

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Document heading

doi:10.12980/JCLM.2.2014B1026

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Geographic variation in cypermethrin insecticide resistance and morphometry in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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PEER REVIEW

Peer reviewer

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Comments

The authors have written the manuscript with standard language and the objective of paper covered with standard methodology. The study revealed significant results which will help the crop protection manufacturers to develop or improve pesticide formulation to manage resistant pest population.

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ABSTRACT

Objective: To find out the most resistant strain of *Spodoptera litura* (*S. litura*) from Tamil Nadu, Kerala and Karnataka by synthetic insecticide treatment.

Methods: Using leaf disc no-choice method, the insects were tested with different doses for pesticides. The LC₅₀ and LC₉₀ values were calculated by probit analysis.

Results: In the insect bioassay, the cypermethrin insecticide showed significantly higher LC₅₀ values of 14.699 g/L and 15.738 g/L against the Tamil Nadu and Kerala *S. litura* insect cultures respectively. The body length of 2nd, 3rd and 4th instar larvae were significantly higher ($P \leq 0.05$) in TTP insect population [(19.2±2.3) mm, (28.05±3.20) mm, (36.1±2.0) mm], when compared with KTK [(18.5±2.7) mm, (23.38±2.00) mm, (31.75±2.70) mm] and control, KBB [(15.65±2.30) mm, (23.65±2.70) mm, (33.2±2.2) mm] populations. The body breadth of 3rd instar larvae was significantly higher ($P \leq 0.05$) in TTP insect population (4.9±1.1) mm, when compared with KTK (3.93±0.80) mm and control, KBB (3.65±0.70) mm populations.

Conclusions: The present study clearly showed that field collected strains were highly resistant when compared to control. Based on our study, we conclude that reduced use of synthetic pesticides is necessary and IPM is a better way to reduce the development of pesticide resistance among strains of *S. litura*.

KEYWORDS

Cypermethrin, Geographic variation, Morphometry, *Spodoptera litura*, Insecticidal resistance

1. Introduction

Spodoptera litura (*S. litura*) (Fabricius) (Lepidoptera: Noctuidae) commonly known as tobacco caterpillar, devastates a large host range of more than 120 plants worldwide. In India, it is a major pest that attacks a wide variety of economically important crops such as cotton, groundnut, tomato, chilli, bhendi and green leaves tobacco, jute, lucerne, maize, rice, soybeans, tea, cauliflower, cabbage, capsicum, potato and castor[1,2]. Humans have been trying to control insect and other arthropod pests, plant pathogens weeds, rodents, and other vertebrate pests for thousands of years. However, in the last 50 years, significant progress has been made in controlling pests due to synthetic

pesticides. Pesticide research and development has brought a large number of chemicals in protecting the crop against insect pests. Pyrethroids are among the most commonly used synthetic insecticides worldwide, accounting for more than 30% of global use. Cypermethrin is a synthetic, pyrethroid insecticide that is extremely effective against a wide range of insect pests.

The evolution of pesticide resistance has been identified worldwide as the most serious threat to the development of sustainable integrated pest management practices[3]. Previous exposure and selection with insecticides can confer resistance to newly introduced insecticides through cross-resistance reducing the effectiveness of new insecticides[4]. Resistance to insecticides is a major problem associated

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Foundation Project: Supported by Tamil Nadu State Council for Science and Technology (Grant No. TNSCST/STU PROJ/AR/2011–2012/AS 10).

Article history:

Received 10 Jan 2014

Received in revised form 16 Jan, 2nd revised form 22 Jan, 3rd revised form 27 Jan 2014

Accepted 22 Feb 2014

Available online 28 Mar 2014

with the chemical control of insect pests. In India, synthetic pyrethroids have been used as dominant insecticides in army worm management programs. In India, in the early 1980s, mid–1990s and early 2000s, the pest populations in Andhra Pradesh and Tamil Nadu were highly resistant to synthetic pyrethroids, lindane, endosulfan, carbaryl and malathion[5,6]. In India, *S. litura* is the first agricultural pests to develop resistance against synthetic insecticides. The problem of development of resistance to insecticides is more acute, due to its polyphagous nature and rapid multiplication[5]. The variations in geographical location may result in alteration among phenotypic characters in any insect population[7]. The proper identification and recognition of diverse pest populations may create effective and cheap pest management methods[8]. Based on these reasons, the present study was undertaken to demonstrate the cypermethrin resistance and morphometric variations among *S. litura* collected from different geographical locations in India.

