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Baseline Susceptibility and Stability of Insecticide Resistance of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) in the Absence of Selection Pressure

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Abstract.- A population of *Spodoptera litura* collected from Dunyapur was reared for eleven generations under laboratory conditions without any insecticide exposure. The LC_{50} data was recorded through diet incorporation method against four insecticides such as emamectin benzoate, spinosad, imidacloprid and profenofos. For new chemistry insecticides the larval mortality data was taken after 72 hrs while in case of conventional insecticides the mortality data was taken after 48 hrs. Emamectin benzoate (1.59 ppm) was found to be most toxic on the basis of LC_{50} values followed by spinosad (7.77 ppm), profenofos (686.5 ppm) and imidacloprid (258.75 ppm) at generation 1. The decrease in the LC_{50} values after 11 generations as compared to the field population of *S. litura* was 4.81, 9.83, 9.3 and 13.82 folds against emamectin benzoate, spinosad, imidacloprid and profenofos, respectively. The estimated decrease in resistance was 11.36, 11.11, 16.67 and 9.61 for imidacloprid, spinosad, emamectin benzoate and profenofos, respectively. The results suggest that spinosad can be included in the control program of *S. litura*, due to its lower stability and higher reversion rate with insecticides bearing novel modes of action and this baseline susceptibility data could be very helpful in future monitoring of insecticide resistance in *S. litura*.

Key words: Insecticide, resistance, spinosad, insecticide susceptibility, emamectin benzoate, imidacloprid, profenofos, *Spodoptera litura.*

INTRODUCTION

The tobacco cutworm, *Spodoptera litura* (Fab.), is one of the most destructive insect pests of vegetables and field crops (Lightfield, 1996; Ogden and Podleckis, 2000) that may cause 26-100% yield loss depending upon the severity of the attack (Dhir *et al.*, 1992). This pest has wide host range with more than 120 host plants including cotton, ground nut, tobacco, soybean and vegetables (Qin *et al*., 2004). The main crops which are attacked by *S. litura* in Pakistan are cotton, alfalfa, berseem, maize, tobacco, groundnut, summer legumes, and vegetables such as cucurbits, brinjal, potato, sweet potato, brassica, and capsicum (Ahmad *et al*., 2007). It can be found in those regions which have dry, tropical and temperate climates *i.e*., Asia and Oceania (IIE, 1993; Zhang, 1994; CAB, 2003).

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 reducing the effectiveness of new insecticides. *S. litura* is also notorious for developing insecticide resistance. The problem of development of resistance to insecticides is more acute in this pest because of its polyphagous nature and rapid multiplication (Ramakrishnan *et al*. 1984).

High resistance in this pest to various insecticides including organochlorines, organophosphates, carbamates, synthetic pyrethroids and *Bacillus thuringiensis* have been reported in China and India (Ramakrishnan *et al*., 1984; Wu *et al*., 1984; Zhou, 1984; Armes *et al*., 1997; Kranthi *et al*., 2001, 2002; Shi *et al*., 2003). In Pakistan *S. litura* has developed high resistance to conventional and new chemistry insecticides, due to their extensive use (Ahmad *et al*., 2007, 2008).

The presence of this pest on different crops throughout the year has widely exposed it to insecticides and resulted in the rapid development of resistance to a range of these insecticides (Sayyed *et al*., 2008). During 2001-2002 its outbreaks had been more common in South Asia, mainly due to its

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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 (Ahmad *et al*., 2007). Different insecticides from new chemistry insecticides and IGR's *i.e*, lufenoron, methoxyfenozide, emamectin benzoate and indoxacarb are being used for the control of *S. litura*.

Some new insecticides are now being used for successful control of this pest in the field (Shi *et al*., 2003). Keeping in view the problems associated with this insect pest, a study was planned with the following objectives; To investigate the stability of insecticide resistance of *S. litura* to different insecticides in the absence of selection pressure, to generate a baseline susceptibility data of *S. litura* against these insecticides and to observe the susceptibility of succeeding generations of *S. litura* under laboratory conditions.

