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IN VITRO COMPATIBILITY ASSESSMENT OF *BACILLUS THURINGIENSIS* WITH COMMONLY USED INSECTICIDES

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ABSTRACT

In the present investigation efforts were made to study the compatibility between *Bacillus thuringiensis*, entomopathogenic bacterium and insecticides at different lethal and sublethal concentrations (R, R/2, R/4, R/8 and R/16) under *in-vitro* conditions. Compatibility study showed that as the concentration of the insecticides increased bacterial growth decreased indicating reduced compatibility over increased concentration. *Bt* was significantly highly compatible (Compatibility index 1) with chlorantraniliprole 18.5% SC at all the tested concentrations as compared to other treatments. Maximum percent inhibition was observed at recommended dose (46ppm) of chlorantraniliprole 18.5% SC (21.663). However, lambda cyhalothrin 5%EC and neem 10000ppm were found non compatible with *Bt* at all the concentrations with compatibility index 3.

Keywords **:** *Bacillus thuringiensis*, Chlorantraniliprole, Lambda cyhalothrin, Compatibility.

Introduction

Bacillus thuringiensis is gram positive, soil dwelling bacteria, produces the toxins which act on a greater number of insects. There are many types of *Bt*, each target on different group of insects includes caterpillars, moths, mosquitoes, beetles etc.

Present day's *B. thuringiensis (Bt)* is most successful bacterial microbial insecticide with the world-wide application for protection of agricultural crops, forest trees and human health. Worldwide commercialization of *Bacillus thuringiensis sub sp. kurstaki* was preceded by its development for the management of many insect pest (Van Frankenhuyzen, 2013) *Bacillus thuringiensis (Bt)* has long been recognized as a valuable bio pesticide due to its ability to produce insecticidal proteins lethal to specific groups of insect pests. The widespread adoption of *Bt*-based formulations in integrated pest management (IPM) strategies has led to substantial reductions in chemical pesticide use and associated environmental risks. However, the efficacy of *Bt* formulations can be influenced by various factors, including interactions with chemical insecticides commonly used in agriculture. The in vitro compatibility of *Bacillus thuringiensis (Bt)* with insecticides is a critical aspect of integrated pest management (IPM) strategies aimed at controlling agricultural pests while minimizing environmental impacts. In vitro studies allow researchers to assess the interactions between *Bt* formulations and chemical insecticides under controlled laboratory conditions, providing valuable insights into their combined efficacy and potential synergistic or antagonistic effects.

Understanding the compatibility between *Bt* and chemical insecticides is essential for optimizing pest management practices and minimizing the risk of resistance development. In vitro compatibility studies provide valuable insights into the potential interactions between these agents at the molecular level, informing decisions regarding their concurrent or sequential use in pest control programs.

Overall, in vitro compatibility studies provide valuable insights into the potential synergies and trade-offs associated with integrating *Bt* and chemical insecticides in pest management programs. By elucidating the factors that influence compatibility, researchers can optimize the design and implementation of IPM strategies that effectively control pests while minimizing environmental risks.

Materials and Methods

Procurement of pure culture of *B. thuringiensis var. kurstaki*

The pure culture of *B. thuringiensis* was procured from Division of Entomology, IARI New Delhi.

Nutrient agar composition

Ingredients	Quantity
Beef extract	2.0 g
Peptone	5.0 g
Agar	20.0 g
Sodium chloride	5.0 g
Yeast extract	5.0 g
Distilled water	1000 ml

Note: Nutrient agar without agar called as nutrient broth

Mass multiplication of *B. thuringiensis* by growing of *Bt* on nutrient agar media from the cells mixed with acetone powder, powder of acetone containing *B. thuringiensis* cells was dissolved in a drop of distilled sterilized water under the laminar air flow on a sterilized watch glass. Then dissolved cells spread over the sterilized nutrient agar media by streaking method with the help of heat sterilized bacterial loop. These culture plates were kept inside BOD incubator in stable condition with temperatures maintained at 28 ± 5 ^{0}C cross check it and RH 85 % (Vimala, 2018).

