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Resistance risk analysis to acetamiprid and other insecticides in Acetamiprid-Selected population of *Phenacoccus solenopsis*

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Abstract Acetamiprid is a new chemical insecticide recommended for the control of a number of insect pests, including cotton mealybug *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae). We report the risk of resistance evolution to acetamiprid and three other insecticides (imidacloprid, chlorpyrifos and deltamethrin) in *P. solenopsis*. After 24 generations of selection with acetamiprid, *P. solenopsis* developed a high level of resistance (10631-fold) compared to the susceptible strain. Realized heritability of resistance for acetamiprid, imidacloprid, chlorpyrifos and deltamethrin was 0.21, 0.12, 0.11 and 0.09, respectively. The projected rate of resistance development indicated that if mortality is 30% at each generation then a ten-fold increase in resistance was to be expected after 24 and 20 generations for acetamiprid (h^2 = 0.1, slope = 1.16) and imidacloprid (h^2 = 0.12, slope = 1.20), respectively, and after 29 generations for chlorpyrifos (h^2 = 0.1, slope = 1.44) and deltamethrin (h^2 = 0.08, slope = 1.13). Therefore, *P. solenopsis* has the ability to develop resistance under continuous selection pressure. This study will contribute to the design of a pest management strategy for *P. solenopsis*.

Keywords Cotton · Mealybug · Pest · Invasive · Neonicotinoid · Honeydew

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Introduction

The spread of insect pests has increased with the increased trade of plant species. Scale insects especially remain undetected during quarantine inspections due to their cryptic nature. Scale insects become more damaging when they are introduced in a new area in the absence of their natural enemies. Invasive species can cause economic losses to existing plant biodiversity (Muniappan 2011). Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) is a very damaging and wide spread pest of more than 200 species of plants including agricultural crops, vegetables, weeds and ornamentals of economic importance for about 24 countries including Pakistan and India (Abbas *et al.* 2005, 2010; Vennila *et al.* 2010a; Wang *et al.* 2010; Nagrare *et al.* 2011; Arif *et al.* 2012). Sudden epidemics of *P. solenopsis* have been reported on numerous host plants (Jhala & Bharpoda 2008; Jhala *et al.* 2008; Dhaliwal *et al.* 2010; Zhu *et al.* 2011). Cotton has been adversely affected by the invasion of *P. solenopsis* all over the world (Anonymous 2005). *P. solenopsis* reduces cotton yields by sucking cell sap from tender plant parts, which leads to poor plant development and undersized bolls (Aheer *et al.* 2009). Deposition of honeydew on plant parts promotes the development of sooty mold that hampers the process of photosynthesis and leads to significant crop losses (Meyerdirk *et al.* 2001). An estimated yield loss of 3.1 million bales which is about 40% of the cotton crop has been reported from Pakistan due to this pest (Abdullah 2009; Mahmood *et al.* 2011).

Various conventional and new chemical insecticides have been reported for the control of *P. solenopsis*. Most farmers rely upon chemical control and they may apply several treatments to suppress *P. solenopsis* due to its cryptic nature and high reproductive power (Nagrare *et al.* 2011; Nikam *et al.* 2010; Suroshe 2011). However, the over reliance on and repeated applications of these chemicals decrease the efficacy of chemicals and lead to the evolution of resistance (Abbas *et al.* 2007). Also less used and newly introduced insecticides may rapidly lose their efficacy due to cross-resistance mechanisms (Kranthi *et al.* 2001). In addition to the evolution of resistance, these toxins are persistent in nature and increase the problem of biotype development. Also, destruction of their natural enemies may result in sudden induced outbreaks of the pest that may lead to a complete crop failure (Campiche *et al.* 2006; Peng *et al.* 2010). Neonicotinoids are novel and broad spectrum insecticides (Yamamoto & Casida 1999). Acetamiprid is one of the effective members of this class used for the control of insects belonging to the order Coleoptera, Lepidoptera, Homoptera and Thysanoptera. It is especially recommended for sucking pests like *P. solenopsis*. It has systemic, contact as well as osmotic activity in insects (Takahashi *et al.* 1998). It blocks nerve transmission across the nicotinergic acetylcholine receptor site by depolarizing the action potential in the central nervous system of insects. It has low mammalian toxicity and is safer for natural enemies and other non-target organisms (Elbert *et al.* 2008). Recently, concern has been raised regarding the negative impact of a common neonicotinoids such as acetamiprid and imidacloprid on honey bees and native bee pollinators (Williams et al. 2015). Despite its novel mode of action, a number of resistance reports have been published for different insect pests such as *Bemisia tabaci* Gennadius, *Aphis gossypii* Glover, *Cydia pomonella* L., and *Plutella xylostella* L. (Sayyed & Crickmore 2007; Knight 2010; Basit *et al.* 2011; Herron & Wilson 2011).

