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Larvicidal toxicity of various insecticides against Tobacco caterpillar, *Spodoptera litura*, Fabricius (Lepidoptera: Noctuidae)

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Abstract
The present study was envisaged to check the toxic effect of certain insecticides (chlorpyriphos, spinosad, novaluron, acetamiprid and thyme oil) on third instar larvae of *Spodoptera litura* by using three different bioassays i.e. topical treatment, leaf dip and artificial diet method at different concentrations *viz*., 5, 25,125 and 625 ppm. Observations on larval mortality were recorded from each treatment at 24, 48 and 72 hours intervals. Spinosad was found to be most toxic on the basis of larval mortality followed by chlorpyriphos, novaluron, thyme oil and acetamiprid in topical bioassay. But in leaf dip bioassay, thyme oil at 5 ppm caused 100 percent larval mortality. Acetamiprid proved as poor insecticide against this pest causing 26.66, 16.66 and 10 percent mortality after 72 hours of treatment in topical, leaf dip, and artificial diet bioassay at highest concentration, respectively. In artificial diet method, highest mortality percent was recorded in chlorpyriphos and spinosad i.e. 86.66 percent after 48 and 72 hours of treatment, respectively.

Keywords: Insecticides, *Spodoptera litura*, spinosad, chlorpyriphos, novaluron

Introduction
The tobacco caterpillar, *Spodoptera litura* Fabricius is ubiquitous, polyphagous, multivoltine, lepidopterous insect pest of agricultural crops in the Asian tropics [23]. It is also known as cluster or tobacco caterpillar, common cutworm, cotton leaf worm, grey streaked moth and tropical armyworm [24]. It is widely distributed throughout tropical and temperate Asia, Australasia and the Pacific Islands [21, 25]. Due to its migration ability over long distances and high reproductive capacity, *S. litura* has an enormous potential to invade new and wide range of ecological niches. More than 389 species belonging to 109 families of economically important plants including corn, peanut, soybean, castor, sunflower, mash, moong, eggplant, tobacco, cabbage and so on are host of this pest, making it one of the most damaging agricultural pests in Asian countries [31, 3]. The host plant range for *S. litura* can vary due to their higher level of feeding on almost all parts of plants and on different species of plants. Based on the crop stage and its infestation level in the field, it causes economic losses ranging from 25.8 to 100% [5].

From the insect control point of view, various strategies were implemented such as use of insecticides, chemosterilants, cultural and environmental practices, growing resistant varieties, pheromone-based tactics (mating disruption, lure and kill, mass trapping, attractants) and genetic control methods etc. [17]. Use of insecticides for controlling major insect pest was the most efficient and inexpensive way during past years. These chemicals are used primarily to control disease-carrying insects or to control pests that infest plants in specific areas. Complete reliance control on various insecticides (chemical control) of this pest was due to higher multiplication rate of *S.litura*.

Synthetic organic insecticides are now the leading agents in insect control having different mode of actions. The organophosphates (OPs) work by inhibiting acetylcholinesterase of the nervous system which results in the accumulation of acetylcholine (ACh) at the neuromuscular junctions or synapses, causing disruption of nervous activity [14]. Neonicotinoids are the synthetic versions of nicotine and interact with nicotinic acetylcholine receptors (nAChR) at the central and peripheral nervous systems, resulting in excitation and paralysis and finally death of the organism [44].

Among alternative to the conventional insecticides, some novel insecticides are there like Spinosad, IGR and botanicals. Spinosad is a bacterial formulation which is a mixture of the two most active naturally occurring metabolites (spinosyns A and D) produced by *Saccharopolyspora spinosa* [41]. It is having primary target binding site nAchRs and secondary binding site as GABA [37] causing excitation of the insect nervous system, further leads to involuntary muscle contractions, prostration with tremors and paralysis. IGRs are new class of bio-rational compounds for insect pest control. These compounds cause abnormal morphogenesis of insect's integument by imitating juvenile hormone and moulting hormone or by interfering with chitin synthesis [28]. Extracts derived from plants are currently one of the most promising methods [29]. Plants are great sources of natural substances possessing pesticidal properties that can be used in the development of environmentally safe methods for insect control [33]. Therefore, in the present study we investigated the toxicity of different insecticides against 3rd instar larvae of *S.litura.*

Materials and Methods

The egg masses of *S.litura* and larvae were collected and reared in the Insect culture laboratory of Plant Protection Division, PG Department of Agriculture, Khalsa College Amritsar. The larvae of S. *litura* were reared in the laboratory on castor (*Ricinus communis*) leaves in sterilized glass jars (20cm x 15cm) at 25±2 ºC and 65±5% RH in B.O.D incubator [26]. The leaves were used after sterilization by sodium hypochlorite solution and air dried. The freshly laid egg batches were placed on these leaves. Eggs hatched into larval instars which when fully grown starts to pupate. Then the pupae were transferred to glass jars containing thick layer of moist sterilized sand covered with filter paper. The moths so emerged were collected and transferred to clean jars wrapped with filter paper from inside and containing suspended cotton swabs soaked with 10% honey solution.

