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RESEARCH ARTICLE

Enhanced bioefficacy of *Bacillus thuringiensis* var. *kurstaki* against *Spodoptera litura* (Lepidoptera: Noctuidae) through particle size reduction and formulation as a suspension concentrate

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ABSTRACT

Technical powder of DOR Bt-127, a local isolate of *Bacillus thuringiensis* (Bt) var. *kurstaki*, containing 105 µm particles was subjected to milling in a planetary ball mill. Dynamic light scattering of samples drawn at 30 min intervals revealed reduced particle sizes ranging 1171–210 nm. Alkali-soluble protein was higher at 153–175 mg/g in milled Bt powders over 140 mg/g in unmilled Bt powder. All milled powders gave higher mortality of *Spodoptera litura* (Fabricius) larvae in comparison to the unmilled Bt powder. Bt powder from 60 min milling containing 559 nm particles with a low polydispersity index of 0.338 gave a lower LC_{50} value of 1.35 mg/ml against third instar *S. litura* larvae in comparison to 2.04 mg/ml for unmilled Bt powder. LC_{50} value of the suspension concentrate (SC) formulation developed with 559 nm Bt particles was 2.84 µl/ml containing only 0.95 mg Bt. Field evaluation of the SC formulation against *S. litura* on castor revealed highest per cent larval reduction of 92.4 and 96.2 at concentrations of 2.5 and 3.0 ml/l, respectively.

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Bacillus thuringiensis var. *kurstaki*; ball milling; *Spodoptera litura*; particle size; suspension concentrate

1. Introduction

Castor, *Ricinus communis*, is a non-edible oilseed crop of industrial importance. Globally castor is being cultivated in 1.45 million ha with an average production of 1.95 million MT and productivity of 1345 kg/ha. Cultivated areas in India are 1.04 million ha with an average production of 1.72 million MT and productivity of 1666 kg/ha during 2014 (Sarada, Alivelu, Sambasiva Rao, Sudhakar Babu, & Varaprasad, 2015). *Spodoptera litura* is a polyphagous pest of castor in India (Sujatha, Vimala Devi, & Reddy, 2010) in addition to several agricultural crops in Southeast Asia, China and Japan (Hummelbrunner & Isman, 2001) and throughout the world (EI-Aswad, Abdelgaleil, & Nakatani, 2003). It has developed resistance to many of the commonly used insecticides (Aydin & Gurkan, 2006; Huang, Xu, & Han, 2006).

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The insecticidal bacterium *Bacillus thuringiensis* (Bt) has been employed globally for insect pest management on several crops. Bt toxins have been employed as topical pesticides against pests like *Helicoverpa armigera*, *Plutella xylostella*, *Ostrinia nubilalis*, *Agrotis ipsilon*, *Achaea janata*, *Spodoptera Exigua*, etc. (George & Crickmore, 2012; Pandey, Joshi, & Tiwari, 2009; Sanchis & Bourguet, 2008; Vimala Devi & Sudhakar, 2006; Vimala Devi & Vineela, 2015; Vimala Devi, Ravinder, & Jaidev, 2005). Although Bt *cry* toxins are effective insecticidal proteins, several economically important insects such as black cut worm, *A. ipsilon* and *Spodoptera* spp. are less sensitive to their action (Abdullah, Moussa, Taylora, & Adang, 2009). The Egyptian cotton leaf worm, *S. littoralis*, is not susceptible to majority of the delta endotoxins (Alotaibi, 2013).

Development of innovative formulations aiming to decrease particle size has been the subject of intense research over the past decade in pharmaceuticals, cosmetics and crop protection (Lav, Sindhuja, Joe, Reza, & Edmund, 2012). Bioavailability of the active ingredient can be increased through the use of additives or by means of particle size reduction (Horn & Rieger, 2001). One of the oldest strategies for improving the solubility of drugs has been through particle size reduction since solubility is intrinsically related to particle size. Particle size and particle size distribution are critical factors in the performance of nanoparticles, as batches with wide particle size distribution show significant variation in efficacy (Kharia, Singhai, & Verma, 2012).

Micronisation is a conventional approach adopted in drug development employing techniques such as jet milling, ball milling and high pressure homogenisation for conversion of coarse powder to an ultrafine powder with the mean particle size in the range of 2–5 μm and a small fraction of the particles below 1 μm size range (Khadka et al., 2014). Feasibility of this approach for increasing the efficacy of Bt, that acts through solubilisation of the crystal toxin in the alkaline gut fluid of the target insect followed by proteolytic activation, has not received much attention. Information available on increasing the efficacy of Bt through particle size reduction of Bt technical powders is scanty and limited to hammer milling, air milling and high pressure homogenisation (Kim & Jae, 2012; Murthy, Vineela, & Vimala Devi, 2014). Another approach employed effectively in particle size reduction is the planetary ball mill, wherein balls made of zirconium, agate, ceramic, stainless steel, nylon, alumina, etc. have been used for grinding powders of several materials including aluminium, silicon, nickel, copper, carbonates of cadmium and zinc, etc. for obtaining particles of nanometer scale (Ksiaiek, Wacke, Gorecki, & Gorecki, 2007; Ramezani & Neitzert, 2012) but not exploited for Bt.

