Plant Protection, 08 (01) 2024. 121-133 DOI: 10.33804/pp.008.01.5051

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

TOXICITY ASSESSMENT OF VARIOUS INSECTICIDES ON *OXYCARENUS HYALINIPENNIS* COSTA (LYGAEIDAE: HEMIPTERA) IN LABORATORY SETTINGS ACROSS VARIOUS DISTRICTS OF PUNJAB, PAKISTAN

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ARTICLE INFO

Article history

Received: 31st January, 2024

Revised: 13th March, 2024

Accepted: 16th March, 2024

Keywords

Toxicity

Dusky cotton bug

Feeding bioassay

Location

ABSTRACT

Cotton contributes 0.6% to the value added in GDP and 3.1% to the value added in the agriculture sector of Pakistan. The dusky cotton bug (DCB), *Oxycarenus hyalinipennis* (Hemiptera: Lygaeidae), is among the insect pest species of highest economic significance in cotton production in the country. Adults and nymphs feed on seeds and cotton bolls, affecting seed germination. The dusky cotton bug is a polyphagous insect that feeds on a variety of host plants. Its adults and nymphs typically feed on squares of the cotton plant, resulting in a pale yellow color of squares and subsequent shedding of infested squares. Insecticide resistance in cotton insect pests is an emerging threat to sustainable cotton production in Pakistan. A thorough understanding of resistance might be a valuable tool for implementing pest control approaches. In the present study, Clothianidin, Fipronil, Bifenthrin, Triazhophos, Deltamethrin, and Clothianidin + Imidacloprid insecticides were used in different cotton-growing districts of Punjab. Among all the insecticides used for controlling the DCB population, Clothianidin + Imidacloprid was found to be the most effective in all districts, while Deltamethrin was the least effective in District Khanewal, Bifenthrin in Dera Ghazi Khan, Rahim Yar Khan, and Vehari, and Clothainidin in Multan. Therefore, we recommend the use of a pesticide mixture (Clothianidin + Imidacloprid) as a beneficial strategy against the dusky cotton bug in various cotton-growing districts of Punjab.

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INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important cash crop and a substantial source of foreign exchange in Pakistan. Cotton contributes 0.6% of Pakistan's GDP and 3.1% of its agricultural value (Anonymous, 2021). The value-added by cotton amounts to 37.1% of total agricultural revenues (Farooq, 2011, 2012). India is set to become the world's largest cotton grower in 2021-22, having produced 28 million bales, which is 0.4 million bales more than in 2020-21. Worldwide cotton output in 2021-22 is expected to reach an all-time high of 811 kg per hectare, with global cotton production anticipated at 121.6 million bales (Meyer, 2021).

Pakistan is the world's fourth-largest producer of cotton, the third-largest consumer of cotton, and the seventh-largest maker of garments (Shuli et al., 2018). Cotton

accounts for 18.9% of agricultural GDP and 10% of the national GDP (Azumah et al., 2019). In Pakistan, cotton produces an average of 730 kg/ha, with 10,671 million bales produced, which is 1.5-2% lower than the global average (Nadeem et al., 2014; Shabbir and Yaqoob, 2019). The value of Pakistan's and the world's cotton exports increased from 2008 to 2013 but then began to fall until 2018. According to the Government of Pakistan, the overall value of cotton exports from Pakistan in 2018 was US$ 3498.997 million, with China, the United Kingdom, the United States, Germany, Italy, Spain, Australia, and several other countries being the key overseas markets. Cotton and cotton-based products exports account for 10% of Pakistan's GDP and 55% of total export revenues (Maqbool, 2019).

Oxycarenus hyalinipennis is now considered a major pest of cotton crops in Pakistan (Hafeez et al., 2021), as well as in other countries such as China, the Turks and Caicos Islands, the Bahamas, Caicos, and the Cayman Islands (Stocks and Pea, 2013; Colmenárez et al., 2020). Although it is a well-known pest of Malvaceae crops, it has also been observed infesting other crops such as guava, moringa, mango, okra, chilies, and lemon (Naeem-Ullah et al., 2020). When mature cotton seeds become accessible, *O. hyalinipennis* begins feeding, mating, and depositing eggs. Both adults and nymphs consume seed oil. Females deposit eggs on cotton lint and consume seeds (Sweet, 2000). Its adults and nymphs feed socially on cotton plant squares, causing them to turn pale yellow and subsequently shed (Rajendran et al., 2018). A variety of factors may influence the population parameters of the dusky cotton bug, including the adaptation of novel cotton crop techniques such as early sowing of Bt-cotton and the excessive use of insecticides (Rocha-Munive et al., 2018). Such circumstances are establishing a niche for the survival of the dusky cotton bug, and this insect pest is quickly becoming one of Pakistan's most economically important pests (Naeem-Ullah et al., 2020).

In Pakistan and other developing nations, the use of pesticides is regarded as a reliable means of controlling this insect problem (Hashmi and Khan, 2011). The dusky cotton bug has also developed resistance against many insecticides, including bifenthrin, chlorpyrifos, cypermethrin, deltamethrin, lambda-cyhalothrin, methomyl, profenofos, triazophos, acetamiprid, emamectin benzoate, fipronil, spinosad, spirotetramat, imidacloprid, and nitenpyram (Banazeer et al., 2021). However, information on the most recent efficacy of clothianidin, fipronil, bifenthrin, triazhophos, deltamethrin, and clothianidin + imidacloprid against the dusky cotton insect is limited. Therefore, the present study was conducted to molecularly identify the species of the dusky cotton bug, monitor insecticide resistance in different field populations of the dusky cotton bug, and identify the resistance detoxification enzymes involved in conferring resistance in strains of the dusky cotton bug across five different districts of Pakistan, namely Rahim Yar Khan, Vehari, Multan, Dera Ghazi Khan, and Khanewal.

MATERIALS AND METHODS

Collection of dusky cotton bug (DCB)

The population of DCB was collected from different cotton-growing areas of Multan, Rahim Yar Khan, Dera Ghazi Khan (D.G Khan), Vehari, and Khanewal. The nymphs were then transferred to a sterile Petri dish (1.20 cm × 1.50 cm × 6.00 cm, Diameter: Length: Width) with the temperature set to 27°C and relative humidity at 55% before commencing the experiment.

