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Resistance of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) to endosulfan, organophosphorus and pyrethroid insecticides in Pakistan

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ABSTRACT

Field populations of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), from Pakistan were assessed for their resistance to the chlorinated hydrocarbon endosulfan, the organophosphates chlorpyrifos and quinalphos, and the pyrethroids cypermethrin, deltamethrin, bifenthrin and fenpropathrin. Using a leaf-dip bioassay, resistance to endosulfan was high during 1998–2000 but declined to very low, to low levels during 2001–2007, following a reduced use of the insecticide. Organophosphates and pyrethroids were consistently used over the past three decades, and the resistance had been increasing to these insecticide classes. Generally, the resistance to chlorpyrifos and pyrethroids remained low from 1998 to 2002–2003, but resistance increased to moderate to high levels from 2003–2004 to 2006–2007. For deltamethrin, resistance was very high during 2004–2007. Quinalphos resistance remained low during 1998–2006. Correlation analysis of LC_{50} and LC_{90} values showed a positive correlation between organophosphates and pyrethroids, but no correlation between endosulfan and organophosphates or pyrethroids tested herein. These results suggest that the conventional chemistries should be replaced with new chemistries for the successful management of *S. exigua*.

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1. Introduction

Beet armyworm, *Spodoptera exigua* (Hübner), is polyphagous and infests a wide range of crops in the tropical and sub-tropical regions (Metcalf and Metcalf, 1992). It is an important insect pest of cotton, potato, tomato, soybean, okra, onion, chilli, and clovers in Pakistan. On Pakistani cotton it is usually found early in the season. It is also called lesser armyworm, pigweed caterpillar and small mottled willow. It has the potential of causing outbreaks under favourable conditions. Although a closely related species *Spodoptera litura* (F.) had frequent outbreaks in Pakistan, yet it remained localized and never occurred as an outbreak in large areas.

S. exigua is an invasive pest species in Pakistan, which has been observed since late 1980s. However, it was in late 1990s when complaints of its control failures by insecticides were reported by growers and insecticide resistance was implicated as the potential cause of this poor control. Insecticide resistance has been documented in *S. exigua* from China (Chen et al., 2002), Taiwan (Chou et al., 1984), Guatemala (Delorme et al., 1988), Mexico (Teran-Vargas, 1997), and the USA (Brewer and Trumble, 1989, 1994; Kerns et al., 1998; Mascarenhas et al., 1998). The present study was initiated to determine the existence of resistance to endosulfan, organophosphates (OPs) (chlorpyrifos, quinalphos) and pyrethroids (cypermethrin, deltamethrin, bifenthrin, fenpropathrin) in *S. exigua* in Pakistan.

2. Materials and methods

2.1. Insects

Fifth or sixth instar larvae of *S. exigua* were collected primarily from various locations within 50 km radius of Multan in the southern Punjab, Pakistan during 1998–2007. Each collection of about 300 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 consisted of chickpea flour (300 g), ascorbic acid (4.7 g), methyl-

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4-hydroxybenzoate (3 g), sorbic acid (1.5 g), streptomycin (1.5 g), corn oil (12 ml), vitamin mixture (10 ml), yeast (48 g), and agar (17 g). Yeast and agar were dissolved in 800 ml of boiling water and added to other constituents premixed in 500 ml of water. Adults were fed on a solution containing sugar (50 g), vitamin mixture (10 ml), methyl-4-hydroxybenzoate (1 g), and distilled water (500 ml).

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), Ekalux (quinalphos, 250 g/l EC; Syngenta, Basle, Switzerland), Arrivo (cypermethrin, 100 g/l EC; FMC, Philadelphia, PA, USA), Decis (deltamethrin, 25 g/l EC; Bayer), Talstar (bifenthrin, 100 g/l EC; FMC), and Danitol (fenpropathrin, 100 g/l EC; Sumitomo Chemical Company, Osaka, Japan).

