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

Mujahid Tanvir¹, Muhammad Asam Riaz¹*, Muhammad Zeeshan Majeed¹, Mazhar Iqbal Zafar², Muhammad Tariq³ and Muhammad Bilal Tayyab¹

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

**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 *Mueara arenaria* Forsk, *Nerium indicum* Mill., *Suaeda fruticosa* (L.) Delile, *Olea ferruginea* Wall., *Adiantum capillus-veneris* L. and *Dicliptera bupleuroides* Nees exhibiting 87, 84, 81, 79, 78 and 77% larval mortality, respectively with minimum LC₅₀ and LC₉₀ values. Among the microbial and synthetic insecticides, the highest larval mortality was recorded by *Bacillus thuringiensis* 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 mosquitoes (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,
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 \textit{Culex quinquefasciatus} mosquitoes and resulted in suppression of the population of native Hawaiian honeycreepers (Atkinson and LaPointe, 2009).

Mosquitoes are primarily controlled by extensive applications of persistent synthetic insecticides such as DDT, malathion, chlorpyrifos, deltamethrin etc. and many field populations of mosquitoes including \textit{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 mosquitoes (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 \textit{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 (\textit{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 F₃ 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 \textit{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±2°C 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 from 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 pilot 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%),

| Insecticides               | Trade name  | Formulation | Company          |
|----------------------------|-------------|-------------|------------------|
| Indoxacarb                 | Steward®    | 15 SC       | FMC              |
| Pyriproxyfen               | Admiral®    | 10 EC       | FMC              |
| Permethrin                 | Rid®        | 10 EC       | Bayer            |
| Lambda-cyhalothrin         | Karate®     | 2.5 EC      | Syngenta         |
| *Bacillus thuringiensis* NCIM 2514 | Lipel®   | WP (18000 IU/mg) | AgriLife, India |
| *Metarhizium anisopliae* NCIM 1311 | Pacer®    | WP (1×10$^6$ cfu/g) | AgriLife, India |
| *Beauveria bassiana* NCIM 1216 | Racer®    | WP (1×10$^6$ cfu/g) | AgriLife, India |
| *Isaria fumosorosea* PFA 011 | Paecilomite® | WP (1×10$^6$ cfu/g) | AgriLife, India |

*Table II. Selective microbial and synthetic insecticide formulations bioassayed against *Culex quinquefasciatus* larvae.*
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* ≤ 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         | Lahhra                | Leaves / Stem    | 81±5                      | A-D               |
| 5       | *Olea ferruginea* Wall. ex Aitch.      | Zatoon                | Fruit            | 79±7                      | B-E               |
| 6       | *Adiantum capillus-vernis* L.          | Khati booti           | Leaves           | 78±4                      | B-E               |
| 7       | *Diplipera buupleoides* 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.          | Kabal                 | 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      | *Mellitosis 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.                  | Harmoli               | 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 smithii* 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                |
Comparative Efficacy of Selected Biorational Insecticides

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).

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).

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...
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-constituents 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, larvalcidal and repellent 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., 2015).

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, stigmastereos, 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 (Diaz 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 Teresa et al. (2019) showing 60% mortality in Anopheles mosquito larvae by the extract of O. europaea plant. Similarly, 0.03% hexane extract of A. capitatus-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 mosquitocticial formulations.

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

| Plant species               | Plant Parts extracted | LC₅₀ (%) (95% CI) | LC₉₀ (%) (95% CI) | Significance (ANOVA; HSD at p ≤ 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                                     |
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.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

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

Mujahid Tanvir¹, Muhammad Asam Riaz⁎, Muhammad Zeeshan Majeed¹, Mazhar Iqbal Zafar², Muhammad Tariq³ and Muhammad Bilal Tayyab¹

¹Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan
²Department of Environmental Sciences, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan
³Department 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 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) |

