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Time Trends in Mortality for Conventional and New Insecticides Against Leaf Worm, *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract: To determine time trends in mortality for various insecticides, which are being used against cotton pests, the fourth instar larvae of *Spodoptera litura* was collected from Muzaffar Garh and tested for pyrethroids, organophosphate and new chemistry insecticides. The efficacy of the insecticides was examined by time-oriented mortality at LC_{50}, through leaf-dip bioassays in the laboratory. In sodium channel agonists, endosulfan was the most efficient insecticide. The cholinesterase inhibitors tested, chlorpyrifos showed high efficiency while phoxim performed better in time-oriented mortality. Emamectin benzoate proved to be the most efficient insecticide in new chemistry insecticides tested. Spinosad and indoxacarb had almost similar LC_{50} and LT_{50} values. The least effective insecticide found was abamectin. The results are discussed in relation to Integrated Pest Management (IPM).

Key words: *Spodoptera litura*, lethal time, lethal concentration, organochlorine, organophosphates, carbamates, pyrethroids and new chemistry insecticides

INTRODUCTION

Insecticides have widely been used for more than 50 years. Although the goal of insecticides application is to kill all target pests, however survivors are common as target species develop resistance to particular compounds (Armes *et al*., 1997; Byrne and Toscano, 2001; Kranthi *et al*., 2002; Wei *et al*., 2004). In response to control failures, growers have habitually switched to newly registered insecticides, which have generally provided effective control. In the past decade, however, the availability of new insecticides has become increasingly difficult to predict and, coupled with product withdrawals, cancellations and regulation based on toxicological or environmental standards, it has become important to retain the efficacy of existing materials.

Several methods have been used to define time trends in insects after exposure to insecticides. For example, Stringer *et al.* (1964) defined the delayed toxicity of bait toxicants for *Solenopsis invicta* as <15% mortality at 24 h and >89% at the end of the experiment. Using Weibull function, Dell *et al.* (1983) define time trends to a changed physiological state (e.g., ecdysis) of the proportion of an insect population. Using this technique, Haverty and Dell (1984) estimated the time required to achieve 90% mortality for fast acting compound at fixed concentrations. Such values can be used to characterise slow-acting compounds (Remmen and Su, 2005).

The leaf worm, *Spodoptera litura* (Fab.) is a serious and polyphagous insect, its reproductive capacity and migration ability over long distances has made it an economically important pest of many agricultural crops. It has been reported to attack 112 cultivated species (Mallikarjuna *et al*., 2004). In the last few decades it has extended its host range to other crops such as cotton, mungbean, soya bean, cabbage and leafy vegetables and causes economic losses of crops from 26-100% (Dhir *et al*., 1992). *Spodoptera litura* is one of the first insect pests of agricultural importance in sub-continent to develop resistance to insecticides (Kranthi *et al*., 2002). It has developed resistance to variety of insecticides used (Armes *et al*., 1997; Byrne and Toscano, 2001; Kranthi *et al*., 2002; Sudhakaran, 2002).

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Effective time trends of a pesticide, neem Azal-T/S in combination with nuclear polyhydrosis virus has been studied for *Spodoptera litura* (Kim *et al*., 2001), yet little is known about the role of conventional and new insecticides. The objective of this study was to examine time trends in mortality for various insecticides, which are being used against the pest of cotton and other economical crops in Pakistan and also to define the time required to reach >50% mortality.

MATERIALS AND METHODS

Insects: Full-grown larvae of *S. litura* were collected from arum crop from Muzaffar Garh in August, 2004 and were reared at 25+2°C and 60±10% relative humidity with light: darkness (14:10 h) on a semi-synthetic diet (Ahmad *et al*., 1995).

Insecticides tested: Commercial formulations of endosulfan (Thiodon® 35EC, Bayer Crop Science), cypermethrin (Arrivo® 10EC, FMC), deltamethrin (Decis Super® 10.5EC, Bayer Crop Science), esfenvalerate (Sumi Alpha®, Sumitomo), chlorpyrifos (Lorsban® 40EC, Dow Agro Sciences), triazophos (Hostathion® 40EC, Bayer Crop Science), phoxim (Volaton™ 40EC, Bayer Crop Science), methomyl (Lannate® 40WP, DuPont), thiodicarb (Larvin® 80DP, Bayer Crop Science), spinosad (Tracer®, Dow Agro Sciences), indoxacarb (Steward®15SC, DuPont), abamectin (Agrimec™ 1.8EC, Syngenta), emamectin benzoate (Proclaim® 1.9EC, Syngenta), lufenuron (Match® 5EC, Syngenta), diflubenzuron (Teflon™ 7.5WP, Helb Pesticides), methoxyfenozide (Runner® 24SC, Dow Agro Sciences) and fipronil (Regent® 36EC, Bayer Crop Science) were obtained from their respective manufacturers.

