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Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan

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Abstract

Field populations of *Spodoptera litura* from Pakistan were evaluated for their resistance to conventional insecticidal chemistries viz. organochlorine (endosulfan), organophosphates (chlorpyrifos, phoxim, quinalphos, profenofos), carbamates (methomyl, thiodicarb) and pyrethroids (bifenthrin, cyfluthrin) during 1997–2005 using a leaf-dip bioassay method. Generally, resistance levels were very low to low to endosulfan, chlorpyrifos, phoxim, quinalphos, profenofos, bifenthrin and thiodicarb, and moderate to high to methomyl and cyfluthrin. Correlation analysis indicated that insecticides belonging to the same class such as organophosphate, carbamate or pyrethroid exhibited a positive cross-resistance in *S. litura*. Positive correlation was also found between endosulfan and carbamates. Except methomyl and bifenthrin, which were negatively correlated, there was no cross-resistance between organophosphate or carbamate or pyrethroid insecticides in the resistant populations of *S. litura*. Integrated pest management tactics aimed at reducing pesticidal applications, rotating chemistries of diverse modes of action and conserving natural enemies are recommended.

Keywords: *Spodoptera litura*; Insecticide resistance; Pakistan; Endosulfan; Chlorpyrifos; Phoxim; Quinalphos; Profenofos; Methomyl; Thiodicarb; Bifenthrin; Cyfluthrin

1. Introduction

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a polyphagous insect pest (Holloway, 1989). It is an indigenous pest of a variety of crops in South Asia and was found to cause 26–100% yield loss in groundnut (Dhir et al., 1992). It is variously known as Indian leafworm, cluster or tobacco caterpillar, and common or tobacco cutworm. Under favourable conditions, its populations increase in large numbers and move across fields like an army, and hence called "armyworm." In recent years its outbreaks have been more common in South Asia, mainly due to its development of insecticide resistance (Armes et al., 1997; Kranthi et al., 2001, 2002) and subsequent control failures. In 2003, its outbreak occurred in Pakistan throughout the cotton belt and it devastated the crop. Most of the insecticides, especially pyrethroids and carbamates, failed to provide its adequate control. Insecticide resistance was implicated as the major cause of its control failures. The present studies investigated the susceptibility of Pakistani populations of *S. litura* to selected insecticides of diverse chemistries, such as organochlorine (endosulfan), organophosphorus (chlorpyrifos, phoxim, quinalphos, profenofos), carbamate (methomyl, thiodicarb) and pyrethroid (bifenthrin, cyfluthrin) insecticides, commonly used for controlling this pest.

2. Materials and methods

2.1. Insects

Fifth or sixth instar larvae of *S. litura* were mostly collected from various locations within 50 km radius of Multan in the southern Punjab, Pakistan during 1997–2005. Each collection of about 400 larvae was made by walking through a 2-ha block of a particular host crop in a zigzag manner to randomize collections. Larvae were fed in the laboratory on a semi-synthetic diet, which

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consisted of chickpea flour (300 g), ascorbic acid (4.7 g), methyl-4-hydroxybenzoate (3 g), sorbic acid (1.5 g), streptomycin (1.5 g), corn oil (12 ml), yeast (48 g), agar (17 g) and distilled water (1300 ml) with a vitamin mixture. Adults were fed on a 5% sugar solution with the addition of vitamins and methyl-4-hydroxybenzoate.

2.2. Insecticides

Commercial formulations of insecticides used in bioassays were: Thiodan (endosulfan, 350 g/l EC [emulsifiable concentrate]; Bayer CropScience, Leverkusen, Germany), Lorsban (chlorpyrifos, 400 g/l EC; Dow AgroSciences, Indianapolis, IN, USA), Baythion (phoxim, 400 g/l EC; Bayer), Ekalux (quinalphos, 250 g/l EC; Syngenta, Basle, Switzerland), Curacron (profenofos, 500 g/l EC; Syngenta), Lannate (methomyl, 400 g/kg SP [water soluble powder]; DuPont Agricultural Products, Wilmington, DE, USA), Larvin (thiodicarb, 800 g/kg DF [dry flowable]; Bayer), Talstar (bifenthrin, 100 g/l EC; FMC, Philadelphia, PA, USA), and Baythroid (cyfluthrin, 50 g/l EC; Bayer).

