



Insecticide Resistance Development and Detoxification Enzyme Activities of *Spodoptera litura* (Fabricius) in Soybean from Kumaon Himalayas

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ABSTRACT

Background: Soybean is an important crop of Uttarakhand both in hills as well as in plains and *Spodoptera litura* is an important pest of soybean in Uttarakhand. Several insecticides have been in use for its management and resistance has been observed against different group of insecticides. Thus, to study the level of infestation and insecticide resistance from soybean fields of hill farmers of Kumaon Himalaya, survey and experiments were conducted.

Methods: Survey was conducted and scale of infestation was determined. Chemical and biochemical assay were conducted to study insecticide resistance. For chemical assay IRAC Method No. 7 using insecticides indoxacarb 14.5% SC, chlorantraniliprole 18.5% SC, fipronil 5% SC, chlorpyrifos 50%+cypermethrin 5% EC and profenofos 40%+cypermethrin 4% EC and for biochemical assay the specific activity of Carboxylesterase, Acetyl choline esterase, Mono-oxygenase and Glutathione-S-transferase was studied.

Result: The infestation level from different regions showed the presence of *S. litura*, ranging from mild to severe infestation. The resistance ratio of different insecticides used ranged from a low to an extremely high resistance level with LC₅₀ ranging from 0.37 µg/ml to 1648.84 µg/ml. The biochemical analysis of carboxylesterase, acetylcholinesterase, monooxygenase and glutathione-s-transferase showed an overproduction of these detoxification enzymes in the resistant population.

Key words: Detoxification enzymes, India, Insecticide-resistance, *Spodoptera litura*.

INTRODUCTION

Spodoptera litura is one of the most important polyphagous lepidopteran pests in agriculture and belongs to the Noctuidae family (Wang *et al.*, 2018; EFSA PLH Panel, 2018). This pest has enormous economic importance for field and horticultural crops (Murthy *et al.*, 2006) and is widespread throughout tropical and temperate Asia (Kranz *et al.*, 1977). Severe outbreaks of *S. litura* have been reported in Kota (Rajasthan) and Marathwada and Vidarbha (Maharashtra) in India, causing total damage of about US\$ 4.5 crores and US\$22.5 crores, respectively (CROPSAP, 2012). Soybean, which is an important crop of India and the country ranks fifth in world soybean production (SOPA, 2020), suffered about 24.7% damage from *S. litura* (Higuchi *et al.*, 1991). Uttarakhand is a Himalayan state located in the north-western part of India (Sati, 2020). *S. litura* is one of the major defoliator moths that occur in different regions of Uttarakhand and cause significant crop losses. Singh and Sachan (1992) reported that defoliating moths play an important role at the pod stage and thereafter in soybean. Several insecticides were recommended for use against this pest, which exerted high selection pressure on populations of *S. litura*. This selection pressure was responsible for the development of resistance of *S. litura* to many insecticides (Shi *et al.*, 2019). In the 2017 IRAC Newsletter, it was documented that *S. litura* was ranked 7th among top 20 arthropods in terms of number of resistance cases. Resistance to insecticides is a complex process and may

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result mainly from four mechanisms: i) increased metabolism to non-toxic products, ii) decreased sensitivity of the target site, iii) decreased rate of penetration of the insecticide, iv) increased rate of excretion of the insecticide (Sakine, 2012). The simplest and most conventional detection of resistance is dose-mortality experiments conducted in the laboratory under controlled conditions (Brown, 1976). Another approach is the biochemical mechanism, which, when used in addition to basic toxicity studies, gives the best results. Resistance to insecticides has been found to be essentially due to two mechanisms, *i.e.*, enhanced enzymatic detoxification of an insecticide or reduced sensitivity of a

target enzyme to inhibition by the insecticide; out of the four mechanisms discussed (Brown, 1987). The farmers from locations surveyed were using insecticides such as Profenofos 40%+Cypermethrin 4% EC, Indoxacarb 14.5% SC, Chlorantraniliprole 18.5% SC, Carbofuran 3G, Chlorpyrifos 50%+ Cypermethrin 5% EC, Fipronil 3.5%+Chlorpyrifos 35% etc. which were provided by government agencies involved in agricultural work as per information provided by farmers themselves. Therefore, the present study investigated the resistance level of five different insecticides and five *S. litura* field populations collected during 2019 and 2020 from soybean fields of Kumaon, Himalayas. The results of this study can help in developing effective and timely management practises.