* Corresponding author: asam.riaz@uos.edu.pk

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| 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) | ![Picture](image1.png) |
| 4      | *Petrophytum caespitosum* | Mat rock spiraea | Khura | Leaves and stem | Rosaceae | Not available                                                                   | ![Picture](image2.png) |
| 5      | *Astragalus Spp.*    | Koohni      | Khura    | Leaves       | Fabaceae| Not available                                                                   | ![Picture](image3.png) |
| 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) | ![Picture](image4.png) |
| Sr. No. | Scientific name | Common name | Locality | Part(s) used | Family | Phytochemical (s) | Picture of plant |
|---------|----------------|-------------|----------|--------------|--------|-----------------|------------------|
| 7       | *Dicliptera bupleuroides* | Kaalu and Pipri 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 Sharif | Leaves | Lamiaceae | Alkaloids, Flavonoids, Saponin, Terpenoids, Tannins (Mojab et al. 2010; Amessis-Ouchemoukh et al. 2014) |
| 9       | *Fagonia indica* | Dhamasa 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 | |

Comparative Efficacy of Selected Biorational Insecticides
| Sr. No. | Scientific name | Common name | Locality | Part(s) used | Family | Phytochemical(s) | Picture of plant |
|--------|----------------|-------------|----------|--------------|--------|-----------------|-----------------|
| 11     | *Mentha longifolia* | Desipodina | Daep Sharif | Leaves and stem | Lamiaceae | Essential oils, Flavonoids | ![Picture](image1.png) |
| 12     | *Solanum surattense* | Kanda kari/Choti Kateri | Daep Sharif | Leaves and fruit | Solanaceae | Alkaloids, Flavonoids, Glycosides, Sterols, Tannins, Triterpenoids | ![Picture](image2.png) |
| 13     | *Nerium indicum* | Kanera | Daep Sharif | Leaves | Apocynaceae | Alkaloids, Carbohydrates, Glycosides, Lipids, Proteins, Sterols, Saponins, Tannins, Triterpenoids | ![Picture](image3.png) |
| 14     | *Nerium indicum* | Kanera | Daep Sharif | Fruit | Apocynaceae | Alkaloids, Carbohydrates, Glycosides, Lipids, Proteins, Sterols, Saponins, Tannins, Triterpenoids | ![Picture](image4.png) |
## Comparative Efficacy of Selected Biorational Insecticides

| Sr. No. | Scientific name | Common name | Locality | Part(s) used | Family | Phytochemical(s) | Picture of plant |
|---------|-----------------|-------------|----------|--------------|--------|------------------|-----------------|
| 15      | *Acacia melanoxylon* | Hickory | Sharif | Leaves and stem | Fabaceae | Alkaloids, flavonoids, Phenols (Luis et al. 2012) | ![Picture](https://example.com/acacia.png) |
| 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) | ![Picture](https://example.com/datura.png) |
| 18      | *Suaeda fruticosa* | Lahnra | Uchhali | Leaves | Amaranthaceae | Anthraquinons, Alkaloids, Carbohydrates, Flavonoids, Phenol, Saponins, Steroids, Terpenoids, Tannins (Ullah et al. 2012; Munir et al. 2014) | ![Picture](https://example.com/suaeda.png) |
| 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 | Amaranthaceae | Alkaloids, Anthocyanosides, Anthraquinons, Carbohydrates, 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, Terpenoids Tannins (Pooja and Vidyasagar 2016) |
| 21      | *Murraya koenigii* | Jangli curry Patta | Kanhati Garden Leaves and stem | Rutaceae | Alkaloids, Anthraquinons, Carbohydrates, Flavonoids, Proteins, Phytosterols, Saponins, Tannin, Volatile oil, (Handral and Prashanth 2010) |
| 22      | *Periploca aphylla* | Bata | Kanhati Garden Stem and leaves | Apocynaceae | Anthraquinons, Alkaloids, Carbohydrates, Flavonoids, Proteins, Phytosterols, Steroids, Saponins, Terpenoids (Khan et al 2012) |
### Comparative Efficacy of Selected Biorational Insecticides

