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Integrating biocontrol agents with farmer's practice: impact on diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) and cabbage yield

Omprakash Navik[1*], R. S. Ramya[2], Richa Varshney[2], S. K. Jalali[2], T. M. Shivalingaswamy[2], R. Rangeshwaran[2], Y. Lalitha[2], Jagadeesh Patil[2] and Chandish R. Ballal[2]

Abstract

A field trial was conducted in a farmer's field by integrating biocontrol agents, a multiple insecticide tolerant strain (MITs), *Trichogramma chilonis* and formulation of *Bacillus thuringiensis* (NBAIR *Bt*G4) with reduced insecticidal spray as a biocontrol-based IPM compared to insecticidal application as a farmer's practice, for the management of the diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) infesting cabbage. Six *T. chilonis* releases of 100,000 parasitized eggs ha^{-1} were applied. Along with parasitoid release, a liquid formulation of *B. thuringiensis* (2%) was applied after third and fifth releases of *T. chilonis*. The number of *P. xylostella* larvae were significantly reduced in the field treated with biocontrol-based IPM as compared to farmer's practice after 30 and 45 days after treatment. After the 45 days, the holes on cabbage leaves were 2.2/plant in the field treated with biocontrol, opposed to 8.0 holes per plant were recorded in the farmer's practice filed. Only 7% of cabbage head damage was recorded in the field treated with biocontrol, whereas, in farmer's practice field, those were 32.2%. The cost-benefit analysis showed that integrating these biological control agents along with a reduced number of insecticidal sprays could reduce DBM population and percent head damage with an eventual increase in the yield.

Keywords: *Plutella xylostella*, Cabbage, *Trichogramma*, *Bacillus thuringiensis*, Integrated management

Background

Cabbage (*Brassica oleracea var. capitata* L.) is one of the extensively grown vegetable crops in India. Its cultivation is spread across tropical to temperate climatic conditions and constitute the most important component in the diets of various cultures (Shelton 2004). In India, cabbage is grown in an area of 0.39 m ha with a production of 8.8 MT with an average productivity of 22 t ha^{-1} (NHB 2016). The production of healthy and insect pest-free cabbage to fetch a remunerative price in the market, more attention has been paid towards the plant protection measures. Under extensive cultivation, cabbage suffers from the diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), and leaf webber, *Crocidolomia binotalis* Zeller (Nagarkatti and Jayanth 1982), that affects the production and quality of the yield. Among them, *P. xylostella* is the most destructive insect pest of cabbage and even more difficult pest to control. The loss in crop yield caused by this pest varies from 31 to 100% (Lingappa et al. 2004). Cultivation of crop round the year provides regular availability of host plant that helps DBM to complete 16–20 generations (Talekar and Shelton 1993). The mainstay of control measure under intensified farming is the frequent use of insecticides. The sole reliance on insecticides control of DBM has led a selection pressure that did help in the rapid build-up of resistance to almost all groups of insecticides (IRAC 2017). Development of DBM resistance to relatively selective compounds from the newer insecticide groups also have been reported (Ramya et al. 2016). Moreover, redundant

* Correspondence: omnavikm@gmail.com
[1]Division of Germplasm Collection and Characterization, ICAR–National Bureau of Agricultural Insect Resources, P. B. No. 2491, H. A. Farm Post, Bellary Road, Bengaluru 560 024, India
Full list of author information is available at the end of the article

use of broad-spectrum insecticides for its control not only increased the cost of production, but also led to environmental pollution through toxic residue (Guo et al. 1999). The worldwide efforts have been made to develop an efficient, integrated pest management approach with the incorporation of non-chemical methods for DBM management (Sarfraz et al. 2005). Egg parasitoids, *Trichogramma* and *Trichogrammatoidea*, have been reported for DBM control and were found promising in the management under glasshouse conditions and field studies (Singh and Jalali 1993).

An Austro-Asian origin species, *Trichogramma chilonis* Ishii, is the most widely used in integrated pest management (IPM) in India against lepidopterous pests of various crops. The development of insecticide tolerant strain has a potential to tolerate the pressure of frequent insecticidal applications by farmers and enables to suppress the insect pest's infestations in farmers' fields (Jalali et al. 2016). However, utilization of *T. chilonis* in combination with *Bacillus thuringiensis* (*Bt*) for DBM control was reported in a net house and under laboratory conditions (Singh et al. 2000). Several formulations of *Bt* were evaluated either in the greenhouse or field conditions in the form of dust, wettable powder, or emulsion from different parts of countries against DBM. (Justin et al. 1990 and Asokan and Mohan 1996). However, the research on the combination of these biocontrol agents with farmer's practices has been limited.