2. Materials and methods

2.1. Insects collection and culture

S. litura larvae were collected from groundnut fields at Vengatapuram (Tiruvallur district, Tamil Nadu), and from banana plantations at Moorkanikara (Thrissur district, Kerala) and the laboratory reared control larvae were received from National Bureau of Agriculturally Important Insects, Bangalore district, Karnataka. The larvae were reared on castor leaves and were kept till larvae attained pupal stage under laboratory conditions [(27±2) °C and (75±5)% relative humidity]. Sterilized soil was provided for pupation. After pupation, the pupae were collected from the soil and placed inside the cage for emergence of adults. Cotton soaked with 10% honey solution mixed with a few drops of multivitamins was provided for adult feeding to increase the fecundity. Potted groundnut plant was provided for inside the adult emergence cages, for egg laying. After hatching, the larvae were collected from the cage and tender castor leaf was provided[9]. The latitude and longitude of the collection sites were recorded using Juno SB Handheld global positioning system equipment. A study area map was constructed using the latitude and longitude of the locations by ArcGIS Desktop software version 9.0.

2.2. Morphometric analysis

The morphometric parameters *viz.* the length and breadth of larvae, pupae and adults were measured with the help of ordinary millimeter scale[10].

2.3. Insecticide

Commercial formulation of the insecticide SUNCYPER 25 emulsifiable concentrate (EC) (Cypermethrin, 250 g/L EC; Sun agro chemical industries, Madurai, India) was used in bioassays. The required concentrations (0 g/L, 4.5 g/L,

6 g/L, 7.5 g/L, 9 g/L, 12 g/L, 18 g/L, 24 g/L and 30 g/L, 36 g/L, 42 g/L) were prepared by serially diluting the formulation in distilled water.

2.4. Insect bioassay

Fresh, second instar larvae from laboratory cultures were exposed to cypermethrin insecticide using the leaf–dip no–choice method recommended by the Insecticide Resistance Action Committee (2009) accessing the larvicidal activity. Three and half centimeter castor (*Ricinus communis* L.) leaf discs were cut and dipped into the diluted formulations for 10 seconds with gentle agitation, then allowed to dry. Four larvae were released onto four leaf discs placed in a 9 cm–diameter Petri dish. Ten replicates ($N=40$) used for each concentration and 6–8 serial concentrations were used for the cypermethrin. The same number of leaf discs per treatment was dipped into distilled water used as control. The larvae were allowed to starve for 3 h. The larvae were maintained at a constant temperature of 25 °C with a photo–period of 14 h. Based on the percentage of mortality, the isolates were designated as resistant, moderately resistant and susceptible. Whenever needed, Abbot’s formula was used to correct mortality[11].

Based on the LC_{50} values of cypermethrin 25 EC against second instar larvae in the leaf–dip bioassays, the resistance of the two insect cultures were classified as low resistance (>1 to <1.5 fold control LC_{50}), moderate resistance (>1.5 to <2 fold control LC_{50}) and high resistance (>2 fold control LC_{50}) after comparing their LC_{50} with control insect culture.

2.5. Statistical analysis

Dosage–mortality and time–data were subjected to probit analysis as prescribed by Finney[12]. Analysis of variance was carried out by one way ANOVA. Significant differences between treatments were determined using Tukey’s multiple range tests ($P\leq 0.05$) using SPSS v.17.0.

3. Results

3.1. Study area

A survey was conducted in two different states *viz.*, Tamil Nadu, Kerala for incidence of *S. litura* during August 2011–February 2012. The latitude and longitude of the sample collection sites are summarised in Table 1. Base map of the study area showed three different collection sites in three different states *viz.*, Tamil Nadu, Kerala and Karnataka.

