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Endospore morphology and efficacy of indigenous *Bacillus thuringiensis* strains against okra fruit and shoot borer, *Earias vittella*

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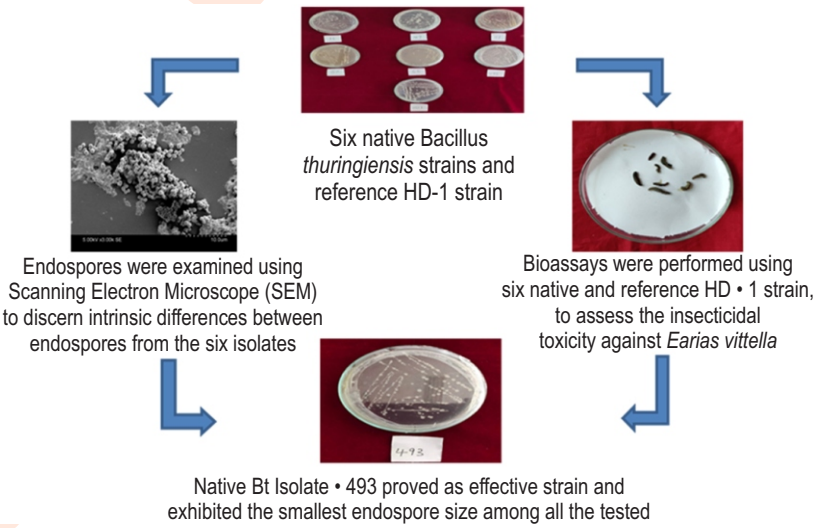
Abstract

Aim: The aim of this study was to determine the size of endospores of native *Bacillus thuringiensis* strains and identify an effective strain for managing the okra fruit and shoot borer, *Earias vittella*.

Methodology: Six native *Bacillus thuringiensis* strains were cultured on T3 agar plates and incubated at 30^{0}C for 96 hrs. Subsequently, detailed examination of the endospores was conducted using a NanoSem 450 scanning electron microscope (SEM) to discern intrinsic differences between endospores from six isolates. Bioassays were performed using six native *Bacillus thuringiensis* cultures at various concentrations. A fruit dip bioassay experiment was conducted with the reference strain, *Bacillus thuringiensis* subsp *Kurstaki* (HD 1), to assess the insecticidal toxicity of six native Bacillus thuringiensis isolates against the okra fruit and shoot borer, *Earias vittella*.



Results: Scanning electron microscope studies on *Bt* endospores revealed a range of sizes, with a notable proportion being spherical. Endospore sizes were quantified using Image J software, showing a normal distribution. Qualitative insecticidal bioassays were used to predict their insecticidal activity. In laboratory evaluations against *Earias vittella* larvae with the fruit dip method, all native *Bt* isolate treatments resulted in over 50% mortality. The highest mortality was observed with native *Bt* isolate 493 (90.00%), while the lowest mortality was recorded with isolate 49 treated larvae (60.00%).

Interpretation: In summary, isolate 493 emerged as the most effective native Bt isolate among those tested, exhibiting the smallest endospore size compared to other native *Bt* isolates.

Key words: *Bacillus thuringiensis*, Endospores, *Earias vittella*, Okra

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Introduction

Bacillus thuringiensis (*Bt*) is a Gram-positive, rod-shaped, aerobic bacterium known for its entomopathogenic properties. It is commonly found in diverse environments such as soil, grain dust, dead insects, and water sources (Lambert and Peferoen, 1992). *Bt* has emerged as one of the most successful bioinsecticidal agents due to its broad-spectrum toxicity against various insect pests, such as Dipteran, Lepidopteran, and Coleopteran orders (Federici *et al.,* 2006; Lacey *et al.,* 2015). This widespread efficacy is due to the production of crystalline endotoxins, known as Cry proteins, during sporulation. When insects ingest these proteins, they disrupt the gut cells, leading to insect's death (Bravo *et al.,* 2013; Jha *et al.,* 2021). *Earias vittella* (Lepidoptera: Noctuidae), cause huge damage to okra (*Abelmoschus esculentus*) crop across regions of South Asia, South east Asia, and parts of Africa. Larvae of *E. vittella* bore into the tender shoots and fruits of okra plant, leading to substantial crop damage, reduced yields, and economic losses, which can reach up to 69% under severe infestation (Sharma *et al.,* 2022).

This insect has ability to attack okra during both the vegetative and reproductive phases which further complicates its management (Choudhury *et al.,* 2021). Larval feeding on young shoots and leaves can lead to significant defoliation and stunted growth. It indicates that larvae can cause up to 40% reduction in leaf area, which negatively impacts overall plant health and productivity (Srinivasan *et al.,* 2018). Infestation during fruiting phase results in damage up to 50% of the okra pods, depending on the intensity of pest population and timing of infestation (Nair *et al.,* 2019). Infested fruits often exhibit feeding scars, which can lead to secondary infections and rotting. The economic losses associated with *E. vittella* infestations are significant. Management of *E. vittella* heavily relies on chemical insecticides. However, the extensive use of these chemicals has resulted in several negative outcomes, including the development of insecticide resistance, environmental contamination, and adverse effects on non-target organisms, including beneficial insects and human health (Deshmukh *et al.,* 2023; Dhawan *et al.,* 2021). The resistance of *E. vittella* is particularly concerning, as it reduces the efficiency of these chemical control methods (Rao and Devi, 2020). Moreover, given that okra is consumed directly as a vegetable, minimizing chemical insecticide use is crucial to avoid harmful residues.