We report here results of studies undertaken for increasing the efficacy of a local isolate of Bt *kurstaki* DOR Bt-127 through particle size reduction to submicron-scale employing a planetary ball mill followed by formulation and evaluation of the product against larvae of the major lepidopteran pest of castor, *S. litura* in the laboratory and field.

2. Materials and methods

2.1. Bt multiplication

DOR Bt-127 isolate was multiplied on a solid substrate based on wheat bran flakes (150 g) in plastic tubs (Vimala Devi et al., 2005). After 72 h incubation at 30°C, substrate with Bt spores and crystals from each tub was added to 800 ml of sterile distilled water, mixed well

and filtered through a muslin cloth. The filtrate was centrifuged at 10,000 rpm for 10 min. The resulting pellet was spread thinly on a sterile polythene sheet and dried overnight at 30°C in a laminar airflow chamber and powdered. Each tub yielded 20 g of Bt technical powder which was passed through a sieve (Jayanth test sieve) of 105 µm mesh size to obtain a coarse powder of Bt technical.

2.2. Milling

Bt-127 technical powder (105 µm) was milled in a planetary ball mill, XQM-1-A, Changsha Tianchuang Powder Technology Co. Ltd, China. The mill contains four grinding tanks made of zirconium oxide and is equipped with a cooling system to provide cooling temperatures 2–10°C. In each jar, 30 g of Bt technical powder and 300 g of 3 mm zirconium balls were added (1:10). Jars were fixed on the turntable and milling was carried out at 300 rpm for 120 min period. Samples were withdrawn at 30 min intervals and used for further studies.

2.3. Particle sizing of Bt milled particles

Milled samples of Bt-127 drawn at different intervals were subjected to dynamic light scattering (DLS) in a HORIBA Nano particle analyzer SZ-100 for particle size determination. A small amount of milled material (0.25 mg/ml) was suspended in 0.01% Tween-80 water, sonicated to get a clear suspension and transferred to the sample dispersion unit. Size was measured through number weighted distribution using a laser beam at 25°C at a scattering angle of 173 and viscosity 0.892 mPa/s of the dispersion medium. Polydispersity index (PI), which is a parameter for studying the particle size distribution, was also measured for each sample.

2.4. Development of suspension concentrate formulation

Bt-127 powder from 60 min milling containing 559 nm Bt particles was used for development of the suspension concentrate (SC) formulation. Two wetting agents viz., 14.6 ml of Tween-80 (polyoxyethylene sorbitan mono-oleate) and 9.6 ml of Span-20 (sorbitan monolaurate) were taken in a sterile glass conical flask, mixed well and vortexed in a vortex mixer. To this mixture, 213.8 ml of light mineral oil (liquid paraffin light from Fischer Scientific) was added, and the contents of the flask were vortexed for 10 min to obtain a uniform mixture. Initially, 50 ml of this wetting agent + mineral oil mixture was added to 100 g of Bt powder, mixed well with a glass rod to form a uniform paste without any clumps followed by the addition of the remaining mixture to get the SC formulation. This SC formulation was then stirred well at 300 rpm in a magnetic stirrer for 30 min to get a free flowing SC formulation. The formulation was transferred into ahigh-density polyethylene bottle and used for further studies.

2.5. Determination of heat viable spore count and total protein estimation

Samples of pre- and post-milled Bt powders (1 g/100 ml) and SC formulation (1 ml/100 ml) were given heat shock at 80°C for 3 min for determination of the heat viable

spore count (Travers, Martin, & Reichelderfer, 1987). Samples were then subjected to serial dilution up to 10^{14}, and 100 µl of each sample was plated on nutrient agar in sterile glass Petri plates. Six replicates were maintained for each sample. Observations on the number of Bt colonies per plate were recorded at 48 h after inoculation, and the heat viable spore count per g Bt powder or ml of the SC formulation was calculated using the formula $N \times 10^3 \times D$ (N = number of colonies per plate and D = dilution).

For total protein estimation, 100 mg of each Bt powder was suspended in 10 ml of 1 M sodium chloride and centrifuged at 17,000 rpm in a Heraeus Biofuge Strauss followed by water washes. Resultant pellet was suspended in 10 ml of 100 mM sodium hydroxide and incubated at 30°C on a shaker (200 rpm) for 2 h followed by centrifugation at 17,000 rpm for 10 min at 5°C. Soluble protein in the supernatants was estimated (Lowry, Rosenbrough, Fair, & Randall, 1951). In case of SC formulation, 100 µl of the formulation was initially given two washes with 10 ml of 1% Tween-80 water to remove oil at 17,000 rpm, and protein was extracted from the sample by the above procedure.