Insecticides

Clothianidin, fipronil, bifenthrin, triazophos, deltamethrin, and clothianidin + imidacloprid insecticides were purchased from the market under multinational brands such as Bayer and Syngenta. The concentration of each insecticide was prepared based on the toxicity levels mentioned in previous literature. In the present study, five concentrations were prepared in µg/ml for each insecticide, with one control designated as 0.00. The concentrations for clothianidin were 2441.40, 976.56, 390.63, 156.25, and 62.50; for fipronil, they were 10.00, 4.00, 1.60, 0.64, and 0.26; for bifenthrin, they were 2441.40, 976.56, 390.63, 156.25, and 62.50; for triazophos, they were 62.50, 25.00, 10.00, 4.00, and 1.60; for deltamethrin, they were 976.56, 390.63, 156.25, 62.50, and 25.00; and for clothianidin + imidacloprid, they were 10.00, 4.00, 1.60, 0.64, and 0.26.

Bioassay

The adults of DCB were used for toxicological studies. The insecticidal bioassay was conducted using the leaf dip method. The experiment involved 7 treatments, including a control treatment, and each treatment had a further 15 replications. The data were recorded at 24 h, 48 h, 72 h, and 96 h. The adults of DCB were collected from the cotton fields of the Central Cotton Research Institute in Multan. They were then shifted to Petri dishes containing treated leaves, with each dish containing five adults of DCB. After applying all treatments, the treated Petri dishes were placed at a temperature of 27-28ºC and a relative humidity

of 60-70%. Data were recorded after 24 h for the 1st day, after 48 h for the 2nd day, after 72 h for the 3rd day, and after 96 h for the 4th day.

Statistical Analysis

The percentage (%) mortality of lethal doses of the insecticides was calculated using MS Excel 2019. The toxicity results analysis of the different insecticides was calculated using Probit software in SPSS. The average mortality in each experimental unit was determined using the Abbott's Formula.

RESULTS

LC50 of insecticides on adults of DCB in Rahim Yar Khan

In district Rahim Yar Khan, the LC50 of clothianidin + imidacloprid at 72 h showed the highest mortality in adult dusky cotton bugs at the highest concentration, which was maximum compared to other chemicals because the concentrations of clothianidin + imidacloprid were minimum compared to other insecticides. The LC50 of fipronil at 96 h showed maximum mortality of third instar dusky cotton bugs at 0.355 ug/ml. The LC50 of bifenthrin at 96 h showed maximum mortality at 360.358 ug/ml. The LC50 of deltamethrin at 96 h showed maximum mortality at 5.795 ug/ml. The LC50 of triazhophas at 96 h showed maximum mortality at 4.862 ug/ml. The LC50 of clothianidin at 96 h showed maximum mortality at 224.887 ug/ml (Table 1).

Table 1. LC50 of different insecticides against dusky cotton bug (adults) in Rahim Yar Khan.

Insecticides	Time interval (h)	Total Population	LC_{50} and 95% confidence limit (µg/ml)	Slope ± SE	Chi Square
Clothianidin	24	240	1317.662 (834.620–2701.109)	1.043 ± 0.187	0.888
	48	240	1055.717 (669.181–2093.465)	0.989 ± 0 .178	2.099
	72	240	715.603 (450.624–1334.005)	0.916 ± 0.172	1.657
	96	240	224.887 (129.710–346.531)	0.985 ± 0.176	0.827
Fipronil	24	240	26.394 (7.047–25073.644)	0.471 ± 0.172	0.180
	48	240	1.133 (0.329–2.635)	0.520 ± 0.162	0.060
	72	240	0.527 (0.063–1.178)	0.519 ± 0.165	0.043
	96	240	0.355 (0.041–0.790)	0.577 ± 0.167	0.467
Bifenthrin	24	240	2183.180 (660.828–2.573E8)	0.364 ± 0.162	0.70
	48	240	1224.634 (432.15–223022.910)	0.390 ± 0.161	0.049
	72	240	1709.335 (503.795–1.352E12)	0.336 ± 0.161	0.120
	96	240	360.358 (35.080–2443.155)	0.367 ± 0.160	0.191
Triazhophos	24	240	22.773 (11.453–83.693)	0.599 ± 0.165	0.458
	48	240	12.672 (5.219–39.835)	0.517 ± 0.162	1.166
	72	240	6.992 (1.832–16.876)	0.500 ± 0.161	0.656
	96	240	4.862 (1.399–94.450)	0.593 ± 0.164	0.248
Deltamethrin	24	240	0.114.611 (28.895–293.302)	0.483 ± 0.161	0.001
	48	240	67.780 (23.080–125.836)	0.673 ±0.166	0.326
	72	240	22.210 (1.545–54.419)	0.582 ± 0 .170	0.210
	96	240	5.795 (0.000–27.028)	0.453 ± 0.174	0.102
Clothianidin + Imidacloprid	24	240	0.246 (0.000–0.877)	0.349 ± 0.162	0.137
	48	240	0.094 (0.000-0.678)	0.306 ± 0.164	0.431
	72	240	0.076 (0 .000–0.357)	0.407 ± 0.170	0.499
	96	240	0.106 (0.004–0.308)	0.638 ± 0.183	0.409

LC50 of insecticides on adults of DCB in Multan

As regards district Multan, the LC50 of clothianidin + imidacloprid at 72 h (Table 2) showed the highest mortality in adult dusky cotton bugs at the highest concentration, which was maximum compared to other chemicals because the concentrations of clothianidin + imidacloprid were minimum compared to other insecticides. The LC50 of fipronil at 96 h showed

maximum mortality of adult dusky cotton bugs at 1.441 ug/ml. The LC50 of bifenthrin at 96 h revealed maximum mortality at 289.111 ug/ml. The LC50 of deltamethrin at 96 h displayed maximum mortality at 45.520 ug/ml. The LC50 of triazhophas at 96 h showed maximum mortality at 6.365 ug/ml. The LC50 of Clothianidin at 96 h exhibited maximum mortality at 294.726 ug/ml (Table 2).

Table 2. LC_{50} of different insecticides against dusky cotton bug (adults) in Multan.