In light of these challenges, there is an increasing focus on integrated pest management (IPM) strategies that utilize biological control agents, such as *Bt*, to manage *E. vittella*. Recent research has underscored the potential of indigenous Bt strains, which are often better adapted to local environmental conditions and may produce unique Cry proteins effective against specific pests like *E. vittella* (Kumar *et al.,* 2022). These indigenous strains are also more likely to remain viable and effective under field conditions, where they must withstand environmental stresses such as temperature fluctuations and UV exposure (Setlow, 2019). Despite the established efficacy of *Bt* in pest management, there remains a critical gap in understanding the role of endospore morphology in the bioefficacy of different *Bt* strains. Endospore is a highly resistant, dormant structure that enables the bacterium to survive harsh environmental conditions and persist in the soil for extended periods. Variations in endospore morphology, including size, shape and structural characteristics can influence the persistence and insecticidal potential of *Bt* strains (Setlow, 2019). Consequently, the study of endospore morphology in indigenous *Bt* strains is essential to optimize their use in pest management programs. Researchers worldwide continuously explore various ecologies in search of novel *Bt* isolates for potential applications (Campanini *et al.,* 2012; Soares-da-Silva *et al.,* 2015; El-Kersh *et al.,* 2016). This study focus on morphological analysis of endospores and insecticidal efficacy of six native *Bt* isolates, along with a standard reference strain, *Bacillus thuringiensis* var. *kurstaki* (HD 1), against the okra fruit and shoot borer, *E.vittella.*

Materials and Methods

Study Area: A laboratory experiment was conducted at the Insect Pathology Laboratory, Department of Entomology, Agricultural College, Bapatla, Andhra Pradesh (coordinates: 80.4671 °E longitude and 15.9039 °N latitude). The experimental area is characterized by a tropical humid climate with low rainfall.

Crystal Morphology Identification: Each sample was cultured on T3 agar plates and then incubated at 30^{0}C for 96 hrs. Following sporulation, the spore crystal mixture was examined under a light microscope to determine the morphology of endospores. Subsequently, detailed examination of the endospores was conducted by Scanning Electron Microscope (Nano Sem 450) to identify intrinsic characteristics and differences between endospores from different isolates. The reference strain used was HD-1 (*Bacillus thuringiensis kurstaki*) (Lane, 2020).

Insect rearing: The Lepidopteran larvae used in the bioassays were obtained from the Insect Pathology Laboratory, Department of Entomology, Agricultural College, Bapatla). As shown in Fig. 1, the eggs of *E. vittella* were small, round and exhibited a greenish or bluish tint. The surface of the eggs were textured with fine ridges and they were typically laid in clusters. Fine hairs or fibers surrounded the eggs, offering additional protection. The neonates, or newly hatched larvae were characterized by a black head and a pale, almost translucent body. Their segmented bodies were covered with sparse, fine hairs, giving them a slightly fuzzy appearance. As the larvae matured, a distinct brown and white banded pattern developed along the segments, which became increasingly prominent as the larvae reached full maturity. Fully developed larvae are dark brown in colour. The larvae also become thicker and more robust, with well-defined segmentation. Upon reaching the pupal stage, the larvae spin a silk cocoon that is oval in shape, beige in colour and rough in texture. This cocoon serves as a protective barrier, shielding the pupa during its metamorphosis into an adult moth. The adult moth, as depicted in Fig. 1, distinctively marked with an inverted

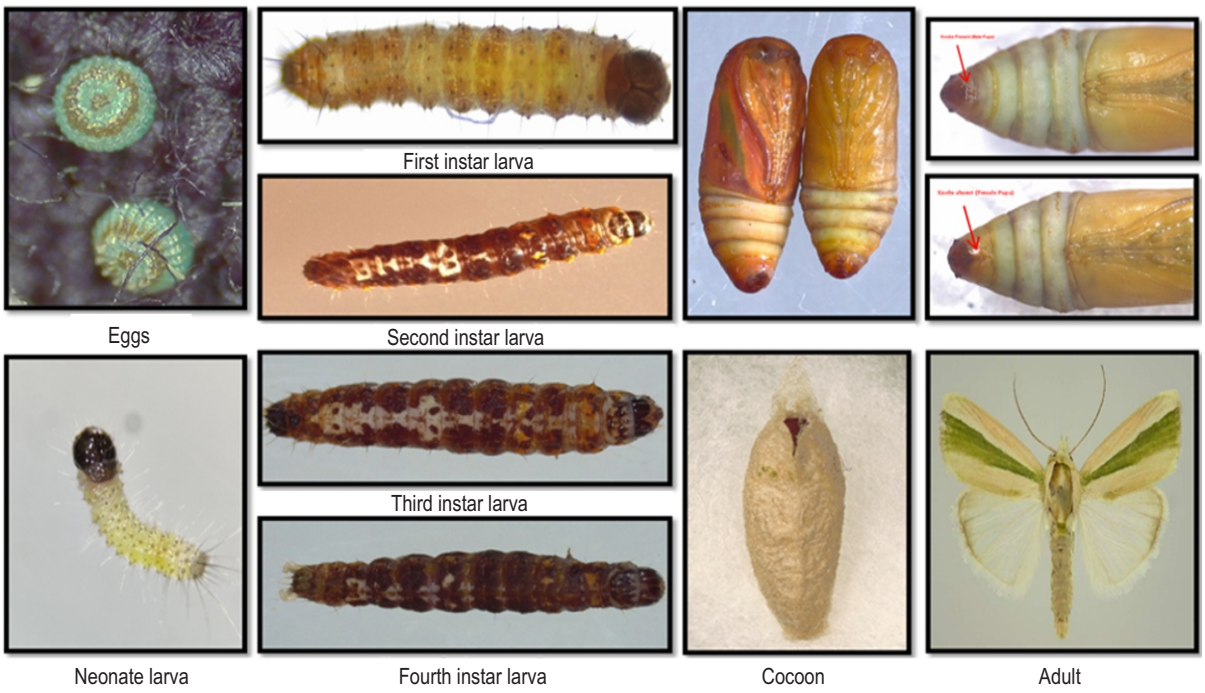

Fig. 1: Different life stages of *E. vittella* reared on okra fruits under laboratory conditions.

V-shaped green band on its forewings. This band is one of the most recognizable features of the moth, extending from the leading edge of the wing and tapering downward towards the center. The band is typically a vibrant green, contrasting sharply with pale yellowish-green or creamy background colour of the rest of the wing. This inverted V-shaped band is positioned near the middle of the forewing, creating a noticeable pattern that is important for species identification (Dhawan *et al.,* 2021; Rao *et al.,* 2020; Sharma *et al.,* 2019 and Vennila *et al.,* 2018).