| 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) | ![Plant 1] |
| 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) | ![Plant 2] |
| 25      | *Cassia occidentalis* | Bana Chakunda | Kanhati Garden | Leaves     | Fabaceae      | Alkaloid, Flavonoid, Glycosides, Steroid, Saponin, Tannin (Saganuwan and Gulumbe 2006; Yadav et al. 2010) | ![Plant 3] |
| 26      | *Cassia occidentalis* | Bana Chakunda | Kanhati Garden | Fruit      | Fabaceae      | Anthraquinons, Flavonoids, Glycosides, Phenols, Steroid (Yadav et al. 2010) | ![Plant 4] |
| Sr. No. | Scientific name       | Common name               | Locality Part(s) used | Family   | Phytochemical (s)                                                                 | Picture of plant                                      |
|--------|-----------------------|---------------------------|-----------------------|----------|----------------------------------------------------------------------------------|------------------------------------------------------|
| 27     | *Adiantum capillus-veneris* | Venus hair fern/ Khati booti | Kanhati Garden        | Pteridaceae | Alkaloids, Carbohydrates, Fiber, Fats and waxes, Flavonoids, Glycosides, Phenolics, Saponins, Steroids, Terpenoids, Tannins (Ibraheim et al. 2011; Rajjurkar and Gaikwad 2012; Ishaq et al. 2014) | ![Picture of plant](image1.png)                      |
| 28     | *Justicia adhatoda*    | Dhodhak Booti, Vaheakar/ Baikarr and Vasaka | Kanhati Garden        | Acanthaceae | Alkaloids, Anthraquinones, Flavonoids, Glycosides, Phenols, Polyphenols, Phytosterols, Saponins, Triterpenoids (Chanu and Sarangthem 2014; Jayapriya and Shioba 2015) | ![Picture of plant](image2.png)                      |
| 29     | *Salvia virgata*       | Khabikki Flower Meadow Sage | Kanhati Garden        | Lamiaceae | Amino acids, Alkaloids, Carbohydrates, Flavonoids, Glycosides, Phenolic compounds and Proteins, Saponins, Terpenoids (Koşar et al. 2008) | ![Picture of plant](image3.png)                      |
| 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) | ![Picture of plant](image4.png)                      |
## Comparative Efficacy of Selected Biorational Insecticides

| 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) | ![Picture of plant](image1) |
| 32      | *Melilotus officinalis* | Yellow sweet clover | Kanhati Garden | Leaves | Fabaceae | Flavonoids, Phenol, Saponins, Tannin, Terpenoids (Govindappa and Poojashri 2011) | ![Picture of plant](image2) |
| 33      | *Salvia officinalis* | Khalatra Angah | Leaves | Lamiaceae | Alkaloids, Diterpenes, Flavonoids, Polyphenols, Saponins, Triterpenic acids (Kontogianni et al. 2013; Hernández-Saavedra et al. 2016) | ![Picture of plant](image3) |
| 34      | *Solanum incanum* | Mahori Angah Fruit | Solanaceae | Alkaloids, Carbohydrates, Cardiac glycosides, Cyanogenic glycosides, Flavonoids, Phenols, Resins Oxalates, Steroids, Saponins, Tannins (Auta et al. 2011; Indhumathi and Mohandass 2014; Sambo et al. 2016) | ![Picture of plant](image4) |
| Sr. No. | Scientific name          | Common name | Locality | Part(s) used | Family     | Phytochemical (s)                                                                 |
|--------|--------------------------|-------------|----------|--------------|------------|----------------------------------------------------------------------------------|
| 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, 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, Cardiac glycosides, Cyanogenic glycosides, Carbohydrates, 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 | Leaves, fruits | Solanaceae | Alkaloids, Amino acids, Carbohydrates, Organic acids, Phenolic compounds, Proteins, Steroids, Saponin, Tannins, (Mathur et al. 2011) | ![Picture](image1.png) |
| 40      | Eruca sativa | arden rocket/Jamahoon | Flower | Brassicaceae | Allyl isothiocyanate, 3-butenyl isothiocyanate, 4-methylsulfinybutyl isothiocyanate, sulforaphane, 2-phenylethyl isothiocyanate and bis(isothiocyanatobutyl) disulphide, fatty acids (Khoobchandani et al. 2010) | ![Picture](image2.png) |

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