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Risk Assessment of Flonicamid Resistance in *Musca domestica* (Diptera: Muscidae): Resistance Monitoring, Inheritance, and Cross-Resistance Potential

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Abstract

Flonicamid is a chordotonal modulator and novel systemic insecticide that has been used frequently for controlling a broad range of insect pests. The risk of flonicamid resistance was assessed through laboratory selection and determining inheritance pattern and cross-resistance potential to five insecticides in house fly, *Musca domestica* L. Very low to high flonicamid resistance in *M. domestica* populations was found compared with the susceptible strain (SS). A flonicamid-selected (Flonica-RS) *M. domestica* strain developed 57.73-fold resistance to flonicamid screened for 20 generations compared with the SS. Overlapping 95% fiducial limits of LC_{50} of the F_1 and $F_1^{\ddagger}$, and dominance values (0.87 for F_1 and 0.92 for $F_1^{\ddagger}$) revealed an autosomal and incomplete dominant flonicamid resistance. The monogenic model of resistance inheritance suggested a polygenic flonicamid resistance. The Flonica-RS strain displayed negative cross-resistance between flonicamid and sulfoxaflor (0.10-fold) or clothianidin (0.50-fold), and very low cross-resistance between flonicamid and flubendiamide (4.71-fold), spinetoram (4.68-fold), or thiamethoxam (2.02-fold) in comparison with the field population. The estimated realized heritability (h^2) value of flonicamid resistance was 0.02. With selection mortality 40–90%, the generations required for a 10-fold increase in LC_{50} of flonicamid were 94–258 at h^2 (0.02) and slope (3.29). Flonicamid resistance was inherited as autosomal, incomplete dominant, and polygenic in the Flonica-RS. Negative or very low cross-resistance between flonicamid and sulfoxaflor, clothianidin, flubendiamide, spinetoram, and thiamethoxam means that these insecticides can be used as alternatives for controlling *M. domestica*. These data can be useful in devising the management for *M. domestica*.

Key words: house fly, insecticide selection, resistance evolution, heritability, vector management

Musca domestica Linnaeus (Diptera: Muscidae), which is commonly known as the house fly, is responsible for the spread of more than one hundred diseases in humans and livestock, including diarrheal diseases (Chavasse et al. 1999, Graczyk et al. 2001, Barin et al. 2010) and avian influenza (Nielsen et al. 2011). Moreover, the larvae of *M. domestica* are voracious feeders, and they damage livestock animals and poultry. Additionally, adults of *M. domestica* decrease the aesthetic worth of livestock commodities, resulting in economic losses (Abbas et al. 2016a). Insecticides are considered very effective for controlling *M. domestica*. However, inappropriate insecticidal applications result in the development of resistance to insecticides, such as

pyrethroid, organophosphate, and new chemistry classess (Scott et al. 2000; Shono et al. 2004; Acevedo et al. 2009; Kaufman et al. 2010; Khan et al. 2013; Abbas et al. 2015b,c), side effects on nontarget natural enemies (Soares et al. 2019), and environmental pollution (Hladik et al. 2014, Ippolito et al. 2015). Therefore, to avoid the problem of resistance and environmental pollution due to the usage of high doses, new insecticides should be included in integrated pest management plans (Abbas et al. 2016a).

Flonicamid, a chordotonal organ modulator (IRAC 2020), is a novel systemic insecticide that shows excellent efficacy for controlling a broad range of insect pests (Morita et al. 2014, Roditakis et al. 2014, Kodandaram et al. 2017). It limits or blocks the feeding activity of insects immediately after treatment and eventually leads to death. Due to a lack of cross-resistance to conventional insecticides and minimal negative impacts on beneficial insects, flonicamid has become the most promising chemical for pest management (Colomer et al. 2011, Jansen et al. 2011, Roditakis et al. 2014, Wang et al. 2018, Jiang et al. 2020). It also has a comparatively safe toxicological profile against birds, bees, fish, and mammals (Taylor-Wells et al. 2018, Wang et al. 2018). Flonicamid has been launched against cotton and rice sucking pests in Pakistan since 2017. Normally, 2–3 numbers of sprays of this insecticide are recommended on cotton and rice (personal communication). This insecticide might be used against *M. domestica* by poultry farmers because in developing countries including Pakistan, the usage of left over amount of insecticides, originally applied for agricultural crop pests, is a common practice for the control of medical pests like *M. domestica* (Khan et al. 2013, Abbas et al. 2015b). Moreover, in Pakistan, most poultry farms are surrounded by agricultural field crops (Abbas et al. 2015b), so indirect exposure of *M. domestica* is also common (Khan 2020). Flonicamid resistance has been reported in *Aphis gossypii* Glover (Hemiptera: Aphididae) (Gore et al. 2013, Koo et al. 2014), *Amrasca devastans* (Distant) (Hemiptera: Cicadellidae) (Abbas et al. 2018), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Roy et al. 2019), and *M. domestica* (Khan 2020).

