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Dose-Response relationship of some insecticides with *Helicoverpa armigera* hübner (Lepidoptera; Noctuidae) under laboratory conditions

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Abstract
Dose response of some insecticides against *H. armigera* were checked under laboratory conditions, following the guidelines recommended by the Insecticide Resistance Action Committee (IRAC). Five different concentrations were used i.e. Field Recommended Concentration (FRC), quarter, half, double and quadruple of the FRC of all the insecticides viz, emamectin benzoate (Emamectin benzoate® 1.9 EC), lufenuron (Match® 50EC), flubendiamide (Belt 480® SC), spinosad (Tracer® 240 SC), indoxacarb (Steward® 150 EC), deltaphos (Deltaphos® 36 EC), thiodicarb (Larvin® 80 DF) and a botanical product (Neem oil) against 2nd instar *H. armigera* larvae. Results showed that emamectin benzoate is most potent, followed by flubendiamide, lufenuron, spinosad, indoxacarb and neem oil. Spinosad, closely followed by indoxacarb and flubendiamide were the quickest in exerting their lethal effect on the larvae. The pest expressed mild level of tolerance against deltaphos and thiodicarb with LC50 and LT50 values much higher than their respective FRCs and the rest of insecticides.

Keywords: *Helicoverpa armigera;* Insecticide' sefficacy; Insecticide Resistance; New Chemistry Insecticides.

1. Introduction
Helicoverpa armigera is considered as one of the major biotic constraints faced bytoday's intensive agriculture [1]. Its wide dissemination and pest status has been attributed to its polyphagy and its ability to undergo both facultative diapause and seasonal migration [2]. The species is migratory and a key pest on all continents [3], with minimum of 200 reported hosts [4]. Manjunath [5] reported 180 plant species from 45 different families as hosts of *H. armigera* from India. In Pakistan, the pest has been recorded attacking 65 plant species [6], including a number of important crops like cotton, tobacco, tomato, gram, grain, sorghum, maize, lucerne seed, sunflower, wheat and pea [7, 8]. The common name of the pest changes with the host it attacks; therefore it is also attributed as cotton bollworm, tobacco budworm, gram pod borer, tomato fruit worm and corn earworm.
H. armigera has received a great attention due to its status as a serious pest of anumber of important crops. The use of insecticides has been the mainstay for the control of this pest. Almost 30 percent of the total world insecticides are used against *H. armigera*; thereby it is under intense selection pressure with a range of insecticides [9]. Indiscriminate application of pesticides during 1980s and 1990s has contributed a lot in heavy outbreaks of *H. armigera* [10, 11]. There are now several pesticide resistant biotypes active in various cropping systems worldwide and in Pakistan. The insect has developed substantial and often uncontrollable levels of resistance to all major groups of insecticides (organochlorines, organophosphates, carbamates and pyrethroids) directed against it worldwide [9, 12-16].
The present study was therefore, planned to determine the efficacy and dose-response relationship of some new insecticides in comparison with those having a history of widespread use against the pest.

2. Materials and Methods
2.1 Insect Rearing: The experiment was conducted in year 2008.Prior to start, disinfection of all the related materials was assured by using locally available detergents in the laboratory for both insect rearing and bioassys with 27 + 2 ^{0}C temperature,60+5% relative humidty and 14:10 L:D period. The moths were collected from tobacco and tomato growing areas of Mardan,

Swabi and Peshawar, and. were brought to the laboratory on the same day and kept in cages (L: 18 cm, W: 10 cm, H: 12 cm). made up of wooden frame and glass walls and having a removable top and an opening at the front covered with muslin cloth. Diet (sucrose solution with the addition of vitamins and methyl-4- hydroxybenzoate) was provided in soaked cotton swabs to feed adult moths [17]. A piece of nappy liner (8x8 cm) was hung inside the cage in order to collect eggs laid by the female moths. The eggs were collected with a soft camel-hair brush or a sharp razor daily and kept for incubation in petri dishes (8.5 cm dia, 1.5 cm depth) and labeled with the date. The hatched larvae were reared singly in plastic cups (0.15L volume) and fed with a semi-synthetic diet modified by Ahmad [21] from Shorey [22], consisting of chickpea flour (300 g), ascorbic acid (4.7 g), methyl-4-hydroxybenzoate (3 g), sorbic acid (1.5 g), streptomycin(1.5 g), corn oil (12 ml), yeast (48 g), agar (17 g) and vitamin mixture (10 ml). Yeast and agar were dissolved in 800 ml of boiling water and added to the other constituents premixed in 500 ml of water. The cups also offered sterilized soil for pupation and were covered with organdie cloth. On emergence, the moths were collected and shifted to the above-mentioned wooden chambers for mating and oviposition.

