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TOXICITY OF DIAMIDE AND SPINOSYN INSECTICIDES AGAINST TOBACCO CATERPILLAR *SPODOPTERA LITURA* (F.)

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ABSTRACT

The stomach and contact toxicity of diamide (chlorantraniliprole and flubendiamide) and spinosyn (spinetoram and spinosad) insecticides was tested against 4d old larvae of *Spodoptera litura* (F.) by leaf smear and larval atomization methods on castor leaf disc under laboratory conditions (25 ± 2°C, R.H. 75 ± 5%, Sept.-Oct, 2018). The order of toxicity was observed as: chlorantraniliprole (LC_{50}= 0.00086) > spinetoram (0.0034) > flubendiamide (0.005) > spinosad (0.02%) at 48 hours after exposure (HAE) by larval atomization method and chlorantraniliprole (LC_{50}= 0.0042 and 0.0009) > spinetoram (0.0047 and 0.0032) > spinosad (0.0316 and 0.0182%) at 24 and 36 hours after feeding (HAF) by leaf smear method. Chlorantraniliprole exhibited 1.24 times more stomach toxicity by leaf smear method (LC_{50}= 0.0009%) than contact toxicity by larval atomization method (LC_{50}= 0.00112%) at 36 HAF/HAE. The stomach toxicity (LC_{50}= 0.0047 and 0.0032%) of spinetoram was 2.57 and 2.66 times more than the contact toxicity (0.0121 and 0.0085%) at 24 and 36 HAF/HAE. Spinosad also showed 1.24 and 1.82 times more stomach toxicity (LC_{50}= 0.0182 and 0.0110%) than the contact toxicity (0.0225 and 0.02%) at 36 and 48 HAF/HAE, respectively. All the four insecticides showed varying contact and stomach toxicity against *S. litura*.

Key words: Insecticides, spinosyns, diamides, efficacy, *Spodoptera litura*, stomach and contact toxicity, bioassay,

The tobacco caterpillar *Spodoptera litura* (F.) is one of the destructive insect pests causing a heavy yield loss (Cheng et al., 2017). It is widely distributed throughout tropical and temperate Asia, Australasia and the Pacific Islands (CABI, 2019). Wide host range is considered to be an important factor for better survival during evolution (Simpson et al., 2002). *Spodoptera litura* can feed on more than 389 species of economic plants belonging to about 109 families (Lin et al., 2019). In India 60 plant species were found to be its host (Garad et al., 1984). It has become a major pest of soybean (Glycine max L.) throughout India (Fand et al., 2015). It's incidence on several crops has shown an increasing trend (Dhaliwal et al., 2010).

The resistance within the insecticides exposed population of the pest is a result of selection pressure exerted by insecticide (Tong et al., 2013). *Spodoptera litura* was the first lepidopteron pest known to develop insecticide resistance (seven fold) in India against HCH on cauliflower (Srivastava and Joshi, 1965). It is also known to be resistant to various organochlorines, organophosphates, carbamates and synthetic pyrethroids (Kranthi et al., 2002; Ahmad et al., 2009; Saleem et al., 2008). The continuous, extensive and unscheduled applications of insecticides have led to the development of resistance (Kranthi et al., 2002) and which has been fastened in the last two decades (Venkateshwarlu et al., 2006). The polyphagy and rapid multiplication behaviour of *S. litura* have added to the problem of development of resistance (Ramakrishnan et al., 1984). Diamide (viz. flubendiamide and chlorantraniliprole) and spinosyn (viz. spinosad and spinetoram) are among the novel group of insecticides (Singh, 2013). The present study evaluates the bioefficacy of these against Pantnagar population of *S. litura*.

MATERIALS AND METHODS

A running culture of *S. litura* was maintained in the laboratory by standard rearing technique following Shankarganesh et al. (2009) and Thodsare and Srivastava (2015). Egg masses were collected from wildly grown castor, *Ricinus communis* plants at Norman E. Borlaug Crop Research Centre (NEBCRC) of the University and kept in a petri dish (9 cm dia.) lined with a moist filter paper. Upon hatching, neonate larvae were transferred to plastic tubs (dia 35 cm, ht. approx 15 cm), containing fresh tender castor leaves having a water soaked cotton swab wrapped to petiole. Good hygienic conditions were provided to the growing larvae and fresh food was provided daily. Egg masses obtained from laboratory reared adults were used for developing the further culture and for the experiments.