MATERIALS AND METHODS

Insects

The larvae of *S. litura* were collected from Dunyapur (Lodhran, Punjab, Pakistan) and brought to laboratory. These larvae were reared in glass vials on improved artificial diet at controlled laboratory conditions of 25±2°C, 65-70% relative humidity and a photoperiod of L: D, 14:10 (Ahmad *et al*., 2007). The pupae were collected from the rearing glass vials on alternate days and kept in a plastic jar. Moths emerged from the pupae were shifted into glass jars with 1:1 male and female ratio. Adults were fed on 10% honey solution. Thirty pairs of moth were kept in one glass jar and tissue papers hanged in the glass jars to serve as egg laying substrate.

Insecticides

Commercial formulations of insecticides used in this experiment were: Tracer (spinosad, 240EC, Dow Agro Sciences), Proclaim (emamectin benzoate, 019EC, Syngenta), Curacuron (profenofos, 500EC, Syngenta) and Confidor (imidacloprid, 200SC, Bayer Crop Sciences).

Diet incorporation bioassay

Diet incorporation bioassay method was used for this experiment. The insecticide concentrations were prepared by serial dilution. The insecticide solution was thoroughly blended in diet and 2nd instar larvae due to their good response against insecticides were used for bioassay. There were six treatments including control and each treatment was replicated six times. Mortality data was recorded after 72 h for new chemistry insecticides and after 48 h in case of profenofos.

Data analysis

The LC_{50} value of each insecticide was calculated by using the probit analysis method through POLO-PC Program (LeOra, 2003). Resistance Factor (RF) was calculated for each generation as described by Wearing and Catherine (2005).

RESULTS

Spinosad

Spinosad is an insecticide which is derived from naturally occurring bacteria, *Saccharopolyspora spinosa*. It acts on GABA-gated channels and causes excitation of insect nervous system (Salgado, 1998). The bioassay performed at 1st generation of field collected population of *S. litura* shows that the initial LC_{50} value for spinosad was 7.779 ppm which decreased with succeeding generations and the final LC_{50} value at 11th generation was 0.791 ppm after 72 hrs of insecticide exposure (Table I). The rate of decrease of insecticide resistance of *S. litura* to spinosad was -0.090 and estimated 10-fold decrease in resistance was 11.11 (Table II). The base line susceptibility value of *S. litura* to spinosad was 0.79 (Table I).

Emamectin benzoate

Emamectin benzoate is a semi-synthetic chemical which interferes with neurotransmitters of target organism causing a loss of cell function and disruption of nerve impulses (Dybas and Babu, 1989; Dybas *et al.*, 1989). The LC_{50} value of emamectin benzoate at 1st generation for the field population of *S. litura* was 1.59 ppm which reduced to 0.33 ppm at 11th generation after 72 h of exposure (Table I). The rate of decrease of resistance to emamectin benzoate was -0.060 and estimated 10-fold decrease in resistance was 16.67 (Table II),

Table I.- Toxicity of spinosad, emamectin benzoate, profenofos and imidacloprid to different generations of *Spodoptera litura* under lab. conditions.

