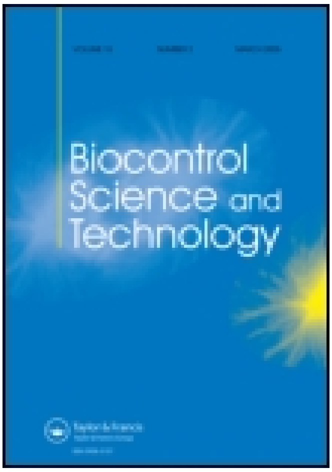

Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbst20

Suspension concentrate formulation of Bacillus thuringiensis var. kurstaki for effective management of Helicoverpa armigera on sunflower (Helianthus annuus)

P.S. Vimala Devi[a] & V. Vineela[a]

[a] Directorate of Oilseeds Research, Hyderabad, Telangana, India

Accepted author version posted online: 20 Oct 2014.Published online: 18 Nov 2014.

To cite this article: P.S. Vimala Devi & V. Vineela (2015) Suspension concentrate formulation of Bacillus thuringiensis var. kurstaki for effective management of Helicoverpa armigera on sunflower (Helianthus annuus), Biocontrol Science and Technology, 25:3, 329-336, DOI: 10.1080/09583157.2014.977846

To link to this article: http://dx.doi.org/10.1080/09583157.2014.977846

Biocontrol Science and Technology, 2015
Vol. 25, No. 3, 329–336, http://dx.doi.org/10.1080/09583157.2014.977846

RESEARCH ARTICLE

Suspension concentrate formulation of *Bacillus thuringiensis* var. *kurstaki* for effective management of *Helicoverpa armigera* on sunflower (*Helianthus annuus*)

P.S. Vimala Devi* and V. Vineela

Directorate of Oilseeds Research, Hyderabad, Telangana, India

(*Received 11 August 2014; returned 11 September 2014; accepted 13 October 2014*)

A local strain DOR Bt-1 belonging to *Bacillus thuringiensis* var. *kurstaki* (Bt.k) was multiplied through solid state fermentation and the resulting technical powder was milled and sieved to obtain particles of various sizes. Efficacy of Bt.k. against larvae of *Helicoverpa armigera* was found to increase with the decrease in particle size. Boric acid was found to be synergistic to DOR Bt-1 technical powder. LC_{50} of the Bt and boric acid mixture (75:25) was lower at 89.63 mg/100 mL in comparison to Bt alone at 116.75 mg/100 mL. A suspension concentrate formulation with DOR Bt-1 technical was developed using boric acid as an adjuvant. The formulation was found to be highly effective against *H. armigera* in laboratory bioassays with an LC_{50} of 185 μL (containing 53.36 mg Bt). The formulation gave effective control of *H. armigera* on sunflower within 3 days after spray even at the lowest dose of 1.0 mL/L under field conditions.

Keywords: *Bacillus thuringiensis* var. *kurstaki*; boric acid; formulation; *Helicoverpa armigera*; suspension concentrate; sunflower

Introduction

Bacillus thuringiensis var. *kurstaki* (Bt.k.) has proven itself to be a valuable tool for the control of lepidopteran insects on vegetables, cotton, soybean, hardwood and coniferous forests. The expansion of Bt from the original vegetable crop applications to field crops and forest insect control programmes has occurred along with significant advances in technology involving the culture, mass production, formulation and application of this unique microbe (Cibulsky, Devisetty, Melcghior, & Melin, 1993).

Formulations are the end product of blending the microbial component with carriers and adjuvants aimed at protection from unfavourable environments, enhancing survival, improving activity, shelf life and stability. Chemical additives viz., boric acid (H_3BO_3), zinc sulphate ($ZnSO_4$), magnesium sulphate ($MgSO_4$), calcium carbonate ($CaCO_3$) etc., have been found to improve the potency of the formulation of *B. thuringiensis* var. *kurstaki* (HD-234) against *Phthorimaea operculella* and *H. armigera* (Sabbour, Abdel-Hakim, & Abdou, 2012).

Suspension concentrates (SCs) are stable suspensions of solid pesticides in a fluid generally intended for dilution with water before spray. The active ingredient in these

*Corresponding author. Email: vimaladevi@gmail.com; vimaladevi@dor-icar.org.in

formulations is a solid that does not dissolve in either water or oil. These formulations are non-dusty, easier to apply and disperse in water. There are requirements of particle size to ensure proper bioactivity, chemical activity etc. If the particles are pre-milled to the required size, they are easily dispersed in the liquid phase. Like wettable powders (WPs), when SCs are sprayed onto a sorptive surface the insecticidal particles remain on the surface of the substrate where they are readily available to the target pest (Gašić & Tanović, 2013). These formulations therefore provide a good level of residual control on a wide range of surfaces.

