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Evaluation of lethal response of biorational insecticides against *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract
The lethal effects of biorational insecticides (methoxyfenozide (Runner 240 SC), spinosad (Tracer® 480 SC), emamectin benzoate (Logo 2.15%EC), indoxacarb (Avaunt15.8% EC) and lufenuron (Match 5% EC) as well one Control treatment against different life stages of *Spodoptera litura* were examined by Leaf Dip Bioassay method. The least possible mortality was caused by lufenuron (Match 5% EC) (18.4%) while highest mortality level was observed in (methoxyfenozide (Runner 240 SC) treatment (25%), so the highly toxic insecticide against the *Spodoptera litura* is methoxyfenozide at its higher concentration after 48 hours. The insecticides with moderate toxicity were spinosad (Tracer® 480 SC), indoxacarb (Avaunt15.8% EC) and emamectin benzoate (Logo 2.15%EC). Order of insecticides toxicity on the basis of mortality was methoxyfenozide > spinosad > indoxacarb > emamectin > lufenuron.

Keywords: Lethal response, Biorational insecticides, Toxicity, *Spodoptera litura*

Introduction
Pakistan is fundamentally an agricultural country and in Pakistan economy it is significant shareholder. GDP Support by agriculture sector is nearly 25% and the total labour force that is openly involved with agriculture is about 45% [7]. For the growth of Pakistan, significance of agriculture sector can be supervised from the facts that the foreign exchange earned through marketed exports is nearly 43% of the total exports and all the small and major agro based industries like sugar and cotton are directly dependent on agriculture sector [6]. In case of cotton crop 20-40% loss is just because of insect pests attack annually [5]. *Spodoptera exigua* and *Helicoverpa armigera* are significant polyphagous pests in the tropical and subtropical regions of cultivated crops [10]. Army worm (*Spodoptera litura)* belongs to order Lepidoptera and family Noctuidae. *S. litura* is famous leaf feeding insect pest of more than 100 plants around the Asia-Pacific region. According to host plant survey by three different areas in the cotton belt exposed 27 different plant species as host of *S, litura* which belongs to 25 genera of 14 families including cultivated crops, weeds, vegetables, fruits and ornamental plants. Mostly its population and damage found on *Gossypium hirsutum* L., *Brassica oleracea var*. *botrytis* L., *Colocasia esculenta* L., *Ricinus communis* L., *Trianthema portulacastrum* L. and *Sesbania sesban* L [4]. Because of significant migration capability makes it to population increase over the universe [2]. In Pakistan its migration and resistance is comparatively low as compare to universe [19]. It can attack wheat, peas, rice and other cruciferous vegetables but cotton and cabbage are two major hosts of *Spodoptera (litura and exigua)* in Pakistan [32]. Resistance in *S. exigua* is caused by many factors but of them the important factor is the excessive use of insecticides over a longer period of time so that's why it has become resistance to organophosphates, chlorinated hydrocarbons, carbamates, benzoylphenylureas and pyrethroides [11, 9, 34]. Methoxyfenozide is new chemistry insecticide, the latest and most persuasive member of the moult-accelerating compounds (MACs) against Lepidoptera [30]. MACs directly binding to the same natural hormone receptors stimulates the molting hormone receptor and cause an anticipated lethal moult [12]. Earlier studies findings about methoxyfenozide revealed that topical or oral administration of this biorational insecticide can cause long term toxic effects to the adults of the target pest and a negative effect on the fertility and fecundity of many Lepidoptera and Coleoptera pest species [15, 22, 31, 33, 25, 26]. Biorational control agents, based on naturally derived compounds that disturb the Physiological

Functions of insects have attracted specific attention. Several new chemistries with unique modes of action spinosad and methoxyfenozide are useful for *S. litura* control [26]. Spinosad and methoxyfenozide can use as an important pest control option for integrated pest management (IPM) because of their low eco-toxicological effects and short time persistence in the environment [8]. The main objective of the study was to find out the best bio rational insecticide against *Spodoptera litura*.

Materials and Methods

The adults of *S. litura* were collected from different fields of cabbage and cotton crop from Ayub Agriculture Research Institute, Faisalabad (AARI) in the month of March-July 2015. Collected larvae were reared on the leaves of Cabbage and Cotton plant. After successfully pupation, emerged adults were reared in cages on artificially prepared diet (Sugar solution) containing essential nutrients and by keeping in view the food and flight requirements of adults.