2.2 Insecticides: The tested insecticides (Table 1) were obtained from local authorized dealers and stored in a refrigerator in the laboratory. Based on the contradictory information obtained from the cited literature regarding thiodicarb efficacy against the pest, we decided to include it in our studies. We also deemed it appropriate to induct deltaphos (a combination of organophosphate and pyrethroid), as it was reported efficacious against the pest by Martin [20] and Gunning [21]. Rest of the test insecticides were relatively modern and locally used by the farmers against *H. armigera* in various crops. A control treatment (distilled water) was included in the test to assess the natural mortality of the insects. Stock solutions of formulated insecticides were prepared in distilled water according to their respective doses (Table 2).

Table 1: List of insecticides with their common names, trade names, chemical class, mammalian toxicity and dose per hectare.

Common Name	Trade name	Chemical class	Acute oral LD50- male Rat (mg kg^{-1})	Dose ha^{-1}
Emamectin	Emamectin ® 1.9EC	Avermectins	76-89	494ml
Lufenuron	Match ® 50 EC	Acyl Urea	2000	494ml
Flubendiamide	Belt ® 480 SC	Diamide	>2000	49.4ml
Spinosad	Tracer ® 240 SC	Spynosyns	3783	250ml
Indoxacarb	Steward ® 150 EC	Oxidizines	1732	375ml
Deltaphos	Deltaphos ® 36 EC	Pyrethroid+ Organophosphate	500	1482-1976ml
Thiodicarb	Larvin ® 80 DF	Carbamate	66	741g
Neem oil	-	Botanical insecticides	Non-toxic	4940ml

Table 2: List of insecticide Active ingredient (A. i.) names, quantities in formulations (g L^{-1} or g Kg^{-1}) and recommended concentrations(mg L^{-1} or mlL^{-1}) used in the experiment.

Name and Quantity of A. i. (g L^{-1} or g Kg^{-1}	A.i(gha^{-1})	R.C*.A.i.(mg L^{-1}ormlL^{-1})	Qrt**. R.C. A.i. (mg L^{-1}ormlL^{-1})	Hlf***. R.C. A.i.(mg L^{-1}ormlL^{-1})	Dbl****. R.C. A.i. (mg L^{-1}ormlL^{-1})	Qdr*****.R.C A.i.(mg L^{-1}ormlL^{-1})
Emamectin benzoate 1.9g L^{-1}	0.95	3.2	0.8	1.6	6.4	12.8
Lufenuron 50 g L^{-1}	25	83.3	20.82	41.65	166.6	333.2
Flubendiamide 480 g_L-1	24	80.0	20.0	40.0	160.0	320.0
Spinosad 240g L^{-1}	36	120.0	30.0	60.0	240.0	480.0
Indoxacarb 150g L^{-1}	56	186.7	46.67	93.35	373.4	746.8
Deltamethrin+Triazophos10g+350g L^{-1}	450	1500.0	375.0	750.0	3000.0	6000.0
Thiodicarb800g kg^{-1}	592	1973.3	493.32	986.65	3946.6	7893.2
Neem oil		16.46	4.12	8.23	32.93	65.86

* Recommended Concentration
** Quarter of the RC
*** Half of the RC
**** Double of the RC
***** Quadruple of the RC

2.3 Test Procedure: Five different concentrations, i.e. field recommended, quarter, half, double and quadruple of the recommended concentrations were tested against newlymoulted 2nd instar larvae of *H. armigera*. A cohort of test insects was obtained from the stock culture and exposed to different insecticides using the leaf-dip technique as recommended by the IRAC [22]. Tomato (Roma Variety) leaves were collected from the unsprayed field and washed up with tap water. Five-centimeter diameter tomato leaf discs were cut and dipped into the test solutions for 10 seconds with gentle agitation, allowed to dry on blotting paper at room temperature and then placed in a 5-cm-diameter glass petri dish covered with organdiecloth and tied with a rubber band. Moistened filter papers were placed beneath the leaf discs to avoid desiccation of leaves in the petri dishes. The experiment was carried out in completely randomized design (CRD), repeated 25 times and each petri dish was allotted with the help of moist end camel hair brush a single larva to avoid cannibalism. The same number of leaf discs per treatment was dipped into distilled water as untreated check. The test units were kept in a controlled environment as mentioned above for rearing of the insects.

