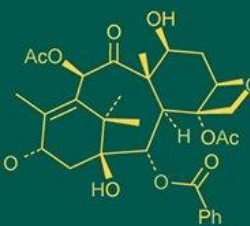
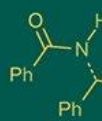


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Performance of different PDKV *Bt* formulations under shelf storage

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Abstract

The present study was conducted to evaluate the shelf stability and insecticidal activity of PDKV *Bacillus thuringiensis* formulations against pink bollworm, *Pectinophora gossypiella* (Saunders). Eleven PDKV *Bt* strains were evaluated in suspension concentrate (SC) and wettable powder (WP) formulations for viable spore count and toxicity at 0, 60, 120 and 180 days of storage. All formulations retained viable spores throughout the six-month storage period, although a gradual decline in spore count was observed with increasing storage duration. Among the SC formulations, PDKV *Bt*-SY-4-75% SC recorded the highest initial spore count (17.80×10^8 CFU/ml), while among the WP formulations, PDKV *Bt*-SY-4-25% WP recorded the highest count (5.91×10^8 CFU/ml). The formulations retained relatively high viable spore populations after 180 days, indicating satisfactory shelf stability. Toxicity against *P. gossypiella* larvae varied among strains and formulations and declined gradually during storage. Among the SC formulations, mortality ranged from 53.24 to 84.36 percent at 0 days and from 39.42 to 69.32 percent after 180 days. Among WP formulations, mortality ranged from 64.36 to 94.28 percent at 0 days and from 45.24 to 79.26 percent after 180 days. PDKV *Bt*-SY-4-25% WP recorded the highest mortality (94.28%) at 0 days and maintained 79.26 percent mortality after 180 days, indicating superior persistence of insecticidal activity. The WP formulations generally exhibited higher mortality than their corresponding SC formulations despite the higher spore counts recorded in SC formulations, suggesting that formulation characteristics may influence biological efficacy in addition to viable spore concentration.

Keywords: *Bacillus thuringiensis*, indigenous formulations, wettable powder, suspension concentrates

Introduction

Bacillus thuringiensis (*Bt*), a gram-positive bacteria occur naturally in soil, guts of moths and butterflies, aquatic environment, under surface of leaves, in dust and marine sediments (Maeda *et al.* 2000) [2]. During sporulation *Bt* produces parasporal crystals containing one or more Cry proteins (δ -endotoxins) that are specifically toxic to insect orders such as Lepidoptera, Diptera, and Coleoptera and also to some nematodes, mites, and protozoa. When the parasporal crystals are ingested by insect larvae, the insecticidal proteins are activated by proteases in insect midgut, which typically has alkaline pH (pH 8-10.5). The active Insecticidal Crystal Protein (ICP) then traverses the peritrophic membrane and binds to specific receptors on the midgut epithelium. This results in pores formation, loss of the trans-membrane potential, cell lysis, leakage of the midgut contents, paralysis, and death of the insect. (Nester *et al.* 2002) [6]. Attempts were made to control several lepidopteran insects with bio-control agents but *B. thuringiensis* has been proved to be potentially toxic because of its ability to produce a variety of insecticidal toxins. The present investigation aimed at preparing various formulations of *Bt* strains isolated from the Department of Entomology, PDKV, and evaluating the spore count of different formulations and the storability of various strains under different formulations after 2 months, 4 months and 6 months respectively and also their insecticidal activities by conducting a bioassay on pink bollworm *P. gossypiella*.

Materials and Methods

The study was conducted to evaluate the "Performance of different PDKV *Bt* formulations under shelf storage".

The strains isolated and cultured from Department of Entomology were used in the study. The study was conducted in Laboratory of Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.) during the year 2025-2026.

Culturing of *Bt* strains

Local 11 *Bacillus thuringiensis* isolates previously isolated and screened for insect toxicity in the Department of Entomology were sub cultured and maintained on Luria broth medium are used in present study. The novel PDKV *Bacillus thuringiensis* strains were developed into required formulation at Department of Entomology, Dr. PDKV, Akola. Luria broth medium (Travers *et al.*, 1987) [7] was used for culturing of all *Bt* isolates used under study.

Preparation of *Bacillus thuringiensis* formulations

Two formulations from the 11 *Bt* strains viz., Wettable powder and Suspension concentrate were prepared. The wettable powder (WP) formulation of PDKV *B. thuringiensis* strains were prepared according to the method developed by Marzaban *et al.* (2021) [4] with slight modification. To formulate the PDKV *Bt* WP formulations, 25% of the *Bt* biomass was used with 75% of the additives. About 60% from 75% additives was allocated to the kaolin and 3% to one of the suspension materials (Tween-80) and 12% to moisturizer (titanium dioxide).