Integration of highly efficient insecticides is a necessary component of an effective and comprehensive approach of integrated pest management (IPM) against resistance evolution. An assessment of the resistance risk to different insecticides is an important tool prior to the wide spread use of the insecticides (Lai & Su 2011). Resistance risk assessement benefits can provide key information about resistance development before its onset in the field, and facilitate the implementation of protective strategies to maintain susceptibility in a field population that overcome the development of resistance. This can be done either by selection for resistance development in the field, and laboratory conditions or by quantitative genetic analysis (Falconer & Mackay 1996; Jutsum *et al.* 1998). Quantitative genetic models estimate the heritability of resistance by the analysis of selection data as a continuous genetic variable (Firkoi & Hayes 1990). Estimation of realized heritability allows a direct comparison among selection histories that vary in terms of duration and intensity (Falconer 1981; Tabashnik 1992). It also facilitates understanding the rate and direction of resistance as a critical factor for resistance management (Firkoi & Hayes 1990; Tabashnik 1992).

The objectives of this study were to assess resistance risk to acetamiprid in *P. solenopsis* in terms of heritability in different selected generations to develop different levels of resistance for better understanding of resistance evolution in future.

Materials and methods

Rearing of P. solenopsis About 400 *P. solenopsis* adults and nymphs were collected from cotton fields at the Central Cotton Research Institute, Multan (30°12'N, 71°25'E). A number of insecticides are being used for the control of different sucking insect pests which attack cotton (Ahmad *et al.* 2007). The *P. solenopsis* population was reared in the laboratory in plastic jars (12 cm×24 cm) covered with muslin cloth. They were fed with soft, clean twigs and leaves of the China rose, *Hibiscus rosasinensis* L. The culture was refreshed at 2-3 day intervals by removing the dried branches and leaves and providing fresh ones that were maintained under standard laboratory conditions at 27 ± 2 °C and 60 ± 5% RH with a 14:10 hr light:dark photoperiod (Afzal *et al.* 2015). The susceptible population was obtained by rearing mealy bugs without any insecticidal exposure in the laboratory for more than three years.

Insecticides The commercially available conventional and new chemicals used in the bioassays were: acetamiprid (Mospilon®, 20 SP; Arysta Life Sciences, Pakistan) imidacloprid (Confidor®, 200 SL; Bayer Crop Sciences, Pakistan), chlorpyrifos (Lorsban®, 40 EC; Dow Agro Sciences, Pakistan) and deltamethrin (Decis Super®, 10 EC; Bayer Crop Sciences, Pakistan).

Selection response Aceta-Sel strain was derived from an already established strain of *P. solenopsis* by Afzal *et al.* (2015) as follows. The population collected from the field was continously selected in the laboratory with acetamiprid from generation (G_3) to G_7 and was named Aceta-Sel. This resistant strain was taken for the study of resistance risk assessment to different insecticides and selection was carried out with acetamiprid from G_7-G_{26} on China rose leaves. About 50-400 nymphs were exposed to different concentarions during different generations of selection. Mortality was assessed 72 hr after exposure to acetamiprid and the next generation was obtained by rearing the survivors of the selected generation.

