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Bioassay for evaluating the resistance level of dusky cotton bug, *Oxycarenus hyalinipennis* Costa against insecticides sprayed on cotton crop

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Dusky cotton bug (DCB), *Oxycarenus hyalinipennis,* a serious economic sucking insect pest of cotton, is a cosmopolitan polyphagous pest with the potency to develop resistance against insecticides. The resistance was evaluated against insecticides of different chemical nature against DCB population from three cotton fields *i.e.,* unsprayed population (Un-sp), field exposed to sucking sprays (SSp) and field exposed to bollworms sprays (BSp) using semi-opened bolls dip method.The DCB population showed differential range of resistance level against tested insecticides. These RR ratios were among susceptibility (1 fold) and tolerance to low resistance (2–10 fold) for all tested insecticides except for deltamethrin with moderate resistance (11–30 fold) for the populations of both sprayed fields. In the order of resistance for the SSp population, deltamethrin (24.4 fold) was followed by imidacloprid (6.4 fold), profenofos (4.0 fold), cypermethrin (3.2), endosulfan (2.8 fold), chlorfenapyr (2.7 fold) and chlothianidin (2.5 fold). For BSp population, RR ratios from high to low were: deltamethrin (21.2 fold) followed by imidacloprid (4.9 fold), chlothianidin (3.7 fold), cypermethrin (2.8 fold), endosulfan (2.7 fold), triazophos (2.4 fold) and profenofos (2.2 fold). Acephate was highly effective with least LC_{50} followed by spinosad and dimethoate, whereas, lambda-cyhalothrin was least effective. Relative toxicity (RT) order from high to low for SSp population was acephate (1.00) followed by spinosad (1.33), chlorfenapyr (2.69), acetamiprid (2.77) and dimethoate (2.85). For BSp population, RT were sequenced as acephate (1.00) followed by spinosad (1.46), chlorfenapyr (1.67) and dimethoate (3.32). In control unsprayed population, RT were in order as acephate < deltamethrin < spinosad < chlorfenapyr (1.0, 1.20, 1.28 and 1.58, respectively). Lambda-cyhalothrin was the least toxic against DCB population of all tested fields. From results, it can be suggested that regular insecticide resistance monitoring is crucial to avoid incidence(s) in *O. hyalinipennis*, and to adopt the rotational application of appropriate insecticides.
Key words: Dusky cotton bug; insecticide; Resistance; bioassays; cotton.

INTRODUCTION

The dusky cotton bug (DCB) (*Oxycarenus* spp.) also known as cotton strainer is a serious insect pest that feeds on reproductive parts of Malvaceous seeds in different countries over the globe including Southeast Asia and Pakistan (Henry 1983, Raman 1987, Schaefer and Panizzi 2000, Smith and Brambila 2008). By the cultivation of *Bt*-cotton in Pakistan with the liberty to expand sowing period from February to end June, the infestation of DCB has become a significant threat for cotton crops (Shah *et al.*, 2016, Ahmad *et al.*, 2019). The early sowing of *Bt*-cotton provided a favourable environment and led to the appearance of DCB infestation during May (Ahsan and Altaf 2009, Shahid *et al.*, 2014; Iqbal *et al.*, 2018). Six out of 55 species of *Oxycarenus* are listed as pest on different crops. *Oxycarenus hyalinipennis* Costa (Lygaeidae: Hemiptera) has been identified using molecular DNA barcoding in Pakistan (Ahmad *et al.*, 2019).

DCB is polyphagous and flourishing rapidly due to wider range of host plants. It causes premature dropping of growing part of the cotton such as flowers, squares and bolls (Srinivas and Patil 2004a). It has become the major insect pest due to its significant qualitative and quantitative losses to conventional cotton crop and *Bt*-cotton because of its heavy infestation (Sewify and Semeada 1993, Schaefer and Panizzi 2000, Srinivas and Patil 2004b, Patil and Rajanikanth 2005,

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Smith and Brambila 2008, Ahmed *et al.,* 2015). In Pakistan, its population has increased significantly, which is severely affecting the lint and seed quality of cotton in various geographical locations. It causes heavy losses in seed cotton weight, oil content, seed germination, seed weight and lint discoloration due to crushed insects (Abbas *et al.,* 2015, Ahmed *et al.,* 2015).

The female DCB lay eggs at various places such as in rind of half-opened bolls, on the seeds, in the lint of fully opened bolls and in between calyx (Srinivas and Patil 2004b, Srinivas and Patil 2004a). The expansion of *Bt*-cotton, early sowing and the change in plant protection measures have made it as a major pest with severe losses as high rate of shedding of flowering buds (squares) and shifting to other fruiting bodies especially to open bolls during the later months of the crop season that significantly reduce seed germination of the cotton. In conventional sowing time of cotton during May, it appears at the end of the crop before harvest. However, due to early sowing of *Bt*-cotton, DCB is emerging earlier in the field and lay a serious threat for other conventional late growing cotton varieties (Iqbal *et al.,* 2018, Shahzad *et al.,* 2022).

