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Assessment of resistance risk to fipronil and cross resistance to other insecticides in the *Musca domestica* L. (Diptera: Muscidae)

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ABSTRACT

Fipronil, a phenyl-pyrazole insecticide has been used frequently for the control of disease vector house flies, *Musca domestica* L., (Diptera: Muscidae) worldwide including Pakistan. This experiment was performed to determine the selection and assessment of fipronil resistance evolution along with cross resistance to other three insecticides. After 26 generations of selection, the house fly strain developed 430-fold resistance to fipronil compared to a susceptible strain. Realized heritability (h^2) of resistance to fipronil was 0.05. The projected rate of resistance development revealed that if 30–90% house flies were selected then a tenfold increase in lethal concentration 50 happened after 95.51–26.59 generations for fipronil (h^2 = 0.05, Slope = 2.34). At similar slope, if h^2 = 0.15, then 31.84–8.86 generations are required for tenfold increase in LC_{50} at 30–90% selection intensity, respectively. Likewise, if h^2 = 0.25, then similar would occur in 19.10–5.32 generations. Differences in any of the variable would affect the rate of resistance development. Selection with fipronil did not increase the level of resistance to lambda-cyhalothrin, profenofos and indoxacarb, suggesting no cross resistance to these insecticides. The results of our study concluded that house flies have the potential to develop resistances following continued selection pressure with fipronil.

1. Introduction

The house fly, *Musca domestica* L., is a vector of diseases in livestock and responsible for the transmission of more than 100 pathogens of humans, poultry and livestock (Abbas et al., 2014b; Förster et al., 2007). Moreover, outbreaks of diarrheal diseases are associated with seasonal abundance of house flies in urban and rural settings of various countries including Pakistan (Graczyk et al., 2001; Khan and Akram, 2014). It is also a vector of avian influenza (bird flu), a serious problem in poultry throughout the world (Barin et al., 2010; Wanaratana et al., 2011). It has been reported that millions of domestic poultry flocks are affected by avian influenza virus and this has resulted in more than 150 deaths of humans globally (Otte et al., 2007). In areas of poultry farming, like Punjab Pakistan, uncovered poultry manure provides an ideal condition for the growth and reproduction of house flies. A high density of flies irritates the workers, stresses the chicks and reduces the aesthetic value of livestock products leading to economic losses (Abbas et al., 2014a; Acevedo et al., 2009).

Carbamates, organophosphates, pyrethroids and new chemistries have been used to control house flies worldwide. Insecticide applications can be very helpful in conditions where a high density of house flies is associated with avian influenza in poultry (Nielsen et al., 2011) and diarrhea in humans (Chavasse et al., 1999) but resistance to insecticides limits their efficacy. A new chemical introduced for the control of house flies may lose its efficacy due to inappropriate use as well as previous insecticidal exposure can result in cross resistance to alternative insecticides. These factors can facilitate the spread of avian influenza and diarrhea diseases in poultry and humans, respectively, in developing countries like Pakistan. Resistance of house flies to various chemicals has been repeatedly reported (Abbas et al., 2015, 2014a,b; Kaufman et al., 2010; Khan et al., 2014b; Scott et al., 2000; Shah et al., 2015c,d; Shono et al., 2004).

Fipronil is a phenyl-pyrazole insecticide which inhibits chloride ion flow by disruption of gamma-amino butyric acid (GABA) receptors in the central nervous system of insects (Ikeda et al., 2003; Ratra and Casida, 2001). These receptors are well-known especially in the context of insecticide resistance, where the first good case of target site resistance were shown with the resistance of Diedrin gene (rdl) (Hansen et al., 2005). Fipronil is a relatively new insecticide and is effective against an array of insect pests of

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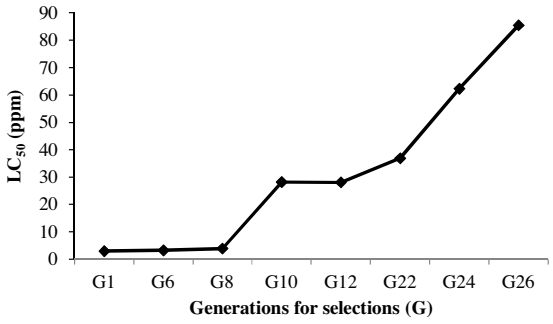

Fig. 1. The development of fipronil resistance in house fly.