Table 1

Latitude and longitude of the insect collection sites.

No.	Location	Latitude and longitude
1	Vengatapuram village	13°06'28" N and 80°03'42" E
2	Moorkanikara village	10°32'10" N and 76°19'23" E
3	Bengaluru	13°01'36" N and 77°35'05" E

3.2. Insect

The insects collected from Tamil Nadu, Kerala and

Karnataka were named based on their places of collection as TTP, KTK and KBB respectively. The distances between the different locations are summarized in Table 2.

Table 2

Distances between the various insect collection sites.

No.	Location		Distance
1	Vengatapuram village	Moorkanikara village	~ 503 km
2	Moorkanikara village	Bengaluru	~ 632 km
3	Bengaluru	Vengatapuram village	~ 334 km

3.3. Morphometric analysis

3.3.1. Larvae

Table 3 shows the body length and breadth of larvae (1st instar, 2nd instar, 3rd instar and 4th instar), pupae and adults of *S. litura*. The maximum body length and breadth of (8.1±1.7) mm and (2.5±0.8) mm, (8.1±2.0) mm and (2.3±0.7) mm of the 1st instar larvae was observed in insect cultures TTP and KTK respectively. While a body length and breadth of (6.2±1.8) mm and (2.0±0.8) mm was observed in the control insect culture KBB (Table 3).

In the case of 2nd instar larva, body length and breadth of (19.2±2.3) mm and (3.3±1.0) mm were observed in insect culture TTP. The body length and breadth of (15.6±2.3) mm and (3.7±0.7) mm were observed in the control insect culture KBB. The body length and breadth of KTK were (18.5±2.7) mm and (3.5±1.0) mm respectively (Table 3).

Body length and breadth of (28.0±3.2) mm and (4.9±1.1) mm of the 3rd instar larvae were observed in insect culture TTP. While a body length and breadth of (23.3±2.0) mm and (3.9±0.8) mm were observed in the control insect culture KTK. KBB recorded a body length and breadth of (23.6±2.7) mm and (3.6±0.7) mm (Table 3).

In the 4th instar larvae, body length and breadth of (36.1±2.0) mm and (5.2±0.9) mm were observed in insect culture TTP. Body length and breadth of (33.2±2.2) mm and (4.9±0.9) mm were observed in the insect culture KBB. Body length and breadth of insect culture KTK was (31.7±2.7) mm and (5.2±0.4) mm respectively (Table 3).

3.3.2. Pupae

Lesser morphometric variations in body length and breadth were observed between the pupae of the three insect cultures. The body lengths and breadths of the pupae of the three insect cultures TTP, KTK, KBB were (16.1±1.9) mm and (4.0±0.7) mm, (16.4±1.0) mm and (3.7±0.4) mm, (16.5±1.3) mm and (4.3±0.4) mm respectively.

3.3.3. Adult

Body length and breadth of (34.9±2.2) mm and (15.3±1.6)

mm were observed in the adult of KTK insect culture. Body lengths and breadths of adults in insect cultures TTP and KBB were (30.6±9.8) mm and (14.5±1.4) mm, (33.9±2.5) mm and (15.1±1.6) mm respectively (Table 3).

3.4. Larvicidal activity

The results of the larvicidal activity of the cypermethrin against the 2nd instar larvae insect cultures of TTP and KTK are summarised in Table 4. Both the insect cultures TTP and KTK exhibited high resistance (>2 fold control LC₅₀) against cypermethrin when compared with control insect culture KBB. The LC₅₀ and LC₉₀ values of control were 6.991 g/L and 9.660 g/L respectively. The LC₅₀ and LC₉₀ values of insect culture TTP were 14.699 g/L and 23.978 g/L respectively. The LC₅₀ value of TTP was 2.10 fold greater when compared to control. Similarly the LC₅₀ value of KTK was 2.25 fold greater when compared to that of control. The LC₅₀ and LC₉₀ values of insect culture KTK were 15.738 g/L and 37.410 g/L respectively. These results clearly suggest that the two insect cultures TTP and KTK were very highly resistant against the insecticide cypermethrin when compared with control.

