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Cross-resistance, genetics and stability of resistance to deltamethrin in a population of *Chrysoperla carnea* from Multan, Pakistan

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ABSTRACT

Broad-spectrum insecticides are still widely being used. *Chrysoperla carnea* has been shown to develop resistance to the insecticides in the field. Knowledge of the evolution and genetics of resistance to insecticides in natural enemies could enable to explain how effectively natural enemies can be implemented in different IPM systems. To examine this, a population of *C. carnea* from Multan Pakistan was collected and was subjected to deltamethrin selection in the laboratory. Bioassays at generation G_1 gave resistance ratios of 47, 86, 137, 76 and 110 for deltamethrin, alphamethrin lambdacyhalothrin, chlorpyrifos and profenofos respectively compared with susceptible Lab-PK. Bioassays at G_4 with a deltamethrin-selected population (Delta-SEL) showed that selection gave resistance ratios of 896 and 30 for deltamethrin compared with the Lab-PK and UNSEL, respectively. Cross-resistance to other insecticides tested was observed in the selected population. A notable feature of the Delta-SEL strain was that resistance to deltamethrin, alphamethrin, lambdacyhalothrin, chlorpyrifos and profenofos did not decline over the course of four generations. Synergism tests with microsomal oxidase (MO) and esterase-specific inhibitors indicated that the deltamethrin resistance was associated with MO and, possibly, esterase activity. Reciprocal crosses between the Delta-SEL and Lab-PK strains indicated that resistance was autosomal and incompletely dominant. A direct test of monogenic inheritance suggested that resistance to deltamethrin was controlled by more than one locus. The broad spectrum of resistance, cross resistance, incompletely dominant mode of inheritance and stability of resistance to insecticides suggest that Delta-SEL population of *C. carnea* could be compatible with most spray programs.

1. Introduction

Evolution of insecticide resistance by insects threatens human welfare through its impact on crop protection and disease transmission. Insecticide resistance is now an immense practical problem, challenging the management of agriculturally and medically important insect pests. Pesticide exposure has resulted in ca. 600 arthropod species being documented as resistant to at least one insecticide or acaricide [1]. In contrast to insect pests, there are very few documented cases of resistance to insecticides in insect natural enemies [2,3]. For example, the generalist predators, common green lacewing, *Chrysoperla carnea* (Steph) has been shown to develop resistance to insecticides in the field [3]. Recently we also have shown that the various populations of the predator collected from five different areas of Pakistan can develop resistance to pyrethroids and organosphates [3]. Resistance to insecticides in natural enemies, however, has generally been shown to develop slowly because of a combination of biological, ecological, and biochemical (lower detoxification capacity) factors [4]. A recent study with pyrethroids and organophosphate resistant populations of *C. carnea* has revealed a significantly higher intrinsic rate, survival rate to adulthood, doubling time and predation rate of the resistant populations compared with the susceptible population [5]. *Chrysoperla carnea* is widely distributed in Indo-Pakistan subcontinent [6] therefore this fact, adds to its preference for open habitats, makes the predator of great potential as a biological control agent in Pakistan where chemical insecticides are still the main tools for insect pest management. They are the most efficient predators of several important agricultural insect in the region such as *Thrips tabaci, Bemisia tabaci, Aphis gossypii, Phenacoccus solenopsis, Amrasca devastans, Tetranynchus urticae* and several other important pests of agriculture crops [7].

The biorational insecticides (relatively innocuous against non target insects) are an alternative to conventional insecticides because of their selectivity against pests and lower impact on beneficial organisms. However, in developing countries including Pakistan, broad-spectrum insecticides (pyrethroids and organophosphates) are still being widely used. Pyrethroids and organophosphates act on the function of voltage-sensitive sodium

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channel and acetylcholinesterase, respectively [8–10]. Esterases, mixed function oxidases and glutathione *S*-transferases have been implicated as broad spectrum insecticide resistance mechanisms to insecticides in numerous insect pests[8,11,12] owing to their ability to hydrolyze insecticide and to sequester xenobiotics (e.g. Li et al. [13]). The cross-resistance mechanism between organophosphates and pyrethroids in *C. carnea* could therefore also be due to the presence of esterases and monooxygenases enzymes. Given the importance of *C. carnea*, we were, however, interested to investigate whether selection with a particular insecticide could also increase resistance to other insecticides and is the cross-resistance between different insecticides conferred by known resistance mechanisms. This could have important implications in integrated pest management (IPM), which advocates both chemical and biological control in agricultural systems, since natural enemies are one of the key components of IPM, and they are often recommended as the first line of defense in an IPM programme [12].

The use of pesticide-resistant natural enemies may prevent pest resurgences and secondary pest outbreaks in several cropping systems in which chemical control of pests is a common practice. Knowledge of the evolution and genetics of resistance to insecticides in natural enemies could enable the development of IPM programs that minimize the use of insecticides [14]. The genetic basis of insecticide resistance in insect pests populations has been extensively studied [15,16]. To the best of our knowledge, however, there is no documented case of genetics of resistance to insecticide in natural enemies. The genetics of insecticide is particularly important to understand the number of genes involved and whether resistance trait(s) dominant or recessive. Knowledge of the mode of inheritance and stability of resistance in the absence of exposure to insecticides can also help to explain how effectively natural enemies can be implemented in different IPM systems. To examine this, a population of *C. carnea* from Multan Pakistan with a history of exposure to various insecticides, including pyrethroids and organophosphates [3] was collected and was subjected to deltamethrin selection in the laboratory.

