Effect of different additives on the persistence and insecticidal activity of native strains of *Bacillus thuringiensis*

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Abstract The persistence and insecticidal activity of native strains of *Bacillus thuringiensis* was evaluated in formulations containing different additives such as arrow-root powder, carboxy methyl cellulose (CMC), gum acacia, non-food grade (NFG) starch, and soluble starch. Persistence of *B. thuringiensis* varied with different additives used in the formulations. Among the different additives used, NFG starch provided maximum protection to *B. thuringiensis* and native strain 42 showed maximum persistence (83%) which was higher than that obtained in commercial formulation. In commercial formulation, the persistence of *B. thuringiensis* was 47% only after 3 d of spray. The feeding trials conducted on second instar larvae of *H. armigera* using leaves sprayed with NFG starch formulation revealed 70% larval mortality while commercial formulation showed 50% mortality during the same period.

Key words: *Bacillus thuringiensis* · *Helicoverpa armigera* · insecticidal activity · persistence · sprayable-formulations

Introduction

Indiscriminate use of synthetic insecticides to control insect pests of various crop plants has resulted in environmental pollution, contamination of ground water and the development of pesticide resistance in insect pests. Therefore, the development and use of biodegradable alternate plant protection agents including *Bacillus thuringiensis* (Bt) has become a necessity in integrated pest management program (IPM) of all the major crop plants. The use of 'cry' gene based bioinsecticide produced by *B. thuringiensis* (Bt) offers advantages over harmful chemical insecticides because they are target specific and economical. Further Bt formulations, being proteinaceous in nature, are easily degraded and persist very little in soil, leading to negligible effect on soil health.

B. thuringiensis is a spore forming bacterium and various types of sprayable formulations are prepared using spores. One of the environmental factors such as exposure to sunlight inactivates bacteria on the plants and reduces their bioinsecticidal efficacy. Further, wash off of spray formulation occurs very fast if it rains after application. To overcome these problems, insecticidal formulations have been improved by addition of various additives such as stickers, spreaders or protectants. Many types of spreaders such as milk powder, casein and other proteins are used to ensure wetting of leaves. Dextran, gum and flour are also added to the formulation for making a thin film on the foliage^{1–8}. These also protect the bacteria as well as active toxin (if ICPs are used) from sunlight. We therefore, carried out a study to examine the effect of different additives such as arrow-root powder, carboxy methyl cellulose, gum-acacia, non-food grade (NFG) starch and soluble starch on the persistence and insecticidal activity of *B. thuringiensis* against *H. armigera*.

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Materials and Methods

B. thuringiensis strains were isolated from soil, collected from cotton field at Research Farm, CCS HAU Hisar, using acetate selection procedure⁹. The stock cultures were stored on LB slants at 4°C. The cultures were identified by using PCR according to the procedure given by Carrozi *et al.*¹⁰ and modified by Gupta *et al.*¹¹ Persistence of *B. thuringiensis* isolates on cotton leaves was determined by adding various additives such as arrow-root powder, carboxy methyl cellulose, gum acacia, NFG starch and soluble starch to bacterial cell suspension. Sodium dodecyl sulphate was used as a spreader to ensure proper wetting of leaf surface. Formulations were prepared using three native strains of *B. thuringiensis* (strain 21, 42 and strain 43). *B. thuringiensis* K was isolated from 'Dipel' and used as positive control. All the cultures were grown in LB broth for 72 h on a rotary shaker at 28°C (200 rpm). Cells were collected by centrifugation at 7000 rpm for 10 min and a homogenous cell suspension containing 5×10^8 cells mL⁻¹, 0.04% additive and 0.01 % sodium dodecyl sulphate was prepared⁵. Using different combinations of additives and isolates of *B. thuringiensis*, 20 formulations were prepared. Commercial formulation 'Dipel' was used as a control.

The formulations were sprayed on cotton plants grown in pot-house and allowed to dry for 1 h on the leaves before colony forming units (CFUs) of bacterial culture were determined or used as feed. The formulation after drying on the leaves formed a uniform thin film.

