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Toxicity of conventonal insecticides to fourth instar larvae of tobaccocaterpillar, *Spodoptera litura* (Fab.) in different generations

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Abstract
Studies on development of resistance in tobacco caterpillar against some insecticides were carried out in the laboratory, Division of Entomology, Udheywalla, Jammu. The differential susceptibility level of parental and susceptible strains of tobacco caterpillar to conventional insecticides revealed that the level of susceptibility in 4th instar larvae of this insect has decreased considerably in parental strain as compared to recommended concentrations of insecticides. The highest resistance factor of 9.33 was observed with monocrotophos whereas malathion encountered lowest resistance factor of 1.50. Comparison of the LC_{50} values obtained in succeeding generations with the 1st generation in 4th instar larvae of *S. litura* revealed that the resistance developed in an increasing order in all the six generations. The resistance ratio in the 6th generation as compared to the 1st generation was 3.97- fold in endosulfan, 3.11 in malathion and 3.45 in carbaryl.

Keywords: *Spodoptera litura*, Resistance factor, LC_{50}

INTRODUCTION

Spodoptera litura (Fabricius) is an important polyphagous pest infesting so many agricultural crops but this pest has developed resistance to many commercially available pesticides [1]. Moreover, outbreaks of secondary pest and the effect of pesticides on non-target organisms are becoming increasingly common. Because of these reasons, the control of arthropod pests is becoming increasingly difficult. Thus, when a population of insects is challenged with an insecticide, a greater proportion of those individuals, which possess resistance genes to survive and reproduce. After every cycle of selection, more insects with resistance survive and breeds fast compared to those with susceptible one. As the prevalence of resistance is noticed, insect population increases with the result it is very difficult to manage that pest and insecticides appear to be less effective against the target pest. This kind of problem is being experienced in *S. litura* commonly known as the Tobacco caterpillar, which is an economically serious polyphagous pest with high reproductive capacity. Its ability to migrate over large distance in the adult stage have resulted in its becoming a pest of many agricultural crops distributed throughout tropical and temperate Asia, Australasia and Pacific Islands [2]. The problem of this pest is further magnified due to its direct attack on fruiting structures, voracious feeding habits, high mobility and fecundity, overlapping generation, nocturnal behaviour, and propensity for acquiring resistance against insecticides [3].

The distribution of this pest is ubiquitous in all the states of India including Jammu and Kashmir, yet no work has been undertaken to assess the level of resistance in it despite of the fact that at many occasions in the past, the insecticidal control failures against this pest even after spraying repeatedly at dosages of insecticides recommended for its control in various crops like tomato, cauliflower, beans and pea etc have been reported. The pest is also indicating the learning instinct in term of its shifting behaviour from one crop to other through biological adaptations. Also, it has developed tendency to move from treated to non-treated hosts at many occasions. It, therefore, becomes pertinent to generate the toxicitydata of conventional insecticides against *S. litura* in Jammu to study the potential of development of resistance in the pest.

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MATERIALS AND METHODS

The culture of test insect tobacco caterpillar was initiated by collecting larvae from the farmer's field nearby Udheywalla, Jammu. The field-collected adults served as nucleus culture. The egg masses obtained from wild parents were surface sterilized using mercuric chloride (1%) for 10 minutes. After thoroughly washing with running water, the eggs were air dried and kept for hatching. After emergence the neonates were fed on tender castor leaves which were previously washed and air dried before being fed to larvae. Uneaten food along with faces was removedregularly in order to maintain hygiene in the rearing containers. The feed was changed daily and rearing space was increased regularly by using more number of jars for avoiding overcrowding of the larvae for promoting uniform growth and development of the larvae. Care was taken for NPV disease symptoms in the larvae, mould growth (if any) and drying up of leaves in the course of larval growth. Daily observation on the growth and development of larvae were taken. The head capsule width of the larvae was recorded to fix the number of instars. When the larvae became pre-pupae they were transferred to fine sterilized sandy soil to facilitate proper pupation. Adults male and females emerged from the pupae were released in glass jars (25cm height × 15cm diameter) and were provided 10 per cent sucrose

solution soaked in cotton as food, white paper strips were provided inside the ovipositional jars to facilitate egg laying. The eggs were clipped off, sterilized and kept for hatching in Petri dishes after subsequent washings. This way the culture was maintained in BOD incubator at 28 ± 2ºC temperature, 70 ± 2ºC relative humidity and 16:8 L/ D period regimes.