Table 4

Effective concentrations of LC₅₀-LC₉₀ and χ^2 -values of cypermethrin on *S. litura*.

Insect	LC ₅₀ (g/L)	95% confident limit		LC ₉₀ (g/L)	95% confident limit		Chi-square
		Lower	Upper		Lower	Upper	
TTP	14.699	8.507	17.284	23.798	21.136	31.100	0.808*
KTK	15.738	13.250	20.760	37.410	26.120	87.259	0.010*
KBB	6.991	6.205	7.779	10.944	9.660	13.124	2.364*

* χ^2 values were significant at $P \leq 0.05$.

4. Discussion

4.1. Morphometric analysis

The earlier reports on morphometric analysis of *S. litura* were very less. In the present study, the body length of 1st instar larvae of TTP and KTK insect populations were significantly higher when compared with 1st instar larvae of control culture KBB. The body lengths of 2nd, 3rd and 4th instar larvae were significantly higher in TTP insect population, when compared with KTK and KBB populations.

No significant difference was found among the body lengths of pupae of TTP, KTK and KBB insect populations. Similarly, no significant difference was found among the body lengths of adults of TTP, KTK and KBB insect populations.

Table 3

Morphometrics of the various larvae, pupae and adults collected from Tamil Nadu, Kerala and Karnataka.

No.	Insect	1st instar		2nd instar		3rd instar		4th instar		Pupae		Adult	
		Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
1	TTP	8.1±2.0*	2.3±0.7	19.2±2.3*	3.3±1.0	28.0±3.2*	4.9±1.1*	36.1±2.0*	5.2±0.9	16.1±1.9	4.0±0.7	30.6±9.8	14.5±1.4
2	KTK	8.1±1.7*	2.5±0.8	18.5±2.7*	3.5±1.0	23.3±2.0*	3.9±0.8*	31.7±2.7*	5.2±0.4	16.4±1.0	3.7±0.4	34.9±2.2	15.3±1.6
3	KBB	6.2±1.8*	2.0±0.8	15.6±2.3*	3.7±0.7	23.6±2.7*	3.6±0.7*	33.2±2.2*	4.9±0.9	16.5±1.3	4.3±0.4	33.9±2.5	15.1±1.6

Values are showed as mean±SD. * Values are significant at $P \leq 0.05$.

The body breadth of 3rd instar larvae was significantly higher in TTP insect population, when compared with KTK KBB populations. But the body breadth of 1st, 2nd and 4th instar larvae, pupae and adult of TTP, KTK and KBB insect populations were statistically similar.

The studies on morphometrics done by Thiti *et al.* on *S. litura* reared by feeding different cotton varieties *viz.*, CB9, CB10 and SR05 suggested that, no significant difference was found among the body length of 1st, 2nd, 3rd and 4th instar larvae, pupae and adult^[10]. Similarly, no statistical difference was found among the body breadth of 1st, 2nd, 3rd and 4th instar larvae and adult. But statistical difference was found in the body breadth of pupae.

The differences in plants external structural defenses may discourage insects feeding. Plant morphology has been shown to influence the biology of herbivores through feeding preferences^[13]. The optimal growth of plant feeding insects depends on their ability to acquire essential amino acids from dietary protein^[14]. Simt *et al.* reported that insects are able to grow and develop on a variety of host plant species which characteristic effect on larval growth and survival, pupal development, adult emergence, adult size and weight^[15]. In our study, the insects TTP and KTK had been collected from groundnut fields and banana plantations respectively, whereas KBB insect cultures were reared in castor (*Ricinus communis*) leaves. The differences in the external morphology and nutritive value (*viz.*, essential amino acids) of the plants would have played a major role in variations in body lengths and breadths of larvae, pupae and adult of TTP, KTK and KBB insect cultures of *S. litura*.

4.2. Insect bioassay

S. litura has developed resistance against a variety of commercially available insecticides belonging to almost all the insecticide groups even against new chemistry insecticides like lufenuron^[6,16].