2.4 Data Collection: The insects were examined for mortality, 12, 24, 36, 48 and 72hours after release. Larvae were considered dead if they failed to respond to stimulation by touch. Toxicity of the insecticides was judged on the basis of Lethal Concentration (LC50) and Lethal Time (LT50).

2.5 Statistical Analysis: The data were analyzed through Probit analysis using SPSS v.16. for calculation of the Lethal Concentration (LC50) and Lethal Time (LT50) [23].

3. Results and Discussion

It is evident from the data (Table 3) regarding the LC50 values of the insecticides for the second instar *H. armigera* larvae that the insecticides with novel modes of action recordedLC50 values at less than half of the Field Recommended Concentration (FRC).Neem oil though a botanical product, also recorded slightly lesser LC50 value than the FRC. It could be concluded from the results that the test insect demonstrated moderate level of resistance against the conventional insecticides i.e. deltaphos and thiodicarb with LC50 values much higher than the FRCs. Spinosad was closely followed by indoxacarb and flubendiamide in expressing their swiftness in exerting its lethal effects against the larvae at MFRC. The CSI (lufenuron) and emamectin benzoate though efficacious, yet proved much slower in expressing their potency against the larvae. Neem oil exhibited maximum reaction time of all the candidate insecticides.

Results regarding the toxicity of insecticides in terms of LC and LT (50s) (Table 3 and 4 respectively) revealed that emamectin benzoate recorded minimum LC50 value (1.650 mg a.i L^{-1}) but a relatively longer lethal time (LT50> 57hrs). The chemical was reported efficacious against the lepidopterous pests in general by Vijaykumar [24] at relatively higher rates of 28-56 mg a.i L^{-1}. Similarly, Brevault [25] evaluated emamectin for its initial and residual activity against *H. armigera* by transferring larvae on leaf discs collected from sprayed plots. Emamectin-benzoate (33.33 mg a.i L^{-1}) was recorded to have caused a high level (99.3± 0.8%) of the 2nd instar larval mortality. While, Hirooka [26] calculated a much lower LC50 value (0.049 mg a.i. L^{-1}) for emamectin against a laboratory reared susceptible strain of the pest. Concurring opinions were also reported by Gupta [37] who evaluated the toxicity of different insecticides against the 2nd instar *H. armigera* larvae under laboratory conditions and concluded that emamectin benzoate was more toxic than indoxacarb and spinosad. Supporting observations regarding avermectin toxicity to the larval stage of the pest were also reported by Wang [28]. The relatively longer time taken by emamectin at FRC to kill the target insects could be attributed to the fact that the larvae ceased feeding within a few hours after their exposure to the chemical as also reported by Vijaykumar [24]. The ingestion of the chemical is also halted with feeding cessation, thereby, resulting in the slowing down of the poisoning process and death of the affected insect is ultimately brought about by the combination of poisoning and starvation. This conclusion is supported by the fact that double and quadruple doses of the chemical caused 96% mortality of the test insects within 48 hrs post exposure.

Our results regarding lufenuron toxicity (Lethal time) against the test insect are in conformity with that of Emmanuel [29] who reported that first and second instar larval stages exposed to treated surfaces recorded high mortality. He further observed that the larval mortality occurred during or after moulting. We are also in agreement with the author regarding the poisoning symptoms of the CSI as nearly all the dead or dying larvae had black shriveled bodies, ruptured exoskeleton and failed to shed the old larval skin. Similar poisoning symptoms were also reported by Butter [30] after examining the toxic effects of lufenuron on different larval instars of *H. armigera.* We differ with the authors regarding the lethal concentration as they calculated LC90 value of the insecticide at 7.89 mg a.i L^{-1} for the 2nd instar larvae which is approximately five and ten times lower than the LC50 we recorded and the RFC respectively. Concurring opinions regarding growth-regulatory insecticides were also recorded by Bueno and Freitas [31] and Sechser [32] for *Chrysoperla externa* and Chrysoperla carnea larvae respectively.

