafar[2], Muhammad Tariq[3] and Muhammad Bilal Tayyab[1]

[1]*Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan*

[2]*Department of Environmental Sciences, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan*

[3]*Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46000, Pakistan*

Article Information
Received 05 July 2021
Revised 26 August 2021
Accepted 02 September 2021
Available online 12 November 2021

Authors' Contribution
MAR conceived and designed the experimental protocols. MT and MBT performed experiments. MZM and MT provided technical assistance in experimentation. MIZ and MAR performed statistical analysis. MAR and MZM prepared the manuscript.

Key words
Biorational insecticides, *Culex quinquefasciatus*, Entomopathogenic insecticides, Larvicidal bioassays, Phytoextracts

ABSTRACT

Mosquitoes are unquestionably the important arthropod vectors of diseases such as malaria, dengue, filariasis and systemic allergic reactions in humans. Southern house mosquito *Culex quinquefasciatus* Say is found in tropical and subtropical regions of the world and transmits many zoonotic diseases in humans and in wild and domestic animals. It is primarily controlled by the extensive use of conventional synthetic insecticides against most of which it has developed resistance. This study was aimed at determining the toxicity of selected microbial and synthetic insecticide formulations and botanical extracts against *C. quinquefasciatus* larvae. Among the n-hexane extracts of 40 indigenous plant species collected from Soon Valley and surrounding salt range of Pakistan bioassayed against *C. quinquefasciatus* larvae, eighteen botanicals exhibited more than 50% larval mortality in 48 h exposure. The most effective botanical extracts were *Maerua arenaria* Forsk, *Nerium indicum* Mill., *Withania coagulans* Dunal, *Suaeda fruticosa* (L.) Delile, *Olea ferruginea* Wall., *Adiantum capillus-veneris* L. and *Dicliptera bupleuroides* Nees exhibiting 87, 84, 83, 81, 79, 78 and 77% larval mortality, respectively with minimum LC_{50} and LC_{90} values. Among the microbial and synthetic insecticides, the highest larval mortality was recorded by *Metarhizium anisopliae* NCIM 1311 (83%) and *Bacillus thuringiensis* subsp. *israelensis* (63%), and by pyriproxyfen (86%) and indoxacarb (85%), respectively. Hence, these botanical, microbial and synthetic insecticides are recommended for the efficient control of *C. quinquefasciatus* larvae in field to reduce the environmental pollution caused by persistent synthetic insecticides.

INTRODUCTION

Many arthropod species vector direct and indirect transmission of different bacterial, viral, and protozoan diseases in humans. The most common vector borne diseases which affect humans are typhus transmitted by human louse, plague caused by fleas, enteric diseases caused by houseflies, sleeping sickness caused by tsetse fly, chagas disease vectored by triatomine bugs (Manguin and Boëte, 2011; Dacey and Chain, 2020). Similarly, several mosquito species belonging to *Aedes*, *Anopheles* and *Culex* genera are medically important and vector many viral diseases such as chikungunya, malaria and dengue fever (Mullen and Durden, 2009; Benelli and Mehlhorn, 2016; Salam *et al*., 2018). One-third of the world population is at risk of mosquito transmitted diseases. Every year more than one million people die due to the transmission of various causative agents of infectious diseases by mosquitos (Becker *et al*., 2020).

The global dispersion and distribution of mosquitoes pose threats to health status, biosecurity as well as the economy of countries worldwide (Manguin and Boëte, 2011). This has been boosted by the extensive use of sea, land and air transport networks, and the global trade of used car tyres (Tatem *et al*., 2006). Different mosquito species transmit about 28 viruses of major public health. *Aedes* is responsible for transmitting yellow and dengue fever and filariasis is transmitted by *Anopheles* and *Culex*. Many types of encephalitis are spread by mosquitoes of *Culex* and *Aedes* genera (Vythilingam *et al*., 1997; Lounibos,

* Corresponding author: asam.riaz@uos.edu.pk
0030-9923/2021/0001-0001 $ 9.00/0

2002; Paily *et al*., 2007; De Wispelaere *et al*., 2017). In early 19th century, transmission of malaria and avian pox virus in Hawaiian bird populations was caused by *Culex quinquefasciatus* mosquitoes and resulted in suppression of the population of native Hawaiian honeycreepers (Atkinson and LaPointe, 2009).

Mosquitos are primarily controlled by extensive applications of persistent synthetic insecticides such as DDT, malathion, chlorpyrifos, deltamethrin etc. and many field populations of mosquitoes including *C. quinquefasciatus* have attained high resistance against these synthetic insecticides (Tikar *et al*., 2008; Senthil-Nathan, 2020). Therefore, there is a dire need of searching for biorational mosquito control methods such as botanical, microbial and reduced-risk synthetic insecticides (Rose, 2001; Benelli, 2015). Plant based pesticides usually have low mammalian toxicity and have been emerging as promising alternatives to synthetic insecticides for the control of mosquitoes (Sukumar *et al*., 1991; Isman, 2008; Zhu *et al*., 2008; Senthil-Nathan, 2020). Similarly, microbial pesticides are usually based on entomopathogenic fungal, bacterial or viral strains and have been demonstrated as safe and effective against a wide range of insect pest species including mosquitos (Federici, 1995; Regis *et al*., 2000; Bukhari *et al*., 2013; Dacey and Chain, 2020). This research work was hence aimed to determine the effectiveness of selected microbial and synthetic insecticides and indigenous botanical extracts against the larvae of *C. quinquefasciatus*.

MATERIALS AND METHODS

Collection and preparation of plant samples

Indigenous flora consisted of stems, leaves, flowers and fruits of local plant species (including herbs, shrubs, bushes and trees) were collected from six different sites of Soon Valley located in North-West of district Khushab (Punjab, Pakistan) (Table I). Collected samples were labeled and were brought to the Laboratory of Entomology at College of Agriculture, University of Sargodha, Pakistan. These samples were cleaned manually to remove all foreign material followed by washing with distilled water and were shade-dried at room temperature (27°C). Dried samples were weighed and ground to fine powder with an electric blender. Powdered samples were stored in hermetic plastic zipped-locked bags to avoid any contamination.

Botanical extraction

As the ordinary method of extraction was not efficient to yield good amount of phyto-constituents, Soxhlet extractor (DAIHAN Scientific North America Inc., USA) was employed for the extraction of prepared plant samples using n-hexane as extraction solvent following a previously described protocol (Majeed *et al*., 2020). In brief, extractor thimble was filled with a known amount (50 g) of ground plant material of each sample and was plugged with a piece of cotton to stop the entry of crude extract into the siphoning tube. A known volume (500 ml) of n-hexane (purity ≥ 99.0%) was filled into the flask (1 L) installed on the mantle of heating device. The temperature of heating mantle was maintained at 68±5°C. The extraction process took 5 to 6 h for each sample. The crude botanical extract obtained from Soxhlet apparatus was concentrated by evaporating excess of solvent using rotary evaporator (DAIHAN Scientific North America Inc., USA). Final concentrated extracts were preserved in hermetic dark glass vials in a refrigerator at 4°C until their downstream use in toxicity bioassays.

Table I. Geographical coordinates of sites for the collection of indigenous flora of Soon Valley and surrounding Salt Range situated in district Khushab, Punjab, Pakistan.

Localities	Latitude N	Longitude E	Elevation (m)
Khura	32.23° N	72.11° E	866
Daip 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
Anga	32.35° N	72.05° E	821
Khabbeki	32.35° N	72.12° E	774

Collection of mosquitoes

Mosquito (*C. quinquefasciatus*) larvae were collected from the water pound near the College of Agriculture (32°06' N to 72°39' E) with the help of an aquatic net. It was ensured that collection site was never exposed to any insecticide application. These larvae were brought to the laboratory for identification and were reared up to F_3 to get a homogeneous population.