B. thuringiensis has been traditionally multiplied through submerged fermentation. However successful cost-effective production of Bt.k through solid state fermentation (SSF) has also been reported on different agricultural wastes including waste water sludge (Smitha, Jisha, Pradeep, Josh, & Benjamin, 2013; Vimala Devi, Ravinder, & Jaidev, 2005; Zhuang, Zhou, Wang, Liu, & Xu, 2011). W.P. formulation of a local isolate of Bt (DOR Bt-1) multiplied through SSF has been reported effective against the castor semilooper *Achaea janata* on castor (Vimala Devi & Sudhakar, 2006; Vimala Devi et al., 2005), leaf folder of rice *Cnaphalocrocis medinalis* (Kaur, Virk, & Joshi, 2008; Kandibane, Kumar, & Adiroubane, 2010) and gram pod borer *H. armigera* on pigeon pea (Sreekanth & Seshamahalakshmi, 2012). This paper presents results of studies undertaken subsequently to develop a SC formulation of DOR Bt-1 along with a suitable synergist for effective management of *H. armigera* (capitulum borer) on sunflower.

Materials and methods

A local isolate of Bt.k. (DOR Bt-1) was multiplied through SSF (Vimala Devi et al., 2005) and the resulting Bt flakes were powdered in a blender to obtain the technical powder used for further studies.

Generation of Bt particles of different sizes

Bt.k. technical powder was sieved through meshes to obtain 210, 105 and 70 μm particles. Bt powder with 105 μm particles was further subjected to milling in an End Perger mill at the Indian Institute of Chemical Technology, Hyderabad, India, to obtain 70 μm particles and a High Pressure Homogenizer (Mini Dee Bee) at the Central Institute for Research on Cotton Technology, Mumbai, India to obtain submicron particles. High pressure homogenisation was conducted at a pressure of 10,000 PSI in five cycles for 2 h at 15°C.

Larval bioassays

Laboratory bioassays were carried out against 6-day-old *H. armigera* larvae by the diet surface treatment technique. Larvae of *H. armigera* were maintained in the laboratory on a semi-synthetic diet (Vimala Devi & Hari, 2009). For the bioassays, the diet was poured as a thin layer into 12 well tissue culture plates, approximately 4 mL per well with a surface area of 3.14 cm^2. Test suspensions of Bt at 100 μL were overlaid on the diet surface in each well for all concentrations. One larva was released in each well. A total of 30 larvae were used for each concentration @10 larvae/replication. Observations of larval mortality were recorded at 24 h intervals. Data were subjected to probit analysis using the statistical package SPSS-11.5.

Identification of suitable synergist for DOR Bt-1

Bt (technical-70 µm particles) was bioassayed against 6-day-old *H. armigera* larvae in combination with the inorganic salts H_3BO_3, $ZnSO_4$, ZnO, $MgSO_4$ and $CaCO_3$ that have been reported to be synergistic to Bt (Sabbour et al., 2012). Bt was mixed with the chemicals in ratios of 75:25 and 88:12 and 100 mg of the mixtures were suspended in 100 mL of sterile distilled water containing 0.02% Tween-80. Positive controls were maintained for each chemical at the higher dose of 25 mg/100 mL. All chemicals used in the study were of analytical grade and purchased from Qualigens and SD-Fine Chem. Ltd.

Development of SC formulation

Seventy five grams of Bt (technical-70 µm particles) and 25 g of boric acid were mixed well with a sterile stainless steel spatula in a 500 mL sterile glass beaker. Tween-80 was added to light mineral oil in 1:6.84 ratio and vortexed to get a uniform mixture (T-M mixture). Fifty millilitres of this mixture was added initially to the Bt and boric acid mixture and mixed with a sterile spatula to obtain a fine paste. This paste was further ground in a mortar and pestle to obtain a fine slurry. This was followed by addition of 142 mL of the T-M mixture to the slurry and mixing to get a fine suspension. This suspension was then poured in a mixie jar and blended for 2–3 minutes to ensure proper mixing of the components for obtaining a uniform SC formulation of DOR Bt-1. The final volume of the suspension was 261 mL. The suspension when added to water formed a clear uniform suspension.