Insecticides	Time interval (h)	Total Population	LC_{50} and 95% confidence limit (µg/ml)	Slope ± SE	Chi Square
Clothianidin	24	240	1782..019 (795.984 – 15666.149)	0.572 ± 0 .167	0.025
	48	240	1083.143 (540.193 – 4595.135)	0.606 ± 0.166	0.046
	72	240	608.396 (340.698 – 1598.122)	0.623 ± 0.164	0.018
	96	240	294.726 (130.046 – 567.622)	0.656 ± 0.164	0.070
Fipronil	24	240	19.329 (5.069 – 519540.581)	0.404 ± 0.166	0.022
	48	240	9.606 (3.120 – 10630.925)	0.403 ± 0.163	0.105
	72	240	3.599 (1.546 – 24.730)	0.490 ± 0.162	0.675
	96	240	1.441 (0.578 – 3.283)	0.596 ± 0.163	0.287
Bifenthrin	24	240	1513.175 (719.278 – 9028.019)	0.601 ± 0.165	0.254
	48	240	684.225 (366.381 – 175.768)	0.662 ± 0.165	0.275
	72	240	390.509 (119.831 – 1273.267)	0.466 ± 0.166	0.201
	96	240	289.111 (80.630 – 711.487)	0.501 ± 0.162	0.235
Triazhophos	24	240	25.273 (15.571 – 53.029)	0.902 ± 0.176	1.611
	48	240	19.751 (12.142 – 39.217)	0.868 ± 0.172	0.647
	72	240	11.639 (5.486 – 27.570)	0.602 ± 0.163	0.039
	96	240	6.365 (1.829 – 14.025)	0.537 ± 0.163	0.031
Deltamethrin	24	240	272.623 (138.108 – 795.691	0.608 ± 0.164	0.450
	48	240	244.082 (96.130 – 1269.791)	0.468 ± 0.161	0.933
	72	240	167.036 (46.031 – 705.618)	0.433 ± 0.161	1.042
	96	240	45.520 (4.722 – 103.439)	0.519 ± 0.164	0.062
Clothianidin + Imidacloprid	24	240	0.411 (0.000 – 1.433)	0.325 ± 0+.161	0.001
	48	240	0.179 (0.000 – 0 .605)	0.416 ± 0.166	0.065
	72	240	0.106 (0.000 – 0 .477)	0.369 ± 0.166	0.131
	96	240	0.129 (0.000 –0 .429)	0.483 ± 0.171	0.652

LC50 of insecticides on adults of DCB in Vehari

In district Verhari, the LC50 of clothianidin + imidacloprid at 96 h exhibited the highest mortality among adult dusky cotton bugs at the highest concentration, which was maximum compared to other chemicals because the concentrations of clothianidin + imidacloprid were minimum compared to other insecticides. The LC50 of fipronil at 96 h showed maximum mortality of adult dusky cotton bugs at 5.100 ug/ml. The LC50 of bifenthrin at 96 h revealed maximum mortality at 435.450 ug/ml. The LC50 of deltamethrin at 96 h displayed maximum mortality at 30.057 ug/ml. The LC50 of triazhophas at 96 h showed maximum mortality at 3.826 ug/ml. The LC50 of clothianidin at 96 h exhibited maximum mortality at 81.487 ug/ml (Table 3).

LC50 of insecticides on adults of DCB in D.G. Khan

As far as district D.G. Khan is concerned, the LC50 of clothianidin + imidacloprid at 72 and 96 h resulted in the highest mortality among adult dusky cotton bugs at the highest concentration, which was greater than that of other chemicals. This difference can be attributed to the lower concentrations of clothianidin + imidacloprid compared to other insecticides. Additionally, the LC50 of fipronil at 72 h showed a maximum mortality rate of 0.227 ug/ml for adult dusky cotton bugs. Similarly, the LC50 of bifenthrin at 96 h resulted in a maximum mortality rate of 121.984 ug/ml. Deltamethrin exhibited

a maximum mortality rate of 13.720 ug/ml at 72 h. Triazhophas, at 96 h showed a maximum mortality rate of 2.102 ug/ml. Lastly, clothianidin at 72 h had a maximum mortality rate of 32.281 ug/ml (Table 4).

Table 3. LC_{50} of different insecticides against dusky cotton bug (adults) in Vehari.

Insecticides	Time interval (h)	Total Populations	LC_{50} and 95% confidence limit (µg/ml)	Slope ± SE	Chi Square
Clothianidin	24	240	456.173 (95.333 – 3962.063)	0.383 ± 0.160	0.175
	48	240	261.574 (8.214 – 1051.154)	0.367 ± 0.160	0.106
	72	240	133.551 (10.924 – 316.591)	0.477 ± 0.163	0.090
	96	240	81.487 (10.502 – 179.169)	0.599 ± 0.169	0.121
Fipronil	24	240	44.190 (12.530 – 4079.237)	0.651 ± 0.197	0.938
	48	240	62.507 (9.796 – 6.268E12)	0.385 ± 0.172	0.018
	72	240	11.295 (3.969 – 933.943)	0.471 ± 0.167	0.242
	96	240	5.100 (2.377 – 33.060)	0.556 ± 0.165	0.230
Bifenthrin	24	240	1578.858 (871.327 – 4925.742)	0.803 ± 0.177	0.875
	48	240	1307.940 (681.423 – 4935.403)	0.679 ± 0.169	0.120
	72	240	789.687 (404.123 – 2517.149)	0.614 ± 0.165	0.335
	96	240	435.450 (190.526 – 1088.623)	0.567 ± 0.163	0.293
Triazhophos	24	240	31.940 (15.864 – 147.436)	0.616 ± 0.167	0.727
	48	240	25.735(13.169 – 94.450)	0.624 ± 0.166	0.395
	72	240	8.241 (5.654–23.760)	0.302 ± 0.159	0.112
	96	240	3.826 (0.000 – 13.071)	0.377 ± 0.160	0.259
Deltamethrin	24	240	114.835 (8.800 – 451.470)	0.383 ± 0.160	0.114
	48	240	84.579 (0.007 – 353.929)	0.335 ± 0.159	0.104
	72	240	52.921 (0.009 – 165.918)	0.355 ± 0.160	0.285
	96	240	30.057 (0.007 – 93.834)	0.381 ± 0.162	0.210
Clothianidin	24	240	1.233 (.262 – 3.565)	0.451 ± 0.161	0.036
+	48	240	0.808 (0.223 – 1.646)	0.577 ± 0.164	0.227
Imidacloprid	72	240	0.487 (0.062 – 1.075)	0.537 ± 0.165	0.378
	96	240	0.321 (0.028 – 0.745)	0.559 ± 0.168	0.021

LC50 of insecticides on adults of DCB in Khanewal

The LC50 values in district Khanewal for clothianidin + imidacloprid at 96 h exhibited the highest mortality among adult dusky cotton bugs at the highest concentration. This was attributed to the fact that the concentrations of clothianidin + imidacloprid were comparatively lower than those of other insecticides. Specifically, the LC50 for fipronil at 96 h resulted in a maximum mortality of adult dusky cotton bugs at 2.026 ug/ml. The LC50 of bifenthrin at 96 h showed a maximum mortality of 222.539 ug/ml, while that of Deltamethrin at 96 h led to a maximum mortality of 72.465 ug/ml. Triazhophas demonstrated an LC50 at 96 h with a maximum mortality of 9.459 ug/ml. Additionally, Clothianidin's LC50 at 72 h exhibited a maximum mortality of 32.281 ug/ml (Table 5).