Toxicological bioassays Leaf dip bioassays were performed on the Aceta-Sel and susceptible populations of *P.solenopsis* (Ahmad *et al.* 2007). China rose leaves were dipped in serial dilutions of insecticides for 10 s with little agitation and air dried at room temperature before transferring the treated leaves to Petri dishes. For the bioassay, five serial dilutions were made and each treatment was replicated five times. Leaves for the control treatment were dipped in tap water. Five 2nd instar nymphs were exposed to each replication. Response of insects was assessed after 48 and 72 hr exposure to the conventional and new chemicals, respectively (Sayyed & Crickmore 2007; Ninsin 2004). Inactive nymphs were considered dead when touched, with a fine hair brush (Afzal *et al.* 2015).

Statistical analysis of bioassays Bioassay data were analyzed by probit analysis using Polo Plus software (LeOra 2002) to estimate different lethal concentrations, slopes and their respective confidence intervals (CIs).

Estimation of realized heritability (h^2) Realized heritability of resistance to insecticides was determined in two phases of selection according to the formula given by Tabashnik (1992).

$$h2 = Rsponse\ \ to\ \ Selection\ \ (R)/Selection\ \ Differential(S)$$

The response to selection (R) was calculated as

$$R = log(final\ \ LC50) - log(initial\ \ LC50)/n$$

Here, the final LC_{50} is the LC_{50} of survivors after concluding selection of respective generation and initial LC_{50} is the LC_{50} of the parental generation.

Selection differential was calculated as follows

$$S = intensity\ \ of\ \ selection(i) \times phenotypic\ \ variance(\sigma p)$$

Where i is the selection intensity estimated by using the following formula

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

In the above mentioned formula P is the average percent survival of the resistant strain after selection (Tabashnik & McGaughey 1994).

The phenotypic deviation was determined as follows:

$$\sigma p = [0.5(initial\ \ slope + final\ \ slope)] - 1$$

Response to selection (R) is the product of heritability (h^2) and selection differential (S).

$$R = h2S$$

The number of generations (G) required for a ten-fold increase in resistance based on the selection of *P. solenopsis* to acetamiprid is the reciprocal of R. While, mean slope for the projected rate of resistance was calculated by taking the average of the slopes of respective generations.

$$G = R - 1 = (h2S) - 1$$

Results

Selection history and development of resistance Selection of *P. solenopsis* with acetamiprid for 24 generations on concentrations ranging from 46 to10,276 ppm decreased the susceptibility of the selected population and increased survival up to 90%. In our study evolution of resistance to acetamiprid was divided in two phases of selection. In the first phase, the resistance ratio (RR) was 3,943-fold after selection from G_3 to G_{14} while in the next 12 generations (G_{15} to G_{26}) the RR increased to 10,631-fold (Table 1).

Estimation of realized heritability The overall mean estimated value of h^2 for acetamiprid resistance (G_8-

Table 1 History of selection of *P. solenopsis* to develop an acetamiprid resistant strain

