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Genetics, cross-resistance and realized heritability of resistance to acetamiprid in generalist predator, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae)

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Abstract

The common green lacewing, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) has a remarkable role in biological control programs being used to control insect pests of economic significance. This study aimed to investigate the potential of *C. carnea* against commonly used insecticides, especially acetamiprid. Selection with acetamiprid resulted in 31,070.69- and 13.34-fold resistance when compared with Lab-PK and Field strains, respectively. Selection also induced a very low cross-resistance to buprofezin, pyriproxyfen, and spinosad in Aceta-SEL strain. Realized heritability (h^2) was 0.24 showed a remarkable genetic variant for resistance. Resistance to acetamiprid in *C. carnea* was incompletely dominant, autosomal, and polygenic. These outcomes are helpful to employ the acetamiprid-resistant *C. carnea* in fields.

Keywords: *Chrysoperla carnea*, Insecticide resistance, Acetamiprid, Cross-resistance, Genetics, Realized heritability

Background

There is a demand to notice the impact of insecticides not only on the targeted agricultural pests but also on non-targets, i.e., predators and parasitoids (Biondi et al. 2012). The agronomic worth of numerous insecticides has been reduced because of resistance development in pest species (Whalon et al. 2012). However, it is evident that certain populations of natural enemies given frequent insecticide exposure can develop resistance in a similar approach as the pests themselves (Rodrigues et al. 2013). Resistance evolution or development is usually influenced by different intrinsic factors including behavior patterns, physiology, metabolic, and genetic structure of species as well as extrinsic or operational factors that depend on insecticide coverage, application frequency and properties (Rosenheim and Tabashnik 1990).

The common green lacewing, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) is known to have a wide prey range such as mites, whiteflies, aphids, thrips, and caterpillars (Pathan et al. 2010). This cosmopolitan species has revealed a significant resistance against organophosphates, pyrethroids and new chemistry insecticides with prominent involvement of detoxification enzymes (Mansoor et al. 2017; Mansoor and Shad 2019b). In recent years, different studies from various fields or locations confirmed that this species has a low susceptibility to commonly used insecticides (Abbas et al. 2014; Mansoor et al. 2013, 2017; Mansoor and Shad 2019a, 2019b).

Neonicotinoids have a novel mode of action thus classified as an advanced class of insecticides. These insecticides have made a key status in Integrated Pest Management (IPM) programs because of their high efficacy against a wide range of insect pests (Yamamoto and Casida 1999). Acetamiprid is a neonicotinoid to control sucking insect pests of plants. It has osmotic, systemic, and contact action (Takahashi 1998). Resistance to acetamiprid has been reported in different insect pests including *Plutella xylostella* (Linnaeus) (Sayyed and

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Crickmore 2007), *Bemisia tabaci* (Gennadius) (Basit et al. 2011), *Leptinotarsa decemlineata* (Say) (Mota-Sanchez et al. 2006), *Aphis gossypii* (Glover) (Herron and Wilson 2011), *Frankliniella occidentalis* (Pergande) (Minakuchi et al. 2013), and *Phenacoccus solenopsis* (Fernald) (Ijaz et al. 2016). To author's best knowledge, there is no report of resistance to acetamiprid in *C. carnea*.

The use of natural enemies with the pesticide-resistant feature may foil common issues such as secondary pest outbreak and pest resurgence in cropping systems where pesticides are used as chemical control priority (Sayyed et al. 2010). Knowledge of genetics and evolution of resistance to insecticides could support device IPM programs with an aim to minimize the utilization of pesticides (Landis et al. 2000). Insecticide resistance and its genetic basis have been extensively studied in insect pest populations (Ffrench-Constant et al. 2004). To the best of our information, however, genetics of acetamiprid resistance in *C. carnea* has not been reported yet. Studying genetics is mainly essential to identify a number of genes responsible for resistance development as a dominant or recessive trait. This knowledge also provides strong opinions to utilize natural enemies in various IPM systems (Mansoor et al. 2017).