The test insecticides viz., chlorantraniliprole (Coragen 18.5 SC, Du Pont India Pvt. Ltd.), flubendiamide (Fame 480 SC, Bayer Crop Science), spinetoram (Delegate 11.7 SC, Dow Agro Sciences) and spinosad (Tracer 45 SC, Dow Agro Sciences) were purchased from market and stored in a refrigerator. Experiments for the determination of contact and stomach toxicity were conducted against 4d old larvae (mean larval weight = 0.01g) by larval atomization and leaf smear methods under laboratory conditions (temp. 25 ± 2°C, R.H. 75 ± 5%, Sept.-Oct, 2018).

The contact toxicity of insecticides was tested by larval atomization method (Kumar and Srivastava, 2016; Negi and Srivastava, 2018) by taking different dilutions of respective insecticide prepared in tap water by serial dilution method. The larvae in each treatment were atomized with different dilutions with the help of corning glass hand atomizer (Capacity: 100ml) in a glass petri plate (dia. = 15 cm) and given the contact exposure for 30 min. (Kumar and Srivastava, 2016). The control larvae were sprayed with water alone. Treated larvae were transferred into a corning glass petri dish (dia. = 9 cm) lined with the moist filter paper and containing a castor leaf disc for feeding.

The stomach toxicity was tested by leaf smear method (Srivastava et al., 1990) by taking different dilutions of respective insecticides prepared in tap water by serial dilution method. With the help of an Eppendorf pipette 200µL of each of the dilutions were applied and smeared onto the surface of a castor leaf disc (5x5 cm^2), the control leaf disc was smeared with water alone and thereafter air dried. Each treatment was replicated five times. One such leaf disc was put into a corning glass petri dish (dia. = 9 cm) lined with a moist filter paper and six prestarved larvae were released in each of the petri dishes. Freshly treated leaves were provided at every 24h up to 72 HAF.

Two experiments were conducted to determine the contact and stomach toxicity of chlorantraniliprole and contact toxicity of flubendiamide. The contact and stomach toxicity of chlorantraniliprole was tested by larval atomization and leaf smear methods (as described above), taking five dilutions each (range: 0.01 - 0.0005% and 0.03- 0.0001%), respectively. The contact toxicity of flubendiamide was tested by larval atomization method, taking ten dilutions (range: 0.04 to 0.0005%). Thirty larvae (6 per replication) were taken in a treatment and each treatment was replicated five times. The observations on mortality and morbidity were taken at 12h interval from 12 to 120 hours after exposure/ hours after feeding (HAE/HAF), moribund larvae were counted as dead (Sharma and Pathania, 2014; Kumar and Srivastava, 2016).

Two experiments were conducted to determine the contact and stomach toxicity of spinetoram and spinosad by larval atomization and leaf smear methods (as described above). The contact toxicity of spinetoram was tested by larval atomization method, taking nine dilutions (range: 0.05-0.0001%) and stomach toxicity was tested by leaf smear method, taking five dilutions (range: 0.02- 0.001%). The contact and stomach toxicity of spinosad was tested by larval atomization and leaf smear methods, respectively taking five dilutions each (range: 0.05-0.007%). Thirty larvae (6 per replication) were taken in a treatment and each treatment was replicated five times. The observations on mortality and morbidity were taken at 12h interval from 12 to 108 hours after exposure/ hrs after feeding (HAE/HAF). Moribund larvae were counted as dead.

The observations on mortality were corrected using Abbott's formula (Abbott, 1925). The data so obtained was subjected to probit analysis for obtaining regression equation and computing LC and LT values following Finney (1971). LC and LT values were computed using probit analysis based computer programme STPR718 at the Computer Center, College of Basic Science and Humanities of this University.

RESULTS AND DISCUSSION

Contact and stomach toxicity- chlorantraniliprole and contact toxicity- flubendiamide

Concentration-mortality response: The LC_{50} values of chlorantraniliprole were 0.00112 and 0.00086%, respectively at 36 and 48 HAE by larval atomization method (contact toxicity), however were 0.0042 and 0.0009%, respectively at 24 and 36 HAF by leaf smear method (stomach toxicity), showing 1.24 times higher stomach toxicity than contact toxicity at 36 HAF/HAE. The contact toxicity of flubendiamide in terms of LC_{50} values were 0.005 and 0.0038%, respectively at 48 and 72 HAE by larval atomization method. The contact action of chlorantraniliprole (LC_{50}= 0.00086%) was 5.8 times more toxic than flubendiamide (LC_{50}= 0.005%) at 48 HAE. It is evident from the data that chlorantraniliprole exhibited higher stomach than the contact toxicity and also has higher contact toxicity than flubendiamide against *S. litura* (Table 1).