A suspension concentrate (SC) formulation of PDKV *Bt* strains was prepared according to the method developed by Vimala Devi *et al.* (2014) [8]. The suspension concentrate was prepared by adding seventy-five (75 g) grams of *Bt* and 25 g of boric acid in a 500 ml sterile glass beaker. Tween-80 was added to a light mineral oil in 1:6.84 ratio and vortexed to get a uniform mixture (T-M mixture). Fifty milliliters of this mixture was added initially to the *Bt* and boric acid mixture and grounded to obtain fine paste. This was followed by addition of 142 ml of T-M mixture. This suspension was added in to a mixer jar and blended for 2-3 min to ensure proper mixing of the components for obtaining a uniform SC formulation.

Estimation of CFU counts

The required formulation was dissolved as per the recommended dose in 100 ml of sterile distilled water. The resulting suspension was serially diluted using sterile techniques. An appropriate dilution was selected and plated onto LB-agar medium. The plates were incubated overnight at 30 ± 2 °C and RH of 75%. After incubation, the number of colony-forming units (CFUs) was counted using a colony counter to determine the spore concentration (Mohamed *et al.*, 2010) [5]. The formula used to determine spore count is given below:

$$\text{CFU/ml} = \frac{\text{(Number of colonies} \times \text{Dilution factor)}}{\text{Volume of culture plate (in ml)}}$$

Bioassay of selected native isolates of *Bt*

In order to determine the lethal concentrations of selected endotoxins from *Bt* isolates against *Pectinophora gossypiella*, diet incorporation assays such that 1mg of *Bt* formulation in 1 ml of diet as per active ingredient is used for the toxicity estimation and were followed as described by Bhalla *et al.* (2005) [1] for *H. armigera*. The diet for

Pectinophora gossypiella was prepared by the method described by Malthankar *et al.* (2014) [3]. Molten diet mix was cooled to 50 °C and mixed thoroughly with desired concentrations of parasporal preparations from selected native *Bt* isolates. The diet was then immediately poured in the 12-well culture plate (1 ml/well) and allowed to solidify. Ten 3rd instar larvae (8 hrs. starvation) were released in each well. A set of 10 such larvae was tested for each concentration of parasporal preparations at one time. The plates were covered and kept in the insect culture room at 28 ± 2 °C, $60 \pm 5\%$ RH and 14L:10D photoperiod. Mortality was recorded after 3 days.

Results and Discussion

Spore count of PDKV *Bt* formulations in shelf storage

The results presented in Tables 1 and 2 indicate that all the PDKV *Bacillus thuringiensis* formulations retained viable spores throughout the six-month storage period, although a gradual decline in spore count was observed with increasing storage duration. The extent of reduction varied among formulations, indicating differences in their storage stability and ability to maintain viable *Bt* spores.

Among the suspension concentrate (SC) formulations, the initial spore count ranged from 2.98×10^8 to 17.80×10^8 CFU/ml. The highest spore count was recorded in PDKV *Bt*-SY-4-75 SC (17.80×10^8 CFU/ml), followed by PDKV *Bt*-SA-20-75 SC (16.40×10^8 CFU/ml) and PDKV *Bt*-SGn-4-75 SC (15.00×10^8 CFU/ml). The lowest spore count was observed in PDKV *Bt*-SGd-1-75 SC (2.98×10^8 CFU/ml). After six months, the same formulations retained relatively high spore counts of 17.70, 16.28 and 14.87×10^8 CFU/ml, respectively, indicating comparatively better stability during storage. The reduction in spore count was generally small, suggesting that the SC formulations were able to maintain good viability under the storage conditions.

The WP formulations showed an initial spore count ranging from 1.63×10^8 to 5.91×10^8 CFU/ml. The highest count was recorded in PDKV *Bt*-SY-4-25 WP (5.91×10^8 CFU/ml), followed by PDKV *Bt*-SA-20-25 WP (5.54×10^8 CFU/ml) and PDKV *Bt*-SGn-4-25 WP (5.10×10^8 CFU/ml). The lowest initial spore count was recorded in PDKV *Bt*-SGd-1-25 WP (1.63×10^8 CFU/ml). After six months, these formulations recorded 5.87, 5.50 and 5.01×10^8 CFU/ml, respectively, demonstrating relatively good retention of viable spores.