Generation	Concentration (ppm)	n[a]	% survival	LC_{50} (ppm)	Slope±SE	RR[b]
G_3	46	200	42	_	_	_
G_4	250	200	60	_	_	_
G_5	510	200	68	_	_	_
G_6	903	300	70	_	_	_
G_7	2104	100	78	_	_	_
G_8	10276	100	81	14901 (983.69 - 3157.66)	1.24 ± 0.31	901
G_9	10276	250	90	_	_	
G_{10}	10276	800	93	2620 (1862.61 - 4355.50)	1.40 ± 0.31	1584
G_{11}	10276	300	84	_	_	
G_{12}	10276	600	84	1805 (1022.53 - 2781.94)	1.03 ± 0.28	1091
G_{13}	10276	400	88	_	_	
G_{14}	2104	200	82	6522 (4135.60 - 15941)	1.18 ± 0.31	3943
G_{15}	2104	300	77	_	_	
G_{16}	903	200	89	7129 (4783.60 - 14353)	1.30 ± 0.312	4310
G_{17}	903	200	87	_	_	
G_{18}	903	300	83	10313 (5922.40 - 38473)	0.94 ± 0.29	6235
G_{19}	903	250	88	_	_	
G_{20}	250	400	84	10721 (7469.50 - 19530)	1.47 ± 0.33	6482
G_{21}	250	200	60	_	_	
G_{22}	250	100	85	11786 (6797.30 - 42951)	0.92 ± 0.29	7126
G_{23}	250	100	80	_	_	
G_{24}	250	50	90	11399 (7834.90 - 21102)	1.34 ± 0.31	6892
G_{25}	250	100	83	_	_	
G_{26}	250	60	83	17583 (10215 - 60590)	1.04 ± 0.30	10631

[a] n = Number exposed for the selection.

[b] RR was calculated by dividing LC_{50} of Aceta-SEL Pop/LC_{50} of Susceptible (LC_{50} = 1.654 ppm)

G_{26}) in *P. solenopsis* was 0.21, with an average value of R and S of 0.06 and 0.26, respectively. S was lower (0.24) in the first phase of selection than in the second half of the selection experiment (0.28), but R was higher (0.08) in the first than in the second phase (0.04). So the estimate of heritability (h^2) was lower in the second phase (0.14) than in the first phase of selection (0.32). The higher initial h^2 value indicated a decrease in the phenotypic variations of acetamiprid resistance during the selection experiment. The mean estimated h^2 values for imidacloprid, chlorpyrifos and deltamethrin in the acetamiprid resistant population were 0.12, 0.11 and 0.09, respectively (Table 2).

Projected rate of acetamiprid resistance evolution The effect of different heritability values on the development of ten-fold increases in acetamiprid resistance on different % mortalities at a constant slope value (1.16) in *P. solenopsis* is given in Fig. 1A. The projected rate of evolution of resistance is directly proportional to heritability and selection intensity (Fig 1). Considering h^2 = 0.2, 12-5 generations are required for a ten-fold increase in resistance at a 30-70% selection intensity. Similarly, 24-10 generations are required at the same selection intensity when h^2= 0.1. If h^2 = 0.3, similar increase in resistance would occur in 8/3 generations at a 30-70% selection intensity (Fig. 1A).

The projected rate of resistance development is inversely proportional to the slope. Considering the heritability constant (h^2 = 0.2), and slope = 3.16, 32-14 generations are required at a selection intensity of 30-70%. If the slope = 1.16, 12-5generations are required for similar conditions (Fig. 1B).

Table 2 Trend of estimated realized heritability of acetamiprid and other insecticides in the development of Aceta-Sel strain of *P. solenopsis*

Generations	Insecticides	Estimation of selection response			Estimation of selection differential				
		Initial LC_{50} [ppm] (log)	Final LC_{50} [ppm] (log)	R	I	Mean slope	σp	S	h^2
09(G_8-G_{16})	Acetamiprid	3.17	3.85	0.08	0.29	1.23	0.81	0.24	0.32
10(G_{16}-G_{26})	Acetamiprid	3.85	4.25	0.04	0.32	1.13	0.89	0.28	0.14
19(G_8-G_{26})	Acetamiprid	3.17	4.25	0.06	0.30	1.16	0.86	0.26	0.21
19(G_8-G_{26})	Imidacloprid	3.07	3.67	0.03	0.30	1.20	0.83	0.25	0.12
19(G_8-G_{26})	Chlorpyrifos	2.81	3.25	0.02	0.30	1.44	0.69	0.21	0.11
19(G_8-G_{26})	Deltamethrin	2.82	3.27	0.02	0.30	1.13	0.88	0.27	0.09

Projected rate of imidacloprid resistance evolution If slope = 1.20 (mean value of the slope for imidacloprid observed in our study) and $h^2 = 0.12$, 20-9 generations are required for a ten-fold increase in the LC_{50} but the same would occur in 11-5 generations if $h^2 = 0.22$ at 30-70 % mortality. Similarly, if the $h^2 = 0.32$, then 8-3 generations are required at 30-70% selection intensity (Fig. 2A).