Mortality of *O. hyalinipennis* adults exposed to various pesticides in Khanewal

In district Khanewal, the highest percentage of mortality among all (87.5%) was observed in clothianidin at a concentration of 2441.4 (ug/ml) after 96 h. In the case of fipronil, 80% mortality was observed with a concentration of 10 (ug/ml). Bifenthrin at 96 h, at a concentration of 2441 (ug/ml), was observed with maximum mortality of 70%. Triazophos had the highest percentage mortality at a concentration of 62.5 (ug/ml) after 96 h, which was 65%. In the case of deltamethrin, the highest percentage of mortality was 77.5% after 96 h at a concentration of 976.5625 (ug/ml). The mixture of clothiandin and imadacloprid recorded maximum mortality (67.5%) at 96 h at a concentration of 10 (ug/ml) as shown in Figure 1.

Table 4. LC_{50} of different insecticides against dusky cotton bug (adults) in D.G. Khan.

Insecticides	Time interval (h)	Total Population	LC_{50} and 95% confidence limit (µg/ml)	Slope ± SE	Chi Square
Clothianidin	24	240	155.991 (.071 – 501.658)	0.354 ± 0.160	0.055
	48	240	74.731 (.307 – 215.601)	0.419 ± 0.163	0.124
	72	240	32.281 (.240 – 102.855)	0.504 ± 0.171	0.325
	96	240	35.686 (4.818 – 80.968)	0.802 ± 0.194	0.667
Fipronil	24	240	5.599 (2.346 – 84.997)	0.485 ± 0.164	0.598
	48	240	1.213 (.537 – 2.330)	0.659 ± 0.165	0.550
	72	240	0.227 (.024 - 0.525)	0.639 ± 0.174	0.096
	96	240	0.229 (.035 - 0.497)	0.719 ± 0.179	1.566
Bifenthrin	24	240	631.780 (169.903 – 27994.388)	0.387 ± 0.160	0.183
	48	240	361.116 (49.627 – 1957.405)	0.383 ± 0.160	0.337
	72	240	230.657 (.055 – 1069.069)	0.335 ± 0.150	0.285
	96	240	121.984 (.013 – 380.927)	0.356 ± 0.160	0.032
Triazhophos	24	240	7.335 (1.849 – 18.771)	0.483 ± 0.161	0.001
	48	240	5.183 (1.012 – 11.739)	0.504 ± 0.162	0.060
	72	240	3.214 (.242 – 7.646)	0.478 ± 0.163	0.095
	96	240	2.102 (.120 – 5.129)	0.510 ± 0.165	0.620
Deltamethrin	24	240	32.643 (.000 – 1.05.155)	0.361 ± 0.161	0.029
	48	240	18.316 (.000 – 73.755)	0.334 ± 0.162	0.106
	72	240	13.720 (.000 – 56.935)	0.359 ± 00164	0.085
	96	240	13.976 (0.018 – 47.714)	0.447 ± 0.168	0.435
Clothianidin + Imidacloprid	24	240	0.160 (0.000 – 674)	0.337± 0 163	0.071
	48	240	0.098 (0.000 – 0.366)	0.471 ± 0.172	0.021
	72	240	0.065 (0.002 – 0.198)	0.762 ± 0.212	0.952
	96	240	0.065 (0.002 – 0.198)	0.762 ± 0.212	0.952

Table 5. LC_{50} of different insecticides against dusky cotton bug (adults) in Khanewal.

Insecticides	Time interval (h)	Total Population	LC_{50} and 95% confidence limit (µg/ml)	Slope ± SE	Chi Square
Clothianidin	24	240	155.991(.071 – 501.658)	0.354 ± 0.160	0.055
	48	240	74.731 (.307 – 215.601)	0.419 ± 0.163	0.124
	72	240	32.281 (.240 – 102.855)	0.504 ± 0.171	0.325
	96	240	35.686 (4.818 – 80.968)	0.802 ± 0.194	0.667
Fipronil	24	240	32.554 (11.316 – 745.753)	0.746 ± 0.202	0.359
	48	240	212.441 (6.832 – 3223.816)	0.517 ± .173	0.062
	72	240	2.509 (1.607 – 4.372)	0.950 ± .173	1.396
	96	240	2.026 (1.296 – 3.356)	0.961 ± 0.173	1.643
Bifenthrin	24	240	1762.474 (750.331 – 22487.670)	0.530 ± 0.166	0.65
	48	240	9270.681 (426.018 – 5201.845)	0.526 ± 0.163	0.153
	72	240	368.723 (131.240 – 968.652	0.516 ± 0.161	0.225
	96	240	222.539 (58.008 – 482.122	0.537 ± 0.162	0.089
Triazhophos	24	240	41.681 (19.808 – 253.584)	0.615 ± 0.169	1.230
	48	240	25.119 (13.119 – 85.465)	0.644 ± 0.167	1.118
	72	240	15.615 (7.990 – 40.739)	0.625 ± 0.164	0.890
	96	240	9.459 (3.369 – 24.774)	0.517 ± 0.162	0.916
Deltamethrin	24	240	329.336 (198.189 – 696.465)	0.832 ± 0.171	1.245
	48	240	236.705 (118.159 – 496.122)	0.868 ± 0.171	0.475
	72	240	125.633 (98.121 – 234.165)	0.856 ± 0.169	1.771
	96	240	72.465 (38.189 – 196.465)	0.784 ± 0.169	1.662
Clothianidin + Imidacloprid	24	240	3.040 (0.917 – 256.496)	0.370 ± 0.160	0.024
	48	240	2.438 (0.540 – 247.404)	0.352 ± 0.160	0.154
	72	240	1.068 (0.407 – 123.123)	0.287 ± 0.159	0.039
	96	240	0.499 (0.000 – 1.564)	0.324 ± 0.161	0.091