Toxicity tests and Bioassays: Bioassays were performed using all *Bt* cultures at varying concentrations. A fruit dip bioassay experiment was conducted using the reference strain *B. thuringiensis* sub sp. *Kurstaki* (HD 1) to evaluate the insecticidal activity of six native *B. thuringiensis* isolates (Shelton *et al.,* 2000). Okra fruits were cut into uniform pieces and air-dried after being dipped in specific concentrations of *Bt*. The treated fruit pieces were then placed in petri dishes, and larvae were introduced. Twenty number of third instar larvae were used for each of the three replications. Larval mortality was recorded every 24 hrs for 7 days post-treatment. Mortality in the control group was also recorded, and the corrected mortality was calculated by Abbott formula (Abbott, 1925).

Statistical Analyses: Endospore sizes were calculated using Image J software. Additionally, spore diameter results were graphically represented using Origin Pro software to illustrate the distribution and variability of endospore sizes and morphological data. Laboratory data were statistically analyzed using Two Factorial Completely Randomized Design (CRD). Data were subjected to ANOVA following arcsine transformation, and the means were compared by Tukey's HSD (Honestly Significant Difference) post hoc test (Tukey, 1949), utilizing ADEL-R (Analysis and Design of Experiments with R-3.2.0 for Windows) version 2.0 software (Angela *et al.,* 2017). LC_{50} and LT_{50} values were determined through Probit Analysis (Finney, 1971).

Results and Discussion

Bt has been isolated from various ecologies and studied extensively since its discovery by Ishiwata in 1902. In this study, the morphologies and size distributions of the spores of seven different strains of *Bacillus thuringiensis* were analyzed. The results showed that, the Isolate 493 produced the least spore area, while the Isolate 49 produced the highest spore area. Nevertheless, it should be noted that a change of culture medium does affect the production of *B. thuringiensis* crystals and their size (Scherrer, 1973).

The normal distribution is used to create histograms of the size distribution of spore length and area for seven strains. Following observations under a SEM microscope, confirmed the surface view of rod-shaped bacterial cells in chains and cluster arrangement. *Bt* isolates tended to have sub-terminal spores. *Bt* isolates showed endospore architectures of spherical and marginal (Fig. 2-8). Spore area was lowest in the Isolate 493 (0.091 nm), which proved as highly efficient strain against *E. vittella* among all other native *Bt* isolates. While the highest spore

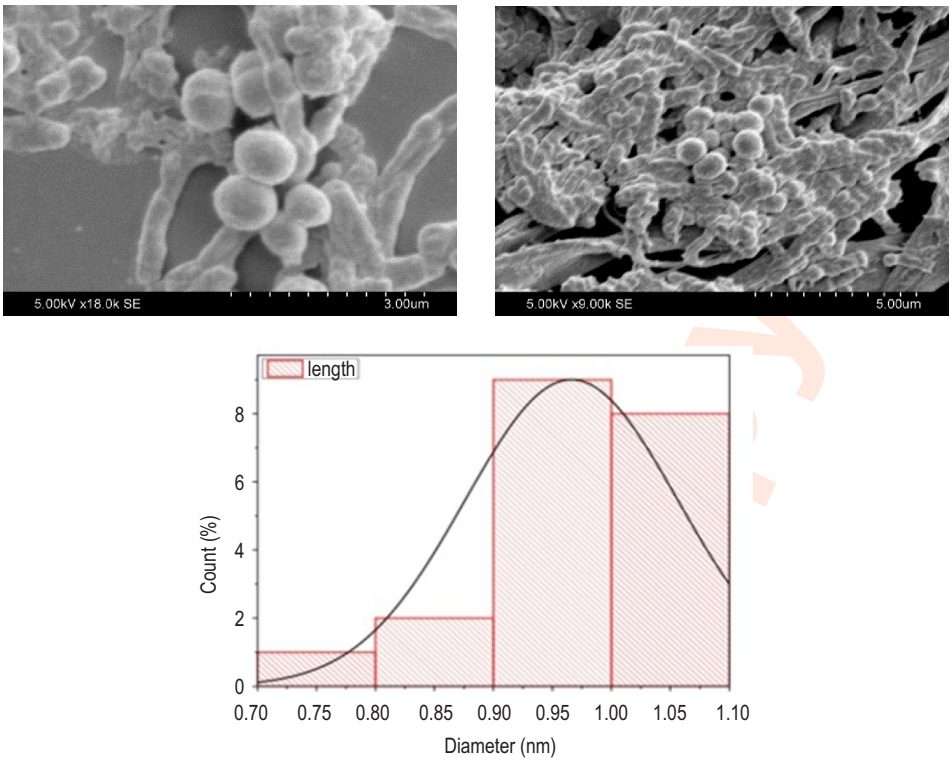

Fig. 2: (a, b) SEM image of the I-16 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