G	Hrs.	LC_{50} (ppm)	FL 95%	Slope ± SE	n	DF	$\chi 2$	P	RF
Spinosad									
1	72	7.77	6.23- 9.69	2.66 ± 0.35	180	4	6.748	0.149	9.83
4	72	4.57	3.63- 5.62	2.91± 0.41	180	4	5.537	0.236	5.79
5	72	4.72	3.58 - 6.94	2.08 ± 0.34	180	4	1.397	0.844	5.97
7	72	3.95	2.57- 8.16	1.14 ± 0.26	180	4	0.813	0.936	5.01
8	72	2.53	1.89 - 3.53	1.74± 0.28	180	4	4.384	0.356	3.21
9	72	1.66	1.18 - 2.26	1.66 ± 0.28	180	4	2.225	0.694	2.10
10	72	1.14	0.78 - 2.07	1.42± 0.28	180	4	0.424	0.980	1.44
11	72	0.79	0.24 - 1.34	0.964±0.26	180	4	0.245	0.993	1.00
Emamectin benzoate									
1	72	1.59	0.83-1.84	1.77±0.29	180	4	0.90	0.92	4.81
4	72	1.37	0.95-1.80	1.87±0.30	180	4	3.81	0.432	4.15
5	72	1.14	0.54-1.74	1.19±0.26	180	4	1.87	0.759	3.45
7	72	1.19	0.76-1.86	1.19±0.26	180	4	1.65	0.799	3.60
8	72	1.13	0.80-1.57	1.60±0.27	180	4	2.80	0.591	3.42
9	72	0.70	0.43-0.99	1.45±0.27	180	4	0.83	0.934	2.12
10	72	0.40	0.26-0.56	1.50±0.27	180	4	0.40	0.982	1.21
11	72	0.33	0.22-0.45	1.75±0.29	180	4	2.86	0.581	1.00
Profenofos									
1	48	258.757	165-356	1.6±0.29	180	4	1.547	0.818	13.8
4	48	136.497	110-168	2.8±0.38	180	4	4.348	0.360	7.29
5	48	52.073	40.7-65.7	2.3±0.33	180	4	0.673	0.954	2.78
7	48	49.817	34.4-81.7	1.3±0.26	180	4	0.719	0.948	2.66
8	48	40.719	27.4-65.3	1.2±0.26	180	4	2.024	0.731	2.18
9	48	24.623	12.4-41.1	0.96±0.25	180	4	0.782	0.940	1.32
10	48	19.619	12-35.6	1.03±0.25	180	4	0.569	0.966	1.05
11	48	18.714	13.6-26.2	1.6±0.28	180	4	6.792	0.147	1.00
Imidacloprid									
1	72	686.5	449-1020	1.30±0.26	180	4	6.77	0.148	9.34
4	72	359.3	265-514	1.68±0.28	180	4	1.57	0.814	4.89
5	72	314.7	211-449	1.42±0.27	180	4	5.28	0.259	4.28
7	72	134.6	103.6-175.8	2.08±0.30	180	4	2.07	0.722	1.83
8	72	131.8	88.9-197	1.33±0.26	180	4	1.86	0.761	1.79
9	72	96.1	51.1-155	1.03±0.25	180	4	0.059	0.999	1.31
10	72	75.14	52.8-110.8	1.44±0.27	180	4	0.34	0.987	1.02
11	72	73.5	54.8-111.5	1.91±0.32	180	4	2.56	0.633	1.00

G= generation number of *S. litura*
n = number of larvae used in the bioassay including control.
RF = Resistance factor (RF) was calculated for each generation as LC_{50} of test generation divided by LC_{50} of susceptible generation

while the baseline susceptibility value of *S. litura* for emamectin benzoate was 0.33 (Table I).

Profenofos

Profenofos is an anticholinesterase chemical which belongs to organophosphate (OP) group of insecticides. For profenofos the LC_{50} value of field collected population of *S. litura* was 258.75 ppm at 1^{st} generation which reduced to 18.71 ppm at 11^{th} generation after 48 hrs of exposure when reared in the laboratory under controlled conditions without any selection pressure (Table I). The rate of decrease of insecticide resistance of *S. litura* was -0.104 with an estimated 10-fold decrease in

Table II.- Stability of insecticide resistance of *Spodoptera litura* against different insecticides.

TG	Insecticide	Initial LC_{50} (log)	Final LC_{50} (log)	R	GR
11	spinosad	7.77 (0.89)	0.79(-0.102)	-0.090	11.11
11	emamectin benzoate	1.59 (0.02)	0.33 (-0.481)	-0.060	16.67
11	profenofos	258.76 (2.41)	18.71 (1.27)	-0.104	9.61
11	imidacloprid	686.5 (2.83)	73.5 (1.86)	-0.088	11.36

TG= Total generations of *S. litura*
R= rate of decrease in LC_{50} [log (final LC_{50} – initial LC_{50})/*N]*, where *N* is number of generation populations reared without insecticide exposure.
GR= estimated number of generations required for a tenfold decrease in LC_{50}

resistance of 9.61 for profenofos (Table II). The base line susceptibility value of *S. litura* to profenofos was 18.71 (Table I).