Field testing of DOR Bt-1 SC formulation

The experiment was conducted at the Directorate of Oilseeds Research (DOR), Rajendranagar, Hyderabad on sunflower hybrid KBSH-53 in 3 × 3 m plots adopting 60 × 30 cm spacing. Sowing was undertaken in the third week of August, 2013. The experiment was laid out in a randomised complete block design with seven treatments and three replications. Natural incidence of neither *H. armigera* larvae nor natural enemies was observed before spray. Hence, inundative releases of 6 days old *H. armigera* larvae were undertaken at two larvae per plant at the star bud stage and the treatments were made 24 h later to ensure larval establishment on the capitulum. Treatments included five doses of DOR Bt-1 SC formulation at 1.0, 1.5, 2.0, 2.5 and 3.0 mL/L of spray suspension, one insecticidal check (0.01% profenophos @ 1.0 mL/L) and a water sprayed control. Treatments were made in the third week of October, 2013. Formulations were suspended in water and sprayed with a high volume knapsack sprayers on the sunflower crop at the star bud stage. Incidence of the *H. armigera* larvae and natural enemies was recorded 3 and 6 days after spray (DAS) from 10 tagged plants from each replication. Data were subjected to analysis of variance (RCBD Factor-1) using the statistical package MSTAT-C.

Results and discussion

Studies on efficacy of different sized Bt particles through larval bioassays against 6 days old *H. armigera* larvae revealed that larval mortality increased with decrease in particle size. Feeding cessation in larvae was observed within 24 h after treatment with Bt particles 70 µm and lower while low larval feeding was observed with Bt

Table 1. Efficacy of *B. thuringiensis* var. *kurstaki* strain DOR Bt-1 of different particle sizes against *H. armigera*.

	% Larval mortality[a]	
Bt particle size (μm)	3 DAT	4 DAT
210	37.0 (36.6)	42.9 (46.6)
105	37.6 (38.3)	51.0 (60.0)
70	47.5 (53.3)	60.3 (71.6)
0.5–1.0	68.1 (83.3)	90.0 (100)
S.E. Mean ±	3.9	4.2
C.D. (P = 0.05)	11.9	12.6

[a]Values are angular transformed, values in parenthesis are actual mean.
DAT, days after treatment.

particles of 105 μm. However, larval feeding was greater with Bt particles of 210 μm. Impact of the feeding is reflected in the larval mortality 3 and 4 days after treatment (DAT). Complete larval mortality was observed with sub-micron Bt particles 4 DAT followed by 71.6%, 60% and 46.6% larval mortality with 70, 105 and 210 μm Bt particles, respectively (Table 1). Previously, Bt suspensions with smaller particles were reported to have a higher control efficacy against diamondback moth (Kim & Je, 2012).

Out of the various inorganic salts bioassayed with Bt (70 μm particles) against 6-day-old *H. armigera* larvae, boric acid was found most promising. Bt and boric acid (75:25) resulted in cumulative larval mortalities of 73.3% and 100% at 3 and 4 DAT, respectively while Bt and boric acid (88:12) resulted in cumulative larval mortalities of 70% and 100% at 3 and 4 DAT, respectively. Bt alone (100 mg) resulted in lower mortalities of 65.4% and 73.3% at 3 and 4 DAT, respectively. All other chemicals were found to cause lower larval mortalities in comparison to Bt alone (Figure 1). Thus boric acid was found synergistic to Bt and was further used for development of the SC formulation. The formulation was a brown viscous liquid which was easily miscible with water to give a clear suspension.

Larval bioassays were conducted with Bt (technical), Bt technical and boric acid mixture (75:25) and SC formulation (containing Bt and boric acid mixture) against 6-day-old *H. armigera* larvae. Feeding cessation was observed within hours after treatment followed by mortality 3 DAT. LC_{50} values 3 DAT were 116.8 mg for Bt (technical-70 μ), 89.63 mg for Bt and boric acid (containing 67.22 mg Bt) and 185 μL/100 mL for SC formulation (containing 53.36 mg Bt). Thus, requirement of Bt against *H. armigera* was lowered by 43% when used in combination with boric acid and further lowered by 54% when formulated along with boric acid as a SC (Table 2).

