

Baseline data for insecticide resistance monitoring in tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) on cole crops in Punjab, India



Agriculture

KEYWORDS : Chlorantraniliprole, Emamectin benzoate, Indoxacarb, Resistance, *Spodoptera litura* Fabricius, Spinosad, Toxicity.

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ABSTRACT

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an agricultural important pest species. *S. litura* has a wide host range of approximately recorded 150 plant species worldwide (Chari & Patel, 1989). In Punjab, this pest attains sporadic status primarily on cauliflower, Brassica oleracea (L.). This pest destroys vegetable crop and particularly prefers the cruciferae family (Charoensak et al., 2009). However, it is also observed feeding on other crops such as arbi, *Colocasia esculenta* (L.), mung bean, *Vigna radiata* (L.), sunflower, *Helianthus annuus* (L.), cotton, *Gossypium hirsutum* (L.), castor, *Ricinus communis* (L.), etc. (Kumar, 1992). Larvae of this pest completely devour the leaves of infested plant resulting in huge crop losses which ranges from 50 to 70 per cent. Indiscriminate and continuous use of insecticides has contributed in development of insecticide resistance in insects and caused the environmental degradation as well. Moreover, a base line data regarding the toxicity of the newer insecticides would help in understanding the level of resistance developed in this pest and any possible cross-resistance there in, which could be assessed in advance. Therefore, present studies on development of resistance in *S. litura* against four new chemistry insecticides (emamectin benzoate, chlorantraniliprole, indoxacarb and spinosad) were carried out in the Toxicology laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India during the year 2011-12. Various stages of *S. litura* (eggs, larvae) were collected from four different locations (Malerkotla, Hoshiarpur, Amritsar and Samrala) of Punjab. Resistance is developed in third instars of lepidopterous pests (Anand et al., 2011). Therefore, larval bioassays were conducted to estimate the response of field populations of thirty third-instar larvae of *S. litura* under laboratory conditions at 25±2°C and 65±5 per cent relative humidity. Leaf dip bioassay technique with diluted insecticide formulations recommended by Insecticide Resistance Action Committee (IRAC) was performed in the laboratory with seven to ten treatments depending on the insecticide class, respectively. LC50 values were estimated by probit analysis after correction to record control mortality data which was used to calculate the resistance ratios (RR). The LC50 values worked out for emamectin benzoate, chlorantraniliprole, indoxacarb, spinosad are 0.081, 0.088, 0.380, 40 parts per million (ppm) against pest populations collected from Malerkotla; 0.051, 0.060, 0.250, 30 ppm of Amritsar; 0.040, 0.029, 0.140, 20 ppm for Samrala and 0.037, 0.029, 0.100, 20 ppm against pest population of Hoshiarpur, respectively. The LC50 values for populations collected from these four locations were in the order Malerkotla>Amritsar>Samrala>Hoshiarpur for the insecticides (emamectin benzoate, chlorantraniliprole, indoxacarb and spinosad) tested. Based on LC50 values obtained, emamectin benzoate (0.037 ppm) was found to be the most toxic among all the tested populations, followed by chlorantraniliprole (0.029 ppm), indoxacarb (0.100 ppm) and spinosad (20 ppm), respectively. The pairwise correlation coefficients of LC50 values indicated that there was lack of cross resistance for emamectin benzoate, chlorantraniliprole, spinosad, indoxacarb in populations of *S. litura* from Punjab. These insecticides may prove to be promising substitutes for the effective control of insecticide resistant populations of *S. litura* which were collected from Malerkotla region of Punjab state, India.

Introduction

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera, Noctuidae) is recognized as a serious cosmopolitan pest with considerable host range of economically agricultural crops such as cotton, groundnut, soybean, tomato and many other crops (Matsura and Naito, 1997) causing higher economic losses to crops at their blossoming and vegetative stages with 70 to 100% yield loss (Ahmad et al., 2007). This pest has been surveyed to infest about 150 plant species (Chari and Patel, 1989) and in Punjab this pest arises in sporadic form principally on cauliflower but is also observed feeding on other crops like arbi, mash, moong, sunflower, cotton, castor, etc. (Kumar, 1992). For the control of this pest in the field, a number of insecticides belonging to different groups viz., organochlorines, organophosphates, carbamates and pyrethroids have been registered in the country and proved to be effective but the continuous and indiscriminate use may result in the development of resistance in *S. litura* to these insecticides (Kranthi et al., 2002).

