Prediction of resistance and its stability of cowpea aphid, *Aphis craccivora* (Koch) to chloropyrifos-methyl

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Abstract

Resistance risk assessment is of great important to predict the probability of resistance development in response to pesticide selection, and consequently insecticides resistance management. Pest's susceptibility to insecticides may change depending on population's selection pressure. In order to verify the extent changes in heritability in a field strain of *Aphis craccivora* to chloropyrifos-methyl, experiments were carried out throughout twenty four generations of laboratory selection, resulted in 105-fold increase in median lethal concentration (LC_{50}) compared to the parent level. In general, the estimated realized heritability of chloropyrifos-methyl resistance was (0.35).The projected rate of resistance development indicated that, if slope (3.38) and h^2 (0.35), then 11–5 generations are required for tenfold increase in LC_{50} at 50–95% selection intensity. Resistance reverse of chloropyrifos- methyl in the absence of selection was studied to investigate its stability**.** After ten generations, resistance reverted to approximately the parent level. These findings suggest that the pest has the potential to develop resistance to chloropyrifos-methyl and it was unstable. The study provided some information contributing in understanding resistance characteristics in *Aphis craccivora* which facilitate its resistance management.

Keywords: *Aphis craccivora*; chloropyrifos-methyl; realized heritability; resistance reverse.

1. Introduction

Cowpea aphid, *Aphis craccivora* (Koch) is a serious legume pest in Egypt **(El-Ghareeb *et al.*, 2002)**. Its damage is due to direct feeding and its ability to transmit virus diseases **(Schepers, 1988)**.The control of aphids mainly relied on insecticides application; different insecticides were used to combat the pest. This strong selection pressure has led to widely distributed insecticide resistant populations **(Devonshire *et al.*, 1989)**.

As a result to pesticides resistance potential, resistance risk assessment has become of great importance because the results can be used to avoid or at least postpone resistance problems **(Anonymous, 1986; Keiding, 1986)**.Realized heritability (h^2) is an index to quantify pushing degree to a trait in a population by selection. It's defined as the ratio of genetic variance to total phenotypic variance; this number can range from 0 (no genetic contribution) to 1 (all differences on a trait reflect genetic variation). It provides effective mean for prediction future evolutionary response to selection **(Tabashnik, 1992)**.

Insecticides overuse caused a serious problems beside resistance include pest resurgence and insecticides pollution, to overcome these disadvantages there's great need to reduce pesticides application **(Lu *et al.*, 2012)**. From the perspective of resistance management, reduction or temporary stop of insecticide to promote the construction of refuge, which often was aimed to protect susceptible insect to dilute the recessive resistant individuals **(Crowder and Carrie` re, 2009 ; Lu *et al.*, 2012)**. No exposure to insecticides could provide the opportunity to combat resistance **(Wilson *et al.*, 2007)**. Temporary stop or rotation of insecticide application was one of alternatives that were put into use to cope with pest resistance **(Yang *et al.*, 2014)**.

In order to determine the heritability of resistance in *A. craccivora* field strain, selection experiment was carried out through twenty four generations. Then, stability of chloropyrifos-methyl resistance was characterized by rearing the resistant strain in the absence of selection and testing it over successive generations.

2. Material and methods

2.1. Insecticides

Chloropyrifos-methyl (Reldan 50% EC, the National Company for Agrochemicals &Investment, Egypt)

2.2. Insects

Field strain of *A. craccivora* which originated from Sharkia Governorate, Egypt was used. In laboratory, aphids were reared on faba bean seedlings, (*Vicia fabae*) grown in a rearing chamber. Faba bean seedlings were maintained in another chamber without exposure to any insecticides until needed. The collected aphid designated as parent (F1) at the first assessment of this study and the next generation after selection was designated F2, followed by F3, etc.

2.3. Bioassay

The leaf-dipping bioassay method corresponded to that described by **Moores *et al.* (1996)** was used. Faba bean leaves were dipped in the aqueous solution of the respective insecticides for about 10 sec., and allowed to dry on a paper towel. Leaves were then placed upside down on an agar bed in small Petri dishes (60mm diameter). Ten apterous adults of *A. craccivora* were placed on the treated leaf surface, while leaves dipped in water served as controls. Five replicate batches of aphids were used per each insecticide concentration, and serial concentrations were used. Petri dishes containing aphids were kept in the rearing chamber until mortality was scored after 48 hrs.