Our observations vis-à-vis flubendiamide efficacy (LC50 36.5 mg a.i L^{-1}) differ with Hirooka [26] who conducted a series of laboratory and extended laboratory experiments and recorded much lower EC50 (0.24 mg a.i. L^{-1}) and LC50 (0.079 mg a.i. L^{-1}) values for the chemical against 3rd instar *H. armigera* larvae. While our observations regarding the fast acting efficacy of the chemical as compared to emamectin benzoate are in concurrence with that of the aforementioned authors. The difference in the lethal concentration values could be attributed to the fact that Hirooka [26] used laboratory reared susceptible strain of the insect.

We observed spinosad to be highly toxic to 2nd instar *H. armigera* larvae as it recorded an LC50 value of 57.304 mg a.i L^{-1} which is lower than the half of the FRC (120 mg a.i. L^{-1}). A relatively inferior performance of the compound was reported by Ramasubramanian [15] who reported 77.5-80% mortality of pyrethroid resistant *H. armigera* at the recommended dose (200 mg a.i L^{-1}) under laboratory conditions. Higher toxicity levels were determined by Nirmal [36] (LC50 value: 0.4 mg a.i L^{-1}) and Hirooka [26] (LC50 value: 2.1 mg a.i. L^{-1}) for the chemical against third instar *H. armigera* larvae, following the method we used in our experiment. Highly escalated toxicity level was also reported by Dastjerdi [34] with LC50 values of, 0.13 and 0.2 mg a.i L^{-1}, in dietary and leaf disc methods respectively. We are somewhat in agreement with the authors regarding the time to mortality (24 h) of the test insects. The comparatively lower LC50 values and almost equal time to mortality could be attributed to the fact that they used 1st instar *H. armigera* larvae as compared to the 2nd instar in our case which are relatively tolerant than the former. Similarly, Ahmed [35] found spinosad (1 ppm) toxic to 2nd instar *H. armigera* larvae under laboratory conditions. The observed variation in spinosad toxicity to *H. armigera* could be attributed to the fact that the pest has been reported to have developed variable level of resistance against the chemical [36, 37].

As observed in case of the aforementioned insecticides, indoxacarb recorded an L C50 value (91.87 mg a.i L^{-1}) slightly lesser than half the recommended concentration (187mg a.i L^{-1}). Our observations are in accordance with that of Ramasubramanian [38] who observed more than 70% and 92.5% *H. armigera* larval mortality at the rate of 33.33 mg a.i L^{-1} (10% of the recommended dose) and 333.3 mg a.i L^{-1} (recommended dose) respectively. Indoxacarb efficacy has also been reported by workers like Brevault [25] who evaluated the insecticide for its activity against different larval instars of *H. armigera* in laboratory by exposing the test insects to leaf discs collected from sprayed plots. Indoxacarb had high initial activity against *H. armigera*, regardless of larval instars but was more effective on large than on small larvae; Indicating that the higher quantity of food ingested by large larvae may have increased their exposure to the insecticide as it is mostly active by ingestion [39]. Much lower LC 50 (0.25 mg a.i. L^{-1}) was recorded against a laboratory reared susceptible strain of *H. armigera* by Hirooka [26]. The larvicidal properties of indoxacarb have been confirmed against other lepidopteran pests as well, as Liu [40] revealed that indoxacarb was highly toxic to the larvae of *Trichoplusiani*.

The conventional insecticide deltaphos appeared relatively nontoxic to the test insect as the recorded LC50 value (9035.21 mg a.i L^{-1}) is approximately six times higher than the FRCs (1500 mg a.i L^{-1}). The inefficacy of the chemical is also reflected in the much higher LT50 value (124 hrs) as compared to the much shorter time recorded by the new chemistry insecticides. A number of workers have confirmed *H. armigera* resistance against organophosphates and pyrethroids when used separately. But a few studiesfound the combination of both thegroups highly effective against the pest leading to the subsequent development of a commercial formulation. This mixture is marked in Pakistan by the name of Deltaphos 36 EC (deltamethrin 10,000 mg + 350,000 mg triazophos L^{-1}). Deltaphos was initially effective against the pest in tomato and tobacco but gradually lost its efficacy like its parent compounds within a few years of its widespread use (personal communication with tomato and tobacco growing farmers in three districts of Khyber Pakhtunkhwa i.e. Peshawar, Mardan and Swabi). No citable literature could be gathered regarding the efficacy of this product against the subject pest except for one study Khattak [41] who recorded an intermediate effect of the insecticide on the damage and incidence of cotton bollworms in cotton.