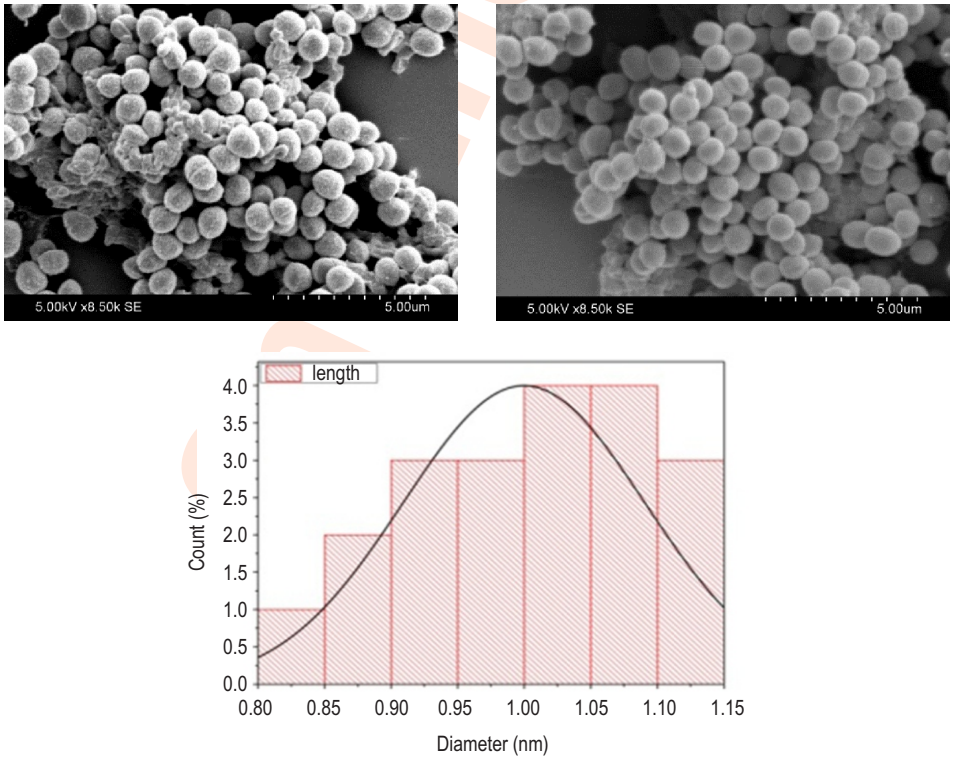

Fig. 3: (a, b) SEM image of the I-49 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

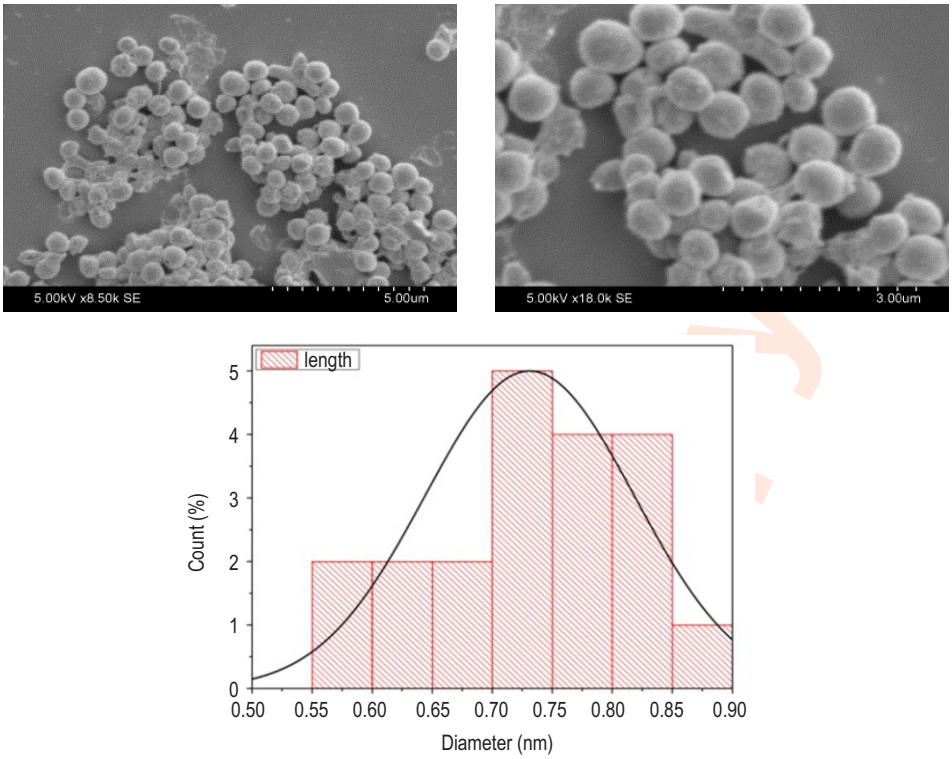

Fig. 4: (a, b) SEM image of the I-51 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

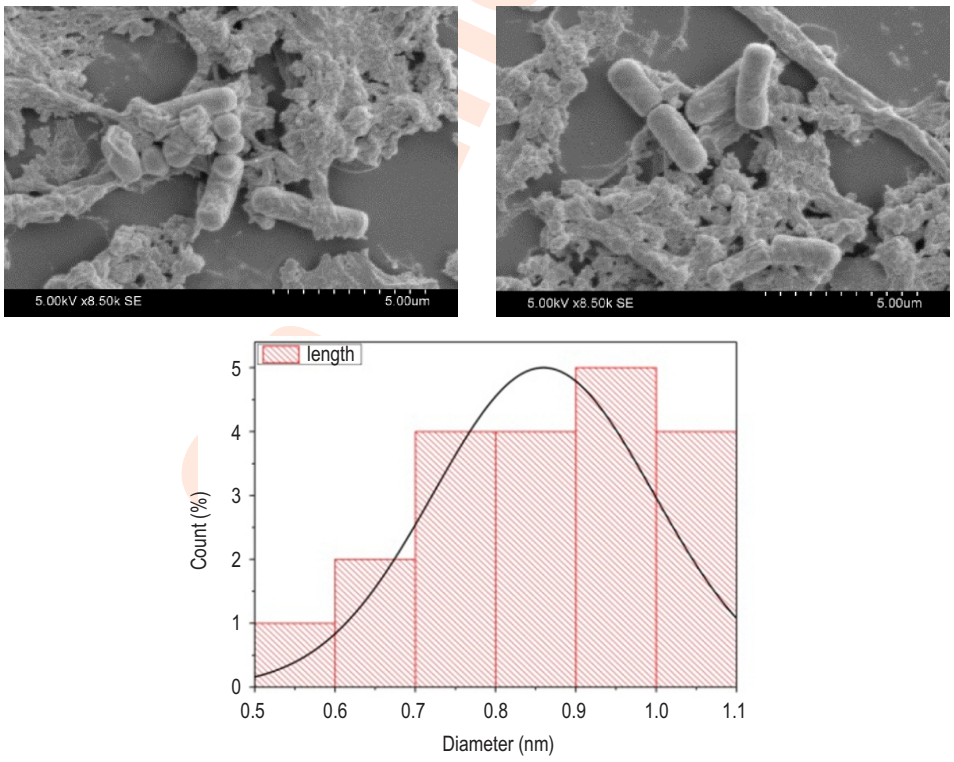

Fig. 5: (a, b) SEM image of the I-52 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