Therefore, in the present study, adaptation of the biocontrol-based IPM, using an insecticide tolerant strain of *T. chilonis* (NBAII-MP-TRI-13) and liquid formulation of *B. thuringiensis* (NBAIR *Bt*G4) for the management of DBM in cabbage field in comparison with farmer's applications of insecticides were assayed.

Materials and methods

Source of *Trichogramma chilonis* and *Bacillus thuringiensis*

The multiple insecticide-tolerant egg parasitoid strain, *T. chilonis* (NBAII-MP-TRI-13) was obtained from the Division of Genomic Resources, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India, where it is maintained under selection pressure of 5 groups of insecticides (organo-chlorine, organophosphate, synthetic pyrithroid, oxa-dizinon, and spinosyn) for the last 10 years and mass-produced in the Insectary of the Division of Germplasm, Conservation, and Utilization, ICAR-NBAIR, Bengaluru. The parasitoids were mass produced on the eggs of rice grain moth, *Corcyra cephalonica* Stainton. A liquid formulation of *B. thuringiensis* (NBAIR *Bt*G4) developed in the Division of Genomic Resources, ICAR-NBAIR, Bengaluru, was used for the field experiment.

Insecticides

The commercial formulations of recommended chemical insecticides for the management of DBM were obtained for field experiment from the local market.

Field trial

A field experiment was conducted on naturally infested cabbage with DBM in the Chikballapur, Karnataka, located at (13° 43′ N, 77° 72′ E and 915 m above sea level), India, during July–September 2017. The experiment was conducted on 2-weeks-old seedlings, planted in red sandy loam soil. Ten-days-old cabbage (cv. Saint) seedlings were procured from the local nursery and transplanted by following ridges and furrow method. A recommended dose of fertilizer (25 kg N; 150 kg P; 25 kg K) was applied as a basal dose. One month after planting, a nitrogenous fertilizer was applied (50 kg) during the earthing up operation (50 kg). Crop was irrigated through drip irrigation. During the experimental period, except DBM, no other pest species were observed on cabbage plants. Hence, the management strategies were based on the occurrence of DBM and suitable biocontrol inputs that were integrated with farmer practices. The experiment was conducted in a cabbage field of 2000 m^2, with a plant spacing of (45 × 60 cm). The experimental layout was a randomized complete block design with two treatments. There were 15 replicates (blocks of 66 m^2) per treatment (1000 m^2) and approximate 245 plants per replicate (a total of 3675 plants per treatment). To estimate the larval populations of DBM, three plants were selected randomly within each block. By thorough inspection, the number of larvae/plant was counted and the population density of larvae was estimated. The number of holes on the leaves caused by DBM was also counted. To estimate the number of holes/plant, the inner leaves of cabbage were observed, excluding the old leaves. However, to estimate the percent head damage, 10 plants were randomly selected and observed for the damage due to larval scrapping and also feeding holes made by larvae on the leaves. Based on healthy and damaged heads, the percentages of head damage were calculated.

The release of *T. chilonis* was initiated 15 days after transplanting, then parasitoids were released weekly at 9.30 a.m. (at the rate of 100,000 parasitized eggs ha^{-1}). A total of six releases were made at weekly intervals. The parasitoids were released immediately after observing about (5%) emergence in the laboratory. The tricho-cards, each having approximate 15,000 parasitized eggs, were cut into small pieces (16 bits; 4 × 1.5 cm) before release. Thus, for an area of (2000 m^2 a total of 24 pieces) were used (3 tricho-cards). These small pieces of tricho-cards were stapled to the lower surfaces of cabbage leaves and were uniformly distributed in the field. Then, the liquid formulation of *B. thuringiensis* (NBAIR *Bt*G4) was applied after third and fifth releases of parasitoids (10 and 20 days after transplanting) depending on the larval population. A liquid *Bt*G4 formulation was thoroughly mixed, using a sticker (gum acacia 1%) in 350 l capacity tank containing 300 l of tap water. Then, this *B. thuringiensis* (NBAIR *Bt*G4) suspension was sprayed on cabbage plants at 5.00 pm by using tractor-drawn Horizontal Triplex Power (HTP) sprayer. Insecticidal application was carried out by farmers at 15 days after transplantation. Insecticides were applied through tractor-drawn HTP sprayer with the recommended concentration of each insecticide either single application or in combination in a similar volume of water.