2.6. Larval bioassay

Unmilled and milled Bt powders were evaluated through bioassays at 27 ± 1°C against third instar *S. litura* larvae by leaf disc technique at a concentration of 1.5 mg/ml using castor leaves (Ravi charan, Lakshmi Narasu, & Vimala Devi, 2013). Leaf discs (95 cm^2) were treated on each surface with 0.5 ml of test suspensions at 1.5 mg/ml prepared in 0.02% aqueous Tween-80, dried in laminar flow and transferred to Petri plates lined with moist cotton. Ten larvae were released in each plate. Four such replicates were maintained for each treatment and leaf discs treated with Tween-80 water were used as controls. Larval mortality was recorded at 24 h intervals till 72 h after treatment. Data were subjected to analysis of variance (ANOVA) using statistical package SAS 9.3 (SAS Institute). Mortality data were angular transformed before analysis, and Tukey's HSD test was used to test for differences among Bt treatments.

For LC_{50} determination, bioassays were carried out against third instar larvae of *S. litura* at five concentrations of Bt unmilled and 60 min milled samples (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) and Bt SC formulation (1.0, 1.5, 2.0, 2.5 and 3.0 µl/ml). Observations of larval mortality were recorded at 24 h intervals till 72 h after treatment. Data were subjected to probit analysis using the statistical package SPSS-16.0.

2.7. Field testing

Bt SC formulation was field tested at the Indian Institute of Oilseeds Research (IIOR), Rajendranagar, Hyderabad on castor hybrid DCH-519 in 5.4 × 3.0 m plots with 90 × 60 cm spacing. Sowing was undertaken during *kharif* (rainy season) in the second week of July 2014. The experiment was laid out in a randomised complete block design with seven treatments and three replications. Treatments were imposed in the first week of September against larval masses of *S. litura* larvae. Bt-127 SC formulation was tested at four concentrations viz., 300, 400, 500 and 600 ml per acre (corresponding values – 1.5, 2.0, 2.5 and 3.0 ml/l) along with the commercial Bt *kurstaki* formulation Delfin at 200 g (1.0 g/l) and an insecticidal check Profenofos at 200 ml (1.0 ml/l) per acre, respectively, in 200 l of water and an untreated control. Sprays were undertaken with a knapsack sprayer.

Observations of *S. litura* larvae were recorded before spray and 5 days after spray (DAS). Data were subjected to ANOVA using statistical package SAS 9.3 (SAS Institute).

3. Results

3.1. Milling and particle size analysis

Milling of Bt technical powder-105 µm resulted in powders with mean particle sizes of 1171, 559, 252 and 210 nm for samples drawn at 30, 60, 90 and 120 min, respectively, as revealed by DLS (Table 1). Unmilled Bt powder was brown in colour while milling resulted in light brown coloured powders. Extent of decolorisation of powders increased with duration of milling (Figure 1). Particle size distribution was monomodal (single peak) in all samples indicating a single size range of particles. Bt sample drawn at 60 min of milling has a low PI of 0.338 (Figure 2).

3.2. Heat viable spore count and protein quantification

Heat viable spore count and total protein content of the unmilled Bt powder were 7.2×10^{17} CFU/g and 140 mg/g, respectively. CFU of milled powders showed a decrease to 3.3×10^{16} g^{-1} till 120 min, while the total protein content increased after milling to 153–175 mg/g (Table 1).

3.3. SC formulation

The SC formulation was a brown coloured free flowing liquid forming a clear suspension when added to water that passed through a 70 µm sieve without clogging the pores. This makes the formulation highly suitable for field application using power sprayers which give a better coverage of the foliage. Total volume of the formulation using 100 g Bt powder was 300 ml thus making it a 33.3% SC formulation. Heat viable spore count and total protein content of the SC formulation were 3.3×10^{17} CFU/ml and 157 mg/ml, respectively.

3.4. Larval bioassays

Bioassays with unmilled and milled Bt powders against third instar larvae of *S. litura* at 1.5 mg/ml revealed significant differences in larval mortalities. Unmilled Bt powder resulted in 52.5% mortality at 48 h after treatment while samples drawn from 30 to

Table 1. Particle size, heat viable spore count/g and total protein content of Bt-127 powders obtained from ball milling at 300 rpm and ~10°C for different time intervals.

