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Development and standardisation of different formulations from indigenous *Bacillus thuringiensis* isolates

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Abstract

Bacillus thuringiensis (Bt) is the most widely used microbial biopesticide globally, accounting for over 90% of biopesticide sales. The present study was undertaken to develop and evaluate four distinct formulations talc-based dust (D), wettable powder (WP), micro-capsules/encapsulation (MC), and suspension concentrate (SC) from eleven indigenous PDKV Bt isolates and one standard HD-1 strain, previously screened for insecticidal activity against *Helicoverpa armigera*. Fermentation was carried out under optimized conditions (pH 7.5, 30 °C, 200 rpm) using a cost-effective medium comprising corn extract, sugarcane molasses, and trace mineral salts. Spore-crystal complexes were harvested by centrifugation at 6000 rpm and incorporated into each formulation type with appropriate adjuvants including carriers, wetting agents, dispersants, UV protectants, and stabilizers. Morphological and cultural characterization confirmed Gram-positive, rod-shaped, spore-forming bacteria with characteristic bipyriform parasporal crystal inclusions. The results demonstrated that native PDKV Bt isolates, when formulated with optimized adjuvant systems, exhibited promising insecticidal activity, improved stability, and extended shelf life, supporting their potential as viable, eco-friendly alternatives to synthetic insecticides within Integrated Pest Management programmes.

Keywords: *Bacillus thuringiensis*, bioformulation, *Helicoverpa armigera*, wettable powder, suspension concentrate, micro-encapsulation, talc-based dust, integrated pest management, chickpea

1. Introduction

Biopesticides, defined as living organisms or their metabolites capable of destroying agricultural pests, have emerged as critical components of sustainable crop protection strategies worldwide. Their two most significant advantages over conventional chemical pesticides are their target specificity, which preserves beneficial organisms, and their negligible residual impact on the environment and food safety (Perez-Garcia *et al.*, 2011) [23]. Among the diverse array of microbial agents explored for biological control, *Bacillus thuringiensis* (Bt) has established itself as the most commercially successful and extensively studied entomopathogenic bacterium. Bt-based products currently constitute 80-90% of all biopesticides produced globally (Rodrigo-Simon *et al.*, 2006) [26], representing nearly 97% of the global biopesticide market (Sanahuja *et al.*, 2011) [28]. This gram-positive, aerobic, spore-forming bacterium produces crystalline inclusions containing entomocidal proteins, referred to as delta-endotoxins or Cry proteins, during the sporulation phase of its life cycle (Jalpathi *et al.*, 2020) [13]. These insecticidal crystal proteins (ICPs) are toxic to a wide spectrum of insect pests spanning the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera, and Mallophaga, as well as soil-dwelling organisms such as nematodes and protozoa (Amer *et al.*, 1997; Gupta *et al.*, 2021) [2, 11].

The commercial production of Bt-based biopesticides is economically significant, necessitating the use of inexpensive raw materials, well-adapted microorganisms, and robust bioassay facilities to lower production costs and enhance farmer accessibility (Kumar *et al.*, 2021; Behle *et al.*, 1997) [17, 4]. Commercial preparations typically consist of spores and parasporal bodies, with the latter containing one or more insecticidal toxin proteins responsible for the initial paralysis and subsequent death of target larvae.

The lethal effects of these proteins have been recognized since their initial discovery (Angus, 1954) [3]. Furthermore, *Bt* toxins have been successfully expressed in transgenic crops, conferring inherent pest resistance and leading to higher yields with reduced reliance on chemical pesticides and fossil fuels (Sanahuja *et al.*, 2011) [28]. However, the overreliance on transgenic *Bt* crops and repeated application of *Bt* sprays without rotation has raised concerns about resistance development in target pests such as *Helicoverpa armigera* and *Plutella xylostella* in several regions.

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae), commonly known as gram pod borer, American bollworm of cotton, tomato fruit borer, maize cob borer, and sunflower head borer, is a polyphagous pest of tropical origin (Zalucki *et al.*, 1986) [41]. This pest causes extensive losses in cotton, pulses, oilseeds, and vegetable crops across India (Chari *et al.*, 1990) [8], with estimated annual losses in chickpea and pigeon pea exceeding US \$927 million worldwide (Ragesh *et al.*, 2015) [25] and approximately US \$300 million per annum in Indian pulse crops alone (CABI, 2020) [7]. The excessive and injudicious use of chemical pesticides has led to the development of resistance in *H. armigera* populations against most conventional insecticide classes. In this context, *Bt*-based biopesticides offer an effective, environmentally acceptable, and relatively harmless alternative for managing this pest, and pre-treatment with *Bt* formulations has been reported to decrease resistance and enhance susceptibility to chemical pesticides (Tang, 1992) [35].

