Bangladesh J. Bot. **45**(1): 39-45, 2016 (March)

BASE LINE TOXICITY OF FUNGICIDES AND INSECTICIDES TO *SPODOPTERA LITURA* (FAB.)

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Key words: *Spodoptera litura*, Base line toxicity, Fungicides, Insecticides

Abstract

Base-line toxicity of fungicides *viz.* mancozeb, propaconazole, carbendazim, azoxystrobin and ridomil MZ and insecticides viz. spinosad 45SC, lufenuron 5EC, flubendiamide 48 EC and acetamiprid 20 SP were evaluated against *Spodoptera litura*. The LC_{50} values showed that flubendamide was most toxic against *S. litura* than all the chemicals. The order of toxicity was flubendiamide (8.37) > acetamipirid (9.58) > spinosad (23.22) > lufenuron (23.46) > propaconazole (90.98) > carbendazim (413.51) > azoxystrobion (597.77) > mancozeb (681.39) > ridomil (1193.00). The LT_{50} values showed that flubendamide was most toxic while azoxystrobion was found to be least toxic against *S. litura*. The order of toxicity was flubendiamide (39.00) > acetamipirid (44.00) > spinosad (68.00) > propaconazole (108.00) > carbendazim (112.00) > lufenuron (120.00) = Ridomil (120.00) > mancozeb (148.00) > azoxystrobion (192.00). Responsiveness of *S. litura* larvae provides important information with dose- and time-mortality for selection of insecticides in field for better pest management. Information based on these results would help in avoiding economic loses because of insecticide dosage concentration and also helps in better integration of insecticides and fungicides into IPM and IRM program for the control of major pests and pathogens.

Introduction

The lepidopteran *Spodoptera litura* (Fabricius) is a serious polyphagous insect-pest reported to attack over 112 cultivated plants belonging to 44 families worldwide (Garad *et al.* 1984) and 63 plants species belonging to 22 families in India (Mallikarjuna *et al*. 2004). The full grown caterpillars are the most voracious feeders and cause extensive damage by defoliation. Use of insecticides for controlling this pest is on the rise and it has the ability to develop resistance to many insecticides (Ahmad *et al*. 2011). Various pesticides *viz.* herbicides, fungicides have been reported to have destructive on different aspects of life of the *S. litura* (Singh and Bhattacharya 2004). Therefore, it is essential to know the role of agrochemicals (fungicides, herbicides and plant growth regulators) on the developmental profile of *S. litura*. Moreover, a base line data regarding the toxicily of the insecticides against this economically important insect pest would help in understanding the level of resistance developed and any possible cross resistance therein, could be assessed in advance. Information on this interesting area of pest management is scanty. Therefore, keeping the things in view, the present studies were contemplated to explore the possibilities of integrating fungicides and insecticides to manage tobacco caterpillar, *S. litura* as well as to reduce the pesticide load.

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Materials and Methods

The larvae and egg patches of *S. litura* was collected from castor plant and reared in laboratory condition at the Division of Entomology, SKUAST-Jammu. The bioassays were kept at a temperature of 26°C, 60 - 70% relative humidity and 16 : 8 (light : dark) photoperiod. Mass rearing was done according to the methodology of Britto (1980). These laboratory-reared larvae were used for bioassays and the cultures were maintained throughout the study period. Leaf residue dipping (LRD) method developed by Tabashnik and Cushing (1987) and modified by Dhingra and Sarup (1990) was used. Castor leaves were collected from unsprayed plants, washed and air-dried and made 5 cm diameter leaf discs with the help of a leaf cutter. Stock solution of each tested insecticide was made from the formulation available with different concentrations were prepared by calculation the field doses from the available insecticides in the market. The leaf disc were dipped in each concentration for 20 seconds and allowed to dry at ambient temperature for about 15 - 20 min in a fume hood. Air-dried leaf discs were then placed in individual plastic Petri dishes (5 cm diameter) containing moistened filter paper. Each treatment (concentration) including controls was replicated three times. Ten larvae of *S. litura* (2nd instar), of uniform age, were exposed to three different concentrations of insecticides, using a leaf-dip technique, as recommended by the Insecticides Resistance Action Committee (IRAC) of GIFAP (Anon. 1990). The larvae were fed on treated leaves for 48 hrs, thereafter fresh leaves were provided for feeding. Sterilized sand was provided at the bottom of the vials to fully grown larvae to facilitate pupation in case of *S. litura*. Observations were recorded at 24 hrs interval. Median lethal concentrations (LC_{50}) were calculated to observe dose mortality relationship. LT_{50} was also calculated for each concentration. Per cent mortality observed in the control groups if any were corrected by using Abbott’s formula (Abbott 1925). Data recorded on larval mortality were subjected to probit analysis to understand the dose mortality and time mortality relationship. Probit analysis was carried out using software programme developed by Mondal *et al.* (2001).