For evaluating compatibility between insecticides and *Bt*, double strength concentrated nutrient broth was prepared. Insecticides (Azadirachtin 10000ppm, Chlorantraniliprole 18.5SC, Lambda cyhalothrin 5EC), at field recommended dose *i.e.* 50 % RD, 25 % RD, 12.5 % RD, 6.25 % RD and RD each at 4 replications were evaluated using poison food (media) technique. The poisoned medium was inoculated with 0.1ml of spore suspension ($1.2x10^{7}$ cells ml^{-1}) with the help of micropipette under aseptic conditions. Un-inoculated poisoned medium was considered as control and blank. Absorbance was noted down at 625 nm wavelength using spectrophotometer. Blank reading was calibrated to read zero and treatments absorbance was recorded at 0, 2, 4, 6, 12, 24 and 36 hrs, after inoculation. Growth pattern of *Bacillus thuringiensis var kurstaki* was observed by measuring OD spectrophotometrically at 625 nm in comparison to control (Harley & Prescott., 1996; Li *et al*., 2012)

The compatibility of *B. thuringiensis* with chemicals insecticides estimated under following index.

Per cent inhibition over zero hours	Compatibility index.
0-25	1
25-50	2
50-75	3
75-100	4
>100	5

Results and Discussion

Compatibility refers to the ability to combine different pesticides without causing physical or chemical interactions or changes, resulting in improved biological effects. For the integration and concurrent use of chemical and biological pest control technologies in an Integrated Pest Management (IPM) programme, compatibility studies are required.

The result showed that all concentrations of chlorantraniliprole 18.5SC were found compatible with *B. thuringiensis var kurstaki,* with compatibility index 1.

The values in the table 2, it clearly showed that reducing concentration of lambda cyhalothrin 5EC insecticide there was an increase in absorbance value over a period of time as compared to non-poisoned (Control) media inoculating *Bacillus thuringiensis.* The mean maximum percent inhibition (63.63) was recorded at recommended dose of (50 ppm). However, all other concentrations showed more than 50 percent inhibition in bacterial population over control, as all concentrations of lambda cyhalothrin had compatibility index 3. The data in the table 3 revealed that azadirachtin 10000ppm affected the growth of *B. thuringiensis*, with the compatibility index of 3 in all concentrations. The data clearly indicated that azadirachtin inhibited the *B. thuringiensis* colony and had antagonistic effect with *B. thuringiensis.*

Table 1: Compatibility of *Bacillus thuringiensis* Chlorantraniliprole 18.5SC.

CHLORANTRANILIPROLE 18.5SC																	
	ABSORBANCE HOURS (B)								PERCENT INHIBITION IN BACTERIAL POPULATION HOURS (B)								
CONC (ppm)(A)	0	2	4	6	12	24	36	Mean	0	2	4	6	12	24	36	Mean	COMPATI-BILITY INDEX
46	0.012	0.019	0.038	0.052	0.082	0.168	0.244	0.088	23.39	34.718	27.255	20.883	16.085	14.748	14.56	21.663	1
23	0.012	0.02	0.041	0.055	0.085	0.172	0.251	0.091	23.048	30.343	21.028	17.095	13.013	12.01	15.433	18.853	1
11.5	0.011	0.022	0.044	0.056	0.087	0.175	0.256	0.093	28.265	23.32	16.738	14.818	10.978	11.06	10.293	16.496	1
5.75	0.012	0.023	0.046	0.058	0.089	0.178	0.258	0.095	21.83	18.975	11.485	12.163	8.925	9.405	9.793	13.225	1
2.87	0.012	0.026	0.048	0.06	0.093	0.181	0.261	0.097	21.83	11.303	8.118	8.37	5.355	7.75	8.658	10.198	1
CONT	0.015	0.029	0.052	0.066	0.098	0.197	0.286	0.106									
Mean	0.012	0.023	0.045	0.058	0.089	0.178	0.259		23.673	23.732	16.925	14.666	10.871	10.995	11.747		
Factors	**C.D.**		**SE(m)**						**Factors**	**C.D.**		**SE(m)**					
Factor (A)	0.06		0.002						Factor(A)	1.993		0.71					
Factor (B)	0.012		0.004						Factor(B)	2.358		0.84					
Factor (A X B)	0.002		0.001						Factor (A X B)	5.273		1.878					

Table 2: Compatibility of *Bacillus thuringiensis* with lambda cyhalothrin 5EC.