Larvicidal bioassays with botanical extracts

In initial screening bioassays, only one concentration (0.5%) of each plant extract was used. Twenty five late 3rd or early 4th fourth instar larvae of *C. quinquefasciatus* were released in 30 ml of 0.5% aqueous solution of each plant extract in disposable glasses (100 ml). The experimental layout was CRD with five replications for each treatment and was performed under controlled condition (25±2ºC and 60±5% RH) with 16:8 light and dark hours, respectively. The mortality of mosquito larvae was recorded at 24 and 48 h post-exposure. Ten plants exhibiting significant larvicidal activities in screening bioassays were further bioassayed to determine their detailed toxicity. A volume of 30 ml of following concentrations (2.0, 1.0 and 0.5%) were prepared form stock solution of plant extracts in disposable plastic glasses (100 ml). Late 3rd or early 4th instar larvae (n = 25) of *C. quinquefasciatus* were released in these plastic cups with the help of a dropper. The mortality of larvae was observed at 12, 24 and 48 h post-exposure. Each treatment was replicated four times.

Larvicidal activities of synthetic and microbial insecticides

Larvicidal activities of synthetic and microbial insecticides were determined by performing bioassays according to WHO protocol with insecticidal formulations detailed in Table II. One drop of Tween 80 was used to solubilize the microbial insecticides in water. Three concentrations (800, 400 and 200 ppm) of microbial insecticides were used and water with Tween 80 was used as control. However, four concentrations (5.0, 2.5, 1.25 and 0.62 ppm) of synthetic insecticides, causing mortality from 10 to 90%, were employed and only water was used as control. Late 3rd or early 4th instar larvae (n = 25) of *C. quinquefasciatus* were tested in disposable glasses. The mortality of mosquito larvae was recorded at 24 and 48 h post-exposure for synthetic and microbial insecticides, respectively. The experiment was repeated four times and was performed under controlled condition (25±2°C and 60±5% RH) with 16:8 light and dark hours, respectively.

Statistical analysis

Prior to statistical analysis, data regarding the mosquito larval mortality were corrected using Abbott's formula (Abbott, 1925). Lethal concentration (LC_{50} and LC_{90}) values were calculated by Probit analysis using POLO® Plus version 2.0 (LeOra Software). Mortality data was subjected to one-way ANOVA and the treatment means were compared by Tukey's HSD at 95% level of significance.

RESULTS

Identification of plants

Botanical extracts are world widely used for insect control. They are effective against insects without considerable deleterious effects on the environment. This study focuses on the identification of plants from salt range to assess their toxicity potential against mosquito larvae. The plants were collected from different locations of Soon Valley and its surrounding salt range (Punjab, Pakistan). These plants were identified up to species level with the help of botanists from the Department of Botany, University of Sargodha, Sargodha. The vernacular names provided by the native inhabitants, botanical names and literature-based phyto-constituents of collected plants are given in Supplementary Table SI. Interestingly, all plants collected from salt range constitute of alkaloids, flavonoids, terpenoids, tannins and saponins in common, showing their anti-insect potential. This plant collection and characterization would serve as baseline data about the indigenous flora of study area.

Initial screening of botanical extracts against C. quinquefasciatus *larvae*

N-hexane extracts of 40 plant species were bioassayed initially against *C. quinquefasciatus* larvae. The result of these piolet screening bioassays (Table III) revealed that most of plant extracts showed significant mortality of mosquito larvae as compared to control ($p \leq 0.05$). Out of 40 botanical extracts, 18 showed more than 50% mortality of mosquito larvae. The extract of *M. arenaria* exhibited highest larvicidal activities (87%) against *C. quinquefasciatus*, followed by *N. indicum* (84%), *W. coagulans* (83%), *S. fruticosa* (81%), *O. ferruginea* (fruit) (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%),

Table II. Selective microbial and synthetic insecticide formulations bioassayed against *Culex quinquefasciatus* larvae.

Insecticides	Trade name	Formulation	Company
Indoxacarb	Steward®	15 SC	FMC
Pyriproxyfen	Admiral®	10 EC	FMC
Permethrin	Rid®	10 EC	Bayer
Lambda-cyhalothrin	Karate®	2.5EC	Syngenta
Bacillus thuringiensis NCIM 2514	Lipel®	WP (18000 IU/mg)	AgriLife, India
Metarhizium anisopliae NCIM 1311	Pacer®	WP (1×10^8 cfu/g)	AgriLife, India
Beauveria bassiana NCIM 1216	Racer®	WP (1×10^8 cfu/g)	AgriLife, India
Isaria fumosorosea PFA 011	Paecilomite®	WP (1×10^8 cfu/g)	AgriLife, India

Table III. Percent corrected mortality (mean ± S.D.) of *Culex quinquefasciatus* larvae at 48 h post-exposure to 0.5% extracts of different plant species. Treatment means sharing different alphabets of homogenous group are significantly different each other (one-way ANOVA; HSD at $p \leq 0.05$).

Sr. no.	Plant species	Vernacular names	Plant parts used	Mean mortality (%) ± S.D.	Homogenous groups
1	*Maerua arenaria* Hook	Hemkand	Leaves	87±6	A
2	*Nerium indicum* Mill.	Kanera	Leaves	84±4	AB
3	*Withania coagulans* (Stocks) Dunal	Paneer booti	Leaves	83±4	ABC
4	*Suaeda fruticosa* (L.) Delile	Lahnra	Leaves / Stem	81±5	A-D
5	*Olea ferruginea* Wall. ex Aitch.	Zatoon	Fruit	79±7	B-E
6	*Adiantum capillus-veneris* L.	Khatti booti	Leaves	78±4	B-E
7	*Dicliptera bupleuroides* Nees	Kaalu and Pipri	Leaves / Stem	77±7	B-E
8	*Astragalus* spp. L.	Koohni	Leaves	73±5	B-F
9	*Solanum surattense* Burm. f.	Kanda kari	Leaves	73±6	B-F
10	*Eruca sativa* Mill.	Jamahoon	Leaves	72±7	C-H
11	*Cynodon dactylon* (L.) Pers.	Khabal	Leaves	69±10	C-G
12	*Marrubium vulgare* L.	Pahari gandana	Leaves	69±7	C-H
13	*Buxus papillosa* Schneid.	Shamshad	Leaves	69±13	D-H
14	*Trichodesma indicum* (L.) Lehm.	Juri	Fruit	68±10	D-H
15	*Datura alba* L.	Dhatura	Leaves	66±10	E-I
16	*Opuntia dillenii* (Ker Gawl.) Haw.	Thor	Leaves	61±4	F-J
17	*Chenopodium album* L.	Bathuwa	Leaves	57±13	H-K
18	*Solanum incanum* L.	Mahori	Leaves	53±12	K-O
19	*Dodonaea viscosa* (L.) Jacq.	Santha	Leaves	49±8	J-M
20	*Periploca aphylla* Decne.	Bata	Stem	49±7	I-M
21	*Melilotus officinalis* (L.) Pall.	Yellow sweet clover	Leaves	49±7	J-M
22	*Salvia officinalis* L.	Khalatra	Leaves	49±14	I-L
23	*Justicia adhatoda* L.	Dhodak booti	Leaves	48±7	J-N
24	*Mentha longifolia* (L.) Huds.	Desi podina	Leaves	48±10	J-N
25	*Portulaca oleracea* L.	Loonak	Leaves	46±7	J-M
26	*Salvia virgata* Jacq.	Meadow sage	Leaves	42±7	L-O
27	*Rumex dentatus* L.	Toothed dock	Leaves	42±10	L-O
28	*Amaranthus viridis* L.	Jangli cholai	Leaves	40±14	L-P
29	*Sonchus asper* (L.) Hill	Bhattal	Leaves	40±10	J-M
30	*Petrophytum caespitosum* Rydb.	Mat rock spiraea	Leaves	39±4	M-P
31	*Ricinus communis* L.	Harnoli	Leaves	36±4	M-Q
32	*Dryopteris filix-mas* (L.)	Male fern	Leaves	34±4	N-R
33	*Cassia occidentalis* L.	Bana chakunda	Fruit	33±7	O-R
34	*Fagonia indica* Burm.f. and Thomson	Dhamasa	Leaves	29±13	G-K
35	*Murraya koenigii* (L.) Spreng.	Jangli curry patta	Leaves	28±8	P-S
36	*Nerium indicum* Mill.	Kanera	Leaves	27±0	P-S
37	*Rhamnus smithi* Greene	Buck thorn	Leaves	23±8	QRS
38	*Alternanthera pungens* Kunth	Kandaa booti	Leaves	21±7	RST
39	*Cassia occidentalis* L.	Bana chakunda	Leaves	21±4	RST
40	*Acacia melanoxylon* R.Br.	Hickory	Leaves	19±10	ST