Insecticides Tested- Following insecticides were tested for their efficacy against 3rd instar larvae of *S. litura*-

Class of Insecticides	Name of Insecticides	Trade name
Organophosphate (OPs)	Chlorpyriphos	Aldrin 20 EC
Neonicotinoid	Acetamiprid	HEME 20 SP
Spinosyn	Spinosad	Tracer 45 SC
IGR	Novaluron	Rimon 10 EC
Botanical	Thyme oil	Allin exporters

Four concentrations of each insecticide i.e. 5, 25, 125 and 625 ppm were used. Untreated larvae were included as a control to assess the natural mortality rates of the test insect species. The different bioassay methods *viz*. topical bioassay, leaf dip bioassay and artificial diet bioassay were used to evaluate the toxicity of these insecticides against larvae of *S. litura.*

Topical method-

The toxicity of selected chemicals was tested using micropipette. 3rd instar larvae were placed in petri dish and placed in the spray tower and sprayed with the insecticides at different concentrations mentioned before. In control, no treatment was given to larvae. After application, the larvae were allowed to dry for 10 minutes and transferred to artificial diet in rearing containers individually. Each treatment was replicated three times with 10 larvae/replication. The observations were recorded after 24, 48 and 72 hours on larval mortality.

Leaf Dip Bioassay

Fresh castor leaves were washed, sterilized and air dried. Afterwards, the leaves of uniform size (approx. 6 cm in size) were dipped for 10 minutes in different insecticidal concentrations. Leaves were again air dried and placed in petri plates having moist cotton swab so as to avoid desiccation of leaves. The 3rd instar larvae were released on the treated leaves subsequently. Each treatment was replicated three times with 10 larvae/replication. The mortality was recorded after 24, 48 and 72 hours of treatment for each insecticidal treatment at each concentration.

Artificial Diet Bioassay

The artificial diet was prepared as per methodology given by [19] and was supplemented with different concentrations of each insecticide. Diet without insecticide served as control. The 3rd instar larvae from culture maintained on artificial diet were selected and fed on treated as well as untreated diet. Each treatment was replicated thrice with 10 larvae/ replication. The mortality count was taken after 24, 48 and 72 hours of treatment.

Statistical analysis

The larval mortality was analyzed by using ANOVA at different intervals to find out the significant difference if any in the observations obtained from different treatments at $p<0.05$ for variance.

Results

Toxicity of test insecticides against *S. litura* using topical bioassay

The larvicidal toxicity of different insecticides using topical bioassay is presented in (Table-1). The toxicities of chlorpyriphos and spinosad were higher than the other insecticides (novaluron, acetamiprid and thyme oil). It is evident from the results that as the time interval increased, the mortality also increased and maximum mortality was observed after 72 hours of treatment in all concentrations. Highest mortality (80 percent) was observed at 625 ppm after 72 hours of treatment in spinosad and 73.33 percent when treated with chlorpyriphos and as compared to control, significant results in treated larvae was observed at highest concentrations of 125 and 625 ppm after 24 and 48 hour of treatment in both chlorpyriphos and spinosad whereas all the treatments found to be significant after 72 hours of treatment. In treatment with novaluron, at every concentration and interval, significant results were obtained with highest percent mortality of 63.33 percent after 72 hours of treatment. In the case of acetamiprid, only highest concentration was significant at 24 hours after treatment and at 125 and 625 ppm was found to be significant after 48 and 72 hours of treatment. Lastly in thyme oil, at highest concentration, 60 percent mortality was recorded also compared to control and results were significant at all concentration after 72 hours of treatment.

Table 1: Larvicidal toxicity of different insecticides against *S. litura* (3rd instar larvae) under laboratory conditions by topical application bioassay.

