RESEARCH NOTE

Studies on genetics, stability and possible mechanism of deltamethrin resistance in *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) from Pakistan

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Introduction

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) is a serious worldwide polyphagous pest causing serious damage to the cotton crop. This study was conducted to investigate the mode of inheritance and possible mechanism of deltamethrin resistance in *P. solenopsis*. After 10 rounds of selection (G_3–G_{12}) with deltamethrin, *P. solenopsis* had a 740-fold level of resistance compared to the laboratory susceptible population (lab pop). The 740-fold resistant strain was reared further for next seven generations (G_{13}–G_{19}) without exposure to deltamethrin and bioassayed at G_{20} which revealed that the resistance was unstable. There was no significant difference in the LC_{50} values of progenies of both reciprocal crosses (F_1 and F_1') and their degree of dominance values were 0.63 and 0.71, respectively. Monogenic model of inheritance and Lande's method revealed that more than one factor was involved in deltamethrin resistance. Bioassays, piperonyl butoxide expressed synergism with deltamethrin but S,S,S-tributyl phosphorotrithioate did not. Hence, deltamethrin resistance in *P. solenopsis* is unstable, autosomal, incompletely dominant, polygenic and mono-oxygenases based. These results provide basic information for designing and planning fruitful management programmes to control *P. solenopsis*.

Deltamethrin is a pyrethroid that causes inhibition of activated voltage-sensitive sodium channels in the axon of neurons. This results in prolonged permeability of the nerve to sodium ions and produces a series of repetitive nerve signals in sensory organs, nerves and muscles and ultimately death (Joy 1994). Deltamethrin is broad spectrum insecticide (Tomlin 2006) registered for the control of different pests of economic importance on cotton (http://www.toxipedia.org/display/toxipedia/Deltamethrin). In the Indo-Pakistan subcontinent, the broad spectrum use of conventional insecticides such as pyrethroids, organochlorines and organophosphates creates an ideal atmosphere for insecticide resistance development in insect pests (Ahmad *et al.* 2007).

Cotton mealybug, *P. solenopsis* (Homoptera: Pseudococcidae) has been a major pest in cotton in India since 2003–2004 (Jhala *et al.* 2008) and in Pakistan, since 2005 (Abbas *et al.* 2007). *P. solenopsis* feeds and reproduces on many host plants and has adapted to various biotic and abiotic environmental factors (Vennila *et al.* 2013). *P. solenopsis* is a serious threat to sustainable cotton production in cotton growing areas of Pakistan (Afzal *et al.* 2015a) and has been reported from 35 locations in different ecological zones in the world (Ben-Dov *et al.* 2009). *P. solenopsis* is phloem feeder (Meyerdirk *et al.* 2001). The pest sucks cell sap from the cotton plant (Miller and Gimpel 2006; Saeed *et al.* 2007), excretes honeydew (Godfrey *et al.* 2002) and inserts toxic saliva into the plant (Williams and Granara de Willink 1992). The black sooty mould that grows on honeydew interferes with photosynthesis (Saeed *et al.* 2007). Infested plants are stunted and may die as well (Lysandrou *et al.* 2012).

Pesticide resistance is a genetic phenomenon and a complete understanding of resistance development requires knowledge on how it is inherited (Roush and Daly 1990). It is very important to study types of resistance inheritances in the laboratory for every insecticide used in the field against different pests because it helps to develop a better resistance management strategy in the field (Abbas *et al.* 2014). The genetics of resistance to various insecticides in different insects has been studied extensively (Germano *et al.* 2010; Shi *et al.* 2011). The genetics of deltamethrin resistance has

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been reported in *Cydia pomonella* Linnaeus (Bouvier *et al.* 2001), *Plutella xylostella* Linnaeus (Sayyed *et al.* 2005), *Triatoma infestans* Klug (Germano *et al.* 2010; Gomez *et al.* 2015) and *Spodoptera litura* Fabricius (Ahmad *et al.* 2007).

