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Performance of novel PDKV *Bacillus thuringiensis* formulations on L.B. medium

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Abstract
The colonies of novel PDKV *Bacillus thuringiensis* strains from the wettable powder and suspension concentrate formulations raised on L.B. medium were counted using colony counter. Among the suspension concentrate formulation, the highest spore count was recorded in PDKV *Bt* SY-4-75 SC with 17.6×10^8 CFU/ml, followed by PDKV *Bt* SA-20-75 SC with 16.5×10^8 CFU /ml, PDKV *Bt* SGn-4-75 SC with 14.7×10^8 CFU/ml and PDKV *Bt* SGn-5-75 SC with 13.2×10^8 CFU/ml. Among the wettable powder formulations, the highest spore count was recorded in PDKV *Bt* SY-4-25 WP with 5.85×10^8 CFU/ml, followed by PDKV *Bt* SA-20-25 WP with 5.5×10^8 CFU/ml, PDKV *Bt* SGn-4-25 WP with 4.9×10^8 CFU/ml and PDKV *Bt* SGn-5-25 WP with 4.4×10^8 CFU/ml. All the formulations recorded CFU counts above the standard *Bt kurstak*i 5% WP (5×10^7 spore/mg) commercial formulation. The studies revealed suitability of L. B. medium for culturing of all the novel PDKV *Bt* strains.

Keywords: *Bt* formulations, LB medium, CFU

Introduction
Bacillus thuringiensis (*Bt*), a gram positive bacteria occurs naturally in soil, guts of moths and butterflies, aquatic environment, under surface of leaves, in dust and marine sediments (Maeda *et al.* 2000) [7]. During sporulation *Bt* produces parasporal *cry*stals 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 *cry*stals 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) [10]. 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 their performance on L.B. Medium. Consequently, it is very important to explore new *Bacillus thuringiensis* strains and formulations for the effective control of agricultural insect pests. With this aim, a study was conducted to evaluate the performance of various PDKV *Bacillus thuringiensis* strains on LB medium and to know the CFU count in two indigenously prepared wettable powder and suspension concentrate *Bt* formulations from novel PDKV *Bt* strains.

Materials and Methods
The study was conducted to evaluate the "Performance of novel PDKV *Bt* formulations on L.B. Medium". 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 2024-2025.

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) was used for culturing of all *Bt* isolates used under study. After formulation preparation, the spore count (CFU/ml) was calculated.

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) 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) [12]. 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. After incubation, the number of colony-forming units (CFUs) was counted using a colony counter to determine the spore concentration (Mohamed *et al.*, 2010) [9]. The formula used to determine spore count is given below:

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

Results and Discussion

The CFU count (CFU/ml) of the respective PDKV *Bt* formulations have been depicted in Tables 1 & 2. Among the suspension concentrate formulations (Table 1), the highest spore count was recorded in the formulation of PDKV *Bt* SY-4-75 SC which recorded 17.6×10^8 CFU/ml, followed by PDKV *Bt* SA-20-75 SC with 16.5×10^8 CFU /ml, PDKV *Bt* SGn-4-75 SC with14.7×10^8 CFU/ml, PDKV *Bt* SGn-5-75 SC with 13.2×10^8 CFU/ml, PDKV *Bt* SAk-9-75 SC with 12.23×10^8 CFU/ml, PDKV *Bt* SBn-2-75 SC with 8.63×10^8 CFU/ml, PDKV *Bt* SA-6-75 SC with 7.88×10^8 CFU/ml, PDKV *Bt* I-3-75 SC with 4.8×10^8 CFU/ml, PDKV *Bt* SA-18-75 SC with 4.73×10^8 CFU/ml, PDKV *Bt* SGd-1-75 SC with 3×10^8 CFU/ml and PDKV *Bt* SAk-6-75% SC with 3×10^8 CFU/ml respectively.

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

Sr. No	PDKV *Bt* SC formulations	Spore count (CFU/ml)
1	PDKV *Bt*-SA-6-75 SC	7.88×10^8
2	PDKV *Bt*-SA-18-75 SC	4.73×10^8
3	PDKV *Bt*-SA-20-75 SC	16.5×10^8
4	PDKV *Bt*-SAk-6-75 SC	3×10^8
5	PDKV *Bt*-SAk-9-75 SC	12.23×10^8
6	PDKV *Bt*-SGd-1-75 SC	3×10^8
7	PDKV *Bt*-SGn-4-75 SC	14.7×10^8
8	PDKV *Bt*-SGn-5-75 SC	13.2×10^8
9	PDKV*Bt*-SBn-2-75 SC	8.63×10^8
10	PDKV*Bt*-I-3-75 SC	4.8×10^8
11	PDKV *Bt*-SY-4-75 SC	17.6×10^8