T. indicum (68%), *D. alba* (66%), *O. dillenii* (61%), *C. album (57%)* and *S. incanum* (53%), whereas the remaining plant extracts showed less than 50% larval mortality.

Toxicity bioassay with the most effective plant extracts against C. quinquefasciatus *larvae*

Based on the results of initial screening bioassays, ten plants, exhibiting significant mortality (more than 70%), were further evaluated against *C. quinquefasciatus* larvae. Results of this toxicity bioassay (Table IV) revealed that the extracts of *M. arenaria* and *N. indicum* were most effective showing lowest LC_{50} values *i.e.* 0.116 and 0.176%, respectively, and were significantly different from all other plant extracts (Fig. 1). The extract of *E. sativa* leaves showed the highest LC_{50} and LC_{90} values of 2.58 and 15.9%, respectively, and caused minimum larval mortality as compared to all other plant extracts (Table IV).

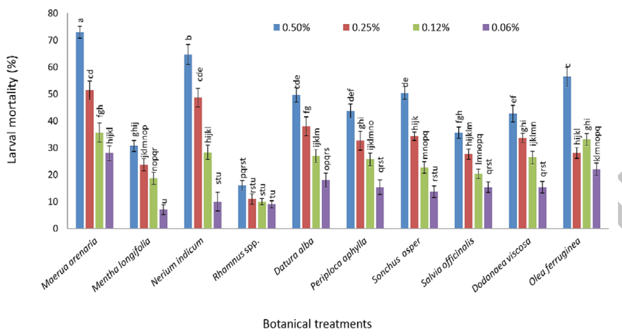


Fig. 1. Percent corrected mortality (mean ± S.D.) of *Culex quinquefasciatus* larvae bioassayed against selected microbial (A) and synthetic insecticides (B). Asterisk symbols indicate the significant difference among LC_{50} and LC_{90} values of microbial or synthetic insecticides due to non-overlapping of their C.I.

Larvicidal activities of microbial and synthetic insecticides against C. quinquefasciatus *larvae*

The results of larvicidal bioassay conducted with microbial insecticides (Fig. 2A) showed that all insecticidal formulations caused significant larval mortality ($p \leq 0.05$) as compared to control. *M. anisopliae* was the most effective larvicidal treatment exhibiting significantly highest mortality (83%), followed by *B. thuringiensis* (60%), *B. bassiana* (58%), while the lowest larval mortality was recorded for *I. fumosorosea* (50%) at 800 ppm at 48 h post-exposure. Similarly, entomopathogenic fungi *M. anisopliae* had the lowest LC_{50} value *i.e.* 325 ppm and was the most toxic larvicide as compared to other three microbial insecticides (95 % CI did not overlap). *B. thuringiensis*, *B. bassiana* and *I. fumosorosea* showed similar toxicity against *C. quinquefasciatus* larvae (Fig. 2A). Larvicidal evaluation of synthetic insecticides against *C. quinquefasciatus* showed that permethrin exhibited 70% mortality at 0.62 ppm. Indoxacarb showed 86% mortality at 5 ppm. Lambda-cyhalothrin displayed 73% at 2.5 ppm and pyriproxyfen showed 86% mortality at 200 ppm at 24 h post-exposure. Indoxacarb had the lowest LC_{50} value *i.e.* 0.14 ppm, and was the most toxic synthetic insecticide as compared to other three tested insecticides (95 % CI did not overlap). Permethrin and lambda-cyhalothrin were moderately toxic larvicide as compared to pyriproxyfen which was proved to be the least toxic synthetic chemical (Fig. 2B).

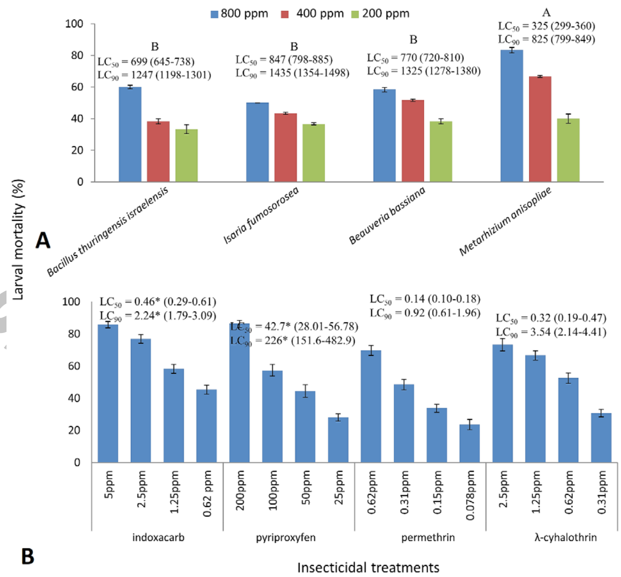


Fig. 2. Percent corrected mortality (mean ± S.D.) of *Culex quinquefasciatus* larvae bioassayed against different concentrations of selected botanical extracts. Treatment means sharing different alphabets are significantly different from each other (one-way ANOVA; HSD at $p \leq 0.05$).

DISCUSSION

Mosquitoes are responsible to transmit world's most severe life-threatening diseases (Benelli and Mehlhorn, 2016). Mosquitoes in the larval stage are more susceptible targets for chemical control because they breed in water making it easy to control in this habitat. The use of conventional pesticides in the water sources is highly risky to humans and their environment. Better alternative control means are required due to the continuous increase in resistance of mosquitoes to commonly used conventional synthetic insecticides (Tikar *et al*., 2008). Pakistan, particularly salt range (study area), has diverse ecological zones, rich natural resources and flora with more than

Table IV. Lethal concentration values of the most potent botanical extracts bioassayed against *Culex quinquefasciatus* larvae.