Insecticides are commonly used against different insect pest of cotton (Iqbal *et al.,* 2014). DCB is being controlled with the commercially available insecticides especially those used against bollworms in *Bt*-cotton (Ahmed *et al.,* 2015). Their extensive and repetitive use in conventional and *Bt*-cotton has resulted in occurrence of insecticide resistance in different insect pests including DCB (Roger *et al.,* 1997, Kannan *et al.,* 2004, Jan *et al.,* 2015, Ullah *et al.,* 2016). Therefore, it is imperative to evaluate the commercially available insecticides of different chemical groups for their resistance and efficacy against DCB especially from three different cotton fields i.e. unsprayed, field where sucking pest were controlled with insecticides, and a field where bollworms were controlled with insecticides.

MATERIALS AND METHODS

The experiments were conducted at Central Cotton Research Institute (CCRI), Multan (30.19 ºN, 71.46 ºE), Punjab, Pakistan. Three experimental cotton fields (30×30 sq ft each) in CCRI were used for the collection of *O. hyalinipennis*. In field-1, the crop was kept unsprayed. In field-2, sucking insect pests (whitefly, jassid and thrips) were controlled with imidacloprid, flonicamid (Ulala), diafenthiuron (Polo) and spinosad. In field-3, bollworms especially pink bollworm and armyworm were controlled with triazophos, deltamethrin, lambda-cyhalothrin and cypermethrin.

The insect population from each field were named as Un-sp (unsprayed population), SSp (exposed to sucking sprays) and BSp (exposed to bollworm sprays) collected randomly from each field before harvesting of the crop for monitoring of insecticidal resistance.

Insect collection and rearing**:** Approximately 1000 adult DCB were collected randomly from each of the three fields, brought into the laboratory, placed in the plastic jars (12×24 cm) and provided with semi-opened cotton bolls from the unsprayed field. The insect culture was weekly refreshed by replacing the fed-bolls with the fresh semi opened bolls. The homogeneous population were obtained by rearing the DCB for one generation under laboratory conditions at 27±2 ºC (temperature), 60±5% (relative humidity), and a photoperiod of 14:10 h (L:D).

Reference Laboratory Population**:** The DCB population collected from unsprayed cotton field (field-1) was considered as reference population for comparing with other field's populations.

Insecticides**:** The population of *O. hyalinipennis* of each respective field (field-1, field-2 and field-3 were tested for evaluating the insecticidal resistance against commercial insecticides of different groups as presented in Table 1.

Bioassays**:** Bioassays were performed on adult DCB (2–3 days old) using the semi opened bolls dip method (Afzal *et al.,* 2015) adopted at Entomology Section of CCRI, Multan, Punjab, Pakistan. The leaves of China rose and cotton were tested in preliminary screening but the results were not promising because most of the *O. hyalinipennis* escaped from the insecticides and move towards the roof of the Petri dishes. Therefore, semi-opened fresh bolls were used where all the bugs gathered around bolls for feeding. The small jars of 500 g capacity were utilized for these analyses.

Five serial concentrations of each insecticide were prepared and replicated for five times from their stock solutions (Ullah *et al.,* 2016). Fresh semi-opened bolls were dipped in each concentration for 10 s and air dried for 2–3 h at room temperature. Two treated bolls were placed in each glass jar and moist filter paper was placed in the jar to prevent them from desiccation (Afzal *et al.,* 2015). Ten adults of DCB were added in each jar and a total of 250 adults were exposed in five replications of each concentration. For the control, semi-opened bolls were used and replicated five times (50 DCB adults). Bioassays were conducted under laboratory conditions at 27±2 ºC (temperature), 60±5% (relative humidity), and a photoperiod of 14:10 h (L:D) similar as utilized for insect rearing. The response of *O. hyalinipennis* in terms of mortality was assessed 48 h after exposure to conventional insecticides and 72 h after exposure to novel chemistry insecticides. If adults showed no movement after touching with fine camel hair brush, they were considered as dead.

Concentrations of insecticides**:** The commercial formulations of insecticides were used for the laboratory bioassay. Serial dilutions (parts per million of active ingredients) of each insecticide were formulated using 50 mL distilled water through the following equation

$$\text{ppm} = \frac{\text{Field dose}/1000 \times 50 \text{ mL distilled water}}{\%\,\text{formulation}/100}$$

Table 1. Insecticides and their details.