2. Material and methods

2.1. Insects

Adults *C. carnea* were collected from Multan Pakistan as described previously [3] from cotton with an insect sampling device (John W. Hock Company, Gainesville, FL). The collected adults were kept in plastic jars ($12 \times 12 \times 20$ cm) with artificial diet (yeast, honey, and distilled water; 1:2:4 ratios) and were transported to the laboratory of IPM Station PARC, University College of Agriculture, B.Z. University, Multan in air conditioned vehicle to avoid mortality due to temperature fluctuation. The laboratory susceptible population of *C. carnea* was collected from Multan in 1999 and it was designated as Lab-PK [3]. The population was in the laboratory for over 10 years without exposure to insecticides before start of this study. In the laboratory adults were kept at 25 °C, 60 ± 5% RH and a photoperiod of 18:6 (L:D) h. in a plastic rearing cage ($23 \times 38 \times 38$ cm) with muslin cloth on two sides. Kitchen foil (10×4 cm) was hung in the cage for egg laying. To rear larvae and also to treat with insecticides, the eggs were collected on alternate days by removing Kitchen foil from the rearing cages. An individual egg was placed in a vertical well (4×3 mm) of perpex cell chamber with the help of forceps. After two to three days eggs were hatched in these cells and usually more than 90% hatching occurred from the harvested eggs. Frozen eggs (kept for 15 min at 0 °C) of laboratory reared *Sitotroga cerealella* were provided to newly hatched larvae of *C. carnea* in the individual well.

2.2. Insecticides

The formulated insecticides used were Curacron EC (profenofos 500 g/l, Sygenta Crop Protection, Basil, Switzerland), Lorsban EC (chlorpyrifos 400 g/l, Dow Agro Sciences, Hitchin, United Kingdom), Karate EC (lambdacyhalthrin 25 g/l, Syngenta Limited, Jealot Hill, United Kingdom), Bestox EC (alphamethrin 50 g/l, FMC, Philadelphia, PA), and Decis Super EC (deltamethrin 100 g/l, Bayer Crop Sciences, Montpellier, France).

2.3. Bioassays

Bioassays were conducted on 2- to 3-d-old larvae of *C. carnea* populations by using a topical application assays method as described previously [3]. Briefly larvae were directly treated with insecticide by using an auto-microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, England) equipped with a 1-ml glass syringe; 0.5 μl of an insecticide solution in water was applied onto the pro-thorax of individual larva. The treated larvae were kept as described above (Section 2.1). Each insecticide was tested with six concentrations to determine the LC_{50} value, and each concentration was replicated three times. One larva was placed in each cell of rearing plates and 10 larvae were used per replication therefore 30 larvae were treated for each insecticide concentration, and 30 control larvae were treated with water only. The bioassays were kept at a temperature 25 °C, 65% RH, and a photoperiod of 16:8 (L:D) h. Mortality was assessed after 120-h exposure to insecticides. Larvae were considered dead if they failed to make a coordinated movement when prodded with a probe.

2.4. Selection of C. carnea with deltamethrin

The field population was divided into two sub-populations at G_1. One subpopulation was left unselected (UNSEL), and the other was selected with deltamethrin (Delta-SEL) from G_1 to G_4 at which point selection was stopped. Selection was achieved by exposing L_2 larvae to various concentrations (500, 800, 1000 and 1500 μg ml^{-1} in G_1, G_2, G_3 and G_4, respectively) of deltamethrin in topical bioassays [3]. The number of larvae selected per generation ranged between 300 and 500. The bioassays were kept at a temperature 25 °C, 65% RH, and a photoperiod of 14:10 (L:D) h. Mortality was assessed after 48-h exposure to insecticides. The mean survival of larvae for four generations of selection was 39%.

2.5. Realized heritability of deltamethrin resistance in C. carnea

Estimation of heritability, the proportion of phenotypic variation accounted for by additive genetic variation, is a standard method to summarize insecticide selection experiments [17]. This approach relies on the assumption that the logarithm of resistance to insecticide is normally distributed similar to probit analysis [18]. The normal distribution of insecticide resistance in a population could be due to environmental variation and resistant genes, or interactions between genes and environment. Thus, estimation of heritability makes no assumptions about mode of inheritance and may be useful when resistance is controlled by one or many loci [19]. We used selection experiment data (LC_{50} and slope before and after selection or average mortality per generation caused by selection) to estimate the realized heritability.

2.6. Stability of resistance in Delta-SEL and UNSEL populations

The UNSEL and Delta-SEL sub-populations, which were cultured in the absence of selection pressure, were assayed at G_5 and G_9, respectively. A decline or increase in resistance to different insecticides in the populations was measured by calculating an *R*