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Original article

Effects of temperature on baseline susceptibility and stability of insecticide resistance against *Plutella xylostella* (Lepidoptera: Plutellidae) in the absence of selection pressure

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ABSTRACT

Plutella xylostella L. (Lepidoptera: Plutellidae) is an important pest causing significant losses to vegetables worldwide. Insecticides resistance in *P. xylostella* is a serious issue for scientists since last 30 years. However, deltamethrin and *Bt Cry1Ac* are commonly used insecticides against *P. xylostella* but studies involving development of resistance in *P. xylostella* against these two insecticides at different temperatures are lacking. The current study was aimed to find out the toxicity of deltamethrin and *Bt Cry1Ac*, and resistance development in *P. xylostella*. Results showed that the positive correlation between the temperature and toxicities of deltamethrin and *Bt Cry1Ac*. The results indicated −0.051, −0.049, −0.047, and −0.046 folds of deltamethrin resistance at 15 °C, 20 °C, 25 °C, and 30 °C temperatures, respectively from 1st to 12th generations. The toxicity of *Bt Cry1Ac* after 24 h was 2.2 and 4.8 folds on 1st generation at 20 °C and 25 °C temperatures, respectively compared to the toxicity recorded at 15 °C (non-overlapping of 95% confidence limits). Based on the results of this study, it is concluded that the temperature has a positive correlation with the toxicity of deltamethrin and *Bt Cry1Ac* against the larvae of *P. xylostella*. This study suggests that deltamethrin and *Bt Cry1Ac* can be included in the management

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program of *P. xylostella* on many vegetable crops. The baseline susceptibility data might be helpful to understand the resistance mechanisms in *P. xylostella*.

1. Introduction

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is an important insect pest of cruciferous crops worldwide (Furlong et al., 2013; Jaleel et al., 2017; Saeed et al., 2019). Worldwide yield losses and the cost of management associated with the diamondback moth is estimated to be about 4–5 billion dollars annually (Zalucki et al., 2012). Chemical control is a most common and effective way to manage *P. xylostella*. But the serious concern to use different insecticides for *P. xylostella* is the development of insecticides resistance (Talekar and Shelton, 1993; Troczka et al., 2017). The extensive use of different insecticides results in the more selection pressure on *P. xlostella* leading to the development of resistance to more than 95 different insecticides. This situation is quite threating and considered as the biggest implications for Integrated Pest Management (IPM) of this pest (Steinbach et al., 2017). Among insecticides, pyrethroid and bacterial insecticides are most commonly use insecticides against of *P. xylostella* in Asia especially Pakistan (Gong et al., 2010; Zamojska et al., 2011). Deltamethrin is the most important pyrethroid used against the *P. xylostella* (Zamojska et al., 2011). The susceptibility against pyrethroids has been studied against the field population of *P. xylostella* (Sayyed et al., 2008). Several studies have reported high resistance in *P. xylostella* to several conventional, new chemistry insecticides and bacterial insecticides due to their extensive use in vegetables or agricultural crops (Sayyed et al., 2008; Shelton, 2007).

The *Bt Cry1Ac* is one of the most important bio-pesticides used against many Lepidopterans pests like *P. xylostella* (Gong et al., 2010; Nian et al., 2015; Zhou et al., 2011). This toxin has also been used in many toxicity studies against *P. xylostella* but studies showing the change in toxicity at different temperatures are lacking. According to Mohan and Gujar (2002) *Bt* kurstaki was found to be the safer and effective bio-pesticides against the *P. xylostella*.

Among abiotic factors, the temperature significantly affects the metabolism and survival rate of insect pests (Arrese and Soulages, 2010). Temperature affects the efficacy of insecticides, the correlation between temperature and insecticides is an important study to establishing the IPM strategies (Ahn et al., 2012; Notter-Hausmann and Dorn, 2010; Saeed et al., 2016). Detailed studies involving the effect of temperature on toxicity and insecticides resistance in *P. xylostella* are lacking from Pakistan.

Considering the importance of *P. xylostella*, this study was aimed to find out the stability of deltamethrin and *Bt Cry1Ac* resistance in *P. xylostella* in the absence of selection pressure at different temperatures. Baseline susceptibility data were generated and the susceptibility was observed in succeeding generations at different temperature under laboratory conditions.