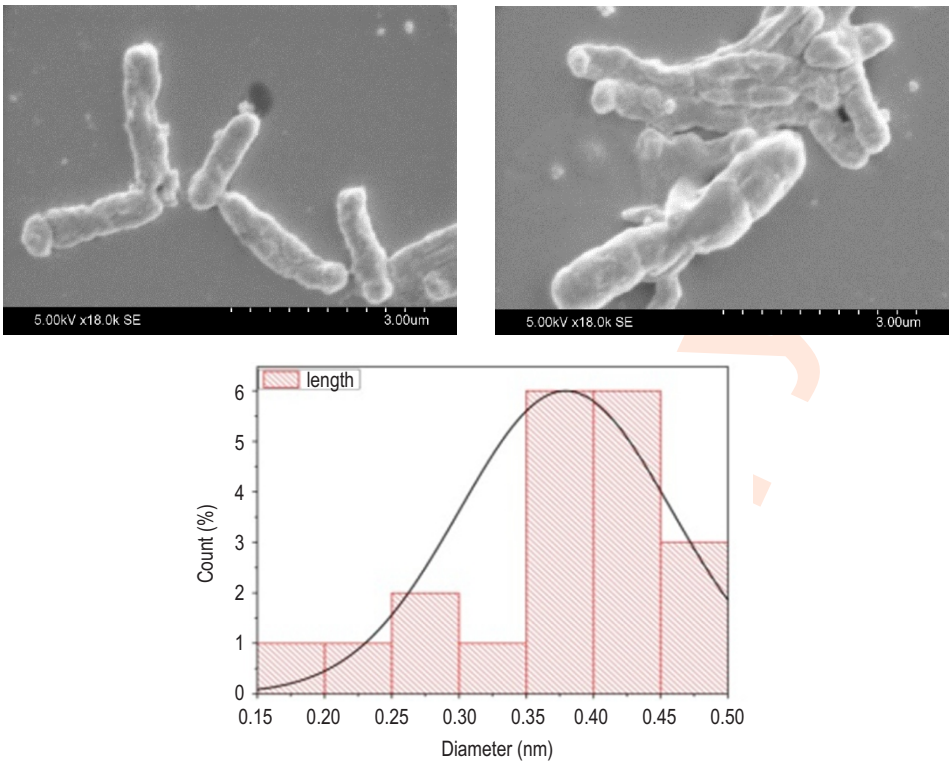

Fig. 6: (a, b) SEM image of the I-493 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

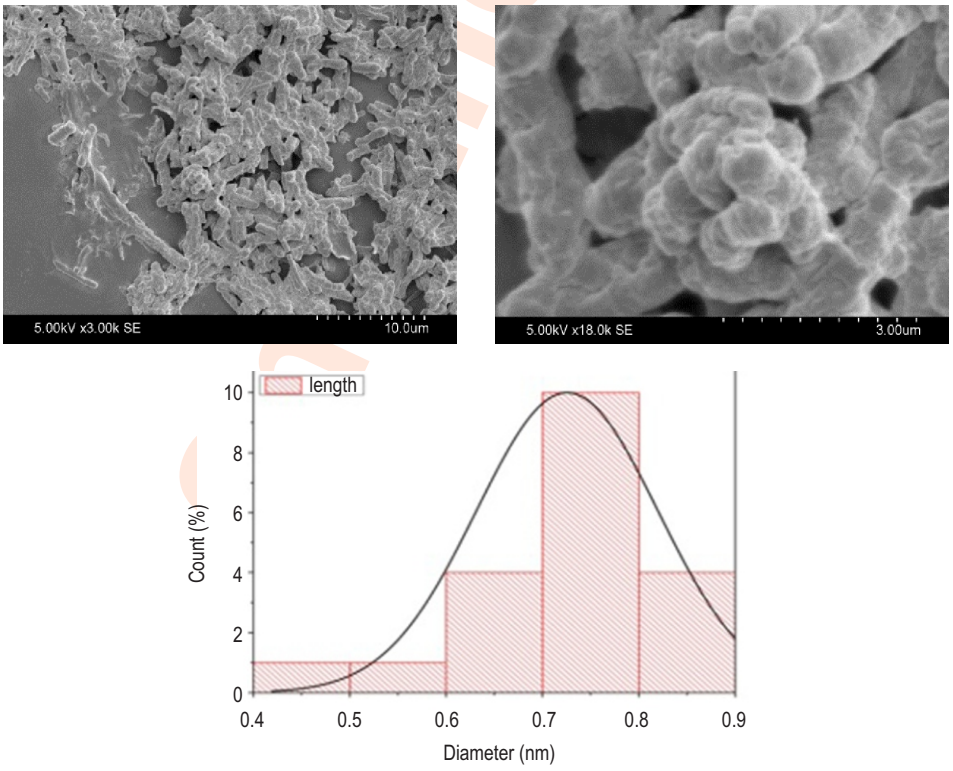

Fig. 7: (a, b) SEM image of the HD 1 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

