

Research Article

Cross-negative effects of selective insecticides against different life stages of non-target pests, *Dysdercus koenigii* and *Oxycarenus hyalipennis* on transgenic cotton under laboratory conditions

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Abstract

Sucking insect pests, dusky cotton bug; *Oxycarenus hyalinipennis* Costa and red cotton bug; *Dysdercus koenigii* Walk has been great threat to transgenic (Bt) cotton worldwide. Present research was carried out to determine effect of some new chemistry insecticides [Steward[®] 150 EC (indoxacarb), Match[®] 50 EC (lufenuron), Coral[®] 36% SC (chlorfenapyr), Scatter[®] 15% SC (indoxacarb), Orchard[®] 13.3% EW (imidachloprid + abamectin) and Snap[®] 10% WDG (emamectin benzoate + tebufenozide)] under laboratory conditions (28 ± 2 °C, 65-70 % relative humidity and at photoperiod of 16:8 h of Light: Dark) to determine either field recommended dose rates have contact toxicity against non-target pests *Dysdercus koenigii* (Pyrrhocoridae: Hemiptera) and *Oxycarenus hyalipennis* (Lygaeidae: Hemiptera). Insecticides having contact mode of action that are recommended against chewing insects pest have also possess toxicity against sucking insect pests for which these pesticides are not recommended. Therefore the main objective of this research was to determine the contact toxicity against *O. hyalinipennis* and *D. koengii*. Laboratory bioassay results exhibit that Match[®] 50 EC was most toxic against nymphs, female and male of *O. hyalinipennis* and *D. koengii* and also showed maximum results in population reduction percentage for both under investigated pests. However, Orchard[®] has been caused minimum population reduction of both non-target pests among the tested insecticides. While remaining four insecticides Scatter[®], Steward[®], Coral[®] and Snap[®] showed moderate level of toxicity against tested insect pests.

Keywords: Bioassay; *Dysdercus koenigii*; Insecticides; Life stages; *Oxycarenus hyalinipennis*; Toxicity

Introduction

Gossypium hirsutum L. (Cotton) is a main oilseed and fiber crop [1]. Cotton has significant contribution in earning through foreign exchange. In Pakistan, cotton has been attacked by chewing and sucking insect pests [2]. To control insect pest different methods are used especially conventional chemical insecticides. Globally Bt. Cotton is cultivated on large scale to manage the bollworms population [3, 4]. In Pakistan, chemical control is considered as an immediate and effective control of insects pests [5-8]. Every year about 80 % of the total agro-chemicals are applied against insect pests of cotton that cause various problems [9-12].

With arrival of Bt cotton and changing in sowing time season has effect the status of all cotton pests. Sudden emergence of minor pest as a major or common pest is huge risk for cotton of Pakistan e.g.; cotton stink bugs and dusky cotton bugs [13, 14]. First time dusky cotton bug (*Oxycarenus hyalipennis*) was reported in May, 2010 in Israel, on Bt-Cotton and resulted in prematured fallings of the flowers along with small bolls and squares / brackets. In Pakistan as well as in several other countries of the world, Red cotton bug and Dusky cotton bug have gained the status of pest. In Israel adults of the dusky cotton bugs caused damages to fruit trees comprising grease spots on fruits as well as cause deformity of the fruits [15]. In Africa, dusky cotton bugs are known as major

pests of cotton and okra crops, in South-east Asia and India as well [16]. In Egypt, Dusky cotton bugs were reported as major cotton pests, where it has caused weight losses in cotton seed and also caused decrease in oil seed quality and germination [17]. Dusky and Red cotton bugs feed on seeds and unopened or opened bolls [18]. When they crawls, a colored liquid excretes from bodies of these bugs on cotton lint which results in staining like yellow spots that ultimately reduces market value and quality of lint or textiles products. A bacteria is also present in saliva that results in rotting of bolls and thus produced seeds from these damaged bolls has germination and viability issues. Fungus infestation occurs on the hole made due to feeding of these bugs and results in higher contents of the aflatoxin in the seed cake that is produced from such seeds which renders it not fit for the purpose of animal feeds [19].

Materials and methods

For the determination of cross negative impacts of selective insecticides (Table 1), as contact toxic against two non-target insect pests, red cotton bug and dusky cotton bug; research was performed in IPM (Integrated Pest Management) laboratory, department of Entomology, University of Agriculture Faisalabad, Faisalabad during 2019. The research experiments were planned as these selected pesticides have not been examined before against these test insects for their contact toxicity.