Materials and methods

Adults of *C. carnea* (field strain) were collected in early spring from the fields of District Muzaffargarh (30.0703° N, 71.1933° E), Pakistan and brought to the laboratory. The adults were kept in plastic cages (23 × 38 × 38 cm) and fed on a mixture of honey, yeast, and water in ratio of 2:1:4, respectively. Black glossy papers were hung horizontally on the ceiling of cages for egg deposition. After hatching, every larva was placed in a Petri dish (5 cm) to avoid cannibalism. The larvae were given frozen eggs of Angoumois grain moth, *Sitotroga cerealella* Oliver (Sattar et al. 2007). A strain of susceptible population was obtained from Multan in 1999 and designated as Lab-PK (Mansoor et al. 2013). It was reared without any insecticide exposure to be used as control.

Insecticides

Acetamiprid (Mospilon 20 WP, Dow Agro-Sciences), buprofezin (Fuzin 25 WP, Four Brothers), pyriproxyfen (Admiral 10 EC, FMC), and spinosad (Tracer 240SC, Arysta Life Sciences) were commercial formulations used for the experiments.

Selection with insecticide

The field-collected population was divided into 2 groups at first generation. One group of around 300 larvae were selected by acetamiprid and named Aceta-SEL, while the second was reared without any exposure to insecticide and named UNSEL. Selection was continued from G1 to G15, using the 1st instar larvae of *C. carnea*. Varying levels of acetamiprid solutions were topically applied, using a Handheld Micro applicator as described (Mansoor and Shad 2019a).

Concentration-response bioassays

The 1st instar larvae (2–3 days old) of *C. carnea* were used for the bioassays (Mansoor et al. 2017). Four serial concentrations of each insecticide were prepared and replicated 4 times (Robertson and Preisler 1992). Each replication contained 20 larvae, while 30 larvae were used as control. Eggs of *S. cerealella* were provided to treated larvae (Pathan et al. 2008) while mortality results were recorded after 72 h.

Genetic crosses

Crosses were done between Aceta-SEL and Lab-PK strains to recognize the genetics of resistance. The F_1 progeny was obtained by crossing 30 Aceta-SEL and 30 Lab-PK adults. The F_2 was obtained by crossing males (♂) of F_1 and females (♀) of Lab-PK strains. Backcrosses were also done in order to obtain BC_1(F_1♀ × Lab-PK Pop ♂), BC_2 (F_1 ♂ × Lab-PK Pop ♀), BC_3 (F_1'♀ × Lab-PK Pop ♂) and BC_4 (F_1' ♂ × Lab-PK Pop ♀) (Sayyed et al. 2010).

Degree of dominance (D_{LC})

The D_{LC} of acetamiprid resistance was calculated as mentioned by Bourguet and Raymond (1998) and Stone (1968). The resistance is considered completely recessive if $D_{LC} = 0$ and completely dominant if $D_{LC} = 1$.

$$D_{LC} = (\log LC_{RS} - \log LC_S)/(\log LC_R - \log LC_S).$$

where $\log LC_{RS}$, $\log LC_S$, and $\log LC_R$ are logs of LC_{50} of F_1, Lab-PK and Aceta-SEL strains.

The effective dominance (D_{ML}) was calculated (Bourguet et al. 2000) as

$$D_{ML} = (MT_{RS} - MT_{SS})/(MT_{RR} - MT_{SS})$$

while MT_{RS} (F_1), MT_{RR} (Aceta-SEL) and MT_{SS} (Lab-PK) were percent mortalities on a single dose of insecticide. The resistance is considered completely recessive if $D_{ML} = 0$ and completely dominant if $D_{ML} = 1$ (Mansoor et al. 2019).

Gene frequency involved

Goodness of fit test (Chi-square) was used to test the monogenic resistance hypothesis. Based on this test, the null hypothesis of monogenic resistance was calculated as:

$$\chi^2 = (F-pn)^2/pqn.$$

where F is mortality in the population (BC_1) against a specific dose, n = total number of individuals exposed to a specific dose, p = expected mortality (Georghiou 1969) while q = 1_p. Significant difference ($p < 0.05$) between 50 % of observed and expected mortalities would reject the null hypothesis of monogenic resistance.

Secondly, the number of genes controlling acetamiprid resistance was estimated using the given equation (Lande 1981).

$$\eta E = (X_{RR}-X_{SS})^2/(8\sigma^2 S)$$

where X_{RR} or X_{SS} = Log LC_{50} of Aceta-SEL or Lab-PK Strain.