Duration- mortality response: The LT values at

Table 1. Concentration-mortality response of insecticides against larvae of *S. litura*- larval atomization and leaf smear methods.

Insecticide (Trade name)	HAE/ HAF	LC Values (%)		Heterogeneity X^2	Regression equation Y = a +bx	Fiducial Limits at LC_{50}	
		LC_{50}	LC_{90}			Upper	Lower
Larval atomization method							
chlorantraniliprole (Coragen 18.5 SC)	36	0.00112	0.03160	2.132	y = 4.374 + 0.291x	0.00209	0.000601
	48	0.00086	0.00893	3.333	y = 4.240 + 0.424x	0.00137	0.000537
Flubendiamide (Fame 480 SC)	48	0.0050	0.1312	0.274	y = 3.980 + 0.268x	0.0081	0.0031
	72	0.0038	0.1629	0.366	y = 4.137 + 0.268x	0.0069	0.0021
Spinetoram (Delegate 11.7 SC)	24	0.0121	0.2748	2.175	y = 3.719 + 0.397x	0.0204	0.0072
	36	0.0085	0.1141	2.352	y = 3.613 + 0.487x	0.0133	0.0054
	48	0.0034	0.0439	2.902	y = 3.986 + 0.517x	0.0058	0.0020
	72	0.0018	0.0158	6.190	y = 3.653+ 0.473x	0.0026	0.0012
Spinosad (Tracer 45 SC)	36	0.0225	0.150	1.625	y = 3.940 + 0.287x	0.0311	0.0166
	48	0.0200	0.140	0.786	y = 4.050 + 0.279x	0.0273	0.0144
	72	0.0185	0.100	2.020	y = 3.946 + 0.328x	0.0241	0.0138
Leaf smear method							
chlorantraniliprole (Coragen 18.5 SC)	24	0.0042	0.3500	2.825	y = 3.627 + 0.438x	0.00894	0.00200
	36	0.0009	0.0176	0.100	y = 3.718 + 0.621x	0.00174	0.000507
Spinetoram (Delegate 11.7 SC)	24	0.0047	0.0634	2.149	y = 3.856+0.380x	0.0072	0.0030
	36	0.0032	0.0574	5.885	y = 3.257 + 0.767x	0.0140	0.0015
Spinosad (Tracer 45 SC)	24	0.0316	0.175	2.696	y = 3.581 + 0.319x	0.0460	0.0243
	36	0.0182	0.092	2.809	y = 3.907 + 0.345x	0.0234	0.0137
	48	0.0110	0.0535	1.824	y = 4.262 + 0.362x	0.0150	0.0081

HAE/HAF = Hours after exposure/ after feeding; Mean larval weight = 0.01g (4d old); No. of larvae/ treatment (n) = 30; 25±2°C, R.H.: 75±5%

0.005 and 0.002% concentration of chlorantraniliprole were 25.05 and 25.18h, respectively in larval atomization method (Table 3). The difference in the LT values in the two concentrations was not pronounced. In leaf smear method at 0.001% concentration of chlorantraniliprole the LT_{50} and LT_{90} values were 28.03 and 74.97h, respectively, and at a lower concentration of 0.0001% the higher LT_{50} values were observed i.e., 53.22h.

Contact and stomach toxicity- spinetoram and spinosad

Concentration-mortality response: The LC_{50} values of spinetoram were 0.0121, 0.0085, 0.0034 and 0.0018% at 24, 36, 48, and 72 HAE, respectively in case of larval atomization method; and were 0.0047 and 0.0032% at 24 and 36 HAF, respectively in leaf smear method, showing 2.57 and 2.66 times more stomach toxicity than contact toxicity at 24 and 36 HAF/HAE. The LC_{50} values of spinosad were 0.0225, 0.02 and 0.0185% at 36, 48 and 72 HAE, respectively by larval atomization method and; 0.0316, 0.0182 and 0.011% at 24, 36 and 48 HAF, respectively by leaf smear method. The stomach toxicity of spinosad (LC_{50}= 0.0182 and 0.0110%) was 1.24 and 1.82 times more than the contact toxicity (0.0225 and 0.02%) at 36 and 48 HAF/HAE, respectively.