Hundred pupa of susceptible strain of *S. litura* were procured from Division of Entomology, IARI, New Delhi and reared in the laboratory as cited above for further studies. Sub-lethal concentration of insecticides prepared on the basis of recommended concentration of various insecticides were applied to the thoracic dorsum of each 4th instars of susceptible as well as field collected strain of *S. litura* separately with the help of a micropipette @1.0 µ1/ larva. Ten larvae per replicate were treated with one concentration of each insecticide housed in the petriplates (9cm diameter) individually and were kept in BOD incubator (28 ± 2ºC). Fourth instars larvae were exposed to various concentrations of three insecticides *viz.* endosulfan, malathion and carbaryl. Six concentration each of the three insecticides were utilized. A set of control (with acetone alone) was also maintained with each exposure to work out the correct mortalities. These were 10 larvae per replicate and in all three replications for each concentration were maintained. The mortality data was recorded at 24 ,48 and 72 hours after treatment. The surviving larvae were reared as defined above. The progeny of the first surviving lot was termed as F_1 generation corresponding to the insecticide to which it was exposed in the same way, the exposure up to six generations were conducted. The parental strain was also maintained all through without exposure to obtain the mortality data.

The degree of development of resistance to the three insecticides through different generations was determined by working out LC_{50} values in each generation by SPSS 13.0.0 to obtain the resistance ratio (RR). The resistance ratio for respective generation was worked out by dividing LC_{50} value of a given generation with LC_{50} of the parental strain. The observations on larval mortality were recorded by considering the larvae as dead if it would not move in a coordinated manner when prodded with a fine camel hair brush.

Note: Experiment was conducted to evaluate the ill effects and resistance developed by *S. litura* to conventional insecticides used in India as few of them has been banned by government of India still some farmers are using these products. Present investigations were carried out for technical knowhow of the extension workers which ultimately reach those farmers.

RESULTS AND DISCUSSION

Comparison of the LC_{50} values obtained in succeeding generations with the 1st generation in 4th instar larvae of *S. litura* revealed that the resistance developed in an increasing order in all the six generations (1 and 2). The resistance ratio in the 6th generation as compared to the 1st generation was 3.97- fold in endosulfan. Though several workers [4,5,6] have come out with the report of development of resistance to endosulfan in *S litura,* yet the available literature is devoid of any information which matches the generation-wise study on the potential of development of resistance in this pest. However, it could be inferred that the potential of resistance development to endosulfan is extremely high in Jammu strain of *S litura* and any increase in the use of this insecticide for the control of this pest in field may lead to control failures which would not be economical for the farmers. In case of malathion, the LC_{50} value increased from 0.077 in the 1st generation to 0.240 in the 6th generation with a resistance ratio of 3.11 in the 6th generation as compared to the 1st generation which indicated the faster development of resistance in test insect. This kind of increasing trend in resistance ratio from 1st to 6th (*i.e.,* 3.45 fold in the 6th generation) was also observed in carbaryl. Though the information in development of resistance to carbaryl in S.*litura*is available from the field collected strains, yet no systematic study has been conducted so far to detect the resistance levels in the laboratory bred generations.28-fold resistance has been observed in Thailand strain of *H. armigera*to carbaryl [7]. It has been advocated that upto 80 per cent carbaryl consumption on cotton was responsible for the multiple cross resistance in this pest to carbamates with a magnitude of 3.36 to 4.83-fold [8] and 4.58-fold [9].Similarly, an increase in the resistance level of *H. armigera* to carbaryl (9.10-fold) has been detected in Gujaratwhich has increased to more than 10-fold in further reports [10].

