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Resistance Status of *Musca domestica* L. Populations to Neonicotinoids and Insect Growth Regulators in Pakistan Poultry Facilities

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Abstract.- House flies, *Musca domestica* L. (Diptera: Muscidae) are mechanical vectors of many diseases on poultry facilities and have the ability to develop resistance to different insecticides. The aim of the present study was to assess the resistance status of house flies to neonicotinoids and insect growth regulators (IGR) from poultry facility populations in Punjab, Pakistan. *M. domestica* populations from five poultry facilities were studied for their resistance status to selected neonicotinoids and IGRs. For three neonicotinoids, the range of resistance ratio was 4.9-16-fold for acetamiprid, 2-14-fold for imidacloprid and 9.7-35-fold for nitenpyram when compared with the susceptible population. For four IGRs, the range of resistance ratio was 0.3-6.6-fold for pyriproxyfen, 0.8-18-fold for cyromazine, 1-22-fold for lufenuron and 1-14-fold for methoxyfenozide. Positive significant correlations were found among the toxicities of acetamiprid, nitenpyram and cyromazine. Regular insecticide resistance monitoring and integrated management plans on poultry facilities are required to prevent resistance development, field control failures and environmental pollution.

Key words: House fly, imidacloprid, acetamiprid, nitenpyram, pyriproxyfen, cyromazine, lufenuron, methoxyfenozide.

INTRODUCTION

House fly, *Musca domestica* L. (Diptera: Muscidae) is pest of poultry (Scott *et al.*, 2000) and transmits more than 100 pathogens such as protozoan, bacterial, helminthic and viral agents (Förster *et al.*, 2007; Khan *et al.*, 2012; Abbas *et al.*, 2014). *M. domestica* are also mechanical vectors of zoonotic diseases on poultry *e.g.*, *Campylobacter, Salmonella* and avian influenza (Hald *et al.*, 2007, 2008; Nielsen *et al.*, 2011). Poultry manure which is exposed to high humidity and temperature provides an ideal condition for *M. domestica* growth in poultry farms (Khan *et al.*, 2012). High densities of *M. domestica* irritate and stress poultry workers, hens and reduce the value of poultry products (Acevedo *et al.*, 2009).

Pesticide resistance is a major problem in the management of agricultural and public health pests (Scott *et al.*, 2000; Saleem *et al.*, 2010) and results in response to increased frequency and application rates of insecticides. This resistance development is also promoted by a number of biological traits such as adaptability to different environments, high fecundity, short developmental time and cross resistance (Kaufman *et al.*, 2010a). Ultimately, the effectiveness of insecticides is lost. Therefore, resistance management programs are necessary to decrease the use of pesticides and limit their harmful impacts on human and environment. Producers in Pakistan typically have no concern about the specificity of pesticides and use left-over pesticides for the control of ticks, fleas, and flies in poultry farms. Moreover, chemical control practices are used on the basis of a farmer's own or other local fellow producer's experiences without asking entomologists or veterinarians. Such type of practices can exert selection pressures on different insect pests exposed to insecticides on or around poultry.

Numerous scientific reports regarding resistance to carbamates, organophosphates, pyrethroids and newly developed chemical insecticides in *M. domestica* are available worldwide (Scott *et al.*, 2000; Kristensen and Jespersen, 2003; Kristensen *et al.*, 2004; Deacutis *et al.*, 2006; Acevedo *et al.*, 2009; Kaufman *et al.*, 2010b; Memmi, 2010; Khan *et al.*, 2013; Abbas *et*

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al., 2015b). Resistance to neonicotinoids (imidacloprid) and IGR's (pyriproxyfen, cyromazine) have been reported previously in *M. domestica* (Tang *et al.*, 2002; Acevedo *et al.*, 2009; Bell *et al.*, 2010; Khan *et al.*, 2014; Abbas *et al.*, 2015a; Shah *et al.*, 2015). In the present study, we monitored the susceptibility of *M. domestica* populations from five poultry facilities in Punjab, Pakistan to neonicotinoid and IGR insecticides. The purpose of our work was to provide baseline data for future monitoring efforts and to define a control strategy for resistance management of *M. domestica* in poultry facilities.