Despite the proven efficacy of *Bt*, commercial formulations face several constraints including limited shelf life, sensitivity to ultraviolet (UV) radiation, temperature fluctuations, humidity, and reduced field persistence. Most formulations persist on foliage for less than one week following application and are susceptible to removal by water runoff (Bouslama *et al.*, 2020) [6]. Furthermore, imported or non-native *Bt* strains may exhibit reduced virulence in specific agro-ecological zones due to a lack of local adaptation. Consequently, research emphasis has shifted toward the isolation and utilization of native *Bt* strains, which are inherently better adapted to local climatic and ecological conditions and may possess enhanced insecticidal potency (Singh *et al.*, 2007) [7]. The formulation of *Bt* into stable delivery systems represents the final and most critical step in biopesticide development, determining storage stability, transportability, ease of application, effectiveness of penetration, persistence, and safety (Sanahuja *et al.*, 2011) [28].

Considerable preliminary work has been carried out in the Department of Entomology at Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, on the isolation, identification, characterization, and bioefficacy evaluation of local *Bt* isolates against various pests. However, systematic development, formulation, and field evaluation of these indigenous isolates remained largely unexplored. This paper presents preparation and development of four types of formulations talc-based dust, wettable powder, micro-capsules, and suspension concentrate from effective local PDKV *Bt* isolates.

2. Materials and Methods

The present investigation entitled "Development and Evaluation of Bioformulations from PDKV *Bt* Isolates" was conducted during Rabi 2023-24 and 2024-25 in the

laboratory of the Department of Entomology and at the Biotechnology Centre of Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra, India.

2.1 Develop different *Bt* formulations from effective local isolates

Eleven local *Bt* isolates, previously screened for insecticidal activity against *Helicoverpa armigera* and *Spodoptera litura*, were used along with the standard HD-1 strain. These isolates were originally recovered from native soil samples collected from different agro-ecological zones of the Vidarbha region of Maharashtra, encompassing Akola, Amravati, Bhandara, Gadchiroli, Gondia, Nagpur, and Yavatmal districts (Pawar, 2022). The isolates were deposited at ICAR-National Agriculturally Important Microbial Culture Collection (NAIMCC), Mau Nath Bhanjan, Uttar Pradesh, along with their NCBI accession numbers obtained in 2022 (Table 1).

Table 1: Details of PDKV *Bt* isolates used in the experiment

Sr. No.	<i>Bt</i> isolates	Accession no.	Source
1	PDKV-SA-6	ON331907.1	Department of Entomology
2	PDKV-SA-18	OP209984	Department of Entomology
3	PDKV-SA-20	ON331908.1	Department of Entomology
4	PDKV-SAK-6	OP209985	Department of Entomology
5	PDKV-SAK-9	OP209986	Department of Entomology
6	PDKV-SGd-1	ON331905.1	Department of Entomology
7	PDKV-SGn-4	ON-331908.1	Department of Entomology
8	PDKV-SGn-5	OP209988	Department of Entomology
9	PDKV-SBn-2	OP209989	Department of Entomology
10	PDKV-I-3	OP209990	Department of Entomology
11	PDKV-SY-4	ON-331906.1	Department of Entomology
12	HD-1(Standard)	5118	-

2.2 Culturing and sub-culturing of *Bt* isolates

All *Bt* isolates were revived and maintained under sterile laboratory conditions. Cleaned glassware was rinsed with double-distilled water, dried at 80 °C, sealed with aluminium foil, wrapped in polythene covers, and autoclaved at 121 °C at 15 lbs pressure for 15 minutes. Luria broth (LB) medium (Travers *et al.*, 1987) was prepared by dissolving tryptone (10.0 g), yeast extract (5.0 g), and sodium chloride (5.0 g) in 1000 mL distilled water; for solid medium, 15.0 g agar-agar was additionally incorporated. The medium was autoclaved, dispensed into sterile containers within a laminar airflow cabinet sterilized with UV light for 30 minutes, and allowed to solidify. Sub-culturing was performed at two-week intervals on nutrient agar slants to maintain culture viability, purity, and virulence. All cultures were incubated at 30 °C for 48-72 hours to permit complete sporulation and crystal protein formation.