Insect and Insecticides

2nd larval instar of F_2 generation were used in the experiment under Completely Randomized Design (CRD). Five biorational insecticides (methoxyfenozide (Runner 240 SC), Spinosad (Tracer® 480 SC), Emamectin benzoate (Logo 2.15%EC), Indoxacarb (Avaunt15.8% EC) and lufenuron (Match 5% EC) along with various concentrations and water as a control treatment were applied on *S. litura* larvae by using leaf dip bioassay method [23] to study the lethal and sub-lethal effects. Different lethal and sub-lethal field concentrations of the insecticides were used for the experiment under standard constant environment conditions (27± 2°C, 65 ± 5 RH and L: D 16:8 h).

Leaf Dip Bioassay Method

Fresh cotton leaves were collected from the fields and washed under available tap water. Leaf disks were prepared of 2 inches of diameter with the help of disk cutter, then disks dipped in the prepared insecticides concentration (5 leaves per concentration per insecticide) for 9-10 seconds and then air dry. Disk leaves for controlled treatment were dipped on in tap water.

Data Analysis

Leaves were observed to check the feeding behaviour on treated as well controlled experiment, in addition, adults and larvae were examined to check the sub-lethal effects of insecticides after 3, 6, 12, 24, 48, 72 and 144 hours of treatments. Abbott's formula [1] was used to correct the mortality on untreated leaves where it was necessary. Data was analysed by using Probit Analysis two-way analysis of the variance (ANOVA) [16].

Evaluation of lethal effects

In lethal effects mortality of 2nd instar larvae was observed at 3, 6, 12, 24 and 48 hours after exposure for each insecticide individually.

Result and Discussion

(Table. 1) Scored data regarding the percentage mortality of *Spodoptera Litura* larvae was subjected to statistical software and mentioned results was observed. Methoxyfenozide showed minimum mortality 9% against minimum concentration of 1500ppm after 3 hours of insecticide application and maximum mortality 25% against maximum concentration of 24000ppm after 24 hours of insecticide application. Spinosad shows 8.2% and 23.8% after 3 and 24 hours of insecticide application at 960ppm and 15360ppm respectively. After 3 hours of insecticide application 8% mortality were recorded at 656.25ppm and 22.3% mortality after 24 hours at 10500ppm concentration of indoxacarb. Emamectin benzoate shows minimum 5.8% mortality at 9.5ppm and 20.2% mortality at 152ppm after 3 and 24 hours of insecticide application correspondingly while only 4.4% mortality after 3 hours of insecticide application was observed at 250ppm concentration of lufenuron and maximum 18.4% mortality against 4000ppm after 24 hours. Overall mean mortality response of each insecticide at its minimum and maximum concentration is given in fig. 1.

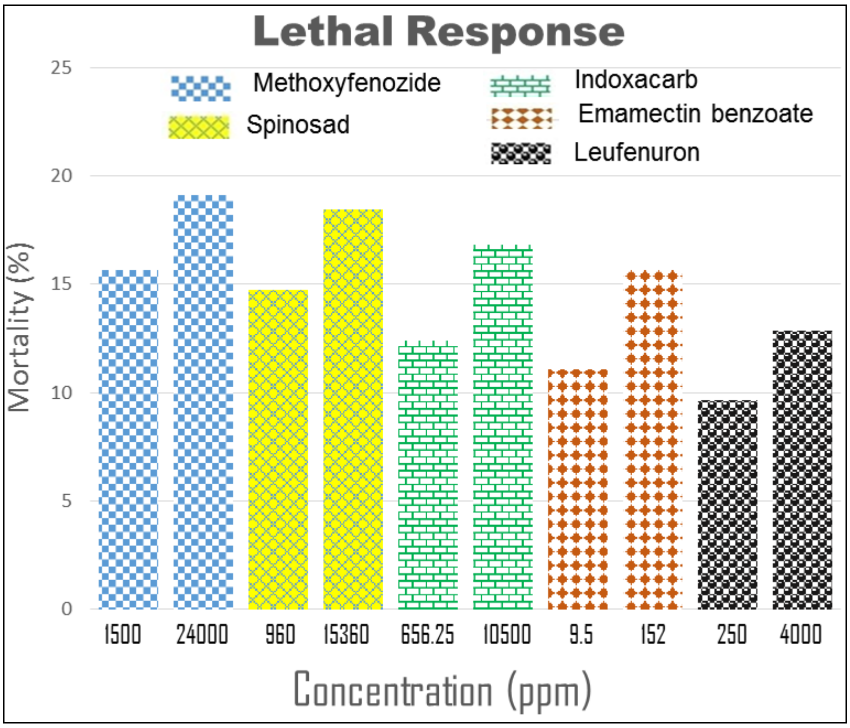

Fig 1: Minimum and maximum percentage mortality response of different insecticides at their lowest (after 3 hours) and highest (after 24 hours) and concentrations.

The conventional insecticides are being operational on large scale to control large no. of insect pests. It is measured that the use of insecticides is effective and swift control method for insect pest but they have also negative influence on the environment and along with harmful insect's theses insecticides also kill the non-target beneficial insects. So that's why its need of today to use bio rational and target specific insecticides to use against targeted harmful insect pests' control.