Milling time (min)	Particle size (nm) ± SD[a]	Heat viable spore count/g	Total protein mg/g ± SD[a]
Unmilled	105,000	7.2×10^{17}	140 ± 3
30	1171 ± 479	6.7×10^{17}	153 ± 2
60	559 ± 169	5.7×10^{17}	175 ± 5
90	252 ± 175	8.3×10^{16}	173 ± 3
120	210 ± 101	3.3×10^{16}	170 ± 1

[a]SD, standard deviation.

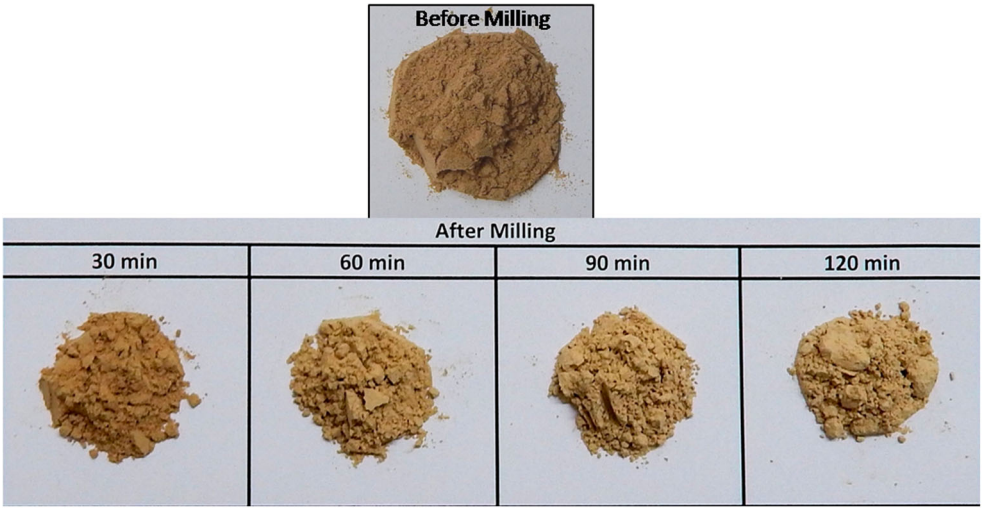

Figure 1. Powders of Bt-127 drawn after different milling durations.

120 min post-milling resulted in significantly higher mortalities ranging 72.5–77.5% (Table 2). Larval mortality showed a positive correlation to protein content. Cumulative larval mortality 72 h after treatment due to sample post-milling were significantly higher (77.5–85.0%) over unmilled Bt (55.5%). In general, larval mortality of *S. litura* caused by milled Bt powders was higher than that caused by the unmilled Bt powder.

With unmilled Bt powder, high feeding was observed in the lower concentrations 0.5, 1.0 and 1.5 mg/ml and medium feeding in the higher concentrations 2.0 and 2.5 mg/ml while LC_{50} was 2.04 mg/ml at 72 h after treatment. However, an immediate feeding cessation was observed at 24 h after treatment with milled Bt powders in all the concentrations except for the lowest concentration of 0.5 mg/ml while LC_{50} was 1.35 mg/ml at 72 h after treatment. In the case of Bt SC formulation, feeding cessation was observed in all the concentrations at 24 h after treatment. LC_{50} of the SC formulation was 2.84 µl (containing 9.5 mg of Bt) at 72 h after treatment (Table 3). Bt requirement for effective kill of *S. litura* larvae has thus been lowered by milling and further lowered by developing SC formulation with the milled powder.

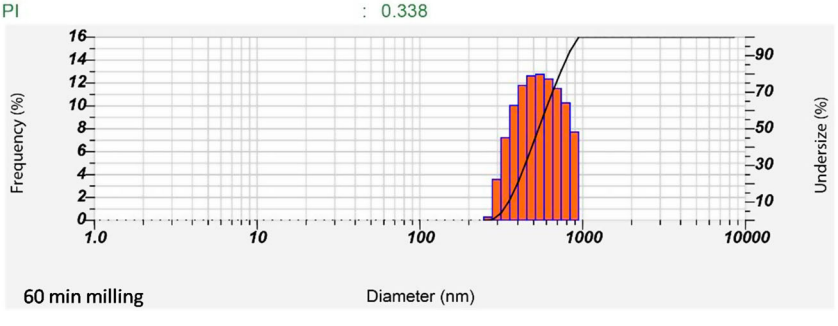

Figure 2. DLS of 60 min milling Bt particles – number weighted size distribution.

Table 2. Laboratory efficacy of Bt powders of different particle sizes against third instar larvae of *S. litura*.