The $\sigma^2 S$ was estimated as given:

$$\sigma^2 S = \sigma^2_{B1} + \sigma^2_{B2} - [\sigma^2 F_1 + 0.5\sigma^2 X_{SS} + 0.5\sigma^2 X_{RR}]$$

where $\sigma^2_{B1} + \sigma^2_{B2} - [\sigma^2 F_1 + 0.5\sigma^2 X_{SS} + 0.5\sigma^2 X_{RR}]$ were variances of BC_1, BC_2, F_1, Lab-PK and Aceta-SEL Strain.

Realized heritability (h^2)

Realized heritability was computed as described by (Tabashnik 1992) as

$$h^2 = \text{response to selection } (R)/\text{selection differential } (S).$$

Response to selection was calculated as

$$R = [\text{Log final } LC_{50} \text{ of Aceta-SEL strain} - \text{Log initial } LC_{50} \text{ of field strain}]/n,$$

Here, n is the number of generations exposed with acetamiprid.

Selection differential was calculated as

$$S = \text{intensity of selection } (i) \times \text{phenotypic standard deviation } (\sigma p).$$

Intensity of selection was as

$$i = 1.583 - 0.0193336p + 0.0000428p2 + 3.65194/p,$$

where p is the average survival of the Aceta-SEL strain.

The phenotypic standard deviation was calculated as

$$\sigma p = [1/2(\text{final slope} + \text{initial slope})]^{-1}.$$

Statistical analysis

Mortality data obtained was corrected using Abbot's formula (Abbott 1925). Concentration-response data was analyzed with POLO Software (Software 2005) by using probit analysis (Finney 1971) to calculate LC_{50} (Median Lethal Concentration), 95% Fiducial limits (FLs), slopes with standard errors and Chi-square ($\chi 2$). The LC_{50} values were considered similar if their 95% FLs overlapped (Litchfield and Wilcoxon 1949). Insecticide resistance level was defined as: no resistance if (RR = < 2-fold), very low resistance if (RR = 2 to 10-fold), moderate resistance if (RR = 21-50-fold) and high resistance if (RR > 100) (Abbas et al. 2015).

Results and discussion

Toxicity response of multiple insecticides to Lab-PK, field, UNSEL and Aceta-SEL strains

The response of acetamiprid was different from all other tested insecticides (non-overlapping of 95% FLs), except spinosad (overlapping of 95% FLs) on Lab-PK strain. Both buprofezin and pyriproxyfen were significantly less toxic than acetamiprid and spinosad (non-overlapping of 95% FLs) (Table 1). Acetamiprid was less toxic to field strain, followed by spinosad but pyriproxyfen and buprofezin were highly toxic (non-overlapping of 95% FLs). Buprofezin was more toxic than other tested insecticides (non-overlapping of 95% FLs). Field population showed a very high level of resistance to acetamiprid, spinosad, and pyriproxyfen, while moderate level of resistance to buprofezin (Table 1)

To UNSEL strain, acetamiprid and pyriproxyfen were less toxic (overlapping of 95% FLs) when compared with spinosad and buprofezin (non-overlapping of 95% FLs). Buprofezin showed different toxicity than that of spinosad (non-overlapping of 95% FLs). Aceta-SEL strain was 31,070-fold and 13.34-fold resistant than Lab-PK and Field strains, respectively. Toxicity to acetamiprid in Aceta-SEL strain was different from all other tested insecticides (non-overlapping of 95% FLs) (Table 1). Pyriproxyfen and spinosad were less toxic than buprofezin (non-overlapping of 95% FLs).

Green lacewings are very important in the IPM systems (Tauber et al. 2000). The availability and performance of these general predators in the field crops heavily depend on different factors including exposure to insecticides. This apprehends the need to study the genetics of insecticides resistance in green lacewings because these are commonly recommended and employed for control of various insect pests. Pre-testing of green lacewing strain collected from the field showed a moderate level of resistance to buprofezin, but a very high level of field evolved resistance to acetamiprid, spinosad, and pyriproxyfen. Acetamiprid resistance significantly amplified in field strain due to selection pressure from G1 to G15. Bioassays at G1 and G16 indicated 2327.87-fold and 31,070.19-fold resistance to acetamiprid, respectively, when compared with Lab-PK strain. There are reports about the significant increase of resistance development in *C. carnea* under laboratory conditions against deltamethrin (Sayyed et al. 2010), emamectin benzoate (Mansoor et al. 2013), spinosad (Abbas et al.