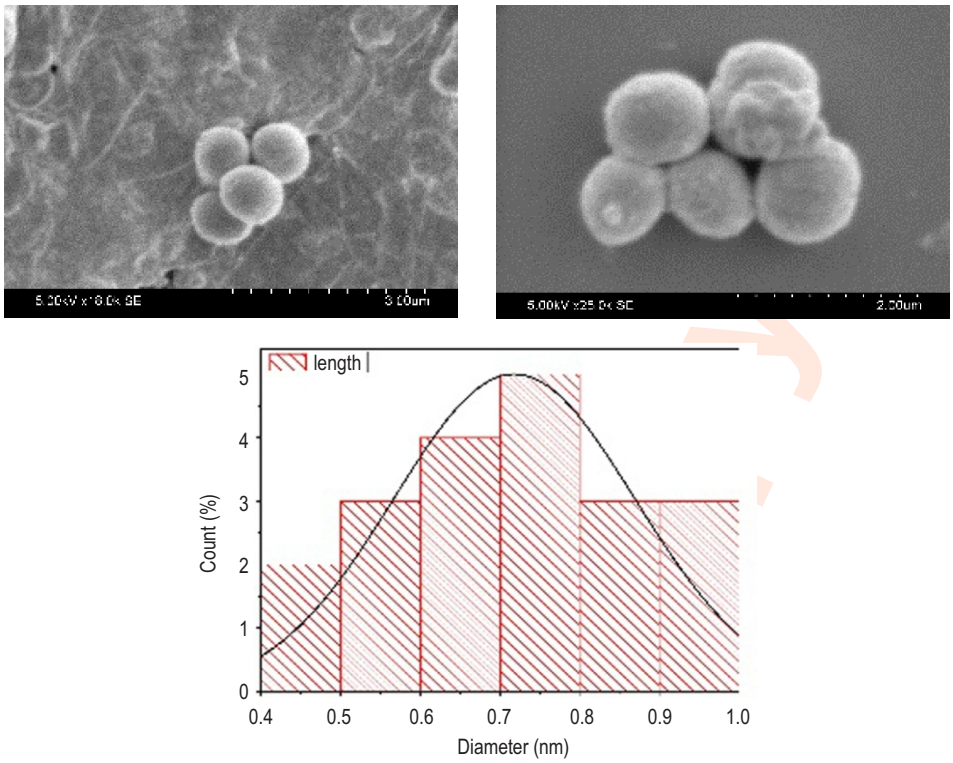

Fig. 8: (a, b) SEM image of the I-55 Spore, (c) Graph representing the size distribution in terms of count, along with the diameter of the spore.

Table 1: Endospore morphology of native *B. thuringiensis* strains and a reference HD 1 strain (*B. thuringiensis* var. kurstaki)

Strains	No. of measurements (N)	Endospore length (nm)		Endospore area (nm)	
		Average	Range	Average	Range
I-16	20	0.966 ± 0.088	0.723-1.086	0.529 ± 0.113	0.263-0.721
I-49	20	1.000 ± 0.089	0.837-1.143	0.799 ± 0.145	0.263-0.721
I-51	20	0.731± 0.085	0.555-0.858	0.448± 0.054	0.352-0.525
I-52	20	0.860 ± 0.134	0.581-1.038	0.721± 0.190	0.358-1.094
I-55	20	0.718± 0.148	0.484-0.980	0.353± 0.142	0.151-0.654
I-493	20	0.379± 0.077	0.245-0.483	0.091 ± 0.025	0.051-0.163
HD 1	20	0.726 ± 0.092	0.486-0.860	0.600± 0.114	0.380-0.889

area was observed in the Isolate 49 (0.799 nm). Based on the morphological data presented in Table 1, similar variations were also observed in the average spore length and area among each of the seven *B. thuringiensis* strains.

A selective bioassay with multiple doses revealed that all the tested *Bt* isolates were extremely toxic to *E. vittella* larvae (100% mortality). The mean per cent mortalities at different concentrations for all the isolates tested are present in Table 2. The mean per cent mortality recorded varied from 28.33 to 90.00 per cent at different concentrations tested ($1x10^8$ to $1x10^{12}$ CFU ml^{-1}). Among all the isolates tested, the reference strain HD1 showed the best mortality response, ranging from 55.00 per cent at $1x10^8$ CFU ml^{-1} to 88.33 per cent at 1x1012 CFU ml^{-1}. Among the native isolates, the highest mortality was recorded by Btisolate 493 which was on par with isolate HD1 at $1x10^{12}$ CFU ml^{-1} concentration (90.00 and 88.33 per cent, respectively). The next effective treatments were isolate 16 and HD1 which were on par with each other at $1x10^{12}$ CFU ml^{-1} and 1x1011 CFU ml^{-1} concentration (81.66 and 83.33 per cent). However, isolate 16 proved as the second-best treatment among the native Bt isolates with a mortality response of 45.00 to 81.66 per cent at $1x10^8$ to $1x10^{12}$ CFU ml^{-1} concentrations, respectively. From the current study, it was evident that the larval mortality increased with an increase in the concentration of *B. thuringiensis,* showing a positive correlation.

Table 2: Efficacy of native Bt isolates against spotted boll worm, *E.vittella*

Concentration/Isolate	I-16	I-49	I-51	I-52	I-55	I-493	HD1
1×10^{8}CFU ml^{-1}	45.00 (42.13)^{mnoppqr}	31.66 (34.23)st	35.00 (36.27)^{rst}	28.33 (32.14)^{t}	31.67 (34.23)st	48.33 (44.043)^{lmnopq}	55.00 (47.88)^{ijklm}
1×10^{9} CFU ml^{-1}	55.00 (47.87)^{ijklm}	38.33 (38.243)^{pqrs}	43.33 (41.163)^{nopqr}	36.67 (37.257)^{qrst}	41.67 (40.197)^{opqr}	60.00 (50.77)^{ghijk}	70.00 (56.79)^{def}
1×10^{10} CFU ml^{-1}	63.33 (52.743)^{fghij}	43.33 (41.163)^{nopqr}	50.00 (45.00)^{klmno}	45.00 (42.13)^{mnopqr}	46.67 (43.087)^{lmnopq}	71.67 (57.86)^{def}	76.67 (61.143)^{cde}
1×10^{11} CFU ml^{-1}	68.33 (55.77)^{efg}	51.66 (45.957)^{klmno}	60.00 (50.79)^{ghijk}	53.33 (46.913)^{jklmn}	56.67 (48.837)^{hijkl}	78.33 (62.287)^{cd}	83.33 (65.95)^{abc}
1×10^{12} CFU ml^{-1}	81.66 (64.807)^{bc}	60.00 (50.77)^{ghijk}	68.43 (55.77)^{efg}	65.00 (53.73)^{fghi}	66.67 (54.75)^{fgh}	90.00 (71.57) a	88.33 (70.117)^{ab}
Tween 80 - 0.1%				0.00%			
Control				0.00%			
		Isolates		Concentrations		Isolate x concentrations	
SEm ±		0.468		0.395		1.046	
CD (p=0.05)		1.319		1.115		2.95	
CV (%)		3.677					
Standard Error for Comparison		1.4858					
Critical Q Value		5.648					
Critical Value for Comparison		5.9336					

* Mean of three replications (N=20); Values in parentheses are arc sin transformed; Mean values followed by same alphabet in a column are not significantly different by Tukey's HSD (P=0.05).