Table 1. List of the formulations of insecticides, chosen for current experiments

Insecticides formulation	Dose (gm or ml/acre)
Match [®] 50 EC; (leufenuron)	250
Coral [®] 36 % SC; (chlorfenapyr)	150
Scatter [®] 15 % SC; (indoxacarb)	175
Orchard [®] 13.3 % EW; (abamectin+ imidachloprid)	200
Steward [®] 150 EC; (indoxacarb)	175
Snap [®] 10% WDG (tebufenozide+emamectin benzoate)	100

Collection of test insects; dusky cotton bug and red cotton bug from infested fields

The population of red cotton bug and dusky cotton bug; adults and nymphs, was taken from different fields of cotton at University of Agriculture Faisalabad and taken to IPM

laboratory of the University in order to acclimatize the specimen with laboratory conditions.

Layout of Experiment

Different six insecticides; Scatter[®] (indoxacarb) 15% SC, Match[®] (lufenuron) 50 EC, Orchard[®] (abamectin+imidachloprid) 13.3% EW,

Steward[®] (indoxacarb) 150 EC, Snap[®] (tebufenozide+emamectin benzoate) 10% WDG and Coral[®] (chlorfenapyr) 36% SC were evaluated against selected test insect pests. As per recommended doses for field application five concentrations of every insecticide were formed (Table 2) by applying Charles formula; $C_1V_1=C_2V_2$.

Table 2. List of concentrations applied for presented laboratory bioassays

Concentrations	Match [®]	Coral [®]	Scatter [®]	Orchard [®]	Steward [®]	Snap [®]
T ₁	00.05	00.0625	00.04375	00.05	00.04375	00.025
T ₂	00.1	00.125	00.0875	00.1	00.0875	00.05
T ₃	00.2	00.25	00.0175	00.2	00.175	00.1
T ₄	00.4	00.5	00.35	00.4	00.35	00.2
T ₅	00.8	1	00.7	00.8	00.7	00.4
Control	-	-	-	-	-	-

Bioassay

For the purpose of bioassay of the selected insecticides, method of filter paper treatment was applied. Highest dose of stock solutions were formed of the selected insecticides with accurate dilutions. A scissor was used for the cutting of sterilized filter papers accordingly petri dish diameter that was (90 x 15 mm). Then the dilutions of insecticides were applied by spraying on filter paper. Later on the treated filter paper were arranged in the petri dishes after drying. Filter paper treated with water was arranged in petri dishes for control. For each stage ten individuals (nymph, adult female and adult male) of both bugs were released at edges of the separated petri dishes with tender fresh cotton boll set at the center of every petri dish as bug food. All petri dishes were placed at 28 ± 2 °C temperature and 65-70 % relative humidity in laboratory. Three repeats were made for whole experiment with CRD. Data for mortality was collected after three days of the post treatment interval. Henderson and Tilton formula was used for transforming mortality data into percent corrected mortality [20].

Results

In vitro screening of different selective pesticides recommended against the

chewing insects pest showed cross negative impact on different non target insect pests. All formulations of selected insecticides cause inevitable population reduction of under investigated insect pests after 3 days of insecticides treatment (Table 3-8).

Among tested insecticides Match[®] 50 EC (lufenuron) showed diverse impact and cause considerable reduction in population of Male, female and nymphs of under investigated pests. Highest concentration (0.8%) caused reduction in population by 86.18 (males), 81.76 (females), 85.61 (numphs), 85.77 (males), 85.00 (females) and 87.00% (numphs) for both insects (*D. koenigii* and *O. hyalinipennis*) respectively. Mean mortalities of *D. koenigii* and *O. Hyalinipennis* gradually increased with increase in concentration. During all these experiments, there has been performed a comparative evaluation of some selective pesticides with different mode of action and founded that Match[®] caused considerable and significant reduction in population of non target insects of cotton (Table 3). These results shown that contact toxicity of Match[®] (Leufenuron) 50 EC enhanced with the increase in the concentrations (Table 3).

Table 3. Means mortality of *Dysdercus Koenigii* and *Oxycarenus hyalinipennis*, Nymph, Male and Female exposed to various concentrations of Match®

