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Studies on dosage-mortality response of diamondback moth, *Plutelaxylostella* L. larvae to different insecticides in combination with Streptocycline

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ABSTRACT

The toxicity of insecticides with bactericide and individual insecticides to test insect was quantified by adopting leaf dip bioassay method and the compatibility was assessed based on the median lethal concentrations(LC_{50}). The results clearly revealed that in some combinations toxicity was enhanced while in others the toxicity was lowered. Among seven insecticides in combination with bactericide, streptocycline tested for efficacy, four insecticides (chlorantraniliprole, flubendiamide, Proton® and profenophos) showed synergistic effect (SF 1.18, 1.07, 1.03 and 1.01, respectively), whereas three insecticides (indoxcarb, novaluron and Hamla®) were antagonistic (SF 0.83, 0.89 and 0.93, respectively) against *P. xylostella* larvae.

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INTRODUCTION

Among various insect pests, diamondback moth is the most serious in causing economic loss on cole crops. Though, the moth originated in the Mediterranean area, it has surpassed all the natural barriers and is believed to have become a cosmopolitan pest (Meyriche, 1928). Diamondback moth, *P. xylostella* is one of the most destructive pests of cruciferous vegetables in the world and has been reported from at least 128 countries. In recent years, DBM acquired serious dimension and has become major limiting factor for successful cultivation of cabbage in India (Sexena *et al.*, 1989, Srinivasan and Krishnamoorthy, 1991). Diamondback moth is known to cause yield loss in cabbage from 31 per cent (Abraham and Padmanabhan, 1968) to 100 per cent (Cardleron and Hare, 1986) and the annual cost for managing this pest is estimated to be US $1 billion (Talekar, 1992).

The number of chemicals involved in plant protection is too many and the information on compatibility of individual chemical is scattered in the literature. Common growers find difficulty in ascertaining the compatibility of agro-chemicals. Hence, based on experience, Gray (1914) prepared a chart showing compatibility of some insecticides, fungicides and bactericides. Later several charts were developed or updated by Frear (1979), Gruzdyed *et al.* (1983) for the chemicals in use with additional information regarding incompatibility under certain crops, season, aging of mixtures and many other factors. Later, Baicu (1980) suggested studying the compatibility in different stages including determination of chemicals and physical properties, biological activity of compounds, field tests of effectiveness, phytotoxcity and yield after treatment. Several insecticide molecules are available in market, but many of them are not tested for the compatibility or recommended by reputed research institutes. Hence, it is necessary to investigate the compatibility of the most common agro-chemicals with respect to insect pest management in cabbage ecosystem. Therefore, the present research was planned with the following objectives :

– Insecticidal property of bactericide.
– Influence of bactericide on the bio-efficacy of insecticides.

MATERIAL AND METHODS

Seven insecticides and one bactericide were selected for the expirements and these are presented in Table A.

Insecticidal action of Streptocycline against *P. xylostella* :

A study was carried out to know the insecticidal property of selected bactericide. The *P. xylostella* larvae collected from cabbage field around Chikmagalur were reared to first generation on mustard seedlings. The third instar larvae were exposed to different concentrations of bactericide streptocycline.

Bioassay :

For every insecticide and bactericide mixture and individual insecticide, keeping the company's recommendation or farmer's practice as the base, five concentrations in geometric progression were used for each bioassay experiment. For every concentration, three replications of 30 third instar larvae were maintained and the leaves treated with water served as control. Fresh and uniform sized cabbage leaves were dipped in insecticide dilutions for thirty seconds and dried under room temperature. The cut ends of petioles of treated leaves were provided with wet cotton wads to retain the vigour. The treated cabbage leaves were placed in Petridishes. Thirty early third instar larvae of *P. xylostella* were released on treated leaves in each Petridish. The treated larvae were maintained at room temperature and the mortality was recorded at 6, 12, 24, 36, 42 and 48 hours after the treatment.

Observed mortality data were converted to percentages and corrected for control mortality according to Abbott (1925). Observed mortality data were converted to percentage and were subjected to probit analysis (Finney, 1971) for obtaining regression equations for dosage mortality response and to determine the LC_{50} values.