	% larval mortality of *S. litura* larvae at 1.5 mg/ml[a]	
Particle size (nm)	48 h	72 h
105,000	46.4 (52.5)[b]	47.9 (55.5)[b]
1171	61.8 (77.5)[a]	61.8 (77.5)[a]
559	61.8 (77.5)[a]	67.5 (85.0)[a]
252	61.8 (77.5)[a]	65.8 (82.5)[a]
210	60.1 (72.5)[a]	63.4 (80.0)[a]
Control	0.52 (0.0)	0.52 (0.0)
SE mean ± CD ($P = .05$) CV (%)	2.2	3.2
	4.5	6.6
	6.3	8.7

[a]Values are angular transformed, values in parenthesis are original means; means followed by the same lower-case letter in a column did not differ significantly according to Tukey's HSD ($P = .05$).

Table 3. Probit analysis of DOR Bt-127 technical powder and SC formulation against third instar larvae of *S. litura*.

Treatment	Hours after treatment	LC_{50} value	Confidence limits		Regression equation (Y)
			Lower	Upper	
Unmilled Bt powder	72	2.04 mg/ml	1.74	2.73	$-1.99 + 0.70X$
60 minutes milled Bt powder (559 nm)	72	1.35 mg/ml	0.96	1.84	$-0.92 + 0.007X$
SC formulation with milled Bt	72	2.84 µl/ml[a]	2.47	3.15	$-1.99 + 0.70X$

[a]2.84 µl of formulation contains 0.95 mg of Bt.

3.5. Field testing of Bt-127 SC formulation

Field testing of Bt-127 SC formulation resulted in feeding cessation within 24 h after spray in all treatments while high feeding was observed in the control. Pre-treatment larval count was 4–6 larvae per plant. Post-treatment count ranged from 1.0 to 3.4 larvae per plant in the treatments while it was 26.2 in the control. Larval reduction in the SC formulation sprayed plots ranged from 87.0% to 96.2% at 5 DAS in tune with the concentration. Bt-127 SC formulation at applications of 2.5 and 3.0 ml/l resulted in highest population reduction of 92.4% and 96.2% on par with 94.2% larval reduction with profenophos and over 89.7% higher reduction by the commercial Bt formulation Delfin. Per cent defoliation was below 10% in Bt SC formulation sprayed plots (2.5 and 3.0 ml/l) and

Table 4. Field efficacy of DOR Bt-127 SC formulation against *S. litura* on castor during *kharif* 2014.

Treatment	Number of larvae/ plant 5 DAT	Per cent reduction over control 5 DAT	Per cent defoliation
Bt-127 SC formulation @ 1.5 ml/l	3.4 (1.85)[a]	87.0	11–25
Bt-127 SC formulation @ 2 ml/l	3.1 (1.76)	88.2	11–25
Bt-127 SC formulation @ 2.5 ml/l	2.0 (0.81)	92.4	<10
Bt-127 SC formulation @ 3 ml/l	1.0 (0.71)	96.2	<10
Delfin @ 1 g/l	2.7 (0.91)	89.7	11–25
Profenophos @ 1 ml/l	1.5 (0.71)	94.2	<10
Control	26.2 (5.11)	–	51–75
CD ($P = 0.5$)	0.30	–	

DAT, days after treatment.
[a]Values in parenthesis are square root transformed values.

profenophos (1.0 ml/l), 11–25% with 1.0 and 1.5 ml/l of SC formulation and Delfin (1.0 g/l) while very high feeding of 51–75% was observed in control (Table 4).

4. Discussion

Our studies show that milling of Bt powder in a planetary ball mill with cooling facility is a promising approach for particle size reduction to the submicron-scale. Milled Bt powders gave higher mortality of the less susceptible *S. litura* when compared to unmilled Bt powder although heat viable spore count decreased slightly with milling. However, Bt sample drawn at 60 min of milling was more effective than samples drawn at 30, 90 and 120 min, and contained Bt particles of 559 nm with a low PI of 0.338 (Figure 2). Distribution width of the particles which is described using the PI, ranges from 0.01 for mono dispersed particles and up to values of 0.5–0.7 (Nidhin, Indumathy, Sreeram, & Balachandran, 2008). Thus, low PI of 60 min milled Bt powder in our study indicates monodisperse nature of the Bt particles which is an essential parameter for proper dispersion of active ingredient in formulations. This sample also had a high total protein content of 175 mg/g coupled with a higher CFU of 5.7×10^{17} g^{-1} when compared to other milled Bt powders.

Kim and Jae (2012) reported particle size reduction of Bt *aizawai* spray dried powder from 37.1 to 34.8 and 5.3 μm with hammer and air milling, respectively, involving two rounds of milling. They also reported reduction of particle sizes to 35.9–1.9 μm through homogenisation at high rpm ranging 2000–8000 with no decrease in CFU. Bt *aizawai* technical powder with smaller particles (16.9 μm) had higher control efficiency against diamondback moth but excessively small particles (5.3 μm) reduced the insecticidal activity. Murthy et al. (2014) reported an average particle diameter of 813 nm through high pressure homogenisation of Bt *kurstaki* powder (105 μm) for 120 min, particle size distribution showing three peaks with sizes ranging 32–1106 nm with a slight decrease in CFU from 2×10^{14} to 8×10^{12} g^{-1}. They also reported an increase in efficacy of Bt *kurstaki* powder against *H. armigera* after high pressure homogenisation with effective dose reduction by 50%.