Resistance risk assessment provides valuable information for devising proactive and effective resistance management strategies, sustaining the susceptibility of insect pests to a particular insecticide (Lai and Su 2011, Shah et al. 2015c). The risk of insecticide resistance and rate of genetic variations can be predicted using estimated realized heritability values following quantitative genetics theory from the selection experiments (Firkoi and Hayes 1990, Tabashnik 1992, Jutsum et al. 1998, Abbas et al. 2016a). The assessment of resistance risk to various insecticides using laboratory selection experiments and realized heritability estimations have been described for *M. domestica* (Abbas and Shad 2015; Shah et al. 2015a,c; Abbas et al. 2016a), *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Ismail et al. 2017, Afzal et al. 2020), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Lai and Su 2011), and *Choristoneura roseceana* (Harris) (Lepidoptera: Tortricidae) (Sial and Brunner 2010).

Analyses of resistance inheritance in terms of the number of genes governed, expression of dominance, and cross-resistance potential of insecticides with similar or different modes of action are crucial for determining their efficacy and informing sustainable pest management (Abbas et al. 2014a,b). Inheritance of resistance and cross-resistance analyses have been conducted for various insecticide-resistant *M. domestica* strains (Abbas et al. 2014a,b, 2015a; Khan et al. 2014a; Shah et al. 2015b, 2017; Ma et al. 2017; Khan 2019) and in other pests, including imidacloprid- and emamectin benzoate-resistant *Dysdercus koenigii* (Fabricius) (Hemiptera: Pyrrhocoridae) (Saeed and Abbas 2020, Saeed et al. 2020). But the inheritance and cross-resistance of flonicamid resistance in *M. domestica* are still unexplored worldwide.

The characterization of flonicamid resistance can help build effective resistance management strategies to sustain the effectiveness of flonicamid. Therefore, we assessed the possibility of *M. domestica* developing resistance to flonicamid, the inheritance pattern of resistance, and the potential for cross-resistance to alternative insecticides. Moreover, we monitored the status of flonicamid resistance in different populations of *M. domestica*.

Materials and Methods

Insecticides

The insecticides used for the bioassay were flonicamid (Ulala 50WG, ICI), flubendiamide (Belt 48SC, Bayer Crop Sciences), sulfoxaflor (Transform 50WG, Dow Agro Sciences), spinetoram (Radiant 120SC, Dow Agro Sciences), clothianidin (Telsta 20SC, FMC), and thiamethoxam (Actara, 25WG, Syngenta).

Musca domestica Populations

Approximately 100–150 *M. domestica* adults of mixed-sex were captured using plastic jars (33 × 19 cm) from nine poultry facilities including Multan (30.1575°N, 71.5249°E), Kot Addu (30.4685°N, 70.9606°E), Taunsa Sharif (30.7046°N, 70.6574°E), Muzaffargarh (30.0736°N, 71.1805°E), Kabirwala (30.4011°N, 71.8631°E), Vehari (30.0442°N, 72.3441°E), Shujabad (29.8717°N, 71.3231°E), Toba Tek Sing (30.9709°N, 72.4826°E), and Lodhran (29.6869°N, 71.6673°E) in Punjab province of Pakistan. An adult diet (sugar + powdered milk at 1 g:1 g ratio) and water-soaked cotton wick were placed in plastic Petri dishes, which were provided to the adults of each population. The adult food was replenished every 2 d, and the cotton wicks were moistened daily using a microsyringe (5 ml). An artificial larval medium (wheat bran 20 g, yeast 5 g, sugar 1.5 g, milk powder 1.5 g, and water 70 ml) in plastic cups (300 ml) was placed in the adult jars to obtain the eggs of the F_1 progeny (Abbas et al. 2014b). The plastic cups containing eggs were removed from the adult jars daily and covered with a muslin cloth to prevent larval escape. When the larvae consumed the food in the plastic cups, they were moved into glass jars containing fresh larval medium to feed until the pupal stage.

The population collected from a poultry farm located in Multan was divided into two lines. The one line was continuously reared in the laboratory for 20 generations without any contact with insecticides, and it was named the susceptible strain (SS). The second line was selected using flonicamid for 20 generations to establish a flonicamid-resistant strain, named Flonica-RS. Each generation (G_2 to G_{21}) was screened using 256–800 ppm concentrations of flonicamid, and these concentrations were screened on the basis of sufficient survival of adult flies to produce the next generation. Two- to three-day-old adults were treated with flonicamid by providing cotton wicks saturated in insecticide solution for every generation of selection. Averagely 449 adults of mixed-sex were screened in each generation. After 72 h of flonicamid treatment, the surviving flies were moved to clean plastic jars to produce the next generation. All populations (field, SS, and Flonica-RS) were reared in the laboratory at 27 ± 2°C temperature, 60% ± 5% RH, and 12:12 (L:D) h photoperiod.

Crosses for Inheritance of Flonicamid Resistance

The reciprocal crosses; F_1 (SS♀ × Flonica-RS♂) and F_1† (SS♂ × Flonica-SEL♀) between the Flonica-RS and SS strains were made to know the dominance level of flonicamid resistance. To determine the number of genes involved in flonicamid resistance, the F_1

progeny was backcrossed with the resistant parents as BC (F_1♀ × Flonica-RS♂). Ten pairs of adults (1:1 ratio) were used in each reciprocal and backcross to produce the offspring (Abbas et al. 2014b). These strains were maintained according to aforementioned rearing protocol and laboratory conditions.

