Research Article

Insecticidal Potential of Indigenous Flora of Soon Valley against Asian Citrus Psyllid *Diaphorina citri* Kuwayama and Cotton Aphid *Aphis gossypii* Glover

Muhammad Bilal Tayyab[1], Muhammad Zeeshan Majeed[1*], Muhammad Asam Riaz[1], Muhammad Anjum Aqueel[2], Sylvain Nafiba Ouedraogo[3], Muhammad Luqman[4], Kanwer Shahzad Ahmed[1] and Mujahid Tanvir[1]

[1]Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan; [2]Department of Entomology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan; [3]Institut des Sciences de l' Environnement et du Développement Rural (ISEDR), Université de Dédougou, Burkina Faso; [4]Department of Agricultural Extension, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan.

Abstract | Sap-sucking insect pests have been a severe threat to horticultural and agricultural crops all over the world. Asian citrus psyllid *Diaphorina citri* Kuwayama (Psyllidae: Hemiptera) and cotton aphid *Aphis gossypii* Glover (Aphididae: Hemiptera) are destructive sap-sucking pests of citrus and cotton, respectively. Extensive use of persistent synthetic insecticides against these pests poses issues of environmental contaminations and health hazards and suggests looking for alternate biorational plant protection measures such as botanical pesticides. This study evaluated the potential toxicity of acetone extracts of 40 indigenous plant species of Soon valley and surrounding salt range (Punjab, Pakistan) against *D. citri* and *A. gossypii* using standard twig-dip and leaf-dip bioassay methods, respectively. Results of initial screening bioassay showed the highest mortality of *D. citri* by 10% extracts of *Mentha longifolia* (L.) Huds. (93%), *Melilotus officinalis* (L.) Pall. (91%), *Nerium indicum* Mill. (89%), *Datura alba* L. (88%) and *Salvia officinalis* L. (81%). Second bioassay conducted against *A. gossypii* using different concentrations (5, 10, 20 and 40%) of the most effective botanical extracts revealed that the extract of *S. officinalis* was most toxic (LC_{50} = 18.59%), followed by *N. indicum* (LC_{50} = 20.27%) and *M. longifolia* (LC_{50} = 20.73%). Similar trend of effectiveness was observed regarding their LT_{50} values. Overall study results demonstrated the biocidal potential of the extracts of indigenous plant species of Soon valley against *D. citri* and *A. gossypii*, and suggest their further biochemical characterization and practical implication in future IPM programs against these and other sap-feeding insect pests.

Received | April 29, 2021; **Accepted** | July 19, 2021; **Published** | November 01, 2021
***Correspondence** | Muhammad Zeeshan Majeed, Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan; **Email:** zeeshan.majeed@uos.edu.pk
Citation | Tayyab, M.B., M.Z. Majeed, M.A. Riaz, M.A. Aqueel, S.N. Ouedraogo, M. Luqman, K.S. Ahmed and M. Tanvir. 2022. Insecticidal potential of indigenous flora of soon valley against asian citrus Psyllid *Diaphorina citri* kuwayama and cotton aphid *Aphis gossypii* glover. *Sarhad Journal of Agriculture,* 38(1): 26-39.
DOI | https://dx.doi.org/10.17582/journal.sja/2022/38.1.26.39
Keywords | Botanical pesticides, Phytoextracts, Phloem-feeding pests, *Diaphorina citri, Aphis gossypii*, Toxicity evaluation, Local flora

Introduction

Insect pests adversely affect the world food production. Among these, sap-feeding insect pests have been a serious threat to horticultural and agricultural crops all over the world including Pakistan (Gavloski, 2018). Asian citrus psyllid *Diaphorina citri* Kuwayama (Psyllidae: Hemiptera) and cotton aphid *Aphis gossypii* Glover (Aphididae: Hemiptera) are destructive sap-sucking pests of citrus and cotton, respectively.

Asian citrus psyllid (ACP) *D. citri* Kuwayama (Psyllidae: Hemiptera) is a destructive pest of citrus. It is native to Asia (Halbert and Nunez, 2004) and has been most notorious sap-feeding pest of citrus all over the world including Pakistan (Boykin *et al.*, 2012; Mahmood *et al.*, 2014; Augier *et al.*, 2017). Both nymphs and adults of ACP desap young twigs and foliage resulting in the withering of twigs and curling of leaves and flowers and premature fruit drop (Grafton-Cardwell *et al.*, 2013; Ahmad *et al.*, 2014). Furthermore, ACP acts as an important vector in the transmission of bacteria *Candidatus liberibacter asiaticus* Jagoueix, *C. l. africanus* Jagoueix and *C. l. americanus* Texeira that cause Huánglóngbìng (citrus greening) disease (Teixeira *et al.*, 2005; Bove, 2006; Grafton-Cardwell *et al.*, 2013; Hall *et al.*, 2013). Citrus greening causes substantial economic loss to citrus production all over the world and its prevention solely relies on the control of its vector *i.e.* ACP (Halbert and Manjunath, 2004; Bove, 2006).

Cotton aphid *A. gossypii* is a polyphagous sap-feeding pest found ubiquitously around the globe (Kocourek *et al.*, 1994). It has a wide host range including plant species from the Malvaceae, Cucurbitacae, Solanaceae and Rutaceae families (Ebert and Cartwright, 1997; Satar *et al.*, 1999). It is one of the most destructive pests of cotton in the world including Pakistan (Xia *et al.*, 1999; Henneberry *et al.*, 2000; Razamjou *et al.*, 2006; Ashfaq *et al.*, 2011; Majeed *et al.*, 2016; Eid *et al.*, 2018; Siddiqui *et al.*, 2021). Both nymph and adults of *A. gossypii* damage directly by desaping plant foliage and sprouts and indirectly by interrupting normal photosynthetic activity due to sooty mold growth on their honeydew secretions and by transferring various viruses in plants including cotton crop (Ebert and Cartwright 1997; Henneberry *et al.*, 2000).