MATERIALS AND METHODS

Field populations of *S. litura* egg masses and larvae in soybean fields were collected from four hill districts, namely Ramgarh (Nainital district), Bhujan (Almora district), Kameri, Bageshwar and Ganai (Pithoragarh district) in Kumaon, Uttarakhand in 2019 and 2020. Other crops observed during survey in farmers field were Soybean, Cabbage, Cauliflower, Colocasia, Capsicum and French bean. Larvae were reared on artificial diet (Ballal, 2003) and adults were fed on 10% honey solution under laboratory conditions ($27\pm1^{\circ}\text{C}$ and $65\pm5\%$ relative humidity) with a photoperiod of 16:8 hours light: dark. Adults were kept in jars with butter paper attached for oviposition by females and reared to F1 generation. A susceptible population of *S. litura* was obtained from NBAIR, Bangaluru, for comparative studies. Based on the survey information on insecticides used by farmers in the selected regions, five insecticides were used in the study, namely

indoxacarb 14.5% SC, chlorantraniliprole 18.5% Sc, fipronil 5% SC, chlorpyrifos 50%+cypermethrin 5% EC and profenofos 40%+cypermethrin 4% EC. The bioassay method of IRAC Method No. 7 (IRAC, 2010) was performed on third instar larvae of *S. litura* in Pulse Entomology Lab, Deptt of Entomology, College of Agriculture, GBPUAandT, Pantnagar. The insecticide solution was prepared from a 1% stock solution by serial dilution. Fresh castor leaves of uniform size i.e., 5×5 cm were taken and dipped in the insecticide solution for 10 seconds. Excess liquid was drained off and the leaves were then air dried for half an hour. Ten third instar larvae from the F1 progeny (after being starved for 6 hours) were transferred to each petri dish and mortality was monitored at 24, 48 and 72 hours post exposure. Tests were conducted at a controlled temperature of $27\pm 1^{\circ}\text{C}$, $65\pm5\%$ relative humidity and a 16:8 L:D photoperiod. To investigate the potential of the detoxification enzymes, biochemical analysis was performed to test the total protein and estimate the activity of Carboxylesterase (CarE) (Devonshire, 1977 and Van, 1962); Acetyl choline esterase (AChE) (Kranthi, 2005); Mono-oxygenase (P450) (Kranthi, 2005) and Glutathione-S-transferase (GST) (Van, 1962 and Booth *et al.*, 1961). Polo Suite Leora Software LLC was used to estimate the LC_{50} values of the chemical bioassays and the specific activities of the biochemical studies were analysed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The survey to study the distribution and collection of *S. litura* was conducted in 2019 and 2020 in different districts of Kumaon region of Uttarakhand (Table 1, Fig 1). Visual estimation was used to determine the infestation level. The

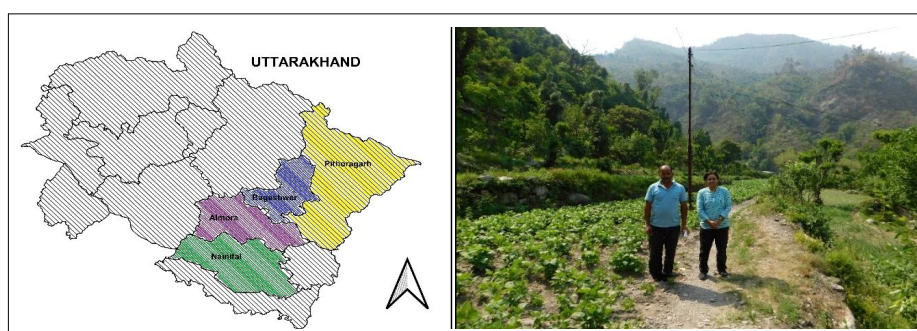


Fig 1: Map of Uttarakhand and districts surveyed shown in shaded colour viz., Pithoragarh, Bageshwar, Almora and Nainital (on left side) and image from farmer field surveyed Ramgarh, District Nainital (on right side).

Table 1: Geographical dimensions and meteorological data of areas from where *S. litura* population was collected for study.