MATERIALS AND METHODS

Insects and rearing

About 200 adults of *M. domestica* were collected from each poultry farm by sweep-netting located in five cities of Punjab, Pakistan: Multan (30°5'11N, 71°39'15E), Khanewal (32°4'50N, 73°38'10E), Lodhran (29°15'25N, 71°32'60E), Muzaffar Garh (30°4'0N, 71°12'0E) and Bhakar (31°37'60N, 71°4'0E). These collected flies from different locations were brought to the laboratory and reared separately in meshed plastic jars (34×17cm). Adults were kept in meshed plastic jars and powdered milk mixed with sugar (1:1) was provided as food. A cotton wick soaked with water was provided in a separate Petri dish. Cotton wicks were hydrated with water by micro syringe daily to prevent drying. Larvae were reared on artificial diet (Abbas *et al.*, 2014). All insects were reared at laboratory conditions (Abbas *et al.*, 2014). Adults of F_1 generation of field population were used for toxicological bioassays. The susceptible strain was collected from an urban area (Multan) where no insecticide was used in 2011 and cultured in the laboratory without exposure to insecticides for 27 generations.

Insecticides

Commercial formulated insecticides were used for bioassays including acetamiprid (Mospilan® 70WP, Arysta Life Sciences), imidacloprid (Confidor® 20SL, Bayer Crop Science), nitenpyram (Paranol® 10EC, Kenzo Agro Chemicals), pyriproxyfen (Admiral® 10EC, FMC), cyromazine (Trigard® 75WP, Syngenta), lufenuron (Match® 050EC, Syngenta) and methoxyfenozide (Runner® 240SC, Arysta Life Sciences).

Adult bioassays

The toxicities of the neonicotinoid insecticides were determined by using a feeding bioassay (Kaufman *et al.*, 2006). Following Marçon *et al.* (2003), ten 2-3-day-old randomly collected both sex flies were placed into plastic jars (14×9cm) and provided a 3cm cotton wick piece moistened with 20% sugar solution having different concentrations of insecticides. Five concentrations were prepared as serial dilutions for each insecticide and each concentration was replicated three times. A total of 180 flies including control were used for each bioassay. Cotton wicks soaked in 20% sugar solution without insecticide were provided to control flies. Cotton wicks were hydrated with tap water at 24 h and 48 h to prevent from drying (Kaufman *et al.*, 2006). All treated flies were kept at standard laboratory conditions mentioned previously. Mortality was assessed at 72 h after treatment. Ataxic flies were assumed as dead.

Larvicidal bioassays

A diet incorporation method was used to determine the toxicities of IGRs (Kristensen and Jespersen, 2003). The larval rearing medium containing wheat bran, grass meal, yeast, powdered milk, and sugar (20:5:5:1.5:1.5 ratio by weight) was treated with different concentrations of insecticides. Five concentrations for each bioassay were prepared through serial dilution and each concentration was replicated three times. Ten first instar larvae were used in each replication and thirty larvae were used in one concentration. A total of 180 larvae were used for each bioassay and kept at standard laboratory conditions as described above. Three weeks after bioassay, the data of adult emergence was recorded (Cetin *et al.*, 2006). All the larvae that were unable to develop into adults were considered dead.

Data analysis

Control mortality was corrected by the Abbott's formula (Abbott, 1925), if occurred. The concentration response data was analyzed by probit

analysis (Finney, 1971) with POLO software (Software, 2005) to determine the LC_{50} values, their standard errors, slopes and 95% confidence intervals (CI). Resistance Ratio (RR) and its 95% CI were determined by dividing the LC_{50} value and its 95% CI of test strains with the LC_{50} of the susceptible strain (Ban *et al.*, 2012). LC_{50} values of populations were not significantly different if their 95% CI overlapped. The RR was considered significantly different if their 95% CI did not overlap with value of 1 (Robertson and Preisler, 1992). An alpha level of 0.05 was used for all comparisons. The levels of insecticide resistance were scaled on following criteria: no resistance (RR = <2), very low resistance (RR = 2-10), low resistance (RR = 11-20), moderate resistance (RR = 21-50), high resistance (RR = 51-100) and very high resistance (RR > 100).

RESULTS

Resistance to neonicotinoids in Musca domestica

Acetamiprid

Very low to low level resistance to acetamiprid was observed in tested *M. domestica* populations (Table I). Populations collected from Multan, Khanewal, Lodhran and Bhakar had very low resistance levels and a population collected from Muzaffar Garh had a low resistance level when compared with the susceptible population. Slopes of all the tested populations against acetamiprid were similar when compared with the susceptible population.