Table 3: Mean lethal concentration and lethal time response at 50 and 90 per cent mortality of third instar larvae of *E. vittella* with native Bt isolates

BtIsolates	LC_{50} (CFU ml^{-1})	LC_{90} (CFU ml^{-1})	LT_{50} (hpi) at 1×10^{12} CFU ml^{-1}	LT_{90} (hpi) at 1×10^{12} CFU ml^{-1}
I-16	3.664×10^{8}	9.052×10^{13}	116.908	198.021
I-49	5.246×10^{10}	6.868×10^{17}	141.824	284.415
I-51	7.531×10^{9}	7.136×10^{15}	132.616	232.154
I-52	3.090×10^{10}	9.239×10^{15}	135.082	255.337
I-55	1.44×10^{10}	1.020×10^{16}	129.661	232.916
I-493	1.528×10^{8}	1.991×10^{12}	110.768	175.745
Hd1	1.947×10^{7}	1.542×10^{12}	106.992	170.942

LC – Lethal Concentration; LT – Lethal Time; CFU – Colony Forming Units and hpi – Hour post Inoculation

Native *Bt* isolates showed LC_{50} and LC^{90} (Table 3) in the range of $5.246x10^{10}$ to $1.947x^{107}$ CFU ml^{-1} and $6.868x10^{17}$ to $2.766x10^{11}$ CFU ml^{-1}, respectively. The reference strain HD1 recorded the lowest median lethal concentration of $1.947x10^{7}$ CFU ml^{-1}. Among the native *Bt* isolates, the lowest LC_{50} value was recorded by the isolate-493 ($1.528x10^{8}$ CFU ml^{-1}), whereas highest LC_{50} value was obtained with the isolate 52 ($3.090x10^{10}$ CFU ml^{-1}) and thus, was found to be the least effective of all the native isolates tested. Of all the native *Bt* isolates, the isolate-493 achieved the fastest lethal action on the third instar larvae of *E. vittella* with 110.768 hpi (Hour post Inoculation). The insecticidal efficacy of *Bacillus thuringiensis* (Bt) has been well-documented in prior studies. DeLucca *et al.* (1981) were among the earliest to report the distribution of Bt in soils across the United States and its insecticidal activity. Following this, Ohba and Aizawa (1986a, 1986b) found widespread Bt toxicity in Japanese soils, with significant effects on lepidopteran larvae. Later, Martin and Travers (1989) expanded this understanding, demonstrating Bt's global abundance and insecticidal diversity.

In subsequent years, Iriarte *et al.* (1998) demonstrated that most Bt isolates showed insecticidal activity, achieving over 25% mortality against various lepidopteran species. Specific studies on Earias vittella followed, with Pandey (2002) reporting mortality rates between 76.67% and 83.33% for Bt kurstaki formulations targeting this pest. Narendran *et al.* (2013) also observed 100% mortality in *E. vittella* larvae using fruits from transgenic lines expressing a single Bt transgene, segregating in a 3:1 ratio.

More recently, Elyass *et al.* (2017) tested local Bt isolates and the reference strain Bt kurstaki HD-1 against *E. vittella*, achieving larval mortalities of 75%, 87.3%, and 100% at spore-crystal concentrations of 1.3×10^7, 4.5×10^8, and 2.4×10^9 CFU, respectively, by 72 hours. Adding to these findings, Chilcott and Wigley (2020) showed that soil-derived Bt isolates retained high toxicity against lepidopteran larvae, with mortality rates ranging from 37% to 88%.

In the current study, isolate 493 demonstrated superior performance among all tested native Bt isolates, showing the smallest endospore size and highest insecticidal efficacy against *E. vittella*. This result aligns with previous research on the potential of indigenous Bt strains as effective, locally adapted biocontrol agents.

The study on Endospore Morphology and Efficacy of Indigenous *Bacillus thuringiensis* Strains against Okra Fruit and Shoot Borer, Earias vittella is highly relevant for Integrated Pest Management (IPM) in agriculture. *Earias vittella* is a major pest of okra, causing severe crop losses and relying predominantly on chemical control methods, which lead to environmental contamination, insecticide resistance, and negative impacts on non-target species and human health. This study explores the potential of indigenous *Bacillus thuringiensis* (Bt) strains as biological control agents, which offer a more sustainable alternative by reducing chemical pesticide reliance.

The research emphasizes the role of endospore morphology in Bt strains' efficacy, which can affect persistence and insecticidal effectiveness. Indigenous Bt strains are often better suited to local environmental conditions, potentially enhancing pest control outcomes in field applications.

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Authors' contribution: A.J. Reddy: Conducted the experiment and collected data, performed the statistical analysis, wrote the paper; **D.V.S.R. Kumar:** Designed the experiment, monitored experiments, contributed data analysis and corrected the manuscript; **S. Rao, V.C. Prasannakumari and V. Roja:** Monitored experiments and corrected the manuscript.

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Research content: I declare that the submitted research paper is my original work and no part of it has been published anywhere else in the past. I take full responsibility, that if in future, the paper is found invalid according to basic rules, the last decision will be of the authorities concerned.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interests.

Data availability: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

References

Abbott, W.S.: A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.,* **18**, 265-267 (1925).

Angela, P., R. Francisco, A. Gregorio, C. Jose and B. Juan: ADEL-R (Analysis and design of experiments with R for Windows) Version 2.0, International Maize and Wheat Improvement Center (2017).

Bravo, A., S.S. Gill and M. Soberón: Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon.,* **49**, 423-435 (2013).

Campanini, E.B., C.C. Davolos, E.C. Alves and M.V.F. Lemos: Characterization of new strains of *Bacillus thuringiensis* for the control of important insect pests in agriculture. *J. Appl. Microbiol.,* **71**, 362-369 (2012).