Bioassays for LC_{50}: Bioassays were conducted on newly moulted second instar larvae (L_2) of *S. litura* using a standard leaf disc bioassay (Anonymous, 1990). The population was tested at the first generation (G_1) following field collection. Test solution was made in distilled water. Leaf discs of five centimetres diameter were cut from unsprayed cotton leaves, which were washed and dried before using. These leaf discs were dipped in the test solutions for ten seconds with gentle agitation and were placed on tissue papers for drying with adaxial surface upward. On drying, these were placed in 5 cm plastic Petri dishes having moist filter papers underneath to avoid desiccation. Five L_2 larvae were placed on each leaf disc. Each treatment was replicated 8 times, including controls. Mortality was assessed after 48 h of exposure. Larvae failed to show movement after a gentle touch with a blunt lead pencil head were considered dead.

Time to effect (LT_{50}): Time trends in mortality were determined by using serial dilution in leaf dip bioassay (Anonymous, 1990). For lethal time experiment, newly moulted L_2 larvae of G_1 were exposed to treated leaf discs at lethal concentrations (LC_{50} values). Forty larvae per insecticide were exposed in eight replications with five larvae per Petri dish. Mortality for dose-mortality relationship at serial dilutions of each insecticide was recorded after 48 h of exposure and for lethal time mortality relationship at 12 h interval after 72 h.

Data analysis: Mortality data was corrected by Abbott's formula and analysed by Probit analysis (Finney, 1971) using the software POLO-PC (Le Ora Software, 1987) for dose-and time-mortality regression lines. Significant difference was inferred by non-overlapping of 95% fiducial limits.

RESULTS

Sodium channel agonists: Endosulfan and three synthetic pyrethroids viz., cypermethrin, deltamethrin and esfenvalerate, which act on sodium channel, were tested. Endosulfan was significantly more toxic ($p<0.01$) than pyrethroids (Table 1). The most rapid mortality was with deltamethrin with LT_{50} 19.8 h, which was significantly less compared with cypermethrin (30.2 h) and esfenvalerate (33.8 h) and endosulfan (42 h; Table 2). The correlation between LC_{50} and LT_{50} of endosulfan (Fig. 1) was better (R^2 = 0.96) compared with deltamethrin (R^2 = 0.87), esfenvalerate (R^2 = 0.93) and cypermethrin (R^2 = 0.94), respectively. It is possible that the variability in deltamethrin and cypermethrin, esfenvalerate or endosulfan activity was the result of differences in feeding status of *S. litura*, which had previously fed, would be able to withstand a longer period without feeding in the feeding inhibitor treatment. However other factors such as behavioural effects not tested in these studies might also have contributed in feeding.

Cholinesterase inhibitors: Phoxim is relatively an uncommon organophosphate in Pakistan and therefore has significantly less ($p<0.01$) LC_{50} compared with chlorpyrifos and triazophos, which reflect its lower use in Pakistan (Table 1). When these insecticides were used in bioassays at LC_{50}, the LT_{50s} for all three organophospate

Table 1: Lethal concentration response at 48 hours interval of some conventional and new chemistry insecticides against leafworm, *Spodoptera litura* of arum crop collected from Pakistan