It is important to understand a mechanism by which an insect develops resistance to an insecticide. The use of synergists to study mechanisms of insecticide resistance is well-documented in different insects (Ahmad *et al.* 2007; Ishtiaq *et al.* 2012; Askari-Saryazdi *et al.* 2015), including *P. solenopsis* (Afzal and Shad 2015; Afzal *et al.* 2015b). Traditionally, synergism studies have been used to identify possible resistance mechanisms (Askari-Saryazdi *et al.* 2015). Four general mechanisms for insecticide resistance have been identified in insects. They are metabolic detoxification, reduced penetration of toxicants, target site mutations and behavioural resistance (Brattsten *et al.* 1986). As deltamethrin is widely used by farmers against *P. solenopsis* in cotton fields, it is important to study possible mechanisms of deltamethrin resistance in *P. solenopsis*.

There is a need to study the inheritance pattern and possible mechanisms of resistance to deltamethrin in *P. solenopsis* due to its extensive use in the field against sucking and chewing pests of cotton. This study was designed to investigate the mode of inheritance and possible mechanisms of deltamethrin resistance in *P. solenopsis*. The study will help in formulating rational resistance management tactics against this invasive species.

Materials and methods

Insects

P. solenopsis population (∼200–400 individuals) was collected from cotton fields of the Central Cotton Research Institute (CCRI) in the Multan region of Punjab, Pakistan. The field-collected population was continuously reared on China rose, *Hibiscus rosasinensis* leaves and its soft branches under laboratory conditions at 27±2°C and 60±5% relative humidity with a 14 : 10 h light : dark photoperiod using the method of Afzal *et al.* (2015a).

Insecticide

Deltamethrin (Decis Super, 10 EC; Bayer Crop Sciences, Pakistan) was used for bioassays and selection of *P. solenopsis*.

Concentrations, response bioassays and selection

The field-collected population was reared for one generation to increase the number of individuals. A bioassay with deltamethrin was conducted on the second generation (G_2) and then it was divided into two subpopulations. One subpopulation was kept unexposed to any insecticide and was continuously reared as the susceptible population (lab population). The second subpopulation (delta–SEL pop) was exposed to deltamethrin at different lethal concentrations (LCs) ranging from 21.21 to 1046.70 μg/mL of bioassay done at G_2 for 10 consecutive generations from G_3 to G_{12}. The number of insects selected per generation ranged from 100 to 800 depending upon the availability. The survivors of each selection were grouped together for rearing and further selection. Three-day old 2nd instar nymphs of *P. solenopsis* were used for bioassays as well as selection. A standard leaf dip method was used to determine the LC_{50s} of the different tested populations (Afzal *et al.* 2015a). In each bioassay, China rose leaves were immersed for 10 s in newly prepared solutions with little agitation. Treated leaves were air dried for ∼1 h at room temperature before placing them on the Petri dishes. Five replicas of each concentration and control were made. At each concentration, 25 nymphs (five per replicate) were exposed and in control treatment, insects were given leaves dipped in tap water alone. In each bioassay, 150 insects were tested. The bioassays were conducted in the laboratory conditions as described above. Insect response (mortality) was assessed 48 h after exposure to deltamethrin.

Deltamethrin resistance stability

The selection pressure was removed on deltamethrin-selected (DEL-SEL) population from G_{13}–G_{19} by rearing it continuously for seven generations, to determine whether the resistance remained stable or unstable. A decrease in resistance (DR) to insecticide was determined by the following formula:

$$\mathrm{DR} = [\log(\text{final } \mathrm{LC}_{50}) - \log(\text{initial } \mathrm{LC}_{50})]/n,$$

where n is the number of insect generations reared without insecticide exposure.

Genetic crosses

To determine the inheritance mode of deltamethrin resistance, a mass mating was made between the lab pop and the delta-SEL pop to obtain two lines: F_1 (15 females of lab pop × three males of delta-SEL) and F'_1 (three males of lab pop × 15 females of delta-SEL). Similarly, two backcrosses populations were also obtained by crossing females of F_1 with males of the lab pop (BC_1) and delta-SEL pop (BC_2) in the same male female ratio as given above.

Synergism test

For synergism bioassays, piperonyl butoxide (PBO), a cytochrome P-450 monooxygenase inhibitor, and S,S,S-tributyl phosphorotrithioate (DEF), an esterase inhibitor were used. Before testing PBO and DEF with the insecticide, both synergists were alone dissolved at different concentrations in 1 mL of acetone to identify a nontoxic concentration. A nontoxic concentration of 5 mg/mL of PBO and 2.5 mg/mL of DEF per mL of acetone were mixed with each concentration of insecticide. Bioassays were performed with the leaf dip protocol on both the lab pop and delta-SEL pop.