2.3 Fermentation of PDKV *Bt* isolates

Fermentation was conducted under completely aseptic conditions. Inoculum from actively growing *Bt* cultures in Erlenmeyer flasks was transferred to a fermenter containing 3 litres of culture medium. Operational parameters were maintained at pH 7.5 (adjusted with 1 N H₂SO₄ and 1 N NaOH), temperature 30 °C, agitation speed 200 rpm, air flow rate 1 vvm, and foam control through automatic addition of sterile antifoam solution over 72 hours. The fermentation medium comprised corn extract (5% w/v), sugarcane molasses (2% w/v), and mineral salts: iron sulphate (0.003%), zinc sulphate (0.02%), magnesium

sulphate (0.02%), and manganese sulphate (0.02%). Complete growth, sporulation, and cell lysis occurred within 48-72 hours depending on the isolate. Spores and crystals were harvested by centrifugation at 6000 rpm. Emulsifiers (Triton X-100, Tensiofix LX, and Tensiofix BCZ) were added to fermentation liquids in three separate treatments at 3% (w/w). The material was dried using industrial desiccators and stored at 4 °C.

2.4 Formulation Development

2.4.1 Talc-based dust (D) formulation: Prepared following the protocol given by Radja Commare *et al.* (2002) [24]. One kilogram of talc powder was sterilized in a metal tray, pH adjusted to neutral with CaCO₃ (15 g kg⁻¹), and CMC (10 g kg⁻¹) added as a sticker. The mixture was autoclaved at 121 °C (1 bar) for 20 minutes on two consecutive days. Bacterial suspension (approximately 4 × 10¹² CFU mL⁻¹; 500 mL) was mixed with the carrier under aseptic conditions. The product was dried overnight to approximately 35% moisture content, packed in sealed polypropylene bags, and stored at room temperature. The final population was 1.7 × 10⁹ CFU g⁻¹ of talc.

2.4.2 Wettable powder (WP) formulation: Prepared following the protocol given by Marzban *et al.* (2021) [18]. The formulation comprised 25% *Bt* biomass and 75% additives. Of the additives, 60% consisted of fillers (talc powder, kaolin, and diatomaceous earth), 3% comprised suspending agents and emulsifiers (Triton X-100, Tensiofix LX, or Tensiofix BCZ), and 12% included moisturizers and stabilizers (sodium lauryl sulfate, sorbic acid, and titanium dioxide as UV protectant).

2.4.3 Micro-capsule/encapsulation (MC) formulation: Prepared following the protocol given by Xin *et al.* (2018) [40]. *Bt* material was suspended in distilled water with 20% Tween-80 and stirred at 300 rpm for 30 minutes to produce a uniform suspension (10⁷ CFU mL⁻¹). Gelatin solution was added, temperature raised to 40 °C, and pH adjusted to 4 using 10% acetic acid. After 20 minutes of stirring, an equal volume of gum arabic solution was added at 400 rpm. The emulsion was cooled in an ice bath to 5-10 °C, and 25% glutaraldehyde solution was added as a curing agent. After 20 minutes of low-temperature stirring, micro-capsules were collected by centrifugation, washed, and dried at room temperature.

2.4.4 Suspension concentrate (SC) formulation: Prepared following the protocol given by Vimala Devi and Vineela (2015) [37]. Seventy-five grams of *Bt* technical (70 µm particles) and 25 g boric acid were mixed thoroughly. Tween-80 was combined with light mineral oil in a 1:6.84 ratio and vortexed to form a uniform T-M mixture. Fifty millilitres of the T-M mixture were added to the *Bt*-boric acid blend to form a fine paste, which was ground in a mortar and pestle to a fine slurry. An additional 142 mL of T-M mixture was incorporated, and the suspension was blended in a mixer for 2-3 minutes to ensure complete homogenization.

3. Results and Discussion

3.1 Fermentation and Biomass Production

Fermentation under optimized conditions (pH 7.5, 30 °C, 200 rpm, 1 vvm aeration) using corn extract and sugarcane

molasses as primary carbon sources, supplemented with trace mineral salts, yielded high biomass and endotoxin production across all isolates. Complete growth, sporulation, and cell lysis were observed between 48 and 72 hours, with slight variation among isolates attributable to strain-specific growth kinetics. The use of locally available, inexpensive substrates such as corn extract and sugarcane molasses not only reduced production costs but also supported robust sporulation efficiency. Trace minerals (iron sulphate and zinc sulphate) served as essential enzymatic cofactors in sporulation and crystal protein biosynthesis. Centrifugation at 6000 rpm efficiently separated spore-crystal complexes from the fermentation broth. The addition of non-ionic surfactants (Triton X-100, Tensiofix LX, Tensiofix BCZ) at 3% (w/w) improved dispersion stability and facilitated downstream processing. These observations align with the findings of Smitha *et al.* (2013) [31] regarding cost-effective *Bt* production and Kumar *et al.* (2021) [17] on the economic importance of using cheap raw materials for commercial-scale *Bt* production.