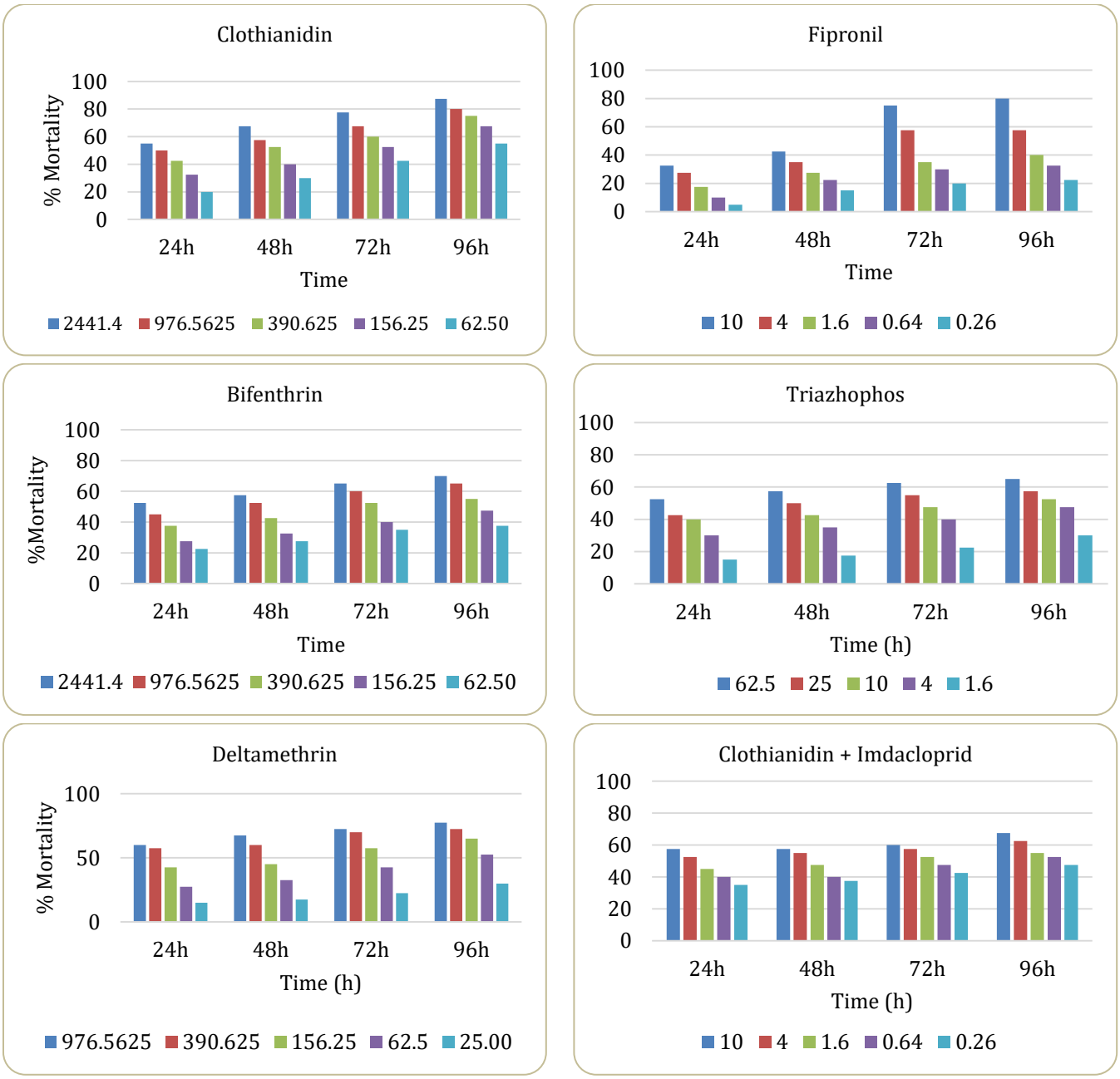

Figure 1. Percent mortality of *O. hyalinipennis* adults collected from Khanewal, Punjab, Pakistan against five different concentrations of five different pesticides.

Mortality of *O. hyalinipennis* adults exposed to various pesticides in D.G. Khan

In the case of district D.G. Khan, the highest percentage of mortality among all (95%) was observed in clothianidin at a concentration of 2441.4 (ug/ml) after 96 h. For fipronil, a 90% mortality rate was observed at a concentration of 10 (ug/ml). Bifenthrin at 96 h, with a concentration of 2441 (ug/ml), showed maximum mortality of 67.5%. Triazophos had the highest percentage of mortality at a concentration of 62.5 (ug/ml) after 96 h, reaching 75%. Deltamethrin had the highest percentage of mortality, which was 80% after 96 h at a concentration of 976.5625 (ug/ml). The mixture of clothianidin and imidacloprid recorded maximum mortality of 97.5% after 96 h at a concentration of 10 ug/ml (Figure 2).