Morris, Converse, and Kanagaratnam (1995) reported that boric acid alone was safe to *Mamestra configurata* larvae causing very low larval mortality up to 4.3%. They also reported that boric acid increased the efficiency of the endotoxin of Bt.k. (Dipel 2×) by 3.5, 3.5 and 1.1 fold at 0.10%, 0.05% and 0.01% concentration, respectively. In our studies, boric acid resulted in a low larval mortality (3.3%) in *H. armigera* larvae (Figure 1). Boric acid has been reported to be a synergist as well as a lubricant for Bt (Bernhard, Holloway, & Burges, 1998). Boric acid caused an

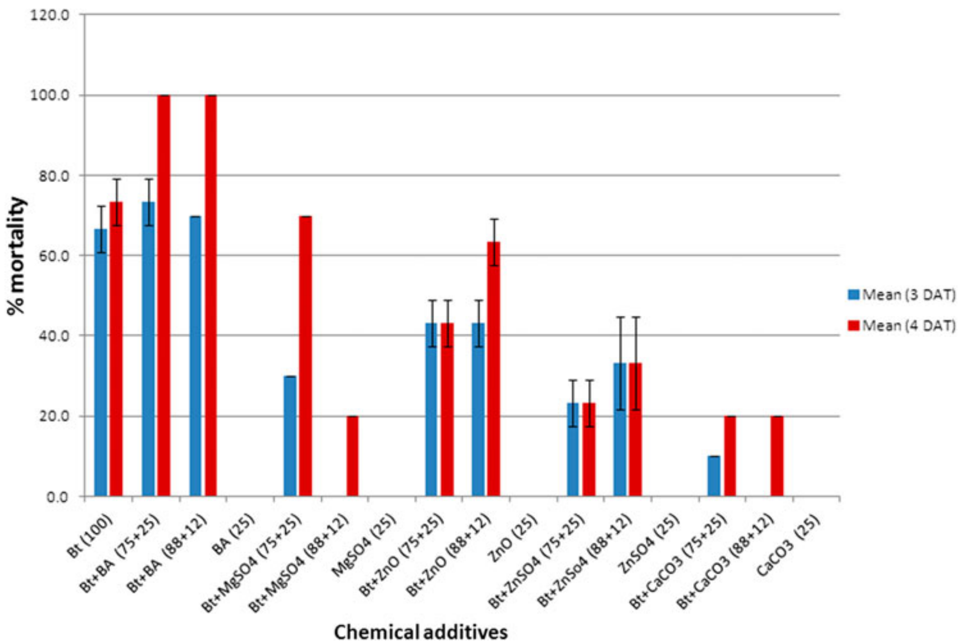

Figure 1. (Colour online) Testing of different chemicals along with DOR Bt-1 (technical) against *H. armigera* larvae.

enhancement in the activity of *B. thuringiensis* (HD-234) by increasing mortality from 51% for Bt alone to 90% against *H. armigera* larvae, causing 1.76 fold of increase in the potency (Sabbour et al., 2012).

Larval bioassays with Bt-1 SC formulation against 6-day-old *H. armigera* larvae showed a dose related response with larval mortality ranging 33.3–73.3% for doses 1–3 mL/L 3 DAT. However the formulation at 1.5 mL/L (containing 576.9 mg Bt technical) resulted in larval mortality of 46.7% that was on par with mortality of 43.3% caused by Bt (technical) at 1 g/L. All the treatments, Bt SC formulation as well as Bt (technical), resulted in 100% mortality of the larvae 4 DAT showing that even the lowest dose of the SC formulation at 1 mL/L containing only 384.6 mg Bt

Table 2. Probit analysis of *B. thuringiensis* var. *kurstaki* strain DOR Bt-1 (technical) and SC formulation against 6-day old *H. armigera.*

Treatment	LC50value (µl/mg per 100 mL) 3DAT	Confidence limits		Regression equation
		Lower	Upper	
DOR Bt 1 Technical	116.75 mg	102.31	137.28	*Y* = –1.718 + 0.0147x
DOR Bt 1 Technical and boric acid (75:25)	89.63 mg (contains 67.22 mg Bt)	73.95	102.34	*Y* = –1.454 + 0.0162x
SC formulation with DOR Bt 1	185.00 µl (contains 53.36 mg Bt)	1.228	2.322	*Y* = –0.899 + 0.485x

DAT, days after treatment.