Insecticide group	Common name	Trade name	Company name
Organophosphate (OP)	profenofos	Curacron® 500 EC	Syngenta, Pakistan
	triazophos	Triazophos® 40 EC	Arysta Life Science, Pakistan
	acephate	Commando Plus® 97% DF	FMC United (Pvt.) Ltd
	dimethoate	Sanitox® 40 EC	Pakistan Agro Chemicals (Pvt.) Ltd
Pyrethroid	cypermethrin	Arrivo® 10% EC	FMC United (Pvt.) Ltd
	deltamethrin	Decis Super® 10 EC	Bayer Crop Sciences, Pakistan
	bifenthrin	Talstar® 10 EC	FMC United (Pvt.) Ltd
	esfenvalerate	Sumi-alpha® 110 EC	FMC United (Pvt.) Ltd
	lambda-cyhalothrin	Karate® 2.5 EC	Syngenta, Pakistan
Neonicotinoid	imidacloprid	Confidor® 20 SL	Bayer Crop Sciences, Pakistan
	acetamiprid	Mospilon® 20 SP	Arysta Life Science Pakistan
	chlothianidin	Telsta® 20 SC	FMC United (Pvt.) Ltd
Organochlorine (OC)	endosulfan	Thiolix® 35% EC	Arysta Life science Pakistan
Naturalyte	spinosad	Tracer® 240 SC	Arysta Life Sciences, Pakistan
	emamectin benzoate	Proclaim® 019 EC	Syngenta, Pakistan
Halogenated Pyrroles	chlorfenapyr	Pyrate® 360 SC	BASF, Pakistan

***Evaluation of relative toxicity of insecticides*:** The relative toxicity (RT) of different insecticides over highly effective insecticides was calculated using the following formula of Awasthi *et al.,* (2013):

$$\text{Relative toxicty (RT)} = \frac{\text{LC}_{50}\text{ of less toxic compound}}{\text{LC}_{50}\text{ of high toxic compound}}$$

Scale of Resistance Ratio (RR): The scale for grading of resistance levels of insecticides was determined as described by Ahmad *et al.,* (2008) (Table 2).

Table 2. Scale of resistance ratio (RR) for grading the resistance levels of insecticides

Resistance Ratio (RR)	Resistance level
< 2 fold	Susceptibility, no resistance
2–10 fold	Tolerance to low resistance
11–30 fold	Moderate resistance
31–100 fold	High resistance
>100 fold	Very high resistance

***Data Analysis*:** The concentration dependent data was analysed with POLO Software (LeOra 2005) using Probit analysis (Finney 1971) to calculate LC_{50} values, chi-square (χ^2), 95% fiducial limits (FL) and slopes with standard error. Abbott's formula (Abbott 1925) was used to correct the data for control mortality. The LC_{50} values were considered significantly similar if their 95% FLs overlapped. The resistance ratios (RR) were calculated as: RR= LC_{50} of the DCB population from SSp or BSp/ LC_{50} of Un-sp. The 95% FLs for the RRs were determined according to (Robertson and Preisler 1992).

RESULTS

***Monitoring of insecticidal resistance in DCB*:** The present study presented the results regarding the resistance level in *O. hyalinipennis* against insecticides belonging to different chemical groups.

Organophosphates (OP): The concentration response bioassay revealed two resistance levels (susceptibility and tolerance to low resistance) at LC_{50} for OPs in tested DCB populations of SSp and BSp compared to Un-sp (unsprayed population). The high resistance ratio (RR) was tolerance to low resistance (RR =2–10 fold) for profenofos (4.0 fold) in SSp, 2.2 fold in BSp, for triazophos (2.4 fold) in BSp population. Among others, acephate and dimethoate were stable and more effective with lowest LC_{50} value (5.59–8.71 ppm) and (22.62–24.81 ppm), respectively. Profenofos and triazophos exhibited LC_{50} value range of 27.20–107.64 ppm and 27.32–65.77 ppm, respectively (Table 3).

***Pyrethroid*:** The resistance in DCB was increased to moderate level (RR = 11–30 fold) against deltamethrin in SSp (24.4 fold) and BSp (21.2 fold). All other pyrethroids showed equal level of resistance (susceptibility: RR < 2 fold) in all the tested populations of DCB (Un-sp, SSp and BSp) with RR values for bifenthrin (1.0, 0.3 and 1.4, respectively), esfenvalerate (1.0, 1.1 and 1.1, respectively) and lambda-cyhalothrin (1.0, 1.1 and 1.6, respectively). Cypermethrin presented tolerance to low resistance level (RR: 2–10 fold) in SSp (3.2 fold) and BSp (2.8 fold) insect population, respectively. The lower and upper range of LC_{50} were 33.97–149.06 ppm, 46.22–149.91 ppm, 6.71–163.70 ppm and 264.60–426.5 ppm for cypermethrin, deltamethrin, bifenthrin and lambda-cyhalothrin, respectively. These insecticides showing high ranges of LC_{50} against DCB were unstable except esfenvalerate (114.83–122.60 ppm) which was not affected by the general field insecticide applications against DCB in SSp and BSp (Table 3).