Imidacloprid

No to low resistance levels to imidacloprid were observed in tested *M. domestica* populations (Table I). Populations collected from Multan and Khanewal had no resistance levels, populations collected from Bhakar and Muzaffar Garh had very low resistance levels (5.3 and 7.5-fold) and a population from Lodhran had a low resistance level (14-fold) when compared with the susceptible population. Slopes of all the tested populations against imidacloprid were steeper than that of the susceptible population.

Nitenpyram

Very low to moderate resistance levels to nitenpyram were observed in tested *M. domestica* populations (Table I). Populations collected from Multan and Khanewal had very low resistance levels (9.7 to 9.8-fold), a population collected from Bhakar had a low resistance level (12-fold) and populations collected from Lodhran and Muzaffar Garh had moderate resistance levels (22 to 35-fold) when compared with the susceptible population. Slopes of all the tested populations against nitenpyram were similar when compared with the susceptible population.

Resistance to insect growth regulators in Musca domestica

Pyriproxyfen

No to very low resistance levels to pyriproxyfen were observed in tested *M. domestica* populations (Table II). Populations collected from Multan, Muzaffar Garh and Khanewal had very low resistance levels (5.9 to 6.6-fold) and populations collected from Lodhran and Bhakar had no resistance levels when compared with the susceptible population. Slopes of Multan, Khanewal, Lodhran and Bhakar populations tested against pyriproxyfen were shallower than that of the susceptible population. However, slope of Muzaffar Garh population was similar.

Cyromazine

No to low resistance levels to cyromazine were observed in tested *M. domestica* populations (Table II). Populations collected from Khanewal and Multan had very low resistance levels (5.5-6.5-fold), populations collected from Lodhran and Bhakar had no resistance levels and a population collected from Muzaffar Garh had a low resistance level (18-fold) when compared with the susceptible population. Slopes of Multan, Khanewal, Lodhran and Bhakar populations were similar than that of the susceptible population. However, slope of Muzaffar Garh was steeper.

Lufenuron

No to moderate levels of resistance to lufenuron were observed in tested *M. domestica* populations (Table II). Populations collected from Multan and Khanewal had moderate resistance levels (22-fold) and populations collected from

Table I.- Toxicity of neonicotinoids to adults of *Musca domestica* from five poultry facilities in Punjab, Pakistan.

Insecticide	Population	N	LC_{50} (95% CI) [ppm]	Slope (±SE)	RR (95% CI)
Acetamiprid	Susceptible	180	3.2 (2.4-4.2)	2.6 ±0.4	1
	Multan	180	16 (7.7-22)	2.1 ±0.5	4.9 (2.4-6.9)*
	Khanewal	180	18 (9.2-25)	2.0 ±0.5	5.6 (2.9-7.9)*
	Lodhran	180	25 (19-32)	2.3 ±0.3	7.8 (6.0-9.9)*
	Muzaffar Garh	180	50 (39-64)	2.3 ±0.3	16 (12-20)*
	Bhakar	180	17 (14-21)	3.0 ±0.4	5.3 (4.3-6.5)*
Imidacloprid	Susceptible	180	6.7 (3.8-19)	0.9 ±0.3	1
	Multan	180	13 (9.9-16)	2.8 ±0.5	2.0 (1.5-2.4)
	Khanewal	180	14 (9.6-18)	2.2 ±0.4	2.0 (1.4-2.7)
	Lodhran	180	97 (67-184)	1.6 ±0.3	14 (9.9-27)*
	Muzaffar Garh	180	50 (39-64)	2.3 ±0.3	7.5 (5.8-9.5)*
	Bhakar	180	35 (26-54)	1.8 ±0.3	5.3 (3.9-8.1)*
Nitenpyram	Susceptible	180	2.5 (1.8-3.3)	2.0 ±0.3	1
	Multan	180	25 (18-32)	2.5 ±0.4	9.7 (7.1-13)*
	Khanewal	180	25 (15-34)	1.8 ±0.3	9.8 (6.0-14)*
	Lodhran	180	55 (44-72)	2.5 ±0.4	22 (17-28)*
	Muzaffar Garh	180	89 (71-115)	2.4 ±0.3	35 (28-46)*
	Bhakar	180	30 (22-45)	1.7 ±0.3	12 (8.7-18)*

Resistance ratio (RR) = LC_{50} of poultry population / LC_{50} of Susceptible population.
* Significantly different from 1.0, when 95 % CI did not overlap (Robertson and Preisler, 1992).