LAMBDA CYHALOTHRIN 5EC																	
	ABSORBANCE HOURS(B)								PERCENT INHIBITION OF BACTERIAL POPULATION HOURS(B)								
CONC (ppm)	0	2	4	6	12	24	36	Mean	CONC.	0	2	4	6	12	24	36	Mean	COMPTI-BILIY INDEX
50	0.01	0.015	0.018	0.028	0.034	0.05	0.066	0.031	50	43.851	55.255	67.717	61.832	68.108	72.58	76.132	63.639	3
25	0.008	0.017	0.022	0.032	0.04	0.056	0.071	0.035	25	54.906	49.167	59.511	56.682	61.679	69.891	74.128	60.852	3
12.5	0.009	0.019	0.025	0.034	0.044	0.06	0.075	0.038	12.5	52.202	42.297	54.044	53.922	58.584	67.736	72.673	57.351	3
6.25	0.01	0.023	0.029	0.035	0.046	0.062	0.078	0.04	6.25	42.389	31.699	48.163	51.533	56.683	66.532	71.674	52.668	3
3.125	0.01	0.025	0.031	0.037	0.047	0.062	0.081	0.042	3.125	46.474	25.472	44.052	49.468	55.227	66.934	70.58	51.172	3
CONT	0.018	0.033	0.055	0.073	0.105	0.186	0.275	0.106										
Mean	0.011	0.022	0.03	0.04	0.052	0.079	0.107		**Mean**	47.964	40.778	54.697	54.687	60.056	68.735	73.037		
Factors	**C.D.**	**SE(m)**							**Factors**	**C.D.**	**SE(m)**							
Factor(A)	0.006	0.002							Factor(A)	1.651	0.588							
Factor(B)	0.012	0.004							Factor(B)	1.953	0.696							
Factor (A X B)	0.003	0.001							Factor (A X B)	4.368	1.555							

NEEM AZADIRACHTIN 10000ppm																	
	ABSORBANCE HOURS(B)								PERCENT INHIBITION OF BACTERIAL POPULATION HOURS(B)								
CONC (ppm)(A)	0	2	4	6	12	24	36	Mean	0	2	4	6	12	24	36	Mean	COMPATI-BILITY INDEX
10000	0.009	0.01	0.012	0.014	0.023	0.041	0.052	0.023	41.963	66.771	76.281	81.466	77.881	78.766	81.833	72.137	3
5000	0.008	0.01	0.014	0.016	0.026	0.043	0.053	0.024	48.678	66.049	73.337	79.465	75.175	77.737	81.48	71.703	3
2500	0.008	0.012	0.016	0.017	0.029	0.045	0.054	0.026	45.449	60.982	68.932	78.113	71.5	76.835	80.864	68.954	3
1250	0.008	0.012	0.017	0.018	0.033	0.047	0.056	0.027	43.421	60.037	66.476	76.788	68.337	76.061	80.247	67.338	3
625	0.009	0.013	0.018	0.02	0.035	0.048	0.058	0.029	36.811	55.856	65.012	74.13	66.147	75.16	79.63	64.678	3
CONT	0.015	0.03	0.051	0.076	0.103	0.194	0.284	0.107									
Mean	0.009	0.014	0.021	0.026	0.041	0.07	0.093		**Mean**	43.264	61.939	70.008	77.992	71.808	76.912	80.811		
Factors	**C.D.**		**SE(m)**						**Factors**	**C.D.**		**SE(m)**						
Factor(A)	0.009		0.003						Factor(A)	2.173		0.774						
Factor(B)	0.018		0.006						Factor(B)	2.571		0.916						
Factor (A X B)	0.002		0.001						Factor (A X B)	N/A		2.048						