2.3. Bioassays

Newly moulted second instar larvae from F_1 laboratory cultures were exposed to different insecticides using the leaf-dip method recommended by the Insecticide Resistance Action Committee (IRAC; http://www.irac-online.org/resources/methods.asp) (Anonymous, 1990). Serial dilutions as ppm of the active ingredient of the test compounds were prepared using distilled water. Five-centimeter cotton (*Gossypium hirsutum*) leaf discs were cut and dipped into the test solutions for 10 s with gentle agitation, then allowed to dry on paper towel on both sides. Five larvae were released on to each leaf disc placed in a 5-cm-diametre Petri dish with adaxial side up. Eight replicates of five larvae were used for each concentration and 5–11 serial concentrations were used for each test insecticide. The same number of leaf discs per treatment was dipped into distilled water as an untreated check. Moistened filter papers were placed beneath leaf discs to avoid desiccation of leaves in Petri dishes. Before and after treatment, larvae were maintained at a constant temperature of 25($\pm$2) °C with a photoperiod of 14 h.

2.4. Data analysis

Larval mortalities were recorded after 48 h. Larvae were considered dead if they failed to make a coordinated movement when prodded with a probe. Data were corrected for control mortality using Abbott's (1925) formula and analysed by probit analysis (Finney, 1971) using Poloplus programme (LeOra, 2003). The lethal concentrations (LC) were calculated and any two values compared were considered significantly different if their respective 95% confidence limits (CL) did not overlap. Resistance factors (RF) were determined at LC_{50}s and LC_{90}s by dividing the LC values of each insecticide by the corresponding LC values for the Shershah-2 population for phoxim and the Multan-1 population for rest of the insecticides. To interpret cross-resistance spectra among the insecticides tested, pairwise correlation coefficients of log LC values of the common populations for each insecticide were calculated by MSTAT statistical computer programme (MSTAT-C, 1989).

2.5. Interpretation of resistance levels

In our laboratory using second instar larvae in the leaf-dip bioassays, we generally considered <10-fold RF as very low resistance, 11–20-fold as low resistance, 21–50-fold as moderate resistance, 51–100-fold as high resistance, and >100-fold as very high resistance. Our field experience showed that there was no control failure of an insecticide when insect pest's resistance against it was still very low, provided the insecticidal application was proper and targeted at the insect's habitat. Low-resistance levels to a particular insecticide found in bioassays gave a poor control in the field. Moderate to high or very high levels of insect resistance resulted in the field failure of the insecticide in question. Poor control was observed in Australia, where *Helicoverpa armigera* (Hübner) showed tolerance of up to 12-fold to fenvalerate, 15-fold to cypermethrin (Gunning et al., 1984), and 21-fold to endosulfan (Kay, 1977).

3. Results

3.1. Baselines

The Multan-1 population of *S. litura*, collected in July 1997 from arum, exhibited the lowest LC values (Table 1), and hence used as reference strain to determine RFs for all the insecticides except phoxim. It may not be a true susceptible strain. However, in the absence of a laboratory susceptible strain, it can serve as a good reference strain because its LC values were reasonably low and slopes steep. For phoxim, Muzafargarh-2 population, which was collected in April 2000 from berseem, exhibited the lowest LC_{50}s, and therefore it was used as a reference strain for this insecticide.

3.2. Endosulfan

Out of the 20 populations of *S. litura*, 15 populations showed a very low resistance to endosulfan (Table 1). Khokhran-1 and Muzafargarh-1 populations, collected in December 1997 and November 1998, respectively, had a low resistance. There was a moderate resistance to endosulfan in Lar-1 population collected in April 1998 from berseem, and a very high resistance in Muzafargarh-3 population collected in December 2003 from cauliflower. So, no particular trend was discernible in the extent of resistance among different years. It may probably be due to