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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(8): 990-993

www.thepharmajournal.com
Received: 01-05-2023
Accepted: 08-06-2023

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Efficacy of *Bacillus thuringiensis* var. *kurstaki* microcapsules for eco-friendly management of castor semilooper, *Achaea janata* (Noctuidae: Lepidoptera)

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Abstract
Insect pests constitute major biotic stresses in crop production, inflicting 17.5% yield losses in major field crops. Due to their immediate effects, chemical pesticides have become essential in the management of insect pests. However, increasing environmental concerns, health consciousness, and the demand for organic produce free of residue have necessitated a search for green technology for pest management. However, high dose recommendations, short shelf-life and less persistent nature are major weaknesses of such microbial formulations. Recent research has focused on microencapsulating microorganisms in natural polymers to boost formulation durability and efficacy while lowering dose requirements. The present study was conducted to develop *Bacillus thuringiensis* var. *kurstaki* microcapsules by a complex coacervation process and evaluate their effectiveness on a polyphagous pest *viz*., castor Semilooper (*Achaea janata*). Pot culture study on castor revealed that the two promising *Bacillus thuringiensis* var. *kurstaki* microcapsules *viz*., CC-1+Btk (Chitosan + lignosulphonate + manganous sulphate + Btk) and CC-2+Btk (Chitosan + lignosulphonate + carboxymethylcellulose + manganous sulphate + Btk) were found effective and recorded higher percent larval mortality (91.66 to 100%) of semilooper (*A. janata*) as compared to commercial Btk formulations (90.0 to 95.00%). Additionally, the Btk microcapsules showed promising results with a lower percent of active ingredient (a.i.), indicating their potential for use in bio-pesticides. The current study will help to develop eco-friendly and low volume biopolymer formulations of microbial pesticides with increased efficacy and field persistence for effective management of lepidopteran pests.

Keywords: *Bacillus thuringiensis* var. *kurstaki*, eco-friendly pest management, efficacy, microcapsules, semilooper

Introduction
Castor (*Ricinus communis*) is an important non-edible industrial oilseed crop grown in India. With a productivity of 1937 kg/ha, the country produces 16.1 lakh metric tonnes of castor on 8.3 lakh hectares (Anonymous, 2022). The excessive damage caused by lepidopteran pests, the most significant of which is the Semilooper (*Achaea janata* L.), is one of the main factors that restrict castor productivity (Duraimurugan and Sujatha, 2023) [3]. Semiloopers are common throughout the vegetative and early reproductive stages of the crop, and it feeds voraciously on leaves and tender capsules (Duraimurugan and Lakshminarayana, 2014; Madhuri *et al*., 2022) [5, 9]. An estimated yield loss of 30–50% due to semilooper alone has been reported in castor (Rao *et al*., 2012) [12]. So far, synthetic chemical insecticides have been used to manage the pest problem in castor. Broad-spectrum chemical insecticides with a similar mechanism of action, however, should not be used repeatedly since this could lead to insect resistance development, pest resurgence, and harmful effects on natural enemies.

The most significant parasitoids of the castor semilooper are *Trichogramma chilonis* and *Snellenius maculipennis*, with up to 92% parasitization documented (Prabhakar and Prasad, 2005; Duraimurugan and Lakshminarayana, 2018) [11, 4]. Microbial insecticides can offer a different environmentally safe solution for controlling these insect pests while conserving natural enemies (Vimala Devi *et al*., 2020; Vimala Devi *et al*., 2021) [14, 13]. Among all the microbial products used for pest management, *Bacillus thuringiensis* (Bt) is popular and accounts for around 50% of the global market. However, high dose recommendations, short shelf-life and less persistent nature are major weakness of such microbial formulations. Numerous studies have shown that UV exposure has a negative impact on Bt performance, primarily causing the loss of spore viability and toxin integrity and, as a result, reduced insecticidal effectiveness (Zogo *et al*., 2019; Zhou *et al*., 2018) [17, 16].

Microencapsulating microorganisms in polymers has been the subject of recent study to increase formulation durability and efficacy while reducing dose requirements (Bashir *et al*., 2016; Jhones *et al*., 2021) [1, 8]. Hence, the present study was conducted to assess the effectiveness of biopolymer-based *B. thuringiensis* var. *kurstaki* microcapsules for the environmentally friendly management of semilooper in castor.

