



In vitro Interactions of *Bacillus thuringiensis* with Biorationals and Synthetic Insecticides

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Abstract: Managing crop pests using microorganism is one of the key component and eco-friendly alternative to reduce chemical inputs in integrated farming systems. Biorationals and chemical pesticides can exert either antagonistic or synergistic effects on the efficacy of entomopathogens potentially impacting natural epizootic outbreaks. The present study was therefore undertaken to evaluate the in vitro compatibility between *Bacillus thuringiensis* with biorationals and synthetic insecticides. The findings revealed that *B. thuringiensis* MTCC 9025 recorded minimum growth percent reduction over control with *Beauveria bassiana* PCP-6 (5.33%) and *B. bassiana* MTCC 6097 (12.76%) and were highly compatible. However, *B. thuringiensis* MTCC 8481 recorded maximum growth percent reduction and was least compatible. All *B. thuringiensis* isolates were compatible with azadirachtin 5% w/w (@0.2ml/l and @0.4ml/l) and synthetic insecticide Chlorantraniliprole 18.5% SC (@0.2ml/l and @0.4ml/l). However, all *B. thuringiensis* isolates were incompatible with Emamectin benzoate 5% SG and exhibited maximum growth percent reduction over control. The results indicated that *B. thuringiensis* isolates could be successfully integrated into Integrated Pest Management strategies with *Beauveria bassiana* isolates, azadirachtin 5% w/w and chlorantraniliprole 18.5% SC.

Keywords: Azadirachtin, *Bacillus thuringiensis*, *Beauveria bassiana*, Chlorantraniliprole, Emamectin benzoate, Compatibility

Microbial pest management is an ecofriendly method to reduce chemical inputs in agricultural systems. Indiscriminate and excessive use of chemical insecticides has negative effects and there is need to reduce their use and introduce various ecofriendly biorationals. Various biocontrol agents viz. entomopathogens, botanicals, natural enemies are exploited for the management of crop pests, may have antagonistic or synergistic effects on the potentiality of entomopathogens and may influence natural epizootics (Garcia-Riano et al 2022). Entomopathogens like *B. thuringiensis*, *Metarhizium anisopliae*, *M. rileyi*, *B. bassiana* are mainly exploited for management of insect pest (Matcha et al 2021). *B. thuringiensis* is important soil bacterium that produces a variety of bioactive compounds, including insecticidal protein and thuringinsin (Savini and Fazii 2016). The entomopathogenic fungus, *B. bassiana* naturally exists in the soil and has good epizootic potential, infecting the insect by adhesion to their cuticle by adhesion proteins. Entomopathogenic fungi act as immunosuppressive compounds, leading to mycosis of crop insects (Shashikala et al 2023). Botanicals offer a safer alternative, posing minimal risk to humans and allowing easy combination with various other biological agents. Neem based formulations are environmentally safe and disrupts the insect growth and development and prevents insect resistance development (Bharti et al 2023). Compatibility studies between various bioagents are important as incompatible biocontrol agents may negatively affect the efficacy of integrated pest

management programme (Sarkhandia et al 2023). Therefore, it is important to determine the interactions of *B. thuringiensis* with other biorationals to maximize their combined efficacy.

MATERIAL AND METHODS

Five *Bacillus thuringiensis* isolates viz. *B. thuringiensis* MTCC 9025, *B. thuringiensis* MTCC 868, *B. thuringiensis* MTCC 8481, *B. thuringiensis* MD4, *B. thuringiensis* (commercial formulation) and two *Beauveria bassiana* isolates *B. bassiana* MTCC 6097 and *B. bassiana* PCP-6 along with azadirachtin 5% w/w, emamectin benzoate 5% SG and chlorantraniliprole 18.5% SC were selected for compatibility studies. Three *B. thuringiensis* isolates viz. *B. thuringiensis* MTCC 9025, *B. thuringiensis* MTCC 868, *B. thuringiensis* MTCC 8481 and one *B. bassiana* MTCC 6097 procured from Institute of Microbial Technology (IMTECH), Chandigarh were grown on their respective growth media and maintained at 4°C till further use.