For the control of both these sap-feeding pests, citrus and cotton growers around the globe rely exclusively on the extensive applications of highly persistent synthetic insecticides including organochlorines, organophosphates, carbamates and pyrethroids (Ahmad and Arif, 2008; Setamou *et al.*, 2010; Yan *et al.*, 2013; Boina and Bloomquist, 2015; Nazir *et al.*, 2017). However, many environmental and health issues are being manifested by this irrational and widespread use of synthetic chemicals such as soil and water contaminations (Kumari *et al.*, 2008; Edwards, 2013; Deng *et al.*, 2020), eradication of non-target fauna including predators and parasitoids (Desneux *et al.*, 2007; Halstead *et al.*, 2015; Haddi *et al.*, 2020), insect pests resistance (Herron *et al.*, 2001; Tiwari *et al.*, 2011; Yan *et al.*, 2013; Naeem *et al.*, 2016) and human health hazards (Kim *et al.*, 2017; Dhananjayan *et al.*, 2020).

This situation demands for searching novel biorational pest management strategies which would be more environment-friendly and safer than synthetic chemical insecticides. For instance, plant-based pesticides appear as promising alternative control measures (Copping and Menn, 2000; Isman, 2020). Many plant essential oils and phytoextracts are well-known regarding their effectiveness against different species of sap-feeding and chewing insect pests including ACP and aphids (Borad *et al.*, 2001; Rossetti *et al.*, 2008; Regnault-Roger *et al.*, 2012; Majeed *et al.*, 2018). Moreover, botanical pesticides usually exhibit low mammalian toxicity and get degraded in the environment rapidly as compared to conventional synthetic insecticides (Turek and Stintzing, 2013).

As local flora of any biogeographical zone may be composed of certain bioactive and toxic constituents against local insect pest species (Isman, 2020), this study was conducted to explore the toxicity potential of local flora (including herbs, shrubs and trees) of Soon valley and surrounding Salt Range (Punjab, Pakistan) against *D. citri* and *A. gossypii*. This valley is located in a Salt Range of district Khushab in between latitudes 32°25 and 32°45 N and longitudes 72°00 and 72°30 E and covers an area of about 300 km^2. It is usually rich in floral diversity including many valuable medicinal plants (Ahmad *et al.*, 2002; Ahmad *et al.*, 2009).

Material and Methods

The study was conducted to evaluate the biocidal potential of local plants of Soon valley and surrounding Salt Range of the Punjab province of Pakistan against *D. citri* and *A. gossypii*. The study was performed in the Department of Entomology, College of Agriculture, University of Sargodha, Pakistan.

Collection sites of plant material

Samples of indigenous flora (including trees, shrubs and herbs) were collected from six selected sites (Angah, Dape Shareef, Kenhatti Garden, Khabeki, Khoora and Uchhali) of Soon valley and surrounding Salt Range of district Khushab (Punjab, Pakistan) during