Materials and Methods

A native *Bacillus thuringiensis* var. *kurstaki* strain (Btk) preserved at ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad was cultured as per the methodology reported by Vimala Devi *et al*. (2021). Two biopolymer based Btk microcapsules (CC-1+Btk and CC-2+Btk) were developed using complex coacervation process and evaluated for their efficacy against castor semilooper (*Achaea janata*). Semilooper larvae collected during *kharif* season of 2022 from castor fields at Research Farm, ICAR-IIOR, Hyderabad were used to initiate the mass rearing. After that, the larvae were raised in a lab on castor leaves (cv. DCH-519) in accordance with Duraimurugam *et al.* (2015) [6]. Caged pot culture studies were carried out to evaluate the effectiveness of different doses of two Btk microcapsules *viz*., CC-1+Btk (Chitosan + Lignosulphonate + Manganous sulphate + Btk) and CC-2+Btk (Chitosan + Lignosulphonate + Carboxy Methyl Cellulose + Manganous sulphate + Btk) along with Btk technical, commercial Btk formulations and conventional insecticides against semilooper. The experimentation was conducted in a Completely Randomized Design (CRD) with thirteen treatments and replicated thrice. The treatments included three doses each for the two Btk microcapsules namely CC-1+Btk and CC-2+Btk (0.75 g/l, 1.0 g/l and 1.5 g/l), Btk-technical, commercial formulation of Btk (Delfin®) (1 g/l), Btk-WDG (1g/l), Bt-127 SC (3 ml/l), flubendiamide (0.2 ml/l), profenofos (2 ml/l) and control (only water spray) (Table 1). Castor hybrid (ICH-66) seeds were sown in pots, and the treatments were levied when the plants were 45-days-old. The plants were sprayed using a Ganesh hand compression sprayer. After the spray fluids had dried, 5-days old larvae of semilooper, *A. janata* were released onto the plants at a rate of 20 larvae per potted plant using a hairbrush. The plant and pot were enclosed with a nylon net cage that allowed for sufficient ventilation and aeration while also preventing the larvae from escaping. For up to 5 days after treatment, observations on larval mortality were observed at 24-hour intervals. The percent mortalities were converted to arcsine percentages and then statistical analyses were performed using CRD, with the least significant difference (LSD) method for mean comparisons (P = 0.05).

Results and Discussion

Effectiveness of the Btk microcapsules (CC-1+Btk and CC-2+Btk), commercial formulations of Btk, Btk technical and conventional insecticides against the 5-days old larvae of castor semilooper, *A. janata* is presented in the Table 1. Among the three doses of Btk microcapsules evaluated, CC-1+Btk microcapsules @ 1.5 g/l showed higher larval mortality of 100% followed by CC-2+Btk microcapsules @ 1.5 g/l (98.33% larval mortality) at 5 days after treatment. CC-1+Btk and CC-2+Btk microcapsules @ 1.0 g/l showed mortality of 91.66 to 96.66% mortality. The Btk microcapsules (CC-1Btk and CC-2Btk) @ 0.75 g/l caused mortality of 91.66 to 95.00%. The Btk technical and commercial Btk formulations (Btk-WDG, Btk-SC and Btk-Delfin) showed larval mortality of 90.00, 90.00, 95.00 and 91.66%, respectively. The conventional chemical insecticides *viz*., flubendiamide (0.2 ml/l) and profenofos (2ml/l) showed 100% larval mortality, while there was no mortality recorded in untreated control resulted complete defoliation of castor leaves (Fig. 1).

In recent years, the search for novel methods and formulations that can boost Bt's effectiveness has gained popularity. Micro/nanotechnology advancements have created new opportunities for the creation of more effective formulations that can get around some of the challenges that arise with using it in the field (Vimala Devi *et al*., 2021) [13]. The method that offers the greatest protection for biopesticides is encapsulation, which is safe and practical. It does this by polymer-coating the Bt spores and crystals, which increases their durability against UV radiation (Myasnik *et al*., 2001; Jhones *et al*., 2021) [10, 8]. Our studies demonstrated the effectiveness of encapsulated Btk with less (30%) active ingredients showed higher larval mortality of 98.33 to 100% as compared to other commercially available formulations (Fig. 2).

In the management of pests, Bt has been used as a successful alternative to chemical insecticides. However, due to vulnerability to environmental influences, the application of spore-crystal mixture is constrained in field situations. To address this issue effectively, microencapsulation has emerged as an excellent solution. Microencapsulated formulations have proven to enhance the firmness of Bt and protecting from photo degradation (Khorramvatan *et al.*, 2014; Vimala Devi *et al*., 2019) [7, 15]. From our study the encapsulated Bt showed higher mortality compare to non-encapsulated formulations. The present study will be useful to develop low volume biopolymer formulations of microbial pesticides with increased efficacy and field persistence for the efficient and environmentally acceptable management of lepidopteran pests.