Bioassays

The toxicity of aforementioned insecticides was evaluated using a feeding bioassay following Abbas et al. (2014b) with some modifications. Five to six concentrations (causing mortality from >0 to <100%) were prepared in a 20% sugar solution using serial dilutions. For each bioassay, three replications were made of each concentration. Thirty mixed-sex adults were used for each concentration, and 180 mixed-sex adults (2- to 3-d old) were used for each bioassay, including the control. The adults were moved into an aerated plastic jar (15 × 11 cm) and covered with a muslin cloth to prevent escape. A ~3 cm length of cotton wick soaked in an insecticide solution of each concentration was placed in a Petri dish and placed in each plastic jar for adult feeding. For the control treatment, adults were only exposed to a 20% sugar solution. The cotton wicks were hydrated daily using a microsyringe to prevent them from drying. All bioassay experiments were conducted under the aforementioned laboratory conditions. Mortality was recorded after 72 h of treatment with the insecticides.

Flonicamid Resistance Dominance (D_{LC})

The D_{LC} values of flonicamid resistance were estimated following Bourguet et al. (2000) as:

$$D_{LC} = \frac{(\log LC_{50F_1 \text{ or } F_1\dagger} - \log LC_{50SS})}{(\log LC_{50\ \text{Flonica-RS}} - \log LC_{50\ SS})}$$

If the D_{LC} = 0, it shows complete recessive flonicamid resistance, if the D_{LC} = 1, it shows complete dominant flonicamid resistance, if the D_{LC} >0 and ≤0.5, it shows incomplete recessive flonicamid resistance, and if the D_{LC} >0.5 and <1, it shows incomplete dominant flonicamid resistance.

Frequency of Genes Controlling Flonicamid Resistance

Chi-square analysis of backcross was used to examine the null hypothesis of monogenic resistance inheritance following Tabashnik (1991) as:

$$\chi^2 = \frac{(F - pn)^2}{pqn}$$

'*F*' is the observed response in backcross progeny to a concentration, *n* is the number of *M. domestica* adults exposed to a concentration, *p* is the expected response, and $q = 1 - p$. Significant differences among observed and expected mortalities ($P \leq 0.05$) against three concentrations out of five concentrations would reject the null hypothesis of monogenic resistance.

Realized Heritability (h^2) Estimation

For flonicamid resistance, the realized heritability (h^2) was estimated following Tabashnik (1992):

$$h^2 = R/S$$

Here '*R*' is the selection response to treatment and '*S*' is the selection differential.

'*R*' was determined as:

$$R = [\log(\text{final } LC_{50}) - \log(\text{initial } LC_{50})]/n$$

Here, final LC_{50} is the LC_{50} of flonicamid in the Flonica-RS (G_{21}), and initial LC_{50} is the LC_{50} of flonicamid in the field population (G_1).

'*S*' was determined using an equation:

$$S = i \times \sigma p$$

Here '*i*' is selection mortality and is calculated following Tabashnik and McGaughey (1994):

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

Here, '*p*' is the survivorship of the Flonica-RS after 20 generations of selection.

'σ*p*' was assessed as:

$$\sigma p = \frac{1}{\text{mean slope } (G_2 - G_{21})}$$

The numbers of generations (G) were calculated as:

$$G = \frac{1}{h^2 S}$$

The effect of h^2 and slope on the projected rate of resistance was evaluated between selection intensity and generations at calculated and assumed values of h^2 and slope.

Bioassay Data Analyses

Bioassay data were analyzed following probit analyses by Finney (1971) using POLO PLUS Software (LeOra 2003) to calculate the lethal concentration 50 (LC_{50}), fiducial limits (FLs), and slopes and their standard errors (SEs). The LC_{50} values were considered to be significantly different when their 95% FLs did not overlap (Litchfield and Wilcoxon 1949). Resistance ratios (RRs) were assessed as:

$$\frac{LC_{50} \text{ of insecticide in the Flonica} - \text{RS or field population}}{LC_{50} \text{ of insecticide in the SS}}$$

The confidence limits of RR were calculated following Robertson and Preisler (1992). Resistance and cross-resistance (CR) levels were categorized as very high RR or CR > 100, high RR or CR = 51–100, moderate RR or CR = 21–50, low RR or CR = 11–20, very low RR or CR = 2–10, and no RR or CR = 1 (Abbas et al. 2015b).

Results

Field-Evolved Resistance to Flonicamid in *M. domestica* Populations

Compared with the SS, *M. domestica* populations collected at poultry facilities had very low to high levels of flonicamid resistance. Kabirwala and Lodhran populations had high resistance levels (50.79- to 54.40-fold) while populations collected from Multan, Kot Addu, Vehari, and Shujabad had moderate resistance levels (32.03- to 48.44-fold). Taunsa Sharif and Muzaffargarh populations also had moderate resistance levels (24.41- to 28.50-fold) and the Toba Tek Sing population had very low resistance (4.18-fold) to flonicamid (Table 1).

