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BIOCHEMICAL MECHANISM OF INSECTICIDE RESISTANCE IN *SPODOPTERA LITURA* (F) POPULATIONS FROM UTTARAKHAND

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ABSTRACT

***Spodoptera litura* populations were collected from different lowland areas and doon valley situated in Uttarakhand and insecticide resistance effect was studied for indoxacarb 14.5% SC, chlorantraniliprole 18.5%SC, fipronil 5% SC, chlorpyriphos 50%+cypermethrin 5% EC and profenofos 40%+cypermethrin 4% EC, also the specific activity of carboxylesterase, acetyl choline esterase, mono-oxygenase and glutathione-s-transferase was studied for biochemical analysis of detoxification enzymes. The scale of infestation was measured and related to temperature and humidity. High infestation has been observed in areas with optimum temperature and humidity. Highest resistance in the study was found against profenofos 40%+chlorpyriphos 4% and fipronil 5%SC with LC_{50} 243.63 µg/ml and 214.22 µg/ ml respectively. On biochemical analysis of detoxification enzymes, it was observed that the highest activity of all the enzymes was observed in Mota Haldu population. Hence, overproduction of detoxification enzymes was found to be responsible for insecticide resistance in *S. litura* populations studied.**

Key words: *Spodoptera litura*, carboxylesterase, acetyl choline esterase, mono-oxygenase, glutathione-S-transferase, Uttarakhand, insecticide resistance, Himalayan lowlands, LC_{50}, detoxification enzymes.

Spodoptera litura (F) is an economically important polyphagous pest on field crops and horticultural crops and is widespread in tropical and subtropical regions (Ferry et al., 2004). Losses from *S. litura* are estimated to vary from 26% to 100% under field conditions (Su et al., 2012). According to datasheet of CABI (2022), *S. litura* is a polyphagous pest and covers about 120 host range species, soybean having one of the important hosts. In Uttarakhand, *S. litura* is an important defoliator moth, causing significant crop losses (Singh and Sachan, 1992; Joshi et al., 2022). Chemical method of management has been the main control method till now, but recently most of the insecticides are ineffective on *S. litura* due to the development of insecticide resistance (Vengateswari et al., 2020). It has been documented in IRAC Newsletter 2017 that India ranks 7th among the top 20 countries and *S. litura* ranks 7th among the top 20 arthropods, in terms of number of cases of resistance. The most basic and classical detection of resistance is the dose-mortality experiments carried out in the laboratory in a controlled environment (Brown, 1976). There are primarily three types of assays, namely bioassays, biochemicals and molecular assays, which focus on phenotypic, biochemical, and genetic modifications (R4P Network, 2016). In biochemical analysis it was revealed that the main enzyme families involved in pesticide catabolism and/or sequestration are cytochrome P450-dependent mixed-function oxidases (MFOs), glutathione-S-transferases (GST), carboxylesterases (CarE) and UDT-glycosyltransferase (UGT) (Kranthi, 2005). So far, there has been no systematic study of *S. litura* in lowlands of Himalayas to study baseline resistance data from different geographical areas. The current study was conducted in 2019-2020 and 2020-2021 to study the insecticide resistance in *S. litura* in different areas situated in lowlands and valley of N-W Himalayas, India. The results of this study will facilitate insecticide resistance management practices.

MATERIALS AND METHODS

Field populations of *S. litura* egg masses and larvae were collected from four different lowland regions of Uttarakhand, namely Mota Haldu (District Nainital, 29.1473N, 79.5352E, 424 masl), Bichai, Tanakpur (District Champawat, 29.0875N, 80.0133E, 255 masl), Mandawar (District Haridwar, 30.039N, 77.7327E, 314 masl) and Bimora, Dakpathar (District Dehradun, 30.4691N, 77.7931E, 452 masl) in 2019-2020 and 2020-2021. Larvae were reared on artificial diet (Ballal, 2003) and adults were fed on 10% honey solution