If $h^2 = 0.12$ and slope = 3.2 then a ten-fold increase in resistance would occur in 54-23 generations. Changing the slope = 2.2 the same would occur in 37-16 generations at a 30-70% selection intensity. However if the slope = 1.2 and selection intensity was 30-70%, 20-9 generations are required for a ten-fold increase in resistance at 30-70 % selection intensity (Fig. 2B).

Projected rate of chlorpyrifos resistance evolution If the slope is 1.44 (mean slope for chlorpyrifos observed in our study) and $h^2 = 0.1$, then 29-12 generations are required for ten-fold increase in the LC_{50} at a 30-70% selection intensity. Conversely, the same would require 15-6 generations and 10-4 generations at the same selection intensities when h^2 is 0.2 and 0.3, respectively (Fig. 3A).

At a constant $h^2 = 0.1$ (heritability of chlorpyrifos resistance observed in our study) and slope = 1.44, 29-12 generations would be expected for a ten-fold increase in the LC_{50} at the selection intensity of a 30-70%. However, at slope = 2.44 it would require 50-21 generations at 30-70% selection intensities. Similarly, using the slope = 3.44 the same would be achieved in 70-30 generations at the same selection intensity (Fig. 3B).

Projected rate of deltamethrin resistance evolution If the slope = 1.13 (mean slope for deltamethrin resistance observed in our study) and $h^2 = 0.08$, then 29-12 generations are required for a ten-fold increase in resistance when the selection intensity is 30-70 % at each

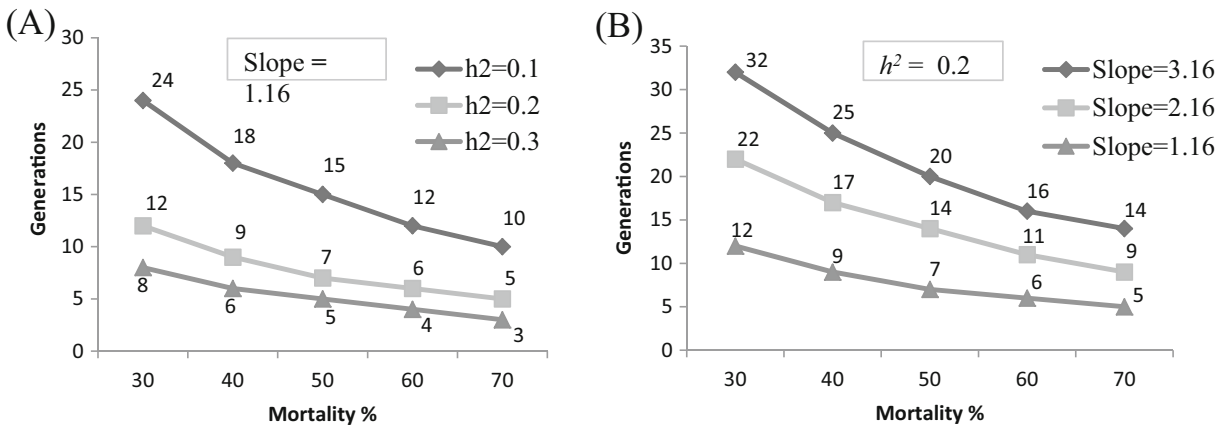

Fig. 1 Effect of heritability (h^2) and slope on the number of generations of *P. solenopsis* required for a ten-fold increase in LC_{50} of acetamiprid at different selection intensities