Though the development of resistance by the pest to these insecticides may be delayed, the possibility for development of resistance cannot be totally denied. Thus the insecticide resistance must be continuously monitored and must form an integral part of chemical control to enable the detection of resistance as early as possible and to take necessary measures. Baseline data on the susceptibility of the target pest to the toxicant is the most important factor for insecticide use especially for monitoring the development of resistance. Keeping these things in view, the present studies were contemplated. The LC50 values obtained would serve as ready reckoner for the selection of insecticide for field strains. Also, such base line data could be used as critical inputs in the deployment of new insecticides and insecticide re-

sistance management programmes. This study is expected to be helpful in devising management strategies to overcome the resistance problems and to control *S. litura* under field conditions in the future.

Materials and methods

Insect collection and rearing

The egg masses of *S. litura* and larvae were collected from different locations of Punjab viz Amritsar, Hoshiarpur, Samrala and Malerkotla during 2010 which is predominantly a vegetable growing area. Each collection of larvae was made by walking through a 3 ha block of a particular host crop randomly from various areas (Fig. 1). Wherever possible, farmers were interviewed to determine the number of insecticides sprays applied to their crop until sampling. Samples were brought to the laboratory and were reared on fresh castor leaves at 25 ± 2°C and 65±5 % RH in glass jars (Ahmad et al., 2008). Diet was replaced after 24 h and pupae were collected on alternate days. Moths were shifted to glass jars and fed on a solution containing 10 % honey soaked into cotton wool ball (Ahmad et al., 2007). The field collected populations were reared in the laboratory to accommodate to laboratory conditions and to obtain sufficient insect numbers for bioassays.

Insecticides

Commercial formulations of different insecticides used for bioassays comprised Emamectin benzoate 5SG, Spinosad 2.5 SC, Indoxacarb 15.8 EC, Chlorantraniliprole 18.5 SC.

Bioassays

Newly moulted third instars from F1 laboratory cultures were exposed to different insecticides using the leaf-dip method

(Sayyed et al., 2008) recommended by the Insecticide Resistance Action Committee (IRAC; <http://www.irc-online.org/resources/methods.asp>) (Anonymous, 1990). Serial dilutions as part per million of the active ingredient of the test compounds were prepared using distilled water. The hatched larvae were further reared on castor (*Ricinus communis*) leaves to complete their life cycle. Castor leaves were collected at morning time, washed thoroughly with tap water and air dried. Leaf discs (10 cm diameter) of well grown castor leaves were cut and cleaned with wet cotton swab and dipped in required concentrations of insecticides for twenty seconds with gentle agitation. Castor leaves dipped in water alone were used as control. Leaf surface was allowed to air dry for some time and then leaves were transferred to glass jars. Castor leaves were changed daily till the pupation started. Pupae were kept individually in separate jars lined with filter papers. Newly emerged adults were placed in large jars having cotton balls soaked in 10% honey solution as adult diet. Leaf of castor was suspended with the help of pins in the jars as egg substrate below the surface of leaf and were collected and kept in batches separately in jars. Thirty third instar larvae were released in each glass jar, which was kept at a constant temperature of $25 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ relative humidity.



Figure 1: Sampling sites of *Spodoptera litura* in various regions of Punjab. The survey was carried out in the season of 2011–2012. Surveyed province is highlighted in a coloured shades (Color figure online).

Data analysis

The mortality data were recorded at 24 and 48h after treatment. A larva was considered 'dead' if it failed to move in a coordinated manner, when probed with blunt needle. Data obtained were corrected for control mortality using Abbott's formula (1925) (Abott, 1925) where necessary, and were analyzed by probit analysis through POLO (Leora, 2003) to estimate LC50 values and their 95% Fiducial limits (FLs). A cross resistance mechanism was determined among the tested insecticides by pairwise correlation coefficients of log LC50 values of the population by the Pearson correlation with the help of computer program XL-Stats. The values of relative toxicity of different insecticides were calculated by taking values of median lethal concentration of spinosad as unity.

Results and discussion

Toxicity of newer insecticides against different populations of *S. litura*

All the insecticides tested in the present study caused concentration-dependent mortality in 3rd instar larvae of field population of *S. litura*. The results showing the response of different populations of *S. litura* populations collected from four different locations i.e. Amritsar (AMT.), Malerkotla (MAL.), Hoshiarpur (HSP) and Samrala (SAM.) of Punjab to different insecticides

viz chlorantraniliprole, emamectin benzoate, indoxacarb, spinosad using leaf disc residue bioassay technique are summarized in Table 1. The larval mortality of *S. litura* populations collected from different locations when exposed to different concentrations of tested insecticides was found to range from 10 to 90 per cent. The chi-square values indicated good fit to probit regression. The LC50 values worked out for emamectin benzoate, chlorantraniliprole, indoxacarb, spinosad are 0.081, 0.088, 0.380, 40.0 ppm against pest populations collected from Malerkotla; 0.051, 0.060, 0.250, 30.0 ppm of Amritsar; 0.040, 0.029, 0.140, 20 ppm for Samrala and 0.037, 0.029, 0.100, 20 ppm against pest population of Hoshiarpur (Table 1).