The carbamate insecticide (thiodicarb) was also found as highly inefficacious against the pest as it recorded an LC50 value of 8843.1 mg L^{-1} which is 4.5 times higher than the FRC (1973 mg L^{-1}). The lethal time (157 hrs) observed for the compound was much longer than that of the rest of the test insecticides including deltaphos. Our findings regarding thiodicarb resistance in *H. armigera* are in conformity with that of Gunning [42] Ahmad [43] Armes [44] and Dastjerdi [34] who reported it from Australia, Pakistan, India and Iran respectively. Low level of thiodicarb resistance was reported by Ahmad [45], despite of the fact that the same study reported high levels of *H. armigera* resistance against some other carbamates. Contrary to our observations, thiodicarb was reported efficacious against the pest when used together with *Bt.* under laboratory conditions Khalique [46] and alone under field conditions Brickle [47].

Our results showed that neem oil recorded almost similar LC50 value (15.27 ml L^{-1}) as the recommended concentration (16. 5ml L^{-1}). The lethal time (70 hrs) was though much longer than spinosad, indoxacarb and flubendiamide and slightly longer than emamectin and lufenuron yet it was found much quicker than deltaphos and thiodicarb. The longer lethal time could be justified due to the fact that neem is known to exert phagodetterance and growth regulatory effects on the lepidopterans including *H. armigera* Schmutter [48] thereby; killing its target insects through starvation and growth inhibition slowly, like the IGRs. Similar observations were reported by Ma [49] after conducting a detailed study on the survival, development and feeding responses of *H. armigera* on neem leaflets. Neem leaflets provided to starving fourth-instar larvae were expectorated; weight of surviving larvae decreased and could not complete development on neem leaflets alone. Larvae resumed feeding when transferred to cotton leaves after 5 days of feeding on neem leaflets. Fourth instars strongly discriminated between neem leaflets and cotton leaves when offered a choice. Early sixth instars decreased in weight and had delayed development when fed only on neem leaflets. More than one-half lived for more than 2 weeks and some completed development to adult stage.

Table 3: Toxicity of different insecticides against 2nd instar *H. armigera* larvae under laboratory conditions expressed in terms of Lethal Concentration (LC50).

Treatments	Slope ± SE	LC_{50}(mg/L or ml/L)	95% confidence limits		Chi-square (P-Value)
			LL*	UL**	
Emamectinbenzoate	7.887±4.085	1.650	NA***	NA	0.758(0.685)
Lufenuron	7.276±3.646	41.232	0.016	55.490	0.677(0.713)
Flubendiamide	4.816±2.169	36.506	0.473	52.860	0.022(0.989)
Spinosad	6.621±2.771	57.403	21.079	73.431	0.910(0.635)
Indoxacarb	8.610±4.215	91.872	14.992	115.176	0.131(0.937)
Deltaphos	1.341±0.602	9035.215	2394.807	175428.752	1.702(0.427)
Thiodicarb	1.382±0.876	8843.111	NA	NA	0.285(.867)
Neem oil	0.035±0.006	24.561	5.422	62.335	12.283(0.015)

* Lower limit
** Upper limit
*** Not available

Table 4: Toxicity of different insecticides against 2nd instar *H. armigera* larvae under laboratory conditions expressed in terms of Lethal Time (LT50)

Treatments	Slope ± SE	LT50(hrs)	95% confidence limits		Chi-square(P-value)
			LL	UL	
Emamectin benzoate	0.077±0.025	57.44	42.26	66.73	2.195 (0.533)
Lufenuron	0.110±0.026	59.76	52.69	64.02	0.372 (0.946)
Flubendiamide	0.047±0.07	33.11	NA*	NA	13.357(0.004)
Spinosad	0.033±0.009	15.06	NA	NA	6.674(0.083)
Indoxacarb	0.30±0.009	19.23	-54.844	45.433	4.984(0.173)
Deltaphos	0.025±0.015	124.025	NA	NA	3.167(0.367)
Thiodicarb	0.018±0.022	157.177	NA	NA	2.744(0.433)
Neem oil	0.046±0.011	70.393	60.377	83.154	2.409(.492)

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