2. Material and methods

2.1. Plant materials

The cabbage plants were used in the study. For this purpose, seeds of *Brassica oleracea* var. *capitata* were purchased from the market and sown in plastic boxes ($6 \times 10 \times 20$ cm) 5–7 weeks before the start of experiment.

2.2. Collection and rearing of P. xylostella

The larvae of *P. xylostella* were collected from cabbage fields located at Multan, Pakistan in 2017 and shifted to laboratory in plastic bottles (15×25 cm). The larvae were reared at different temperatures, 15 °C, 20 °C, 25 °C, and 30 °C, $65 \pm 5\%$ relative humidity, and 14 h :10 h (L : D) photoperiod in incubators (Model VELP # 90E Japan, Model SANYO # 153MIR) before the beginning of the experimental study.

2.3. Insecticides

Deltamethrin (Super Delta 10 EC) and *Bt Cry1Ac* (BTK formulation 2000 iu/μL) were purchased from the local market and used in this experiment.

2.4. Bioassays

Second instar larvae of *P. xylostella* were treated with deltamethrin and *Bt Cry1Ac* for toxicological studies at each temperature on 1st, 4th, 8th, and 12th generations. The insecticides bioassay was conducted using the leaf-dip method. The experiment involved six treatments including control and eight replications of each treatment. Mortality data were recorded for 72 h for *Bt Cry1Ac* and 48 h for deltamethrin after each 24 h. as suggested by Mohan and Gujar (2002) and Saeed et al. (2016).

2.5. Statistical analysis

The LC_{50} values of each insecticide at each temperature were calculated by using the probit analysis method through POLO-PC Program (LeOra, 2003). A resistance factor (RF) was calculated according to the method of Wearing and Colhoun (2005). Temperature coefficients of each insecticide were calculated by following the methodology of Musser and Shelton (2005). The temperature coefficient was considered positive if lower LC_{50} value was at higher temperature and negative when lower LC_{50} at lower temperature.

3. Results

The evaluation of toxicity for deltamethrin and *Bt Cry1Ac*, showed increase toxicity with the increase of temperature

3.1. Deltamethrin

There was a positive correlation between the toxicity of deltamethrin and different temperature ranges (15 °C, 20 °C, 25 °C, and 30 °C). At 20 °C and 25 °C temperatures, the toxicity of deltamethrin (in the term of LC_{50} values) was 1.34 and 2.27 times higher as compared to that recorded at 15 °C (i.e. non-overlapping of 95% CI). Similarly, the toxicity of deltamethrin was four times higher for 1st generation of *P. xylostella* at 30 °C. The toxicity values of 4th, 8th and 12th generations of *P. xylostella* showed a positive correlation with temperature. There was a successive decrease in the insecticide resistance from 1st to the 12th generations of *P. xylostella* to deltamethrin with values of −0.051, −0.049, −0.048, and −0.047 folds at 15 °C, 20 °C, 25 °C and 30 °C, respectively. The LC_{50} value of deltamethrin observed after 24 h in 1st generation of *P. xylostella* at 15 °C

Table 1
Toxicity of deltamethrin to different generation of *Plutella xylostella* at different constant temperatures.