Concentrations	Mortality of <i>D. koenigii</i> (Mean ± SE)			Mortality of <i>O. hyalinipennis</i> (Mean ± SE)		
	Male	Female	Nymph	Male	Female	Nymph
0.8	86.18 ^A ±0.55	81.76 ^A ±0.41	85.61 ^A ±0.29	85.77 ^A ±0.25	85.00 ^A ±0.91	87.00 ^A ±0.97
0.4	67.66 ^B ±0.69	70.23 ^B ± 0.69	58.69 ^B ± 0.89	62.53 ^B ±0.89	69.00 ^B ± 0.87	69.00 ^B ±0.88
0.2	52.5 ^C ± 0.76	43.30 ^C ± 0.56	39.46 ^C ± 0.81	43.30 ^C ±0.81	57.00 ^C ± 0.81	45.00 ^C ±0.90
0.1	30.63 ^D ±0.83	31.76 ^D ± 0.83	24.07 ^D ± 0.70	27.92 ^D ±0.70	37.00 ^D ± 0.69	25.00 ^D ±0.73
0.05	19.51 ^E ±0.69	8.69 ^E ± 0.59	8.69 ^E ± 0.51	7.33 ^E ± 0.57	17.00 ^E ± 0.44	7.00 ^E ± 0.56
Control	7.33 ^F ± 0.39	8.33 ^F ± 0.39	8.00 ^F ± 0.32	4.84 ^F ± 0.32	8.00 ^F ± 0.38	5.00 ^F ± 0.27

Different letters in column indicates statistical significance (at $P \leq 0.05$) among the treatments

Second most toxic insecticide against non target insects was Coral®, where the maximum concentration (1%) caused 72.42 (males), 67.66 (females), 86.85 (nymphs), 66.51 (males), 72.42 (females) and 87.54%

(nymphs) for both insects (*D. koenigii* and *O. hyalinipennis*) respectively. Mean mortality gradually decreased with decrease in concentration (Table 4).

Table 4. Means mortality of *Dysdercus Koenigii* and *Oxycarenus hyalinipennis*, Nymph, Male and Female exposed to various concentrations of Coral®

Concentrations	Mortality of <i>D. Koenigii</i> (Mean ± SE)			Mortality of <i>O. hyalinipennis</i> (Mean ± SE)		
	Male	Female	Nymph	Male	Female	Nymph
1.0	72.42 ^A ± 0.81	67.66 ^A ± 0.79	86.85 ^A ± 0.75	66.51 ^A ± 0.87	72.42 ^A ± 0.93	87.54 ^A ± 0.86
0.5	51.00 ^B ± 0.66	49.14 ^B ± 0.57	58.14 ^B ± 0.87	45.82 ^B ± 0.61	58.14 ^B ± 0.81	75.14 ^B ± 0.76
0.25	40.28 ^C ± 0.76	34.33 ^C ± 0.83	40.28 ^C ± 0.76	32.03 ^C ± 0.76	43.85 ^C ± 0.75	64.45 ^C ± 0.51
0.125	29.57 ^D ± 0.57	19.51 ^D ± 0.91	26.00 ^D ± 0.63	28.58 ^D ± 0.51	40.28 ^D ± 0.73	46.21 ^D ± 0.48
0.625	15.28 ^E ± 0.61	15.81 ^E ± 0.64	15.28 ^E ± 0.55	18.24 ^E ± 0.45	26.00 ^E ± 0.67	36.38 ^E ± 0.41
Control	4.66 ^F ± 0.49	5.66 ^F ± 0.51	5.33 ^F ± 0.39	5.33 ^F ± 0.34	5.66 ^F ± 0.47	5.33 ^F ± 0.37

Different letters in column indicates statistical significance (at $P \leq 0.05$) among the treatments

In case of scatter®, the highest concentration (0.7%) cause reduction in population by 52.85 (males), 62.53 (females), 57.00 (nymphs), 58.14 (males), 54.84 (females) and 59.62% (nymphs) for both insects (*D. koenigii* and *O. hyalinipennis*) respectively. Results have also shown that the contact toxicity of

Scatter® (Indoxacarb) 15 % SC enhanced with increase in concentrations (Table 5). Statistical analysis showed that Orchard® was least toxic and caused minimum population reduction of under investigation insects among tested insecticides. Maximum concentration of Orchard® (0.8%) caused 45.44 (males), 43.85 (females), 43.85 (numphs), 41.74 (males),

40.28 (females) and 40.28% (nymphs) reduction in population of examined insects (*D. koenigii* and *O. hyalinipennis*) (Table 5). Mean data showed that average mortality of Orchard[®] was less than 50%. Moreover Steward[®] and Snap[®] caused moderate reduction in population of tested insects (Table 6-8).

Before extensive adaptation of genetically improved varieties of cotton, the application of synthetic and conventional pyrethroids were considered the efficient control against attack of bollworms on conventional varieties of cotton. At present

decrease use of such insecticides on transgenic cotton varieties increased the occurrence of damage by minor pest and ultimately those minor pests have gain the status of major pests of cotton crop. Earlier 5% damage was reported by dusky cotton bug on conventional varieties when mostly bolls of cotton got opened, consequently, the injury produced by bugs was insignificant. When peoples begun growing the Bt. Cotton initially; these bugs developed very thoughtful danger for cotton crop [21].