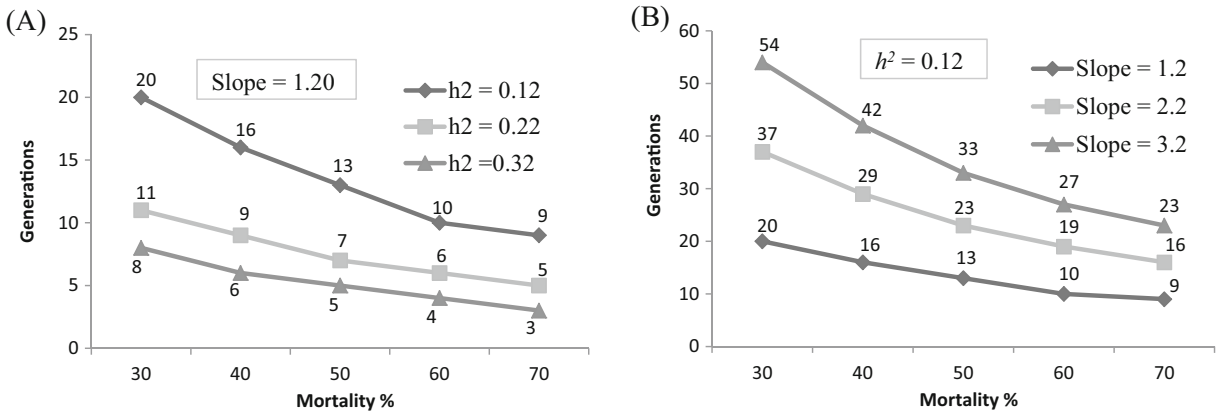

Fig. 2 Effect of heritability (h^2) and slope on the number of generations of *P. solenopsis* required for a ten-fold increase in LC_{50} of imidacloprid at different selection intensities

generations. When $h^2 = 0.18$, then 13-5 generations are required at a 30-70 % selection intensity. But if $h^2 = 0.28$ the same would be expected in only 8-3 generations at a 30-70 % selection intensity (Fig. 4A).

If $h^2 = 0.08$ and slope = 1.13 then 29-12 generations are expected for a ten-fold increase in resistance at a 30-70 % selection intensity. However, by changing the slope = 2.13, 54-23 generations are required at the same selection intensity. Likewise, if the slope = 3.13, then 80-34 generations are required for a similar increase in resistance at a 30-70 % selection intensity (Fig. 4B).

Discussion

Neonicotinoids have been used widely against sucking pests, ornamentals and vegetable crops, etc. Resistance to acetamiprid has been reported in numerous pests, including *P. solenopsis* (Ninsin 2004; Sayyed & Crickmore 2007; Afzal *et al.* 2015). However, there was no literature available on resistance risk assessment to any neonicotinoid in any insect to the best of the authors' knowledge. Initial bioassays of acetamiprid revealed a low level of resistance but exposure to acetamiprid for 24 generations of selection resulted in a high level of resistance (10631-fold) compared to the susceptible strain, signifying that selection had an obvious effect on the evolution of resistance. Evolution of resistance becomes faster under high selection pressure when susceptible genes are replaced with resistant genes leaving a high number of resistant individuals in a given population (Matsumuura 1985). The average survival was 84% in different generations as the population was exposed to increasing concentrations of acetamiprid. However, a sufficient number of the survivors were used for the next generation.