A comparison between the two formulations showed that the SC formulations generally contained higher spore counts than the WP formulations. This difference may be attributed to the formulation type, concentration of viable spores and the protective effect of formulation ingredients on spore survival during storage. In both formulations, the reduction in viable spore count was gradual rather than abrupt, indicating that the formulations maintained reasonable shelf stability.

Among all the formulations, PDKV *Bt*-SY-4 exhibited consistently high spore counts in both SC and WP formulations and showed only a marginal decline during the six-month storage period. Similarly, PDKV *Bt*-SA-20 and PDKV *Bt*-SGn-4 maintained comparatively higher viable spore populations. These formulations therefore appeared to possess better storage stability than formulations having lower initial spore concentrations.

The observed gradual decline in spore count during storage may be associated with natural loss of viability of *B.*

thuringiensis spores, changes in moisture content, temperature fluctuations, or gradual degradation of formulation components. However, the relatively small reduction observed in most formulations suggests that the PDKV *Bt* formulations were capable of retaining viable spores for at least six months under the conditions evaluated.

Toxicity performance of PDKV *Bt* formulations under shelf storage conditions:

The data presented in Table 3 revealed considerable variation in the toxicity of PDKV *Bacillus thuringiensis* formulations against *Pectinophora gossypiella* larvae during different periods of shelf storage studies. The mortality varied with the *Bt* strain, formulation type and storage duration. In general, a gradual decline in mortality was observed with increasing storage period from 0 to 180 days, indicating a reduction in the biological activity of the formulations during prolonged storage.

Among the SC formulations, mortality at 0 days ranged from 53.24 to 84.36 percent. The highest mortality was recorded with PDKV *Bt* SY-4-75% SC (i.e. 84.36%), followed by SA-20-75% SC (77.18%), SAK-9-75% SC (70.36%) and SA-18-75% SC (69.24%). The lowest mortality was recorded with SGn-5-75% SC (53.24%). After 60 days, mortality ranged from 46.38 to 79.24 percent, with SY-4-75% SC retaining the highest activity. At 120 days, mortality declined further to 40.26-74.18 percent, while after 180 days it ranged from 39.42 to 69.32 percent.

Among the WP formulations, mortality at 0 days ranged from 64.36 to 94.28 percent. The highest mortality was observed with PDKV *Bt* SY-4-25% WP (94.28%), followed by SA-20-25% WP (87.42%), SAK-9-25% WP (81.28%) and SA-18-25% WP (79.36%). The lowest mortality was recorded with SGn-5-25% WP (64.36%). After 60 days, mortality ranged from 58.42 to 89.36 percent, while at 120 days it ranged from 52.18 to 84.42 percent. At 180 days, the mortality ranged from 45.24 to 79.26 percent.

The SY-4 strain exhibited the highest toxicity among all the formulations evaluated. SY-4-25% WP recorded mortality of 94.28, 89.36, 84.42 and 79.26 percent at 0, 60, 120 and 180 days, respectively. The corresponding SY-4-75% SC recorded 84.36, 79.24, 74.18 and 69.32 percent mortality. Thus, although mortality declined during storage, SY-4

retained comparatively high insecticidal activity throughout the six-month storage period.

The decline in mortality with storage was evident in almost all formulations. This reduction may be associated with gradual loss of viability and biological activity of *Bt* during storage. The trend is consistent with the spore-count observations presented in the earlier tables, where a gradual reduction in viable spore counts was observed from the initial storage period to six months. The reduction in viable propagules may consequently reduce the quantity of active *Bt* ingested by the larvae and thereby decrease mortality.

The cumulative mean mortality of *Pectinophora gossypiella* larvae varied considerably among the different *Bacillus thuringiensis* formulations evaluated after 180 days of storage. Among the PDKV *Bt* SC formulations, the cumulative mean mortality ranged from 44.83 to 76.78 percent. The highest cumulative mortality was recorded with PDKV *Bt* SY-4-75% SC (76.78%), followed by PDKV *Bt* SA-20-75% SC (68.30%), while the lowest mortality was observed with PDKV *Bt* SGn-5-75% SC (44.83%). Other formulations such as SA-18-75% SC (61.31%), SAK-9-75% SC (61.11%) and I-3-75% SC (58.86%) exhibited intermediate levels of larval mortality.

Among the PDKV *Bt* WP formulations, cumulative mean mortality ranged from 55.05 to 86.83 percent. The highest cumulative mortality was observed with PDKV *Bt* SY-4-25% WP (86.83%), followed by PDKV *Bt* SA-20-25% WP (79.40%) and PDKV *Bt* SAK-9-25% WP (72.02%). The lowest cumulative mortality was recorded in PDKV *Bt* SGn-5-25% WP (55.05%). The remaining formulations showed intermediate cumulative mortality, with SA-18-25% WP recording 70.90 percent and I-3-25% WP 67.87 percent.