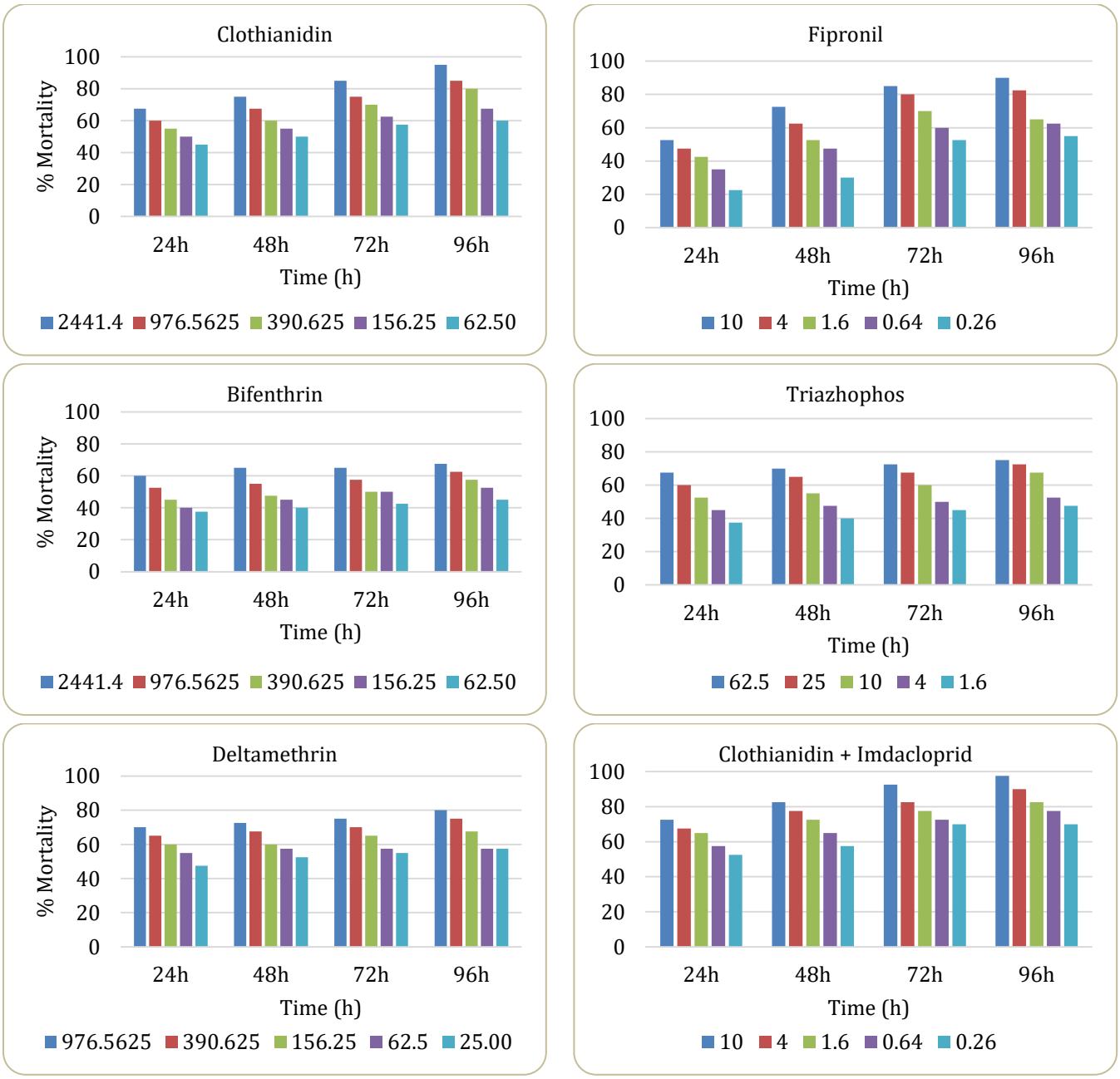

Figure 2. Percent mortality of *O. hyalinipennis* adults collected from DG-Khan, Punjab, Pakistan against five different concentrations of five different pesticides.

Mortality of *O. hyalinipennis* adults exposed to various pesticides in Vehari

Among all tested pesticides, clothianidin exhibited the highest mortality rate (82.5%) at a concentration of 2441.4 µg/ml after 96 h in district Vehari. For fipronil, a 57.5% mortality rate was observed at a concentration of 10 µg/ml. Bifenthrin, at a concentration of 2441 µg/ml after 96 h, showed a maximum mortality rate of 65%. Triazophos had the highest mortality rate (65%) at a concentration of 62.5 µg/ml after 96 h. In the case of deltamethrin, the highest percentage mortality was 72.5% after 96 h at the concentration of 976.5625 µg/ml. The combination of clothianidin and imidacloprid recorded the highest mortality (80%) at 96 h and a concentration of 10 µg/ml (Figure 3).

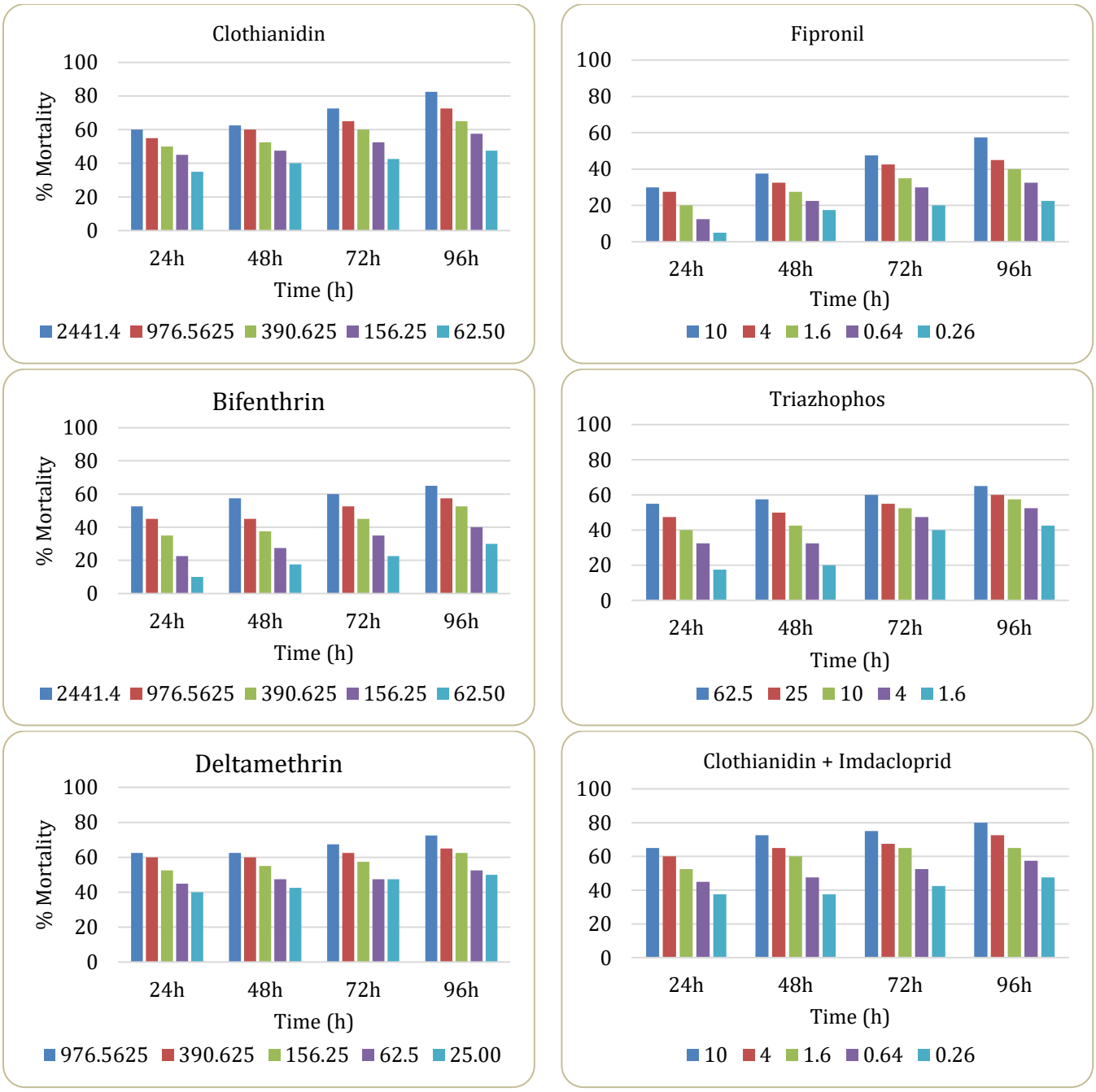

Figure 3. Percent mortality of *O. hyalinipennis* adults collected from Vehari, Punjab, Pakistan against five different concentrations of five different pesticides.