Table 1 Response of various insecticides to Lab-PK, Field, UNSEL and Aceta-SEL populations of *Chrysoperla carnea*

Strain	Insecticide	LC_{50} (95% FL) (μg mL^{-1})	Fit of probit line				N^a	RR^b	RR^c
			Slope (±SE)	χ^2	df	*P*			
Lab-PK (G130)	Acetamiprid	0.72 (0.45-0.95)	2.26 (0.36)	1.48	3	0.86	350	1	
	Spinosad	1.15 (0.94-1.32)	3.21 (0.40)	0.46	3	0.98	350	1	
	Buprofezin	4.27 (3.34-5.10)	2.68 (0.35)	1.88	3	0.51	350	1	
	Pyriproxyfen	5.54 (4.53-6.50)	2.55 (0.30)	4.13	3	0.63	350	1	
Field (G1)	Acetamiprid	1676.07 (1171.58-2867.68)	1.32 (0.26)	1.46	3	0.69	350	2327.87	
	Spinosad	877.29 (607.27-1547.13)	1.31 (0.27)	0.71	3	0.87	350	762.86	
	Buprofezin	113.39 (82.96-160.79)	1.48 (0.26)	5.74	3	0.12	350	26.55	
	Pyriproxyfen	335.77 (274.89- 418.66)	1.68 (0.22)	1.06	3	0.79	350	60.61	
UNSEL (G16)	Acetamiprid	133.10 (111.35-158.30)	2.00 (0.23)	1.72	3	0.63	350	184.86	
	Spinosad	75.40 (57.90-91.26)	2.24 (0.28)	1.52	3	0.68	350	65.56	
	Buprofezin	16.42 (13.68-18.97)	2.94 (0.34)	2.45	3	0.48	350	3.84	
	Pyriproxyfen	162.53 (138.76-186.15)	2.89 (0.30)	3.80	3	0.28	350	29.33	
Aceta-SEL (G16)	Acetamiprid	22370.54 (12089.78-99622.35)	1.25 (0.28)	0.30	3	0.82	350	31070.19	13.34
	Spinosad	1531.44 (953.33-4323.61)	1.12 (0.23)	1.26	3	0.99	350	1331.69	1.75
	Buprofezin	342.52 (221.09-829.50)	1.38 (0.26)	0.92	3	0.74	350	80.21	3.02
	Pyriproxyfen	1322.92 (857.01-3174.71)	1.35 (0.27)	1.62	3	0.65	350	238.79	3.94

[a]RR resistance ratio, LC_{50} of UNSEL, Field and Aceta-SEL strains/LC_{50} of Lab-PK strain
[b]RR resistance ratio, LC_{50} of Aceta-SEL strain/LC_{50} of Field strain
[a]N Number of total larvae exposed in a bioassay including control

2014), nitenpyram (Mansoor et al. 2017), and buprofezin (Mansoor and Shad 2019a).