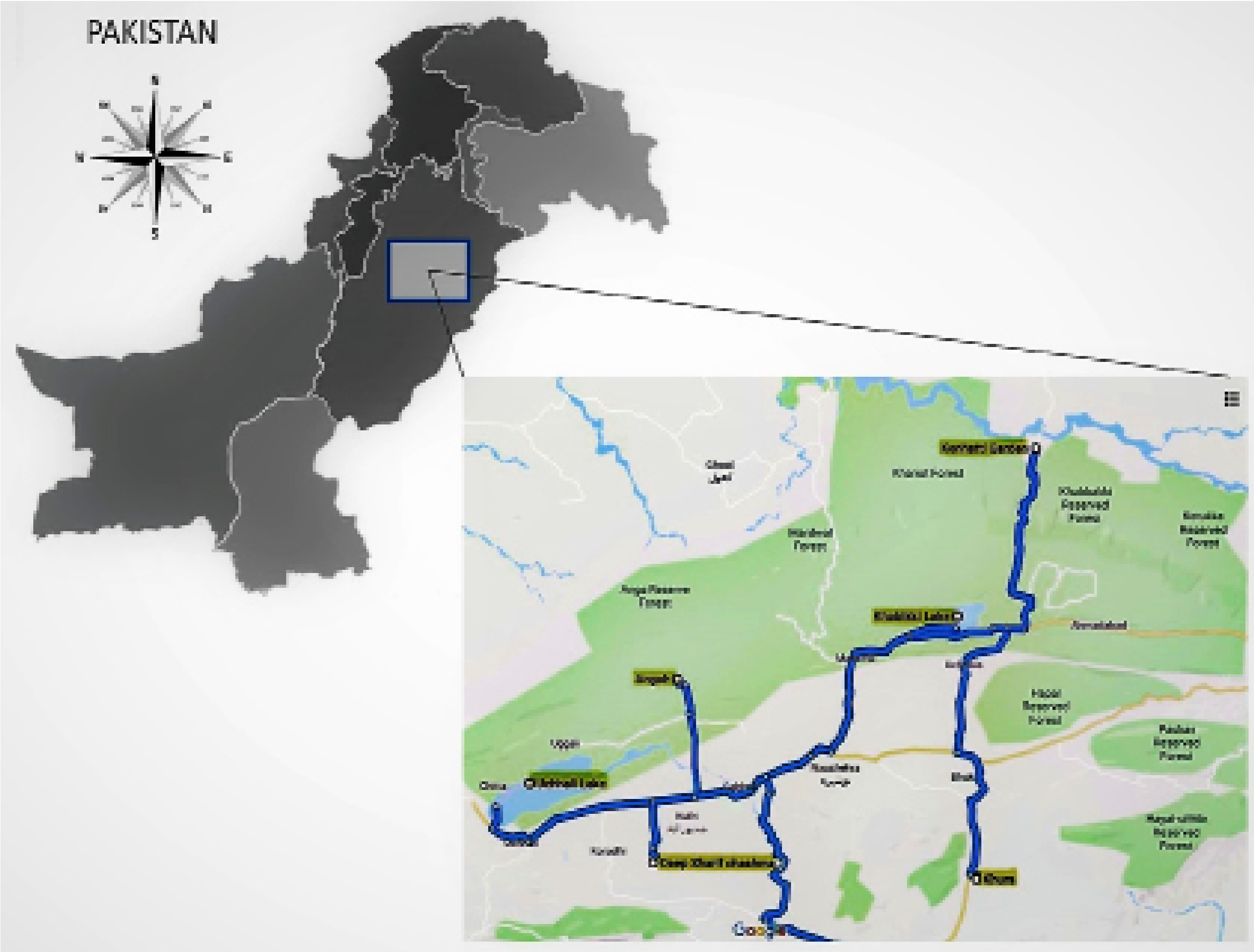

Figure 1: *Sampling sites regarding the collection of local flora of Soon Valley and surrounding Salt Range of Pakistan.*

Table 1: *Geographical coordinates of selected flora collection sites in Soon Valley and surrounding Salt Range of Pakistan.*

Sr. No.	Localities	Latitude N	Longitude E	Elevation (m)
1	Angah	32.35° N	72.05° E	821
2	Dape Sharif	32.30° N	72.04° E	890
3	Kenhatti Garden	32.40° N	72.14° E	783
4	Khabeki	32.35° N	72.12° E	774
5	Khoora	32.23° N	72.11° E	866
6	Uchhali	32.56° N	72.02° E	794

the extensive surveys carried out in spring seasons of 2018 and 2019. Geographic information of these selected sites is given in Figure 1 and Table 1. Samples were composed of plant twigs, stems, leaves, roots, fruits, flowers and seeds. These collected plant samples were identified up to species level by the experts from the Department of Botany, University of Sargodha and by the native ethnobotanical experts.

Plant samples preparation

The plant materials collected were washed with tap-water and were dried under shade for approximately two weeks at room temperature (28°C). After that plant material was grinded into fine powder using a commercial electrical mix-blender (TCB-318; 750W). This powdered material was then extracted by means of Soxhlet apparatus.

Soxhlet extraction

As common method of extraction is usually not efficient to yield good amount of phyto-constituents, Soxhlet extractor (DH.WHM-12393, Daihan Scientific, South Korea) was used to extract the powdered plant materials following the following extraction procedure.

Extraction procedure

Fifty gram of each plant sample was filled in the thimble of Soxhlet apparatus. This thimble was made up of a filter-paper sheet and 500 mL acetone