In our present study with ball milling high particle size reduction from 105 μm to 210 nm was observed. Per cent larval mortality agaist *S. litura* increased from 55.5% to 85% by 72 h in spite of the slight decrease in CFU from 7.2×10^{17} to 3.3×10^{16} which is in concordance with the above reports. Effectiveness of Bt depends on the quality and type of proteins present in the crystal coupled with the insect's ability to digest the crystal and release the active toxic fraction in the midgut. Faster kill of larvae is due to the action of the crystal alone and the spore is at best only a secondary factor in killing insects (Dulmage, 1993). In less susceptible species, effectiveness also depends on the ability of the ingested spores to invade the hemolymph, germinate, multiply and cause a lethal septicaemia indicating that both the spore and crystal are necessary for optimal kill. Murthy et al. (2014) opined that higher mortality of larvae in bioassays with Bt-NPs could be due to improved solubilisation of the crystal toxin in the alkaline midgut fluid owing to their small size followed by proteolytic activation thereby making more amount of toxin available for binding with receptors on the surface of midgut columnar epithelium ultimately leading to rapid paralysis of the gut. Results of our study are in corroboration with the above reports.

Majority of studies with Bt against *S. litura* are confined to neonate larvae (Amonkar, Urmila, & Amardeep, 1985; Puntambekar, Mukherjee, & Ranjekar, 1997). Lalitha and Muralikrishna (2012) reported that a native Bt isolate 375 and reference standard HD-1 multiplied on Luria broth and tested against third instar larvae of *S.litura* gave 76.7% and 70% larval mortality at 6 days after treatment (DAT). Pandey et al. (2009) reported highest mortality of 73.3% for third instar *S. litura* larvae with the commercial Btk formulation Biolep at 10% concentration. In our study, milling of Bt resulted in 85% mortality of third instar *S. litura* larvae within 3 DAT (72 h) at the concentration of 1.5 mg/ml (0.15%).Thus, our studies show that milling in a planetary ball mill is a promising option for increasing efficacy of Bt through particle size reduction.

Limited information is available on field testing of Bt formulations against *S. litura*. Basavaraju, Shashank, Doddabasappa, Vijayakumar, and Chakravarthy (2010) undertook sprays of Bt formulation at 1.0 ml/l in potato crop in 2005 and 2006 after monitoring the moth incidence with pheromone traps. They reported a decrease in larval population per plant from 1.45 to 1.22 and 0.93, respectively, at 7 and 21 DAS, that is, 15% and 35.8% larval reduction. A similar trend was observed by them in 2006 also with 26.3% and 39% larval reduction at 7 and 21 DAS. Suganthy and Sakthivel (2013) reported that in plots sprayed with Bt at 2 ml/l against *S. litura* on *Gloriosa superba* at Vellipalayam, larval population decreased from 2.6 to 1.47 and 0.7 at 7 and 14 DAS respectively, that is, 68% and 73% reduction. However, information pertaining to trade names of the formulation as well as the sub-species of Bt have not been reported by the authors. In our trials with SC formulation of DOR Bt 127, results were promising against *S. litura* on castor with larval reduction above 90% within 5 DAS for 2.5 and 3.0 ml/l concentrations. Reduction in larval population of 87–88% recorded with the lower concentrations of 1.5 and 2.0 ml/l is also higher than the values reported by researchers. Stability of our SC formulation as well as its suspensibility in water was good due to use of a combination of the wetting cum dispering agents Span-20 and Tween-80. The formulation contained fine Bt particles with narrow particle size distribution as reflected by the low PI index. Bt formulations with small and narrow particle size distribution less than 20 μm will improve the physical properties as well as coverage of sprayed foliage (Devisetty et al., 1998; Vimala Devi & Vineela, 2015).