2.4. Selection

Selected strain was reared with selection pressure at each generation. Selection pressure was applied by

using leaf dipping method according to **(Guo *et al.*, 1996)**. Based on preliminary data, the median lethal concentration (LC_{50}) for the tested insecticide was used for the first generation, and a new LC_{50} for insecticide was used based on the resistance level from bioassay results every two generations. Faba bean seedlings were infested with apterous adults for 24 h. before treatment. The plants bearing aphids were dipped in each insecticide dilution for10s. After being completely dried for about 1h, the plants were placed in the rearing room. The surviving aphids were transferred to new plants. Aphids were maintained on the plants and mature apterous adults of the new generation were used for bioassay. Heritability was calculated using the formula presented by **Falconer, 1989** and **Tabashnik, 1992**.

Reversion bioassay was conducted by leaf-dipping as described above per the explained generations. Chloropyrifos-methyl resistant strain was maintained without exposure to insecticides to determine resistance reversion.

2.5. Estimation of realized heritability

Realized heritability (h2) was estimated by using the method described by Tabashnik (1992) as follows:

$$h^2 = \frac{\text{Response to selection(R)}}{\text{Selection differential (S)}}$$

Response to selection (R) was estimated as follows:

$$R = \frac{(\text{Log final } LC_{50} - \text{Log initial } LC_{50})}{n}$$

Where the final LC_{50} is the LC_{50} of population after n generations of selection and initial LC_{50} is for the parental population before selection.

The selection differential (S) was estimated as follows:

$S = i^{\delta}p$,

Where i is the intensity of selection and is calculated according to **Falconer (1989)** and $^{\delta}p$ is the phenotypic standard deviation, calculated as:

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

Or $(\text{mean slope})^{-1}$

To estimate either a change in R, S, and h^2 during the selection pressure, each parameter was calculated for the first and second half of the experiment (12 generations in each half).

The response to selection (R) can be estimated as follows:

$R = h^2S$

The number of generations required for a tenfold increase inLC_{50} was calculated as follows:

$G = R^{-1} = (h^2S)^{-1}$

Effect of heritability on projected rate of resistance increase at constant slope value was assessed by drawing a graph between percent mortality and generations. Three values of h^2were used (one value was calculated from F1 to F24 and other two values were assumed theoretically and same procedure was adopted for effect of slope on projected rate of resistance evolution at calculated constant value of h^2.

2.6. Mortality assessment and statistical analysis

Mortality was scored after 48-h. adults failing to exhibit coordinate forward movement when probed with a soft camel hair brush was considered dead. Mortality was corrected for control using Abbott's formula **(Abbott 1925)**. Data were analyzed by probit analysis **(Finney, 1971)** using the software package EPA probit analysis version 1.5.

3. Results

3.1. Selection and resistance development

The selection of chloropyrifos- methyl was started by exposing the adults of field strain to median lethal concentration at F1 (parent) and selection pressure was maintained for 24 consecutive generations to generate chloropyrifos- methyl resistant strain. Resistance level of chloropyrifos- methyl was monitored at every two generations in respect to field strain. Sequential selection with chloropyrifos- methyl for 24 generations resulted in LC_{50} values increasing from 0.18 to 18.9 (mg $Litre^{-1}$) and the resistance ratio increased to105-fold compared with parental field strain (Table 1).

Table 1. Resistance development in the cow pea aphid *A. craccivora* exposed to laboratory selection with chloropyrifos-methyl.

Generation	Slope ± SE	LC_{50} (mg $Litre^{-1}$)	95% CL (mg $Litre^{-1}$)	RR (fold)
Parent	0.82 ± 28	0 .18	(0.04 – 0.93)	1
F2	2.93±0.85	1.10	(0.60 - 1.55)	6.1
F4	1.72 ± 0.49	1.69	(0.80 - 2.93)	9.3
F6	2.08± 0.40	2.44	(1.35 - 3.60)	13.5
F8	2.51±0.60	4.22	(2.73 - 5.94)	23.4
F10	4.43±1.50	7.37	(4.77 - 9.41)	40.9
F12	2.96± 0.55	7.99	(6.03- 10.15)	44.38
F14	3.52± 0.618	10.4	(8.64- 12.3)	57.7
F16	4.46±1.25	13.15	(8.4- 15.9)	73
F18	4.55±.43	14.4	(10.2- 17.45)	80
F20	4.9 ±. 1.17	15.03	(13.1- 17.4)	83.5
F22	3.22 ± 0.67	16.3	(13.45- 19.8)	90.5
F24	2.51 ± 0.74	18.9	(10.3- 27.4)	105