T°C	(G)	(n)	LC$_{50}$ and 95% confidence limit (μg/mL)	R	Slope ± SE	χ^2	DR	TC		
								5 °C	10 °C	15 °C
15	1st	240	712.32 (512.16–1612.19)	4.12	0.493 ± 0.352	0.234		0.0	0.0	
	4th	240	526.42 (325.464–1392.99)	3.04	1.191 ± 0.252	0.274		0.0	0.0	
	8th	240	313.58 (221.294–539.738)	1.81	1.378 ± 0.247	0.888		0.0	0.0	
	12th	240	172.90 (126.424–251.635)	1	1.367 ± 0.233	0.062	−0.0512	0.0	0.0	
20	1st	240	565.23 (341.149–915.195)	3.92	0.183 ± 0.215	0.656		1.35		
	4th	240	368.24 (249.849–716.295)	2.55	1.288 ± 0.245	0.776		1.53		
	8th	240	251.04 (184.286–386.587)	1.74	1.464 ± 0.243	1.148		1.31		
	12th	240	144.35 (106.333–200.888)	1	1.429 ± 0.234	0.091	−0.0494	1.75		
25	1st	240	402.14 (302.183–765.174)	3.75	0.192 ± 0.228	1.997		1.68	2.27	
	4th	240	306.53 (201.883–637.070)	2.86	1.092 ± 0.229	0.997		1.47	2.25	
	8th	240	197.60 (139.269–316.527)	1.84	1.210 ± 0.220	1.657		1.55	2.04	
	12th	240	107.31 (75.492–148.187)	1	1.347 ± 0.231	0.294	−0.0478	1.60	2.81	
30	1st	240	231.19 (184.484–396.113)	3.63	0.956 ± 0.124	0.321		1.81	3.04	4.12
	4th	240	131.99 (89.984–196.813)	2.07	1.156 ± 0.224	0.400		1.74	2.55	3.92
	8th	240	82.32 (54.290–114.171)	1.29	1.302 ± 0.232	0.827		1.84	2.86	3.75
	12th	240	63.67 (41.868–86.280)	1	1.478 ± 0.245	0.424	−0.0467	1.29	2.07	3.63

T, temperature (°C); G, generation; n, total number of insects; χ^2, chi-square to the observed mortality data * significant ($p < 0.05$); R, resistance factor/fold was calculated for each generation as LC$_{50}$ of test generation divided by LC$_{50}$ of susceptible generation; DR, rate of decrease in LC$_{50}$ [log (final LC$_{50}$ – initial LC$_{50}$)/N], where N is a number of generation populations reared without insecticide exposure; TC, temperature coefficient (Ratio of higher to lower LC$_{50}$ value for 5 °C, 10 °C and 15 °C differences in temperature).

Table 2
Toxicity of *Bt Cry1Ac* to different generation of *Plutella xylostella* at different constant temperatures.