Five *Bacillus thuringiensis* were studied for compatibility with two isolates of *B. bassiana* viz. *B. bassiana* MTCC 6097 and *B. bassiana* PCP-6 according to dual culture technique by Udayababu and Zacharia (2021). In vitro compatibility of *B. thuringiensis* with the neem-based formulation (azadirachtin 5% w/w) and synthetic insecticides (emamectin benzoate 5% SG and chlorantraniliprole 18.5% SC) were also studied. Neem based formulation (azadirachtin 5% w/w) (@ 0.2 and @ 0.4ml/l), emamectin benzoate 5% SG and

chlorantraniliprole 18.5% SC (0.2 and 0.4ml/l respectively), were added individually to the sterilized Luria Bertani agar supplemented with *B. thuringiensis* and this supplemented media was poured aseptically into petriplates and allowed to solidify according to methodology of Dev et al (2021). These petriplates were incubated @32±2°C for 24-48h and compared with control plates (without any supplementation of azadirachtin in growth media) and (without any supplementation of individual insecticides) in growth media. There were four replications per treatment.

The growth of the *B. thuringiensis* was evaluated by determining whether they exhibited inhibitory or supportive interactions. The effect of the bacterial isolates on mycelial development was calculated to assess their influence.

$$\% \text{ growth inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Control = radial growth of *Beauveria bassiana* in control plate

Treatment = radial growth of *Beauveria bassiana* in co-cultivation with *B. thuringiensis* isolates

The viable count of *B. thuringiensis* isolates (CFU/ml) were recorded in control and compared with viable count of *B. thuringiensis* on media supplemented with azadirachtin or insecticide individually according to methodology of Sarkhandia et al (2023) with some little modifications. The Inhibitory effect of biopesticides, percent growth reduction over control was recorded and was classified according to following scale (Derakhshan Shadmehri et al 2016),

RESULTS AND DISCUSSION

Maximum radial growth (8.42±0.15) and the minimum growth percentage reduction over control (5.33%) were observed in *B. bassiana* PCP-6 when inoculated and incubated on growth media supplemented with *B. thuringiensis* MTCC 9025. Conversely, the minimum radial growth (3.64±0.24) and the maximum growth percentage

reduction (59.02%) were in *B. bassiana* PCP-6 supplemented with *B. thuringiensis* MTCC 8481 (Table 1). In co-cultivation studies of *B. thuringiensis* with *B. bassiana* MTCC 6097, maximum radial growth (7.45±0.11) and the minimum growth percentage reduction over control (12.76%) were in *B. bassiana* MTCC 6097 supplemented with *B. thuringiensis* MTCC 9025 and minimum radial growth (4.14±0.09) and the maximum growth percentage reduction (51.50%) were in *B. bassiana* MTCC 6097 supplemented with *B. thuringiensis* MTCC 8481. Compatibility studies of *B. thuringiensis* with *B. bassiana* recorded variation in compatibility. However, least compatibility of *Beauveria bassiana* was recorded with *B. thuringiensis* MTCC 8481.

Luria Bertani agar media supplemented individually with azadirachtin 5% w/w (@0.2ml/l and 0.4ml/l) recorded maximum colony count (9.6x10⁶cfu/ml) and (9.1x10⁶cfu/ml) and minimum growth percentage reduction compared to the control (5.88% and 10.78%) respectively, in *B. thuringiensis* MTCC 9025. In contrast, the minimum colony count (6.4x10⁶cfu/ml) and (6.2x10⁶cfu/ml) and maximum growth percentage reduction over control (26.43% and 28.73%) respectively, were recorded with *B. thuringiensis* MTCC 868. All *B. thuringiensis* isolates were moderate to high compatible with azadirachtin 5% w/w (Table 2).

Compatibility studies of *B. thuringiensis* with chemical insecticide chlorantraniliprole 18.5% SC (@0.2 and @0.4ml/l) recorded maximum colony count (8.1x10⁶cfu/ml) and (7.9x10⁶cfu/ml) and least percent reduction over control (20.58% and 22.54%) respectively in *B. thuringiensis* MTCC 9025 and was moderate compatible with both doses of chlorantraniliprole 18.5% SC, whereas minimum colony count (6.2x10⁶ and 5.9x10⁶cfu/ml) and maximum percent reduction over control (28.73% and 32.18%), respectively in *B. thuringiensis* MTCC 868 and was moderate compatible (Table 3). However, emamectin benzoate 5% SG recorded

Table 1. Co-cultivation of *B. thuringiensis* with *B. bassiana* (Mean± S.E.)