Acetone alone was used as a control. The synergism ratio (SR) was calculated as follows:

$$SR = LC_{50} \text{ of insecticide only / } LC_{50} \text{ of insecticide with synergist.}$$

Statistical analysis

Concentration response bioassays: Probit analysis was used to analyse the concentration response data (Finney 1971) with the EPA Probit Analysis program to determine LC_{50} values and their standard errors, slopes and 95% confidence intervals (CIs). Probability (P) values were estimated based on the chi square (χ^2) distribution. Resistance ratio (RR) was determined as:

$$RR = LC_{50} \text{ of tested population / } LC_{50} \text{ of the lab pop.}$$

The LC_{50} values of populations were considered significantly different ($P > 0.05$) when there was no overlapping of 95% CIs (Litchfield and Wilcoxon 1949).

Analysis of resistance inheritance

Assessment of sex-linked or autosomal inheritance: Sex-linked or autosomal inheritance of deltamethrin resistance in delta-SEL pop was assessed by comparing the 95% CIs of respective LC_{50} values of reciprocal crosses. If the populations' 95% CIs overlap, then, the resistance is autosomal; otherwise it is sex-linked.

Degree of dominance (D): Dominance (D) values of deltamethrin resistance were determined according to Stone (1968) with the following formula:

$$D = [\log LC_{50} \text{ of } F_1 \text{ or } F_1' \text{ or } F_2 - \log LC_{50} \text{ of lab pop}] / [\log LC_{50} \text{ of delta-SEL pop} - \log LC_{50} \text{ of lab pop}].$$

The D values were categorized as 0 = completely recessive and 1 = completely dominant. According to Bourguet *et al.* (2000), effective dominance (D_{ML}) was estimated with the following formula:

$$D_{ML} = [MT_{RS} - MT_{SS}]/[MT_{RR} - MT_{SS}],$$

where MT_{RR}, MT_{RS} and MT_{SS} were the per cent mortalities to a single insecticide dose for the delta-SEL pop, F_1 and lab pop, respectively. If $D_{ML} = 0$, it is a completely recessive type of resistance and if $D_{ML} = 1$, it is a completely dominant type of resistance.

Number of factors / genes involved: Two approaches were used to estimate the number of factors involved in resistance. The first approach was a χ^2 goodness of fit test in which an analysis of a comparison of the observed and expected mortalities at different concentrations were done based on P values (Sokal and Rohlf 1981). If the observed and expected mortalities were found to differ significantly at more than 50% concentrations, then, the null hypothesis of monogenic model was rejected and inheritance is polygenic.

χ^2 for this method was calculated as follows:

$$\chi^2 = [F - pn]^2/pqn.$$

In the above equation, F, observed mortality in the BC population against a particular dose; n, numbers exposed at a particular dose; p, expected mortality calculated according to Georghiou (1969) and q is calculated as 1−p.

The second approach to calculate the number of genes controlling deltamethrin resistance (Lande 1981) used the following formula:

$$nE = [X_{RR} - X_{SS}]^2/[8\sigma^2_S],$$

where X_{RR} and X_{SS} were the log LC_{50} of delta-SEL pop and lab pop, respectively, and σ^2_S was calculated as follows:

$$\sigma^2_S = \sigma^2_{B1} + \sigma^2_{B2} - [\sigma^2_{F1} + 0.5\sigma^2 X_{SS} + 0.5\sigma^2 X_{RR}],$$

where σ^2_{B1}, σ^2_{B2}, σ^2_{F1}, $\sigma^2 X_{SS}$ and $\sigma^2 X_{RR}$ were the variances of the BC_1, BC_2, F_1, lab pop and delta-SEL pop, respectively.