Based on LC50 values obtained, the order of toxicity of these insecticides against insect pest populations of Malerkotla and Amritsar were found to be emamectin benzoate> chlorantraniliprole>indoxacarb>spinosad (Table 1). However in Samrala and Hoshiarpur populations order of toxicity was chlorantraniliprole >emamectinbenzoate>indoxacarb> spinosad (Table 1).

Based on the LC90 values (a point which indicates the concentration required for field recommendation to manage pest population), emamectin benzoate and chlorantraniliprole were most toxic and spinosad was least toxic among the other insecticides against *S. litura*.

Pairwise correlations between log LC50 values of different insecticides

Correlation between newer chemistry insecticides and old generation insecticides was not significant ($P < 0.05$) except deltamethrin which was significant to chlorantraniliprole and indoxacarb, quinalphos but non-significant with others viz., spinosad, emamectin benzoate (Table 2). A significant correlation was observed between chlorantraniliprole, indoxacarb quinalphos and deltamethrin ($P < 0.01$), whereas resistance to quinalphos showed no correlations with resistance to other insecticides except spinosad ($P < 0.05$). There was lack of cross resistance for emamectin benzoate, chlorantraniliprole, spinosad, indoxacarb in populations of *S. litura* from Punjab, India.

The current study, conducted from 2010 to 2012, demonstrate that the *S. litura* populations on cole crops in four regions of Punjab have shown varying degrees of resistance to four newer insecticides. This suggests that populations of *S. litura* have the potential to develop resistance to a wide range of chemicals. Historically, resistance to insecticides has been a limiting factor for effective chemical control of cotton pests in the world (Whalon et al., 2008). Many organochlorines, organophosphates, pyrethroids and carbamates became relatively ineffective because of the resistance evolution in pest populations. The same trend is evident for newer insecticides for which reports already exist in insects of the order Lepidoptera, mainly Noctuidae (Silva et al., 2011). In *S. litura*, non-exposure of the insects to these insecticides in the field may be responsible for their higher toxicity. Chlorantraniliprole proved to be most toxic for populations collected from Hoshiarpur and Samrala areas with similar LC50 value 0.029 ppm (Table 1). Emamectin benzoate, a derivative of abamectin, is quite effective against a number of lepidopteran insect pests including *S. exigua* and *S. litura* (Ahmad et al., 2006). These conclusions were also consistent with the field control effect of these insecticides (Saeed et al., 2012) who reported that emamectin benzoate gave lowest LC50 value i.e., 0.005 mg/l followed by lufenuron (0.65 mg/l). Thus, the established methods and baseline data are valuable and could be recommended for further work.

Spinosad and indoxacarb were introduced at almost the same time against insect pests of cotton in Pakistan which cause paralysis in the larvae on eating them (Tomlin, 2001). Their similar LC50 but low LT50 values revealed the more effective role of the

later one as compared to the former insecticide. In the present studies, the LC₅₀ values worked out for indoxacarb, spinosad are 0.380, 40 ppm against pest populations collected from Malerkotla; 0.250, 30 ppm of Amritsar; 0.140, 20 ppm for Samrala and 0.100, 20 ppm against pest population of Hoshiarpur (Table 1). It shows that indoxacarb and spinosad are less effective insecticides than emamectin benzoate and chlorantraniliprole whereas spinosad is least toxic against *S. litura*.

According to LC₅₀ values, no significant differences were observed between spinosad and methoxyfenozide against *Spodoptera littoralis* after 48 hours of treatment based on the overlap of 95% fiducial limits (Pineda et al., 2004). High toxicity of indoxacarb against *S. litura* with LC₅₀ value of 0.0064% in New Delhi (Gupta et al., 2004) and very strong ovicidal action of indoxacarb and thiodicarb against *S. litura* with 86.6 and 95.5% mortality in Guntur has been reported by Ahmad et al. (2001). Indoxacarb and spinosad showed more lethal and sub-lethal effects on *S. litura* by effecting its survival, development and reproduction with more lethality of indoxacarb as compared to that of spinosad (Kaur et al., 2007).

Shankarganesh et al. (2007) also reported similar conclusions about the susceptibility of *S. litura* to indoxacarb and profenophos with leaf dip bioassay technique. Such a situation is evident in the present investigations as well as were at par.