Treatments	Radial growth of <i>B. bassiana</i> PCP-6 (cm)				Radial growth of <i>B. bassiana</i> MTCC 6097 (cm)			
	Control*	**Media supplemented with <i>B. thuringiensis</i>	Reduction over control (%)	CI	Control*	**Media supplemented with <i>B. thuringiensis</i>	Reduction over control (%)	CI
<i>B. thuringiensis</i> MTCC 9025	8.9±0.02	8.42±0.15	5.33	HC	8.55±0.10	7.45±0.11	12.76	HC
<i>B. thuringiensis</i> MTCC 868	8.9±0.02	6.59±0.17	25.91	MC	8.55±0.10	6.94±0.09	18.79	HC
<i>B. thuringiensis</i> MTCC 8481	8.9±0.02	3.64±0.24	59.02	LC	8.55±0.10	4.14±0.09	51.50	LC
<i>B. thuringiensis</i> MD4	8.9±0.02	8.08±0.15	9.17	HC	8.55±0.10	7.07±0.03	17.30	HC
<i>B. thuringiensis</i> (Commercial formulation)	8.9±0.02	6.74±0.10	24.25	MC	8.55±0.10	6.79±0.14	20.56	MC

#Value of mean± standard error (mean± S.E.) of four replicates

* Radial growth of *B. bassiana* on media without *B. thuringiensis* supplementation

** Radial growth of *B. bassiana* on media supplemented with *B. thuringiensis*

CI= Compatibility Index, HC= Highly Compatible, MC= Moderately Compatible, LC= Least Compatible

minimum colony count and maximum growth percent reduction over control (89.65% and 91.95%) in *B. thuringiensis* MTCC 868 and maximum colony count and minimum growth percent reduction over control (83.33 and 87.25%) in *B. thuringiensis* MTCC 9025 and was incompatible at both doses (@ 0.2 and @0.4 ml/l). Among both insecticides chlorantraniliprole 18.5% SC was compatible whereas Emamectin benzoate 5% SG was incompatible with *B. thuringiensis*.

Interactive studies of *B. thuringiensis* with *Beauveria bassiana* recorded variation in compatibility. An interactive study of *B. thuringiensis* with azadirachtin 5% w/w was safe and compatible with all *B. thuringiensis*. Among both insecticides, emamectin benzoate reduced viable counts of *Bacillus* drastically and was incompatible, whereas, chlorantraniliprole was compatible and could be used in

integrated insect management programme. In vitro compatibility studies between biocontrol bacteria and entomopathogenic fungal strains and growth inhibition of entomopathogenic fungus upto 25% by entomopathogenic bacterial strains was recorded by Siciua et al (2014). The variation in compatibility in different isolates may be due to precipitation compound observed in the area of fungal growth inhibition. These aspects were observed only in co-cultivation of lectin producing fungi with certain bacterial isolates. The activity of *B. thuringiensis* depends on its insecticidal toxins and various other virulence factors that contribute to insect mortality. *B. thuringiensis* carries various transposons, which contribute to the significant genetic variability of its toxin genes and consequently, the diversity of the toxins it produces. This genetic variability accounts for the wide range of *B. thuringiensis* strains. Variation in