Lodhran, Muzaffar Garh and Bhakar had no resistance levels when compared with the susceptible population. Slopes of Multan, Khanewal, Lodhran and Bhakar populations tested for lufenuron were similar than that of the susceptible population. However, slope of Muzaffar Garh population was shallower.

Methoxyfenozide

No to low resistance levels to methoxyfenozide were observed in tested *M. domestica* populations (Table II). Populations collected from Multan and Muzaffar Garh had low resistance levels (14-fold), populations collected from Lodhran and Khanewal had very low resistance levels (2.8-7.4-fold) and a population collected from Bhakar had a no resistance level when compared with the susceptible population. Slopes of Multan, Khanewal, Lodhran and Muzaffar Garh populations were similar than that of the susceptible population. However, slope of Bhakar population was shallower.

Pairwise correlation of different insecticides

The toxicity of acetamiprid had a significant positive correlation with nitenpyram and cyromazine and non-significant positive correlation with imidacloprid, pyriproxyfen and methoxyfenozide; however negatively correlated with lufenuron. The toxicity of imidacloprid showed non-significant negative correlation with IGRs and similarly non-significant positive correlation with nitenpyram. The toxicity of nitenpyram had non-significant positive correlation with pyriproxyfen, cyromazine and methoxyfenozide and non-significant negative correlation with lufenuron. The toxicity of pyriproxyfen had significant positive correlation with methoxyfenozide and non-significant positive correlation with cyromazine and lufenuron. The toxicity of cyromazine was negatively correlated with lufenuron and positively non-significantly correlated with methoxyfenozide. The toxicity of lufenuron was positively non-significantly correlated with methoxyfenozide (Table III).

DISCUSSION

The current study was performed to evaluate the resistance of *M. domestica* collected from five

Table II.- Toxicity of IGRs to larvae of *Musca domestica* from five poultry facilities in Punjab, Pakistan.

Insecticide	Population	N	LC_{50} (95% CI) [ppm]	Slope (±SE)	RR (95% CI)
Pyriproxyfen	Susceptible	180	0.01 (0.007-0.01)	2.5 ±0.5	1
	Multan	180	0.07 (0.03-0.1)	1.8 ±0.4	5.9 (2.9-8.6)*
	Khanewal	180	0.07 (0.04-0.1)	1.8 ±0.4	6.6 (3.5-8.6)*
	Lodhran	180	0.03 (0.01-0.04)	1.4 ±0.3	2.4 (0.7-4)
	Muzaffar Garh	180	0.07 (0.05-0.09)	2.4 ±0.4	6.4 (4.8-8)*
	Bhakar	180	0.003 (0.00-0.01)	1.1 ±0.3	0.3 (0.00-0.7)
Cyromazine	Susceptible	180	0.006 (0.001-0.01)	1.3 ±0.4	1
	Multan	180	0.04 (0.02-0.05)	2.0 ±0.4	6.5 (3.8-8.8)*
	Khanewal	180	0.03 (0.02-0.05)	1.8 ±0.4	5.5 (2.7-7.8)*
	Lodhran	180	0.02 (0.01-0.03)	1.7 ±0.4	3.3 (1.0-5.7)
	Muzaffar Garh	180	0.11 (0.08-0.14)	2.1 ±0.3	18 (13-23)*
	Bhakar	180	0.005 (0.00-0.02)	0.9 ±0.3	0.8 (0.00-3.3)
Lufenuron	Susceptible	180	0.005 (0.001-0.01)	1.6 ±0.5	1
	Multan	180	0.11 (0.05-0.2)	1.3 ±0.3	22 (10-32)*
	Khanewal	180	0.1 (0.07-0.2)	1.6 ±0.3	22 (14-32)*
	Lodhran	180	0.01 (0.003-0.03)	1.1 ±0.3	2 (0.6-6)
	Muzaffar Garh	180	0.01 (0.00-0.02)	0.7 ±0.3	1.8 (0.00-4)
	Bhakar	180	0.005 (0.00-0.01)	0.9 ±0.3	1 (0.00-2)
Methoxyfenozide	Susceptible	180	0.1 (0.06-0.14)	2.0 ±0.4	1
	Multan	180	1.4 (1.0-2.6)	1.6 ±0.3	14 (9.6-26)*
	Khanewal	180	0.7 (0.5-1.2)	1.4 ±0.3	7.4 (5.2-12)*
	Lodhran	180	0.3 (0.2-0.4)	2.0 ±0.4	2.8 (1.6-3.9)*
	Muzaffar Garh	180	1.4 (1.0-2.1)	1.4 ±0.3	14 (9.8-21)*
	Bhakar	180	0.1 (0.01-0.2)	1.1 ±0.3	1 (0.1-2.4)