The comparison was between DBM larval populations, holes on the leaves, and the percentage of damaged heads. These estimations were monitored at 15, 30, and 45 days after treatment (DAT) with farmer's practice and biocontrol-based IPM. The details of these both treatments are given in (Table 1). The effect of treatments on cabbage yield was estimated and recorded and the cost benefit of biocontrol-based IPM and farmer's practice was calculated.

Statistical analysis

The arcsine transformation was used to normalize the percentage of damage before an ANOVA was conducted. Analysis was undertaken on the transformed data and untransformed means ± SE. All data were analyzed using PROC GLM (SAS version 9.3; SAS Institute 2011). Biocontrol agents' rates, insecticides' doses, and time effects, and their interactive effects on the number of larvae plant^{-1}, holes' number plant^{-1}, and percentages of damaged heads were estimated, using PROC GLM (SAS version 9.3; SAS Institute 2011). When ANOVA was significant, comparisons of relevant means were made, using Tukey's post hoc significance test at a significance level of 5%. The cost benefit of both treatments was estimated based on *Bt* (NBAIR *Bt*G4), *T. chilonis* and insecticides' applications. Average heads' yield was calculated in kg ha^{-1}. The cost of the treatments was estimated based on the cost of production of *T. chilonis* MITs, *Bt* (NBAIR *Bt*G4), and retail price of insecticides. Net profit was estimated based on the income of cabbage yield (USD$ 0.22 per kg) and the cost ha^{-1} from both the treatments.

Results and discussion

In order to determine the efficacy of the biological control agents integrated with reduced number insecticidal application in the management of DBM in comparison with farmer's practice, it is important to consider the level of pest reduction, the reduction in crop damage, and net profit to encourage the farming community.

Reduction in DBM larval population

The number of DBM larvae were significantly ($F = 41.55$, df = 1, 84, $P < 0.0001$) reduced in the 2 treatments. In biocontrol-based IPM field, the number of larvae was significantly ($P < 0.05$) lower compared to farmer's practice (Fig. 1). Analysis of variance showed that there was insignificant interaction between the treatments and

Table 1 IPM treatments and their application methods for controlling DBM in cabbage

Treatments	Treatment details	Number of application	Application methods
Biocontrol-based IPM (T1)	*Trichogramma chilonis* MITs-NBAII-MP-Tri 13	6	Stapling the Tricho bits at under surface of leaves
	Bacillus thuringiensis-NBAIR *Bt*G4	2	Foliar spray
	Chlorantraniliprole	1	Foliar spray
	Spinosad	1	Foliar spray
	Spinosad + Fenpropathrin	1	Foliar spray
	Chlorantraniliprole	1	Foliar spray
	Emamectin benzoate + Dichlorvos	1	Foliar spray
Insecticide-treated farmers' practice (T2)	Chlorantraniliprole	1	Foliar spray
	Emamectin benzoate + Dichlorvos	1	Foliar spray
	Chlorantraniliprole	1	Foliar spray
	Emamectin benzoate + Dichlorvos	1	Foliar spray
	Spinosad + Chlorantraniliprole	1	Foliar spray
	Thiodicarb	1	Foliar spray
	Spinosad + Chlorantraniliprole	1	Foliar spray
	Indoxacarb	1	Foliar spray
	Spinosad + Chlorantraniliprole	1	Foliar spray