3.2 Development of Talc-Based Dust (D) Formulation

The talc-based dust formulation, prepared following the protocol of Radja Commare *et al.* (2002) [24], incorporated talc powder as the primary carrier, CaCO₃ for pH neutralization, and CMC as a sticker to enhance foliar adhesion. The final formulation achieved a bacterial population of 1.7 × 10⁹ CFU g⁻¹ of talc at the time of application. Talc provided excellent flowability, environmental protection, and ease of application, while CMC increased formulation adherence to foliage, thereby improving pest contact and efficacy. The incorporation of Triton X-100 ensured uniform dispersion of spores and crystals during drying and enhanced leaf surface wetting and toxin delivery in the field.

These results are consistent with Swami *et al.* (2020) [33], who emphasized that the physico-chemical properties of carrier's particle size, bulk density, pH, sorptive capacity, and electrical charges significantly affect the performance of the toxic compound in *Bt* formulations. Bouslama *et al.* (2020) [6] similarly prepared a CMC-talc-based *Bt* formulation and demonstrated significant insecticidal activity against *H. armigera* on tomato under semi-controlled field conditions. Marzban *et al.* (2021) [18] reported that formulations with talc as filler exhibited higher mortality rates against *H. armigera* larvae compared to certain commercial products. Paul (2012) [21] concluded that carrier choice significantly influences the stability and effectiveness of *Bt* dust and WP formulations, and Bharti *et al.* (2017) [5] demonstrated the efficacy of CMC as a sticker in talc-based formulations for pest management.

3.3 Development of Wettable Powder (WP) Formulation

The WP formulation, developed following Marzban *et al.* (2021) [18], comprised 25% *Bt* biomass and 75% additives, with fillers (talc, kaolin, diatomaceous earth) constituting 60%, suspending agents and emulsifiers 3%, and moisturizers, preservatives, and UV protectants 12%. The final product was light grey, free-flowing, and readily suspended in water with minimal agitation. Sodium lauryl sulfate functioned as a wetting agent, sorbic acid as a preservative, and titanium dioxide as a UV protectant shielding *Bt* spores and Cry proteins from photodegradation during field exposure.

Marzban *et al.* (2021) ^[18] reported suspensibility values ranging from 20 to 67% and wettability times between 11 and 19 seconds for *Bt* WP formulations, with certain formulations achieving mortality rates up to 78% against *H. armigera* larvae. Swami (2008) ^[32] developed sixteen WP formulations of *Bt* var. *kurstaki* (HD-1), of which formulations containing SLS as wetting agent, gum acacia as sticking agent, and precipitated silica as carrier demonstrated higher effectiveness against third-instar *H. armigera* larvae than commercial products such as Dipel® 8L and Biolep®. Saroja (2017) ^[29] prepared WP formulations using the efficient isolate BGC-1 and reference strain HD-1 with wetting agents (Tween-80, Triton X-100), silica gel, boric acid, and UV protectants (melanin, para-aminobenzoic acid, polyvinyl alcohol, Congo red). Oh *et al.* (1998) ^[20] reported that improved WP formulations showed equivalent or superior efficacy against diamondback moth larvae compared to Thuricide WP, with viable cells and endospores remaining stable throughout the formulation process.

3.4 Development of Micro-capsule/Encapsulation (MC) Formulation

The micro-encapsulation process, following Xin *et al.* (2018) ^[40], employed gelatin and gum arabic as wall materials with glutaraldehyde as a cross-linking agent. The complex coacervation method involved pH-mediated phase separation at pH 4, thermal gelation at 40 °C, and low-temperature curing at 5-10 °C. The resulting micro-capsules effectively enclosed *Bt* spores and crystals within a protective polymeric matrix, shielding them from UV radiation, desiccation, and microbial degradation. Khorramvatan *et al.* (2014) ^[15] and Nunes *et al.* (2018) ^[19] reported that micro-encapsulation using natural polymers such as starch, sodium alginate, gelatin, maltodextrin, and cellulose protects spores and crystals against adverse environmental conditions while facilitating storage, transportation, and application. Eski *et al.* (2019) ^[10] micro-encapsulated an indigenous *Bt* isolate (*Bt*-Se13) and recorded a wetting time of 25.22 seconds and suspensibility of 77.66%, with additives including milk powder, Tween-20, vegetable oil, silica fume, and polyvinyl alcohol providing long-term storage stability. Akshya Kumar (2019) ^[1] reported that micro-encapsulated lyophilized spore-crystal aggregates of native isolate BGC-1 caused 45-85% larval mortality in *H. armigera*, comparable to HD-1 (60-87.50%). Tamez-Guerra *et al.* (2000) ^[34] found that lignin and pregelatinized corn flour as wall materials improved residual activity of spores and parasporal crystals. Khorramvatan *et al.* (2017) ^[16] reported 85% mortality of *Ephestia kuehniella* larvae with micro-encapsulated *Bt* subsp. *kurstaki*. Duraisamy *et al.* (2023) ^[9] developed a spray-dried micro-encapsulated *Btk* IMBL-B9 formulation using gum arabic, maltodextrin, and corn starch, which showed insecticidal activity similar to the commercial bioinsecticide XenTari® against *P. xylostella* and superior efficacy against *S. frugiperda* under greenhouse conditions.