Among the wettable powder formulation (WP) (Table 2), the highest spore count was recorded in PDKV *Bt* SY-4-25 WP with 5.85×10^8 CFU/ml, followed by PDKV *Bt* SA-20-25 WP with 5.5×10^8 CFU/ml, PDKV *Bt* SGn-4-25 WP with 4.9×10^8 CFU/ml,, PDKV *Bt* SGn-5-25 WP with 4.4×10^8 CFU/ml, PDKV *Bt* SAk-9-25 WP with 4.08×10^8 CFU/ml, PDKV *Bt* SBn-2-25 WP with 2.88×10^8 CFU/ml, PDKV *Bt* SA-6-25 WP with 2.63×10^8 CFU/ml, PDKV *Bt* I-3-25 WP with 1.6×10^8 CFU/ml, and PDKV *Bt* SA-18-25 WP with 1.58×10^8 CFU/ml respectively. The PDKV *Bt* strains SGd-1-25 WP and SAk-6-25 WP recorded the lowest spore count of 1×10^8 CFU/ml. Although there was a difference in spore count among the *Bt* formulations, their efficacy did not rely solely on it. The efficacy of the strains depends on the presence of potent crystal proteins and other related factors. According to Schnepf *et al.* (1998) [6], the virulence of a *Bt* strain is not determined only by spore count, but also by the composition, concentration, and bioactivity of its toxins.

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

Sr. No	PDKV *Bt* SC formulations	Spore count (CFU/ml)
1	PDKV *Bt*-SA-6-25 WP	2.63×10^8
2	PDKV *Bt*-SA-18-25 WP	1.58×10^8
3	PDKV *Bt*-SA-20-25 WP	5.5×10^8
4	PDKV *Bt*-SAk-6-25 WP	1×10^8
5	PDKV *Bt*-SAk-9-25 WP	4.08×10^8
6	PDKV *Bt*-SGd-1-25 WP	1×10^8
7	PDKV *Bt*-SGn-4-25 WP	4.9×10^8
8	PDKV *Bt*-SGn-5-25 WP	4.4×10^8
9	PDKV*Bt*-SBn-2-25 WP	2.88×10^8
10	PDKV*Bt*-I-3-25 WP	1.6×10^8
11	PDKV *Bt*-SY-4-25 WP	5.85×10^8

Conclusions

Among the two formulations, suspension concentrate (SC) was found to have more CFU count which can be attributed to its higher concentration of active ingredient. It can be concluded that all PDKV *Bt* formulations had higher CFU count (CFU/ml) than the standard *Bt K* formulation, [*Bt kurstaki* 5% WP (CFU 5×10^7 spore/ml)]. Among PDKV *Bt* suspension concentrate (SC) formulations, the highest CFU count was recorded in PDKV *Bt* SY-4-75 SC with 17.6×10^8 CFU/ml whereas, PDKV *Bt* SY-4-25 WP recorded highest spore count among wettable powder formulations with

5.85×10^8 CFU/ml. The studies revealed suitability of L. B. medium for culturing of all the novel PDKV *Bt* strains.

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References

1. Bravo A, Likitvivatanavong S, Gill SS, Soberón M. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochemistry and Molecular Biology. 2011;41(7):423-431.
2. Ishiwata S. Eineneue Krankheit der Seidenraupe [A new disease of the silkworm]. Tokyo: Insect Pathology Society; 1901.
3. Kumar S, Sahai V, Bisaria VS. Application of toxins from *Bacillus thuringiensis* as biopesticides. Biotechnology Advances. 2008;26(4):323-335.
4. Saberi F, Marzban R, Ardjmand M, Shariati FP, Tavakoli O. Optimization of culture media to enhance the ability of local *Bacillus thuringiensis* var. *tenebrionis*. Journal of the Saudi Society of Agricultural Sciences. 2020;19(7):468-475.
5. Saxena D, Sinha RK, Balaraman K. Enhanced UV tolerance and insecticidal activity in melanin-producing mutants of *Bacillus thuringiensis*. Biocontrol Science and Technology. 2002;12(5):521-525.
6. Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Dean DH. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews. 1998;62(3):775-806.
7. Maeda M, Mizuki E, Nakamura Y, Hatano T, Ohba M. Recovery of *Bacillus thuringiensis* from marine sediments of Japan. Current Microbiology. 2000;40(6):418-422.
8. Marzban R, Babaei J, Kalantari M, Saberi F. Preparation of wettable powder formulation of *Bacillus thuringiensis* KD2. Journal of Applied Biological Sciences. 2021;15(3):285-293.
9. Mohamed HALA, Abd El-Fatah MR, Sabbour MM, El-Sharkawey AZ, El-Sayed RS. Genetic modification of *Bacillus thuringiensis* var. *kurstaki* HD-73 to overproduce melanin, UV resistance and their insecticidal potentiality against potato tuber moth. International Journal of Academic Research. 2010;2(6):74.
10. Nester EW, Thomashow L, Metz M. 100 years of *Bacillus thuringiensis*: A critical scientific assessment [Internet]. Washington (DC): American Society for Microbiology; 2002 [cited 2025 Jul 23]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559445/ doi:10.1128/AAMCol.16Nov.2002
11. Travers RS, Martin PAW, Reichelderfer CF. Selective process for effective isolation of *Bacillus* spp. Applied and Environmental Microbiology. 1987;56(6):1263-1266.
12. Vimala Devi PS, Vineela V. Suspension concentrate formulation of *Bacillus thuringiensis* var. *kurstaki* for effective management of *Helicoverpa armigera* on sunflower (*Helianthus annuus*). Biocontrol Science and Technology. 2015;25(3):329-336. doi:10.1080/09583157.2014.977846.