T°C	(H)	(G)	(n)	LC$_{50}$ and 95% confidence limit(μg/mL)	R	Slope ± SE	χ^2	DR	TC		
									5 °C	10 °C	15 °C
15	24	1th	240	156107.93 (39481.36–498692.71)	4.66	0.912 ± 0.298	0.119				
	24	4th	240	136007.92 (36482.36–478699.70)	4.06	0.815 ± 0.283	0.017				
	24	8th	240	50441.23 (23050.96–473766.70)	1.51	1.044 ± 0.276	0.183				
	24	12th	240	33472.53 (17085.96–188804.50)	1.00	0.996 ± 0.249	0.887	−0.0557			
	48	1st	240	91791.74 (47491.32–6902312.11)	4.97	0.916 ± 0.362	0.209				
	48	4th	240	81792.77 (27490.22–6702311.18)	4.43	0.816 ± 0.262	0.207				
	48	8th	240	34295.02 (17267.15–211784.77)	1.86	0.983 ± 0.252	0.130				
	48	12th	240	28476.65 (11599.14–46289.84)	1.00	1.152 ± 0.244	0.580	−0.058			
	72	1st	240	29205.69 (15507.46–67579.42)	3.38	1.141 ± 0.235	0.269				
	72	4th	240	19204.68 (11508.49–57509.46)	2.22	1.041 ± 0.239	0.219				
	72	8th	240	13174.07 (8715.65–27402.74)	1.52	1.134 ± 0.235	0.564				
	72	12th	240	8648.12 (6081.74–14198.75)	1.00	1.212 ± 0.231	0.838	−0.044			
20	24	1st	240	69939.87 (35906.68–3896001.62)	3.92	0.681 ± 0.256	0.245		2.23		
	24	4th	240	66937.86 (23907.69–3796001.63)	3.75	0.789 ± 0.250	0.235		2.03		
	24	8th	240	39321.55 (18264.42–370800.15)	2.20	0.906 ± 0.247	0.173		1.28		
	24	12th	240	17848.91 (10860.96–50174.39)	1.00	1.042 ± 0.236	0.097	−0.0494	1.88		
	48	1st	240	36568.85 (24862.52–289433.97)	3.99	0.853 ± 0.231	0.382		2.51		
	48	4th	240	30558.80 (14872.50–249432.95)	3.33	0.852 ± 0.237	0.381		2.68		
	48	8th	240	16002.41(9842.36–43359.72)	1.74	1.014 ± 0.233	0.145		2.14		
	48	12th	240	9174.67 (6540.82–14808.25)	1.00	1.281 ± 0.234	0.288	−0.05	2.01		
	72	1st	240	14453.15 (8894.00–39493.21)	2.50	1.986 ± 0.228	0.289		2.02		
	72	4th	240	12451.05 (7894.00–29493.29)	2.15	0.986 ± 0.227	0.208		1.54		
	72	8th	240	7692.15 (5309.93–12683.51)	1.33	1.133 ± 0.226	0.069		1.71		
	72	12th	240	5781.33 (3914.17–8932.03)	1.00	1.109 ± 0.223	0.019	−0.0332	1.50		
25	24	1st	240	32223.32 (25262.20–72911.79)	3.07	1.155 ± 0.265	0.065		2.17	4.84	
	24	4th	240	25226.31 (15262.20–71911.78)	2.41	1.255 ± 0.267	0.049		2.65	5.39	
	24	8th	240	18419.51 (11992.53–41187.90)	1.76	1.275 ± 0.255	0.403		2.13	2.74	
	24	12th	240	10481.38 (7283.68–18320.06)	1.00	1.222 ± 0.234	0.040	−0.0406	1.70	3.19	
	48	1st	240	16316.46 (11239.693–43013.82)	2.18	1.284 ± 0.246	0.048		2.24	5.63	
	48	4th	240	15306.56 (10039.693–33013.82)	2.05	1.184 ± 0.241	0.038		2.00	5.34	
	48	8th	240	11929.48 (8129.78–22442.84)	1.60	1.194 ± 0.235	0.052		1.34	2.87	
	48	12th	240	7471.01 (5303.65–11617.83)	1.00	1.235 ± 0.229	0.076	−0.0283	1.23	2.47	
	72	1st	240	9626.07 (6916.42–25214.56)	1.75	1.116 ± 0.235	0.257		1.50	3.03	
	72	4th	240	9126.07 (6406.42–15204.55)	1.65	1.216 ± 0.231	0.237		1.36	2.10	
	72	8th	240	6518.26 (4630.81–9774.67)	1.18	1.245 ± 0.228	0.214		1.18	2.02	
	72	12th	240	5514.57 (4048.83–7632.61)	1.00	1.427 ± 0.233	0.170	−0.0202	1.05	1.57	
30	24	1st	240	16247.34 (9569.84–31559.39)	2.28	1.159 ± 0.231	1.158		1.98	4.30	9.61
	24	4th	240	14277.94 (9369.84–30559.69)	2.00	1.149 ± 0.236	0.158		1.77	4.69	9.53
	24	8th	240	10888.89 (7508.84–19598.73)	1.53	1.201 ± 0.234	0.029		1.69	3.61	4.63
	24	12th	240	7134.15 (5023.65–11108.50)	1.00	1.204 ± 0.227	0.033	−0.0298	1.47	2.50	4.69
	48	1st	240	11821.20 (7354.07–23778.81)	2.25	1.115 ± 0.209	0.211		1.38	3.09	7.77

(*continued on next page*)

Table 2 (*continued*)

T°C	(H)	(G)	(n)	LC_{50} and 95% confidence limit(μg/mL)	R	Slope ± SE	χ^2	DR	TC		
									5 °C	10 °C	15 °C
	48	4th	240	10800.20 (7254.07–20778.89)	2.06	1.105 ± 0.229	0.201		1.42	2.83	7.57
	48	8th	240	7847.05 (5460.22–12817.71)	1.50	1.161 ± 0.227	0.056		1.52	2.04	4.37
	48	12th	240	5242.50 (3814.36–7271.88)	1.00	1.397 ± 0.232	0.063	−0.0294	1.43	1.75	3.52
	72	1st	240	6675.43 (4231.15–9999.94)	2.25	1.162 ± 0.256	0.089		1.44	2.17	4.38
	72	4th	240	6172.46 (4130.05–9920.74)	2.08	1.062 ± 0.222	0.083		1.48	2.02	3.11
	72	8th	240	4324.26 (2798.92–6387.039)	1.46	1.109 ± 0.223	0.019		1.51	1.78	3.05
	72	12th	240	2965.95 (1813.05–4223.83)	1.00	1.202 ± 0.229	0.085	−0.0294	1.86	1.95	2.92