Variance of dominance ($\sigma^2 D$) was calculated using the following formula (Preisler *et al.* 1990):

$$\sigma^2 D = 4/(X_{RR}-X_{SS})^2\{\sigma^2_{RS} + [(X_{RS}-X_{SS})^2/(X_{RR}-X_{SS})^2]\sigma^2_{RR} + [(X_{RS} - X_{RR})^2/(X_{RR} - X_{SS})^2]\sigma^2_{SS}\},$$

where X_{RR}, X_{SS} and X_{RS} were the log LC_{50} values of delta-SEL pop, lab pop and reciprocal crosses, respectively, and σ^2_{RR}, σ^2_{SS} and σ^2_{RS} were the estimated variance values for the delta-SEL pop, lab pop and reciprocal crosses, respectively. Variance (σ^2) was calculated according to Lande (1981) as the inverse of the slope squared (standard deviation).

Results

Deltamethrin resistance selection and reversion

The resistance ratio of deltamethrin in the delta-SEL pop (G_{13}) was 740.11-fold compared to the lab pop after 10 rounds of selection. However, this high level of resistance dropped significantly to 58.72-fold with DR value 0.16, when the same population was reared without selection for seven generations. It suggests that deltamethrin resistance remained unstable (table 1).

Sex-linkage and maternal effects

The resistance ratios of deltamethrin for F_1 and F_1' were 64.55-fold and 106.82-fold as compared to the lab pop. The LC_{50} of the reciprocal crosses were not significantly different due to the overlapping of 95% CIs, suggesting that deltamethrin resistance is autosomal and neither sex linkage nor maternal effects are present in the *P. solenopsis* selected population (table 1).

Degree of dominance

The degree of dominance for F_1, F'_1 and F_2 was 0.63, 0.71 and 0.62, respectively, suggesting an incomplete dominant inheritance of resistance to deltamethrin in *P. solenopsis* (table 1). The D_{ML} of the five tested concentrations showed that deltamethrin resistance was incompletely recessive at higher concentration ($D_{ML} = 0.31$), while resistance was incompletely dominant at the lowest concentration tested ($D_{ML} = 0.72$) (table 2).

Number of genes

The monogenic model of inheritance showed that there was a significant difference ($P < 0.05$) between observed and expected mortalities at three of the five concentrations tested. These significant differences suggest that resistance to deltamethrin in *P. solenopsis* is polygenic (table 3). The minimum number of genes contributing to deltamethrin resistance was calculated to be 7.51, which also suggested that resistance was controlled by multiple factors, hence it is polygenic.

Synergism of deltamethrin resistance

Synergism bioassays of deltamethrin on the delta-SEL pop indicated that PBO significantly increased the toxicity of deltamethrin (95% fiducial limit (FL) did not overlap), with a prominent decline in RR of 3591.87-fold to 448.30-fold and

Table 1. Responses of lab pop, delta-SEL pop, their reciprocal crosses and backcross populations of *P. solenopsis* to deltamethrin.

Population	LC_{50} (95% CI) (ppm)	log LC_{50}	Slope (±SE)	χ^2	df	*P*	RR	D	DR
Lab pop	3.18 (1.47–5.21)	0.50	1.08 (±0.28)	0.17	4	1.00	1.00	–	–
Delta-SEL pop (G_{13})	2353.55 (1288.20–12138.93)	3.37	0.98 (±0.29)	0.24	4	0.99	740.11	–	–
Delta-SEL pop (G_{20})	186.73 (116.05–275.09)	2.27	1.42 (±0.30)	0.65	4	0.96	58.72	–	0.16
F_1 (delta-SEL (G_{13}) ♂ × lab ♀)	205.27 (105.44–408.88)	2.31	0.94 (±0.28)	0.07	4	1.00	64.55	0.63	–
F'_1 (delta-SEL (G_{13}) ♀ × lab ♂)	339.68 (212.38–724.71)	2.53	1.14 (±0.29)	0.17	4	1.00	106.82	0.71	–
F_2 (F_1 ♂ × F_1♀)	196.02 (111.75–339.88)	2.29	1.10 (±0.28)	0.49	4	0.97	61.64	0.62	–
BC_1 (F_1 ♀ × lab ♂)	111.88 (2.31–268.51)	2.04	0.62 (±0.27)	0.29	4	0.99	35.18	–	–
BC_2 (F_1 ♀ × delta-SEL (G_{13}) ♂)	159.83 (79.01–276.19)	2.20	1.02 (±0.28)	0.16	4	1.00	50.26	–	–

P, probability; RR, resistance ratio; D, degree of dominance; DR, decrease in resistance.