Overall, the WP formulations exhibited higher cumulative mean mortality than their corresponding SC formulations, indicating comparatively better retention of insecticidal activity during storage. The superior performance of SY-4 in both formulations was particularly evident, with cumulative mortality of 76.78% in SC and 86.83% in WP. However, the relatively high cumulative mortality maintained by several formulations, particularly the WP formulations, indicates their comparatively better storage stability and persistence of larvicidal activity against *P. gossypiella* larvae.

Table 1: Spore count of PDKV *Bt* suspension concentrate (SC) formulations in shelf storage

Sr. No	PDKV <i>Bt</i> SC formulations	Spore count (CFU/ml) of PDKV <i>Bt</i> formulations in shelf storage after			
		0 Days	60 days	120 days	180 days
1	PDKV <i>Bt</i> -SA-6-75 SC	8×10^8	7.89×10^8	7.85×10^8	7.80×10^8
2	PDKV <i>Bt</i> -SA-18-75 SC	4.79×10^8	4.75×10^8	4.70×10^8	4.68×10^8
3	PDKV <i>Bt</i> -SA-20-75 SC	16.4×10^8	16.34×10^8	16.30×10^8	16.28×10^8
4	PDKV <i>Bt</i> -SAK-6-75 SC	3.01×10^8	3×10^8	2.96×10^8	2.9×10^8
5	PDKV <i>Bt</i> -SAK-9-75 SC	12.13×10^8	12.10×10^8	12.07×10^8	$12. \times 10^8$
6	PDKV <i>Bt</i> -SGd-1-75 SC	2.98×10^8	2.90×10^8	2.84×10^8	2.8×10^8
7	PDKV <i>Bt</i> -SGn-4-75 SC	15×10^8	14.96×10^8	14.9×10^8	14.87×10^8
8	PDKV <i>Bt</i> -SGn-5-75 SC	13.3×10^8	13.28×10^8	13.24×10^8	13.2×10^8
9	PDKV <i>Bt</i> -SBn-2-75 SC	8.71×10^8	8.7×10^8	8.65×10^8	8.54×10^8
10	PDKV <i>Bt</i> -I-3-75 SC	4.69×10^8	4.64×10^8	4.6×10^8	4.58×10^8
11	PDKV <i>Bt</i> -SY-4-75 SC	17.8×10^8	17.76×10^8	17.74×10^8	17.7×10^8

Table 2: Spore count of PDKV *Bt* wettable powder (WP) formulations in shelf storage

Sr. No	PDKV <i>Bt</i> SC formulations	Spore count (CFU/ml) of PDKV <i>Bt</i> formulations in shelf storage after			
		0 days	60 days	120 days	180 days
1	PDKV <i>Bt</i> -SA-6-25 WP	2.78×10^8	2.75×10^8	2.72×10^8	2.7×10^8
2	PDKV <i>Bt</i> -SA-18-25 WP	1.69×10^8	1.65×10^8	1.62×10^8	1.59×10^8
3	PDKV <i>Bt</i> -SA-20-25 WP	5.54×10^8	5.52×10^8	5.50×10^8	5.50×10^8
4	PDKV <i>Bt</i> -SAk-6-25 WP	2.1×10^8	2.08×10^8	2.07×10^8	2.05×10^8
5	PDKV <i>Bt</i> -SAk-9-25 WP	4.1×10^8	4.07×10^8	4.03×10^8	4×10^8
6	PDKV <i>Bt</i> -SGd-1-25 WP	1.63×10^8	1.6×10^8	1.58×10^8	1.55×10^8
7	PDKV <i>Bt</i> -SGn-4-25 WP	5.1×10^8	5.07×10^8	5.04×10^8	5.01×10^8
8	PDKV <i>Bt</i> -SGn-5-25 WP	4.41×10^8	4.38×10^8	4.35×10^8	4.34×10^8
9	PDKV <i>Bt</i> -SBn-2-25 WP	2.59×10^8	2.55×10^8	2.52×10^8	2.50×10^8
10	PDKV <i>Bt</i> -I-3-25 WP	1.72×10^8	1.71×10^8	1.68×10^8	1.65×10^8
11	PDKV <i>Bt</i> -SY-4-25 WP	5.91×10^8	5.9×10^8	5.87×10^8	5.87×10^8

Table 3: Toxicity performance of PDKV *Bt* formulations under shelf storage conditions