Plant species	Plant Parts extracted	LC_{50} (%) (95% CI)	LC_{90} (%) (95% CI)	Significance (ANOVA; HSD at $p \leq 0.05$)
Maerua arenaria	Leaves and stem	0.116 (0.100-0.147)	0.591 (0.469-0.807)	A
Nerium indicum	Leaves	0.176 (0.142-0.204)	0.802 (0.605-1.198)	A
Withania coagulans	Leaves	0.234 (0.210-0.284)	2.053 (1.496-3.109)	B
Suaeda fruticosa	Leaves and stem	0.333 (0.278-0.378)	2.207 (1.648-3.211)	B
Olea ferruginea	Leaves	0.272 (0.245-0.306)	1.879 (1.422-2.684)	B
Adiantum capillus-veneris	Leaves	0.318 (0.281-0.368)	2.666 (1.763-4.778)	B
Dicliptera bupleuroides	Leaves	0.411 (0.351-0.501)	4.702 (2.968-8.850)	B
Astragalus spp.	Fruits	0.311 (0.267-0.374)	2.019 (1.366-3.452)	B
Solanum surattense	Leaves and stem	0.682 (0.510-1.065)	9.550 (4.410-33.583)	C
Eruca sativa	Leaves	2.589 (1.427-7.289)	15.9 (0.89-26.5)	D

6000 plant species (Ahmad *et al*., 2009; Nawaz *et al*., 2012). As native vegetation of a particular area may contain insecticidal properties which need to be evaluated for their potential use in pest control (Isman, 2008), the present study was conducted to evaluate the larvicidal potential of indigenous plant species of Soon valley and surrounding range of Pakistan along with some promising microbial and synthetic insecticide formulations against 3rd and/or 4th instar larvae of *C. quinquefasciatus*. Most of the plant species collected belonged 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 as described in Supplementary Table S1. Our results revealed that the extract of *M. arenaria* was most effective against mosquito larvae. Aqueous extract of this plant species constitutes of alkaloids, phenolics, phytosterols and saponins (Ali *et al*., 2008) which would be responsible for the observed significant mortality of mosquito larvae. Likewise, the extracts of *N. indicum* have different alkaloids and terpenoids which showed anti-feeding, ovicidal, larvicidal and repellant activities against a wide range of insect pests including mosquitoes (Hiremath *et al*., 1997; Srivastava *et al*., 2003; Saxena and Sharma, 2005; Rahuman *et al*., 2008; Dey *et al*., 2017). Acetone and methanolic extracts of *N. indicum* at 0.02 to 0.03% concentrations showed significant mortality (more than 50%) of *C. quinquefasciatus* larvae (PreetiSharma *et al*., 2005).

Similarly, *D. viscosa* and *O. ferruginea* also exhibited significant larvicidal activity. Both these indigenous plant species have ethnomedicinal values (Shah and Rahim, 2017). *D. viscosa* plant constitutes of such phytochemicals as lupeol, stimgasterols, diterpenoids, flavonol-3-methyl ethers and certain fatty acids (Abdel-Mogib *et al*., 2001) 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* constitutes of 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*) (Kisa *et al*., 2018).

In addition, *W. coagulans* and *S. fruticosa* extracts contain 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 (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* caused 80 and 70% mortality in *Plutella xylostella* and *Aphis craccivora*, respectively (Sharma and Sood, 2012). Taken together, the screened plants could provide a baseline for their insecticidal potential. The extract of highly effective plants could be used for the development of organic mosquito repellent at commercial level and their bioactive fractions could be further developed as botanical mosquitocidal formulations.

Nevertheless, microbial pesticides also appear as alternative to chemical insecticides with target specificity and ecological safety so that they are used individually or in combination with other pest management programs. Among entomopathogenic formulations tested, *M. anisopliae* showed significant mortality of *C. quinquefasciatus* larvae. The possible mode of action of this fungus could be the floating conidia come in contact with larvae. Conidia break the water tension with their peri-spiracular valves for air intake. The fungal conidia germinate and penetrate into the siphon which blocks the breathing mechanism. In warm and moist conditions, conidiophores grow on the cuticle and cover the whole insect with conidia (Daoust *et al.*, 1982; Lacey *et al.*, 1988). The presence of different toxic proteins increases the larvicidal activity and suppresses the development of resistance. Unfortunately, there is no ideal mosquito-pathogenic fungal strain presently known which effectively kill the mosquito larvae. Among the synthetic insecticides, indoxacarb showed highest larval mortality. Indoxacarb is a neurotoxic insecticide that blocks voltage-dependent sodium channels, resulting in insect paralysis and death and is considered safe for environment (Wing *et al.*, 2010) and has shown excellent results against pyrethroid resistant mosquitos including *Anopheles* and *Culex* species (N'Guessan *et al.*, 2007; Shah *et al.*, 2016).

CONCLUSIONS

Overall study results provide preliminary database regarding the insecticidal potential of indigenous plant species of Soon valley and surrounding salt range of Pakistan. These above mentioned effective plants extracts along with microbial insecticides are therefore recommended for the biorational management of mosquitoes and to minimize the contemporary issues of environmental contamination and health hazards associated with the use of persistent synthetic insecticides. Further biochemical characterization of effective plant extracts and field evaluation of these selected botanical, microbial and synthetic insecticides against mosquito larvae and their non-target effects on the environment constitute the future perspectives of this study. Sustainable, safe, and environment-friendly control methods should be established that can target different mosquito species.

ACKNOWLEDGEMENT

This research work was financially supported by a research project (No. 6702) funded by the Higher Education Commission (HEC) of Pakistan under its National Research Programme for Universities (NRPU).

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210705100711

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbot, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.*, **18**: 266-267. https://doi.org/10.1093/jee/18.2.265a

Abdel-Mogib, M., Basaif, S.A., Asiri, A.M., Sobahi T.R. and Batterjee, S.M., 2001. New clerodane diterpenoid and flavonol-3-methyl ethers from *Dodonaea viscosa*. *Die Pharm*., **56**: 830–831.

Ahmad, I., Ahmad, M.S.A., Hussain, M., Hameed, M., Ashraf, M.Y. and Koukab, M.Y., 2009. Spatio-temporal effects on species classification of medicinal plants in Soone Valley of Pakistan. *Int. J. Agric. Biol*., **11**: 64–68.

Ali, Y., Riaz, N., Afza, N., Kalhoro, M.A. and Malik, A., 2008. Phytochemical studies on *Maerua arenaria*. *J. chem. Soc. Pak*., **30**: 102–105.

Atkinson, C.T. and LaPointe, D.A., 2009. Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. *J. Avian Med. Surg.,* **23**: 53–63. https://doi.org/10.1647/2008-059.1

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M.B., Dahl, C. and Kaiser, A., 2020. *Mosquitoes: Identification, ecology and control*. Springer International Publishing, Switzerland. https://doi.org/10.1007/978-3-030-11623-1_16

Benelli, G., 2015. Research in mosquito control: current challenges for a brighter future. *Parasitol. Res.,* **114**: 2801–2805. https://doi.org/10.1007/s00436-015-4586-9

Benelli, G. and Mehlhorn, H., 2016. Declining malaria, rising of dengue and Zika virus: Insights for mosquito vector control. *Parasitol. Res.,* **115**: 1747–1754. https://doi.org/10.1007/s00436-016-4971-z

Bukhari, T., Takken, W. and Koenraadt, C.J., 2013. Biological tools for control of larval stages of malaria vectors. A review. *Biocont. Sci. Technol.,* **23**: 987–1023. https://doi.org/10.1080/09583157.2013.810706

Dacey, D.P. and Chain, F.J., 2020. The challenges of microbial control of mosquito-borne diseases due to the gut microbiome. *Front. Genet.*, **11**: 1238. https://doi.org/10.3389/fgene.2020.504354

Daoust, R.A., Ward, M.G. and Roberts, D.W., 1982. Effect of formulation on the virulence of *Metarhizium anisopliae* conidia against mosquito larvae. *J. Invertebr. Pathol*., **40**: 228–236. https://doi.org/10.1016/0022-2011(82)90120-3

De Wispelaere, M., Desprès, P. and Choumet, V., 2017. European *Aedes albopictus* and *Culex pipiens* are competent vectors for Japanese encephalitis virus. *PLoS Negl. Trop. Dis.,* **11**: e0005294. https://doi.org/10.1371/journal.pntd.0005294

Dey, A., Gorai, P., Mukherjee, A., Dhan, R. and Modak, B.K., 2017. Ethnobiological treatments of neurological conditions in the Chota Nagpur Plateau, India. *J. Ethnopharmacol.*, **198**: 33–44. https://doi.org/10.1016/j.jep.2016.12.040

Díaz, M., Díaz, C.E., Alvares, R.G., González, A., Castillo, L., González-Coloma, A. and Rossini, C., 2015. Differential anti-insect activity of natural products isolated from *Dodonaea viscosa* Jacq. (Sapindaceae). *J. Pl. Protect. Res.*, **55**: 2. https://doi.org/10.1515/jppr-2015-0023

Dimetry, N.Z., El-Gengaihi, S., Hafez, M. and Abbass, M.H., 2015. Pesticidal activity of certain plant extracts and their isolates against the cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae). *HerbaPol,* **61**: 77–92. https://doi.org/10.1515/hepo-2015-0024

Federici, B.A., 1995. The future of microbial insecticides as vector control agents. *J. Am. Mosq. Cont. Assoc. Mosq. New*, **11**: 260–268.

