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Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan

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The toxicity of representative newer insecticides, which are being used widely in Pakistan, were investigated against various populations of *Spodoptera litura* (Fabricius) collected from three different districts for 3 consecutive years. For spinosad, resistance ratio compared with Lab-PK were in the range of 7–122-fold, 3–95-fold for indoxacarb, 4–186-fold for abamectin, 2–77-fold for emamectin and 13–224-fold for fipronil. The resistance ratio for insect growth regulator (IGR) tested was in the range of 2–66-fold for leufenuron, 8–56-fold for diflubenuron and 2–153-fold for methoxyfenozide. Paired wise comparisons of the log LC_{50}s of insecticides tested for all the populations showed correlations among several insecticides, suggesting a cross-resistance mechanism. The most probable reason for low toxicity of these insecticides could also be the development of multiple resistance mechanisms; however, further studies are required to establish these mechanisms. When these same products were tested against a susceptible laboratory population (Lab-PK), emamectin and indoxacarb were significantly more toxic than other compounds tested. The results are discussed in relation to integrated pest management (IPM) for the *S. litura* with respect to unstable resistance in the field.

1. Introduction

Spodoptera litura (Fabricius) is a serious pest causing enormous losses to many economically important cultivated crops such as cotton, soybean, groundnut, tobacco and vegetables (Qin et al., 2004). Sometimes it has been found to cause 26–100% yield loss in the field (Dhir et al., 1992). Its control has depended mostly on application of various insecticides. As a result, many field populations of this pest have developed multiple resistances and field control failure has been observed very frequently (Ahmad et al., 2007a, b; Armes et al., 1997; Kranthi et al., 2001).

Resistance to insecticides is a major problem associated with the chemical control of insect pests. Previous exposure and selection with insecticides can confer resistance to newly introduced insecticides through cross-resistance (Bisset et al., 1997), reducing the effectiveness of many new insecticides. The insecticide resistance to newer insecticides has not received considerable attention particularly in *S. litura*. There are few data available on the changes in conventional insecticide susceptibility in *S. litura* from Asian cotton-growing countries such as Pakistan, China and India (Ahmad et al., 2007a; Armes et al., 1997; Huang et al., 2006). The extensive use of conventional insecticides such as organophosphates, carbamates and synthetic pyrethroids against *S. litura* have provided an ideal environment for its evolution of resistance in Pakistan (Ahmad et al., 2007a). The newer insecticides bearing novel modes of action such as spinosad, indoxacarb, abamectin, emamectin benzoate, fipronil; some chitin synthesis inhibitors, such as lufenuron and diflubenzuron and an ecdysone agonist i.e. methoxyfenozide were recently introduced in Pakistan for management of insect pests of cotton. On the contrary, the farmers of this country have also started using them against different insect pests of vegetables including *S. litura*. Conventional insecticides were usually sprayed at 7–8 days interval; however, new chemistry insecticides provide effective control for 10–15 days (M. Ahmad and M. Saleem, unpublished data). Although new chemistry insecticides are expensive than conventional but due to reduced numbers of sprays has kept the input cost of farmers at par to conventional insecticides. Farmers survey (2004–2006) from four different locations in cotton belt of the Punjab, Pakistan suggested that 75% farmers preferred new chemistry insecticides to conventional insecticides (M. Ahmad, unpublished data). In contrast, survey in 1999 showed that farmers opt for conventional insecticides than new chemistry (Khan and Mehmood, 1999).

Following reports of poor efficacy of the new chemistry insecticides against *S. litura* both in cultivated crops and

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vegetables, we surveyed resistance to the new chemistry compounds in *S. litura* from various cotton zones of the Punjab province of Pakistan to ascertain whether or not the resistance was indeed evolving. More often the reasons for poor insecticide efficacy are attributed to adulteration or spurious chemicals (Parthasarathy and Shameem, 1998). Nevertheless, we feel that the data on the levels of resistance to newer insecticides can help in assessing the relative importance of resistance in causing field control failures. We were also interested in investigating whether resistance to different insecticides was increasing or stayed the same during 3 years period of 2004–2006.

2. Materials and methods

2.1. Insects

S. litura infestation in Pakistan generally commences from the end of March and extends until the end of November depending upon the cropping pattern (Ahmad et al., 2007b). The pest is continuously exposed to insecticides from April to early November, as it receives sprays first on vegetables (cauliflower, arum and okra) and then on fodder (berseem). When cotton emerges in June in the field, it moves to cotton crop and remains on it throughout the season. The growers usually undertake more insecticides on cotton than vegetables (Khan and Mehmood, 1999). We therefore collected *S. litura* populations from cotton crop, as there were higher chances of evolution of resistance on cotton than vegetables.