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 test solutions for 10 s with gentle agitation, then allowed to dry on paper towel on both sides. Five larvae were released onto each leaf disc placed in a 5-cm-diametre Petri dish with adaxial side up. Eight replicates of five larvae each were used for each concentration and 5–10 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 temperature of 25 ($\pm$2) °C with a photoperiod of 14 h.

2.4. Data analysis

Larval mortalities were recorded 48 h after treatment. 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 analyzed by probit analysis (Finney, 1971) using POLO-PLUS programme (LeOra Software, 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 Multan-1 population. The 95% CLs for the RFs were determined according to Robertson and Preisler (1992). 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 the Pearson correlation formula according to Snedecor and Cockran (1989) using the MSTAT statistical computer programme (MSTAT-C, 1989). As described previously (Ahmad and Arif, 2009), resistance was generally classified as none ($RF \leq 1$), very low ($RF = 2–10$), low ($RF = 11–20$), moderate ($RF = 21–50$), high ($RF = 51–100$) and very high ($RF > 100$).

3. Results

3.1. Endosulfan

S. exigua is an early-season pest of cotton in Pakistan. Endosulfan was used to be applied early in the season against sucking insect pests, and therefore *S. exigua* was frequently exposed to endosulfan. A moderate to high resistance was found to endosulfan in five field populations of *S. exigua* tested during 1998–2000 (Table 1). The Shershah-1 population rather exhibited a very high resistance to endosulfan. Endosulfan resistance declined to low levels during 2000–2002 and then to very low levels during 2003–2006. This drop in endosulfan resistance may be associated with a decreased use of endosulfan against insect pests of cotton in Pakistan.

3.2. Organophosphates

Resistance to chlorpyrifos remained very low during 1998–2001 and low during 2002–2003 in all nine populations of *S. exigua* tested (Table 1). During 2004–2007, chlorpyrifos resistance increased to moderate to high levels in the five populations tested. Like chlorpyrifos, quinalphos resistance was also very low during 1998–2002 in the ten populations of *S. exigua* (Table 1). From 2002 to 2006, quinalphos resistance was recorded as low in four populations and slightly moderate in three populations.

3.3. Pyrethroids

Pyrethroids have been consistently used against lepidopteran pests of cotton, especially in mixtures with OPs. Resistance to cypermethrin remained very low during 1998–2000 in eight populations and low during 2001–2002 in four populations of *S. exigua* (Table 1). Cypermethrin resistance rose to moderate to high levels during 2003–2007 in all ten populations tested. Like cypermethrin, resistance to deltamethrin was very low during 1998–2000 in seven populations and low to moderate during 2001–2003 in six populations of *S. exigua* (Table 1). Deltamethrin resistance increased to high to very high levels during 2003–2007 in the seven populations tested.

Resistance to bifenthrin remained very low during 1998–2002 in the 11 populations of *S. exigua* tested (Table 1). Subsequently, it progressively increased but at a slower pace than cypermethrin and deltamethrin. Bifenthrin resistance was low in 2003 but moderate during 2004–2005 and 2007 and high in 2006. Resistance to fenpropathrin remained very low during 1998–2001 in eight populations and then low during 2001–2002 in three populations of *S. exigua* (Table 1). During 2003–2006, the fenpropathrin resistance was moderate to high in all six populations tested.

3.4. Correlation between LC values of insecticides

Paired comparisons of the log LC_{50}s and LC_{90}s for the same populations of *S. exigua* showed positive and highly significant correlations between OPs chlorpyrifos and quinalphos, and among pyrethroids cypermethrin, deltamethrin, bifenthrin and fenpropathrin (Table 2). There was also a highly positive correlation between OPs and pyrethroids tested, indicating a cross-resistance within and between these two insecticide classes. On the contrary, no correlation and therefore no cross-resistance existed between endosulfan and OPs or pyrethroids in *S. exigua*.

4. Discussion

The Multan-1 population of *S. exigua* collected from okra in May 1998 exhibited the lowest LC values and reasonably good

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