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Resistance to new insecticides and their synergism in *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan

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ABSTRACT

The beet armyworm *Spodoptera exigua* (Hübner) is an important pest of field and vegetable crops in Pakistan. Due to persistent use of insecticides, it developed high levels of resistance to organophosphates and pyrethroids in the mid 2000s and farmers then switched to new chemistries. In the present study, its resistance to new chemistries such as indoxacarb, spinosad, chlorfenapyr, methoxyfenozide and avermectins was monitored from 1998 to 2017 using a leaf-dip bioassay. There was generally a very low to low resistance to these insecticides during the first 11 years of their introduction (1998-2008). Resistance to methoxyfenozide remained very low throughout the 20 years of monitoring. A moderate resistance was recorded to indoxacarb (2011-2015), spinosad (2009-2012), chlorfenapyr (2011-2017), abamectin (2016-2017) and emamectin benzoate (2010-2013). A high resistance was found to indoxacarb during 2016–2017, spinosad during 2013–2017 and emamectin benzoate during 2014–2017. A rotation of diverse chemistries, having novel modes of action, along with other integrated pest management tactics, is recommended for the management of insecticide resistance in *S. exigua*.

1. Introduction

The beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is cosmopolitan, occurring in Asia, Africa, Australia, Europe, North and Central America (CAB, 1972). It is a polyphagous pest of vegetable and field crops (Metcalf, 1992). Vegetable crops include tomato (*Lycopersicon esculentum* (L.) Mill.), potato (*Solanum tuberosum* L.), okra (*Hibiscus esculentus* L.), bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), cowpea (*Vigna sinensis* (L.) Savi), eggplant (*Solanum melongena* L.), onion (*Allium cepa* L.), pepper (*Capsicum annuum* L.), cabbage (*Brassica oleraceacapita* (L.) Metzger), cauliflower (*Brassica oleraceabotrytis* (L.) Metzger), lettuce (*Lactuca sativa* L.), spinach (*Spinacea oleracea* L.), turnip (*Brassica rapa* L.), and radish (*Raphanus sativus* L.). Among field crops are cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medic.), soybean (*Glycine max* (L.) Merr.), peanut (*Arachis hypogea* L.), watermelon (*Citrullus lanatus* (Thunb.) Mansf.), sugar beet (*Beta vulgaris* L.), alfalfa (*Medicago sativa* L.), and Egyptian clover (*Trifolium alexandrinum* Bory and Chaubard). Its larvae feed on both foliage and fruit. Young larvae feed gregariously and skeletonize foliage. As they mature, larvae become solitary and eat irregular holes in the foliage. Tomato fruit is most susceptible to injury, buds and growing points may be eaten and fruits pierced. *S. exigua* remains active throughout the year in Pakistan, shifting from one host to the other (Ahmad M, personal communication).

As a major pest of important crops in Pakistan, *S. exigua* has been receiving frequent applications of insecticides applied for its control. Consequently, it developed resistance to conventional as well as new chemistry insecticides in the Pakistani populations (Ahmad and Arif, 2010; Ishtiaq and Saleem, 2011; Ishtiaq et al., 2012). Resistance to new insecticides has also been documented in *S. exigua* in USA (Moulton et al., 2000) and China (Zhou et al., 2011; Che et al., 2013; Su and Sun, 2014).

The present study reports the chronological occurrence of resistance to diverse new chemistries such as indoxacarb, spinosad, chlorfenapyr, methoxyfenozide, and avermectins (abamectin, emamectin benzoate) in Pakistani field populations of *S. exigua* from 1998 to 2017. To find out the role of metabolic detoxification in the putatively resistant Pakistani populations of *S. exigua*, synergists viz. PBO (piperonyl butoxide) as a mixed function oxidase inhibitor (Hodgson, 1999) and DEF (*S,S,S*-tributyl phosphorotrithioate; tribufos) as an esterase inhibitor (Jang et al., 1992) were added to insecticides in bioassays.

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2. Materials and methods

2.1. Insects

Fifth or sixth instar larvae of *S. exigua* were mostly collected from various locations in the Punjab province of Pakistan. The host crops, from which larvae were collected, included cotton, corn, Egyptian clover, okra, tomato, potato, pea and cabbage. 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-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 and synergists

Commercial formulations of insecticides used in bioassays were: indoxacarb (Steward, 150 g/l SC [suspension concentrate], DuPont, Wilmington, DE, USA), spinosad (Tracer, 240 g/l EC [emulsifiable concentrate]; Dow AgroSciences, Indianapolis, IN, USA), chlorfenapyr (Pirate, 360 g/l SC; BASF Corporation, Princton, NJ, USA), methoxyfenozide (Runner, 240 g/l SC; Dow), abamectin (Cure, 18 g/l EC; Hebei Vian Biochemical Company limited, Shijiazhuang, China), and emamectin benzoate (Proclaim, 19 g/l EC; Syngenta, Basle, Switzerland). The synergists PBO (91.3% EC) and DEF (70.5% EC) were obtained from Bayer.