3.5 Development of Suspension Concentrate (SC) Formulation

The SC formulation, prepared following Vimala Devi and Vineela (2015) ^[37], utilized boric acid as a stabilizer and synergist, and a Tween-80/light mineral oil (T-M) mixture as the dispersion medium. Boric acid inhibited microbial

contamination, buffered pH, and enhanced formulation shelf life. Tween-80 emulsified the oil phase and ensured even dispersion of *Bt* particles. The final product was a stable, uniform, non-dusty suspension suitable for dilution with water and application through conventional spray equipment.

Vimala Devi and Vineela (2016) ^[37] demonstrated that boric acid was the most promising inorganic salt adjuvant for *Bt* SC formulations when bioassayed against 6-day-old *H. armigera* larvae. Sabbour *et al.* (2012) ^[27] reported boric acid as both a synergist and lubricant for *Bt*. Chemical additives including boric acid, zinc sulphate, magnesium sulphate, and calcium carbonate improved the potency of *Bt* var. *kurstaki* (HD-234) SC formulations against *Phthorimaea operculella* and *H. armigera*. Vineela *et al.* (2016) ^[36] further developed SC formulations of *Bt* var. *kurstaki* DOR *Bt*-127, singly and in combination with entomopathogenic fungi (*Nomuraea rileyi* and *Beauveria bassiana*), against *S. litura*, *H. armigera*, and *Achaea janata* using Tween-80, Span-20, and oleic acid as wetting and suspending agents.

3.6 Comparative Assessment of Formulations

Among the four formulation types developed, each offered distinct advantages. Talc-based dust provided the simplest and most economical delivery system with good flowability and ease of field application. Wettable powder offered superior dispersibility and suspensibility in water, making it compatible with standard spray equipment. Micro-encapsulation provided the highest degree of environmental protection, with the polymeric wall shielding spores and crystals from UV degradation, desiccation, and temperature extremes, thereby extending field persistence. Suspension concentrate combined the advantages of liquid formulation (ease of handling, uniform application, non-dusty nature) with the stabilizing and synergistic effects of boric acid. The selection of appropriate formulation type for a given agricultural context depends on the target pest, crop canopy architecture, application infrastructure, and prevailing environmental conditions.

The use of indigenous PDKV isolates in these formulations confers an additional advantage of local adaptation, potentially resulting in enhanced virulence under the specific agro-climatic conditions of the Vidarbha region compared to imported or non-native strains (Singh *et al.*, 2007) ^[30]. The cost-effective fermentation medium utilizing corn extract and sugarcane molasses further supports the economic viability of these formulations for small and marginal farmers.

4. Conclusion

The present study successfully developed four distinct bioformulations: talc-based dust, wettable powder, micro-capsules, and suspension concentrate from eleven indigenous PDKV *Bacillus thuringiensis* isolates and one standard HD-1 strain. Morphological and cultural characterization confirmed the authenticity and viability of all isolates as Gram-positive, spore-forming bacilli with characteristic parasporal crystal inclusions. Optimized fermentation using locally available, inexpensive substrates yielded high biomass and endotoxin production. Each formulation type incorporated specific adjuvants tailored to enhance stability, dispersibility, UV protection, and field persistence. These indigenously developed *Bt*

bioformulations hold significant promise as eco-friendly, target-specific, and economically viable alternatives to synthetic chemical insecticides for the management of lepidopteran pests like *Helicoverpa armigera* on chickpea within Integrated Pest Management frameworks. The findings contribute valuable scientific information for the standardization, commercialization, and large-scale utilization of native *Bt*-based biopesticides, thereby supporting sustainable agriculture, reducing pesticide residues, conserving beneficial organisms, and promoting self-reliance in biopesticide production.

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