Table 1: Efficacy of Btk microcapsules against castor semilooper larvae on castor plants in pot culture experiment

Treatment	Dose	Percent larval mortality
T_1: *CC-1+Btk	0.75 g/l	95.00 (77.07)^d
T_2: CC-1+Btk	1.0 g/l	91.66 (73.21)^e
T_3: CC-1+Btk	1.5 g/l	100.00 (90.00)^a
T_4: #CC-2+Btk	0.75 g/l	91.66 (73.21)^e
T_5: CC-2+Btk	1.0 g/l	96.66 (79.46)^c
T_6: CC-2+Btk	1.5 g/l	98.33 (82.57)^b
T_7: Btk technical	1.0 g/l	90.00 (71.56)^f
T_8: Btk WDG	1.0 g/l	90.00 (71.56)^f
T_9: Btk SC	1.0 g/l	95.00 (77.07)^d
T_{10}: Btk (Delfin®)	1.0 g/l	91.66 (73.21)^e
T_{11}: Flubendiamide 480SC 39.35% w/w	0.2 ml/l	100.00 (90.00)^a
T_{12}: Profenophos 50% EC	2.0 ml/l	100.00 (90.00)^a
T_{13}: Untreated Control	-	0.00 (0.00)^g
CD (P=0.01)		7.27
S.Em (±)		2.48
CV (%)		4.91

*CC-1+Btk: Chitosan + Lignosulphonate + Manganous sulphate + Btk

#CC2+Btk: Chitosan + Lignosulphonate + Carboxy Methyl Cellulose + Manganous sulphate + Btk

The values in parenthesis are angular transformed values

Figures with the same letter did not differ significantly

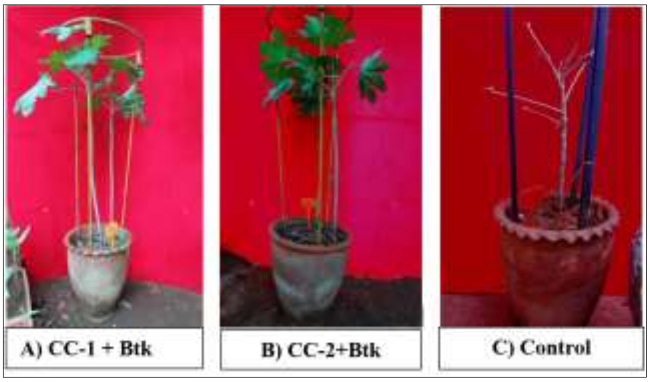

Fig 1: Efficacy of Btk microcapsules against semilooper (*Achaea janata*) larvae

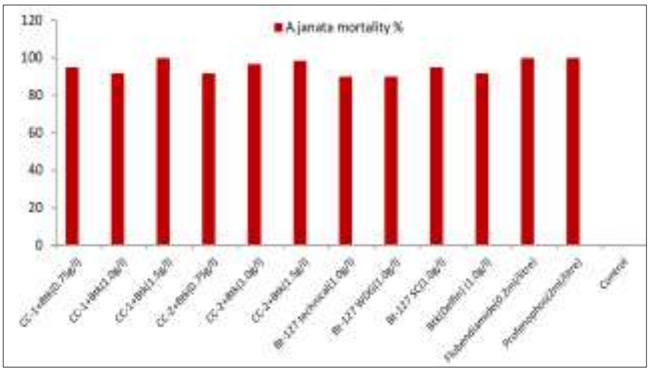

Fig 2: Efficacy of *Bacillus thuringiensis* var. *kurstaki* (Btk) microcapsules on 5-days old larvae of semilooper, *A. janata* (mean mortality percent)

Conclusion

The injury caused by insect pests is one of the main reasons for the low yield of major crops. Microbial bio-agents, in particular entomopathogenic bacteria, have great potential for the management of insect pests in an environmentally acceptable manner. However, high dose recommendations, short shelf-life and less persistent nature are major weaknesses of such microbial formulations. The present findings conclusively demonstrate that the biopolymer based encapsulated *Bacillus thuringiensis* var. *kurstaki* is more promising to be applied under adverse environmental conditions. Further, experiments under different environmental conditions need to be carried out with different UV protectants to increase the effectiveness of biopesticides.

Conflict of Interest: The authors claim that there are no conflicts of interest for this work.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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