Insecticides	LC_{50}[1]	95% FL	Slope	χ^2	df	P
Endosulfan	15.2	12.0-19.2	1.74±0.16	3.22	6	0.78
Cypermethrin	275	225-336	2.26±0.22	3.83	5	0.57
Deltamethrin	87.8	71.8-107	2.33±0.25	1.96	4	0.74
Esfenvalerate	92.1	72.4-116	1.78±0.18	4.16	5	0.53
Chlorpyrifos	7.79	6.13-9.81	1.71±0.16	3.3	6	0.77
Triazophos	98.6	72.4-135	1.66±0.15	6.46	6	0.37
Phoxim	3.78	3.02-4.73	1.87±0.19	2.11	5	0.83
Spinosad	42.1	33.4-52.5	1.89±0.19	1.76	5	0.88
Indoxacarb	42.6	34.2-53.6	1.81±0.17	5.04	6	0.54
Abamectin	235	188-295	1.82±0.17	4.17	6	0.65
Emamectin	1.39	0.99-1.92	1.74±0.16	7.57	6	0.27
Lufenuron	72.7	52.6-101	1.67±0.17	5.06	5	0.41
Diflubenzuron	114	92.6-141	2.06±0.20	2.52	5	0.77
Methoxyfenozide	23.3	18.2-29.8	1.53±0.14	4.97	7	0.66
Fipronil	21.8	17.3-27.3	1.93±0.22	3.49	4	0.48

[1]LC_{50} expressed in $\mu g\ mL^{-1}$

Table 2: Lethal time response at lethal concentration of some conventional and new chemistry insecticides against leafworm, *Spodoptera litura* of arum crop collected from Pakistan

Insecticides	LT_{50}[1]	95% FL	Slope	χ^2	df
Endosulfan	41.8	35.4-48.6	4.50±0.56	4.49	4
Cypermethrin	30.2	27.1-33.1	5.59±0.62	3.03	4
Deltamethrin	19.8	15.8-23.4	2.95±0.38	2.18	4
Esfenvalerate	33.8	29.5-38.1	3.51±0.43	2.21	4
Chlorpyrifos	22.5	10.3-31.6	4.78±0.54	18.5	4
Triazophos	33.4	20.0-47.7	2.63±0.38	9.45	4
Phoxim	20.3	10.3-28.0	2.65±0.37	7.52	4
Spinosad	33.6	25.1-42.6	2.94±0.39	5.63	4
Indoxacarb	38.2	29.6-48.2	3.01±0.41	5.29	4
Abamectin	54.4	46.6-67.0	2.65±0.43	1.61	4
Emamectin	21.8	14.5-27.92	6.46±0.84	5.12	2
Lufenuron	38.3	33.8-43.1	3.57±0.44	2.65	4
Diflubenzuron	20.6	14.3-26.0	2.98±0.38	4.41	4
Methoxyfenozide	28.5	24.4-32.4	3.30±0.40	3.15	4
Fipronil	29.4	22.6-36.1	2.86±0.37	4.14	4

[1]LT_{50} expressed in h., [2]Fiducial limits at 90% probability level

compounds were similar. However, the speed of kill of triazophos was 1.5-fold slower than phoxim and chlorpyrifos (Table 2). The most rapid mortality >20% was detected in the compounds tested after 24 h exposure with 0% mortality in controls.

Similarly, the correlation between LC_{50} and LT_{50} was identical in organophosphate compounds with regression coefficient for phoxim (R^2 = 0.78), chlorpyrifos (R^2 = 0.77) and triazophos (R^2 = 0.83; Fig. 2).

New chemistry insecticides: These insecticides have a short history of use in Pakistan compared with conventional insecticides. Emamectin is significantly ($p<0.01$) more toxic compared with all other new compounds tested. Abamectin was the least effective compound with significantly ($p<0.01$) higher LC_{50}s due to non-overlapping of 95% FL (Table 1). Emamectin benzoate (R^2 = 0.80) and diflubenzuron (R^2 = 0.82)

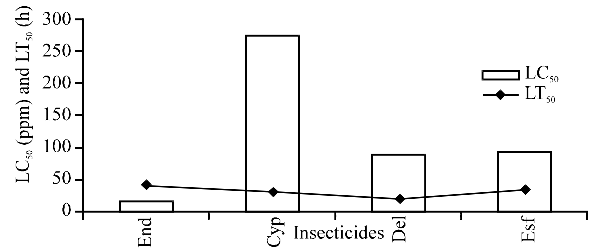

Fig. 1: Relationship betweeen LC_{50} and LT_{50} values of an Organochlorine and Pyrethroids