After the spray of Bt formulations on leaves of cotton plants the persistence of *B. thuringiensis* was determined by estimating the total CFUs at an interval of 3 d. Acetate selection method was used to ensure that only cells of *B. thuringiensis* grew after the LB plates were incubated for 24 h at 28°C. Three leaf discs, each of 3 cm diameter were taken from three different leaves after 0, 3, 6, 9 12 and 15 d of spray, transferred to sterilized centrifuge tubes and total number of CFUs of *B. thuringiensis* were determined⁸. Survival percentage of the cultures was calculated based on the initial CFUs on 0 d.

The bioefficacy of NFG starch formulations was evaluated by using second instar larvae of *H. armigera*. This formulation was sprayed on cotton plants and Bt treated leaves were used as feed for the larvae under laboratory condition. After 72 h of feeding, percent mortality of the larvae was estimated.

Results and Discussion

The type of additive used in different formulation greatly affected the persistence of bacterial cells on cotton leaf

surface and was in the order: NFG starch > soluble starch > CMC > gum acacia > arrow-root (Table 1). In most of the formulations, persistence of *B. thuringiensis* on cotton leaves was higher than the commercial formulation, Dipel. Among the different additives used, persistence of Bt cells was found to be maximum in NFG starch based formulations. In all other formulations, including the commercial formulation there was a gradual decrease in Bt population, which was too steep after 6th day (Table1). The persistence of *B. thuringiensis* cells in soluble starch based formulations was also less than NFG starch. Stickiness of the additive in formulation had no positive effect on bacterial cell survival on leaf surface. In fact when arrow-root was used, the bacterial population was found to be minimum (8-10 %) after 6th day of spray. This could be due to the presence of some toxic metabolites in this formulation. Behle *et al.*⁵ observed that *B. thuringiensis* formulations containing 1 % gluten or 0.5 % casein resisted wash off better than flour/ sucrose when applied to field-grown cabbage. McGuire *et al.*⁶ reported that during a wet year, persistence of insecticidal activity of starch based *B. thuringiensis* formulation were higher than the commercial preparation. Tamez Guerra *et al.*⁷ reported that in starch based formulations the insecticidal activity was higher than the control after artificial solar radiation was given, suggesting that starch provided protection to *B. thuringiensis*.

All the cultures of *B. thuringiensis* showed better persistence on cotton in comparison to *B. thuringiensis* cells in a commercial formulation. It was observed that strain K showed maximum persistence (85 %) on cotton leaves in NFG starch based formulation as compared to 47 % in its original formulation (commercial). Among the native strains of *B. thuringiensis*, the isolate 42 showed maximum persistence (83 %) in NFG starch. It was comparable to the values obtained with Bt K strain. In fact when NFG starch was used as an additive, more than 70% cells of *B. thuringiensis* strain K, isolate 42 and 43, were able to survive on cotton leaves even after 6th day of spray. This was much higher than the value observed in commercial preparation 'Dipel' where 33 % of cells were able to survive during the same period. Similar trend was observed when CMC and gum acacia were used in formulations although persistence of *B. thuringiensis* was much lower in these formulations. After 9 days of spray, 50% population of strain K and native strain 43 could be observed on cotton leaves. From these observation it can be concluded that persistence of bacterial cultures on foliage varied with strains as well as type of additives used. *B. thuringiensis* isolates showed variable persistence on cotton leaves even in same formulation. Strain K and native strain 43 were able to persist maximally on cotton leaves in NFG starch based formulation.

Table 1 Persistence of *Bacillus thuringiensis* on cotton leaves sprayed with different formulations of biopesticide.