Name of place	Latitude	Longitude	Altitude (m) above mean sea level (msl)	Average temperature $^{\circ}\text{C}$		RH (%)		Scale of infestation of <i>S. litura</i>
				(Previous month)	(Collection month)	(Previous month)	(Collection month)	
Ramgarh (Nainital District)	29.4347N	79.5521E	1518	30.93	27.38	38.25	75.70	4
Bhujan (Almora District)	29.51N	79.4776E	1604	24.76	23.51	75.48	84.93	4
Kameri (Bageshwar District)	29.8514N	79.7996E	1004	22.42	20.76	85.96	87.16	3
Ganai (Pithoragarh District)	26.6629N	80.075E	1760	19.43	17.90	85.81	87.85	3

scales for determining infestation were given by Vennila *et al.*, (2010) and used by Singh and Gandhi (2012) in their study on *S. littoralis* on cotton, cabbage, radish and other insect pests. Four scales were given for infestation: no insect/scars appearance (scale 0); scattered appearance of few (scale 1); severe incidence on only one branch (scale 2); severe incidence on more than one branch (scale 3) and complete severe incidence (scale 4). The study found that severe to complete severe incidence of the pest was observed in the regions studied. The *S. litura* samples were collected from different locations and brought to the laboratory for further rearing of the F1 generation, where bioassay tests were carried out (Table 2). Insecticide resistance results according to insecticide used and locations visited were classified according to Shen *et al.*, (1991) insecticide resistance level classification, *i.e.*, $RR < 3.0$ (susceptible), $3.0 < RR < 5.0$ (Decreased Susceptibility); $5.0 < RR < 10.0$ (Low level of resistance); $10.0 < RR < 40.0$ (Moderate level of resistance); $40.0 < RR < 160.0$ (High level of resistance) and $RR > 160.0$ (Extremely high level of resistance).

S. litura populations tested with indoxacarb 14.5% SC were found to be susceptible for Bhujan and Pithoragarh populations; low level of resistance was observed for Ramgarh; moderate level for Bageshwar. Wang *et al.*, (2018) also reported low to moderate level of resistance between 2-31-fold of *S. litura* population in Sichuan, China, to indoxacarb 14.5% SC from 2014-2016. Chlorantraniliprole 18.5% SC showed moderate to high resistance at almost all sites. Muthuswamy *et al.* (2014) reported a resistance ratio of 80.07 with respect to the susceptible NBAIR population, which is in insecticide resistance class 4. The bioassay results showed that Fipronil 5% SC showed resistance at almost all sites, from low to very high levels of resistance. Ahmad and Mehmood (2015) found that Pakistani populations developed moderate resistance every 8 years, increasing from 5.5-5.6-fold in 1998 to 28-35-fold in 2006. Ahmad *et al.* (2008) also reported that 22-fold resistance was observed to Fipronil 5% SC and this high level of resistance was explained by a multiple resistance mechanism. For combination insecticides *i.e.*, profenofos 40%+cypermethrin 4% EC and chlorpyrifos 50%+cypermethrin 5% EC, almost similar results were observed with both insecticides at all locations except Bhujan and the possible reason could be the selection pressure of profenofos 40%+cypermethrin 4% EC since the farmers used only this insecticide in their fields. It was also observed that the population of Ramgarh showed sensitivity to the combination of insecticides, which may have been due to the strict control and regulations of some private companies with which the farmers collaborate for organic crop production in the area as mentioned by farmers in the area and due to the low relative humidity (RH) of the sampling site, as RH is an important factor determining the life span of *S. litura* (Kumar *et al.*, 2013; Khan and Talukder, 2017). Both the profenofos 40%+cypermethrin 4% EC and

chlorpyrifos 50%+cypermethrin 5% EC have an OP to pyrethroid ratio of 1:10 and have good resistance, which Ahmadi (2009), El-Guindy *et al.* (1983), Goebel and Jacquemard (1990) and Forrester *et al.* (1993) reported that profenofos and chlorpyrifos antagonize cypermethrin in a ratio of 1:10.