Table 5. Means mortality of *Dysdercus Koenigii* and *Oxycareus hyalinipennis*, Nymph, Male and Female exposed to various concentrations of Scatter[®]

Concentrations	Mortality of <i>D. Koenigii</i> (Mean ± SE)			Mortality of <i>O. Hyalinipennis</i> (Mean ± SE)		
	Male	Female	Nymph	Male	Female	Nymph
0.7	52.85 ^A ± 0.55	62.53 ^A ± 0.79	57.00 ^A ± 0.94	58.14 ^A ± 0.89	54.84 ^A ± 0.96	59.62 ^A ± 0.78
0.35	38.03 ^B ± 0.61	51.00 ^B ± 0.88	41.00 ^B ± 0.81	51.00 ^B ± 0.58	51.00 ^B ± 0.68	56.17 ^B ± 0.68
0.175	34.33 ^C ± 0.74	31.76 ^C ± 0.76	37.00 ^C ± 0.79	40.28 ^C ± 0.76	31.76 ^C ± 0.83	45.82 ^C ± 0.76
0.875	12.11 ^D ± 0.57	24.07 ^D ± 0.49	11.00 ^D ± 0.68	22.42 ^D ± 0.62	20.23 ^D ± 0.59	32.03 ^D ± 0.82
0.4375	5.00 ^E ± 0.49	8.69 ^E ± 0.53	5.66 ^E ± 0.51	8.14 ^E ± 0.56	8.69 ^E ± 0.41	14.79 ^E ± 0.65
Control	1.00 ^F ± 0.38	6.66 ^F ± 0.59	0.66 ^F ± 0.47	5.66 ^F ±0.41	5.76 ^F ± 0.37	5.33 ^F ± 0.51

Different letters in column indicates statistical significance (at P ≤ 0.05) among the treatments

Table 6. Means mortality of *Dysdercus koenigii* and *Oxycareus hyalinipennis*, Nymph, Male and Female exposed to various concentrations of Orchard[®]

Concentrations	Mortality of <i>D. Koenigii</i> (Mean ± SE)			Mortality of <i>O. hyalinipennis</i> (Mean ± SE)		
	Male	Female	Nymph	Male	Female	Nymph
0.8	45.44 ^A ± 0.83	43.85 ^A ± 0.87	43.85 ^A ± 0.87	41.74 ^A ± 0.96	40.28 ^A ± 0.99	40.28 ^A ± 0.99
0.4	34.33 ^B ± 0.92	36.71 ^B ± 0.94	36.71 ^B ± 0.94	34.33 ^B ± 0.89	36.71 ^B ± 0.89	36.71 ^B ± 0.89
0.2	23.22 ^C ± 0.86	26.00 ^C ± 0.81	26.00 ^C ± 0.81	23.22 ^C ± 0.83	29.57 ^C ± 0.83	29.57 ^C ± 0.83
0.1	12.11 ^D ± 0.83	18.85 ^D ± 0.76	18.85 ^D ± 0.76	19.51 ^D ± 0.72	18.85 ^D ± 0.79	18.85 ^D ± 0.79
0.05	5.33 ^E ± 0.78	6.00 ^E ± 0.68	6.00 ^E ± 0.68	12.11 ^E ± 0.79	15.28 ^E ± 0.69	15.28 ^E ± 0.69
Control	4.70 ^F ± 0.71	4.57 ^F ± 0.56	4.57 ^F ± 0.56	6.33 ^F ± 0.68	5.33 ^F ± 0.52	5.33 ^F ± 0.52

Different letters in column indicates statistical significance (at P ≤ 0.05) among the treatments

Table 7. Means mortality of *Dysdercus Koenigii* and *Oxycareus hyalinipennis*, Nymph, Male and Female exposed to various concentrations of Steward®