S. No	Formulation	Mortality (%) of <i>P. gossypiella</i> larvae after				Cumulative mean
		0 days	60 days	120 days	180 days	
PDKV <i>Bt</i> SC formulations						
1	PDKV <i>Bt</i> SA-6-75% SC	62.18	57.34	51.26	50.18	55.24
2	PDKV <i>Bt</i> SA-18-75% SC	69.24	64.18	59.36	52.47	61.31
3	PDKV <i>Bt</i> SA-20-75% SC	77.18	71.26	65.42	59.36	68.30
4	PDKV <i>Bt</i> SAK-6-75% SC	63.42	57.18	51.36	49.28	55.31
5	PDKV <i>Bt</i> SAK-9-75% SC	70.36	64.42	58.28	51.36	61.11
6	PDKV <i>Bt</i> SGd-1-75% SC	62.36	55.42	48.36	46.28	53.11
7	PDKV <i>Bt</i> SGn-4-75% SC	57.28	51.36	44.28	42.42	48.84
8	PDKV <i>Bt</i> SGn-5-75% SC	53.24	46.38	40.26	39.42	44.83
9	PDKV <i>Bt</i> SBn-2-75% SC	60.24	54.36	48.28	47.36	52.56
10	PDKV <i>Bt</i> I-3-75% SC	68.36	61.28	54.42	51.36	58.86
11	PDKV <i>Bt</i> SY-4-75% SC	84.36	79.24	74.18	69.32	76.78
PDKV <i>Bt</i> WP formulations						
1	PDKV <i>Bt</i> SA-6-25% WP	71.24	68.16	61.38	53.42	63.55
2	PDKV <i>Bt</i> SA-18-25% WP	79.36	73.28	69.42	61.54	70.9
3	PDKV <i>Bt</i> SA-20-25% WP	87.42	82.36	77.28	70.54	79.4
4	PDKV <i>Bt</i> SAK-6-25% WP	72.36	65.24	59.18	52.36	62.29
5	PDKV <i>Bt</i> SAK-9-25% WP	81.28	75.36	69.24	62.18	72.02
6	PDKV <i>Bt</i> SGd-1-25% WP	73.18	66.42	60.36	53.28	63.31
7	PDKV <i>Bt</i> SGn-4-25% WP	68.42	62.18	56.24	49.36	59.05
8	PDKV <i>Bt</i> SGn-5-25% WP	64.36	58.42	52.18	45.24	55.05
9	PDKV <i>Bt</i> SBn-2-25% WP	71.36	65.28	59.42	52.18	62.06
10	PDKV <i>Bt</i> I-3-25% WP	78.42	71.36	64.28	57.42	67.87
11	PDKV <i>Bt</i> SY-4-25% WP	94.28	89.36	84.42	79.26	86.83

Conclusion

The present study demonstrated that the PDKV *Bacillus thuringiensis* formulations retained viable spores and appreciable insecticidal activity against *Pectinophora gossypiella* during six months of shelf storage. All formulations showed a gradual decline in spore count and larval mortality with increasing storage duration, although the extent of reduction varied among strains and formulation types. Among the SC formulations, PDKV *Bt*-SY-4-75% SC recorded the highest spore count and maintained comparatively high viability throughout the storage period. Among the WP formulations, PDKV *Bt*-SY-4-25% WP recorded the highest spore count and showed the highest toxicity against pink bollworm larvae. The WP formulations consistently recorded higher larval mortality than their corresponding SC formulations, indicating better expression of insecticidal activity under the conditions evaluated. The highest mortality was recorded with PDKV *Bt*-SY-4-25% WP (94.28%) at 0 days, which remained relatively high at 89.36, 84.42 and 79.26 percent after 60, 120 and 180 days, respectively. The corresponding SY-4-75% SC recorded 84.36, 79.24, 74.18 and 69.32 percent mortality,

respectively. Thus, SY-4 formulations showed comparatively better retention of biological activity during storage. The results further indicate that spore concentration alone did not determine larval mortality, as the SC formulations generally possessed higher spore counts but showed lower mortality than their corresponding WP formulations. Hence, formulation characteristics and stability may have contributed to differences in insecticidal performance. Overall, PDKV *Bt*-SY-4-25% WP was identified as the most promising formulation, followed by PDKV *Bt*-SA-20-25% WP and other comparatively effective WP formulations. The ability of SY-4-25% WP to maintain high spore viability and insecticidal activity for up to six months, indicates its potential for further evaluation and development as an effective microbial insecticide against *P. gossypiella*.

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