Insecticide used	Control	5ppm	25ppm	125ppm	625ppm	CD (0.05%)
Chlorpyriphos						
24 HAT	0^{c}	6.66^{bc}±3.33	6.66^{bc}±3.33	10^{ab}±0	16.66^{a}±3.33	0.941
48 HAT	0^{b}	10^{ab}±5.77	13.33^{ab}±3.33	20^{a}±5.77	23.33^{a}±3.33	1.458
72 HAT	0^{d}	26.66^{c}±3.33	33.33^{bc}±3.33	53.33^{ab}±8.81	73.33^{a}±14.52	2.319
Spinosad						
24 HAT	0^{b}	13.33^{b}±3.33	13.33^{b}±3.33	33.33^{a}±8.81	46.66^{a}±3.33	1.594
48 HAT	0^{c}	26.66^{bc}±6.66	36.66^{b}±6.66	46.66^{b}±12.01	76.66^{a}±14.52	2.707
72 HAT	0^{c}	43.33^{b}±8.81	50^{ab}±5.77	60^{ab}±20.81	80^{a}±11.54	3.556
Novaluron						
24 HAT	0^{b}	13.33^{a}±3.33	16.66^{a}±3.33	16.66^{a}±3.33	23.33^{a}±3.33	1.002
48 HAT	0^{d}	16.66^{c}±13.33	26.66^{bc}±3.33	30^{b}±5.77	46.66^{a}±8.81	1.114
72 HAT	0^{d}	23.33^{c}±3.33	36.66^{b}±3.33	46.66^{b}±3.33	63.33^{a}±8.81	1.309
Acetamiprid						
24 HAT	0^{b}	0^{b}	3.33^{b}±3.33	6.66^{b}±3.33	20^{a}±5.77	1.140
48 HAT	0^{c}	3.33^{bc}±3.33	6.66^{bc}±3.33	13.33^{b}±3.33	26.66^{a}±3.33	1.002
72 HAT	0^{c}	3.33^{c}±3.33	6.66^{c}±3.33	16.66^{b}±3.33	26.66^{a}±3.33	0.876
Thyme oil						
24 HAT	0^{c}	3.33^{bc}±3.33	13.33^{ab}±3.33	20^{a}±5.77	16.66^{a}±3.33	1.309
48 HAT	0^{d}	13.33^{cd}±3.33	26.66^{bc}±6.66	30^{b}±5.77	46.66^{a}±3.33	1.498
72 HAT	0^{d}	23.33^{c}±3.33	40^{bc}±5.77	46.66^{ab}±8.81	60^{a}±5.77	1.702

All values are given as Mean±SE, CD-Critical Difference, HAT-Hours after treatment
Variables (^{a,b,c}..) significantly differ from each other at 5% level of Significance.

Toxicity of insecticides against *S.litura* using leaf dip bioassay

Third instar larvae of *S.litura* were fed on castor leaves dipped in insecticides at different concentrations. Spinosad at higher concentrations and thyme oil even at lowest concentration of 5 ppm gave complete kill of the larvae (Table 2). Also mortality of 90 and 83.34 percent was reported after 72 hours of treatment with chlorpyriphos and novaluron, respectively. Significant results with control were recorded at all concentrations after 24, 48 and 72 hours after treatment with chlorpyriphos whereas in novaluron only after 48 and 72 hours of treatment results were proved to be significant at all concentrations. However acetamiprid showed very minimal toxicity via this method as no mortality was observed after 24 hours of exposure at all concentrations and at lower concentrations (5 and 25 ppm) even after 48 hours of treatment. Thyme oil after 24 hours of exposure showed larval mortality of 50 percent at highest concentration of 625 ppm and then further to 100 percent after 48 and 72 hours of treatment.

Table 2: Larvicidal toxicity of different insecticides against *S.litura* (3rd instar larvae) under laboratory conditions by leaf dip bioassay.

Insecticide used	Control	5ppm	25ppm	125ppm	625ppm	CD (0.05%)
Chlorpyriphos						
24 HAT	0^{d}	10^{c}±0	23.33^{b}±3.33	16.66^{bc}±3.33	33.33^{a}±3.33	0.909
48 HAT	0^{e}	16.66^{d}±3.3	33.33^{c}±3.33	53.33^{b}±8.81	80^{a}±5.77	1.666
72 HAT	0^{d}	33.33^{c}±6.66	40^{c}±5.77	63.33^{b}±3.33	90^{a}±5.77	1.458
Spinosad						
24 HAT	0^{d}	0^{d}	56.66^{c}±3.33	80^{b}±5.77	100^{a}	0.876
48 HAT	0^{d}	53.33^{c}±3.33	73.33^{b}±3.33	100	100	0.729
72 HAT	0^{d}	63.33^{c}±3.33	83.33^{b}±3.33	100	100	0.729
Novaluron						
24 HAT	0	0	0	0	0	--
48 HAT	0^{b}	56.66^{a}±3.33	43.33^{a}±17.63	43.33^{a}±18.55	66.66^{a}±13.33	3.514
72 HAT	0^{c}	56.66^{b}±3.33	60^{b}±5.77	70^{ab}±5.77	83.33^{a}±3.33	1.353
Acetamiprid						
24 HAT	0	0	0	0	0	--
48 HAT	0	0	0	3.33±3.33	6.66±3.33	--
72 HAT	0^{b}	0^{b}	3.33^{b}±3.33	6.66^{b}±3.33	16.66^{a}±3.33	0.909
Thyme oil						
24 HAT	0^{d}	0^{d}	13.33^{c}±3.33	36.66^{b}±3.33	50^{a}±5.77	0.876
48 HAT	0	100	100	100	100	--
72 HAT	0	100	100	100	100	--