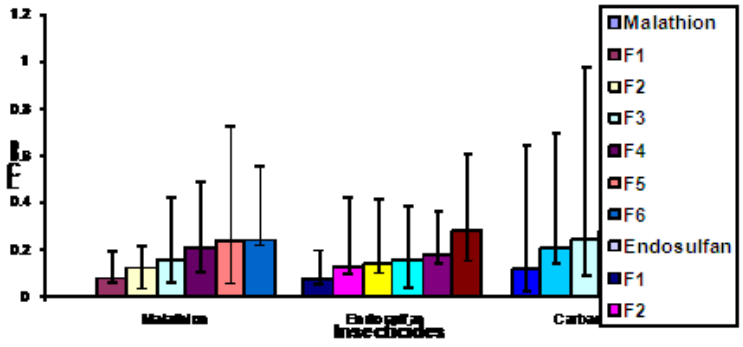

Fig 1. Toxicity of commonly used insecticides against different generations of *S. litura*

Table 1. Selection of residual population of *S. litura* in various generations exposed to endosulfan, malation and carbaryl.

Generation	Residual population R / S	Endosulfan		Malathion		Carbaryl	
		Concentration used (%)	Per cent Mortality	Concentration used (%)	Per cent Mortality	Concentration used (%)	Percent Mortality
F_1	X	0.10	57.00	0.01	30.00	0.05	30.00
	X	0.15	63.00	0.05	36.00	0.10	36.00
	X	0.20	66.00	0.10	51.00	0.15	46.98
	X	0.25	69.00	0.15	60.00	0.20	60.99
	X	0.30	75.00	0.20	78.00	0.25	70.98
	√	0.35	90.00	0.25	90.00	0.30	81.99
F_2	X	0.25	48.00	0.20	48.00	0.30	57.00
	X	0.35	51.00	0.25	54.00	0.35	60.00
	X	0.45	69.00	0.30	57.00	0.40	66.00
	X	0.55	75.00	0.35	60.00	0.45	69.00
	X	0.65	78.00	0.40	63.00	0.50	72.00
	√	0.75	81.00	0.45	66.00	0.55	78.00
F_3	X	0.80	66.00	0.40	57.99	0.50	39.00
	X	0.85	69.00	0.45	60.00	0.55	46.98
	X	0.90	75.00	0.50	63.00	0.60	61.98
	X	0.95	78.00	0.55	66.00	0.65	63.99
	X	1.00	81.00	0.60	69.00	0.70	87.00
	√	1.05	87.00	0.65	72.00	0.75	90.00
F_4	X	1.00	63.99	0.60	57.99	0.70	60.00
	X	1.05	70.99	0.70	57.00	0.80	72.00
	X	1.10	72.00	0.80	60.00	0.90	75.00
	X	1.15	75.00	0.90	63.00	1.00	78.00
	X	1.20	79.98	1.00	66.00	1.10	81.00
	√	1.25	91.98	1.10	69.00	1.20	84.00
F_5	X	1.15	69.00	1.00	21.00	1.10	69.99
	X	1.25	72.00	1.25	24.00	1.20	72.99
	X	1.35	72.00	1.50	33.00	1.30	75.00
	X	1.45	75.00	1.75	42.00	1.40	78.00
	X	1.55	78.00	2.00	48.00	1.50	81.00
	√	1.65	87.00	2.25	60.00	1.60	87.00
F_6	X	1.55	70.98	2.00	36.00	1.50	72.00
	X	1.65	75.00	2.25	39.00	1.60	73.98
	X	1.75	75.99	2.50	44.97	1.70	74.97
	X	1.85	82.98	2.75	54.00	1.80	78.00
	X	1.95	84.00	3.00	60.00	1.90	87.99
	√	2.05	90.00	3.25	69.00	2.00	90.00