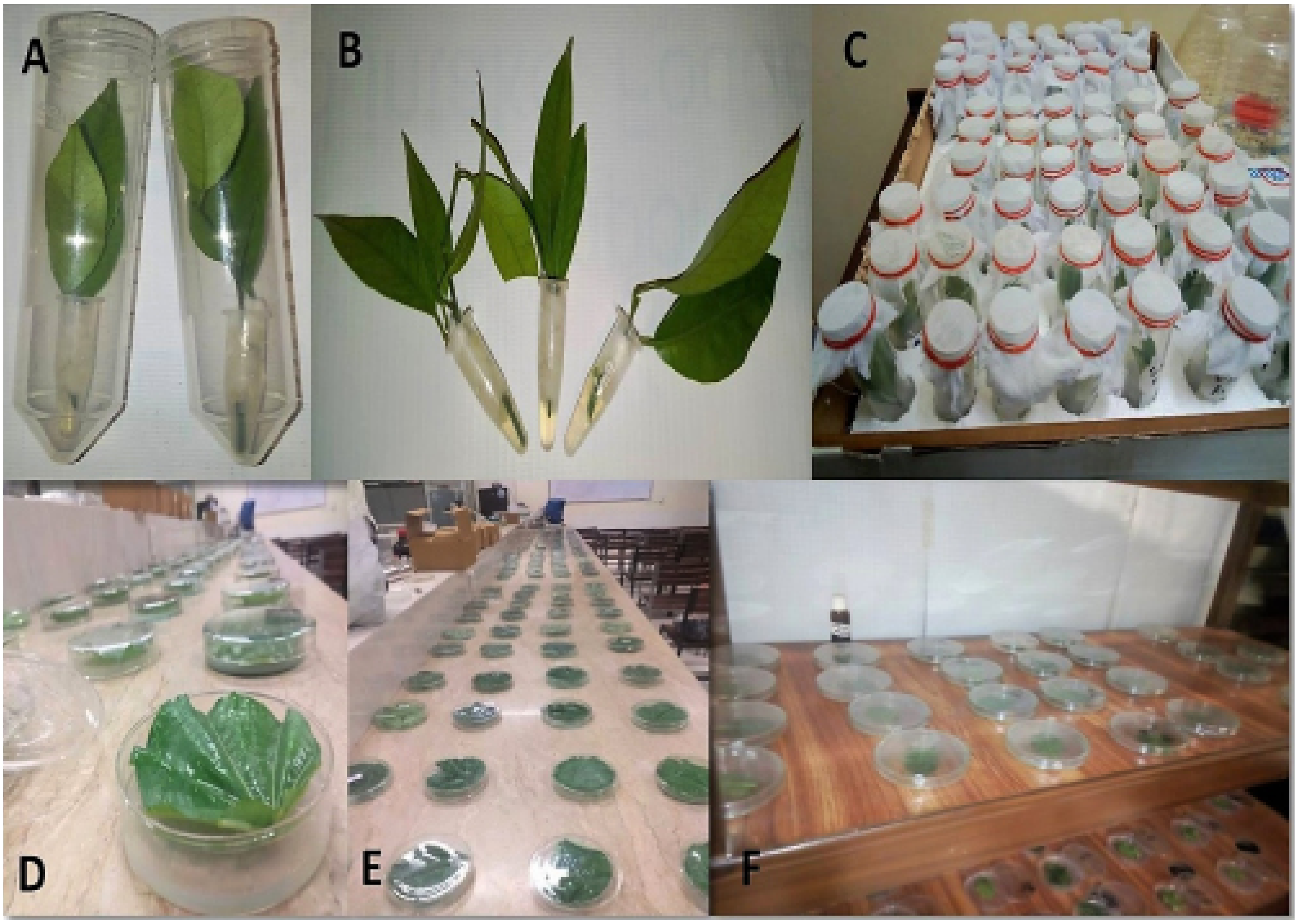

Figure 2: *Twig-dip (A-C) and leaf-dip (D-F) bioassay methods used for the evaluation of toxicity potential of botanical extracts against ACP (D. citri) and cotton aphid (A. gossypii), respectively.*

(99% pure) was filled in the apparatus flask as the extraction solvent. Extraction was done at 60±5°C and the apparatus was connected with the cool water supply of a condenser. The extraction process was carried out for 5 to 6 h for each sample. In order to evaporate the excessive amount of extraction solvent, the crude extract obtained from the Soxhlet extraction process was transferred to the rotary evaporator (WEV-1001L, Daihan Scientific, South Korea) provided with a chiller and vacuum pump. Pure extract obtained from each plant sample was stored in a 50 mL hermetic dark glass vial and was refrigerated until its downstream utilization in the toxicity bioassays.

Insect culture

Rearing of Insects: Active adults of *D. citri* and *A. gossypii* were collected from the citrus (*C. reticulata* Blanco cv. kinnow mandarin) and cotton (*Gossypium hirsutum* L.) plants by means of an aspirator and were reared on the potted citrus jasmine (*Murraya paniculata* (L.) Jack) and cotton plants, respectively. These plants were potted in 500 mL disposable plastic jars, filled with the fine sand and wooden brass (50:50) as potting mixture. Insect populations were reared in laboratory within Plexiglas rearing cages at 60±5% relative humidity, 25±2°C temperature and 16h L: 8h D photoperiod.

Insecticidal bioassays: First bioassay was conducted in order to screen out the most effective botanical extracts from the total collected plant samples for their insecticidal potential. For this screening experiment, we used laboratory reared ACP (*C. citri*) individuals and performed bioassays using twig-dip method. Later on, based on the results of this preliminary toxicity experiment, we conducted second series of toxicity bioassays with detailed experimental parameters using laboratory reared aphid (*A. gossypii*) individuals using leaf-dip bioassay method.

Twig-dip method for ACP (D. citri)

Three to four centimeter twig tips of citrus jasmine (*M. paniculata*) were used in this bioassay. The twigs were immersed for 30 sec in 10% botanical extract solutions. In control, twigs were immersed in pure acetone. After draining them on towel paper, the petioles of these twigs were inserted into 1.5 mL eppendorf tubes filled with 2.0% agar solution to keep them fresh. These tubes with twigs were inserted in 50 mL falcon tubes (Figure 2). Adult psyllids of laboratory reared population were collected with aspirator and were inactivated by keeping them into freezer for 5 min at 4°C, and then 5 psyllid individuals were released on each of the treated twigs with a camel hair brush. These tubes were then covered with a muslin cloth piece, tied by a rubber band to avoid the escape of insect (Figure 2) and were incubated in an environmental chamber at 27±2°C temperature and 60±5% relative humidity. Experimental design was completely randomized with three to five replicates maintained for each treatment. The mortality of exposed psyllids was noted at 1, 2 and 3 days after treatment.

Leaf-dip method for cotton aphid (A. gossypii)

Standard leaf-dip method was used to bioassay most effective botanical extracts against *A. gossypii* adults. In this method, young cotton leaves were dipped for 30 sec in 5, 10, 20 and 40% concentrations of botanical extracts made with acetone. Leaves dipped into acetone were served as control. After that leaves were placed at towel paper to be drained and were placed into 9 cm Petri pates layered with 2% agar solution to keep the leaves fresh till the end of bioassay. Ten freshly molted laboratory reared adult aphids were released in each Petri plate with a camel hair brush and Petri plates were incubated in an environmental chamber at 22±2°C temperature and 60±5% relative humidity. Each treatment was replicated ten times and the experimental design was completely randomized. The mortality of bioassayed aphids was noted at 0.5, 1, 2 and 3 days after treatment.

Statistical analyses of data

Data was analyzed statistically using analytical software Statistix V. 8.1® (Ahmed *et al*., 2004). Apart from the graphical presentation of data regarding the percent mortality of test psyllid and aphid individuals, data were analyzed by factorial analysis of variance (ANOVA) taking time intervals, botanical solutions and their concentrations as factors. Means of treatments were further compared by Tukey's honestly significant difference (HSD) test at standard probability level of 95%. Median lethal time (LT_{50}) and concentration (LC_{50}) values were calculated through probit analysis using statistics regression software IBM SPSS®. Prior to statistical analysis, data of insect mortality were corrected with the help of Abbott's formula (Abbott, 1925).

Results and Discussions

Toxicity of indigenous flora of Soon valley against ACP (D. citri)

Insecticidal potential of acetone extracts of 40 indigenous plant species was tested against *D. citri* using twig-dip bioassay method. Results of this preliminary screening experiment performed with 10% extracts showed that some botanical extracts caused considerable mortality of adult psyllids (F = 44.82; P ≤ 0.01) (Table 2). Among all 40 botanical extracts, maximum mortality of psyllids was exhibited by 10% extracts of *M. longifolia* (93%), followed by *M. officinalis* (91%) and *N. indicum* (89%), while the extracts of *D. alba* and S. *officinalis* showed 88 and 81% mortality, respectively. Whereas about 57% mortality was caused by *R. smithi* and remaining all botanicals caused less than 50% psyllid mortality as shown in Table 3.