T°C, temperature (°C); G, Generation; n, total number of insects; χ^2, chi-square to the observed mortality data * significant ($p < 0.05$); R, resistance factor/fold was calculated for each generation as LC_{50} of test generation divided by LC_{50} of susceptible generation; DR, rate of decrease in LC_{50} [log (final LC_{50} – initial LC_{50})/*N*], where *N* is number of generation populations reared without insecticide exposure; TC, Temperature Coefficient (Ratio of higher to lower LC_{50} value for 5 °C, 10 °C, and 15 °C differences in temperatures).

was 712.32 μg/mL while in 12th generation, the LC_{50} value was decreased to 172.90 μg/mL, indicating an increase in toxicity. The initial LC_{50} value calculated at temperatures of 20 °C, 25 °C, and 30 °C in 1st generation were greater as compared with a final LC_{50} values observed in 12th generation (Table 1).

3.2. Bt Cry1Ac

The relationship between the toxicity of *Bt Cry1Ac* and all tested temperatures (15 °C, 20 °C, 25 °C, and 30 °C) was similar as observed for deltamethrin. The toxicity of *Bt Cry1Ac* was increased with the increase in temperature. The toxicity of *Bt Cry1Ac* at 30 °C was increased 9.61 times once exposed at the 1st generation larvae of *P. xylostella*. Toxicity observed was in positive correlation with temperature coefficient all the generations of *P. xylostella* (Table 2). On 1st generation field population of *P. xylostella*, maximum LC_{50} values of *Bt Cry1Ac* were observed after 24 h (156107.93 μg/mL) of treatment followed by decreasing values after 48 (91791.74 μg/mL), and 72 h (29205.69 μg/mL). However, the same pattern was observed at 12th generation of *P. xylostella* but with quite lower LC_{50} values (Table 2).

4. Discussion

A number of experiments have been performed to better understand interactions between different pathogens used against insect pests (Wraight and Ramos, 2005; Zhou et al., 2011). However, only a few studies are available on the interaction between temperature and insecticides against the *P. xylostella* (Sayyed et al., 2008). Hence, the current study was conducted to define the possible effects of deltamethrin and *Bt Cry1Ac* against the *P. xylostella* at a different temperature. Deltamethrin is one of the best pyrethroid that has been used for 30 years (Zamojska et al., 2011). Several lepidopteran insect pests endure severe stress at a low followed by high temperature and a positive correlation has been observed between the toxicity of deltamethrin and the temperature (Li et al., 2007). With the increase of temperature, the toxicity of deltamethrin was found highest. The coefficient of temperature becomes positive as the temperature increases and resistance increases owing to the high rate of metabolism (Punzo, 1993). According to Toth and Sparks (1990) temperature coefficient correlation with pyrethroids either positive or negative it depends upon the insect. Our results showed positive temperature correlation in case mortality against the deltamethrin and *BtCry1Ac*.

The *Bt* toxin (protein) has been known for its insecticidal properties, and it has the ability to multiply in a suitable temperature (Ferré and Van Rie, 2002; Höfte and Whiteley, 1989). At low temperatures, the activity of *Bt* toxins is inhibited in plants, however, at high temperatures (i.e. 36–42 °C) the crystalline protein is diluted or its effectiveness is reduced in transgenic crops (Hilbert and Piggot, 2004). The results of this experiment summarized that temperature in the range of 20–25 °C is ideal the management program of *P. xylostella*, that is the reason why in unselected population, *P. xylostella* mortality was increased against pyretheroids and *Bt Cry*1*Ac*. Our study concluded that susceptibility of larvae of *P. xylostella* against deltamethrin (pyrethroid) and *Bt Cry*1*Ac* was increased with the increase of temperature because of high feeding and metabolism rate. Deltamethrin and *Bt Cry*1*Ac* must be used an integrated strategy for controlling *P. xylostella* in practice. Moreover, advance studies to know the mechanism involve in toxicity of these insecticides with the combination of Lepidopterans are desirable. Our study will be helpful in the management of *P. xylostella* in many vegetable crops.

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