Results and Discussion

The dose-mortality relationship of an insect to a toxin is typically expressed as an LC_{50} value, which is the toxin concentration required to kill 50 per cent of the population in a specified period. The lower is the LC_{50} value, the greater the toxicity. The results showed that the effect of insecticides and fungicides against *S. litura* larvae varied according to toxicity. All the insecticides tested in the present studies caused concentration-dependent mortality in larval population of *S. litura*. The LC_{50} values showed that flubendamide was most toxic against *S. litura* than all the chemicals tested. Bhatnagar *et al.* (2013) also reported that the relative toxicity ratio (RTR) of novel molecules at LC_{50} value in comparison to cartap hydrochloride at 24 hrs and 48 hrs were: indoxacarb (66.32, 82.5) > flubendiamide (11.45, 49.5) and at 72 hrs the values were flubendiamide (118.33) > indoxacarb (71). Indoxacarb and flubendiamide with low LC_{50} values demonstrated higher toxicity against *S. litura* than cartap hydrochloride ridomil MZ was found to be least toxic. The LC_{50} values among insecticides and fungicides tested ranged from 8.37 for flubendiamide to 1193.00 for ridomil MZ (Table 1). The fungicides, ridomil MZ, mancozeb, azoxystrobin, carbendazim and propiconazole had LC_{50} value ranging from 90.98 to 1193.00 and were generally higher than those observed for the insecticides (lufenuron, spinosad, acetamipirid, and flubendiamide) with LC_{50}’s ranging from 8.37 to 23.46. The larvae of *S. litura* were significantly less susceptible to ridomil MZ than all other fungicides and insecticides. Flubendiamide (8.37) and acetamipirid (9.58) were significantly more toxic as compared with test chemicals. The order of toxicity was flubendiamide > acetamipirid > spinosad > lufenuron > propaconazole > carbendazim > azoxystrobion > mancozeb > ridomil. Significant increase in toxicity was observed as evident from the non overlapping fiducial limits of LC_{50} for the insecticide tested *in vitro*. The slopes of regression lines and the lethal concentration values at 50% kill were significantly different from each other. Slopes of azoxystrobion and mancozeb; flubendamide and lufenuron were non-significant to each other due to overlapping of their regression lines. The LC_{50} values of spinosad and acetamaprid were, however, significant not only from each other but also from other agrochemicals (Table 1). Horowitz *et al*. (1998) also reported that according to LC_{50} and LC_{90} values, acetamiprid was 10- and 18-folds more potent than imidacloprid to whitefly *Bemisia tabaci* (Gennadius) resulting (with the concentration of 25 ml a.i./l) in adult mortality of 90, 93, and 96% and 76, 84 and 76% after 2, 7 and 14 days of application. Spinosad is highly efficient against many lepidopteran pests, but sensibility of the targeted species varies a lot depending on the mode of exposure. Concentrations higher than 0 mg/l of spinosad killed 100% of times more susceptible at higher dose. The present results are in conformity with Aydin *et al.* (2006) who also reported that LC_{50} values for field and susceptible strains were 43.691 and 10.037 ppm, respectively suggesting that spinosad is potentially important in the control of *S. littora*. Lufenuron is an acylurea insecticide under development mainly for the control of lepidopterous pests in field crops, orchards and vegetables (Buholzer and Skillman, 1995). The present study revealed that larvicidal effect of the insecticide was clearly dependent on the concentrations of the insecticide. The base-line susceptibility value of *S. litura* to spinosad was 34.00. Lufenuron required maximum time of 120 hrs to kill 50% population which was due to its mode of action through ingestion and affecting the physiological processes. However, disorders in oogenesis and spermatogenesis have also been main features at their chronic dose rates (Smagghe and Degheele 1994). These results are very similar to those of the xenobionts which affect the hatching success of *S. exigua* eggs (Adamski *et al.* 2009). In the present studies, carbendazim is toxic to *S. litura* with LC_{50} value of 413.51. The present studies are in line with Singh and Bhattacharya (2004) who also reported that larval mortality increased with concentration of carbendazim. The larval mortality started after 24 hrs of feeding only at highest concentrations. On diets containing 0.125 and 0.20% carbendazim, larval mortality occurred after 5 days of feeding which increased gradually up to 9 days of feeding. In the present studies, mancozeb is toxic to *S. litura* with LC_{50} value of 681.30. Nagia *et al.* (1994) also reported that mancozeb used for the control of early blight of potato, *Alternaria solani* also controlled potato aphids, *Myzus persicae* and cotton aphid, *Aphis gossypii.* The present studies are in consonance with Singh and Bhattacharya (2004) who also observed complete larval mortality of *S. litura* at 5 and 7 days after feeding on diets with 1.00 and 0.50 per cent mancozeb, respectively while at field dose of 0.25 per cent, survival was only 5.00 per cent. The base-line susceptibility value of *S. litura* to propaconazole was 90.98. The results are in agreement with those of Zhen *et al.* (2006) who also reported that propiconazole had the highest toxicity against *Spodoptera litura.* The mortality of *S.litura* cells treatment by propiconazole at concentration of 100 µg/ml was 98.08%. The LC_{50} value of propiconazole against SL cells was 20.31 µg/ml 36 hrs after treatmentAfter treatment for 96 and 120 hrs, the LD_{50} values of propiconazole against 4th instar larvae of *S. litura* were 0.59 µg/larva and 0.45 µg/larva, respectively. The high toxicity of propiconazole against cells and larvae of *S. litura* implied the possibility for propiconazole analog in controlling insect pests.