Table 2: *Analysis of variance comparison of different botanical extracts bioassayed against freshly molted adults of Asian citrus psyllid (Diaphorina citri) under laboratory conditions.*

Source	DF	MS	MS	F-value	P-value
Treatment	40	163249	4081.22	44.82	≤ 0.01
Time	2	12450	6225.20	68.37	≤ 0.001
Treatment × Time	80	6550	81.87	0.90	0.708
Error	246	22400	91.06		
Total	368	204649			
GM / CV	37.642 / 25.35				

DF = degree of freedom; SS = sum of squares; MS = mea square; P ≤ 0.001 (highly significant) and P ≤ 0.01 (significant); one-way factorial ANOVA at α = 0.05.

Response of cotton aphid (A. gossypii) to botanical extracts

Detailed toxicological bioassays were carried out against *A. gossypii* using ten most effective plant extracts screened out from the previous bioassay with *D. citri*. Results of these bioassays revealed a differential response of aphid individuals against different plant extracts. According to results, all plant extracts showed considerable mortality of *A. gossypii* and this mortality response was concentration and exposure time dependent as it increased along with the

Table 3: *Percent mortality of Asian citrus psyllids (D. citri) by 10% acetone extracts of different plant species collected from Soon Valley and surrounding Salt Range.*

Botanicals	Common / Vernacular name	Plant part extracted	Mortality [a] ± S.E (%)	Homogenous groups
Chenopodium album L.	Bathu	Leaves	24.44 ± 6.89	F-L
Buxus papillosa Schneid.	Shamshad	Leaves	23.33 ± 2.58	G-L
Cynodon dactylon (L.) Pers.	Khabal	Leaves	20.00 ± 6.56	I-L
Petrophytum caespitosum Rydb.	Mat rock spraea	Leaves	27.78 ± 10.02	E-K
Astragalus spp. L.	Koohni	Leaves	13.33 ± 2.58	L
Trichodesma indicum (L.) Lehm.	Juri	Leaves	18.89 ± 4.16	J-L
Dicliptera bupleuroides Nees	Kaalu	Leaves	30.00 ± 7.67	E-K
Marrubium vulgare L.	Pahari gandana	Leaves	45.56 ± 5.92	B-D
Fagonia indica Burm.f.	Dhamasa	Leaves	37.78 ± 7.65	C-H
Maerua arenaria Hook and Thomson	Hemkand	Leaves/Stem	41.11 ± 6.69	B-F
Mentha longifolia (L.) Huds.	Desi podina	Leaves	93.33 ± 4.85	A
Solanum surattense Burm. f.	Kanda kari	Leaves	44.00 ± 2.50	B-E
Nerium indicum Mill.	Kanera	Leaves	89.00 ± 5.00	A
Nerium indicum Mill.	Kanera	Fruits	47.78 ± 8.58	BCD
Acacia melanoxylon R.Br.	Hickory	Leaves	33.33 ± 5.91	G-J
Rhamnus smithi Greene	Buckthorn	Leaves/Stem	56.67 ± 5.34	B
Datura alba L.	Datura	Leaves/Flowers	87.78 ± 4.37	A
Suaeda fruticosa (L.) Delile	Lahnra	Leaves	37.78 ± 5.20	C-H
Alternanthera pungens Kunth	Phakra	Leaves	30.00 ± 6.56	E-K
Murraya koenigii (L.) Spreng.	Jungli kari patta	Leaves	17.78 ± 6.50	JKL
Periploca aphylla Decne.	Bata	Stem	20.00 ± 4.80	E-K
Dryopteris filix-mas (L.) Schott	Male fern	Leaves	28.89 ± 5.54	D-J
Ricinus communis L.	Harnoli	Leaves	32.22 ± 5.18	D-J
Cassia occidentalis L.	Bana chakunda	Leaves	33.33 ± 5.45	G-K
Cassia occidentalis L.	Bana chakunda	Fruits	22.22 ± 6.04	I-L
Adiantum capillus-veneris L.	Khatti booti	Leaves	26.67 ± 4.62	E-K
Justicia adhatoda L.	Dhodak booti	Leaves	30.00 ± 3.83	E-K
Salvia virgata Jacq.	Meadow sage	Leaves	28.89 ± 5.67	B-F
Amaranthus viridis L.	Jungli cholai	Leaves	41.11 ± 4.29	D-I
Sonchus asper (L.) Hill	Bhattal	Leaves	20.00 ± 5.45	J-L
Melilotus officinalis (L.) Pall.	Yellow sweet clover	Leaves	91.11 ± 4.29	A
Salvia officinalis L.	Sage	Leaves	81.11 ± 6.83	A
Solanum incanum L.	Mahoori	Leaves	33.33 ± 5.45	G-K
Portulaca oleracea L.	Loonak	Leaves	41.11 ± 6.07	D-I
Dodonaea viscosa (L.) Jacq.	Santha	Leaves	24.44 ± 6.89	JKL
Olea ferruginea Wall. ex Aitch.	Kao	Leaves	17.78 ± 6.50	J-L
Rumex dentatus L.	Toothed dock	Leaves	41.11 ± 4.26	D-I
Withania coagulans (Stocks) Dunal	Khamjeera	Leaves	40.00 ± 6.37	E-J
Eruca sativa Mill.	Jamahoon	Leaves	35.56 ± 4.66	F-K
Opuntia dillenii (Ker Gawl.) Haw.	Thor	Leaves	28.89 ± 5.67	KL

a = mean of three to five independent replications.