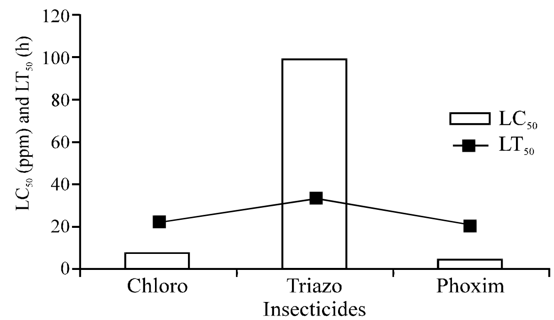

Fig. 2: Relationship betweeen LC_{50} and LT_{50} values of Organochlorines

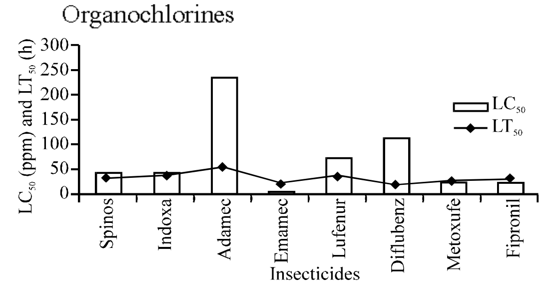

Fig. 3: Relationship betweeen LC_{50} and LT_{50} values of new chemistry insecticides

provided lethal mortality at about 20 h after exposure at concentration equal to LC_{50}, which was significantly lower than other compounds tested; spinosad (R^2= 0.93; 33.6 h), indoxacarb (R^2= 0.95; 38.2 h), abamectin (R^2= 0.97; 54.4 h), lufenuron (R^2= 0.96; 38.3 h), methoxyfenozide (R^2 = 0.94; 28.5 h) and fipronil (R^2 = 0.99; 29.4 h; Table 2; Fig. 3). Although emamectin is derivative of abamectin (Mrozik, 1994), but epi-methyl amino derivative is very effective against a broad spectrum of lepidopteran pests, with good photostability and translaminar movement and lack of cross-resistance with other commercially used pesticides (White *et al*., 1997).

DISCUSSION

The similarities in LT_{50} can be attributed to the mode of action of the products. Synthetic pyrethroids, organophosphates and most of the new products are nerve toxins (Thompson *et al*., 1999) and therefore would be expected to have a relatively rapid action. The synthetic pyrethroids and organophosphates are currently the most widely used products against *S. litura* in Pakistan. Growers are consequently familiar with their rapid action in the field. The presence of live larvae in a treated field one-week post treatment, as could occur with stomach insecticides, would in most situations, be regarded as a control failure and result in a second application. An education programme is therefore essential to inform users of these expected differences.

In term of Integrated Pest Management (IPM), the side effects that an insecticide causes in non-target populations are also important. The product such as chlorpyrifos, which is quite effective against *S. litura,* is also known to have broad-spectrum activity causing mortality in non-target beneficial populations (Corso, 1988) and therefore may not be suitable in terms of IPM. In contrast, although spinosad, indoxacarb and abamectin showed lower levels of mortality in the bioassays, this may not be reflected in differences in post-treatment field populations. However, these products show high levels of selectivity (Nowak *et al*., 2001) and preserve beneficial populations therefore these could lead to enhanced control surviving populations.

One of the basic aspects of resistance management is to devise approaches to minimize reliance on insecticides so that the selection pressure can be alleviated. Development of effective resistance management programme need to be based on information on occurrence and degree of resistance and the local resistance patterns in field populations of insect pests to different insecticides. Because the history of pesticide application varies, resistance patterns also differ. For such differences to be exploited they need to be properly documented. The variation in resistance to the insecticides tested in this study was not large, suggesting the possibility of common resistant mechanism to pyrethroids, organophosphates and some new chemistry compounds. However, further studies are required to confirm the hypothesis. Moreover, the low slopes obtained (Table 1) from the probit assay data suggested that the population was heterogeneous. Heterozygotes are the most common carriers of resistance, they are the most important genotype from a resistance management perspective (Roush and Mckenzie, 1987). The widespread occurrence of heterozygosity in field populations may contribute to rapid increases in resistance levels even as a result of just a few insecticide applications. This phenomenon is exemplified by the transient decline in pyrethroid resistance in several lepidopteran insects, for example *H. armigera* strains following a withdrawal of pyrethroid use for five years until 1987 in Turkey (Forrester *et al*., 1993). Reverted populations were found to maintain a rather high frequency of resistance alleles, which led to the re-establishment of high resistance after only a few selections

The present study suggests that several of the products evaluated could have role in the management of *S. litura* in Pakistan. However, a field evaluation would be required to fully investigate the field efficacy and side effects to determine their field performance and IPM compatibility in Pakistani ecosystem.

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