RR (resistance ratio) = LC_{50} of tested generation/ LC_{50} of parent
CL: Confidence limit

3.2. Realized heritability (h^2)

After 12 generations of selection, LC_{50} values increased from 0.18 to 7.99 ppm. Further selection until (F24) LC_{50} increased to 18.9 ppm (Table1).The heritability of resistance (h^2) estimated over the first 12 generations of selection showed a high value (0.44) decreasing to (0.10) in the second round of selection (F12-F24).Response to selection(R) was higher in the first half than that in the second half. Therefore, the estimated h^2 of resistance to chloropyrifos-methyl was higher for the first half than that of the second half of selection experiment. The higher variance for resistance to chloropyrifos-methyl was lower and additive genetic variance was higher during the first half (Table2).

In general, results of 24 generations of selection elucidated in (Table 2) revealed that realized heritability (h^2) showed a moderate value (0.35). Low h^2 (less than 0.1) occurs when the offspring of the selected parents differ little from the original population, in spite of a big difference between the population as a whole and the selected parents. A high h^2 (greater than .6) occurs when the offspring of the selected parents differ from the original population almost as much as the selected parents do.

Table2.Estimation realized heritability (h^2) of resistance to chloropyrifos-methyl in adults of *Aphis craccivora* (Koch).

Selected generations	No. Selected generations	Estimate of mean responseper generation		R	Estimate of mean selection differential per generation				S	h^2
		Log initial LC_{50}	Log final LC_{50}		P	I	Mean slope	$^{\delta}p$		
(F1–F12)	12	-0.74	0.90	0.14	50.0	0.798	2.49	0.40	0.32	0.44
(F12–F24)	12	0.90	1.28	0.02	50.0	0.798	3.73	0.27	0.21	0.10
(F1–F24)	24	-0.74	1.268	0.09	50.0	0.798	3.38	0.30	0.24	0.35

n, number of generations selected
R, response to selection (R= (initial LC50-final LC50/ n)
i, intensity of selection (Falconer, 1989)
$^{\delta}$p, the phenotypic standard deviation ($^{\delta}p = [1/2(\text{initial slope} + \text{final slope})^{-1}]$
S, selection differential (S=i. $^{\delta}$p)
h2, realized heritability (h^2=R/S).

3.3. Projected rate of resistance evolution

The projected rate of resistance development is proportional to h^2 and intensity of selection (Fig. 1). For example, we assumed that slope = 3.38 (the value of mean slope for the present work) and h^2 = 0.35, then 11–5 generations are required for tenfold increase in LC_{50} at 50–95% selection intensity. However, at similar slope of h^2 = 0.15, then28–11 generations are required for tenfold increase in LC_{50} at 50–95% selection intensity. Likewise, similar would occur in only 9–3 generations at 50–95%selection intensity if h^2 = 0.5. The projected rate of resistance development is inversely proportional to the slope (Fig. 2). For example, if we assumed that h^2 = 0.35 (heritability of chloropyrifos-methyl resistance estimated in the present study), and slope = 3.38 (the value of mean slope for the present work) and h^2 = 0.35, then 11–5 generations are required for tenfold increase in LC_{50} at 50–95% selection intensity. However, at the same h^2 if slope = 4.38, then 17–6 generations are required for tenfold increase in LC_{50} at 50–95% selection intensity, respectively. Similarly if slope = 1.38, then the same would happen in 6–2 generations at 50–95% selection intensity, respectively (Fig 2).

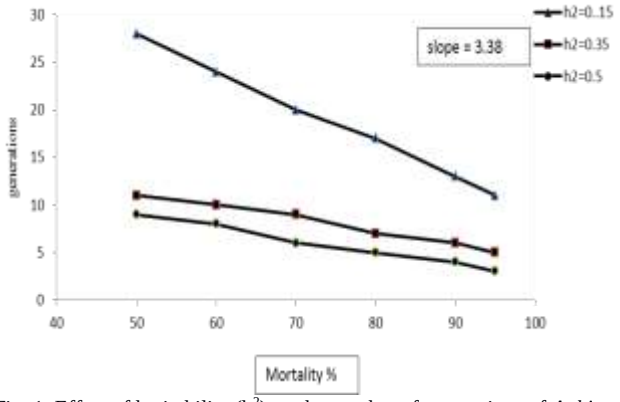

Fig. 1. Effect of heritability (h^2) on the number of generations of *Aphis craccivora required* for a tenfold increase in LC_{50} of chloropyrifos-methyl (slope = 3.38) at different selection intensities.