Table 3. Laboratory bioassay of *B. thuringiensis* var. *kurstaki* strain DOR Bt-1 SC formulation against 6-day-old *H. armigera.*

Treatment	% Larval Mortality[a] 3DAT	4DAT
DOR Bt-1 SC @ 1.0 mL/L (384.6 mg/mL)	35.2 (33.3)	90.0 (100.0)
DOR Bt-1 SC @ 1.5 mL/L (576.9 mg/mL)	43.1 (46.7)	90.0 (100.0)
DOR Bt-1 SC @ 2.0 mL/L (769.2 mg/mL)	45.0 (50.0)	90.0 (100.0)
DOR Bt-1 SC @ 2.5 mL/L (961.5 mg/mL)	50.8 (60.0)	90.0 (100.0)
DOR Bt-1 SC @ 3.0 mL/L (1.15 g/mL)	59.0 (73.3)	90.0 (100.0)
DOR Bt-1 (technical-70 µ) @ 1.0 g/L	41.1(43.3)	90.0 (100.0)
Control	6.1 (3.3)	6.1 (3.3)
SE Mean ±	1.7	1.5
C.D. (*P* = 0.05)	5.0	4.4

[a]values in parenthesis are original means.
DAT, days after treatment.

(technical) could bring about complete larval mortality. Thus boric acid in the formulation could effectively reduce the Bt requirement by synergising its activity (Table 3).

DOR Bt-1 SC formulation resulted in a rapid reduction of *H. armigera* population ranging from 91% to 95% by 3 DAS and 96.5% to 100% by 5 DAS in field trials on sunflower. The population reduction with even the lowest dose of SC formulation at 1 mL/L was on par with the insecticidal check profenophos and was significantly higher than that in unsprayed control. Larval feeding was high in control plots. Spiders were observed in all the experimental plots ranging from 4 to 6 per plot excepting for the profenophos sprayed plots. Thus DOR Bt-1 SC formulation was safe to these natural enemies and decrease of the larval population in Bt formulation sprayed plots was a cumulative effect of Bt and the natural enemies (Table 4).

Table 4. Field testing of SC formulation of *B. thuringiensis* var. *kurstaki* strain DOR Bt-1 against *H. armigera* on sunflower during *kharif* 2013.

Treatment	% decrease in larval incidence[a] 3DAS	5DAS	Spiders/plot 5DAS
DOR Bt-1 SC @ 1.0 mL/L	73.4 (91.5)	85.7 (96.5)	4.3 + 0.58
DOR Bt-1 SC @ 1.5 mL/L	71.6 (91.0)	85.7 (98.0)	4.6 + 0.60
DOR Bt-1 SC @ 2.0 mL/L	79.5 (95.0)	85.7 (96.5)	5.0 + 1.00
DOR Bt-1 SC @ 2.5 mL/L	79.5 (95.0)	83.9 (96.5)	4.3 + 1.53
DOR Bt-1 SC @ 3.0 mL/L	82.4 (95.0)	90.0 (100.0)	4.6 + 1.15
Profenophos @ 1.0 mL/L	83.9 (96.5)	85.7 (98.0)	0.7 + 0.57
Water sprayed control	20.5 (13.0)	22.3 (15.0)	5.6 + 0.58
SE Mean ±	3.6	2.9	–
CD (*P* = 0.05)	10.6	8.4	

[a]values in parenthesis are original means.
DAS, days after spray.

Topical sprays of Bt are advantageous in terms of their safety, specificity and potency compared to chemical sprays, and are also biodegradable, which provides for a large and competitive market. Bt is commercially available primarily as W.P. formulations with effective field dose ranging from 1 to 2 kg/ha for management of insect pests on various crops viz., okra, pigeon pea, chick pea, rice etc. (1.5–2 g/L of spray suspension), requiring 2–3 sprays at 10–15 days intervals (Kandibane et al., 2010; Karim, Zafar, Nasir, & Riazuddin, 2000; Kaur et al., 2008; Santharam, Victoria, Rabindra, & Jayaraj, 1993; Sreekanth & Seshamahalakshmi, 2012). However improved formulations of the active ingredient are essential to make Bt formulations comparable to chemical insecticides in terms of the speed of kill. Our study reveals that use of boric acid to synergise Bt in formulating as a SC could significantly lower the effective Bt requirement.

SC is a formulation containing fine particles of the active ingredient and having the advantage of both an emulsifiable concentrate as well as a wettable powder. Formulations with small and narrow particle-size distribution will improve the coverage of sprays on the foliage. In our study, even the lowest dose of the SC formulation at 1 mL/L gave significant reduction (83.3%) of the target pest within 3 DAS with 96.5% reduction by 5 DAS that was statistically on par with the insecticide profenophos (98%). Thus SC formulation of DOR Bt-1 holds great promise for effective management of *H. armigera* on sunflower. Since *H. armigera* is a polyphagous pest, this formulation will also find use in several other crops.