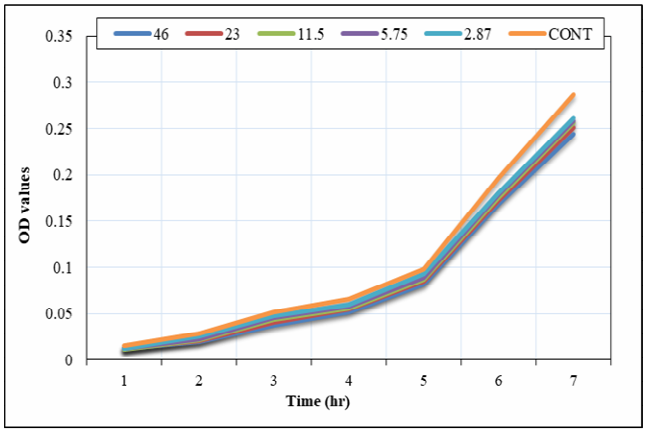

Fig. 1: Effect of chorantraniliprole 18.5 SC on growth of *Bacillus thuringiensis*

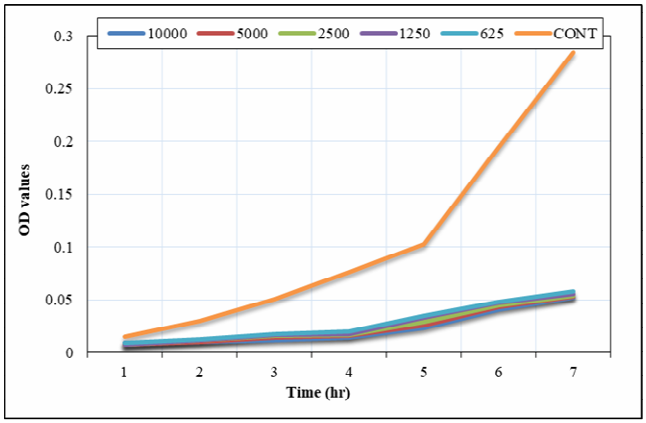

Fig. 2 : Effect of lambda cyhalothrin 5EC on growth of *Bacillus thuringiensis*

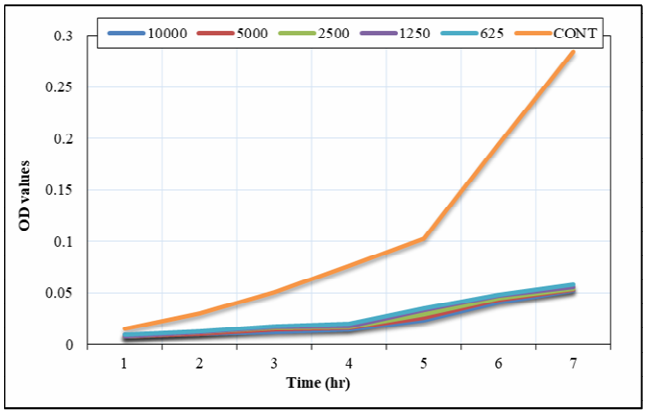

Fig. 3 : Effect of Azadirachtin on growth of *Bacillus thuringiensis*

Conclusion

Study on compatibility of *B. thuringiensis* with chemicals revealed that azadirachtin treated media does not promote growth of *B. thuringiensis* at any of its lethal and sublethal doses and showed antagonistic effect on multiplication of bacterial population. Chlorantraniliprole 18.5SC found highly compatible as there was no inhibition seen at any of their lethal and sublethal doses. An antagonistic interaction observed at recommended dose and half the recommended dose of lambda cyhalothrin 5EC, while at other lower concentrations slight compatibility was recorded. *Bacillus thuringiensis* in combination with these insecticides can be used as an effective management strategy in IPM.

Competing Interests

Authors have declared that no competing interests exist

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