under laboratory conditions (27± 1°C and 65± 5% 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, Karnataka, India 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, chlorpyriphos 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*. The insecticide solution was prepared from a 1% stock solution by serial dilution. Fresh castor leaves of uniform size i.e., 5x5cm 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 hr) were transferred to each petri dish and mortality was recorded at 24, 48 and 72 hours post exposure. Tests were conducted at a controlled temperature of 27± 1 °C, 65± 5 % relative humidity and a 16:8 L:D photoperiod in Pulse Entomology Lab, Deptt of Entomology, College of Agriculture, GBPUA&T, Pantnagar. To investigate the potential of the detoxification enzymes, biochemical analysis was performed to quantity the total protein and estimate the activity of Monooxygenase (P450) (Kranthi, 2005), Glutathione-S-transferase (GST) (Van,1962 and Booth et al., 1961), Carboxylesterase (CarE) (Devonshire, 1977 and Van, 1962) and Acetyl choline esterase (AChE) (Kranthi, 2005). 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 ANOVA.

RESULTS AND DISCUSSION

The present study was conducted in four locations situated in lowlands and valley in the Uttarakhand. The survey was conducted in 2019-2020 and 2020-2021 and the locations selected are important agricultural areas in the region and exposure to various insecticides was observed. To determine the degree of infestation, a visual estimation methodology was used. The scale of infestation was determined based on the analysis of Vennila et al. (2010). Four scales were given for infestation: no insect/scares 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). It was observed that at the time of collection there was complete severe incidence of *S. litura* in Tanakpur; severe incidence on more than one branch in Mota Haldu; severe incidence on only one branch in Dehradun and Haridwar. The infestation scale could be 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* (Fand et al., 2015) (Table 1). Data for the previous month and the month of collection showed that areas such as Tanakpur had constant favourable climatic conditions in addition to host abundance. On the other hand, in areas like Dehradun and Haridwar the lack of availability of host could be the major factor for scarce appearance of *S. litura*.

Samples of *S. litura* collected at different locations and reared in F1 progeny bioassays were subjected to using third instar larvae (Table 2). Insecticide resistance results according to insecticide used and locations visited were classified according to Shen et al. (1991) insecticide resistance level classification. The study found that the degree of resistance varied from susceptible to high. For indoxacarb 14.5%SC, almost all sites were found to be sensitive, except for Mota Haldu, which exhibited a moderate level of resistance. For other insecticides, there was low to high level of resistance in the study areas. Fipronil 5%SC showed resistance at almost all locations ranging from a low level of resistance to an extremely high level of resistance, except

Table 1. Meteorological data and scale of infestation of areas from where *S. litura* was collected

S.No.	Name of place	Average temperature °C		RH (%)		Scale of infestation of *S. litura*
		(Previous month)	(Collection month)	(Previous month)	(Collection month)	
1	Mota Haldu (Nainital)	27.375	25.89	75.70	84.81	3
2	Bichai, Tanakpur, (Champawat)	22.21	21.57	78.47	84.92	4
3	Mandawar, (Haridwar)	25.56	21.45	80.41	64.08	2
4	Bimora, Dakpathar, Dehradun	25.56	21.45	80.41	64.08	2