Populations from different districts of Uttarakhand were subjected to biochemical analysis to identify their levels of detoxification enzymes compared to susceptible populations from NBAIR, Bangaluru (Table 3 and 4). Almora Bhujaan population showed the highest specific activity for both acetylcholinesterase enzyme (6.770 ± 0.140 nmol/min/ml enzyme) and monooxygenase P450 enzymes (2.780 ± 0.630 nmol/min/ml enzyme) and resistance ratio 8.63 and 111.20 were found in the reference laboratory population, respectively. For carboxylesterase enzyme, the highest specific activity was observed in the population of Kameri, Bageshwar, *i.e.*, the formation of 0.505 ± 0.035 μ moles 1-naphthol/min/mg protein and RR (resistance ratio) of 33.67; For glutathione-S-transferase enzyme, the highest specific activity was observed in Ganai, Pithoragarh population *i.e.* 1.910 ± 0.050 μ moles /min/mg protein with a RR of 23.87.

This study was conducted to investigate the level of infection and resistance to five different pesticides preferred by farmers in the region in four different areas of the Kumaon hills, Uttarakhand. The level of *S. litura* infestation was found to be on a scale of 3 and 4, meaning that it ranged from severe presence in more than one branch to full severe presence. According to Chattopadhyay *et al.*, (2019) outbreak of *S. litura* was observed at temperature between 21-27°C and RH above 90%. The infestation scale was related with temperature, humidity and availability of abundant host since the favourable conditions generated due to these factors are important for the growth and development of *S. litura* (Joshi *et al.*, 2022, Fand *et al.*, 2015). The data of previous and collection month showed that Ramgarh and Bhujaan had a constant favourable climatic condition in addition to abundance of host. Also, the chemical bioassay studies showed that the population is resistant against some of the insecticides used implying better survival of insect population in region, in addition to favourable environmental factors. It was observed in the studies that the resistance in Fipronil 5% SC and Chlorantraniliprole 18.5% SC could not be directly correlated with activity of any detoxification enzymes. Arain *et al.*, (2018) reported that detoxification of Fipronil 5% SC is not strongly related to the activity of these detoxification enzymes, as well as Su *et al.* (2012) mentioned similar results for 18.5% SC resistance to chlorantraniliprole and suggested that another mechanism may be responsible for detoxification of this insecticide. The level of resistance to the combination insecticides can be related to high activity of esterase enzymes *i.e.* AChE and CarE, but no such direct relationship with activity was observed for GST and P450 enzymes, also shown by Muthusamy *et al.* (2011) in their studies on insecticide detoxification mechanisms in *S. litura*. Similar

Table 2: Dosage mortality showing susceptibility of *S. litura* against different insecticides.

Insecticides	Population	LC ₅₀ (95%CI)	Chi square	Slope±SE	Resistance ratio
Indoxacarb 14.5%SC	Ramgarh, Nainital	2.48 (1.31-8.12)	0.499	Y=0.48+1.23x	7.09 (Low level of resistance)
	Bhujaan, Almora	0.37 (0.16-0.66)	2.651	Y=0.61+1.44x	1.06 (Susceptible)
	Kameri, Bageshwar	9.28 (5.32-17.94)	0.138	Y=1.39+1.44x	26.51 (Moderate level of resistance)
	Ganai, Pithoragarh	0.79 (0.47-1.32)	1.157	Y=0.25+1.75x	2.26 (Susceptible)
	Control, Lab population	0.35 (0.14-0.64)	0.435	Y=0.60+1.33x	
Chlorantraniliprole 18.5%SC	Ramgarh, Nainital	12.51 (5.48-25.98)	0.238	Y=1.22+1.11x	12.64 (Moderate level of resistance)
	Bhujaan, Almora	146.39 (69.09-621.18)	2.05	Y=2.1+0.98x	147.87 (High level of resistance)
	Kameri, Bageshwar	21.79 (12.15-44.01)	0.307	Y=1.77+1.34x	22.01 (Moderate level of resistance)
	Ganai, Pithoragarh	17.55 (7.95-42.76)	0.303	Y=1.28+1.03x	17.73 (Moderate level of resistance)
	Control, Lab Population	0.99 (0.62-1.64)	0.342	Y=0.05+1.84x	-
Fipronil 5% SC	Ramgarh, Nainital	1648.84 (868.62-5010.72)	0.126	Y= 3.8+1.8x	355.35 (Extremely high level of resistance)
	Bhujaan, Almora	36.34 (21.54-61.26)	0.54	Y=2.55+1.64x	7.83 (Low level of resistance)
	Kameri, Bageshwar	55.73 (31.94-107.68)	0.138	Y= 2.5+1.44x	12.01 (Moderate level of resistance)
	Ganai, Pithoragarh	74.84 (44.29-146.09)	1.347	Y=2.94+1.55x	16.13 (Moderate level of resistance)
	Control, Lab Population	4.64 (2.45-8.78)	0.391	Y=0.89+1.37x	
Chlorpyrifos 50% + Cypermethrin 5%EC	Ramgarh, Nainital	17.19 (8.55-30.77)	0.517	Y=1.7+1.37x	1.81 (Susceptible)
	Bhujaan, Almora	18.99 (10.23-33.33)	0.457	Y=1.91+1.51x	2.00 (Susceptible)
	Kameri, Bageshwar	55.32 (31.69-123.62)	1.634	Y=2.36+1.37x	5.82 (Low level of resistance)
	Ganai, Pithoragarh	115.12 (63.45-215.82)	0.259	Y=2.93+1.41x	12.12 (Moderate level of resistance)
	Control, Lab Population	9.50 (5.48-18.27)	0.075	Y=1.41+1.45x	-
Profenofos 40% + Cypermethrin 4% EC	Ramgarh, Nainital	5.20 (3.07-9.46)	0.087	Y=1.09+1.54x	1.21 (Susceptible)
	Bhujaan, Almora	96.04 (51.75-167.62)	0.142	Y=2.89+1.46x	22.28 (Moderate level of resistance)
	Kameri, Bageshwar	206.25 (119.88-400.93)	0.267	Y=3.52+1.52x	47.85 (High level of resistance)
	Ganai, Pithoragarh	57.99 (21.77-109.45)	0.810	Y=2.26+1.28x	13.45 (Low level of resistance)
	Control, Lab Population	4.31 (2.32-8.29)	0.440	Y=0.83+1.31x	-