Neonicotinoids**:** The resistance level was tolerance to low resistance against acetamiprid (2.1 fold) in BSp and

Table 3. Monitoring of insecticide resistance in dusky cotton bug against various groups of insecticide under lab conditions.

Insecticide	Insect population	LC_{50} (95% FL)[a] (ppm)	Slope ±SE	DF	χ^2	*P*	RR[b]
Organophosphates (OP)							
acephate	Un-sp	5.59 (4.27–7.33)	1.31±0.16	3	6.097	0.11	1.0
	SSp	8.71 (5.9–12.4)	0.93±0.15	3	0.357	0.95	1.6
	BSp	7.02 (5.16–9.24)	1.11±0.15	3	1.729	0.63	1.3
profenofos	Un-sp	27.20 (17.92–46.11)	0.78±0.15	3	2.623	0.45	1.0
	SSp	107.64 (72.3–173.2)	0.83±0.16	3	0.357	0.95	4.0
	BSp	58.44 (38.82–82.83)	0.88±0.15	3	2.548	0.47	2.2
triazophos	Un-sp	27.32 (17.85–42.96)	0.81±0.15	3	0.931	0.82	1.0
	SSp	54.55 (38.3–71.8)	1.11±0.16	3	2.277	0.52	2.0
	BSp	65.77 (46.40–88.25)	1.02±0.15	3	5.350	0.15	2.4
dimethoate	Un-sp	22.62 (16.58–33.38)	1.22±0.18	3	0.318	0.96	1.0
	SSp	24.81 (17.1–36.7)	0.94±0.16	3	5.661	0.13	1.1
	BSp	23.28 (17.05–32.58)	1.01±0.15	3	0.971	0.81	1.0
Pyrethroid							
bifenthrin	Un-sp	33.97 (8.07–62.2)	0.61±0.16	3	1.334	0.72	1.0
	SSp	105.20 (67.9–221.8)	0.86±0.16	3	0.981	0.81	0.3
	BSp	149.06 (107.9–209.8)	0.96±0.15	3	2.484	0.48	1.4
cypermethrin	Un-sp	46.22 (33.35–63.97)	1.07±0.16	3	3.630	0.30	1.0
	SSp	149.91 (11.1–19.1)	1.15±0.16	3	1.371	0.71	3.2
	BSp	129.30 (83.96–186.33)	0.82±0.15	3	0.428	0.93	2.8
deltamethrin	Un-sp	6.71 (13.23–111.87)	1.30±0.29	3	10.46	0.02	1.0
	SSp	163.70 (12.9–28.1)	1.08±0.15	3	3.098	0.38	24.4
	BSp	141.84 (105.3–185.1)	1.20±0.16	3	0.453	0.93	21.2
esfenvalerate	Un-sp	114.83 (85.3–152.0)	1.17±0.16	3	0.812	0.85	1.0
	SSp	122.60 (81.4–187.0)	0.83±0.15	3	1.164	0.76	1.1
	BSp	115.10 (59.9–536.0)	0.61±0.16	3	1.652	0.65	1.0
Lambda-cyhalothrin	Un-sp	264.60 (181.7–453.3)	0.86±0.15	3	1.075	0.78	1.0
	SSp	297.14 (195.4–407.2)	1.04±0.16	3	1.966	0.58	1.1
	BSp	426.5 (290.5–598.8)	0.93±0.15	3	1.321	0.72	1.6
Neonicotinoids							
acetamiprid	Un-sp	14.7 (10.04–23.7)	0.87±0.16	3	2.402	0.49	1.0
	SSp	24.1 (13.6–36.5)	0.74±0.15	3	4.896	0.18	1.6
	BSp	30.28 (21.2–41.8)	0.94±0.15	3	2.529	0.47	2.1
imidacloprid	Un-sp	12.25 (7.3–17.9)	0.80±0.15	3	0.507	0.92	1.0
	SSp	78.30 (52.9–119.3)	0.83±0.15	3	0.831	0.84	6.4
	BSp	59.39 (41.8–81.9)	0.99±0.15	3	3.392	0.34	4.9
chlothianidin	Un-sp	14.21 (8.99–24.97)	0.72±0.14	3	3.738	0.29	1.0
	SSp	35.85 (25.5–49.1)	1.03±0.15	3	0.052	1.00	2.5
	BSp	52.63 (34.13–91.01)	0.69±0.14	3	1.352	0.72	3.7
Organochlorine (OC)							
endosulfan	Un-sp	20.68 (14.4–28.20)	0.98±0.15	3	6.346	0.10	1.0
	SSp	58.28 (41.2–82.1)	1.02±0.16	3	5.757	0.12	2.8
	BSp	55.18 (42.28–71.77)	1.21±0.15	3	3.147	0.37	2.7
Naturalyte							
emamectin benzoate	Un-sp	153.42 (80.39–301.33)	0.55±0.14	3	3.617	0.31	1.0
	SSp	190.92 (124.1–321.1)	0.75±0.15	3	3.695	0.30	1.1
	BSp	173.40 (126.26–243.56)	1.06±0.16	3	4.598	0.20	0.9
spinosad	Un-sp	7.16 (4.13–15.63)	0.61±0.15	3	2.450	0.48	1.0
	SSp	11.57 (7.68–18.5)	0.79±0.15	3	0.770	0.86	1.6
	BSp	10.25 (7.56–14.17)	1.02±0.15	3	1.110	0.76	1.4
Halogenated pyrroles							
chlorfenapyr	Un-sp	8.84 (6.49–11.91)	1.10±0.15	3	4.500	0.21	1.0
	SSp	23.43 (16.4–34.8)	0.98±0.16	3	1.440	0.70	2.7
	BSp	11.72 (7.87–16.29)	0.94±0.15	3	1.800	0.61	1.3