Particle size reduction through planetary ball milling equipped with a cooling system for temperatures below 10°C holds advantage over homogenisation by enabling particle size reduction in dry powders without loss of activity. Our studies reveal that particle size reduction with planetary ball mill is more efficient over hammer and air milling (Kim & Jae, 2012) as particles in submicron-scale could be generated. Physical changes in the secondary or tertiary structure prove lethal for the biological activity of proteins (Wang, 2005). Dry milling of Bt corn hybrids inactivated the protein, that is, delta endotoxin (Dien, Bothast, Iten, Barrios, & Eckhoff, 2002). Since milling is based on attrition, longer periods of milling could damage the spore and active ingredient namely the delta endotoxin. It is therefore pertinent to identify the right rpm and time duration required for milling of Bt powders so as to obtain optimum mortality of target pests at low concentrations. This is the first report of particle size reduction of Bt powder to submicron-scale employing planetary ball mill. Thus, ball milling of Bt *kurstaki* powders is a promising approach for generation of submicron-scale Bt particles essential for development of formulations with increased efficacy and improved coverage on foliage.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abdullah, M. A. F., Moussa, S., Taylora, M. D., & Adang, M. J. (2009). *Manduca sexta* (Lepidoptera: Sphingidae) cadherin fragments function as synergists for cry1A and cry1C *Bacillus thuringiensis* toxins against noctuid moths *Helicoverpa zea*, *Agrotis ipsilon* and *Spodoptera exigua*. *Pest Management Science*, *65*, 1097–1103.

Alotaibi, S. A. (2013). Mortality responses of *Spodoptera litura* following feeding on BT-sprayed plants. *Journal of Basic & Applied Sciences*, *1*, 9–195.

Amonkar, S. V., Urmila, K., & Amardeep, A. (1985). Comparative toxicity of *Bacillus thuringiensis* subspecies to *Spodoptera litura* (F). *Current Science*, *54*, 475–477.

Aydin, H., & Gurkan, M. O. (2006). The efficacy of spinosad on different strains of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Turkish Journal of Biology*, *30*, 5–9.

Basavaraju, B. S., Shashank, P. S., Doddabasappa, B., Vijayakumar, L., & Chakravarthy, A. K. (2010). Efficacy of poison Baits and biopesticides gainst *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) in potato. *Pest Management in Horticultural Ecosystems*, *16*(1), 64–68.

Devisetty, B. N., Wang, Y., Sudershan, P., Kirkpatrick, B. L., Cibulsky, R. J., & Birkhold, D. (1998). Formulation and delivery systems for enhanced and extended activity of biopesticides. In J. D. Nalewaja, G. R. Goss, & R. S. Tann (Eds.), *Pesticide formulation and application systems* (Vol. 18, pp. 242–266). West Conshohocken, PA: ASTM STP 1347. American Society for Testing and Materials.

Dien, B. S., Bothast, R. J., Iten, L. B., Barrios, L., & Eckhoff, S. R. (2002). Fate of Bt protein and influence of corn hybrid on ethanol production. *Cereal Chemistry*, *79*(4), 582–585.

Dulmage, H. T. (1993). Development of isolates of Bacillus thuringiensis and similar aerobic microbes for use in developing countries. In H. S. Salama, O. N. Morris, & E. Rached (Eds.), *The biopesticide Bacillus thuringiensis and its applications in developing countries* (pp. 15–42). Proceedings of an international workshop organized by NRC-Cairo, Agriculture Canada and IDRC, 4–6 November 1991. Qualiub: AL-AHRAM Commercial Press.

EI-Aswad, A. F., Abdelgaleil, S. A. M., & Nakatani, M. (2003). Feeding deterrent and growth inhibitory properties of limnoids from Khaya senegalensis against the cotton leafworm *Spodoptera littoralis*. *Pest Management Science*, *60*, 199–203.

George, Z., & Crickmore, N. (2012). Bacillus thuringiensis applications in agriculture. In E. Sansinenea (Ed.), *Bacillus thuringiensis biotechnology* (pp. 19–39). Brighton, UK: © Springer Science + Business Media B.V.

Horn, D., & Rieger, J. (2001). Organic nanoparticles in aqueous phase: Theory, experiment and use. *Angewandha Chemie International Edition*, *40*, 4331–4343.

Huang, S. J., Xu, J. F., & Han, Z. J. (2006). Baseline toxicity data of insecticides against the common cutworm *Spodoptera litura* (Fabricius) and a comparison of resistance monitoring methods. *International Journal of Pest Management*, *52*, 209–213.

Hummelbrunner, L. A., & Isman, M. B. (2001). Acute, sublethal, antifeedant, and synergestic effects of monoterpenoid essential oil compounds on the Tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Agricultural and Food Chemistry*, *49*, 715–720.

Khadka, P., Jieun, R., Kim, H., Kim, I., Kim, J. T., Kim, H., … Lee, J. (2014). Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian Journal of Pharmaceutical Sciences*, *9*, 304–316.

Kharia, A. A., Singhai, A. K., & Verma, R. (2012). Formulation and evaluation of polymeric nanoparticles of an antiviral drug for gastroretention. *International Journal of Pharmaceutical Sciences and Nanotechnology*, *4*, 1557–1562.

Kim, J. S., & Jae, Y. H. (2012). Milling effect on the control efficacy of spray dried *Bacillus thuringiensis* technical powder against diamond black moths. *Pest Management Science*, *68*, 321–323.