Imidacloprid

Imidacloprid causes interference for the transmission of impulses in the nervous system of target organism. It has both contact and stomach action (Cox, 2001). For imidacloprid the initial LC_{50} value for 1st generation was 686.5 ppm and at 11th generation was 73.5 ppm after 72 hrs of exposure (Table I). The rate of decrease of insecticide resistance of *S. litura* was -0.088 and estimated 10-fold decrease in resistance was 11.36 (Table II). The base line susceptibility value of *S. litura* to imidacloprid was 73.5 (Table I).

DISCUSSION

The results of present study indicates that insecticide resistance in field collected population of *S. litura* to emamectin benzoate in the absence of any selection pressure was more stable as compared to other three insecticides such as spinosad, imidacloprid and profenofos. The reversion rate of insecticide resistance of *S. litura* when reared without insecticide exposure was highest for profenofos (-0.10) and least for emamectin benzoate (-0.06) under controlled laboratory conditions. The rate of decrease in insecticide resistance to spinosad and imidacloprid was equal.

Insecticide resistance in *S litura* to spinosad, imidacloprid and profenofos may involve a common mechanism of resistance. In Pakistan there is intensive use of different insecticides due to high infestation of insect pests on crops, which resulted in evolution of insecticide resistance. The field populations of *S. litura* in Pakistan have developed resistance to both conventional and new chemistry insecticides (Ahmad *et al*., 2007, 2008). The resistance against these insecticides may involve a common mechanism. It has been found that the multiple resistances in *S. litura* is due to enhanced activity of mixed function oxidases and esterases. (Huang and Han, 2007). The high stability of insecticide resistance in *S. litura* to emamectin benzoate as compared to other insecticides may be due to a separate mechanism of resistance (Shad *et al*., 2010) and requires further studies on biochemical mechanism of resistance in *S. litura* to emamectin benzoate.

Fitness cost may be associated with resistance in *S. litura* to spinosad, imidacloprid and profenofos while resistance to emamectin benzoate may not involve any fitness cost. It has been found that relative fitness differences, initial genes frequencies and dominance relation of susceptible and resistant alleles of the phenotype which are from original field population are important factors that may influence on the reversion rate of insecticide resistance in the laboratory (Roush and Croft, 1986). But this aspect is not well clear and requires further investigations. Resistance is a temporal phenomenon and there are many examples of pesticide resistant insects and mites that revert to susceptible ones when reared without any insecticide exposure under laboratory conditions (Abedi and Brown, 1960; Flexner *et al*., 1989; Kristensen *et al*., 2000).

The base line susceptibility of *S. litura* to spinosad, emamectin benzoate, profenofos and imidacloprid was determined by using a population of *S. litura* isolated from insecticides. Initial bioassay performed at first generation showed LC_{50} values of 7.77, 1.59, 258.76 and 686.5 ppm for spinosad, emamectin benozoate, profenofos and imidacloprid, respectively (Table II). The susceptibility of this field collected population of *S. litura* increased with succeeding generations when reared in the laboratory in the absence of any selection pressure. The susceptibility of *S. litura* at G11 incresased 9.83, 4.81, 13.8 and 9.34-fold as compared to first generation. These baseline values may be used for future monitoring of insecticide resistance for S. *litura* in Pakistan.

Based on the findings of present research it can be concluded that for a good management of *S. litura* under the field conditions only those insecticides should be used which have lower stability and high reversion rate of insecticide resistance. Resistance in insects to these kinds of insecticides can be overcome by rotation of insecticides. The insecticides which have high stability and lower reversion rate of insecticide resistance may cause more serious problems and ultimately result in control failure. According to the results of present research, spinosad can be included in control program of *S. litura*, due to its lower stability and higher reversion rate with a novel mode of action.

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