Mortality of *O. hyalinipennis* adults exposed to various pesticides in Multan

In terms of the Multan district, the highest mortality percentage (72.5%) was noted in clothianidin at a concentration of 2441.4 (ug/ml) after 96 h. Fipronil showed a 70% mortality rate at a concentration of 10 ug/ml. Bienthrin exhibited a maximum mortality rate of 70% after 96 h at a concentration of 2441 ug/ml. Triazophos demonstrated the highest mortality percentage of 72.5% at a concentration of 62.5 ug/ml after 96 h. Deltamethrin recorded a mortality rate of 75% after 96 h at a concentration of 976.5625 ug/ml. The combination of clothiandin and imadacloprid achieved the highest mortality rate of 85% after 96 h at a concentration of 10 ug/ml as depicted in Figure 4.

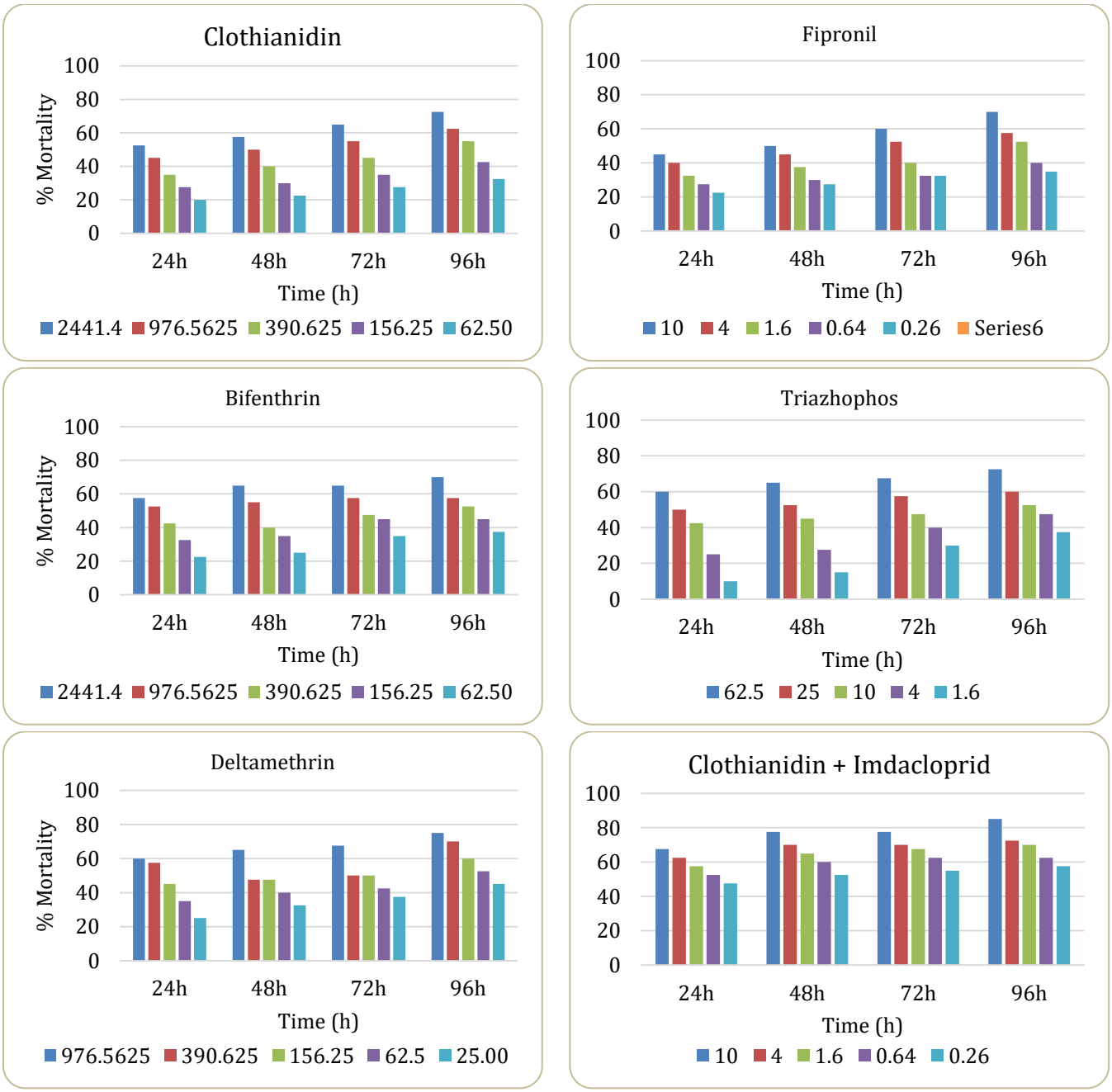

Figure 4. Percent mortality of *O. hyalinipennis* adults collected from Multan, Punjab, Pakistan against five different concentrations of five different pesticides.

Mortality of *O. hyalinipennis* adults exposed to various pesticides in Rahim Yar Khan

In the current study on insecticide effectiveness, the highest mortality rate (87.5%) was observed in Rahim Yar Khan for clothianidin at a concentration of 2441.4 ug/ml after 96 h. For fipronil, the maximum mortality was 82.5% at a concentration of 10 ug/ml. Bienthrin showed a maximum mortality of 62.5% at 96 h and a concentration of 2441 ug/ml. Triazophos achieved its peak mortality of 72.5% at 96 h and a concentration of 62.5 ug/ml. Deltamethrin exhibited the highest mortality rate of 85% after 96 h at a concentration of 976.5625 ug/ml. Finally, the mixture of clothiandin and imadacloprid recorded the maximum mortality of 90% at 96 h and a concentration of 10 ug/ml as shown in Figure 5.