Ksiaiek, K., Wacke, S., Gorecki, T., & Gorecki, C. (2007). Investigation of the effect of intensive ball milling in a planetary ball mill on the thermal decomposition of cadmium carbonate and basic zinc carbonate. *Journal of Physics and Conference Series*, *79*, 012022.

Lalitha, C., & Muralikrishna, T. (2012). Laboratory evaluation of native *Bacillus thuringiensis* isolates against *Spodoptera litura* (Fabricius). *Current Biotica*, *5*(4), 428–435.

Lav, R. K., Sindhuja, S., Joe, M. M., Reza, E., & Edmund, W. S. (2012). Applications of nanomaterials in agricultural production and crop protection: A review. *Crop Protection*, *35*, 64–70.

Lowry, O. H., Rosenbrough, N. J., Fair, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Murthy, S., Vineela, V., & Vimala Devi, P. S. (2014). Generation of nanoparticles from technical powder of the insecticidal bacterium *Bacillus thuringiensis* var. *kurstaki* for improving efficacy. *International Journal of Biomedical Nanoscience and Nanotechnology*, *3*, 236–250.

Nidhin, M., Indumathy, R., Sreeram, K. J., & Balachandran, U. (2008). Synthesis of iron oxide nanoparticles of narrow size distribution on polysaccharide templates. *Bulletin of Materials Science*, *31*, 93–96.

Pandey, S., Joshi, B. D., & Tiwari, L. D. (2009). Relative efficacy of two subspecies of *Bacillus thuringiensis*, available as commercial preparations in market, on different stages of a lepidopteran pest, *Spodoptera litura* (Fabricius). *Archives of Phytopathology and Plant Protection*, *42*, 903–914.

Puntambekar, U., Mukherjee, S., & Ranjekar, P. (1997). Laboratory screening of different *Bacillus thuringiensis* strains against certain lepidopteran pests and subsequent field evaluation on the pod boring pest complex of pigeonpea (*Cajanus cajan*). *Antonie Van Leeuwenhoek*, 71, 319–323.

Ramezani, M., & Neitzert, T. (2012). Mechanical milling of aluminium powder using planetary ball milling process. *Journal of Achievements in Materials and Manufacturing Engineering*, *55*, 790–798.

Ravi charan, S., Lakshmi Narasu, M., & Vimala Devi, P. S. (2013). Genotyping and molecular characterization of local *Bacillus thuringiensis* isolates of DOR by using PCR based methods. *International Journal of Biotechnology & Biochemistry*, *9*, 101–113.

Sanchis, V., & Bourguet, D. (2008). *Bacillus thuringiensis*: Applications in agriculture and insect resistance management. A review. *Agronomy for Sustainable Development*, 28, 11–20.

Sarada, C., Alivelu, K., Sambasiva Rao, V., Sudhakar Babu, S. N., & Varaprasad, K. S. (2015). *Oilseeds statstics – a compendium 2015*. Hyderabad: ICAR-Indian Institute of Oilseeds Research.

Suganthy, M., & Sakthivel, P. (2013). Field evaluation of biopesticides against tobacco caterpillar, *Spodoptera litura* Fab. infesting *Gloriosa superba* (Linn.). *Journal of Biopesticides*, *6*(2), 90–95.

Sujatha, M., Vimala Devi, P. S., & Reddy, T. P. (2010). Insect pests of castor (*Ricinus communis* L.) and their management strategies. In D. R. Vudem, N. R. Poduri, & V. R. Khareedu (Eds.), *Pests and pathogens: Management strategies* (pp. 177–198). Boca Raton: BS Publications/CRC Press. ISBN: 978-0-415-66576-6.

Travers, R. S., Martin, P. A., & Reichelderfer, C. F. (1987). Selective process for efficient isolation of soil Bacillus spp. *Applied and Environmental Microbiology*, *53*(6), 1263–1266.

Vimala Devi, P. S., Ravinder, T., & Jaidev, C. (2005). Cost-effective production of *Bacillus thuringiensis* by solid-state fermentation. *Journal of Invertebrate Pathology*, *88*, 163–168.

Vimala Devi, P. S., & Sudhakar, R. (2006). Effectiveness of a local strain of *Bacillus thuringiensis* in the management of castor semilooper *Achaea janata* on castor (*Ricinus communis*). *Indian Journal of Agricultural Sciences*, *76*, 447–449.

Vimala Devi, P. S., & Vineela, V. (2015). Suspension concentrate formulation of *Bacillus thuringiensis* var. *kurstaki* for effective management of *Helicoverpa armigera* on sunflower (*Helianthus annuus*). *Biocontrol Science and Technology*, *25*, 329–336.

Wang, W. (2005). Protein aggregation and its inhibition in biopharmaceutics. *International Journal of Pharmaceutics*, *289*, 1–30.