increase of concentration of botanicals and exposure time (Figure 3). There was a significant effect of all botanical extracts on aphid mortality (F = 181.30; P ≤ 0.01; Table 4). Overall, the highest average mortality

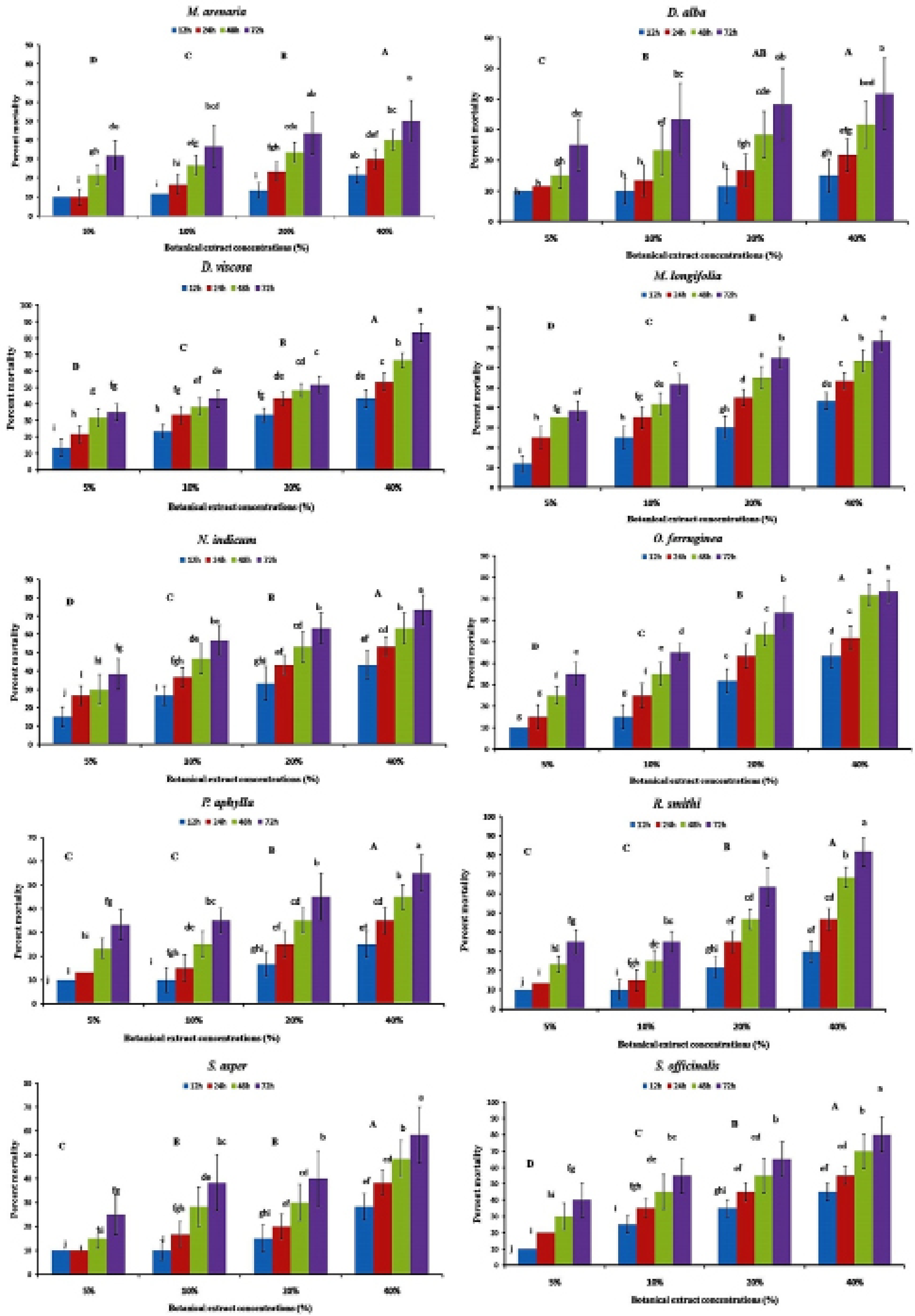

Figure 3: *Percent mortality (mean ± SE; n = 10) of cotton aphid (A. gossypii) individuals exposed to different concentrations of botanical extracts observed at different post-exposure time intervals. For each botanical extract, small alphabets indicate statistical difference among time intervals for each concentration, while capital alphabets are indicating the statistical difference among different concentrations (one-way factorial ANOVA; HSD at α = 0.05).*

Table 4: *Analysis of variance comparison of different botanical extracts bioassayed against freshly molted adults of cotton aphid (Aphis gossypii) under laboratory conditions.*

Source	DF	SS	MS	F-value	P-value
Concentration	3	111399	37132.9	942.95	≤ 0.001
Time	3	106209	35402.9	899.02	≤ 0.001
Treatment	9	64257	7139.6	181.30	≤ 0.01
Concentration × Time	9	2275	252.8	6.42	≤ 0.001
Concentration × Treatment	27	14916	552.4	14.03	≤ 0.001
Time × Treatment	27	2447	90.6	2.30	≤ 0.01
Error	881	34693	39.4		
Total	959	336196			
GM / CV	35.06/ 17.90				

DF = degree of freedom; SS = sum of squares; MS = mea square; P ≤ 0.001 (highly significant) and P ≤ 0.01 (significant); one-way factorial ANOVA at α = 0.05.

(63%) of aphid individuals was observed by the extract of *S. officinalis*, followed by *D. viscosa* and *O. ferruginea* exhibiting 62 and 60% average aphid mortality, respectively, while *M. longifolia* and *N. indicum* both exhibited about 58% aphid mortality for 40% concentrations (Figure 3). Moreover at 20% concentration, the highest mortality (50%) was also observed in case of *S. officinalis* while remaining all phyto-extracts showed less than 50% aphid mortality (Figure 3).