Resistance ratio (RR) = LC_{50} of field collected population / LC_{50} of Susceptible population.
* Significantly different from 1.0, when 95 % CI did not overlap (Robertson and Preisler, 1992).

Table III.- Pairwise comparison of correlation coefficients of LC_{50} values of tested insecticides to the field populations of *Musca domestica.*

Insecticide	Acetamiprid	Imidacloprid	Nitenpyram	Pyriproxyfen	Cyromazine	Lufenuron
Imidacloprid	0.4*					
Nitenpyram	1.0**	0.6*				
Pyriproxyfen	0.3*	-0.4*	0.2*			
Cyromazine	0.9**	-0.002*	0.8*	0.7*		
Lufenuron	-0.5*	-0.8*	-0.7*	0.6*	-0.1*	
Methoxyfenozide	0.5*	-0.4*	0.3*	0.9**	0.8*	0.4*

* P >0.05, ** P <0.05

poultry facilities in Punjab, Pakistan to neonicotinoid and IGR insecticides. No to very low levels of resistance to all insecticides were observed in most poultry collected *M. domestica* populations. Valles *et al.* (1997) suggested that insect populations should be considered resistant if these develop tenfold resistance to insecticides. There was less than 10-fold resistance in *M. domestica* to acetamiprid in four populations, imidacloprid in four populations, nitenpyram in two populations, pyriproxyfen all five populations, cyromazine four populations, and lufenuron and methoxyfenozide three populations. These were considered tolerant rather than resistant. Previous studies demonstrated

that resistance to these insecticides occurs in *M. domestica* worldwide (Zhang *et al.*, 1997; Scott *et al.*, 2000; Kaufman *et al.*, 2006, 2010b; Acevedo *et al.*, 2009; Bell *et al.*, 2010; Pezzi *et al.*, 2011). However, this is the first report of resistance status of *M. domestica* populations to neonicotinoids and IGRs from poultry facilities in Punjab, Pakistan. Pair wise correlation coefficient comparisons of resistance for field populations showed significant positive correlations between acetamiprid and nitenpyram or cyromazine and between pyriproxyfen and methoxyfenozide, suggesting the presence of common resistance mechanism for the insecticides having different modes of action. The insecticides tested in this experiment has different action sites, therefore resistance to these insecticides might be due to multiple resistance mechanism (Ahmad *et al.*, 2008). Further studies are required to confirm the mechanism of resistance in the laboratory selected populations of *M. domestica* with representative insecticides.

The neonicotinoids were introduced in the late 1990s and have been widely used for control of different pests of field crops, dairies and poultry facilities worldwide (Kaufman *et al.*, 2010b; Basit *et al.*, 2011; Khan *et al.*, 2013). However, resistance to neonicotinoids has been reported in various pests such as *Bemisia tabaci* Gennadius (Elbert and Nauen, 2000; Basit *et al.*, 2011), *Leptinotarsa decemlineata* Say (Mota-Sanchez *et al.*, 2006), *Plutella xylostella* Linnaeus (Sayyed and Crickmore, 2007), *Spodoptera litura* Fabricius (Abbas *et al.*, 2012), *Nilaparvata lugens* Stål (Wang *et al.*, 2008) and *M. domestica* (Kaufman *et al.*, 2010a,b). In Pakistan, the poultry farmers use left-over pesticides from crop farming for the management of *M. domestica* (observational data). Therefore, the resistance to nitenpyram might be due to overuse of this insecticide for the control of *M. domestica* in poultry farms. However, low levels of resistance to imidacloprid and acetamiprid are due to the effectiveness of these insecticides against *M. domestica* in poultry farms.