Gupta, L. and Srivastava, M., 2008. Effect of *Withania somnifera* extracts on the mortality of *Callosobruchus chinensis* L. *J. Biopest.,* **1**: 190–192.

Hiremath, G.I., Ahn, Y.J. and Kim, S.I., 1997. Insecticidal activity of Indian plant extracts against *Nilaparvata lugens* (Homoptera: Delphacidae). *Appl. Ent. Zool.*, **32** 1: 159–166. https://doi.org/10.1303/aez.32.159

Isman, M.B., 2008. Botanical insecticides for richer, for poorer. *Pest Manage. Sci.,* **64**: 8–11. https://doi.org/10.1002/ps.1470

Kısa, A., Akyüz, M., Çoğun, H.Y., Kordali, Ş., Bozhüyük, A.U., Tezel, B., Şiltelioğlu, U., Anıl, B. and Çakır, A., 2018. Effects of *Olea europaea* L. leaf metabolites on the tilapia (*Oreochromis niloticus*) and three stored pests, *Sitophilus granarius*, *Tribolium confusum* and *Acanthoscelides obtectus*. *Rec. Nat. Prod.,* **12**: 201–215. https://doi.org/10.25135/rnp.23.17.07.126

Koliopoulos, G., Pitarokili, D., Kioulos, E., Michaelakis, A. and Tzakou, O., 2010. Chemical composition and larvicidal evaluation of mentha, salvia, and melissa essential oils against the West Nile virus mosquito *Culex pipiens*. *Parasit. Res.,* **107**: 327–335. https://doi.org/10.1007/s00436-010-1865-3

Lacey, C.M., Lacey, L.A. and Roberts, D.R., 1988. Route of invasion and histopathology of *Metarhizium anisopliae* in *Culex quinquefasciatus*. *J. Invertebr. Pathol.*, **52**: 108-118.

Lacey, L.A. and Siegel, J.P., 2000. Safety and ecotoxicology of entomopathogenic bacteria. In: *Entomopathogenic bacteria: From laboratory to field application* (eds. J.F. Charles, A. Delecluse and C. Nielsen-Le Roux). Kluwer Academic, Dordrecht, pp. 253–273. https://doi.org/10.1007/978-94-017-1429-7_14

Lai, C.H., Tung, K.C., Ooi., H.K. and Wang, J.S., 2000. Competence of *Aedes albopictus* and *Culex quinquefasciatus* as vector of *Dirofilaria immitis* after blood meal with different microfilarial density. *Vet. Parasitol*., **90**: 231–237. https://doi.org/10.1016/S0304-4017(00)00242-9

Lounibos, L.P., 2002. Invasions by insect vectors of human disease. *Annu. Rev. Ent.,* **47**: 233–266. https://doi.org/10.1146/annurev.ento.47.091201.145206

Majeed, M.Z., Afzal, M., Riaz, M.A., Ahmed, K.S., Luqman, M., Shehzad, M.Z., Tayyab, M.B., Tanvir, M. and Wahid, S., 2020. Comparative toxicity of phyto-extracts of indigenous flora of Soone valley against some insect pests of agricultural and urban importance. *Punjab Univ. J. Zool.*, **35**: 239–253. https://doi.org/10.17582/journal.pujz/2020.35.2.239.253

Malarvannan, S., Giridharan, R., Sekar, S., Prabavathy, V.R. and Nair, S., 2009. Ovicidal activity of crude extracts of few traditional plants against *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). *J. Biopest*., **2**: 64–71.

Manguin, S. and Boëte, C., 2011. Global impact of mosquito biodiversity, human vector-borne diseases and environmental change. In: *The importance of biological interactions in the study of biodiversity* (ed. Lopez-Pujol). Intech Open Access Publisher, Rijeka, Croatia, pp. 27–50. https://doi.org/10.5772/22970

Mathur, D., Agrawal, R.C. and Shrivastava, V., 2011. Phytochemical screening and determination of antioxidant potential of fruits extracts of *Withania coagulans*. *Rec. Res. Sci. Technol.,* **3**: 26–29.

Mohammed, A.K. and Nawar, M.H., 2020. Study of the effect of alcoholic extract of *Dodonaea viscosa* L. leaves on the life performance of the greater wax worm *galleria mellonella* L. (Lepidoptera: Pyralidae). *Pl. Arch.,* **20**: 3449–3454.

Mullen, G.R. and Durden, L.A., 2009. *Medical and veterinary entomology*. Academic Press, New York.

Nawaz, T., Hameed, M., Ashraf, M., Ahmad, F., Ahmad, M.S.A., Hussain, M., Ahmad, I., Younis, A. and Ahmad, K.S., 2012. Diversity and conservation status of economically important flora of the Salt Range, Pakistan. *Pak. J. Bot.,* **44**: 203–211.

N'Guessan, R., Corbel, V., Bonnet, J., Yates, A., Asidi, A., Boko, P., Odjo, A., Akogbeto, M., Rowland, M., 2007. Evaluation of indoxacarb, an oxadiazine insecticide for the control of pyrethroid-resistant *Anopheles gambiae* (Diptera: Culicidae). *J. med. Ent*., **44**: 270–276. https://doi.org/10.1603/0022-2585(2007)44[270:EOIAOI]2.0.CO;2

Paily, K.P., Kumar, B.A. and Balaraman, K., 2007. Transferrin in the mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), up-regulated upon infection and development of the filarial parasite, *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae). *Parasitol. Res.,* **101**: 325–330. https://doi.org/10.1007/s00436-007-0474-2

PreetiSharma, L.M. and Srivastava, C.N., 2005. Larvicidal potential of *Nerium indicum* and *Thuja oriertelis* extracts against malaria and Japanese encephalitis vector. *J. Environ. Biol.,* **26**: 657-660.

Rahuman, A. A., Gopalakrishnan, G., Venkatesan, P., Geetha, K., 2008. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. *Parasitol. Res*., **102**: 981–988. https://doi.org/10.1007/s00436-007-0864-5

Regis, L., da Silva, S.B. and Melo-Santos, M.A.V., 2000. The use of bacterial larvicides in mosquito and black fly control programmes in Brazil. *Mem. Instit. Oswal. Cruz.,* **95**: 207–210. https://doi.org/10.1590/S0074-02762000000700035

Rose, R. I., 2001. Pesticides and public health: Integrated methods of mosquito management. *Emerg. Infect. Dis.,* **7**: 17–23. https://doi.org/10.3201/eid0701.010103

Salam, N., Mustafa, S., Hafiz, A., Chaudhary, A.A., Deeba, F. and Parveen, S., 2018. Global prevalence and distribution of coinfection of malaria, dengue and chikungunya: A systematic review. *BMC Publ. Hlth*., **18**: 1–20. https://doi.org/10.1186/s12889-018-5626-z

Saxena, R., Sharma and A.K., 2005. Insecticidal potentialities of *Ageratum conyzoides* and *Nerium indicum* leaves extracts against *Epilachna 28-punctata* (F.). *Vegetos,* **18**: 43–45.