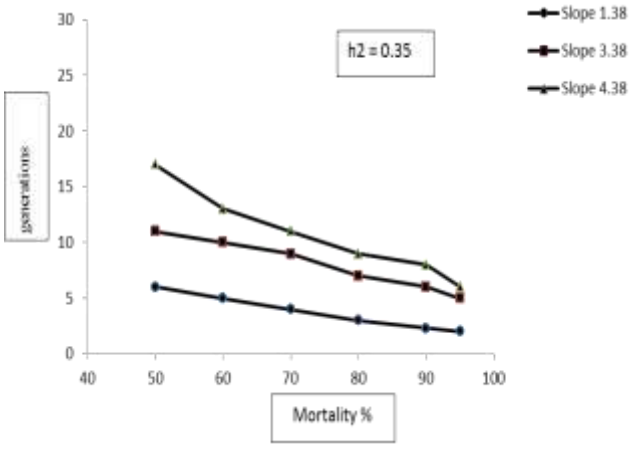

Fig. 2. Effect of slope on the number of generations of *Aphis craccivora* required for a tenfold increase in LC_{50} of chloropyrifos-methyl (h^2 = 0.35) at different selection intensities.

3.4. Resistance reversion

After ten generations without exposure to chloropyrifos methyl, resistance was unstable and LC_{50} decreased from16.34 ppm (91-fold) to 0.43 ppm (2.9-fold).Resistance level of cowpea aphid to chloropyrifos methyl decreased steadily from 1^{-st}generation to 9^{-th}generation with resistance factor of 12 fold. While tended to decrease sharply from 9^{-th}generation to 10^{-th} generation with resistance factor of 2.4- fold (Table3).

Table 3. Resistance reversion of chloropyrifos- methyl in the cow pea aphid, *Aphis craccivora* (Koch).

generation	Slope ± SE	LC_{50} (mg Litre-1) 95% CL	RR
Parent(G1)	0.82 ± 28	0.18(0.04-0.93)	-
1^{-st} Generation	3.60± 0.61	16.34 (13.06 - 19.32)	91
2^{-end} Generation	2.42± 0.35	13.56 (11.12 - 17.24)	75
3^{-ed} Generation	3.79± 0.92	6.94 (4.78- 8.81)	38.5
$4^{-th.}$ generation	1.16± 0.19	3.59 (1.85 - 6.15)	20
5^{-th} generation	2.06± 0.53	3.60(2.07 - 5.380)	20
$6^{th.}$ generation	2.09± 0.40	2.62 (1.87 - 3.53)	14.5
$7^{th.}$ generation	2.19± 0.63	2.54 (1.29 - 3.485)	14
$9^{th.}$ generation	1.74± 0.50	2.15(0.77- 3.569)	12
$10^{th.}$ generation	1.14± 0.35	0.43(0.10- 0.84)	2.4

Resistance ratio (RR) = LC_{50} of the tested generation/ $LC_{50}$of parent (G1)

4. Discussion

Resistance risk assessment to an insecticide provides useful information to devise a pro-active resistance management strategy **(Sayyed *et al.*, 2004)**. Laboratory selection experiments provide essential information to assess the resistance risk in an insect species to a particular insecticide. Moreover, selection experiments data is analyzed by the quantitative genetic techniques to obtain additive genetic variance and realized heritability of resistance **((Jutsum *et al.*, 1998; Firkoi and Hayes, 1990)**.