All values are given as Mean±SE, CD-Critical Difference, HAT-Hours after treatment
Variables (^{a,b,c}..) significantly differ from each other at 5% level of Significance.

Toxicity of insecticides when fed via artificial diet bioassay to *S.litura*

The mortality data of different chemicals at different concentrations given through artificial diet incorporation method are presented in (Table-3). Data showed significant dose-dependent mortality. In chlorpyriphos and spinosad, significant results were found after 24 hours of treatment at highest concentration whereas in chlorpyriphos, spinosad and

novaluron significant results was seen at 125 and 625 ppm after 72 hours of treatment. Chlorpyriphos and spinosad @ 625 ppm was significantly superior to all the treatment with highest mortality (86.66 percent) followed by novaluron in which up to 80 percent mortality was recorded (72 HAT). In acetamiprid and thyme oil, mortality of only 10 and 13.33 percent was noted at highest concentration after 72 hours of treatment, respectively.

Table 3: Larvicidal toxicity of different insecticides against *S.litura* (3rd instar larvae) under laboratory conditions by artificial diet bioassay.

Insecticide used	Control	5ppm	25ppm	125ppm	625ppm	CD (0.05%)
Chlorpyriphos						
24 HAT	0	0	3.33±3.33	13.33±8.81	66.66±6.66	1.458
48 HAT	0	3.33±3.33	10±0	20±5.77	86.66±6.66	1.479
72 HAT	0	16.66±6.66	26.66±16.66	40±10	86.66±6.66	3.342
Spinosad						
24 HAT	0^{b}	0^{b}	3.33^{b}±3.33	3.33^{b}±3.33	10^{A}±0	0.595
48 HAT	0^{b}	0^{b}	6.66^{b}±3.33	20^{ab}±5.77	36.66^{a}±12.01	2.228
72 HAT	0^{c}	40^{bc}±10	36.66^{bc}±17.63	43.33^{b}±18.55	86.66^{a}±13.33	4.328
Novaluron						
24 HAT	0	0	0	6.66±3.33	6.66±3.33	--
48 HAT	0^{c}	3.33^{c}±3.33	6.66^{c}±3.33	36.66^{b}±12.01	63.33^{a}±8.81	2.293
72 HAT	0	13.33^{c}±3.33	16.66^{c}±3.33	43.33^{b}±14.52	80^{a}±0	2.293
Acetamiprid						
24 HAT	0	0	0	0	0	--
48 HAT	0	0	0	3.33±3.33	3.33±3.33	--
72 HAT	0	0	3.33±3.33	6.66±3.33	10±5.77	--
Thyme oil						
24 HAT	0	0	0	0	0	--
48 HAT	0	0	0	3.33±3.33	3.33±3.33	--
72 HAT	0^{b}	0^{b}	0^{b}	3.33^{b}±3.33	13.33^{a}±3.33	0.595

All values are given as Mean±SE, CD-Critical Difference, HAT-Hours after treatment
Variables (^{a,b,c}..) significantly differ from each other at 5% level of Significance.

Discussion

Chlorpyriphos was proved to be toxic in the current study.As chlorpyriphos belongs to OPs, it acts by inhibiting the activity cholinesterase enzyme. When Ahmad *et al.* [4] checked the toxicities of cholinesterase inhibitors, chlorpyriphos showed high toxicity. Overstimulation of the nervous system leads to the insect's death. Toxicity of this insecticide on *Spodoptera* spp. has been reported by several workers [22, 34, 39]. In the field conditions also, Bhadane *et al.* [8] reported upto 70 percent mortality of *S. litura* larvae after 5 days of spray. High toxicity may be due to the low level of resistance against chlorpyriphos as Ahmad and Arif [2] also reported very low level of resistance in another species of *Spodoptera* (*S.exigua*) against chlorpyriphos. Using leaf dip method, mortality of 90 percent was seen when treated with this chemical. Similar results were reported by Saini *et al.* [36] when they observed 90 percent mortality in larvae fed on treated after 48 hours of treatment.