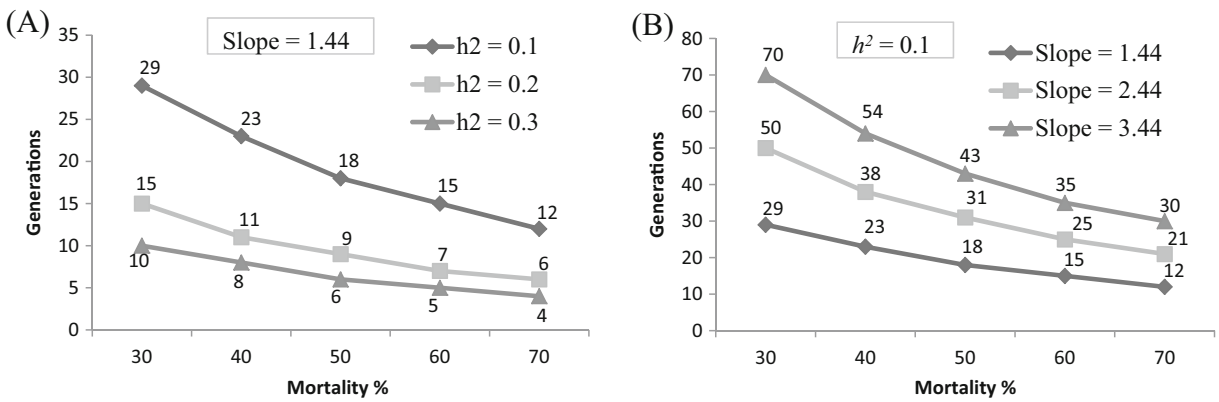

Fig. 3 Effect of heritability (h^2) and slope on the number of generations of *P. solenopsis* required for a ten-fold increase in LC_{50} of chlorpyrifos at different selection intensities

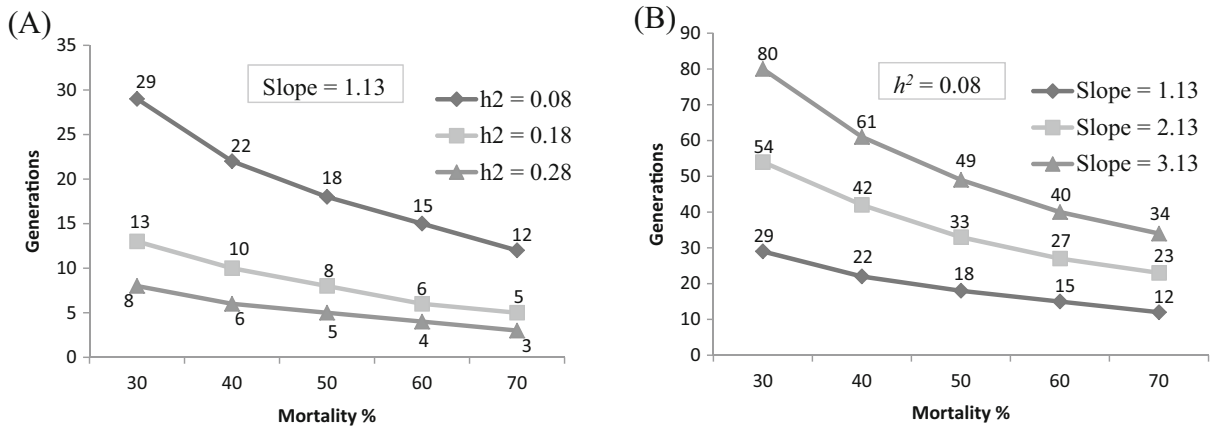

Fig. 4 Effect of heritability (h^2) and slope on the number of generations of *P. solenopsis* required for a ten-fold increase in LC_{50} of deltamethrin at different selection intensities

Estimation of the h^2 provides the standards to compute the results of the selection experiment by integrating the outcomes of the selection intensity and the rate of resistance development. It also places the outcomes of selection in the broader sense of the empirical and theoretical writings of evolutionary biology (Mousseau & Roff 1987; Tabashnik 1992; Falconer *et al.* 1996). The higher h^2 value (0.21) after 26 generations of selection with acetamiprid showed that *P. solenopsis* has a greater potential to develop a high level of resistance and indicated that high phenotypic variations rather than additive genetic variations for acetamiprid resistance occur in *P.solenopsis*. However, R decreased as the selection pressure increased while S increased, leading to a higher h^2 value in the first phase ($G_8 - G_{16}$) of the selection experiment. These findings demonstrated that the genetic variations responsible for acetamiprid resistance were present initially but declined as selection pressure increased. These results are similar to the results of Tabashnik (1992), Sial and Brunner (2010), Lai and Su (2011) and Abbas *et al.* (2014) where alleles responsible for the development of resistance decreased after selection.