Insecticides in experiment were (methoxyfenozide (Runner 240 SC), spinosad (Tracer® 480 SC), emamectin benzoate (Logo 2.15%EC), indoxacarb (Avaunt15.8% EC) and lufenuron (Match 5% EC).

Mortality of *Spodoptera litura* larvae caused by the applied insecticides was checked after 3, 6, 12, 24 and 48 hours. In case of lethal treatments LC_{50} values were calculated along with larval mortality percentage, slope $\pm$standard error and fiducial limits (Upper and lower) against *S. litura* larvae, Toxicity of insecticides and mortality of larvae raised with the rise in treatment time interval and concentration of insecticides.

The most toxic biorational insecticide is (methoxyfenozide (Runner 240 SC) at all-time intervals and concentrations against larvae of *Spodoptera litura*. spinosad (Tracer® 480 SC) and indoxacarb (Avaunt15.8% EC) can also be considered as toxic insecticides again *S. litura* because their mortality percentages near about the mortality percentage of most toxic (methoxyfenozide (Runner 240 SC). Maximum mortality caused by the (methoxyfenozide (Runner 240 SC) is 25%, 24%, 20.2%, 14.8% and 11.6% at its maximum concentration of 24000 ppm against 3, 6, 12, 24 and 48 hours respectively. Moderate toxic insecticides which caused less mortality as compare to (methoxyfenozide (Runner 240 SC) are spinosad (Tracer® 480 SC) and indoxacarb (Avaunt15.8% EC). Mortality caused by spinosad (Tracer® 480 SC) is 23.8%, 22%, 20.2%, 15.6% and 10.6% against different time intervals of 3, 6, 12, 24 and 48 hours respectively, at its maximum concentration of 15360 ppm., whereas, indoxacarb (Avaunt15.8% EC) caused 22.2%, 20%, 17.2%, 14.4% and 10.4% mortality against different time intervals of 3, 6, 12, 24 and 48 hours respectively, at its maximum concentration of 10500 ppm. Minimum mortality is caused by emamectin benzoate (Logo 2.15%EC) 20.2%, 18.8%, 17%, 14.2% and 8.2% against different time intervals of 3, 6, 12, 24 and 48 hours respectively, at its maximum concentration of 152 ppm and lufenuron (Match 5% EC) 18.8%, 16.4%, 13%, 9.2% and 7.2% against different time intervals of 3, 6, 12, 24 and 48 hours respectively, at its maximum concentration of 4000 ppm.

Methoxyfenozide (Runner 240 SC) caused maximum mortality as compare to all other insecticides applied on *Spodoptera litura* 2nd instar larvae. These results resembled to the findings of Ahmad *et al.*, (2005) who study the toxicity of new chemistry insecticides against the *Spodoptera litura* 2nd instar larvae by using three different concentrations of each insecticide at laboratory conditions and scored their LC_{50}values, their results reveal that the maximum mortality scored by emamectin benzoate followed by lufenuron, spinosad, abamectin and indoxacarb. Same results shown by Munir *et al.*, (2005) evaluated the toxicity of new chemistry insecticides included emamectin benzoate, lufenuron, spinosad, abamectin and indoxacarb against 2nd instar larvae of armyworm at laboratory conditions.

Spinosad (Tracer® 480 SC) proved as most toxic insecticide against *Spodoptera litura* larvae with LC_{50} 19.53 before indoxacarb (Avaunt 15.8% EC) and after methoxyfenozide (Runner 240 SC) with 21.85 and 16.04, whereas these results inverse with the finding of Saini *et al.* (2005) tested the different insecticides included thiodicarb, chlorpyriphos, endosulfan, indoxacarb, profenophos, spinosad, cypermethrin, deltamethrin and Neem extract against tobacco caterpillar, *Spodoptera litura* in laboratory and concluded that spinosad is poor insecticide as control agent against *S. litura* while indoxacarb gives 73.3% mortality at 72 hours. Same results scored by Hilal and Gurkan (2006) collected cotton leafworm, *S. litura* from commercial cotton production fields and tested to check the susceptibility for spinosad and concluded that field strains are 4.4 folds less sensitive to spinosad as compare to susceptible strains. Santis *et al.*, (2012)examined the toxicity of spinosad against different larval instars of *Spodoptera exigua* and concluded that spinosad is most toxic to the 3rd instar larvae of *S. exigua* as wellrate of mortality was increase rapidly even after only 3 hours of application the scored mortality % is 71-95.