Acetamiprid selection and Cross-resistance to various insecticides

Resistance to acetamiprid significantly increased from 2327.87-fold to 31,070.69-fold after selection from G1 to G16. Testing cross-resistance specified that selection forced by acetamiprid showed very low cross-resistance to buprofezin, pyriproxyfen, and spinosad when compared with field population (Table 1). In the current experiment, Aceta-SEL strain of *C. carnea* showed very low cross-resistance to buprofezin, pyriproxyfen, and spinosad when compared with field strain. Cross-resistance may happen due to the presence of non-specific enzymes (microsomal oxidases), insecticidal target-site mutation and factors such as delayed cuticular permeation (Luo et al. 2010). Cross-resistance among dissimilar insecticides with the differing mode of action and structures is not predictable but independent genetically linked mechanism or a common mechanism affecting the insecticide could be involved in cross-resistance among unrelated insecticides (Gorman et al. 2010). A particular isoenzyme from an insect acting on various kinds of insecticides could be responsible for cross-resistance among various chemical groups (Ahmad et al. 2007). Contrarily, no cross-resistance to acetamiprid and buprofezin but negative cross-resistance to spinosad in *C. carnea* selected with nitenpyram (Mansoor et al. 2017). Previously, buprofezin selection induced high cross-resistance to pyriproxyfen while no cross-resistance to acetamiprid, spirotetramat, and imidacloprid in *B. tabaci* (Basit et al. 2012). Negative cross-resistance to imidacloprid with no cross-resistance to deltamethrin, indoxacarb, and abamectin has been reported in spinosad selected *M. domestica* (Khan et al. 2014b). No cross-resistance to fipronil, while very low cross-resistance to imidacloprid, endosulfan, and bifenthrin has been documented in an acetamiprid selected strain of *B. tabaci* (Basit et al. 2011). A moderate increase in resistance to deltamethrin and imidacloprid while low cross-resistance to chlorpyrifos has been observed in *P. solenopsis* selected with acetamiprid (Afzal et al. 2015). Furthermore, increase in resistance to nitenpyram, thiacloprid, and thiamethoxam in Aceta-SEL strain of *B. tabaci* has been reported (Basit et al. 2011). Neonicotinoids work as an agonistic on the receptors of postsynaptic nicotinic acetylcholine (Elbert et al. 2007) and shown no cross-resistance to insect growth regulators (Basit et al. 2012). Current results suggest the possibility of careful rotational use of tested insecticides to control pests where this natural enemy is present. Moreover, it could be useful to delay the resistance development in pest populations while keeping the natural enemies alive.

Degree of dominance and maternal effects

Dominance values (D_{LC}) were 0.79, 0.69, and 0.75 for F_1, $F_{1'}$, and F_2 strains, respectively (Table 2). The LC_{50}

Table 2 Response of Lab-PK, resistant, reciprocal crosses and backcross strains of *Chrysoperla carnea* to acetamiprid

Strain	LC_{50} (95% FL) (μg mL^{-1})	Fit of probit line				N^a	RR^b	D_{LC}
		Slope (±SE)	χ^2	df	*P*			
Lab-PK strain	0.72 (0.45-0.95)	2.26 (0.36)	1.48	3	0.86	350	1	1
Aceta-SEL strain	22370.54(12089.78-99622.35)	1.25 (0.28)	0.30	3	0.82	350	31070.19	1
F_1 (Aceta-SEL ♂ × Lab-PK ♀)	7583.75 (5541.965-12992.84)	1.35 (0.23)	0.91	3	0.82	350	10532.98	0.79
$F_{1'}$ (Aceta-SEL♀ × Lab-PK ♂)	4603.76 (3557.35-6620.51)	1.32 (0.22)	0.16	3	0.98	350	6394.11	0.69
F_2 (F_1 ♂ × F_1 ♀)	6285.34 (4051.66-11800.39)	1.14 (0.31)	0.07	3	1.00	160	872963	0.75
BC_1 (F_1♀× Lab-PK ♂)	5754.58 (4360.84-8907.63)	1.92 (0.36)	0.24	3	0.97	160	7992.47	
BC_2 (F_1 ♂ × Lab-PK ♀)	5624.78 (3977.93-10535.84)	1.45 (0.33)	0.17	3	0.98	160	7812.19	
BC_3 ($F_{1'}$ ♀ xLab-PK ♂)	4704.73 (3314.27-8457.31)	1.35 (0.31)	0.34	3	0.95	160	6534.34	
BC_4 ($F_{1'}$ ♂ × Lab-PK ♀)	3050.51 (2299.82-4117.92)	1.76 (0.33)	0.38	3	0.94	160	4236.81	