Table A : Details of agrochemicals selected for the bioassay

Sr. No.	Common name	Chemical name	Trade name and formulation	Manufacturing company
Insecticides				
1.	Chlorantraniliprole	3-bromo-N-[4-chloro-2-methyl-6-[(methylamino)carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide	Coragen®18.5 SC	E.I. Dupont India Pvt. Ltd., Gurgaon, Haryana
2.	Flubendiamide	3-iodo-N'-(2-mesyl-1,1-dimethylethyl)-N-{4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-o-tolyl}phthalamide	Fame®480 SC	Bayer Crop Science India Ltd., Mumbai
3.	Chlorpyriphos + Cypermethrin	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate +	Hamla®505 EC	Gharda Chemicals Ltd., Mumbai
4.	Proton	Cocktail of botanicals *viz.*, Langdu root extract (StellerachamaejasmeL.)- 2.9 (%), CGL extract- 1.50 (%), Brassica campestrisL.- 0.5 (%), Eugenol- 9.0 (%), Siberian cocklour fruit extract-10 (%), Trace elements- 10 (%) (Venkateshalu *et a*l., 2009)	Proton®	United Crop Care, Mumbai
5.	Indoxacarb	Methyl(S)-N-[7-choloro-2,3,4a,5-tetrahydro-4a-Hethoxycarbomyl) indeno [1,2-e]-[1,3,4] oxadiazin-2-ylcarbonyl]-4-(trifluromethoxy)carinilat	Avaunt® 14.5 EC	E.I. Dupont India Pvt. Ltd., Gurgaon, Haryana
6.	Novaluron	1- ∂3-Chloro-4-(1, 1,2-trifluro-2-trifluoromethoxyethoxy) Phenyl	Rimon® 10 EC	Indofil Chemical Company, Mumbai
7.	Profenophos	O-(4-Bromo-2-chlorophenyl)-O-ethyl-S-propyl phosphorothioate	Curacron® 50 EC	Syngenta India Ltd., Mumbai
Bactericide				
1.	Streptocycline	s-Triazine,hexahydro-1,3,5-tris(2,2,3-trichloropropionyl)-(8CI)	Streptocycline®	Hindustan Antibiotics Ltd., Pimri, Pune

Co-toxicity co-efficient (CTC) and synergistic factor (SF) :

The co-toxicity co-efficient (Sarup *et al.*, 1980) and synergistic factor (Kalayanasundaram and Das, 1985) for mixed formulation were calculated after calculating the LC_{50} and LC_{99} for each combination.

$$\text{Co-toxicity co-efficient} = \frac{\text{Toxicity of insectiside alone}}{\text{Toxicity of insecticide + Bactericide}} \times 100$$

$$\text{Synergistic factor} = \frac{\text{Toxicity of insecticide alone}}{\text{Toxicity of insecticide + Bactericide}}$$

A SF value > 1 indicates synergism and an SF value < 1 indicates antagonism.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Insecticidal properties of Streptocycline :

Certain compounds are marketed as bactericides to be used exclusively against bacterial diseases. However, many workers have reported such compounds to possess insecticidal activity also. In this line a study was undertaken to evaluate the insecticidal properties of selected bactericide against third instar larvae of *P. xylostella* in the laboratory at five concentrations. The results revealed that bactericide Streptocycline possessed insecticidal properties and the mortality was significantly more at higher concentrations. Among the bactericide, Streptocycline at 250 ppm caused the highest mortality of 22 per cent (Table 1). Though, no similar studies have been reported in the literature. The available literature envisaged that bactericide namely oxytetracycline possessed insecticidal action on the aphid, *Acyrthosiphon pisum* (Frohlich, 1990). Vekateswarlu and Ramapandu (1992) reported that streptomycine and tetracycline possessed insecticidal actions on citrus leaf miner, *Phyllocnistiscitrella*. Broad spectrum bactericide, Ampicillin sulphate at lower concentrations had effect on soil arthropods, *Anoplodesmus saussurei* and *Porcelliolaevis* (Pramanik *et al.*, 2000). Jim *et al*. (2012) reported that application of bactericides in fruit orchards had affected on European honey bees.

Influence of Streptocycline on the bio-efficacy of insecticides :

Compatibility of pesticides is the behaviour of combination with reference to active component that is, whether it has maintained, reduced or potentiated its insecticidal activity. The changes in chemical contents of individual components, their respective characters, formulation, qualities etc., occurring in the mixtures have not been studied deeply for majority of chemicals. If a new chemical discovered then studying its behaviour in the presence of other chemicals is equally important to exploit utilization of more than one chemical at a time in combination. However, only few attempts have been made to study the compatibility problem in the light of increase in number of chemicals. Hence, attempts were made to study the compatibility of insecticides in combination with bactericide under laboratory conditions by using third instar larvae of *P. xylostella*as test insect. The toxicity of insecticides with bactericide and individual insecticides to test insect was quantified by adopting leaf dip bioassay method and the compatibility was assessed based on the median lethal concentrations(LC_{50}).