Similar trend of toxicity was observed regarding LC_{50} and LT_{50} values of these botanical extracts. According to probit analysis, *S. officinalis* was the most effective at 48 h (LC_{50} = 18.59%), followed by the extract of *N. indicum* (LC_{50} = 20.27%) and *M. longifolia* (LC_{50} = 20.73%), while the extracts of *S. officinalis*, *M. longifolia* and *N. indicum* showed minimum LC_{50} values (*i.e.* 9.24, 9.51 and 10.98%, respectively) at 72 h (Table 5). Similar trend was found in case of median lethal time (LT_{50}) values. The 40% extracts of *S. officinalis* and *O. ferruginea* showed minimum LT_{50} values (*i.e.* 17.73 and 20.05 %, respectively) (Table 6).

Table 5: *Median lethal concentration (LC_{50}) values of different acetone extracts of Soon valley flora bioassayed against freshly molted adults of cotton aphid (Aphis gossypii).*

Treatments	Observation time (h)	LC_{50} (%)	Lower and Upper 95% Fiducial Limits (%)	X^2 (DF = 10)*	P-value
Maerua arenaria Hook and homon	48	63.76	50.29-110.45	52.28	≤ 0.001
	72	58.80	46.71-104.04	47.09	0.001
Mentha longifolia (L.) Huds.	48	20.27	-38.57-42.65	31.59	0.06
	72	10.98	-53.43-34.84	42.51	≤ 0.001
Nerium indicum Mill.	48	20.73	-233.28-52.07	76.59	≤ 0.001
	72	9.51	-192.30-43.54	77.35	≤ 0.001
Rhamnus smithi Greene	48	25.63	-5.3-36.68	53.36	≤ 0.001
	72	15.67	-21.99-29.56	60.05	≤ 0.001
Datura alba L.	48	66.67	NC	83.58	≤ 0.001
	72	72.40	NC	67.28	≤ 0.001
Periploca aphylla Decne.	48	54.17	40.16-91.96	67.92	≤ 0.001
	72	50.03	38.06-75.57	63.27	≤ 0.001
Sonchus asper (L.) Hill	48	41.13	NC	83.23	≤ 0.001
	72	30.11	NC	69.70	≤ 0.001
Salvia officinalis L.	48	18.59	-37.88-37.87	48.74	0.001
	72	9.24	-64.61-32.39	52.07	≤ 0.001
Dodonaea viscosa (L.) Jacq.	48	22.51	-55.11-41.97	17.49	0.012
	72	27.61	19.66-32.07	27.63	0.015
Olea ferruginea Wall. ex Aitch.	48	21.50	-2.12-32.91	46.10	0.001
	72	14.62	-29.37-33.02	45.71	0.001

**Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits. DF = degree of freedom; NC = not calculable.*

Table 6: *Median lethal time (LT_{50}) values of different acetone extracts of Soon valley flora bioassayed against freshly molted adults of cotton aphid (Aphis gossypii).*

Treatment	Botanical Concentration (%)	LT_{50} (h)	Lower and Upper 95% Fiducial Limits (h)	X^2 (DF = 10)*	P-value
Maerua arenaria Hook $ Thomson	20	80.88	72.35-93.52	35.85	0.02
	40	70.16	58.43-93.37	96.97	≤ 0.001
Mentha longifolia (L.) Huds.	20	41.29	37.63-45.06	29.76	0.012
	40	21.63	16.03-26.18	25.33	0.028
Nerium indicum Mill.	20	42.47	35.19-50.49	58.94	≤ 0.001
	40	21.63	10.56-29.21	60.37	≤ 0.001
Rhamnus smithi Greene	20	51.90	48.71-55.49	28.81	0.15
	40	30.55	26.00-34.65	64.08	≤ 0.001
Datura alba L.	20	90.19	78.09-110.94	59.75	≤ 0.001
	40	86.16	69.68-126.09	129.43	≤ 0.001
Periploca aphylla Decne.	20	79.35	65.92-107.27	102.96	≤ 0.001
	40	59.87	49.81-77.61	101.16	≤ 0.001
Sonchus asper (L.) Hill	20	90.51	73.84-128.55	103.87	≤ 0.001
	40	53.02	46.06-62.48	52.03	≤ 0.001
Salvia officinalis L.	20	38.93	34.72-43.14	27.85	0.018
	40	17.73	8.81-24.14	57.99	≤ 0.01
Dodonaea viscosa (L.) Jacq.	20	60	51.87-72.54	25.22	0.028
	40	21.09	17.28-24.37	28.22	0.016
Olea ferruginea Wall. ex Aitch.	20	43.17	39.21-47.39	25.28	0.028
	40	20.05	11.69-26.22	44.63	≤ 0.001

**Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits. DF = degree of freedom.*

This study determined the bioactivity of acetonic extracts of indigenous flora (including 40 species of plants, herbs and shrubs) of Soon valley and surrounding Salt Range of Pakistan against ACP (*D. citri*) and cotton aphid (*A. gossypii*). Among all 40 botanical extracts, maximum mortality of psyllids was exhibited by 10% extracts of *M. longifolia* (93%), followed by *M. officinalis* (91%) and *N. indicum (*89), while the extracts of *D. alba* and S. *officinalis* showed 88 and 81% mortality, respectively. Similarly, 2nd bioassay results revealed that the highest mortality (63%) of *A. gossypii* was exhibited by *S. officinalis*, followed by *D. viscosa* (62%), and *O. ferruginea* (60%), while the extracts of *M. longifolia* and *N. indicum* both exhibited 58% aphid mortality at 40% concentration. Remaining all phyto-extracts showed less than 50% mortality against psyllids and aphids. Overall, the extracts of *M. longifolia*, *M. officinalis* and *N. indicum* and extracts of *S. officinalis*, *D. viscosa* and *O. ferruginea* proved to be most toxic against *D. citri* and *A. gossypii*, respectively, exhibiting minimum LC_{50} and LT_{50} values.