Table 2. Dosage mortality response of *S. litura* against insecticides

Insecticides	Population	LC50 (µg/ ml) (95%FL)	Heterogeneity Chi square ($\chi 2$)	Regression Equation Y=a+bx	Resistance Ratio (RR)
Indoxacarb 14.5%SC	Mota Haldu (Nainital)	3.81(1.84-6.79)	0.378	Y=0.82+1.42x	10.89 (Moderate level of resistance)
	Bichai, Tanakpur, Champawat	0.87(0.44-1.84)	1.154	Y=0.11+1.25x	2.49 (Susceptible)
	Mandawar, Haridwar	0.79(0.47-1.32)	1.157	Y=0.25+1.75x	2.26 (Susceptible)
	Bimora, Dakpathar, Dehradun	0.71(0.38-1.29)	1.842	Y=0.29+1.49x	2.03 (Susceptible)
	Lab population	0.35(0.14-0.64)	0.435	Y=2.97+1.87x	
Chlorantraniliprole 18.5%SC	Mota Haldu (Nainital District)	18.74(8.38-49.23)	0.702	Y=1.28+1.02x	18.93 (Moderate level of resistance)
	Bichai, Tanakpur, Champawat	10.76(4.50-21.70)	0.211	Y=1.16+1.11x	10.87 (Moderate level of resistance)
	Mandawar, Haridwar	12.51(5.48-25.98)	0.238	Y=1.22+1.11x	12.64 (Moderate level of resistance)
	Bimora, Dakpathar, Dehradun	1.74(0.65-3.30)	0.949	Y=0.31+1.22x	1.75 (Susceptible)
	Lab Population	0.99(0.62-1.64)	0.342	Y=0.05+1.84x	-
Fipronil 5%SC	Mota Haldu (Nainital District)	88.51(46.34-180.19)	0.384	Y= 2.43+1.25x	19.08 (Moderate level of resistance)
	Bichai, Tanakpur, Champawat	214.22(108.44-757.29)	0.621	Y=2.51+1.08x	46.17 (High level of resistance)
	Mandawar, Haridwar	81.05(42.51-223.57)	1.668	Y=2.28+1.2x	17.47 (Moderate level of resistance)
	Bimora, Dakpathar, Dehradun	26.24(13.33-46.91)	0.157	Y=1.96+1.38x	5.66 (Low level of resistance)
	Lab Population	4.64(2.45-8.78)	0.391	Y=0.89+1.37x	
Chlorpyriphos 50%+ cypermethrin 5%EC	Mota Haldu (Nainital District)	64.92(28.85-122.82)	0.127	Y=2.21+1.22x	6.83 (Low level of resistance)
	Bichai, Tanakpur, Champawat	280.88(150.05-594.88)	0.586	Y=3.12+1.28x	29.57 (Moderate level of resistance)
	Mandawar, Haridwar	156.61(79.29-286.19)	0.517	Y=3.05+1.39x	16.49 (Moderate level of resistance)
	Bimora, Dakpathar, Dehradun	48.11(26.92-107.43)	0.654	Y=2.23+1.34x	5.06 (Decreased Susceptibility)
	Lab Population	9.50(5.48-18.27)	0.075	Y=1.41+1.45x	-
Profenophos 40%+ cypermethrin 4% EC	Mota Haldu (Nainital District)	243.63(142.06-492.79)	0.616	Y=3.64+1.52x	56.53 (High level of resistance)
	Bichai, Tanakpur, Champawat	35.69(19.16-94.62)	0.773	Y=1.9+1.24x	8.28 (Low level of resistance)
	Mandawar, Haridwar	228.29(123.47-529.69)	0.106	Y=2.98+1.46x	52.97 (High level of resistance)
	Bimora, Dakpathar, Dehradun	103.78(59.12-176.77)	0.442	Y=3.24+1.62x	24.08 (Moderate level of resistance)
	Lab Population	4.31(2.32-8.29)	0.440	Y=0.83+1.31x	

for Dehradun which showed decreased susceptibility. For both the profenophos 40%+cypermethrin 4%EC and chlorpyriphos 50%+cypermethrin 5%EC in which OP and pyrethroids are in a ratio of 1:10 a good level of resistance was observed. Also, chlorantraniliprole 18.5%SC showed a moderate to high level of resistance for almost all locations except for Dehradun which showed susceptibility and *S. litura* population tested with Indoxacarb 14.5%SC was found to be susceptible to almost all populations. It was revealed in the study that fipronil 5%SC showed resistance at almost all locations except Dehradun and the possible cause of such a different outcome in the Dehradun may be the possible absence of a potential host and the selection pressure which is otherwise different from other locations.

The current studies are similar to the studies of Ahmad and Mehmood (2015) where a moderate level of resistance was developed in population of Pakistan in a gap of 8 years and increased from 5.5-5.6 folds resistance in 1998 to 28-35 folds in 2006. Ahmad et al. (2008) also reported 224 folds resistance for fipronil 5%SC and this high level of resistance was explained as result of multiple resistance mechanism. The results of profenophos 40%+cypermethrin 4%EC and chlorpyriphos 50%+cypermethrin 5%EC in the study are in in accordance to the study of Ahmad (2009), El-Guindy et al. (1983), Goebel and Jacquemard (1990) and Joshi et al. (2023) suggested that profenophos and chlorpyriphos show antagonism with cypermethrin in 1:10 ratio. For chlorantraniliprole 18.5% SC, Muthusamy et al. (2014) reported an 80.07 resistance ratio to the susceptible population of NBAIR in insecticide resistance class 4, Che et al. (2023) and Wang et al. (2019) also reported 77.0-fold and 22.3-fold resistance, justifying the present result. Similar findings were reported by Saleem et al. (2016) for Indoxacarb 14.5% SC who found very low to high levels of resistance ranging from 7-87 folds. Babu and Singh (2023) also reported a low to moderate level of resistance ranging from 4-11.88 fold for *S. litura* population of Southern Rajasthan, for indoxacarb from 2017-2019.