√ - Selected population, X- rejected population, R- Resistant, S- Susceptible

The differential resistance reactions at geographically different places could be attributed due to repeated applications of the same insecticides and / or mixing of the different groups of insecticides and / or the quality of the insecticides used [11] It has been postulated that resistance develops at different rates between species and even between populations of the same species due to genetic, reproductive, behavioural/ecological and operational factors [12,13]. These conclusions have efficiently been demonstrated in many pests with three different mechanisms *viz*.Slower rate of pesticide penetration through cuticle. An enhanced rate in pesticide metabolism through mixed function oxidases or estrases or both enzyme systems and insensitivity of voltage-gated sodium channel in nerve membranes which affect pesticide binding or changes in gene expression [14,15]. This has been outlined that increase in resistance was result of high selection with respect to population density, size, crop phenology and weather. Under the genetic changes, the amount gene flow between sprayed and unsprayed hosts of this pest and the way in which selection operated in the field were also considered [16]. Synergism tests with microsomal oxidase (MO) and esterase-specific inhibitors indicated that the deltamethrin resistance was associated with MO and, possibly, esterase activity. Also reciprocal crosses between the Delta-SEL and Lab-PK strains indicated that resistance was autosomal and incompletely dominant [6]. A direct test of monogenic inheritance suggested that resistance to deltamethrin was controlled by more than one locus.

Table 2. Toxicity of endosulfan, malation and carbary to 4th instar larvae of S. litura in different generations.

Insecticide	Generation	Heterogeneity	Regression Equation	Slope ± S.E.	Fiducial Limits	LC_{50}(%)	Resistance Ratio
Endosulfan	F_1	3.582	Y=-0.368+5.191	5.19±1.33	0.018 – 0.127	0.071	1.00
	F_2	2.799	Y=-0.245+1.870	1.87±.61	0.036 – 0.291	0.131	1.84
	F_3	1.767	Y=-0.157+1.127	1.12±1.26	0.037 – 0.281	0.139	1.95
	F_4	1.554	Y=-0.200+1.285	1.28±1.14	0.113 – 0.233	0.156	2.19
	F_5	1.362	Y=-0.148+.835	0.83±1.23	0.032 – 0.191	0.177	2.49
	F_6	2.845	Y=-0.225+.797	0.79±0.64	0.131 – 0.327	0.282	3.97
Malathion	F_1	2.543	Y=-0.242+7.775	7.77±1.47	0.014 – 0.117	0.077	1.00
	F_2	1.250	Y=-0.225+1.783	1.78±1.13	0.092 – 0.321	0.126	1.63
	F_3	1.554	Y=-0.200+1.285	1.28±1.14	0.091 – 0. 267	0.156	2.02
	F_4	1.482	Y=-0.546+2.605	2.60±1.29	0.101 – 0.278	0.210	2.72
	F_5	1.113	Y=-0.184.776	0.77±0.57	0.179 – 0.489	0.237	3.07
	F_6	20.035	Y=-0.492+2.051	2.05±0.42	0.117 – 0.317	0.240	3.11
Carbaryl	F_1	2.469	Y=-0.620+5.278	5.27±1.19	0.092 – 0.532	0.117	1.00
	F_2	1.482	Y=-0.546+2.605	2.60±1.29	0.069 – 0.490	0.210	1.79
	F_3	1.907	Y=-0.071+0.286	0.28±0.22	0.160 – 0.731	0.249	2.12
	F_4	2.845	Y=-0.225+.797	0.79±0.64	0.167 – 1.060	0.282	2.41
	F_5	1.275	Y=-0.504+1.577	1.57±0.67	0.263 – 0.891	0.320	2.73
	F_6	2.196	Y=-0.481+1.189	1.18±0.71	0.219 - 0.598	0.404	3.45

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