Table 2. Effective dominance of resistance to deltamethrin in delta-SEL pop of *P. solenopsis* according to insecticide concentrations.

Concentration (ppm)	Strain	Mortality	D_{ML}
800	Lab pop	100.00	0.31
	Delt-SEL pop (G_{13})	36.00	Incompletely recessive
	F_1	72.00	–
400	Lab pop	100.00	0.43
	Delta-SEL pop (G_{13})	24.00	Incompletely recessive
	F_1	60.00	–
200	Lab pop	100.00	0.54
	Delta-SEL pop (G_{13})	12.00	Incompletely dominant
	F_1	48.00	–
100	Lab pop	100.00	0.60
	Delta-SEL pop (G_{13})	0.00	incompletely dominant
	F_1	40.00	–
50	Lab pop	100.00	0.72
	Delta-SEL pop (G_{13})	0.00	incompletely dominant
	F_1	28.00	–

Table 3. Monogenic model of inheritance for resistance to deltamethrin by comparing observed and expected mortalities of backcross (F_1 ♀ × lab pop ♂) of *P. solenopsis*.

Concentration (ppm)	No. of nymphs tested (*n*)	Observed mortality	Expected mortality (*P*)	χ^2 (df = 1)	*P*
50	25	0.40	0.64	42.25	<0.0001
100	25	0.52	0.70	54.92	<0.0001
200	25	0.56	0.74	66.91	<0.0001
400	25	0.60	0.80	0.00	1.00
800	25	0.72	0.86	0.00	1.00

Table 4. Synergistic effect of PBO and DEF in del for SEL pop and lab pop of *P. solenopsis*.

Population	Treatment	LC_{50} (95% CI) (ppm)	Slope (±SE)	χ^2	df	*P*	*n*	RR	SR
Del-SEL pop	Deltamethrin	3089.01 (1905.52–7606.53)	1.12 ± 0.28	0.06	4	1.00	150	3591.87	–
	Deltamethrin + PBO	434.85 (208.53–1101.24)	0.83 ± 0.27	0.09	4	1.00	150	448.30	7.1
	Deltamethrin + DEF	634.12 (290.96–5860.69)	0.69 ± 0.27	0.03	4	1.00	150	546.66	4.87
Lab pop	Deltamethrin	0.86 (0.34–1.79)	0.82 ± 0.27	0.09	4	1.00	150	1	–
	Deltamethrin + PBO	0.97 (0.489–1.897)	0.94 ± 0.28	0.07	4	1.00	150	1	0.88
	Deltamethrin + DEF	1.16 (0.55–2.93)	0.83 ± 0.27	0.09	4	1.00	150	1	0.74

RR, resistance ratio; SR, synergism ratio; *n*, number of insects.

SR = 7.1. On the other hand, DEF failed to synergize the toxicity of deltamethrin in the delta-SEL pop, where resistance ratios decreased from 3591.87-fold to 546.66-fold and SR = 4.87. Insecticidal activity of deltamethrin in the lab pop was not affected (95% FL overlap) by either synergists (table 4).

Discussion

In the current work, continuous selection pressure with deltamethrin for 10 generations resulted in a resistance of 740.11-fold in *P. solenopsis* compared to that of the lab pop. There are numerous reports of deltamethrin resistance development in insects as a result of selection with deltamethrin such as in *C. pomonella*, 93.7-fold (Bouvier *et al.* 2001); *P. xylostella*, 6736-fold (Sayyed *et al.* 2005); *S. litura*, 63-fold (Ahmad *et al.* 2007); *T. infestans*, 856.84-fold (Germano *et al.* 2010); *Chrysoperla carnea* Stephens, 896-fold (Sayyed *et al.* 2010); *S. exigua*, 976-fold (Ishtiaq *et al.* 2012); *Aedes aegypti* Linnaeus, 91.25-fold (Rodriguez *et al.* 2014); *P. solenopsis*, 100-fold (Saddiq *et al.* 2016). All of these examples support our study that selection of *P. solenopsis* with deltamethrin has a great impact on development of insecticide resistance. Development of very high resistance in *P. solenopsis* might be due to excessive use of deltamethrin by farmers in the field for the control of this pest in cotton growing areas of Pakistan. Various other factors such as high reproductive potential and short life cycle might be responsible for the rapid resistance development in this pest to deltamethrin after limited number of laboratory selections.