Flonicamid Resistance Selection

The average survival rate of *M. domestica* adults was 54.43% in G_2–G_{21} generations at different concentrations of flonicamid. The re-selection of the *M. domestica* field population for 20 generations in the laboratory using flonicamid increased flonicamid resistance from 27.12-fold at G_4 to 57.73-fold at G_{21}, in comparison with the SS. The LC_{50} value of flonicamid increased from 507.90 mg/liter with a

Table 1. Field-evolved resistance to flonicamid in *Musca domestica* populations collected from poultries facilities

Location	Insecticide	N[a]	LC_{50} (mg/liter)[b]	Fiducial limits (95%)	Slope ± SE	df	χ^2	*P*	RR (95% CL)[c]
Susceptible (SS)	Flonicamid	180	18.73	0.09–56.74	0.94 ± 0.33	3	0.51	0.92	1.00
Multan	Flonicamid	180	876.63	675.28–1,110.01	3.03 ± 0.55	3	5.99	0.11	46.80 (7.49–292.74)
Kot Addu	Flonicamid	180	599.88	295.68–1,091.54	2.53 ± 0.39	3	4.64	0.20	32.03 (5.10–201.04)
Muzaffargarh	Flonicamid	180	533.72	363.16–729.22	2.21 ± 0.39	3	1.02	0.80	28.50 (4.49–180.92)
Taunsa Sharif	Flonicamid	180	457.20	242.01–856.89	2.63 ± 0.35	3	6.13	0.11	24.41 (3.91–152.40)
Kabirwala	Flonicamid	180	1,018.94	591.01–1,862.83	3.41 ± 0.45	3	6.74	0.08	54.40 (8.74–338.46)
Vehari	Flonicamid	180	632.29	544.37–729.90	8.59 ± 1.82	3	1.06	0.79	33.76 (5.45–209.06)
Shujabad	Flonicamid	180	907.22	548.06–1,940.91	1.13 ± 0.36	3	0.89	0.83	48.44 (7.35–319.05)
Toba Tek Singh	Flonicamid	180	78.25	7.37–164.29	1.16 ± 0.34	3	2.78	0.43	4.18 (0.50–35.00)
Lodhran	Flonicamid	180	951.30	690.57–1,477.05	1.62 ± 0.29	3	6.59	0.09	50.79 (7.95–324.38)

[a]Number of adults exposed in bioassay.
[b]Median lethal concentration, *P* is the probability value, CL is the confidence limit.
[c]Resistance ratio, calculated as LC_{50} of flonicamid in the field population/LC_{50} of flonicamid in the SS.

Table 2. Laboratory selection of flonicamid resistance in the flonicamid-selected strain of *Musca domestica*

Strain (generation)	Conc.	N[a]	Survival (%)	LC_{50} (mg/liter)[b]	FL (95%)	Slope ± SE	χ^2 (df = 3)	*P*	RR (95% CL)[c]
Susceptible (SS)	—	—	—	18.73	0.09–56.74	0.94 ± 0.33	0.51	0.92	1.00
Flonica-RS (G_2)	256	473	37.21	—	—	—	—	—	—
Flonica-RS (G_3)	256	1,385	28.30	—	—	—	—	—	—
Flonica-RS (G_4)	256	1,268	65.06	508.12	222.38–1,177.02	1.31 ± 0.27	3.15	0.37	27.13 (4.23–173.89)
Flonica-RS (G_5)	500	774	56.85	890.62	637.18–1,219.55	2.24 ± 0.45	2.51	0.47	47.47 (7.52–300.48)
Flonica-RS (G_6)	500	384	20.83	—	—	—	—	—	—
Flonica-RS (G_7)	500	439	19.59	1,036.10	849.50–1,230.26	4.95 ± 0.95	2.09	0.55	55.23 (8.90–343.70)
Flonica-RS (G_8)	500	192	43.23	869.54	682.05–1,161.47	2.28 ± 0.34	0.61	0.89	46.42 (7.40–291.33)
Flonica-RS (G_9)	500	121	97.52	—	—	—	—	—	—
Flonica-RS (G_{10})	600	193	92.23	962.95	780.95–1,143.21	5.09 ± 1.01	0.40	0.94	51.41 (8.27–319.55)
Flonica-RS (G_{11})	650	209	30.62	—	—	—	—	—	—
Flonica-RS (G_{12})	650	103	83.50	—	—	—	—	—	—
Flonica-RS (G_{13})	650	435	35.40	671.10	426.31–1,260.56	1.81 ± 0.50	0.90	0.83	35.83 (5.49–233.70)
Flonica-RS (G_{14})	700	1,044	30.65	579.69	298.70–1,287.90	2.41 ± 0.33	6.59	0.10	30.95 (4.95–193.58)
Flonica-RS (G_{15})	700	177	85.71	655.34	320.39–1,162.12	2.19 ± 0.39	3.23	0.36	34.98 (5.53–221.48)
Flonica-RS (G_{16})	700	278	91.73	—	—	—	—	—	—
Flonica-RS (G_{17})	750	375	87.47	1,022.18	767.11–1,501.91	1.89 ± 0.31	1.61	0.66	54.57 (8.61–345.85)
Flonica-RS (G_{18})	800	411	46.96	783.20	474.96–1,090.34	2.46 ± 0.92	3.77	0.29	41.81 (6.36–274.76)
Flonica-RS (G_{19})	800	312	33.65	—	—	—	—	—	—
Flonica-RS (G_{20})	800	169	65.68	942.62	553.86–4,171.79	1.33 ± 0.45	1.92	0.59	50.25 (7.24–351.01)
Flonica-RS (G_{21})	800	233	36.48	1,081.37	905.95–1,303.05	6.95 ± 2.53	5.53	0.14	57.64 (10.04–389.09)
Average		449	54.43	—	—	—	—	—	—