Un-sp (unsprayed field); SSp (field where sucking pest were controlled); BSp (field where bollworms were controlled). *Significantly different from 1.0, when 95% CL did not overlap. [a] 95% FL= fiducial limits; χ^2 = Chi square; [b] RR= resistance ratio.

imidacloprid (6.4 and 4.9 fold) in SSp and BSp populations, respectively. For chlothianidin, it was 2.5 and 3.7 fold in SSp and BSp populations, respectively (Table 3).

***Organochlorine (OC), naturalyte and halogenated pyrroles*:** Endosulfan (OC) showed increased resistance level from tolerance to low resistance (RR = 2–10 fold) with values 2.8 and 2.7 fold against SSp and BSp population compared to Un-sp population (1.0 fold). In case of naturalyte, DCB populations showed no response against naturalyte and level of resistance persisted as susceptible in the DCB population of different fields (Un-sp, SSp and BSp). The range of LC_{50} was lower for spinosad (7.16–11.57 ppm) and higher for emamectin (153.42–190.92 ppm). For chlorfenapyr, resistance level was 2.7 fold in SSp population compared to BSp (1.3 fold) and Un-sp (1.0 fold) (Table 3).

Collectively, DCB population from SSp field exhibited high level of resistance (RR) against profenofos (4 fold), deltamethrin and cypermethrin (24.4 fold and 3.2 fold, respectively), imidacloprid (6.4 fold), endosulfan (2.8 fold) and chlorfenapyr (2.7 fold) when compared the mortality at LC_{50} of unsprayed (Un-sp) population. Likewise, the higher level of resistance in BSp population was observed against profenofos (2.2 fold), triazophos (2.4 fold), cypermethrin by (2.8 fold), deltamethrin (21.2 fold), imidacloprid by (4.9 fold), chlothianidin (3.7 fold) and endosulfan (2.7 fold) when compared the mortality at LC_{50} in Un-sp population. The higher LC_{50} of insecticide corresponds to less toxicity to DCB and vice versa. Lambda-cyhalothrin were least effective with e highest LC_{50} (264.60, 297.14 and 426.5 ppm) and acephate was highly effective with least LC_{50} (5.59, 8.71 and 7.02 ppm) against Un-sp, SSp and BSp insect populations, respectively. The higher level of LC_{50} corresponds to less toxicity against DCB and vice versa. Lambda-cyhalothrin exhibited maximum LC_{50} values against DCB populations of Un-sp (264.60), SSp (297.14) and BSp (426.5 ppm) fields. The minimum LC_{50} was recorded for acephate (5.59, 8.71 and 7.02 ppm), spinosad (7.16, 11.57 and 10.25), and dimethoate (22.62, 24.81 and 23.28 ppm) for DCB populations of Un-sp, SSp and BSp fields, respectively (Table 3).