2.3. Bioassays

Newly moulted second instar larvae from F_1 laboratory colonies 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 mg l^{-1} of the active ingredient of the test compounds were prepared using distilled water. Synergist solutions were prepared in distilled water as 50 mg l^{-1} for PBO as well as DEF. Based on preliminary bioassays, these were the maximum concentrations of the synergists that could be used without any deleterious effects on the second instar larvae of *S. exigua*.

Five-centimeter cotton leaf disks were cut and dipped into test solutions for 10 s with gentle agitation, then allowed to dry on paper towel. Five larvae were released on to each leaf disk placed in a 5-cm-diametre Petri dish with adaxial side up. Eight replicates of five larvae each were used for each concentration. Five to eleven serial concentrations were used for each test insecticide. The range of concentrations was determined by preliminary bioassays that would give nearly 0-100% mortality. The same number of leaf disks per treatment was dipped into distilled water as an untreated check. To avoid desiccation of leaves in Petri dishes, moistened filter papers were placed beneath leaf disks. Before and after the treatment, larvae were maintained at a temperature of 25(± 2)°C with a photoperiod of 14 h. Larval mortalities were recorded after 48 h.

Multan-1 population of *S. exigua*, collected in May 1998 from okra, was used as a reference population to determine resistance ratios for the remaining populations (Table 1). This population of *S. exigua* was earlier used as a baseline population for endosulfan, organophosphates and pyrethroids (Ahmad and Arif, 2010). Multan-1 population was not exposed to new chemistry insecticides, because their field use had not yet started against armyworms during 1990s.

2.4. Data analysis

Data were analyzed by probit analysis 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 ratios (RR) were determined for 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 of *S. exigua*. To assess the degree of synergism, synergistic ratios (SR) were calculated by dividing the LC_{50} or LC_{90} of the insecticide by the LC_{50} or LC_{90} of the insecticide plus synergist. The 95% CLs for RRs and SRs were computed according to Robertson et al. (2007). As described previously (Ahmad and Gull, 2017), resistance was generally classified as none (RR = 1), very low (RR = 2-10), low (RR = 11-20), moderate (RR = 21-50), high (RR = 51-100) and very high (RR > 100).

3. Results

3.1. Baseline susceptibility

The baseline LC values of emamectin benzoate (LC_{50} = 0.09 mgL^{-1}, LC_{90} = 0.42 mgL^{-1}), chlorfenapyr (LC_{50} = 0.30 mgL^{-1}, LC_{90} = 0.94 mgL^{-1}) and spinosad (LC_{50} = 0.41 mgL^{-1}, LC_{90} = 1.8 mgL^{-1}) determined in Multan-1 population of *S. exigua* were very low, which were followed by indoxacarb (LC_{50} = 3.5 mgL^{-1}, LC_{90} = 17 mgL^{-1}), methoxyfenozide (LC_{50} = 9.6 mgL^{-1}, LC_{90} = 29 mgL^{-1}) and abamectin (LC_{50} = 10 mgL^{-1}, LC_{90} = 56 mgL^{-1}) (Table 1). Methoxyfenozide and abamectin seem to have a low intrinsic toxicity against *S. exigua*.

3.2. Indoxacarb

There was no indoxacarb resistance in both the populations of *S. exigua* tested in 1998 (Table 1). Indoxacarb resistance remained very low (1.8–9.9-fold) for the next 11 years (1999-2009). The population of 2010 showed a low level of resistance to indoxacarb (12-16-fold). Indoxacarb resistance was found to be moderate in 2011–2015 (21-33-fold) and high in 2016–2017 (43-52-fold at LC_{50} and 61-71-fold at LC_{90}).

3.3. Spinosad

Like indoxacarb, both the populations of *S. exigua* evaluated in 1998 were susceptible to spinosad (Table 1). The populations exhibited a very low resistance in 1999–2004 (2.1–7.5-fold) and a low resistance in 2005–2008 (11-20-fold). Spinosad resistance reached moderate levels in 2009–2012 (22-47-fold), high levels in 2013–2015 (55-75-fold at LC_{50} and 52-118-fold at LC_{90}) and very high levels in 2016–2017 (124-153-fold at LC_{50} and 94-147-fold at LC_{90}).

3.4. Chlorfenapyr

The populations of *S. exigua* tested during July 1998 to April 2007 showed a very low resistance (2.2–9.7-fold) to chlorfenapyr (Table 1). The populations in 2008–2010 demonstrated low resistance to chlorfenapyr (14-17-fold at LC_{50} and 13-22-fold at LC_{90}). All the populations assessed during 2011–2017 had moderate levels of chlorfenapyr resistance (20-32-fold at LC_{50} and 17-51-fold at LC_{90}).

3.5. Methoxyfenozide

All 15 populations of *S. exigua* bioassayed during 1998–2017 exhibited very low levels of resistance (1.1–5.2-fold at LC_{50} and 2-9.3-fold at LC_{90}) to methoxyfenozide (Table 1).