[a]Number of selected individuals.
[b]Median lethal concentration, *P* is the probability value, CL is the confidence limit.
[c]Resistance ratio, calculated as LC_{50} of flonicamid in the Flonica-RS/LC_{50} of flonicamid in the SS.

95% FL 333.66–771.19 at Flonica-RS (G_4) to 1081.37 mg/liter with a 95% FL 905.95–1303.05 at Flonica-RS (G_{21}) (Table 2).

Inheritance Patterns

The LC_{50} values of F_1 and F_1‡ were similar with a 95% overlap in FLs, showing an autosomal trait of flonicamid resistance. The degree of dominance (D_{LC}) values of the F_1 (0.87), F_1‡ (0.92), and BC (0.86) hybrids indicated incomplete dominance of flonicamid resistance (Table 3). The monogenic model of inheritance revealed significant differences between observed and expected mortalities at all tested concentrations ($P < 0.05$), suggesting a polygenic mode of inheritance in the development of flonicamid resistance (Table 4).

Cross-Resistance Patterns

The Flonica-RS strain (G_{21}) showed negative CR between flonicamid and sulfoxaflor or clothianidin in comparison with the field population. Very low CR was found between flonicamid and flubendiamide (8.79-fold), spinetoram (4.68-fold), and thiamethoxam (2.02-fold) in the Flonica-RS strain compared with the field population (Table 5).

Realized Heritability (h^2) and Projected Rate of Flonicamid Resistance

The estimated h^2 of flonicamid resistance was 0.02 in the Flonica-RS (G_{21}) *M. domestica* strain (Table 6). The projected rate of flonicamid resistance is inversely proportional to the slope and directly proportional to h^2. With selection mortality ranges from 40 to 90%, the number of generations required for a 10-fold increase in LC_{50} of flonicamid were 94–258, 16–43, and 9–23 at h^2 values of 0.02, 0.12, and 0.22, respectively, with the same slope of 3.29 (Fig. 1). At different slope values of 1.29, 2.29, and 3.29 with a constant h^2 = 0.02, 37–101, 65–179, and 94–258 generations, respectively, would be needed to increase the 10-fold LC_{50} of

flonicamid in the Flonica-RS *M. domestica* (Fig. 2). This suggests that differences in any variable can change the rate of flonicamid resistance.

Discussion

In the present study, field-evolved resistance to flonicamid was assessed in the *M. domestica* populations collected from poultry facilities in Pakistan. We found varying levels of resistance in *M. domestica* populations in comparison with the SS. Very low levels of resistance to flonicamid were detected in the Toba Tek Singh population, moderate resistance levels were detected in the Multan, Kot Addu, Vehari, Taunsa Sharif, Muzaffargarh, and Shujabad populations, and high resistance levels were detected in the Kabirwala and Lodhran populations. This variability in flonicamid resistance could be due to different selection exposure in different populations (Abbas et al. 2015b,c; Saeed et al. 2018). The most probable reason for flonicamid resistance in *M. domestica* populations may be the direct exposure in the poultry farms and/or indirect exposure through intensive use of this insecticide in the cotton and rice crops for the management of different insect pests (Khan 2020). Previous research on flonicamid resistance in different insect pests has found no to very high resistance (0.50- to 250-fold) in *A. gossypii* (Gore et al. 2013, Koo et al. 2014), no resistance (0.56- to 1.23-fold) in *A. devastans* (Abbas et al. 2018), very low to moderate resistance levels (3.09- to 45.92-fold) in *B. tabaci* (Roy et al. 2019), and very low to low resistance (7.83- to 13.28-fold) in *M. domestica* (Khan 2020).