The results clearly revealed that in some combinations toxicity was enhanced while in others the toxicity was

Table 1 : Per cent mortality of *P. xylostella* larvae against Saaf® and Streptocycline formulations

Chemicals	Concentration (ppm)	Per cent larval mortality at different hours after treatment	
		24 (h)	48 (h)
Saaf® (Carbendazim + Mancozeb)	1875	18.89 (25.72)	28.89 (32.49)
	1500	13.33 (21.39)	22.22 (28.09)
	1125	6.67 (14.89)	14.44 (22.27)
	750	3.33 (10.47)	10.00 (18.44)
	375	3.33 (10.47)	5.56 (13.42)
Streptocycline	250	13.33 (21.39)	22.22 (28.09)
	200	10.00 (18.44)	13.33 (21.39)
	150	6.67 (14.89)	10.00 (18.44)
	100	3.33 (10.47)	3.33 (10.47)
	50	3.33 (10.47)	3.33 (10.47)
Control	0.00	0.00 (0.00)	0.00 (0.00)
S.E. ±		0.27	0.72
C D (P=0.05)		1.007	2.41

Figures in parenthesis are arc sine transformed values lowered. The median lethal concentrations of seven insecticides *viz.*, chlorantraniliprole, flubendiamide, novaluron, indoxacarb, Proton®, Hamla® and profenophos were 7.21, 13.99, 74.25, 95.47, 391.74, 420.72 and 907.68 ppm, respectively (Table 2).

Later the extent of loss or gain in toxicity of test insecticides when mixed with bactericide was quantified based on the median lethal concentrations(LC_{50}), co-toxicity co-efficient and synergistic factor to ascertain the compatibility. Some combinations were more toxic and some were less toxic to the test insect compared to insecticides alone. As noticed from the earlier trials, inherent insecticidal properties possessed by the bactericide contributed to the larval mortality. Thus, additive effect of the bactericide with insecticides accounted for increase in mortality over insecticides alone and the antagonistic effect may be due to in-sensitization of insecticidal target sites, which resulted in decrease in the susceptibility of pest species involved.

In combination with streptocycline, LC_{50} values of novaluron, indoxacarb and Hamla® comparatively increased to 81.60, 114.09 and 450.61 ppm, respectively. Streptocycline interacted antagonistically with novaluron (SF 0.89), indoxacarb (SF 0.83) and Hamla® (SF 0.93) where the LT_{50} values of novaluron (SF 0.95), indoxacarb (SF 0.94) and Hamla® (SF 0.92) comparatively increased to 42.62, 34.09 and 30.43 h, respectively. However, in combination with streptocycline, LC_{50} of four insecticides (chlorantraniliprole, flubendiamide, Proton® and profenophos) decreased. These four insecticides when combined with streptocycline, the mortality were increased as compared when insecticides used alone. In combination with streptocycline, the LC_{50} values of four insecticides was Chlorantraniliprole(6.10 ppm) (SF 1.18), flubendiamide (13.06 ppm) (SF 1.07), Proton®(376.90 ppm) (SF 1.03) and profenophos (890.26 ppm) (SF 1.01) which is presented in Table 2.

No specific studies are available in the literature involving the compatibility of above chemical combinations against *P. xylostella* for comparison. However, Raju and Rao (1983) found Agrimycin-100 (streptomycin + oxytetracycline) @ 200 ppm + copper oxychloride @ 0.3 per cent + phosalone @ 0.1 per cent applied in late September and 3 more times at 15 days interval gave good control of *Scirtothrips dorsalis* (Hood) and *Hemi tarsonemus latus* (Banks). Vekateswarlu and Ramapandu (1992) reported on compatibility bactericide with insecticides in the control of citrus canker and leaf miner in acidlime. Plots sprayed with streptomycin + tetracycline + copper oxychloride + fenvalerate recorded lowest intensity of canker and *Phyllocnistis citrella.* Pramanik *et al.* (2000) studied on cumulative effect of bactericide and insecticides on detritivore soil arthropods. The combined effects of ampicillin sulphate (bactericide) with the insecticides (endosulfan 35% EC and