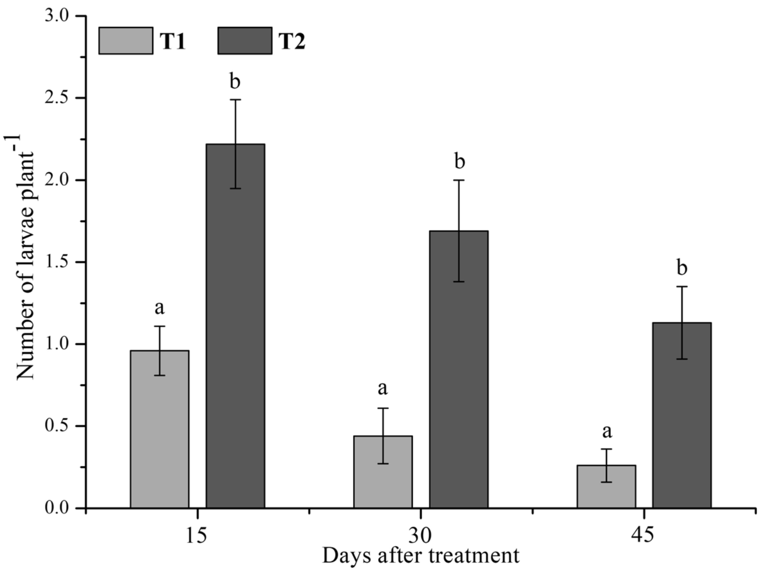

Fig. 1 Effect of biocontrol-based IPM and farmers' practice on larval reduction of DBM in cabbage at 15, 30, and 45 days after treatment. Different letters on the top of error bars indicate statistically different values for different treatments using Tukey's test ($P < 0.05$). Bars = standard error, T1 = biocontrol-based IPM, T2 = farmers' practice

days ($F = 0.54$, df = 1, 84, $P < 0.5854$). It was also found that the number of larvae was significantly ($P < 0.05$) reduced in 30 and 45 days after treatment (DAT) than at 15 DAT (0.44, 0.26, 0.96 larvae at 30, 45, and 15 DAT, respectively). These results indicated that those eggs escaped from parasitism by *T. chilonis* might have hatched and showed less susceptibility to insecticides at 15 DAT. DBM has been difficult to be controlled with synthetic insecticides in many regions of the world because larvae quickly developed resistance to the insecticides (Kianmatee and Ranamukhaarachchi 2007). Another research on DBM showed that larvae were also less susceptible to insecticides, including recently introduced diamides and other groups of compounds (Wang and Wu 2012 and IRAC 2017). Moreover, the parasitoids released to control DBM eggs on the plant is a random process, and the percentage of parasitism sometimes will not always be higher on plants near the release point (Miura 2003). Therefore, frequent mass releases of *T. chilonis* were carried out to control the population of DBM for a wide coverage resulting in fewer larval population in 30 and 45 DAT. Under field conditions, the parasitism percent with *T. chilonis* to the eggs of DBM ranged from 42 to 57% on cabbage and 77.06 to 94.87% on Indian mustard, when used as a trap crop in cabbage (Yadav et al. 2001). Bond and Boyce (1971) demonstrated that when viable DBM eggs on cabbage leaves were dipped in solutions of *Bt kurstaki* (Vectobac®), mortality increased with increase in *Btk* concentration. Apart from releasing *T. chilonis*, *Bt* (NBAIR *Bt*G4) was sprayed after the third and fifth releases of parasitoid. Therefore, the decreased number of larval population occurred at 30 and 45 DAT. Studies confirming the high field efficacy of *Bt* against the larvae of DBM were reported by Singh et al. 2015 and Stemele 2017. These former conclusions agree with those of the present study which showed decrease in DBM population after spraying the *Bt* (NBAIR *Bt*G4).

Number of holes on leaves/plant

The mean number of holes on cabbage leaves were 7.08 per plant in biocontrol-based IPM approach compared to the insecticide-treated farmer practice, (17.72 holes per plant) ($F = 78.61$, df = 1, 84, $P < 0.0001$). The difference in the mean number of holes on cabbage leaves/plant between the 2 treatments, was insignificant at 15 and 30 DAT, whereas, at 45 DAT the numbers of holes were significantly lower (2.21 holes per plant) ($P < 0.05$) in biocontrol-based IPM approach (Fig. 2). Analysis of variances revealed that there was insignificant interaction between treatments and days. In the present study, irrespective of the treatment, higher numbers of holes were observed at 15 DAT. However, significantly ($P < 0.05$) lower number of holes per plant were recorded in biocontrol-based IPM after 45 DAT (2.21 plants^{-1}). The present data showed that in both treatments, the number of holes on cabbage leaves was significantly reduced at 45 DAT. The differences in the larval reduction at 15 and 45 DAT could explain why cabbage plants showed significantly higher leaf damage due to DBM attack at 15 DAT in both treatments. The obtained results indicated that the numbers of holes on