It is evident from the data that the contact toxicity (by larval atomization) of spinetoram (LC_{50}= 0.0085, 0.034 and 0.0018) was 2.65, 5.88 and 10.27 times more than that of spinosad (LC_{50}= 0.0225, 0.02 and 0.0185) at 36, 48 and 72 HAE, respectively and the stomach toxicity (by leaf smear method) of spinetoram (LC_{50}= 0.0047 and 0.0032) was 6.72 and 5.69 times more than

spinosad (LC_{50} = 0.0316 and 0.0182) at 24 and 36 HAF. Also the stomach action of both of the insecticides was more than their contact action (Table 1)

Duration-mortality response: The LT_{50} values of spinetoram at 0.01, 0.005, 0.002% concentrations were 33.91, 37.76 and 51.78h, respectively in larval atomization method; at 0.005% concentration was 27.54h by leaf smear method. On the other hand The LT_{50} value of spinosad at 0.05 and 0.04% concentrations was 23.8 and 30.83h, respectively in larval atomization method; at 0.04, 0.03, 0.02 and 0.01% concentration was 21.11, 28.06, 35.45 and 49.70h, respectively in leaf smear method (Table 2).

The concentration-mortality (LC values) and duration-mortality (LT values) responses of the insecticides against 4d old larvae of *S. litura* by larval atomization and leaf smear methods are not known so far. The toxicity of diamide and spinosyn insecticides by diet overlay bioassay method had been tested against one day old second instar larvae of *S. litura* population of Pakistan collected from different crops by Ahmad and Gull (2017) during 2008-2013. The order of toxicity in 2008, 2009, 2011 and 2013 against the population collected from the cotton, cauliflower, cabbage and berseem crop was chlorantraniliprole (LC_{50} = 0.0001, 0.00031, 0.00059 and 0.0017%) > flubendiamide (0.00018, 0.00066, 0.00059 and 0.0044%) > spinetoram

Table 2. Duration-mortality response of insecticides against larvae of *S. litura*- larval atomization and leaf smear methods

Insecticide (Trade name)	Conc. (%)	LT Values (h)		Heterogeneity X^2	Regression equation Y = a +bx	Fiducial Limits at LT_{50}	
		LT_{50}	LT_{90}			Upper	Lower
				Larval atomozation method			
chlorantraniliprole (Coragen 18.5 SC)	0.005	25.05	73.30	0.570	y = 3.884 + 0.46x	30.12	19.74
	0.002	25.18	78.33	1.108	y = 3.916+ 0.445x	30.55	19.59
				Leaf smear method			
	0.001	28.03	74.97	4.717	y = 3.523 + 0.550x	33.34	22.91
	0.0001	53.22	101.05	2.574	y = 3.117+ 0.524x	60.84	47.62

Insecticide (Trade name)	Conc. %	LT Values (h)		Heterogeneity X^2	Regression equation Y = a +bx	Fiducial Limits at LT_{50}	
		LT_{50}	LT_{90}			Upper	Lower
				Larval atomization method			
spinetoram (Delegate 11.7 SC)	0.01	33.91	81.16	2.924	y = 4.210 + 0.393x	39.46	26.90
	0.005	37.76	200.20	9.454	y = 4.208+ 0.244x	47.35	27.12
	0.002	51.78	583.11	1.779	y = 4.257+ 0.185x	75.11	35.19
spinosad (Tracer 45 SC)	0.05	23.80	85.22	1.582	y = 4.270 + 0.322x	29.27	17.61
	0.04	30.83	100.48	0.864	y = 3.764 + 0.413x	37.87	24.64
				Leaf smear method			
spinetoram (Delegate 11.7 SC)	0.005	27.54	67.65	1.287	y = 3.471 + 0.575x	32.30	22.82
	0.04	21.11	58.24	1.033	y = 3.943 + 0.523x	25.45	16.24
spinosad (Tracer 45 SC)	0.03	28.06	69.36	2.048	y = 3.579 + 0.513x	32.71	23.24
	0.02	35.45	86.60	1.828	y = 3.266 + 0.505x	41.01	30.18
	0.01	49.70	168.61	0.725	y = 3.586 + 0.292x	58.95	41.88

Mean larval weight = 0.01g (4d old); No. of larvae/ treatment (n) = 30; temp.: 25±2°C, R.H.: 75±5%, Sept.-Oct, 2018

(0.001, 0.00085, 0.0069 and 0.0086%) > spinosad (0.0019, 0.0041, 0.0071 and 0.0212%), respectively at 120 HAF. However, the toxicity for tobacco population in 2012 was flubendiamide (0.0012) > chlorantraniliprole (0.0013) > spinetoram (0.0079) > spinosad (0.0115%) at 120 HAF.