It is imperative to obtain knowledge about the stability of insecticide resistance as it aids in developing effective resistance management strategies (Abbas *et al.* 2015). In this study, the significant decrease in deltamethrin resistance in *P. solenopsis* over seven generations without selection suggests that resistance to deltamethrin remained unstable. The susceptibility of resistant individuals may change in the absence of selection pressure under laboratory conditions when insecticide exposure is not a constant factor (Kristensen *et al.* 2000). A reduction in resistance levels may be due to the negative effects of resistance genes on fitness components without insecticide selection pressure (Jan *et al.* 2015). Similar to our study, the reversion of deltamethrin resistance has been previously reported in *Earias vittella* (Jan *et al.* 2015), but in contrast to our study, a stable nature of deltamethrin resistance was reported in *C. carnea* (Sayyed *et al.* 2010), *S. exigua* (Ishtiaq *et al.* 2012) and *Musca domestica* (Khan *et al.* 2015). Discontinued insecticide application with unstable resistance portfolio (on discontinuing the selection pressure) may prove helpful to prolong the effective life span of such chemicals in the field.

In our experiment, the bioassay results of reciprocal crosses showed no significant difference in the LC_{50} between the F_1 and F_1' progenies, indicating that resistance to deltamethrin in *P. solenopsis* was inherited autosomally and that maternal effects were not associated with resistance. Our results of autosomal resistance inheritance are in line with previously reported deltamethrin resistance in *P. xylostella* (Sayyed *et al.* 2005), *S. litura* (Ahmad *et al.* 2007), *C. carnea* (Sayyed *et al.* 2010), *C. pomonella* (Bouvier *et al.* 2001), *A. aegypti* (Rodriguez *et al.* 2014) and *T. infestans* (Germano *et al.* 2010; Gomez *et al.* 2015). In contrast, sex-linked inheritance of deltamethrin resistance has been reported in *Sitophilus zeamais* Mots (Guedes *et al.* 1994). It was suggested that deltamethrin resistance could be expressed as a recessive or dominant trait, depending on the deltamethrin concentrations to which insect pests were exposed. In our study, resistance to deltamethrin was inherited as an incomplete dominant trait. The incomplete dominant nature of deltamethrin resistance has also been documented in *P. xylostella* (Sayyed *et al.* 2005), *S. litura* (Ahmad *et al.* 2007), *T. infestans* (Germano *et al.* 2010; Gomez *et al.* 2015), *C. carnea* (Sayyed *et al.* 2010) and *A. aegypti* (Rodriguez *et al.* 2014). In contrast, a recessive type of deltamethrin resistance has been shown in *C. pomonella* (Bouvier *et al.* 2001) and *S. zeamais* (Guedes *et al.* 1994). The reasons for these differences may be due to variations in selection histories (Shi *et al.* 2011), since bioassay protocols, selection pressure and the number of generations selected were all different. In many cases, selection with the aim of 50% mortality has been carried out under laboratory conditions and could lead to incomplete dominant resistance (Shad *et al.* 2010). Resistance alleles involved in degree of dominance contribute to the distribution and expression of resistance genes. Chemical control will be more difficult to achieve if a dominant gene contributes to resistance because in that case the heterozygotes are also resistant (Bielza *et al.* 2008). Resistance inherited as a dominant trait develops faster as compared to a recessive trait as resistant genotypes along with heterozygotes