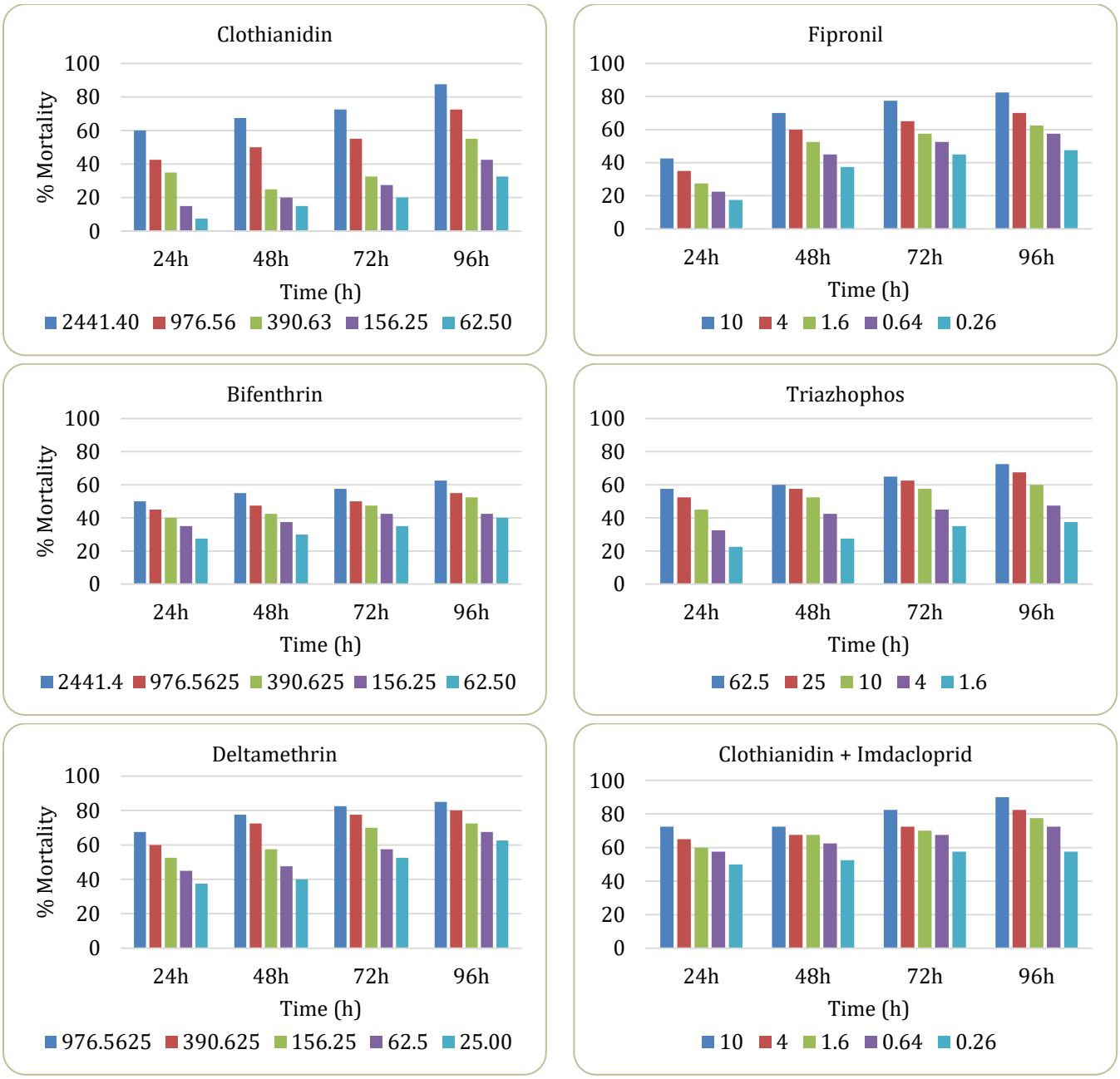

Figure 5. Percent mortality of *O. hyalinipennis* adults collected from Rahim Yar Khan, Punjab, Pakistan against five different concentrations of five different pesticides.

DISCUSSION

Among all insecticides used to control DCB populations, clothianidin + imidacloprid was found to be the most effective in all districts. Delmethrin was found to be the least effective in district Khanewal, bifenthrin in D.G. Khan, Rahim Yar Khan, and Vehari, and clothainidin in Multan after 96 h. These findings differ from those reported by Irshad et al. (2019). The descending order of percentage population reduction after 7 days of spraying was chlorpyriphos > triazophos > lambda cyhalothrin > clothianidin > deltamethrin+ triazophos > bifenthrin > fipronil (Irshad et al., 2019). However, in the current study, the trend varied in different districts, which could be related to differences in growth conditions in each district and the time period of population reduction, as the greatest time period in the current study was 96 h (4 days), whereas they were 7 days. Akram et al. (2013) and Irshad et al. (2019) indicated that the

organophosphate group is better at reducing DCB than other pesticides due to their better contact action, but the current study did not discover the organophosphate group (fipronil) to be very effective (Irshad et al., 2019; Roger et al., 1997). Several organophosphates and pyrethroids were shown to be efficient against DCB; however, pyrethroids (bifenthrin) were found to be the least effective in three districts in the current study. Sanghi et al. (2014) found chlorpyriphos (organophosphate group) to be highly effective, with an 82% reduction in dusky cotton bug population compared to other studied insecticides. The current findings reveal that the insecticide clothianidin from the novel chemical insecticides, in combination with the pyrethroid group (imidacloprid), significantly reduced the population of dusky cotton bugs. These findings are consistent with the findings of Nyman et al. (2013) who stated that after 24 h of spraying, newly induced chemical pesticides maximize death by blocking pest feeding and movement. In the present experiment, the pesticide mixture clothianidin + Imidacloprid also showed good results at 24 h. Therefore, we say that the pesticide mixture of clothianidin and imidacloprid can be very effective in the treatment of dusky cotton bug.

CONCLUSIONS AND RECOMMENDATIONS

The combination of clothianidin + imidacloprid was more toxic to dusky cotton bug adults from all districts, including Rahim Yar Khan, Multan, Vehari, Dera Ghazi Khan, and Khanewal. Without the combination, fipronil was more toxic to dusky cotton bug adults from all districts. The most susceptible population of dusky cotton bug adults was found in district Dera Ghazi Khan. The insecticides clothianidin, imidacloprid, and fipronil should be considered in the management program for dusky cotton bugs.

ACKNOWLEDGMENTS

The authors wish to acknowledge the team and laboratory assistants of Entomology Section, Central Cotton Research Institute, Multan, Pakistan for coordinating the present research work.

AUTHORS' CONTRIBUTIONS

JAK, MI, and ZM conceived and designed the experiments; JAK, MI, NI and ZM performed the experiments; NI, MI, and ZK analyzed the data; JAK and MI contributed materials, analysis, and tools; JAK and MI wrote the paper.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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