Walking through the crops in each field from three districts viz Sahiwal, Vehari and D.G. Khan, about 300–500 larvae were collected. Cropping pattern in Sahiwal district is comparatively complex than D.G. Khan or Vehari districts. The area is under multiple cropping systems with several cultivated crops such as cotton, maize, sorghum, millets, rice, sugarcane, wheat, potatoes, vegetables and fodders. These crops are being grown side-by-side, depending on the season. In D.G. Khan and Vehari districts, growers mostly follow the wheat–cotton–wheat cropping pattern. The temperature in Sahiwal district ranges between 10 and 40 °C (night: day) during the cropping season, while temperature in D.G. Khan district is comparatively higher and ranges from 15 to 50 °C (night: day). The average temperature in Vehari district ranges from 10 to 45 °C (night: day) during the cropping season. Due to differences in temperature and cropping patterns, there is a wide range of *S. litura* infestation, which results in great variation in insecticides spray.

Larvae were reared on semi-synthetic gram-based diet in the laboratory at 25 ± 2 °C and 60–65% relative humidity with a light (14 h):dark (10 h) photoperiod (Ahmad et al., 2007a). Diet was replaced after 24 h and pupae were collected on alternate days. The emerged adults were kept in Perspex ovipostion cages with meshed sides to maintain ventilation. They were fed on a solution containing sucrose (100 g/lit), vitamin solution (20 ml/lit) and methyl-4-hydroxybenzoate (2 g/lit) in a soaked cotton wool ball (Ahmad et al., 2007b). Populations were reared in the laboratory for one generation to obtain sufficient insect numbers for bioassays.

We generated susceptible population in 2005 from a field-collected population. Single pair crosses were set up using individuals from the heterogeneous field population. Bioassays were used to identify families in which the F_1 progeny of these crosses were susceptible to a given insecticide. Those in which 100% mortality was observed using a concentration of insecticide equal to the LC_{20} of the field population were then used in a second round of crossing. F_2 progeny showing 100% mortality at LC_{10} levels of insecticide were then used to make third round of crosses. The family, which showed 100% mortality at LC_1 of field population, was designated as Lab-PK (Ahmad et al., 2007b).

2.2. Insecticides

Commercial formulations of different insecticides used for bioassays comprised of spinosad (Tracer® 24SC, Dow Agro-Sciences, UK), indoxacarb (Steward®15SC, DuPont, Pakistan), abamectin (Agrimec™ 1.8EC, Syngenta, UK), emamectin benzoate (Proclaim® 1.9EC, Syngenta, UK), fipronil (Regent® 36EC, Bayer Crop Science, France), lufenuron (Match® 5EC, Syngenta, UK), diflubenzuron (Teflon™ 7.5WP, Helb Pesticides, China), methoxy-fenozide (Runner® 24SC, Dow AgroSciences, USA) and a non-ionic surfactant (Stapple® Dupont, Pakistan) at 5 $\mu g\,ml^{-1}$ to enhance the adhesiveness of insecticides.

2.3. Bioassays

Bioassays were conducted on newly moulted second instar (3–6 h old) larvae of *S. litura* from F_1 laboratory cultures using a standard leaf disc bioassay method (Sayyed et al., 2000; Ahmad et al., 2007a). Each disc of 4.8 cm diameter was made from the cotton leaves collected from unsprayed fields. These were washed, dried and immersed in a test solution for 10 s and allowed to dry at ambient temperature for 1–1.5 h. Test solutions of insecticides were freshly prepared in distilled water with Stapple (5 $\mu g\,ml^{-1}$) as surfactant. Leaf discs immersed in distilled water and Stapple only were labelled as control. On drying, the leaf discs were placed in individual Petri dishes (5-cm diameter) containing moistened filter paper. Each treatment (concentration) was replicated eight times, including controls. Five-second instar larvae were placed on each leaf disc (replication) and thus total numbers of tested larvae per concentration were 40. The bioassays were kept at a temperature of 25 ± 2 °C, 65% relative humidity and 14:10 (light:-dark) photoperiod. Mortality was assessed after 72 h exposure to insecticides.

2.4. Data analysis

Mortality data were corrected by Abbott's (1925) formula where necessary and analysed by probit analysis (Finney, 1971) using the software POLO-PC (LeOra Software, 2003). The estimates of LC_{50} values and their 95% fiducial limits (FL) were obtained by probit analysis using Polo. Because of the inherent variability of bioassays, pair-wise comparisons of LC_{50} values were made at the 1% significance level (where individual 95% FL for two treatments do not overlap (Litchfield and Wilcoxon, 1949). Resistance ratios were determined by dividing the LC_{50} values of field populations with LC_{50} of Lab-PK. Cross-resistance pattern among insecticides was studied with pair-wise correlation co-efficient of LC_{50} values of the field populations for each insecticide.

Insecticide resistance level was classified by using RRs in terms widely accepted as follows: susceptibility ($RR = 1$), tolerance to low resistance ($RR = 2$–10), moderate resistance ($RR = 11$–30), high resistance ($RR = 31$–100) and very high resistance ($RR > 100$) (Ahmad et al., 2007a).

3. Results

3.1. Toxicity of test chemicals to susceptible population

The results of bioassays with Lab-PK population (Table 1) showed that the emamectin benzoate was significantly ($P<0.01$) more toxic than the insecticides tested viz., spinosad, indoxacarb,