The time-mortality studies for the fungicides and insecticides at their respective LC_{50} values were performed. The LT_{50} value of insecticides and fungicides against *S. litura* larvae varied according to toxicity (Table 2). The order of toxicity was flubendiamide > acetamipirid > spinosad > propaconazole > carbendazim > lufenuron ~ ridomil > mancozeb > azoxystrobion. Among insecticides, flubendamide required the least time to kill the 50% population followed by acetmipirid, spinosad and lufenuron, respectively. Lufenuron, however, required the maximum time of 32 hrs to kill 50% exposed insects (Table 2). The present studies are in conformity with

Table 1. Median lethal concentration (LC_{50}) of insecticides and fungicides against *S. litura* (2nd instar) larvae.

Concentration (ppm)	LC 50	FL 95%	Slope ± SE.	N	χ^2	df	p	Regression (Y = a + bx)
Acetamaprid 20SP	9.58	–18.98 - 24.65	7.61 ± 2.90	30	28.47	19	0.07	6.179 + 0.013x
Azoxystrobin	597.77	392.43 - 1659.9	2.09 ± 1.17	30	9.75	19	0.95	1.404 + 0.001x
Carbendazin	413.509	197.31 - 811.75	4.57 ± 1.70	30	15.15	19	0.71	3.67 + 0.001x
Flubendamide 48 SC	8.37	–30.66 - 20.23	7.28 ± 2.90	30	34.39	19	0..17	5.748 + 0.017x
Lufenuron 5EC	23.46	–17.04 - 49.39	6.89 ± 2.96	30	32.80	19	0.02	5.080 + 0.015x
Mencozeb	681.30	369.15 - 1071.14	4.71 ± 2.07	30	18.87	19	0.46	3.485 + 0.002x
Propoconazol	90.98	74.01 - 113.22	6.42 ± 5.60	30	10.49	19	0.94	2.031 + 0.023x
Ridomil MZ	1193	826.90 - 2537.54	2.14 ± 1.19	30	6.81	19	0.99	1.342 + 0.003x
Spinosad 45SC	23.22	–7.12 - 46.19	5.76 ± 2.73	30	37.41	19	0.07	4.07 + 0.03x

FL = Fiducial limits at 95% confidence level, SE = Standard error. N = Number of larvae. LC_{50} expressed as ppm.

Table 2. Time-mortality (LT_{50}) of insecticides and fungicides against *S. litura* (2nd instar) larvae.