***Relative Toxicity of insecticides*:** Relative toxicity (RT) of insecticides (LC_{50} ppm) was calculated over acephate as it was highly toxic with least LC_{50} value against DCB population of all tested crop fields. For Un-sp population, acephate was the most toxic insecticide with RT values of 1.00 ppm followed by deltamethrin (1.20), Spinosad (1.28) and chlorfenapyr (1.58 ppm). The least toxic insecticides with high LC_{50} values were lambda-cyhalothrin (47.33 ppm) followed by emamectin benzoate (27.45 ppm) and esfenvalerate (20.54 ppm). For SSp population, most toxic was acephate (1.00 ppm) followed by spinosad < chlorfenapyr < acetamiprid <dimethoate (1.33, 2.69, 2.77, 2.85 ppm, respectively). The least toxic insecticides with high LC_{50} values were lambda-cyhalothrin (34.11 ppm) followed by emamectin, deltamethrin and cypermethrin (21.92, 18.79 and 17.21 ppm, respectively). For BSp population, most toxic was acephate (1.0 ppm) followed by < spinosad < chlorfenapyr (1.46 and 1.67 ppm, respectively) but the least toxic were lambda-cyhalothrin (60.76 ppm) followed by emamectin, bifenthrin, deltamethrin, cypermethrin and esfenvalerate (24.70, 21.23, 20.21, 18.42, 16.40 ppm, respectively) (Table 4).

Figure 1 showed the graphical presentations of LC_{50} of different insecticides in individual tested field (Un-sp, SSp and BSp). Lambda-cyhalothrin revealed higher LC_{50}, and acephate was the highly toxic with least LC_{50} in all three tested fields.

Table 4. Evaluation of relative toxicity (RT) of insecticides against dusky cotton bug

Relative toxicity (LC_{50} ppm*)					
Insecticides	**Field 1 (Un-sp)**	**Insecticides**	**Field 2 (SSp)**	**Insecticides**	**Field 3 (BSp)**
lambda-cyhalothrin	47.33	Lambda-cyhalothrin	34.11	Lambda-cyhalothrin	60.76
emamectin	27.45	emamectin	21.92	emamectin	24.70
esfenvalerate	20.54	deltamethrin	18.79	bifenthrin	21.23
cypermethrin	8.27	cypermethrin	17.21	deltamethrin	20.21
bifenthrin	6.08	esfenvalerate	14.08	cypermethrin	18.42
triazophos	4.89	profenofos	12.36	esfenvalerate	16.40
profenofos	4.87	bifenthrin	12.08	triazophos	9.37
dimethoate	4.05	imidacloprid	8.99	imidacloprid	8.46
endosulfan	3.70	endosulfan	6.69	profenofos	8.32
acetamiprid	2.62	triazophos	6.26	endosulfan	7.86
chlothianidin	2.54	chlothianidin	4.12	chlothianidin	7.50
imidacloprid	2.19	dimethoate	2.85	acetamiprid	4.31
chlorfenapyr	1.58	acetamiprid	2.77	dimethoate	3.32
spinosad	1.28	chlorfenapyr	2.69	chlorfenapyr	1.67
deltamethrin	1.20	spinosad	1.33	spinosad	1.46
acephate	1.00	acephate	1.00	acephate	1.00

*Low RT (LC_{50} ppm) values correspond to highly effective insecticides against DCB and vice versa.

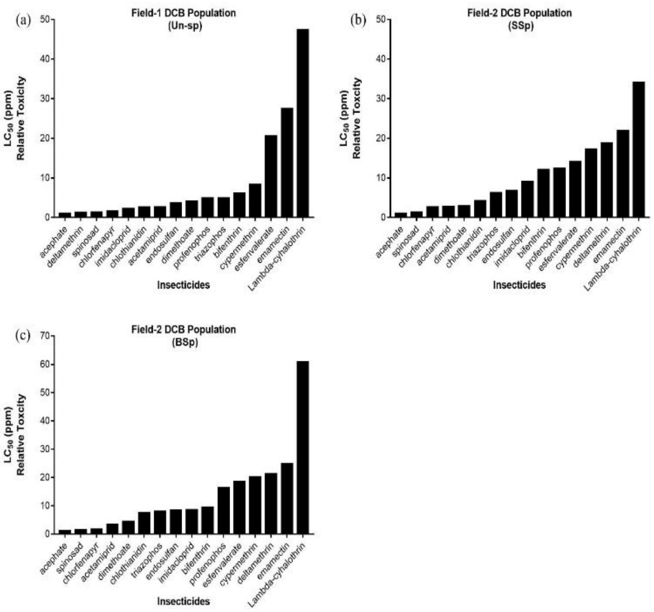

Figure 1. Relative toxicity (LC_{50} ppm) of insecticides against *Oxycarenus hyalinipennis.*

(a) Un-sp (unsprayed Field-1); (b) SSp (field-2 where sucking insects were controlled); (c) BSp (Field-3 where bollworms were controlled). Low RT (LC_{50} ppm) values reflects the highly effective insecticides against DCB and *vice versa*. Each graph shows the relative toxicity which is increasing from acephate to lambda-cyhalothrin.