Estimation of h^2 based on the selection experiments has certain important limitations such as technical mistakes in estimating the parameters and uncertainty about the application of the results to a field population. Tanaka & Noppun (1989) and Falconer *et al.* (1996) resolved the technical complications from selection experiments in estimating heritability, while Tabashnik (1992) encountered them in the context of estimating the h^2 of insecticide resistance. Rosenheim (1991) provided a detailed exploration of factors that may lead to the estimation of a biased selection differential due to the unequal selection of sexes that is minimized by the selection of unmated individuals. Efforts were also made to minimize individual differences in bioassays, but the extent of biasness in estimating the selection differential that occurred due to sublethal effects (which can underestimate S) and unequal exposure of individuals (which can over estimate S) is unknown. In our study, estimation of h^2 and slope was calculated for different insecticides by measuring the selection intensities to project the rate of resistance development. The projected rate of resistance is directly related to the selection intensity and h^2 of resistance. Estimates of h^2 may vary with environmental conditions and changing allele frequency (Tabashnik 1992) therefore, predictions of h^2 based on laboratory selection experiments should be interpreted carefully. Quantitative genetic theory makes predictions on the basis of $G = R^{-1}$ that provides vital information to design management strategies to curtail the evolution of insecticide resistance by reducing the h^2 value (Firkoi & Hayes 1990; Tabashnik 1992). Approximation of h^2 and other factors responsible for the development of resistance are valuable tools to assess resistance risk for different insecticides (Lai and Su 2011). Our results on the selection for acetamiprid resistance suggest that *P. solenopsis* has a tendency to develop

a high level of resistance in the field and could threaten sustainable production. If the laboratory estimates of h^2 are applied to a field population, then only five generations are needed to result in a ten-fold increase in resistance at a mortality of 70% in each generation. Conversely, results should be considered cautiously due the limitations described above since responses in field populations are determined by selection intensities and genetic variations that occur there.

Phenotypic standard deviation provides a better mean slope than the average of the initial and final slopes because the values of the slope undergo change in each generation. The projected rate of resistance is inversely related to the slope. Likewise, in our study if the slope = 3.16 (acetamiprid), slope = 1.2 (imidacloprid), slope = 1.44 (chlorpyrifos) and slope = 1.13 (deltamethrin) then 14, 9, 12 and 12 generations are needed for a ten-fold increase in the LC_{50} even at a mortality of 70% in each generation, whereas, 9, 16, 21 and 23 generations are required for the same to happen at a slope of 2.16, 2.2, 2.44 and 2.13, respectively.

IRM in *P. solenopsis* is a major challenge for cotton growers. Evolution of a high level of resistance to acetamiprid in *P. solenopsis* restricts its usefulness in resistance management programs. Thus, it can only effectively be used in the field by developing an effective resistance management strategy. Relatively higher numbers of generations are required for chlorpyrifos and deltamethrin (18) to increase ten-fold in resistance as compared to acetamiprid (7) and imidacloprid (13) at 50% mortality. Thus resistance risk exists for all the insecticides. However, thecomparatively higher value for chlorpyrifos and deltamethrin suggests that the risk for resistance development is lower and these could be used in a rotation with acetamiprid in resistance management strategies for *P. solenopsis*. These strategies can only be successful in the absence of or weak cross-resistance among insecticides of the same mode of action or different (Caprio & Suckling 2000; Sial & Brunner 2010). Moreover, identification of the resistant genes and cultural and biological control methods should be incorporated into a resistance management program to slow down the evolution of resistance and to increase the effectiveness of already implemented suppression tactics.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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