have more chances of surviving insecticide treatment and have a tendency to amplify (R : S = 3 : 1 for dominant and 1: 3 for recessive) and spread more rapidly in field populations (Denholm and Rowland 1992; Bielza *et al.* 2008). Although in the present study, resistance to deltamethrin in *P. solenopsis* was not completely dominant, in resistance management, it is necessary to take care because heterozygotes have the ability to endure considerably higher deltamethrin doses in comparison to susceptible insects. Although dominance level is a fixed parameter, it may change depending upon the genetic background, environmental conditions and concentrations of the insecticide used (Bourguet *et al.* 2000). Selection of resistant alleles may also lead to evolution in dominance levels (Afzal and Shad 2015). We have also shown that the dominance level of deltamethrin resistance in *P. solenopsis* is concentration-dependent. Resistance at the highest concentration was incompletely recessive, but incompletely dominant at the lowest dose tested. Although dominance level is a fixed parameter, but genetic background of species under study and variation in insecticide selection pressure may influence the dominance levels (Bourguet *et al.* 2000). Dominance of resistance at particular concentrations may also depend upon the life stage of insect; in particular, nymphs, show more susceptibility than adults (Basit *et al.* 2011). The impact of insecticide concentration upon dominance in our study is similar to that of other findings (Horowitz *et al.* 2003; Sayyed and Crickmore 2007; Shad *et al.* 2010; Basit *et al.* 2011). Reduced dominance of resistance (incompletely dominant to incompletely recessive from lower to higher concentrations) decreases the resistance heritability, thus, delaying resistance evolution.

Insecticide resistance may be monogenic or polygenic in insect populations. However, polygenic resistance is most common under laboratory selections due to the absence of rare variants that may be present in large natural insect populations (McKenzie *et al.* 1992). In this study, the frequency of genes involved in resistance calculated by Lande's method and the significant difference between expected and observed mortalities at three concentrations suggest a polygenic nature of deltamethrin resistance in *P. solenopsis*. Similar to our studies, a polygenic nature of deltamethrin resistance was previously reported in *C. pomonella* (Bouvier *et al.* 2001), *P. xylostella* (Sayyed *et al.* 2005), *S. litura* (Ahmad *et al*. 2007), *C. carnea* (Sayyed *et al.* 2010) and *A. aegypti* (Bourguet *et al.* 2014). If resistance was controlled entirely by one locus, then the sole allele would be fixed and a further increase in resistance would not have occurred in selection experiments. However, the observed increase in resistance indicates that the delta-SEL had more than one locus affecting resistance.

Previously, insecticide resistance in many insect species was reported to be mainly due to enhanced activity of detoxification metabolic enzymes (Kang *et al.* 2006; Wang *et al.* 2006). In our study, the use of PBO with deltamethrin significantly reduced deltamethrin resistance in the resistant population of *P. solenopsis*, while DEF along with deltamethrin had no effect on the resistant population. The synergistic effect of PBO in suppressing deltamethrin resistance was previously reported in *S. litura* (Ahmad *et al*. 2007), *S. exigua* (Ishtiaq *et al.* 2012) and *A. aegypti* (Rodriguez *et al.* 2014). It suggested that cytochrome P-450-dependent mono-oxygenases, but not esterases might play an important role in deltamethrin resistance by detoxifying deltamethrin. However, it is important to study the mechanism of deltamethrin resistance in *P. solenopsis* at molecular levels.

It can be concluded from this study that factors like degree of dominance, maternal effects and frequency of genes have strong effects on the development of insecticide resistance. Therefore, it is necessary to determine the inheritance mode of resistance in laboratory experiments for continuous use of an insecticide for the management of pest. The rapid evolution of deltamethrin resistance by *P. solenopsis* stressed the need for appropriate resistance management strategies. The analysis of resistance inheritance and the assessment of resistance risk provide useful information for designing a novel strategy to manage *P. solenopsis* resistance to insecticides. The rapid selection of resistance alleles due to the dominance of resistant genes suggest that alternative chemistry with a different mode of action should be recommended and also the rotational use of deltamethrin rather than the continuous use for a longer time should be adopted. Some researchers have focussed on the use of synergists in combination or in rotation with insecticides as a means for disrupting resistance mechanisms or delaying the development of insecticide resistance (Horowitz *et al.* 1988; Campanhola and Jr 1989; Bagwell and Jr 1992). This supports our conclusion that due to the synergistic action of PBO with deltamethrin in our study, they might be used in cotton fields for the management of *P. solenopsis*. Hence, characterizing the genes and regulatory mechanisms involved in resistance may provide the way to advanced methods of studying resistance, eventually leading to the discovery of the genes responsible for insecticide resistance.

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