The reselection of the *M. domestica* field population with flonicamid for 20 generations under laboratory conditions increased only 57-fold resistance than the SS, suggesting that flonicamid resistance evolves slowly in *M. domestica*. In contrast to our findings, very high levels of resistance to different mode of action insecticides developed rapidly in laboratory-selected *M. domestica*, such as a 106-fold increase in resistance to imidacloprid after 13 generations (Abbas et al. 2015a), 445-fold increase in resistance to lambda-cyhalothrin after 26 generations (Abbas and Shad 2015), 430-fold increase in resistance to fipronil after 26 generations (Abbas et al. 2016a), 5254-fold increase in resistance to methoxyfenozide after 44 generations (Shah et al. 2018), and 750-fold increase in resistance to chlorantraniliprole after eight generations (Shah and Shad 2020). It can be concluded that the development of flonicamid resistance in *M. domestica* is not as rapid as the development of resistance to other insecticides. The possible reasons for slow development of flonicamid resistance may be due to association of independent multiple reistance mechanisms and dominant fitness costs (Abbas et al. 2016b). However, further studies on these factors will explore the related phenanemena.

The estimation of realized heritability (h^2) provides information about the risk of developing resistance to any insecticide in laboratory-induced resistant insect pests (Tabashnik 1992, Abbas et al. 2012, Abbas and Shad 2015). In the present study, the low value of h^2 (0.02) after 20 generations of selection suggested low genetic variation and a low tendency for *M. domestica* to develop flonicamid resistance. Similarly, low h^2 values of resistance to fipronil (0.05), lambda-cyhalothrin (0.06), methoxyfenozide (0.17), and pyriproxyfen (0.03) have been observed in resistant strains of *M. domestica* (Abbas and Shad 2015; Shah et al. 2015a,c; Abbas et al. 2016a). In contrast, a high value of h^2 (0.59) of spiromesifen resistance was observed in a spiromesifen-selected strain of *M. domestica* (Alam et al. 2020). In another study, a spinosad-resistant strain of *M. domestica* also had a high h^2 (0.68) of spinosad resistance (Khan et al. 2014b). However, field conditions do not match with laboratory controlled conditions, although the estimated h^2 of flonicamid resistance mediated with laboratory selection has implications for resistance management plans (Tabashnik and McGaughey 1994). The lower h^2 value in the present study suggests that many generations may be needed before *M. domestica* reaches a significant resistance level, although, flonicamid should be used rotationaly for controlling *M. domestica* to retain its efficacy.

An assessment of resistance risks prior to insecticide application is crucial to establish scientific and rational insecticide resistance management plans (Abbas et al. 2016a, Banazeer et al. 2019). The projected rate of resistance assessed based on $G = 1/h^2S$ provides valuable information for the risk of insect pest resistance to insecticides. These results could be employed to develop strategies for delaying the problem of insecticide resistance (Tabashnik 1992, Lai and Su 2011, Abbas et al. 2016a, Ismail et al. 2017). Previously, we reported a projected rate of resistance to fipronil, lambda-cyhalothrin, methoxyfenozide, and pyriproxyfen in *M. domestica* populations (Abbas and Shad 2015; Shah et al. 2015a,c; Abbas et al. 2016a). Our results reveal that there would be 94, 16, and 9 generations needed for a 10-fold increase in flonicamid resistance

Table 3. Dominance of flonicamid resistance in *Musca domestica*

Strain	Insecticide	LC_{50} (mg/liter)	Fiducial limits (95%)	Slope ± SE	RR (95% CL)	D_{LC}
Susceptible (SS)	Flonicamid	18.73	0.09–56.74	0.94 ± 0.33	1.00	
F_1 (SS ♀ × Flonica-RS ♂)	Flonicamid	647.68	392.58–2,368.81	1.07 ± 0.36	34.58 (5.06–236.17)	0.87
$F_1^{\dagger}$ (SS ♂ × Flonica-RS ♀)	Flonicamid	792.46	174.09–1,506.81	2.15 ± 0.52	42.31 (6.76–264.72)	0.92
BC (F_1 ♀ × Flonica-RS ♂)	Flonicamid	622.98	495.69–791.79	2.47 ± 0.34	33.26 (5.32–207.93)	0.86

D_{LC} = degree of dominance; CL = confidence limit; RR = resistance ratio, calculated as LC_{50} of flonicamid in the F1, $F_1^{\dagger}$, or BC/LC_{50} of flonicamid in the SS.

Table 4. Monogenic model of flonicamid resistance inheritance by comparing observed and expected mortalities of backcross

Concentration (mg/liter)	Number of adults exposed	Observed mortality (proportion)	Expected mortality (proportion)	χ^2 (df = 1)	P^a
128	30	2 (0.07)	5 (0.17)	5.84	0.02
256	30	4 (0.13)	6.5 (0.22)	7.96	0.005
512	30	11 (0.37)	11 (0.37)	16.23	<0.0001
1,024	30	24 (0.80)	14.5 (0.48)	25.05	<0.0001
2,048	30	26 (0.87)	29.5 (0.98)	1,667.53	<0.0001

[a]Mortalities were significantly different at $P < 0.05$.