Emamectin benzoate belongs to the avermectins group and act as chloride channel activators. Therefore, emamectin is still considered as an effective tool for management of *S. litura* for most of the areas. Correlation between newer chemistry insecticides

and old generation insecticides was not significant ($P < 0.05$) except deltamethrin which was significant to chlorantraniliprole and indoxacarb but non-significant with others viz., spinosad, emamectin benzoate (Table 2). A significant correlation was observed between chlorantraniliprole, indoxacarb and deltamethrin ($P < 0.01$), whereas resistance to quinalphos showed no correlations with resistance to other insecticides except spinosad ($P < 0.05$). There was lack of cross resistance for emamectin benzoate, chlorantraniliprole, spinosad, indoxacarb in populations of *S. litura* from Punjab, India.

However, this cannot explain why spinosad resulted in higher resistance compared with other newer insecticides in most of the population in 2010, and pair wise comparisons of the log LC₅₀ values of insecticides tested showed occurrence of correlation within spinosad and quinalphos (Table 2), which suggest that resistance to spinosad might due to a possible cross-resistance mechanism to conventional insecticides. The low application of newer insecticides is also associated with their high price, which many farmers could not afford. These insects have been reported to develop resistance either against different groups or the representative of some group of insecticides. On the other hand, illiteracy can be one of the reasons for indiscriminate insecticides use for the development of insecticidal resistance in the most of major pests of cole crops.

In the present studies, emamectin benzoate and chlorantraniliprole was found to be more toxic and spinosad was found to be least toxic. New chemistry chemicals viz., emamectin benzoate, chlorantraniliprole, indoxacarb and spinosad were found to be more effective than normally used conventional insecticides.

Table 1: Toxicity of various insecticides against different populations of *S. litura* from Punjab, India

Insecticides	Location	LC ₅₀ (ppm)	LC ₉₀ (ppm)	Fit of probit line		df	TR
				Slope	Chi square		
emamectin benzoate	Malerkotla	0.081(0.039-0.137)	1.25(0.73-2.81)	1.079±0.157	3.43	10	493.83
	Amritsar	0.051(0.027-0.082)	1.84(0.49-1.95)	1.048±0.125	1.91	10	588.23
	Samrala	0.040(0.029-0.054)	1.21(0.86-2.12)	0.990±0.190	1.05	9	500.00
	Hoshiarpur	0.037 (0.026-0.052)	1.24(1.03-2.51)	1.140±0.220	1.03	9	540.54
chlorantraniliprole	Malerkotla	0.088(0.041-0.047)	1.70(0.96-3.75)	0.995±0.118	2.71	10	454.54
	Amritsar	0.060(0.032-0.090)	1.60(0.61-2.24)	1.001±0.110	2.59	10	500.00
	Samrala	0.029(0.009-0.035)	1.23(0.58-3.39)	0.850±0.090	1.04	9	689.65
	Hoshiarpur	0.029(0.009-0.035)	1.23(0.58-3.39)	0.850±0.090	1.04	9	689.65
indoxacarb	Malerkotla	0.380(0.220-0.590)	4.85(2.85-10.39)	1.162±0.144	0.84	8	105.26
	Amritsar	0.250(0.150-0.380)	3.01(1.82-6.05)	1.192±0.139	1.28	8	120.00
	Samrala	0.140(0.100-0.190)	2.11 (1.29-4.69)	1.540±0.220	0.65	7	142.85
	Hoshiarpur	0.100 (0.090-0.140)	2.77 (1.62-6.91)	1.460±0.220	0.66	7	200
spinosad	Malerkotla	40(30-60)	280(180-530)	1.561±0.189	0.58	5	1.00
	Amritsar	30(20-50)	340(200-790)	1.293±0.175	1.98	5	1.00
	Samrala	20(20-30)	140(100-250)	1.677±0.210	4.02	5	1.00
	Hoshiarpur	20(20-30)	140(100-250)	1.677±0.210	4.02	5	1.00

Table 2: Pairwise correlation coefficient comparison between log LC₅₀ values of tested insecticides on field population of *S. litura*

	Emamectin benzoate	Chlorantrani-liprole	Indoxacarb	Spinosad	Quinalphos	Deltamethrin
Emamectin benzoate						
Chlorantraniliprole	0.001					
Indoxacarb	0.000	0.000				
Spinosad	0.006	0.002	0.004			
Quinalphos	0.364	0.375	0.368	0.400		
Deltamethrin	0.011	0.005	0.008	0.001	0.414	0

Values in bold are significantly different from 0 with a significance level alpha=0.05

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