Spinosad was also proved to be highly toxic insecticide during this experimentation. Larvae treated with spinosad showed full mortality after 48 hours of treatment. Santis *et al.* [38] tested efficacy of spinosad against *S. exigua* and concluded spinosad as most toxic to the 3rd instar larvae. In current study, spinosad is more toxic than chlorpyriphos is in agreement with Natikar and Balikai [27] who also observed that spinosad is more toxic than the chlorpyriphos. High larval mortality may be due to the susceptibility of *S. litura* larvae to this insecticide. This is confirmed by Stanley *et al.* [43] who reported the high toxicity, high susceptibility and no resistance for emamectin benzoate and spinosad against *S. litura.* Also, no cross-resistance of spinosad was noticed when Rehan and Shoaib [32] tested spinosad with few other insecticides. This implies that spinosad can be used in IPM Programmes.

The main reason for the effectiveness of IGRs is that they disturb the development and metamorphosis of an insect. Application of novaluron to *S.litura* larvae gave higher mortality in leaf dip bioassay then all other bioassays in this study. Also when Tallikoti *et al.* [45] evaluated the toxicity of IGR's, highest toxicity was shown by novaluron after 72 hours of treatment. High mortality was also observed by Dhawan *et al.* [10] using topical application method with LC_{50} value of 0.002%. In topical treatment at 625 ppm, mortality of 63.33 percent was observed. Similar results were scored by Shaila *et al.* [40] at 600 ppm where up to 70 percent mortality was reported. Toxicity of novaluron to some extent is in agreement with reported toxic effects of novaluron on other insects, such as on mosquito, *Culex pipiens* [11], beetles, *Tribolium castaneum* [18], *Leptinotarsa decemlineata* [9] and Moth, *Palpita unionalis* [13]. There has been increase in the mortality of the larvae as time increases and these results are in agreement with Barrania [7] who also observed increase in mortality of *S. littoralis* larvae with increase in time. Toxicity of novaluron is slightly low than spinosad and chlorpyriphos. The results regarding this chemical were also comparable to Sahar *et al.* [35] who reported the order of effectiveness (LC_{10}, LC_{25} and LC_{50}) against third instar larvae of *S.litura* was chlorfluazuron> chlorpyriphos > novaluron > λ- cyhalothrin.

Acetamiprid showed low level of toxicity in the current study. Same results were scored by Ahmed [6] who studied toxicity of some neonicotinoids and found that acetamiprid and thiamethoxam showed lowest level of toxicities. Also when El-Sheikh *et al.* [12] tested efficiency of some neonicotinoids, Acetamiprid was found as least toxic. However, this study is in contradiction with Srivastava *et al.* [42] who observed the order of toxicity as flubendiamide> acetamipirid> spinosad> lufenuron> propaconazole> carbendazim> azoxystrobion> mancozeb> ridomil.

Insects are showing resistance to various synthetic insecticides, thus natural products and plant extracts used as

herbal pesticides seem to resolve the insecticidal resistance development and environmental problems caused by the use of synthetic pesticides. Thyme oil was found toxic in topical and leaf dip bioassays in the current study and seems to be non- toxic in artificial diet bioassay. Jiang *et al.* [16] reported that *T. vulgaris* and *Syzygium aromaticum* was the most active in contact and residual toxicity bioassays against *Trichoplusia ni* (Semilooper). Similar results were reported by Hummelbrunner and Isman [15] and Koul *et al.* [20] when they tested plant essential oils through topical application with highest toxicity was being observed with thyme oil. Also, Pavela [30] tested antifeedant and larvicidal effects of some phenolic components of essential oils against *S. littoralis* in which thymol was most effective. Among thyme, neem and bitter oil, Abdel-Aziz *et al.* [1] found thyme oil as most toxic against 2nd instar larvae of *S. littoralis*.

The results clearly indicated that these chemical insecticides showed different level of toxicity against 3rd instar larvae of *S.litura* in all the bioassays. The botanical (thyme oil) had also shown great results in leaf dip bioassay. Thus, further research is required to find more botanicals which show greater mortality rate in *S.litura*. Moreover, check their insecticidal properties and resistance studies of these eco-friendly insecticides so that they can be efficiently incorporated in IPM programmes.

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