Table 5. Development of cross-resistance to other insecticides in the Flonica-RS of *Musca domestica*

Strain	Insecticide	LC_{50} (mg/liter)[a]	(95% FL)	Slope ± SE	N[b]	df	χ^2	P	RR (95% CL)[c]
Field population	Flubendiamide	33.19	24.98–44.14	1.91 ± 0.29	180	3	2.18	0.07	1.00
Field population	Sulfoxaflor	46.07	25.79–100.50	2.70 ± 0.36	180	3	6.49	0.09	1.00
Field population	Spinetoram	1.08	0.84–1.31	4.33 ± 0.98	180	3	0.75	0.86	1.00
Field population	Clothianidin	2.26	1.63–3.53	3.51 ± 0.55	180	3	3.19	0.36	1.00
Field population	Thiamethoxam	3.74	2.91–4.85	2.06 ± 0.26	210	4	3.12	0.54	1.00
Flonica-RS	Flubendiamide	291.66	221.35–390.78	1.92 ± 0.30	180	3	1.55	0.67	8.79 (5.94–12.99)
Flonica-RS	Sulfoxaflor	4.58	2.28–7.20	1.15 ± 0.22	210	4	0.92	0.92	0.10 (0.06–0.18)
Flonica-RS	Spinetoram	5.05	2.79–8.11	1.18 ± 0.27	180	3	0.41	0.94	4.68 (3.11–7.03)
Flonica-RS	Clothianidin	1.12	0.86–1.41	2.51 ± 0.37	180	3	2.53	0.47	0.50 (0.36–0.68)
Flonica-RS	Thiamethoxam	7.56	6.01–9.49	2.54 ± 0.34	180	3	1.10	0.78	2.02 (1.44–2.84)

[a]Median lethal concentration.
[b]Number of adults exposed in bioassay, P is the probability value, CL is the confidence limit.
[c]Resistance ratio, calculated as LC_{50} of flonicamid in the Flonica-RS/LC_{50} of flonicamid in the field population.

Table 6. Realized heritability (h^2) of flonicamid resistance in the Flonica-RS of *Musca domestica*

Insecticide	Log initial LC_{50}	Log final LC_{50}	G[a]	R[b]	p[c]	i[d]	Mean slope	σp[e]	S[f]	h^2[g]
Flonicamid	2.94	3.03	20	0.005	54.43	0.72	3.29	0.30	0.22	0.02

Initial and final LC_{50s} are in mg/liter.
[a]Number of generations selected with flonicamid.
[b]Selection response.
[c]Average survival of individuals during selection.
[d]Intensity of selection.
[e]Phenotypic variation.
[f]Selection differential.
[g]Realized heritability of flonicamid resistance.

with h^2 = 0.02, 0.12, and 0.22, respectively, at the 90% selection mortality and slope = 3.29. Similarly, there would be 37, 65, and 94 generations needed for a 10-fold increase in flonicamid resistance with slope values of 1.29, 2.29, and 3.29, respectively, at 90% selection mortality and constant h^2 = 0.02. These results suggest that the risk of developing flonicamid resistance is low in *M. domestica*, but there is opportunity to increase flonicamid resistance when h^2 values increase. Therefore, these estimates should be considered tentatively for the judicious use of flonicamid to control *M. domestica*.

Understanding the dominance of the insecticide resistance gene is essential to devising successful resistance management strategies (Bourguet et al. 2000). In the case of complete dominance of resistance, the dominant resistant genes are inherited at three times higher frequencies to the hybrid generations than the recessive genes (Khan et al. 2014a, Saeed et al. 2020). The dominant nature of resistance inheritance has been assumed challenging to manage because the heterozygotes tolerate higher doses of insecticides and would become difficult to kill under field conditions, affecting the durability of insecticides against insect pests (Bourguet et al. 2000, Abbas et al. 2014b, Ma et al. 2017, Saeed and Abbas 2020). However, in the case of the incomplete recessive or dominant resistance trait, dominant resistant genes cannot be passed at higher frequencies to hybrid generations, so this type of resistance can be delayed under field conditions due to the prevalence of susceptible heterozygotes. In this study, the calculated D_{LC} values (0.87 for F_1 and 0.92 for $F_1^{\dagger}$) indicated incomplete dominant resistance to flonicamid in *M. domestica*. This finding is in agreement with incomplete dominant resistance to lambda-cyhalothrin (Abbas et al. 2014b), fipronil (Abbas et al. 2014a), spinosad (Khan et al. 2014b), and imidacloprid (Ma et al. 2017) in *M. domestica*. In contrast to our results, Khan et al. (2014a) found that imidacloprid resistance was inherited incompletely as a recessive trait, while pyriproxyfen resistance was inherited as a completely dominant trait in *M. domestica* (Shah et al. 2015b). Dominant resistant genes can increase the pace of resistance development to any insecticide even if they are rare in the field (Roush and McKenzie 1987). However, in this study, flonicamid resistance showed incomplete dominance but heterozygous individuals tolerated higher doses of flonicamid than the SS. Therefore, flonicamid should be rotated with other insecticides to retain its efficacy in the long term.