The comparative variation in LC_{50} values of different insecticides among the three tested fields (Un-sp, SSp and BSp) are shown in Figure 2. Prominent variation in LC_{50} was shown by bifenthrin, cypermethrin, deltamethrin, endosulfan, imidacloprid, profenofos and lambda-cyhalothrin against DCP population of Un-sp, SSp and BSp.

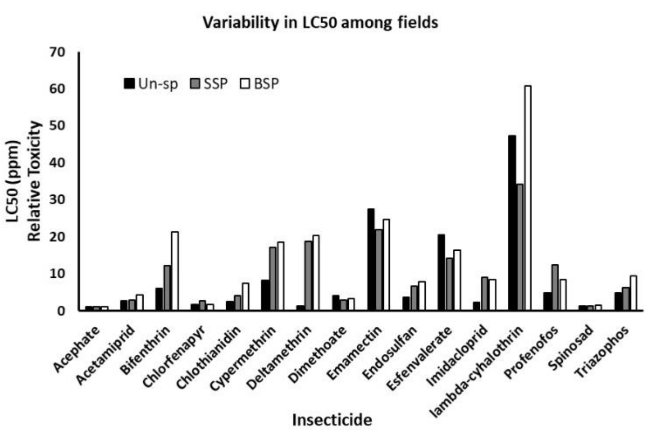

Figure 2. Comparison of variability in LC_{50} (ppm) of insecticides among different fields.

Un-sp (unsprayed Field-1); SSp (field-2 where sucking insects were controlled); BSp (Field-3 where bollworms were controlled).

DISCUSSION

Oxycarenus hyalinipennis, dusky cotton bug (DCB), a major polyphagous insect has developed the resistance against insecticides. The present study determined the level of resistance against insecticides of different chemical groups in DCB populations of three different cotton fields: Un-sp (unsprayed), SSp (sprayed for sucking pest): SSp and BSp (sprayed for bollworm). LC_{50} values were also evaluated which exhibited the toxicity of insecticides. DCB populations revealed the differential level of insecticide resistance against insecticides belonging to different chemical groups. The varying resistance to insecticides is also reported in other insect pests like *Bemisia tabaci* (Homoptera: Aleyrodidae) (Basit *et al.*, 2011), *Spodoptera litura* (Lepidoptera: Noctuidae) (Saleem *et al.*, 2008, Shad *et al.*, 2010, Abbas *et al.*, 2014), *Musca domestica* (Diptera: Muscidae) (Khan *et al.*, 2013), and *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) (Saeed *et al.*, 2018). Many pyrethroids and organophosphates are reported to be effective against DCB, *Oxycarenus* spp. (Roger *et al.*, 1997).

Out of present data, DCB populations of SSp and BSp fields exhibited higher resistant level (>10 fold) against deltamethrin (24.4–21.2 fold), and can be taken as resistant according to the previous report where the populations with 10-fold resistance were considered as resistant to the respective insecticide (Valles *et al.*, 1997). In contrast, Ullah *et al.* (2016) found less resistance (2.14–8.41 fold) for deltamethrin in *O. hyalinipennis.* In present study, DCB showed <10 fold resistance ratio in all other tested insecticides of different chemical groups which is contradictory to a previous study who higher resistant range (> 10 fold) for bifenthrin (14-30 fold), lambda-cyhalothrin (9.12–16 fold), profenofos (12–14 fold), triazophos (9.04–15 fold). emamectin benzoate (9.83 fold), spinosad (6.38–17 fold), imidacloprid (11–22 fold) and chlorfenapyr (16–46 fold) (Ullah *et al.*, 2016). In this contradictory study, DCB population were caught directly from cotton fields in five various cotton regions. Whereas, DCB population in present study was caught from the fields (SSP, BSp and Un-sp) under controlled specific condition. Thus, the difference in experimental setup and DCB population of diversified area may result in diversified resistant level of DCB.

We argued that DCB population (Un-sp) without any insecticide exposure may revealed an increase in the susceptibility to pesticides resistance (Roush and Croft 1986, Kristensen *et al.*, 2000). The mixing of insecticides of different groups (conventional to new novel chemistry) is a common farmers practice to control wide range of cotton pests, and it could resulted in the development of cross or multiple resistance between the insecticides (Abbas *et al.*, 2014). Furthermore, the activity of oxidases and esterase enzymes is enhanced in some insects having insecticidal resistance (Soderlund and Knipple 2003, Huang and Han

2007). The development of cross/ multiple resistance and any potential increase in enzyme activity need further investigation for DCB population.