Table 3: Specific activity of AChE and CarE for *S. litura* collected from different locations.

Places	AChE (nmoles/min/ml of enzyme)		CarE (µmoles of 1-naphthol formed/min/mg of protein)	
	Specific activity	Ratio w.r.t susceptible population	Specific activity	Ratio w.r.t susceptible population
Bhujaan, Almora	6.770±0.140	84.63	0.070±0.010	4.67
Ramgarh, Nainital	1.195±0.015	14.94	0.175±0.015	11.67
Ganai, Pithoragarh	1.790±0.100	22.38	0.265±0.035	17.67
Kameri, Bageshwar	0.985±0.025	12.31	0.505±0.035	33.67
Control, Lab population	0.080±0.010	1	0.015±0.005	1
CD		0.291		0.08
P value		<0.05		<0.05

Table 4: Specific activity of GST and P450 for *S. litura* collected from different locations.

Places	GST (µmoles/min/mg of protein)		P450 (nmoles/min/ml of enzyme)	
	Specific activity	Ratio w.r.t susceptible population	Specific activity	Ratio w.r.t susceptible population
Bhujaan, Almora	1.045±0.065	13.06	2.780±0.630	111.20
Ramgarh, Nainital	0.375±0.075	4.69	0.285±0.035	11.40
Ganai, Pithoragarh	1.910±0.050	23.87	0.260±0.030	10.40
Kameri, Bageshwar	1.475±0.055	18.44	0.070±0.010	2.80
Control, Lab population	0.080±0.010	1.00	0.025±0.005	1.00
CD		0.207		1.050
P value		<0.05		<0.05

results were obtained comparing Indoxacarb 14.5%SC resistance with detoxification enzyme activity, as no correlation was observed between enzyme activity and insecticide resistance, suggesting a possible role for other detoxification mechanisms (Shi *et al.*, 2019). Enzymatic activity indicated that overproduction of detoxification enzymes may be one of the main factors contributing to insecticide resistance in the studied *S. litura* populations (Despres *et al.*, 2007).

CONCLUSION

Soybean is an important crop in Uttarakhand and is important pulse in the diet of people from hills of Kumaon. Development of insecticide resistance in *S. litura* which is one of the major insect-pests of soybean has already been reported from different parts of world. This study found that insecticide resistance could become a major concern for hill farmers in Uttarakhand, India. Multiple mechanisms are responsible for the development of insecticide resistance in insects, which requires further investigation, making it a promising area of research for developing integrated insect control strategies.

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