Senthil-Nathan, S., 2020. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Front. Physiol*., **10**: 1591. https://doi.org/10.3389/fphys.2019.01591

Shah, R.M., Alam, M., Ahmad, D., Waqas, M., Ali, Q., Binyamin, M. and Shad, S.A., 2016. Toxicity of 25 synthetic insecticides to the field population of *Culex quinquefasciatus* Say. *Parasitol. Res.,* **115**: 4345-4351.

Shah, A. and Rahim, S. 2017. Ethnomedicinal uses of plants for the treatment of malaria in Soon Valley, Khushab, Pakistan. *J. Ethnopharmacol*., **200**: 84–106. https://doi.org/10.1016/j.jep.2017.02.005

Sharma, N. and Sood, S., 2012. Effect of *Adiantum capillus-veneris* against *Plutella xylostella* (Lepidoptera: Yponomeutoidae) and *Aphis craccivora* (Homoptera: Aphididae). *Bionfolet*, **9**: 105–110.

Srivastava, V.K., Singh, S.K., Rai, M. and Singh, A., 2003. Toxicity of *Nerium indicum* and *Euphorbia royleana* lattices against *Culex quinquefasciatus* mosquito larvae. *Niger. J. Nat. Prod. Med*., **7**: 61–64. https://doi.org/10.4314/njnpm.v7i1.11712

Sukumar, K., Perich, M.J., Boobar and L.R., 1991. Botanical derivatives in mosquito control: A review. *J. Am. Mosq. Cont. Assoc*., **7**: 210–237.

Tatem, A.J., Rogers, D.J. and Hay, S.I., 2006. Global transport networks and infectious disease spread. *Adv. Parasitol.,* **62**: 293–343. https://doi.org/10.1016/S0065-308X(05)62009-X

Teressa, H., Ersino, W. and Alemayo, T., 2019. Evaluation of larvacidal activity of *Olea europaea* extract against *Anopheles Mosquito* in *in vitro*, *Fogera Woreda*, north western Ethiopia. *Agric. Res. Technol*., **22**: 1–5.

Tikar, S.N., Mendki, M.J., Chandel, K., Parashar, B.D. and Prakash, S., 2008. Susceptibility of immature stages of *Aedes* (Stegomyia) *aegypt*i; vector of dengue and chikungunya to insecticides from India. *Parasitol. Res*., **102**: 907–913. https://doi.org/10.1007/s00436-007-0848-5

Vythilingam, I., Oda, K., Mahadevan, S., Abdullah, G., Thim, C.S., Hong, C.C., Vijayamalar, B., Snniah, M. and Igarashi, A., 1997. Abundance, parity, and Japanese encephalitis virus infection of mosquitoes (Diptera: Culicidae) in Sepang District, Malaysia. *J. med. Ent.,* **34**: 257–262. https://doi.org/10.1093/jmedent/34.3.257

Wing, K.D., Andaloro, J.T. and McCann, S.F., 2010. Indoxacarb and the sodium channel blocker insecticides: Chemistry, physiology and biology in insects. In: *Comprehensive insect physiology, biochemistry, pharmacology and molecular biology* (eds. L. Gilbert, S. Gill and K. Latrou). Elsevier, Amsterdam., pp. 31–55. https://doi.org/10.1016/B0-44-451924-6/00070-3

Zhu, J., Zeng, X., O'neal, M., Schultz, G., Tucker, B., Coats, J., Bartholomay, L. and Xue, R.D., 2008. Mosquito larvicidal activity of botanical-based mosquito repellents. *J. Am. Mosq. Cont. Assoc.* **24**: 161–168.

Pakistan J. Zool., pp 1-9, 2021. DOI: https://dx.doi.org/10.17582/journal.pjz/20210705100711

Supplementary Material

Comparative Efficacy of Selected Biorational Insecticides against Larvae of Southern House Mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae)

Mujahid Tanvir[1], Muhammad Asam Riaz[1]*, Muhammad Zeeshan Majeed[1], Mazhar Iqbal Zafar[2], Muhammad Tariq[3] and Muhammad Bilal Tayyab[1]

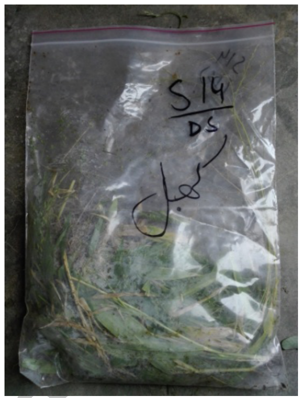
[1]Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan
[2]Department of Environmental Sciences, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan
[3]Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46000, Pakistan

Supplementary Table SI. Taxonomic and vernacular information of indigenous plant samples collected from the different locations of Soon Valley and surrounding Salt Range of Pakistan

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
1	*Chenopodium album*	Bathuwa	Khura	Leaves	Amaranth-aceae	Alkaloids, Flavonoids, Saponin, Tannins (Mojab et al. 2010; Pandey and Gupta 2014)	
2	*Buxus papil-losa*	Sham-shad	Khura	Leaves	Buxaceae	Alkaloids, Flavonoids, Phenols (Parveen et al 2001; Akhtar and Mirza 2018)	

Table continue on next page ....?

* Corresponding author: asam.riaz@uos.edu.pk
0030-9923/2021/0001-0001 $ 9.00/0

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
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	Not available	
5	*Astragalus* Spp.	Koohni	Khura	Leaves and stem	Fabaceae	Not available	
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)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
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)*		Daep Sharif		Not clear yet	Not available	

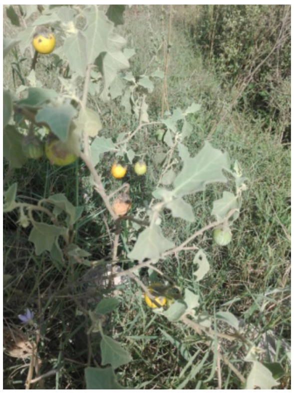
Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
11	*Mentha longifolia*	Desi podina	Daep Sharif	Leaves and stem	Lamiaceae	Essential oils, Flavonoids (Ghoulami et al. 2001)	
12	*Solanum surattense*	Kanda kari/ Choti Kateri	Daep Sharif	Leaves and fruit	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)	

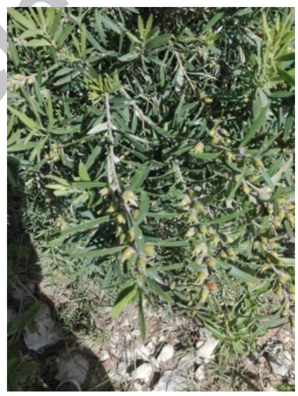
Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
15	*Acacia melanoxylon*	Hickory	Daep Sharif	Leaves and stem	Fabaceae	Alkaloids, flavonoids, Phenols (Luis et al. 2012)	
16	*S-22 (Unidentified)*		Daep Sharif		Not clear yet	Not available	
17	*Datura alba*	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)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
19	*Alternanthera pungens*	Kandaa Booti/ Phakra	Uchhali	Leaves and stem	Amaranth-aceae	Alkaloids, Anthocyano-sides, Anthraquinons, Carbhydrates, Coumarins, Flavonoids, Lipids, Phe-nol, Saponins, Steroids, Triterpenoids, Tannins, (Zongo et al. 2011; Kalpa-na et al. 2018)	
20	*Opuntia dillenii*	Thor	Kanhati Garden	Leaves and roots	Cactaceae	Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Steroids, Terpe-onids Tannins (Pooja and Vidyasagar 2016)	
21	*Murraya koenigii*	Jangli curry Patta	Kanhati Garden	Leaves and stem	Rutaceae	Alkaloids, Anthraquinons, Carbhydrates, Flavo-noids, Proteins, Phytos-terols, 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, Terpe-noids (Khan et al 2012)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
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)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
27	*Adiantum capillus-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)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
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)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
35	*Portulaca oleracea*	Loonak	Angah	Leaves and stem	Portulacaceae	Fatty acids, Organic acids, Phenolic compounds (Oliveira et al. 2009)	
36	*Dodonaea viscosa*	Santha/ Pippar	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	Ligstroside, Oleuropein, Quercetin,β-amyrin (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)	