Table 2. Interactions between *B. thuringiensis* and azadirachtin 5% w/w

Treatments	<i>B. thuringiensis</i> viable count (CFU/mL) supplemented with azadirachtin 5% w/w						
	Control*	0.2 ml/l			0.4 ml/l		
		Colony count	Reduction over control (%)	CI	Colony count	Reduction over control (%)	CI
<i>B. thuringiensis</i> MTCC 9025	1.02 x10 ⁷	9.6 x10 ⁶	5.88	HC	9.1 x10 ⁶	10.78	HC
<i>B. thuringiensis</i> MTCC868	8.7 x10 ⁶	6.4 x10 ⁶	26.43	MC	6.2 x10 ⁶	28.73	MC
<i>B. thuringiensis</i> MTCC 8481	8.5 x10 ⁶	6.7 x10 ⁶	21.17	MC	6.5 x10 ⁶	23.52	MC
<i>B. thuringiensis</i> MD4	9.8 x10 ⁶	8.9 x10 ⁶	9.18	HC	8.6 x10 ⁶	12.24	HC
<i>B. thuringiensis</i> (Commercial formulation)	9.2 x10 ⁶	7.7 x10 ⁶	16.30	HC	7.2 x10 ⁶	21.73	MC

* Viable count of *B. thuringiensis* without any supplementation
 CI= Compatibility Index, HC= Highly Compatible, MC= Moderately Compatible

Table 3. Interactions between *B. thuringiensis* and synthetic insecticides

Treatments	Control*	Viable count of <i>B. thuringiensis</i> (CFU/ml) supplemented with											
		Chlorantraniliprole 18.5% SC						Emamectin benzoate 5% SG					
		0.2 ml/l			0.4 ml/l			0.2 ml/l			0.4 ml/l		
		Colony count	Reduction over control (%)	CI	Colony count	Reduction over control (%)	CI	Colony count	Reduction over control (%)	CI	Colony count	Reduction over control (%)	CI
<i>B. thuringiensis</i> MTCC 9025	1.02 x10 ⁷	8.1 x10 ⁶	20.58	MC	7.9 x10 ⁶	22.54	MC	1.7 x10 ⁶	83.33	IC	1.3 x10 ⁶	87.25	IC
<i>B. thuringiensis</i> MTCC 868	8.7 x10 ⁶	6.2 x10 ⁶	28.73	MC	5.9 x10 ⁶	32.18	MC	9 x10 ⁵	89.65	IC	7x10 ⁵	91.95	IC
<i>B. thuringiensis</i> MTCC 8481	8.5 x10 ⁶	6.5 x10 ⁶	23.52	MC	6.2 x10 ⁶	27.05	MC	1.0 x10 ⁶	88.23	IC	1.0 x10 ⁶	88.23	IC
<i>B. thuringiensis</i> MD4	9.8 x10 ⁶	7.6 x10 ⁶	22.44	MC	7.4x10 ⁶	24.48	MC	1.3 x10 ⁶	86.73	IC	1.1 x10 ⁶	88.77	IC
<i>B. thuringiensis</i> (Commercial formulation)	9.2 x10 ⁶	7.0 x10 ⁶	23.91	MC	6.8 x10 ⁶	26.08	MC	1.1 x10 ⁶	88.04	IC	9 x10 ⁵	90.21	IC

* Viable count of *B. thuringiensis* without any supplementation
 CI= Compatibility Index, MC= Moderately Compatible, IC= Incompatible

compatibility of *B. thuringiensis* strains may be due to difference in their insecticidal toxins or diversity of toxins secreted. Compatibility studies in vitro between chemical insecticides and entomopathogenic bacteria have strong evidence in favour of the utility of that combination under field conditions (Pelizza et al 2014). The variation in compatibility study of *B. thuringiensis* may be attributed to differences in microbial growth parameters and chemistry of insecticides used (Saheb et al 2021). The combination of *B. thuringiensis* with biorationals and insecticides are better practice agents to control pest. *B. thuringiensis* influenced antagonistic activity to the azadirachtin, entomopathogenic fungi and insecticides. Paunikar and Kulkarni (2020) reported that azadirachtin is compatible with all entomopathogens, and there is possibilities of their combinational treatment under IPM against agriculturally importance pests.

CONCLUSION

B. thuringiensis was compatible with *B. bassiana*, azadirachtin and they could be integrated as component in IPM programme. The insecticide chlorantraniliprole 18.5% SC was better option than emamectin benzoate 5% SG. The synergistic effect of beneficial microorganisms, such as *B. thuringiensis* and entomopathogenic fungi and azadirachtin may facilitate integrated treatments to manage pest infestations simultaneously.

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