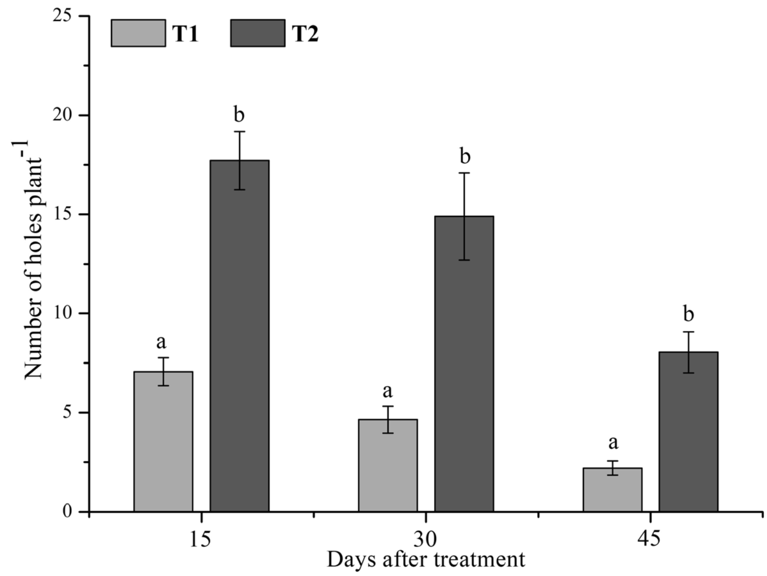

Fig. 2 Effect of biocontrol-based IPM and farmers' practice on a number of holes on cabbage leaves per plant by DBM at 15, 30, and 45 days after treatment. Different letters on the top of error bars indicate statistically different values for different treatments using Tukey's test ($P < 0.05$). Bars = standard error, T1 = biocontrol-based IPM, T2 = farmers' practice

cabbage leaves were also lower (8.04) in the farmers' insecticide practice. Similarly, Freddy (2011) reported that the leaf damage was significantly lower in the fields treated with insecticides than in non-treated. Motoyama et al. (1990) advised that the use of insecticides for control of DBM for longer period is not a good practice because DBM can develop resistance very quickly to insecticides. Integration of biological control treatment(s) with other control methods become necessary for pests' suppression, and this control can be sustained continuously for a longer period (Lim 1992). Therefore, in the present study, when integrated biological control agents with a reduced number of insecticidal sprays were practiced, they gave a very good control of DBM. Similarly, Reddy and Guerrero (2000) reported a decrease in leaves damage continuously after 12 weeks of transplanting in the IPM-based treatment integrated with natural enemies and *Bt*.

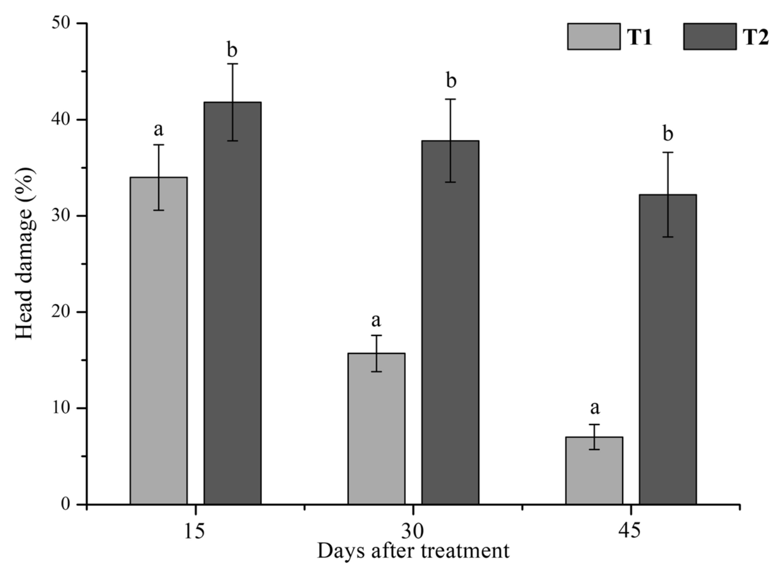

Fig. 3 Effect of biocontrol-based IPM and farmers' practice on percentage head damage of cabbage at 15, 30, and 45 days after treatment. Different letters on the top of error bars indicate statistically different values for different treatments using Tukey's test ($P < 0.05$). Bars = standard error, T1 = biocontrol-based IPM, T2 = farmer's practice