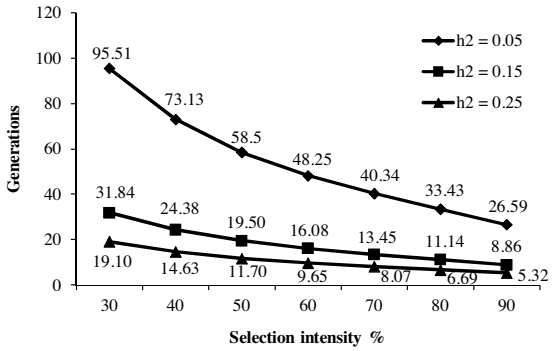

Fig. 2. Effect of heritability (h^2) on the number of generations of house fly required for a tenfold increase in LC_{50} of fipronil (slope = 2.34) at different selection intensities.

agricultural, veterinary and medical importance. Its use is preferred due to its higher toxicity to insects than mammals (Gant et al., 1998; Hainzl et al., 1998). However, resistance to fipronil has been reported previously in various insect pests including *Spodoptera litura* (Fabricius) (Ahmad et al., 2008), *Plutella xylostella* (L.) (Sayyed and Wright, 2004), *Sogatella furcifera* (Horvath) (Tang et al., 2010) and *M. domestica* (Kristensen et al., 2004; Liu and Yue, 2000).

Integration of highly effective insecticides to avoid resistance development is a critical approach in integrated pest management strategies. Therefore, it is important to assess the resistance risk of an insecticide due to its extensive use in the field (Lai and Su, 2011). Such as resistance risk assessment can provide valuable information to help delay resistance development and to maintain susceptibility of insect pest species. There are different ways to assess resistance risk for an insecticide such as selection for resistance (in laboratory or field) and quantitative genetic techniques (Falconer and Mackay, 1996; Jutsum et al., 1998). Quantitative genetic models can be used to analyze the data from selection experiments as a continuous genetic variable and estimate heritability of resistance (Firkoi and Hayes, 1990). Estimation of realized heritability, a measure of phenotypic and additive genetic variation, provides a standard way to summarize data, which helps in understanding of the evolution rate and the direction of resistance (Firkoi and Hayes, 1990; Tabashnik, 1992). It also enables direct comparisons among selection experiments that differ in selection histories in term of intensity and duration (Falconer, 1989; Tabashnik, 1992). Assessment of the risk of resistance to fipronil can help to develop resistance management strategies to maintain susceptibility in field populations of house fly, sustaining the efficacy of this insecticide. We have therefore assessed the likelihood of house flies developing resistance to phenyl-pyrazole insecticide, fipronil and cross resistance to other insecticides.

2. Materials and methods

2.1. Insect rearing

The susceptible strain was originally collected from an urban area (Multan, Pakistan) reared in our laboratory without exposure to any insecticide for more than one year and designated as Susceptible strain. The field strain was collected from a poultry farm located in Multan (30°11′44′N; 71°28′31′E) using a sweep net. The collected adult flies were kept in plastic jars (17 × 34 cm) in the laboratory and fed on powdered milk and sugar (1:1 ratio w/w). Cotton wicks moistened with water were provided in a separate Petri dish. The larvae were reared on an artificial diet according to Abbas et al. (2014a). Plastic cups (7 × 7 cm) containing diet was placed in a plastic jar for egg laying and removed every two days. After feeding in the cups, larvae were shifted to glass jars for pupation. After emergence, adult flies were moved into plastic jars to mate. All strains were maintained in controlled laboratory conditions according to Abbas et al. (2014a).

2.2. Insecticides

The insecticides used for bioassay were fipronil (Regent® 050EC, Bayer Crop Sciences), indoxacarb (Steward® 15SC, DuPont), lambda-cyhalothrin (Karate 2.5EC, Syngenta) and profenofos (Curacron, 500EC, Syngenta).

2.3. Fipronil selection

The field population, after the first generation (G_1), was exposed with fipronil from G_1 to G_{26} to develop a resistant strain designated as Fipro-SEL. The field collected house flies were bioassayed (5–6 concentrations) to know the lethal concentration for desired selection process (i.e. 70% mortality) before starting the selection. Two day old flies were exposed to fipronil by providing cotton wicks soaked in 20% sugar solution for selection. Mortality was assessed 72 h after treatment and the surviving flies were used as parents of next generation.

2.4. Bioassays

The toxicities of the above insecticides were assessed using feeding bioassays according to Kaufman et al. (2001). Briefly, ten 2 to 3-day-old randomly collected male and female flies were placed in plastic jars (250 mL). One piece of cotton wick (3 cm length) moistened with a 20% sugar water solution with different concentrations of insecticide were provided. Five concentrations of each insecticide were used and three technical replicates were set up per concentration. Cotton wicks soaked in 20% sugar solution without insecticide were provided as a control (3 replicates of 10 flies). Cotton wicks were refreshed with tap water after 24 and 48 h to avoid drying (Kaufman et al., 2006). All flies were maintained at the laboratory conditions described above. Mortality was assessed 48 h after treatment for lambda-cyhalothrin and profenofos and 72 h after treatment for fipronil and indoxacarb. All ataxic flies were assumed to be dead.

2.5. Statistical analysis

Bioassay data were analyzed by probit analysis (Finney, 1971) using POLO PC software to determine lethal concentration 50 (LC_{50}), confidence interval (CI) and slopes. Resistance ratio was calculated as follows:

$$RR = \frac{LC_{50}\text{ values of Fipro} - \text{SEL strain}}{LC_{50}\text{ values of Susceptible strain}}$$