N^a Number of total larvae used for bioassay including control
RR^b Resistance ratio calculated as LC_{50} of the Aceta-SEL, F_1, $F_{1'}$, F_2 (reciprocal), and backcross strains/LC_{50} of the Lab-PK strain

values of F_1 and $F_{1'}$ were similar (overlapping of 95% FLs), showing that acetamiprid resistance was neither sex-linked nor there were maternal effects in the development of resistance (Table 2). Resistance to acetamiprid was incompletely dominant from lower to a higher dose (Table 3). Information about resistant genes including its significant factors such as autosomal inheritance, sex linkage, and dominance is usually acquired by crossing individuals from resistant and susceptible strains (Sayyed and Wright 2004). This study concluded that acetamiprid resistance in *C. carnea* took over as autosomal and incompletely dominant (Table 3). Previously, resistance to acetamiprid has been reported autosomal and incompletely dominant in *P. solenopsis* (Afzal et al. 2015). These results are consistent with the previous findings of autosomal and incompletely dominant resistance in deltamethrin selected population of *C. carnea* (Sayyed et al. 2010). However, these results are contradicting to reports about acetamiprid resistance in *B. tabaci* and *P. xylostella*, which showed resistance as autosomal but incompletely recessive trait (Sayyed and Crickmore 2007; Basit et al. 2011).

Resistance alleles engaged in the degree of dominance has a classified role in resistance gene distribution and expression (Sayyed et al. 2004). Development and inheritance of resistance usually takes place faster in the presence of dominant genes than recessive trait. This type of resistance grows quicker in the fields because of the high potential to survive against insecticide applications. So, in light of current results, there are more chances of survival of *C. carnea* if its population continuously exposed to acetamiprid due to incomplete dominance (Bourguet et al. 2000). Furthermore, the level of dominance may experience evolution because of the continuous selection of resistance allele. Selection may also support insecticide resistance alleles making highly dominant phenotypes in a process of allele replacement (Abbas et al., 2014).

These results showed that the category of dominance to acetamiprid in *C. carnea* remained the same with the

Table 3 Effective dominance (D_{ML}) of acetamiprid resistance in Aceta-SEL strain of *Chrysoperla carnea*

Concentration (μg mL^{-1})	Strain	Mortality	Effective dominance (D_{ML})
8000	Lab-PK	100.00	0.69
	Aceta-SEL	27.00	Incompletely dominant
	F_1	50.00	
4000	Lab-PK	100.00	0.75
	Aceta-SEL	18.00	Incompletely dominant
	F_1	38.00	
2000	Lab-PK	100.00	0.90
	Aceta-SEL	10.00	Incompletely dominant
	F_1	18.00	
1000	Lab-PK	100.00	0.91
	Aceta-SEL	3.00	Incompletely Dominant
	F_1	12.00	

Table 4 Direct test of monogenic inheritance of resistance to acetamiprid by comparing expected and observed mortality of the backcross (F_1♀ × Lab-PK ♂) of *Chrysoperla carnea*

Concentration (μg mL^{-1})	Number of larvae tested	Observed mortality	Expected mortality[a]	x^2(df = 1)	P[b]
1000	80	0.075	0.69	175.52	<0.001
2000	80	0.20	0.72	203.02	<0.001
4000	80	0.35	0.82	357.53	<0.001
8000	80	0.62	0.88	550.04	<0.001

[a]Expected mortality at given dose = 0.5 (total number of F_1 larvae killed + number of Aceta-SEL larvae killed)/no. exposed in backcross
[b]Probability values were considered significantly different at $p < 0.05$

change of concentration of insecticide. This contradicts previous reports of acetamiprid resistance in *B. tabaci* (Basit et al. 2011). Increasing insecticide concentration may change the dominance level (Georghiou 1983). Declining dominance level results in the decrease of heritability of resistance, which delays the development of resistance but as *C. carnea* is a beneficial insect, so due to change in concentration of insecticides, its dominance level remains the same which will result in its survival. Incompletely or completely dominant resistant alleles maintain the susceptible alleles for a longer duration in a population and increase the occurrence of interaction between minor and major genes (Sayyed et al. 2000).