Sr. No.	Scientific name	Common name	Locality	Part(s) used	Family	Phytochemical (s)	Picture of plant
39	*Withania coagulans*	Paneer booti/ Kham-jeera	Angah	Leaves, fruits	Solanaceae	Alkaloids, Amino acids, Carbohydrates, Organic acids, Phenolic compounds, Proteins, Steroids, Saponin, Tannins, (Mathur et al. 2011)	
40	*Eruca sativa*	arden rocket/ Jamahoon	Angah	Flower	Brassicaceae	Allyl isothiocyanate, 3-butenyl isothiocyanate, 4-methylsulfinybutyl isothiocyanate, sulforaphane), 2-phenylethyl isothiocyanate and bis (isothiocyanatobutyl) disulphide, fatty acids (Khoobchandani et al. 2010)	

REFERENCES

Akhtar, N. and Mirza, B., 2018. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arab. J. Chem.*, **11:** 1223-1235. https://doi.org/10.1016/j.arabjc.2015.01.013

Amessis-Ouchemoukh, N., Abu-Reidah, I. M., Quirantes-Piné, R., Madani, K. and Segura-Carretero, A., 2014. Phytochemical profiling, in vitro evaluation of total phenolic contents and antioxidant properties of *Marrubium vulgare* (horehound) leaves of plants growing in Algeria. *Ind. Crops Prod.*, **61:** 120-129. https://doi.org/10.1016/j.indcrop.2014.06.049

Anusha, K., Balakrishnan, S., Sindhu, S., Arumugam, P. and Hariram, S.B., 2014. Studies on phytochemical screening and antioxidant potential of *Trichodesma indicum*. *Int. J. Pharmacogn. Phytochem. Res.*, **6:** 536-539.

Auta, R., James, S.A., Auta, T. and Sofa, E.M., 2011. Nutritive value and phytochemical composition of processed *Solanum incanum* (Bitter garden egg). *Sci. World. J.*, **6:** 5-6.

Bhuvaneshwari, L., Arthy, E., Anitha, C., Dhanabalan, K. and Meena, M., 2007. Phytochemical analysis & antibacterial activity of *Nerium oleander*. *Anc. Sci. Life.*, **26:** 24.

Burm, F., 2011. Chemical constituents and biological activities of *Fagonia indica* Burm F. *Res. J. med. Pl.*, **5:** 531-546.

Chanu, W.S. and Sarangthem, K., 2014. Phytochemical constituents of *Justicia adhatoda* linn. found in Manipur. *Indian J. Pl. Sci.*, **3:** 2319-3824.

Eman, A.A., 2011. Morphological, phytochemical and biological screening on three Egyptian species of *Fagonia*. *Acad. Arena*, **3:** 18-27.

Erhirhie, E.O., 2018. Teratogenic effects of ethanol leaf extract of *Dryopteris filix–mas* (L.) Schott. *Alg. J. Nat. Prod.,* **6:** 573-583. http://dx.doi.org/10.5281/zenodo.1336888

Erhirhie, E.O., Emeghebo, C.N., Ilodigwe, E.E., Ajaghaku, D.L., Umeokoli, B.O., Eze, P.M., Ngwoke, K.G. and Okoye, F.B.G.C., 2019. *Dryopteris filix-mas* (L.) Schott ethanolic leaf extract and fractions exhibited profound anti-inflammatory activity. *Avicenna. J. Phytomed.*, **9:** 396-409.

Ghoulami, S., Idrissi, A.I. and Fkih-Tetouani, S., 2001. Phytochemical study of *Mentha longifolia* of Morocco. *Fitoterapia.*, **72:** 596-598. https://doi.org/10.1016/S0367-326X(01)00279-9

Handral, H.K., Jha, P.K. and Shruthi, S.D., 2010. Pharmacognostic and phytochemical studies on the leaves of *Murraya koenigii* L Spreng. *Pharmacophore*, **1:** 231-238.

Hashmi, M.A., Shah, H.S., Khan, A., Farooq, U., Iqbal, J., Ahmad, V.U. and Perveen, S., 2015. Anticancer and alkaline phosphatase inhibitory effects of compounds isolated from the leaves of *Olea ferruginea* Royle. *Rec. Nat. Prod.*, **9:** 164-168.

Hernández-Saavedra, D., Pérez-Ramírez, I.F., Ramos-Gómez, M., Mendoza-Díaz, S., Loarca-Pina, G. and Reynoso-Camacho, R., 2016. Phytochemical characterization and effect of *Calendula officinalis*, *Hypericum perforatum*, and *Salvia officinalis* infusions on obesity-associated cardiovascular risk. *Med. Chem. Res.*, **25:** 163-172. https://doi.org/10.1007/s00044-015-1454-1

Huang, J., Wong, K.H., Tay, S.V., How, A. and Tam, J.P., 2019. Cysteine-rich peptide fingerprinting as a general method for herbal analysis to differentiate *Radix astragali* and *Radix hedysarum*. *Front. Pl. Sci.*, **10:** 973. https://doi.org/10.3389/fpls.2019.00973

Ibraheim, Z.Z., Ahmed, A.S. and Gouda, Y.G., 2011. Phytochemical and biological studies of *Adiantum capillus-veneris* L. *Saudi. pharm. J.*, **19:** 65-74. https://doi.org/10.1016/j.jsps.2011.01.007

Indhumathi, T. and Mohandass, S., 2014. Efficacy of ethanolic extract of *Solanum incanum* fruit extract for its antimicrobial activity. *Int. J. Curr. Microbiol. Appl. Sci.*, **3:** 939-949.

Ishaq, M.S., Hussain, M.M., Siddique Afridi, M., Ali, G., Khattak, M. and Ahmad, S., 2014. In vitro phytochemical, antibacterial, and antifungal activities of leaf, stem, and root extracts of *Adiantum capillus veneris*. *Sci. World. J.*, *1-7*. https://doi.org/10.1155/2014/269793

Jayapriya, G. and Shoba, F.G., 2015. Phytochemical analysis, anti-Microbial efficacy and determination of bioactive components from leaves of *Justicia adhatoda* (Linn.). *Asian. J. Plant. Sci. Res.*, **5:** 43-51.

Kaleeswaran, B., Ilavenil, S. and Ravikumar, S., 2010. Screening of phytochemical properties and antibacterial activity of *Cynodon dactylon* L. *Int. J. Curr. Res.*, **3:** 83-88.

Kalpana, G., Sruthi, K., Banu, Z., Sumakanth, M., Ravindernath, A. and Prakash, D.J., 2018. Screening of analgesic activity of methanolic extract and its fractions of *Alternanthera Pungens*. *IOSR. J. Pharm. biol. Sci.*, **13:** 53-58.