Table 2 : The dosage-mortality response of *P. xylostella* larvae to selected insecticides and in combination with Streptocycline

Treatments	χ^2	Regression equation Y = a ± bx	LC50 (ppm)	Fiducial limits at 95 per cent (ppm)	LC99 (ppm)	CTC	SF	Type of action
Chlorantraniliprole	5.09	2.98±3.47x	7.21	5.71-8.55	33.69	118.19	1.18	Synergistic
Chlorantraniliprole + Streptocycline	5.99	5.73±1.17x	6.10	5.11-8.01	32.11			
Flubendiamide	5.36	3.59±3.14x	13.99	4.18-20.69	77.06	107.12	1.07	Synergistic
Flubendiamide + Streptocycline	5.67	5.89±1.06x	13.06	5.07-30.01	76.56			
Novaluron	1.33	22.13±11.83x	74.25	70.51-77.29	116.77	89.83	0.89	Antagonistic
Novaluron + Streptocycline	4.21	16.01±10.28x	81.60	79.89-90.65	130.33			
Indoxacarb	3.44	10.54±5.32x	95.47	82.13-106.66	261.00	83.67	0.83	Antagonistic
Novaluron + Streptocycline	5.11	11.99±5.02x	114.09	87.32-130.40	298.38			
Proton®	3.45	13.82±5.33x	391.74	337.02-437.63	1070.27	103.93	1.03	Synergistic
Proton® + Streptocycline	3.94	11.63±5.07x	376.90	325.09-422.76	1056.36			
Hamla®	2.82	17.12±6.52x	420.72	378.84-460.18	956.20	93.33	0.93	Antagonistic
Hamla® + Streptocycline	4.98	18.36±7.11x	450.61	398.84-489.13	1006.90			
Profenophos	1.65	19.17±6.48x	907.68	798.81-993.35	2074.43	101.91	1.01	Synergistic
Profenophos + Streptocycline	3.40	18.61±6.98x	890.26	770.89-990.04	2050.75			

heptachlor 20 % EC) were highly inhibitory. Haseeb *et al.* (2000) reported on pesticidal effects of two bactericides (kasugamycin and oxolinic acid) in combination of three chitin synthesis inhibitors (chlorfluazuron, flufenoxuron and teflubenzuron), which were non toxic on *Diadegma semiclausum* Hellen. Jim *et al*. (2012) reported that application of bactericide streptomycine in combination with fungicides and biological in fruit orchards had affected on European honey bees. From the above discussion, present investigations on compatibility can be concluded that insecticides tested were compatible with streptocycline except the novaluron, indoxcarb and Hamla®.

Table 3 : Compatibility for agro-chemicals tested against *P. xylostella larvae*

Agro-chemicals	Streptocycline
Chlorantraniliprole	+
Flubendiamide	+
Novaluron	-
Indoxacarb	-
Proton®	+
Hamla®	-
Profenophos	+

+ = Compatible, - = Incompatible

Compatibility :

From the results of *in vitro* experiments on the interaction of agro-chemicals, a compatibility has been prepared and presented in the Table 3. It may be said that insecticides namely, chlorantraniliprole, flubendiamide, Proton® and profenophos in combination with streptocycline were clearly compatible against test insect. However, novaluron, indoxacarb and Hamla® in combination with streptocycline were clearly incompatible against test insect.

Interestingly, some of the combinations which behaved differently against test insect are to be viewed differently on the basis of desirable action exhibited by the chemicals in the mixture. A mixture of insecticide in combination with bactericide may cause desirable effect on insect or *vice-versa*. If a mixture intended to suppress insect, failed to accomplish and cause adverse effects, such a combination may be rejected. For example, novaluron, indoxacarb and Hamla® in combination with streptocycline resulted in lowering of toxicity to test insect. The literature review also highlighted such variations in compatibility of pesticides and this may be due to variability in test organism or crop. In most of the studies, where compatibility among agro-chemicals tried were too low to exert the desirable effects. Hence, further combination is needed regarding compatibility and bio-efficacy and compatibility of various pesticidal mixtures at their recommended doses in the laboratory and under field conditions. These combinations can be evaluated for phytotoxicity in field conditions. Baseline studies can be undertaken for individual insecticides, so that the folds of resistance can be worked out.

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