Indoxacarb (Avaunt 15.8% EC) with LC_{50} value of 16.04 gives moderate mortality against *S. litura* and same results coated by Ahmad *et al.* (2008) conducted an insecticide resistance experiment against some newer insecticides to check the mechanism of resistance and insecticide resistance strength against *Spodoptera litura.* and observed 3-95-fold resistance for indoxacarb followed by 4-186 fold, 2-77 fold, 13-224 fold 2-66 fold, 8-56 fold and 2-153 fold for abamectin, emamectin, fipronil, lufenuron, diflubenuron and methoxyfenozide respectively. While current research findings were correlated with the results of Gmail *et al.* [17] concluded as 2nd instar larvae of *S. litura* is more susceptible to indoxacarb as compare to 4th instar larvae.

Emamectin benzoate (Logo 2.15%EC) caused minimum mortality of *S. litura* larvae with LC_{50} value 15.95 as compared to methoxyfenozide (Runner 240 SC), spinosad (Tracer® 480 SC) and indoxacarb (Avaunt 15.8% EC), while, greater than lufenuron (Match 5% EC) with LC_{50}21.85, 19.53, 16.04 and 12.01, respectively. These results contradict with the finding of Gupta *et al.*, (2004) who examined the toxicity of conventional and certain novel insecticides against the 5 day old larvae of *S. litura* to check their susceptibility. LC_{50} results reveal that the maximum mortality is caused by the emamectin benzoate (6.93) followed by fenvalerate, indoxacarb, cypermethrin, abamectin, quinalphos, bifenthrin, spinosad, endosulfan and betacyfluthrin with LC_{50} values 1.83, 1.63, 1.00, 0.95, 0.68, 0.52, 0.45, 0.29 and 0.24 respectively. As well Khan *et al.* (2011) coated their findings that emamectin benzoate is most toxic insecticide with 100% mortality of *S. litura* larvae. El-Sheikh (2015) reveals that emamectin benzoate is the most robust insecticide with chronic LC_{90} values of 0.31.

lufenuron (Match 5% EC) with LC_{50} value of 12.01 observed as poor insecticide against *Spodoptera litura* caused minimum mortality of 2nd instar larvae but the results are highly contradicted with the research work of El-Sheikh and Aamir (2011) tested the field persistence and toxicity effects of 3 IGRs lufenuron, flufenoxuron and triflumuron against 2nd and 4th larval instars of *Spodoptera littoralis* in the laboratory. As result they had concluded that lufenuron was the most effective mortality agent on both 2nd and 4th larval instars, as well they have coated that lufenuron kills both larval instars rapidly as compare to flufenoxuron or triflumuron.

Table 1: Percentage mortality (means ± SE) of *Spodoptera litura* against different insecticide'sconcentration

Treatment	Insecticides	Dose (ppm)	Mortality (%) (means ± SE)
T1	methoxyfenozide (Runner 240 SC)	1500	10.20±0.42
T2	methoxyfenozide (Runner 240 SC)	3000	13.04±0.72
T3	methoxyfenozide (Runner 240 SC)	6000	18.92±0.46
T4	methoxyfenozide (Runner 240 SC)	12000	21.6±0.83
T5	methoxyfenozide (Runner 240 SC)	24000	23±0.71
T6	spinosad (Tracer® 480 SC)	960	9.52±0.39
T7	spinosad (Tracer® 480 SC)	1920	14.56±0.45
T8	spinosad (Tracer® 480 SC)	3840	18.24±0.87
T9	spinosad (Tracer® 480 SC)	7680	20.12±0.77
T10	spinosad (Tracer® 480 SC)	15360	21.72±0.78
T11	emamectin benzoate (Logo 2.15%EC)	656.25	7±0.42
T12	emamectin benzoate (Logo 2.15%EC)	1312.5	11.36±1.06
T13	emamectin benzoate (Logo 2.15%EC)	2625	14.56±0.91
T14	emamectin benzoate (Logo 2.15%EC)	5250	16.32±1.00
T15	emamectin benzoate (Logo 2.15%EC)	10500	18.16±0.81
T16	indoxacarb (Avaunt 15.8% EC	9.5	8.96±0.53
T17	indoxacarb (Avaunt 15.8% EC	19	12.16±0.87
T18	indoxacarb (Avaunt 15.8% EC	38	15.28±0.83
T19	indoxacarb (Avaunt 15.8% EC	76	17.64±0.91
T20	indoxacarb (Avaunt 15.8% EC	152	19.44±1.05
T21	lufenuron (Match 5% EC	250	5.68±0.48
T22	lufenuron (Match 5% EC	500	7.76±0.48
T23	lufenuron (Match 5% EC	1000	10.8±0.69
T24	lufenuron (Match 5% EC	2000	14.64±0.52
T25	lufenuron (Match 5% EC	4000	16.84±0.65
T26	Control	---	----

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