Table 2 Cost-benefit of biocontrol-based IPM practices and insecticide-treated farmers' practice for controlling DBM in cabbage

Treatments	Inputs	Rates ha^{-1}	Cost of production (US$ ha^{-1})[a]	Yield (kg ha^{-1})	Income (US$)[b]	Net profit (US$)[c]
Biocontrol-based IPM	*T. chilonis*	100,000 ha^{-1}	55.8	23,590	5413.9	5124.8
	B. thuringiensis	30 l	91.9			
	Chlorantraniliprole	75 ml	18.2			
	Spinosad	187.5 ml	54.8			
	Spinosad + Fenpropathrin	93.5 + 375 ml	30.3			
	Chlorantraniliprole	75 ml	18.2			
	Emamectin benzoate + Dichlorvos	150 g + 562.5 ml	19.9			
Insecticide-treated farmers' practice	Chlorantraniliprole	75 ml	18.2	12,920	2965.1	2749.3
	Emamectin benzoate + Dichlorvos	150 g + 562.5 ml	19.9			
	Chlorantraniliprole	75 ml	18.2			
	Emamectin benzoate + Dichlorvos	150 g + 562.5 ml	19.9			
	Spinosad + Chlorantraniliprole	93.75 + 37.5	36.5			
	Thiodicarb	1125 g	14.0			
	Spinosad + Chlorantraniliprole	93.75 + 37.5 ml	36.5			
	Indoxacarb	265.5 ml	16.1			
	Spinosad + Chlorantraniliprole	93.75 + 37.5	36.5			

Exchange rate used for conversion INR to US Dollar: US$ 1 = 65.15 INR (5 October 2017)
[a]Cost for the *T. chilonis* and *Bt* was estimated based on cost of production and retail price of the insecticides at 2 July 2017
[b]Local purchase price of the cabbage was (US$ 0.23 kg^{-1}) on 5 October 2017
[c]Net profit was calculated based on the only income of cabbage and the cost per hectare from different treatments

Percentages of cabbage damaged heads by DBM

It was found that application of biocontrol-based IPM reduced significantly, the percentage of damaged heads than farmers' practice ($F = 54.45$, df = 1, 84, $P < 0.0001$) (Fig. 3). The percentage of head damage was lower as the trial period progressed in the 2 treatments ($F = 19.60$, df = 2, 84, $P < 0.0001$) (Fig. 3). Significant interaction was also found between treatments and days ($F = 5.82$, df = 2, 84, $P = 0.0043$). The percentage head damage was highest (34%) in biocontrol-based IPM at 15 DAT whereas it was lowest (7%) at 45 DAT. However, in the farmers' practice, highest percentage head damage was observed (41.83%) at 15 DAT and lowest being (32.22%) at 45 DAT.

Crop yield

The cost for using biocontrol-based IPM for the management of DBM was (USD 289.1 ha^{-1}), while in case of farmers' practice, it was (USD 215.7 ha^{-1}). Cabbage yields were 23,590.0 and 12,920.0 kg ha^{-1} and net profit was USD 5124.8 and 2749.3 ha^{-1}, respectively (Table 2).

Conclusions

The present study showed that integration of biological control agents *T. chilonis* and *B. thuringiensis* (NBAIR *Bt*G4) with a reduced number of insecticidal sprays was able to manage the DBM efficiently in the cabbage fields and increased the obtained yield.

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Authors' contributions

First and fourth authors designed the research. All authors conducted the experiments. Six and seventh authors mass produced the biocontrol agents. First and eighth authors analyzed the data and wrote the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials

All data are available at the end of the article and the materials used in this work are of high quality and grade.

Ethics approval and consent to participate

All experimental works were approved by ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India, under the umbrella of AICRP-Biological Control project.

Consent for publication

The agreement of publication was taken, and as a corresponding author, I confirm that.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Division of Germplasm Collection and Characterization, ICAR–National Bureau of Agricultural Insect Resources, P. B. No. 2491, H. A. Farm Post,

Bellary Road, Bengaluru 560 024, India. [2]ICAR- National Bureau of Agricultural Insect Resources, Bengaluru 560 024, India.

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