IGRs were introduced in the late 1970s and have been widely used for control of dipteran pests worldwide. IGRs are commonly used as larvicide for the control of *M. domestica* in livestock units (Scott *et al.*, 2000; Bell *et al.*, 2010). Pyriproxyfen, a juvenile hormone mimic, is potential inhibitor of pupal adult metamorphosis (Seng *et al.*, 2008). Pyriproxyfen is highly selective to target organisms with lower mammalian toxicity and used in integrated pest management of different pests including *M. domestica* worldwide (Geden and Devine, 2012). However, resistance to pyriproxyfen is reported in *B. tabaci* (Ghanim and Kontsedalov, 2007; Ma *et al.*, 2010) and *M. domestica* (Zhang *et al.*, 1997) from different parts of the world. In this study, no to very low levels of resistance to pyriproxyfen were observed in *M. domestica* populations collected from five poultry facilities. Cyromazine, a moulting disruptor insecticide has been used extensively for the control of dipteran pests including *M. domestica*. Resistance to cyromazine has previously been reported in *M. domestica* (Tang *et al.*, 2002; Kristensen and Jespersen, 2003; Acevedo *et al.*, 2009; Bell *et al.*, 2010). In this study, no to low levels of resistance to cyromazine were observed in *M. domestica* populations collected from five poultry facilities. The low level of resistance may be the result of low usage of these insecticides or due to the presence of independent mechanism (Ahmad *et al.*, 2008; Khan *et al.*, 2013). Further studies are required to confirm this mechanism by selecting the population in the laboratory. The experiments on the selection of *M. domestica* with pyriproxyfen are currently under evaluation in our laboratory to study mechanisms of resistance in the regional population.

Lufenuron, a chitin synthesis inhibitor insecticide and have been effectively used against different insect pests. However, some insect pests have developed resistance to this insecticide such as *Drosophila melanogaster* Meigen (Wilson and Cain, 1997) and *S. litura* (Ahmad *et al.*, 2008). In this study, no to moderate resistance levels were observed to lufenuron in *M. domestica* collected from five poultry farms. Methoxyfenozide acts as ecdysone agonist and is used extensively for the management of various insect pests. Resistance to methoxyfenozide has been reported in *Spodoptera exigua* Hübner (Moulton *et al.*, 2002; Gore and Adamczyk Jr, 2004; Mosallanejad *et al.*, 2008) and *S. litura* (Ahmad *et al.*, 2008; Shad *et al.*, 2012). In this study, no to low resistance levels to methoxyfenozide were observed in *M. domestica*

populations collected from five poultry facilities. Most poultry farms are surrounded by field crops in Pakistan. The most probable reason of resistance to lufenuron and methoxyfenozide is heavy usage of these insecticides by the farmers in Pakistan. Such practices results in overuse of these pesticides and might be a major cause of resistance development in poultry pests.

The levels of resistance varied significantly from one facility to another, and generally depend upon the use of insecticides at that facility (Scott *et al.*, 2000) as occurred in present results. The poultry farmers practice chemical approaches for the management of poultry pests on opportunistic and/or hit and trial basis. Thus, the occurrence of insecticide resistance in poultry pests including *M. domestica* is inevitable. No to very low levels of resistance to acetamiprid, imidacloprid, pyriproxyfen and cyromazine suggest that these insecticides are still effective in Pakistan poultry facilities for the control of *M. domestica*. However, the inappropriate and injudicious use of these insecticides with improper application techniques could lead to the development of resistance that might be more severe in the future. Therefore, a systematic plan is needed for the management of poultry pests including flies to delay the development of resistance and to retain efficacy of insecticides for a long period.

Some resistance management strategies such as restricted use of insecticides to which resistance has developed, insecticide mixtures and rotation of insecticides with different mode of action could be helpful for resistance management in *M. domestica* (Kaufman *et al.*, 2001; Memmi, 2010). Monitoring of poultry facility *M. domestica* populations for insecticide resistance should continue for currently used and newly developed insecticides to check the effectiveness of insecticides. Furthermore, a survey of poultry farmer's knowledge, attitudes and practices is necessary for the systematic management of poultry pests. In addition, due to the use of inappropriate pesticides with incorrect application rates, the use of adulterated pesticide formulations and the use of ineffective application methods in Pakistan, the training of poultry farmers have been essential to decrease the development of insecticide resistance in *M. domestica* populations in poultry facilities of Punjab, Pakistan.

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