Gene frequency involved

Monogenic model suggested that there was a significant difference between observed and expected mortalities ($p < 0.05$) when judged against the three concentrations. This clearly suggests that there is involvement of multifactor controlling the acetamiprid resistance. The number of genes engaged in acetamiprid resistance was 71 (Lande 1981). This study indicates that acetamiprid resistance is polygenic in Aceta-SEL population (Table 4). Insect populations may confer monogenic or polygenic resistance to insecticides under high selection pressure and polygenic resistance is more likely to happen in this situation (Roush 1998). This study also showed that resistance to acetamiprid was polygenic in *C. carnea* population (Table 4). Resistance controlled by a single gene increases rapidly in contrast to multiple genes (Barnes et al. 1995). Insecticide resistance in the field populations with a key phenotypic effect may be monogenic or polygenic but the type and geographical origin has an influence on resistance mechanism (Sayyed et al. 2008). The involvement of major and minor genes may promote polygenic resistance and it happens evenly in field and laboratory conditions (Khan et al. 2014a; Sayyed and Wright 2001) but major genes appear quicker than minor genes under high selection pressure. Current outcomes confirm that predators have the potential to get dominant inheritance mode resulting in fitness advantages than susceptible strains (Sayyed et al. 2010). These findings could lead to the initiative of the integration of natural enemies and insecticides in IPM programs.

Realized heritability

The LC_{50} of acetamiprid increased from 1676.07 to 22370.54 μg mL^{-1} after continuous selection of Aceta-SEL strain. The value of realized heritability after selection (G1 to G15) to acetamiprid was 0.24 (Table 5). Natural enemies possess lower realized heritability estimates than their host pests. It suggests a lack of ability to build up resistance which may be due to biochemical, ecological, and biological factors (Roush and Daly 1990). Realized heritability of acetamiprid resistance was calculated to recognize the genetic variation in *C. carnea*. A value of $h^2 = 0.24$ suggested high genetic variation for resistance (Table 5). Previously, high realized heritability ($h^2 = 0.58$) of acetamiprid resistance has been reported in *P. solenopsis* (Afzal et al. 2015) but a low value was also reported ($h^2 = 0.21$) in *P. solenopsis* (Ijaz et al. 2016).

Realized heritability $h^2 = 0.22$ has been reported in a deltamethrin selected strain of *C. carnea* (Sayyed et al. 2010). Realized heritability values higher than current findings have been reported in emamectin benzoate-selected strain of *C. carnea* ($h^2 = 0.34$) (Mansoor et al. 2013), spinosad-selected strain of *C. carnea* ($h^2 = 0.37$) (Abbas et al. 2014), nitenpyram-selected strain of *C.*

Table 5 Estimation of the realized heritability of resistance to acetamiprid in Aceta-SEL strain of *Chrysoperla carnea*.

	Estimation of mean selection response per generation				Estimation of mean selection differential per generation						
N	Insecticide	Initial log LC_{50}	Final log LC_{50}	Response to selection (*R*)	*P*	*I*	Initial slope	Final slope	*σp*	Selection differential (*S*)	h^2
16	Acetamiprid	3.22	4.35	0.08	76	0.41	1.32	1.25	0.78	0.32	0.24

N is the number of generations exposed with acetamiprid
P is the average surviving percentage of green lacewing larvae throughout the selection
i is the intensity of selection
σp is the phenotypic variation

carnea (h^2 = 0.97) (Mansoor et al. 2017) and buprofezin-selected strain of *C. carnea* (h^2 = 0.49) (Mansoor and Shad 2019a). High additive genetic variation rise in LC_{50} values between G1 and G16 for acetamiprid in field strain was noteworthy. This confirms a higher occurrence of resistance alleles in the field strain, which suggested that *C. carnea* would take 12.5 generations to reach 10-fold increase in LC_{50} of acetamiprid (reciprocal of R, Table 5).

Conclusion

This study confirms that *C. carnea* could establish a high level of resistance to acetamiprid ensuring its survival in intense spray programs. Resistance to this neonicotinoid is polygenic and incompletely dominant. Resistance development as incompletely dominant can lead to high efficacy and survival of this beneficial insect. Little cross-resistance to buprofezin, pyriproxyfen, and spinosad can increase the usefulness of acetamiprid in various IPM systems where biological control programs are implemented. However, these insecticides can be used as an alternative but with high care to control different pests.

Abbreviation

µg mL^{-1}: parts per million

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Authors' contributions

The study was planned by MMM and SAS. MMM performed laboratory work, data collection, and analysis. MMM wrote manuscript. SAS helped in the technical write-up and manuscript improvement. All authors read and approved the final manuscript.

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This article does not contain any studies with human participants or animals.

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Competing interests

The authors declare that they have no competing interests.

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