Realized heritability (h^2) is an important indicator for evaluating the sustainability of a chemical on a pest population **(Sayyed *et al.*, 2005)**. Heritability provides a good indication for pest ability to develop resistance to insecticides **(Johnson and Tabashnik, 1999; Roush and Daly, 1990)**. Realized heritability (h^2) is the proportion of phenotypic variation accounted for by additive genetic variation. Lower h^2 reflects higher phenotypic variation and lower additive genetic variation. Phenotypic variation is composed of genetic variation and environment variation **(Yang, 2000)**. Under laboratory conditions, higher phenotypic variation can come from selection stress and gene mutation, but in field conditions, may come from pest migration, alternation of insecticides, selection pressure and environmental factors **(Tabashnik, 1992)**. Phenotypic variation is increased by additive genetic variation and environmental variance **(Abbas *et al.*, 2014)**. Realized heritability (h^2) may decrease either due to the decrease in genetic variance or to the increase in environmental variance **(Falconer *et al.*, 1996)**. The estimated h^2 values provide evidence for the potential of resistance development **(Moulton *et al.*, 2002)**.So, realized heritability (h^2) may of great importance to predict the rate at which a pest population may increase its tolerance to pesticide and consequently managing resistance in field.

In the present study, the higher h^2 (0.44) in the first round of laboratory selection indicate high chance of resistance development to chloropyrifos methyl. In contrast, the second round of selection (F12- F24) showed low h^2 (0.10).Higher h^2 in first round compared with in second round of selection resulted from increased response to selection. Higher heritability in initial generations selected with chloropyrifos-methyl and low heritability with the later ones, suggested that by the 12^{-th} generation the population had approximately stabilized.

The calculated h^2 due to the laboratory selection tests might be higher than in field because of decreased ecological differences **(Zhang *et al.*, 2008)**. Though laboratory trials do not absolutely indicate field circumstances, the approximated h^2 value provides proof for the prospective of further improvement in level of resistance **(Tabashnik, 1992)**.

Estimates h^2 in conjunction with estimates of selection intensity can be used to project rates of resistance development. Prediction based on h^2 must be interpreted cautiously because h^2 of resistance to a particular insecticide can vary between conspecific populations as well as within populations as a result to allele frequencies and environmental variation over time. So, the predictions made from quantitative genetic theory on the basis of $G= R^{-1}$ gives valuable information to develop strategies for managing pesticide resistance **(Tabashnik, 1992)**. Estimating h^2 from laboratory selection experiments for resistance is necessary to assess the risk of insecticide resistance in pests **(Lai and Su, 2011)**.The present results of the selection experiment for resistance to chloropyrifos-methyl showed that *A. craccivora* populations have the ability to develop resistance to this insecticide in the field. If the laboratory estimates of h^2 apply to the field strains and 95% mortality occurred in each generation, then the *A. craccivora* strain can be expected to increase ten-fold resistance after only 5 generations ($h^2 = 0.35$) (Fig. 1).

Owing to the different values of the slope in different generations, the estimation of phenotypic standard deviation (δp = 1/average slope) proposed by **Tabashnik and McGaughey (1994)**, may provide a more reliable estimate of the mean slope than simply the average of the initial and final slopes. Furthermore, the projected rate of resistance evolution is inversely proportional to the slope of the probit line (Fig. 2). For example, assuming that h^2 = 0.35(the heritability of chloropyrifos-methyl resistance observed in this study) and selection mortality = 95%, a tenfold increase in LC_{50} would occur in only 2 generations at a slope of 1.38, whereas, it would take 10 generations for the same to happen at a slope of 4.38.

Studies on resistance reverse in the selected strains by discontinuing the selection pressure may help to

prolong the efficacy of insecticides **(Shah *et al.*, 2015).** In the absence of chloropyrifos-methyl, resistance has reverted from 91 to 2.41- fold (approximately pre-selection level). Reversion could be attributed to the inability of the resistant individuals to compete effectively with the susceptible ones in terms of reproductive potential and other biotic factors **(Georghiou, 1963; Ninsin and Tanaka, 2005)**. Sometimes reversion cited as a prerequisite for the success of rotational strategies for resistance management in the field **(Tabashnik, 1990)**.Reversion of resistance has been previously reported in a variety of insect pests against different insecticides **(Wilson et al., 2007)**.

Generally, realized heritability is a useful tool for predicting the future use of certain insecticide. The currently study suggest that *A. craccivora* has resistance risk to chloropyrifos-methyl, so it's necessary to use this insecticide wisely and using alternative insecticides having different modes of action and without cross-resistance in rotation in a management program **(Sial and Brunner, 2010)**.Resistance reversion of chloropyrifos-methyl suggested the possibility of pesticide reuse after a temporary stop of insecticide application, which could be one of the operational strategies in resistance management.

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