The interactions between resistance and insect ecology becomes an imperative factor because such interaction could reflect the availability of treated and untreated host during different seasons and it also exhibits the migration pattern of DCB among different hosts throughout the year (Denholm *et al.,* 1998). The active migration of insects from protected or controlled environments to unsprayed fields has potential to impart resistance genes in the populations and speed up their damaging incidences (Raymond *et al.,* 1991). The resistance of DCB against insecticides with different chemistry and modes of action revealed the potential presence of phenomenon of cross-resistance and/or multiple resistance that should be tested in future studies.

In the present study, true susceptible DCB population was not developed, and therefore the toxicity level was estimated with relative toxicity or toxicity index of the insecticides. In DCB, the contact residual activity was different among insecticides. High toxicity indices reveals the insecticides were less toxic and low toxicity indices associated with high toxicity against DCB. The insecticides with low indices could be considered most effective with far reaching results. The insecticides with different active ingredients can have different sensitivity to environmental conditions (Tomlin 2009), which could be the reason for the differential level of resistance. Furthermore, the variation in residual activity among different insecticides cannot be avoided. This variation in residual effect suggested that farmers can select suitable insecticides against DCB that exert least negative effects in terms of resistance development.

The development of insecticide resistance in insects are making the them ineffective. Therefore, it is the need of the time to apply different strategies to counter or prevent the development of resistance against commercially used insecticides (Jan *et al.,* 2015). The methods developed by researchers allow the determination of residual toxicity of individual pesticide under laboratory and practical field conditions (Malezieux *et al.,* 1992).

Our data also revealed variation in LC_{50} of different insecticides when compared among Un-sp, SSp and BSp population. Thus, the resistance of the tested insecticides was unstable against DCB in different tested fields. The resistance reversion in the absence of selection pressure is of great importance in resistance management (Mokbel 2018). The reversion or conversion of the insecticides leads towards instability of the insecticides and affect their shelf life (Basit *et al.,* 2011). We observed that DCB showed the high ability to develop resistance when the population collected from sprayed field compared to population from unsprayed field. The possible increase in resistance of DCB population due to field spray regimes may be related to the fitness costs associated with the resistance phenotypes (Carriére and Tabashnik 2001, Kliot and Ghanim 2012).

In many agricultural systems, especially in Pakistan the real challenge is to implement the proper and wise use of pesticides in addition to the ongoing efforts and strategies to counter the insecticidal resistance. It is also reported that resistance to insecticides resulted in fitness costs in many insects (Kliot and Ghanim 2012). Certain crucial points while devising the pest management strategies against DCB must be emphasised such as record of failure due to resistance, reasonable infrastructure, understanding of recommendations and regulations regarding the distribution and effective wise use of insecticides (Denholm *et al.,* 1998, Pretty and Bharucha 2015, Shahzad *et al.,* 2022). The repetitive use of a single insecticide or insecticides from similar chemical group or having same mode of action can increase the risk of rapid build-up of resistance in insect population (IRAC 2020, NIFA n.d.). Therefore, the use of insecticides from different chemical groups may help to slow down the development of resistance in insects (IRAC 2020).

Conclusion: The dusky bug showed exhibited two level of resistance (susceptibility, tolerance to low resistance) except deltamethrin with moderate resistance against DCB population of different cotton fields. The acephate was highly effective insecticides with least LC_{50} value whereas lambda-cyhalothrin was least effective with high LC_{50} values. The outcomes of this study would be helpful to the farmer's choice of the insecticides for the control of DCB. The use insecticides with more toxicity having low LC50 values might reduce the cost of insecticides and insecticide resistance against DCB. The regular monitoring of insecticidal resistance with cautious and rotational application of appropriate insecticides of different chemical nature is crucial to avoid the incidence of insecticidal resistance in *O. hyalinipennis*.

***Conflict of Interest*:** The authors declare that there is no conflict of interest.

***Authors' Contribution Statements*:** Javaid Iqbal, Sohail Akhtar and Muhammad Tahir Jan conceptualized the idea and designed the methodology. Muhammad Ammar Yasir and Sohail Akhtar collected the data and prepare the initial draft. Muhammad Tahir Jan and Sohail Akhtar did the project administrations. Javaid Iqbal, Sohail Akhtar and Muhammad Tahir Jan validate the data. Javaid Iqbal, Khawaja Ghulam Rasool, Mureed Husain and Abdulrahman S. Aldawood did the data analysis, Writing-Reviewing and Editing.

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