Levels of detoxification enzymes were studied in different places and compared to susceptible populations of NBAIR, Bengaluru (Table 3). Mota Haldu, Nainital showed the highest specific activity for all the detoxification enzyme studied i.e., acetylcholinesterase (13.47± 1.24 nmol/ min/ ml enzyme), carboxylesterase (1.48± 0.02 µmoles 1-naphthol/min/mg protein),

Table 3. Specific activity of AChE, CarE, GST and P450 for *S. litura*

S.No.	Places	AChE (nmoles/ min/ ml of enzyme)		CarE (µmoles of 1-naphthol formed/ min/ mg of protein)		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	Specific activity	Ratio w.r.t susceptible population	Specific activity	Ratio w.r.t susceptible population
1	Mota Haldu Nainital	13.47± 1.24	168.38	5± 0.01	62.5	0.41± 0.08	16.4	1.48± 0.02	98.67
2	Bichai, Tanakpur, Champawat	5.115± 0.11	63.94	1.795± 0.05	22.44	0.215± 0.02	8.6	1.3± 0.09	86.67
3	Mandawar, Haridwar	2.355± 0.08	29.44	0.405± 0.11	5.06	0.095± 0.01	3.8	0.06± 0.01	4
4	Bimora, Dakpathar, Dehradun	3.28± 0.04	41	0.18± 0.01	2.25	0.07± 0.01	2.8	0.065± 0.02	4.33
5	Lab Population	1.0.01	1	1.0.01	1	1.0.01	1	0.015± 0.01	1
	CD	2.073		0.156		0.192		0.137	
	p value	<0.05		<0.05		<0.05		<0.05	

glutathione-S-transferase (5± 0.01 µmoles/ min/ mg protein) and monooxygenase P450 enzymes (0.41± 0.08 nmol/min/ml enzyme). It was observed in the studies that the resistance in all the insecticides could not be directly correlated with activity of any detoxification enzymes, however, resistance in chlorantraniliprole could be related with enhanced expression of GST, which has also been reported that detoxification by gluthione-S-transferase might be responsible for chlorantraniliprole resistance in *P. xylostella* (Eziah et al., 2008; Tang et al., 2011, Di et al., 2014). According to Hilliou et al. 2021, Nehare et al. 2010 and Muthusamy et al. 2013 MFO, esterase and GST are significant detoxification enzymes in the metabolism of old and novel insecticides in *S. litura.*

Populations of *S. litura* studied in different lowlands and doon valley regions of Uttarakhand demonstrated widespread spatial variation in insecticide resistance, making it a serious polyphagous pest. A basic knowledge of insecticide resistance is required since there are multiple mechanisms involved in insecticide resistance viz., excretion, sequestration, degradation of toxin, contact and ingestion avoidance and target site mutation (Despres et al., 2007). Different levels of detoxification enzyme production demonstrate the mechanism of insect homeostasis by changing enzyme levels in *S. litura* (Sreelakshmi et al., 2019). However, the resistance phenotypes may be the result of an overproduction of detoxification enzymes or due to an additive effect of multiple loci (ffrench-Constant et al., 2004; Shi et al., 2019). Thus, the detection of insecticide resistance by proper pest scouting and monitoring in the remotest areas is required for timely management of insecticide resistance. Resistance management-based strategies and timely, selective, and controlled use of insecticides in field could ensure successful management of *S. litura*.

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AUTHOR CONTRIBUTION STATEMENT

Dr Rashmi Joshi is the main author and did complete research with Sudha Mathpal in field study, under guidance and mentorship of Dr. Neeta Gaur.

COFLICT OF INTEREST

No conflict of interest.

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