Acknowledgements

The authors thank the Project Director Dr. K.S. Varaprasad for providing the facilities for carrying out the work. The authors also thank Director, CICRCOT, Mumbai for sparing their facility for high pressure homogenisation and Dr. B.V.S.K. Rao, Technical Officer, IICT, Hyderabad for helping in milling.

References

Bernhard, K., Holloway, P. J., & Burges, H. D. (1998). Appendix I: A catalogue of formulation additives: Function, nomenclature, properties and suppliers. In H. D. Burges (Ed.), *Formulation of microbial biopesticides: Beneficial organisms, nematodes and seed treatments*. Dordrecht: Kluwer Academic.

Cibulsky, R. J., Devisetty, B. N., Melcghior, G. N., & Melin, B. E. (1993). Formulation and application technologies for microbial pesticides: Review of progress and future trends. *Journal of Testing and Evaluation*, *21*, 500–503. doi:10.1520/JTE11798J

Gašić, S., & Tanović, B. 2013. Biopesticide formulations, possibility of application and future trends. *Pesticides and Phytomedicine*, *28*, 97–102.

Karim, S., Zafar, A. U., Nasir, I. A., & Riazuddin, S. (2000). Field efficacy of CAMB *Bacillus thuringiensis* biopesticide to control *Helicoverpa armigera* (Hubner) and *Earis vitella* (Fabricius) in okra crop. *Pakistan Journal of Biological Sciences*, *3*, 1296–1298.

Kaur, R., Virk, J. S., & Joshi, N. (2008). Bio-efficacy of DOR Bt, a *Bacillus thuringiensis* formulation against rice leaf folder, *Cnaphalocrosis medinalis* (Guenee) in Punjab. *Journal of Biological Control*, *22*, 475–477.

Kandibane, M., Kumar, K., & Adiroubane, D. (2010). Effect of *Bacillus thuringiensis* Berliner formulation against the rice leaf folder *Cnaphalocrocis medinalis* Guenee (Pyralidae: Lepidoptera). *Journal of Biopesticides*, *3*, 445–447.

Kim, J. S., & Je, Y. H. (2012). Milling effect on the control efficacy of spray-dried *Bacillus thuringiensis* technical powder against diamondback moths. *Pest Management Science*, *68*, 321–3.

Morris, O. N., Converse, V., & Kanagaratnam, P. (1995). Chemical additive effects on the efficacy of *Bacillus thuringiensis* Berliner subsp. *kurstaki* against *Mamestra configurata* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, *88*, 815–824.

Sabbour, M. M., Abdel-Hakim, E. A., & Abdou, W. L. (2012). Role of some additives in enhancing the formulation of bacteria *Bacillus thuringiensis* against *Phthorimaea operculella* and *Helicoverpa armigera*: 2- Chemical additives. *Journal of Applied Sciences Research*, *8*, 2640–2649.

Santharam, G., Victoria, D. R., Rabindra, R. J., & Jayaraj, S. (1993). Microbial control of *Helicoverpa* (= *Heliothis*) *armigera* on chickpea. *Journal of Biological Control*, *7*, 44–46.

Smitha, R. B., Jisha, V. L., Pradeep, S., Josh, M. S., & Benjamin, S. (2013). Potato flour mediated solid-state fermentation for the enhanced production of *Bacillus thuringiensis*-toxin. *Journal of Bioscience and Bioengineering*, *116*, 595–601. doi:10.1016/j.jbiosc.2013.05.008

Sreekanth, M., & Seshamahalakshmi, M. (2012). Studies on relative toxicity of biopesticides to *Helicoverpa armigera* (Hubner) and *Maruca vitrata* (Geyer) on pigeonpea (*Cajanus cajan* L.). *Journal of Biopesticides*, *5*,191–195.

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. doi:10.1016/j.jip.2005.01.013

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., & Hari, P. P. (2009). Identification of a virulent isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, its mass multiplication and formulation for development into a mycoinsecticide for management of *Helicoverpa armigera* (Hübner). *Journal of Biological Control*, *23*, 137–144.

Zhuang, L., Zhou, S., Wang, Y., Liu, Z., & Xu, R. (2011). Cost-effective production of *Bacillus thuringiensis* biopesticides by solid-state fermentation using wastewater sludge: Effects of heavy metals. *Bioresource Technology*, *102*, 4820–4826. doi:10.1016/j.biortech.2010.12.098