Resistance to any insecticide in any insect pest can either be monogenic (controlled by single gene with a major effect) or polygenic (controlled by many genes with a minor effect) (Zhang et al. 2008, Abbas et al. 2014b). The polygenic response of resistance inheritance to any insecticide is more favorable in laboratory-selected individuals due to selection pressure within the population phenotypes with rare variants (McKenzie et al. 1992, Falconer and Mackay 1996). Although it is generally believed that the polygenic nature of insecticide resistance develops slowly and might be diluted speedily by the breeding of susceptible and resistant insects in comparison with the monogenic nature of insecticide resistance (Hoy et al. 1980, Saeed and Abbas 2020, Saeed et al. 2020). In our study, the reciprocal crosses and backcross revealed autosomal and polygenic flonicamid resistance in *M. domestica*. Similarly, polygenic and autosomal inheritance of resistance to different insecticides has been determined for *M. domestica* (Abbas et al. 2014a,b; Khan et al. 2014a; Shah et al. 2015b, 2018). Contrary to our results, a beta-cypermethrin-resistant strain of *M. domestica* has demonstrated an autosomal and monogenic nature of resistance to beta-cypermethrin under laboratory selection pressure (Zhang et al. 2008).

The occurrence of CR affects the potency of any insecticide against insect pests and insecticide resistance management plans. Analyses of CR to other insecticides in the resistant strains could help in scrutinizing the insecticides that have no CR and should be used as rotation for resistance management (Abbas et al. 2014a, 2015a; Afzal et al. 2020; Saeed and Abbas 2020; Saeed et al. 2020). The CR analyses of the Flonica-RS of *M. domestica* in the present study showed negative CR between flonicamid and sulfoxaflor or clothianidin, and very low CR between flonicamid and flubendiamide, spinetoram, or thiamethoxam in comparison with the field population. Selection using flonicamid suggests that the toxicity of sulfoxaflor and clothianidin increased in the Flonica-RS of *M. domestica*. Cross-resistance to these insecticides was unexpected because of different modes of action insecticide classes (IRAC

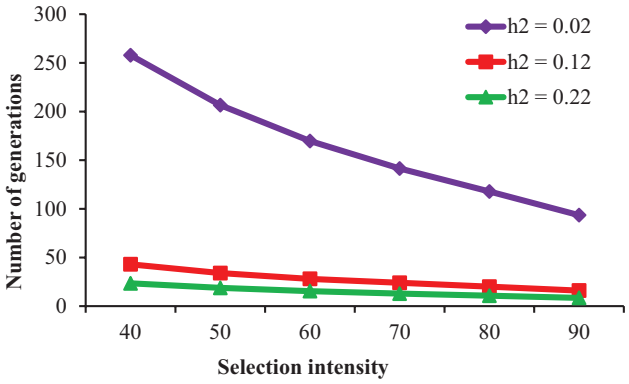

Fig. 1. Effect of heritability on the number of generations of *Musca domestica* needed for a 10-fold increase in LC_{50} of flonicamid at different selection intensities and slope (3.29).

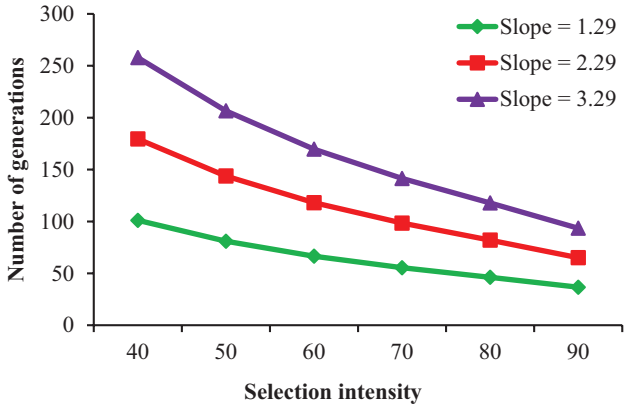

Fig. 2. Effect of slope on the number of generations of *Musca domestica* needed for a 10-fold increase in LC_{50} of flonicamid at different selection intensities and h^2 (0.02).

2020). To date, there has been no record of CR between flonicamid and other insecticides in any flonicamid-resistant strain of insect pest globally. A thiamethoxam-resistant strain of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) has very low CR with flonicamid (Naeem et al. 2019). Conversely, an imidacloprid-resistant strain of *A. gossypii* has high CR with flonicamid (Koo et al. 2014). Negative or very low levels of CR between flonicamid and sulfoxaflor, clothianidin, flubendiamide, spinetoram, or thiamethoxam offers an ultimate situation for these insecticides to rotate with flonicamid to eliminate the resistance problem in *M. domestica*.

In conclusion, insecticide resistance management strategies should be developed for flonicamid to sustain its efficacy for a longer duration. These strategies could include integrated pest management tools, such as biological and cultural control, and reduced selection pressure of this insecticide (Abbas et al. 2016a, Helps et al. 2017). The results of polygenic, incomplete dominant and autosomal mode of inheritance and low h^2 values provide favorable clues for the management of flonicamid resistance. Negative or very low levels of CR between flonicamid and sulfoxaflor, clothianidin, flubendiamide, spinetoram, or thiamethoxam make the rotational use of these insecticides a simple solution for overcoming the resistance problem and environmental pollution. Moreover, flonicamid resistance should be assessed regularly to enable this insecticide to keep controlling *M. domestica*.

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