In this study, the resistance by TTP and KTK *S. litura* insect cultures against the insecticide cypermethrin was significantly high ($P \leq 0.05$) when compared with control KBB insect culture. Similarly, Kranthi *et al.* reported high levels of resistance against cypermethrin insecticide when tested with *S. litura*, strains from both North and South India^[17]. Resistance levels to cypermethrin were high, at 45–148 folds, in the majority of the Andhra Pradesh strains. The Central Indian strain from Mahbubnagar exhibited the highest resistance to cypermethrin. Armes *et al.* reported resistance levels between 0.2 to 197 fold to cypermethrin in *S. litura* strains collected from Andhra Pradesh^[18].

The extensive use of insecticides also plays a major role in development of resistance. In Pakistan, *S. litura* has developed high resistance to conventional and new chemistry insecticides, due to their extensive use^[19].

The development of insecticide resistance is primarily a result of the selection pressure exerted on sprayed population increasing the frequency of resistant individuals. The proportion of the total population sprayed determines the extent to which insecticide resistance may evolve in an area^[20]. Untreated areas may act as refugia providing

susceptible or at least less resistant individuals, which have the potential to dilute insecticide resistance after breeding with resistant individuals, resistant gene frequencies remaining at an acceptable level for successful control^[21]. Moreover, resistant individuals positively selected in sprayed areas are often counter selected in those refugia due to fitness cost associated with the resistance mechanism.

Exposure of *S. litura* to various groups of insecticides throughout the year may also be involved in rapid evolution of resistance to newer insecticides. The development of a broad-spectrum resistance to insecticides has complicated its chemical control. This could be a main obstacle to formulating an integrated pest management programs. Light- or pheromone-traps or prevailing meteorological conditions may help in better timing of control operations. Hand picking of egg masses or small larvae, as has been practiced in Egypt, is feasible at least on a small scale. Birds very much like its last instars, so bird activity should be encouraged. Preservation of its parasitoids such as braconids, encyrtidae, tachinids and ichneumonids is necessary to reduce pesticidal applications. Slow-release pheromone formulations have shown success for mating disruption. Pheromones have also been used for mass trapping by the lure and kill technique^[22]. Strategy of stacking double genes against *Spodoptera* sp., *Helicoverpa* sp. and other bollworm complex holds promise in managing lepidopteran pests of cotton and other crops in future.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Tamil Nadu State Council for Science and Technology for encouraging and funding this project (Grant No. TNSCST/STU PROJ/AR/2011–2012/AS 10) and also thank the Director, NBAII, Bangalore, for providing the insect cultures of *S. litura* for the research work.

Comments

Background

Over the past century, crop protection has relied heavily on synthetic chemical pesticides, but hundreds of insect species have developed resistance to one or more control measures, severely impacting the economics of crop production. Availability of pest control is now declining as a result of new legislation and the evolution of resistance in pest populations. The evaluation of developing resistance in insect is an ongoing concern to crop protection manufacturers. With this background, the present study aimed to find out the most resistant strain of *S. litura* from different geographic locations by using synthetic insecticide treatment.

Research frontiers

The study deals with assessing pesticide resistance in pest population. The study reveals the effect of cypermethrin on morphometric changes of the different stages of the larvae. The manuscript presents useful finding which will be greatly helpful to crop protection manufacturers who develop new synthetic insecticide for the sustainable agriculture.

Related reports

The study showed that it was conducted based on the previous reports of resistant pest against synthetic pyrethroids, lindane, endosulfan, carbaryl and malathion. The manuscript's results and methodology have been discussed with suitable reports which are up to date.

Innovations and breakthroughs

In India, *S. litura* is one of the serious pests that cause severe yield and economic loss in agricultural sector. The authors have chosen different geographic location and compared the resistance level of the pest population. Among the larvae used in the present study, the field collected larvae were highly resistant to cypermethrin when compared to control. The study seems to be a novel finding for pest resistance management.

Applications

The paper is essential, like a bench-mark report for good agricultural practices. The study could be a tool to the pesticide manufacturing companies to improve the formulation.

Peer review

The authors have written the manuscript with standard language and the objective of paper covered with standard methodology. The study revealed significant results which will help the crop protection manufacturers to develop or improve pesticide formulation to manage resistant pest population.

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