Strains	Formulations	% survival of Bt cells (days after spray)						
		0	3	6	9	12	15	mean
K.	Dipel	100	47.0	33.1	16.8	2.2	0.8	33.1
	A	100	40.2	8.0	2.6	0.0	0.0	25.0
	C	100	54.8	41.2	15.9	3.2	0.6	35.2
	G	100	54.6	32.5	10.9	3.1	0.0	32.7
	R	100	85.7	75.0	50.3	31.0	7.3	51.5
43	S	100	66.4	49.5	16.6	6.8	2.2	38.7
	A	100	40.2	8.0	2.6	0.0	0.0	25.0
	C	100	55.0	44.0	19.3	6.6	2.6	37.7
	G	100	53.8	35.0	15.1	5.2	0.4	35.0
	R	100	78.0	70.5	48.9	29.1	7.9	49.7
42	S	100	57.5	43.5	11.8	4.8	2.3	36.6
	A	100	41.6	10.2	5.1	3.8	1.9	29.9
	C	100	60.4	43.8	18.7	4.2	0.5	36.9
	G	100	51.7	32.8	6.3	1.7	1.2	32.7
	R	100	83.3	73.7	40.1	21.1	6.7	49.1
21	S	100	65.3	50.0	16.8	5.7	0.9	38.4
	A	100	35.2	13.2	8.0	3.8	1.4	29.9
	C	100	54.2	32.8	9.9	3.4	0.9	34.0
	G	100	51.6	31.1	7.6	2.3	0.6	32.8
	R	10	71.9	57.0	38.9	9.7	6.0	44.2
	S	00	56.9	41.0	11.0	4.9	0.1	35.1
	CD (P < 0.05)	N.S.	0.5	0.6	0.5	0.5	1.3	

Values average of 9 observations per 3 cm diameter leaf area. P, percent survival; DAYS, days after spray; A, arrow-root; C, carboxy methyl cellulose; G, gum acacia; R, NFG starch; S, soluble starch.

Table 2 Bioefficacy of NFG starch based *B. thuringiensis* formulation sprayed on cotton leaves against second instar larvae of *H. armigera*.

	% mortality of <i>H. armigera</i> larvae				
	days after spray of Bt				Mean
	0	3	6	9*	
Dipel	84	84	50	28	61.5
RK	90	90	80	50	77.5
R43	87	80	60	49	69.0
R42	90	90	77	44	75.2
R21	80	68	56	34	59.5
CD(P < 0.05)	3.1	4.9	2.6	3.4	

Percent mortality of second instar larvae of *H. armigera* after 72 h of feeding Bt sprayed cotton leaves. *cotton leaves fed to larvae

When the bacterial population of all the Bt strains was compared on different days after spray, it was observed that 3 day after the spray the persistence of bacterial cultures

was reduced to 35–55 % of their original number in all additives except in NFG starch. On the other hand, in NFG based formulation, survival of Bt. population was 85.7 % and 75 % after 3 and 6 days of spray, respectively. This clearly indicates the superiority of this additive over others. This may be due to the formation of a thin protective film of NFG starch, encapsulating the *B. thuringiensis* cells on the surface of leaves.

Since the persistence of *B. thuringiensis* cells was maximum in NFG starch formulation, its bioefficacy was determined by feeding second instar larvae of *H. armigera* on Bt-treated cotton leaves grown under pot house conditions. It was observed that larvae showed 100 % mortality in 72 h when 0 day Bt sprayed leaves were fed to the larvae immediately or after 3 d of spray (Table 2). Dipel showed 84 % mortality during the same period. After 6 day of spray Bt formulations containing native strains showed 80% mortality of second instar larvae of *H. armigera*. However, larval mortality was reduced to about 50 % when leaves were used as feed after 9 d of spray. Ridgway et. al. reported

the control of European corn borer by using granular matrix formulations of *B. thuringiensis*⁸.

Thus, it can be concluded that among the various additives evaluated, in NFG starch based formulation, the bacterial cells were able to survive maximally on cotton leaves in comparison to other additives. NFG starch can be used in Bt formulations to increase the persistence of the bio-insecticide on the foliage of different crops with the result bioefficacy of the formulation will also be much higher during field application. Field experiments have been conducted to control *H. armigera* in chickpea and cotton, using NFG starch based Bt formulation and showed 70-80 % control of *H. armigera*¹².

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