Khan, R.A., Khan, N.A., Khan, F.U., Ahmed, M., Shah, A.S., Khan, M.R. and Shah, M.S., 2012. Phytochemical, antioxidant and cytotoxic activities of *Periploca aphyla* and *Mentha longifolia*, selected medicinal plants of District Bannu, Pakistan. *Afr. J. Pharm. Pharmacol.*, **6:** 3130-3135. https://doi.org/10.5897/AJPP12.445

Khoobchandani, M., Ojeswi, B.K., Ganesh, N., Srivastava, M.M., Gabbanini, S., Matera, R., Lori, R. and Valgimigli, L., 2010. Antimicrobial properties and analytical profile of traditional *Eruca sativa* seed oil: Comparison with various aerial and root plant extracts. *Food Chem.*, **120:** 217-224. https://doi.org/10.1016/j.foodchem.2009.10.011

Kontogianni, V.G., Tomic, G., Nikolic, I., Nerantzaki, A.A., Sayyad, N., Stosic-Grujicic, S. and Tzakos, A.G., 2013. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. *Fd. Chem.*, **136:** 120-129. https://doi.org/10.1016/j.foodchem.2012.07.091

Koşar, M., Göger, F. and Can Başer, K.H., 2008. In vitro antioxidant properties and phenolic composition of *Salvia virgata* Jacq. from Turkey. *J. Agric. Fd. Chem.*, **56:** 2369-2374. https://doi.org/10.1021/jf073516b

Kumar, B.A., Lakshman, K., Jayaveea, K.N., Shekar, D.S., Khan, S., Thippeswamy, B.S. and Veerapur, V.P., 2012. Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Amaranthus viridis* Linn in alloxan induced diabetic rats. *Exp. Toxicol. Pathol.*, **64:**75-79. https://doi.org/10.1016/j.etp.2010.06.009

Kumari, P., Kumari, C. and Singh, P.S., 2017. Phytochemical screening of selected medicinal plants for secondary metabolites. *Int. J. Life. Sci. scient. Res.*, **3:** 1151-1157.

Luis, A., Gil, N., Amaral, M.E. and Duarte, A.P., 2012. Antioxidant activities of extracts from *Acacia melanoxylon*, *Acacia dealbata* and *Olea europaea* and alkaloids estimation. *Int. J. Pharm. Pharmaceut. Sci.*, **4:** 225-231.

Mojab, F., Kamalinejad, M., Ghaderi, N. and Vahidipour, H. R., 2010. Phytochemical screening of some species of Iranian plants. *Iran. J. Pharm. Res.*, **2:** 77-82.

Munir, U., Perveen, A. and Qamarunnisa, S., 2014. Comparative pharmacognostic evaluation of some species of the genera *Suaeda* and *Salsola* leaf

(Chenopodiaceae). *Pak. J. Pharmaceut. Sci.*, **27:** 1309-1315.

Muruhan, S., Selvaraj, S. and Viswanathan, P.K., 2013. In vitro antioxidant activities of *Solanum surattense* leaf extract. *Asian Pac. J. trop. Biomed.*, **3:** 28-34. https://doi.org/10.1016/S2221-1691(13)60019-2

Nisa, H., Kamili, A.N., Bandh, S.A., Lone, B.A. and Parray, J.A., 2013. Phytochemical screening, antimicrobial and antioxidant efficacy of different extracts of *Rumex dentatus* L.–a locally used medicinal herb of Kashmir Himalaya. *Asian. Pac. J. trop. Dis.*, **3:** 434-440. https://doi.org/10.1016/S2222-1808(13)60097-3

Pandey, S. and Gupta, R.K., 2014. Screening of nutritional, phytochemical, antioxidant and antibacterial activity of *Chenopodium album* (Bathua). *J. Pharmacogn. Phytochem.*, **3:** 1-9.

Parveen, S., Khalid, A., Farooq, A. and Choudhary, M.I., 2001. Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa*. *Phytochemistry*, **58:** 963-968. https://doi.org/10.1016/S0031-9422(01)00332-6

Perianayagam, J.B., Sharma, S.K., Pillai, K.K., Pandurangan, A. and Kesavan, D., 2012. Evaluation of antimicrobial activity of ethanol extract and compounds isolated from *Trichodesma indicum* (Linn.) R. Br. root. *J. Ethnopharmacol.*, **142:** 283-286. https://doi.org/10.1016/j.jep.2012.04.020

Pooja, S. and Vidyasagar, G.M., 2016. Phytochemical screening for secondary metabolites of *Opuntia dillenii* Haw. *J. med. Pl.*, **4:** 39-43.

Rajurkar, N.S. and Gaikwad, K., 2012. Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris* leaves. *J. Chem. Pharm. Res.*, **4:** 365-374.

Rashid, U., Khan, M.R., Jan, S., Bokhari, J. and Shah, N.A., 2013. Assessment of phytochemicals, antimicrobial and cytotoxic activities of extract and fractions from *Fagonia olivieri* (Zygophyllaceae). *BMC Compl. Altern. Med.*, **13:** 167. https://doi.org/10.1186/1472-6882-13-167

Riaz, T., Abbasi, M.A., Rehman, A., Shahzadi, U., Qureshi, M.Z. and Ajaib, M., 2012. *Dicliptera bupleuroides*: an imperative source for protection from oxidative stress. *J. chem. Soc. Pak.*, **34:** 326-332.

Saboo, S.S., Tapadiya, G.G., Lamale, J.J. and Khadabadi, S.S., 2014. Phytochemical screening and antioxidant, antimitotic, and antiproliferative activities of *Trichodesma indicum* shoot. *Anc. Sci. Life.*, **34:** 113-118.

Saganuwan, A.S. and Gulumbe, M.L., 2006. Evaluation of in vitro antimicrobial activities and phytochemical constituents of *Cassia occidentalis*. *Anim. Res. Int.*, **3:** 566-569.

Sambo, H.S., Olatunde, A. and Kiyawa, A.S., 2016. Phytochemical, proximate and mineral analyses of *Solanum incanum* fruit. *Int. J. chem. Mater. environ. Res.*, **3:** 8-13.

Suresh, K., 2008. Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. *Ethnobotan. Leafl.*, **12:** 1184-91.

Uddin, G., Rauf, A. and Akhtar, S., 2012. Studies on chemical constituents, phytochemical profile and pharmacological action of *Datura alba*. *Mid-East. J. med. Pl. Res.*, **1:** 14-18.

Ullah, S., Bano, A., Girmay, S. and Tan, G., 2012. Anticancer, antioxidant and antimicrobial activities of *Suaeda fruticosa* related to its phytochemical screening. *Int. J. Phytomed.*, **4:** 284.

Venkatesh, S., Reddy, Y.R., Ramesh, M., Swamy, M.M., Mahadevan, N. and Suresh, B., 2008. Pharmacognostical studies on *Dodonaea viscosa* leaves. *Afri. J. Pharm. Pharmacol.*, **2:** 083-088. https://doi.org/10.5897/AJPP.9000220

Wafa, G., Amadou, D. and Larbi, K.M., 2014. Larvicidal activity, phytochemical composition, and antioxidant properties of different parts of five populations of *Ricinus communis* L. *Ind. Crops Prod.*, **56:** 43-51. https://doi.org/10.1016/j.indcrop.2014.02.036

Yadav, J.P., Arya, V., Yadav, S., Panghal, M., Kumar, S. and Dhankhar, S., 2010. *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*, **81:** 223-230. https://doi.org/10.1016/j.fitote.2009.09.008

Yadav, R.N.S. and Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. *J. Phytol.*, **3:** 10-14.

Zongo, C., Savadogo, A., Somda, K.M., Koudou, J. and Traore, A.S., 2011. In vitro evaluation of the antimicrobial and antioxidant properties of extracts from whole plant of *Alternanthera pungens* HB & K. and leaves of *Combretum sericeum* G. Don. *Int. J. Phytomed.*, **3:** 182-191.