Concentrations	Mortality of <i>D. Koenigii</i> (Mean ± SE)			Mortality of <i>O. hyalinipennis</i> (Mean ± SE)		
	Male	Female	Nymph	Male	Female	Nymph
0.7	49.14 ^A ± 0.78	52.85 ^A ± 0.77	72.51 ^A ± 0.87	42.37 ^A ± 0.94	33.00 ^A ± 0.94	47.15 ^A ± 0.98
0.35	34.33 ^B ± 0.66	41.74 ^B ± 0.60	30.63 ^B ± 0.77	32.03 ^B ± 0.87	17.00 ^B ± 0.87	27.92 ^B ± 0.85
0.175	23.22 ^C ± 0.78	26.92 ^C ± 0.76	23.22 ^C ± 0.90	28.58 ^C ± 0.81	9.00 ^C ± 0.81	24.07 ^C ± 0.90
0.875	8.40 ^D ± 0.59	19.51 ^D ± 0.66	19.51 ^D ± 0.94	18.24 ^D ± 0.77	5.00 ^D ± 0.77	16.38 ^D ± 0.86
0.4375	6.66 ^E ± 0.55	5.33 ^E ± 0.78	8.40 ^E ± 0.75	7.89 ^E ± 0.68	4.66 ^E ± 0.68	5.66 ^E ± 0.79
Control	1.00 ^F ± 0.48	4.70 ^F ± 0.57	6.33 ^F ± 0.67	5.00 ^F ± 0.57	1.00 ^F ± 0.57	1.00 ^F ± 0.63

Different letters in column indicates statistical significance (at $P \leq 0.05$) among the treatments

Table 8. Means mortality of *Dysdercus Koenigii* and *Oxycareus hyalinipennis*, Nymph, Male and Female exposed to various concentrations of Snap®

Concentrations	Mortality of <i>D. Koenigii</i> (Mean ± SE)			Mortality of <i>O. hyalinipennis</i> (Mean ± SE)		
	Male	Female	Nymph	Male	Female	Nymph
0.4	33.14 ^A ± 0.80	51.00 ^A ± 0.83	52.72 ^A ± 0.74	43.85 ^A ± 0.543	43.85 ^A ± 0.83	47.15 ^A ± 0.96
0.2	26.00 ^B ± 0.63	47.22 ^B ± 0.71	38.93 ^B ± 0.85	33.14 ^B ± 0.47	36.71 ^B ± 0.76	31.76 ^B ± 0.89
0.1	15.28 ^C ± 0.76	33.14 ^C ± 0.76	32.03 ^C ± 0.79	18.85 ^C ± 0.39	26.00 ^C ± 0.70	24.07 ^C ± 0.76
0.05	11.71 ^D ± 0.68	22.42 ^D ± 0.69	28.58 ^D ± 0.62	11.71 ^D ± 0.76	22.42 ^D ± 0.76	12.53 ^D ± 0.67
0.025	4.57 ^E ± 0.57	15.28 ^E ± 0.51	18.24 ^E ± 0.57	4.57 ^E ± 0.42	11.74 ^E ± 0.68	7.84 ^E ± 0.61
Control	3.66 ^F ± 0.49	3.66 ^F ± 0.43	5.00 ^F ± 0.46	3.33 ^F ± 0.31	4.66 ^F ± 0.55	4.33 ^F ± 0.53

Different letters in column indicates statistical significance (at $P \leq 0.05$) among the treatments

The results of mean mortality indicates that Match® exhibit maximum population reduction of nymph, female and males of *O. hyalinipennis* and *D. koengii* followed by the Coral® and Snap®. However, Orchard® showed minimum reduction in population of tested insect pests that indicate that Orchard® was least toxic against various stages of life of *O. hyalinipennis* and *D. koengii* followed by Steward®. [22, 23] have been reported that Igr's (Polyoxin D or diflubenzuron) showed maximum results and are more efficacious against *D. koengii* and also noted that the Igrs cause negative

impact on various life stages of the *D. koengii* and caused mortalities upto 96%. Against adults, Igr's can result in malformation and produced deformed adults and nymphs as well as inhibit the wing growth. [24-26] founded that Lufenuron showed good chitin synthesis inhibitor effects against *D. koengii*. Direct application of Diflubenzuron inhibited endocuticular deposition when applied on *Mandusa* epidermal. Three sites have been proposed for describing the mode of action of diflubenzuron and other chitin synthesis inhibitors namely: inhibition of chitin

synthetase (or its biosynthesis), inhibition of proteases (or its biosynthesis) and inhibition of UDP-N-acetylglucosamine transport through the membrane and these findings contradict with our results as they have used other products.

Conclusion

The overall findings of present study explained that selective insecticides caused normal to severe population reduction for non-target sucking pests, which could lead to broad spectrum resistance against different groups of pesticides. Pesticides which cause death of non-selected pests can cause major change to pest status as from non-pest to minorpest and from minor to major pests.

Authors' contributions

Conceived and designed the experiments: W Hassan, T Nazir, MD Gogi & MJ Arif, Performed the experiments: W Hassan, T Nazir & B Abid, Analyzed the data: W Hassan, T Nazir & NH Bashir, Contributed materials/ analysis/ tools: W Hassan, T Nazir, T Anwar & S Zaman, Wrote the paper: W Hassan, T Nazir & S Zaman.

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