The overall mortality of psyllid individuals recorded in this study by *M. officinalis*, *N. indicum* and *M. longifolia* might be because of the bioactivity of different phenolic and terpenoid compounds found in these plants (Hiremath *et al*., 1997; Pascual-Villalobos and Robledo, 1998; Lee *et al*., 2001; Thomas *et al*., 2002; Odeyemi *et al*., 2008; Song *et al*., 2009; Hussain *et al*., 2010). Odeyemi *et al*. (2008) evaluated the toxicity of essential oils from *M. longifolia* against maize weevil (*Sitophilus zeamais*) by contact, fumigation and repellency bioassays. Their results showed that this herb contains compounds such as 1, 8-cinole, pulegone and menthone that are toxic to insects. In our study, *M. longifolia* exhibited about 90% mortality of *D. citri* and 58% mortality of *A. gossypii*. The observed psyllid and aphid mortality in this study would be due to such bioactive compounds documented in this plant species as flavonoids, phenol, saponins, tannin and terpenoids (Lee *et al*., 2001; Govindappa and Poojashri, 2011).

Tomczyk and Suszko (2011) demonstrated the biocidal activity of *S. officinalis* essential oil against *Tetranychus urticae* (two spotted spider mites). Likewise, different phyto-constituents found in the extracts of *M. officinalis* such as saponins, flavonoids, terpenoids, phenol, and tannins (Govindappa and Poojashri, 2011) would be the cause of psyllid mortality recorded in this study. Similarly, *N. indicum* extracts have many phyto-constituents such as saponins, triterpenoids, tannins, carbohydrates, lipids, glycosides, proteins, alkaloids and sterols (Bhuvaneshwari *et al*., 2007). The extract of *N. indicum* showed 100% mortality of *Nilaparvata lugens* (Hiremath *et al*., 1997). Mortality of psyllids in this experiment exhibited by the extract of *N. indicum* might be due to presence of these different compounds. Moreover, *D. alba* is well-known for its medicinal value and insecticidal potential. Our results corroborate this fact regarding the insecticidal potential of *D. alba*. Many previous studies have demonstrated significant mortality of aphids (Kuganathan *et al*., 2008) and psyllids (Khan *et al*., 2013) possibly because of different alkaloids found in this plant (Uddin *et al*., 2012).

D. viscosa has a great ethnomedicinal importance (Shah and Rahim, 2017) and its extracts contain many bioactive phytochemicals such as diterpenoids, flavonoids, stimgasterols, lupeol, fatty acids and ethers which have revealed toxicity against many insect pests including homopterous pests (Uddin *et al*., 2012; Díaz *et al*., 2015), coleopterous (Dimetry *et al*., 2015) and lepidopterous (Malarvannan *et al*., 2009; Abbes *et al*., 2016; Mohammed and Nawar, 2020). Likewise, *O. europaea* and many other species of Oleaceae family constitute different terpenes e.g. maslinic acid and phenolic contents showing considerable toxic effects against stored grain pests (*Tribolium confusum* and *Sitophilus granaries*) and aphids (*Myzus persicae*) (Hamouda *et al*., 2015; Alliche and Boughani, 2017; Kısa *et al*., 2018).

Conclusions and Recommendations

In brief, the extracts of *M. officinalis, D. alba*, *M. longifolia*, S. *officinalis* and *N. indicum* are found relatively toxic to ACP (*D. citri*) adults, while the phytoextracts of *S. officinalis*, *O. ferruginea*, *D. viscosa* and *M. longifolia* appeared effective against cotton aphid (*A. gossypii*). Overall study findings suggest the incorporation of the botanical extracts of above mentioned indigenous plant species in future integrated pest management for sucking insect pests. Nevertheless, the biochemical characterization of these plant extracts in order to find out their bioactive constituents responsible for the observed psyllid and aphid mortality and the laboratory and field evaluation of these plant extracts against natural enemies (insect predators and parasitoids) constitute important future perspective of this research work.

Acknowledgements

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

Novelty Statement

This laboratory work evaluated the anti-insect potential of local plant species from Soon valley and surrounding salt range of Pakistan against two destructive sap-feeding insect pests i.e. Asian citrus psyllid (*Diaphorina citri*) and cotton aphid (*Aphis gossypii*). Bioassays revealed that out of 40 plant species, acetone extracts of many plant species, particularly of *Dodonaea viscosa*, *Datura alba*, *Mentha longifolia*, *Melilotus officinalis*, *Nerium indicum*, *Olea ferruginea* and *Salvia officinalis*, exhibited considerable mortality of both insect pest individuals suggesting their biocidal potential against these sap-feeding insect pests.

Author's Contribution

Muhammad Zeeshan Majeed: Conceived the research idea and prepared experimental protocols.
Muhammad Bilal Tayyab, Kanwer Shahzad Ahmed and Mujahid Tanvir: Conducted the bioassays.
Sylvain Nafiba Ouedraogo and Muhammad Luqman: Performed statistical analyses and prepared results.
Muhammad Asam Riaz and Muhammad Zeeshan Majeed: Wrote the first draft of the manuscript.
Muhammad Asam Riaz: Provided technical support.
Muhammad Anjum Aqueel: Technically revised the manuscript.
Sylvain Nafiba Ouedraogo: Proofread the manuscript. Final manuscript has been read and approved by all authors.

Conflict of interest
The authors declare no competing interest regarding

the publication of this research work.

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