Concentration (ppm)	LT_{50}	FL 95%	N	Slope ± S.E	χ^2	Df	p	Regression Y = (a + bx)
Acetamaprid 20SP	44.00	41.95 - 46.04	30	50.64 ± 5.30	40.588	6	0.00	62.63 + (–0.10)x
Azoxystrobin	192.00	156.44 - 227.55	30	189.79 ± 15.96	41.21	6	0.00	238.35 + (–0.43)x
Carbendazim	112.00	105.12 - 118.87	30	119.92 ± 7.00	28.14	6	0.00	139.10 + (–0.62)x
Flubendiamide 48SC	39.00	33.01 - 44.98	30	46.42 ± 5.33	37.59	6	0.00	56.51 + (–0.12)x
Lufenuron 5EC	120.00	60.73 - 179.26	30	105.76 ± 10.59	48.82	6	0.00	140.91 + (–0.78)x
Mancozeb	148.80	133.10 - 164.50	30	143.52 ± 10.47	22.90	6	0.01	159.48 + (–0.02)x
Propiconazole	108.00	84.67 - 31.32	30	109.64 ± 12.19	46.62	6	0.00	147.30 + (–0.33)x
Ridomil MZ	120.00	112.59 - 127.40	30	126.07 ± 13.8	28.14	6	0.00	148.33 + (–0.07)x
	68.00	50.05 - 85.94	30	76.21 ± 10.26	29.398	6	0.00	100.48 + (–0.52)x

FL = Fiducial limits at 95% confidence level. SE = Standard error. N = Number of larvae. LT_{50} expressed in hrs.

Hirooka *et al*. (2007) who also reported that toxicity of flubendiamide to 2nd instar larvae was the highest which were 1.50 - 6.17-folds than the 3rd instar larvae and 22.25, 44.95-folds than 4th instar larvae. Fiducial values of all the insecticides were non-significant to each other; however, it was least in flubendamide making it the most effective. Acetamiprid, (E) - N^1- [(6 - chloro - 3 - pyridyl) methyl] - N^2 - cyano - N^1-methyl acetamidile, is a new-generation novel insecticide with ground and aerial application. It poses low risks to the environment relative to most other insecticides and its use would pose minimal risk to non target plants (Punitha *et al*. 2012). Abdella (2013) evaluated selected neonicotinoid insecticides for their toxicity against cowpea aphid, *Aphis craccivora* Koch and reported that acetamiprid, imidacloprid, thiamethoxam and dinotefuran registered significantly high per cent reduction of the pest at one, seven, 15 and 21 post treatment. The LT_{50} values showed that spinosad was highly toxic against *S. litura*. Topical LD_{50} values for lepidopteran pest species range from 0.1 to 3 mg a.i./l if the compound is applied in earlier instars (Sparks *et al*. 1998). Munir *et al*. (2005) in a similar study also reported that spinosad was highly effective against 2nd instar larvae of leaf worm, *S. litura* under controlled laboratory conditions.

There existed steeper slopes in flubendamide and acetamipirid making them both as effective as other insecticides (Table 2). The LT_{50} values showed that flubendiamide was most toxic while azoxystrobion was found to be least toxic against *S. litura.* Azoxystrobin, a systemic fungicide is effective at low doses against a wide range of fungal pathogens. Cloyd *et al*. (2009) reported that the fungicide, azoxystrobin was not directly toxic to adults of beetle *Atheta coriaria* (Kraatz) (Coleoptera : Staphylinidae) an important natural enemy. Furthermore, the treatments did not inhibit the ability of adult *A. coriaria* to consume fungus gnat (*Bradysia* sp. nr. *coprophila*) larvae in a feeding behavior experiment. The LT_{50} values among insecticides and fungicides tested ranged from 39.00 for flubendamide to 192.00 for azoxystrobion (Table 2). The fungicides, ridomil MZ, mancozeb, azoxystrobion, carbendazim and propaconazole had LT_{50}'s ranging from 108.00 to 192.00 and were generally higher than those observed for the insecticides (acetamipirid, lufenuron, flubendiamide and spinosad) with $LT_{50's}$ ranging from 39.00 to 120.00. Adamski *et al*. (2009) reported that soil microarthropods exposed to the pesticides mancozeb (240 mg/m^2) after a single application showed significant trends in response to pesticide application. They further reported that *A. segetum* larvae when exposed to mancozeb cause 10 - 15 per cent mortality which may be crucial for the exposed population.

This study provides basic information regarding the concentration and time for *S. litura* mortality under laboratory conditions. LC_{50} values are helpful to make suitable formulations for field pest control. Field studies can foster the effectiveness of these and other insecticides for long term and effective management of *S. litura*. By considering statistically analyzed figures farmers can be guided to prepare doses and pest management programme can be designed by fixing number of spray trials to get 100% results. The observations on the influence of fungicides and insecticides will help to understand the shifts in insect pest population on a crop influenced by these fungicides and new insecticides.

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(Manuscript received on 3 March, 2015; revised on 15 April, 2015)