The toxicity of diamide and spinosyn insecticides against 3rd instar larvae of *S. litura* was determined by Potter's tower method by Karuppaiah et al. (2017) for population of Sonepat, Delhi and Varanasi. The order of toxicity against Sonepat and Delhi population was chlorantraniliprole (LC_{50} = 0.0004 and 0.0002) > spinosad (0.0079 and 0.0019) > flubendiamide (0.0122 and 0.0121%), respectively, however against Varanasi population it was chlorantraniliprole (0.0001) > flubendiamide (0.0110) > spinosad (0.0181%) at 48 HAE. The order of toxicity was flubendiamide (LC_{50} = 0.0040) > chlorantraniliprole (0.0044%) against third instar larvae of Ludhiana population by topical application method (Dhawan et al*.,* 2007). In our study the order of toxicity was chlorantraniliprole (LC_{50}= 0.00086) > spinetoram (0.003) > flubendiamide (0.005) > spinosad (0.02%) at 48 HAE by larval atomization method and chlorantraniliprole (LC_{50}= 0.0042 and 0.0009) > spinetoram (0.0047 and 0.0032) > spinosad (0.0316 and 0.0182%) at 24 and 36 HAF by leaf smear method against the 2nd instar (4d old) larvae of Pantnagar population of *S. litura* (Table 1).

The contact and stomach toxicities of diamide and spinosyn insecticides against third instar (7±1 d old) larvae were determined by various workers. The higher stomach than contact toxicity was recorded for chlorantraniliprole and spinosad against Sonepat population by leaf dip (LC_{50} = 0.0001 and 0.0071%) and direct spray method (LC_{50} = 0.0004 and 0.0079%)**,** respectively, however higher contact toxicity was reported for flubendiamide (LC_{50} = 0.0122% by direct spray) than stomach toxicity (LC_{50} = 0.0145% by leaf dip method) and the order of toxicity was chlorantraniliprole > spinosad > flubendiamide by both the methods (Karuppaiah and Srivastava, 2013). The higher stomach than contact toxicity of chlorantraniliprole, flubendiamide and spinosad was observed against Bapatla population by leaf dip (LC_{50} = 0.001, 0.002 and 0.005%) and topical application method (LC_{50} = 0.005, 0.005 and 0.01%), respectively and order of toxicity was chlorantraniliprole > flubendiamide > spinosad by leaf dip and chlorantraniliprole = flubendiamide > spinosad by topical application method (Kanth et al., 2016).

Higher stomach than contact toxicity was also observed for flubendiamide and spinosad against Palampur population at third and fifth instar of *S. litura* by leaf dip and Potters's tower method. The LC_{50} against third instar of *S. litura* for spinosad was 0.00639 and 0.0517% at 72 HAF/HAE and for flubendiamide was 0.0104 and 0.0859% at 24 HAF/HAE, (Sharma and Pathania, 2015); and the LC_{50} against fifth instar larvae of *S. litura* for spinosad was 0.025 and 0.2056% at 72 HAF/HAE and for flubendiamide was 0.0312 and 0.1249% at 24 HAF/HAE (Sharma and Pathania, 2014), respectively by leaf dip and topical application methods**.** In present study higher stomach action compared to contact action was observed for chlorantraniliprole (1.24 times higher stomach (LC_{50} = 0.0009%) than contact toxicity (LC_{50} = 0.00112%) at 36 HAF/HAE), spinetoram (2.57 and 2.65 times more stomach (LC_{50}= 0.0047 and 0.0032%) than contact toxicity (0.0121 and 0.0085%) at 24 and 36 HAF/HAE, respectively) and spinosad (1.24 and 1.82 times more stomach (LC_{50}= 0.0182 and 0.0110%) than contact toxicity (0.0225 and 0.02%) at 36 and 48 HAF/HAE, respectively) against 4d old larvae of *S. litura* of Pantnagar population (Table 1).

The toxicity of flubendiamide and spinosad against third instar of *S. litura* was also reported to vary from host to host by Toke et al. (2015). The LC_{50} of flubendiamide was 0.000031 and 0.000134% and spinosad was 0.00002 and 0.00000029% on castor and cotton, respectively. The toxicity of diamide and spinosyn insecticides have been tested against third instar larvae of *Spodoptera frugiperda* (J E Smith) by Hardke *et al.* (2011) at Department of Entomology, Louisiana state University, Baton Rouge, LA. Spinetoram (LC_{50} = 0.066 µg/ml) and chlorantraniliprole (0.068 µg/ml) were found to be more toxic followed by spinosad (0.557 µg/ml) and flubendiamide (0.930 µg/ml) by diet incorporation method.

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