Chilcott, C.N. and P. Wigley: Efficacy of soil-derived *Bacillus thuringiensis* isolates against lepidopteran pests. *J. Appl. Entomol.,* **144**, 493-505 (2020).

Choudhury, R.A., B. Singh and S. Saxena: Integrated management of okra shoot and fruit borer, *Earias* spp., using biopesticides. *J. Appl. Entomol.,* **145**, 145-155 (2021).

Crickmore, N.: Beyond the spore-past and future developments of *Bacillus thuringiensis* as a biopesticide. *J. Appl. Microbiol.,* **101**, 616-619 (2006).

DeLucca, A.J., J.G. Simonson and A.D. Larson: *Bacillus thuringiensis* distribution in soils of the United States. *Can. J. Microbiol.,* **27**, 865-870 (1981).

Deshmukh, S.G., V.K. Patil and R.A. Jadhav: Integrated pest management: Strategies for sustainable agriculture. *J. Agric. Food Res.,* **13**, 100427 (2023).

Dhawan, A.K., A. Kaur and K. Singh: Integrated pest management strategies for cotton pests in India. *J. Entomol. Zool. Stud.,* **9**, 1670-1677 (2021).

El-Kersh, T.A., A.M. Ahmed, Y.A. Al-Sheikh, F. Tripet, M.S. Ibrahim and A.A.M. Metwalli: Isolation and characterization of native *Bacillus thuringiensis* strains from Saudi Arabia with enhanced larvicidal toxicity against the mosquito vector *Anopheles gambiae* (s.l.). Parasit. *Vectors,* **9**, 647 (2016).

Elyass, M.E., A.A. Mahdi and I.H. Atttalla: Bioassay of efficacy of five isolates of *Bacillus thuringiensis* ssp. Kurstaki against larvae of the spotted bollworm (*Earias vittella*) on okra (*Abelmoschus esculentus* L. Moench). *J. Biol. Sci. Opin.,* **4**, 188–192 (2017).

Federici, B.A., H.W. Park and Y. Sakano: Insecticidal protein crystals of *Bacillus thuringiensis. Appl. Environ. Microbiol.,* **72**, 2612-2621 (2006).

Finney, D.J.: Probit Analysis. 3rd Edn.: Cambridge University Press, London, 333 pages (1971).

Iriarte, J., Y. Bel, M.D. Ferrandis, R. Andrew, J.F. Murillo and P. Caballero:

Environmental distribution and diversity of *Bacillus thuringiensis* in Spain. *Syst. Appl. Microbiol.,* **21**, 97-106 (1998).

Jha, D.K., A. Kumar and B. Choudhary: *Bacillus thuringiensis*: Biopesticide for sustainable agriculture. Biopesticides: *Adv. Bio-Inoculants,* **2**, 345-367 (2021).

Kumar, S., P. Singh and K. Rana: Efficacy of indigenous *Bacillus thuringiensis* strains against major pests of okra and cotton. *J. Pest Sci.,* **95**, 455-468 (2022).

Lacey, L.A., D. Grzywacz, D.I. Shapiro-Ilan, R. Frutos, M. Brownbridge, and M.S. Goettel: Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol.,* **132**, 1–41 (2015).

Lambert, B. and M. Peferoen: Insecticidal promise of *Bacillus thuringiensis. Bioscience,* **42**, 112-122 (1992).

Lane, D.M.: Project Leader, Online Statistics Education: A Multimedia Course of Study. Rice University (Chapter 2 "Graphing Distributions", Section "Histograms"). (2020).

Martin, P. and R. Travers: Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Appl. Environ. Microbiol.,* **55**, 2437-2442 (1989).

Nair, N.S., M. Karthikeyan and P.K. Reddy: Impact of Eariasvittella on okra fruit quality and yield. *Indian J. Entomol.,* **81**, 126-134 (2019).

Narendran, M., S.G. Deole, S. Harkude, D. Shirale, A. Nanote, P. Bihani, S. Parimi, B.R. Char and U.B. Zehr: Efficient genetic transformation of okra (*Abelmoschus esculentus* (L.) Moench) and generation of insect-resistant transgenic plants expressing the cry1Ac gene. *Plant Cell Rep.,* **32**, 1191–1198 (2013).

Ohba, M. and K. Aizawa: Distribution of *Bacillus thuringiensis* in soils of Japan. *J. Invertebr. Pathol.,* **47**, 277-282 (1986a).

Ohba, M. and K. Aizawa: Insect toxicity of Bacillus thuringiensis isolated from soils of Japan. *J. Invertebr. Pathol.,* **47**, 12-20 (1986b).

Pandey, A.K., A.K. Tiwari and P. Mall: Screening of okra (*Abelmoschus esculentus*) for yield performance and susceptibility to fruit borer, E. vittella (Fab.). *Pestology,* **26**, 32-34 (2002).

Rao, N.S. and T.R. Devi: Morphological characterization and molecular phylogeny of *Earias vittella* populations in different agro-climatic zones of India. *Int. J. Pest Manag.,* **66**, 231-242 (2020).

Scherrer, P., P. Luthy and B. Trumpi: Production of endotoxin by *Bacillus thuringiensis* as a function of glucose concentrations. *Appl. Microbiol.,* **25**, 644–646 (1973).

Setlow, P.: Spore resistance properties. *Microbiol. Spectrum,* **7**, 73-90 (2019).

Sharma, A. and D. Kumar: Biology and management of Earias vittella (Lepidoptera: Noctuidae) on okra. *J. Agric. Urban Entomol.,* **35**, 34-41 (2019).

Sharma, R., S. Kumar and R.P. Singh: Economic losses due to *Earias vittella* infestation in okra cultivation. *J. Econ. Entomol.,* **115**, 54-63 (2022).

Shelton, A.M., J.D. Tang, R.T. Roush, T.D. Metz and E.D. Earle: Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnol.,* **18**, 339-342 (2000).

Soares-da-Silva, J., V.C.S. Pinheiro, E. Litaiff-Abreu, R.A. Polanczyk and W.P. Tadei: Isolation of *Bacillus thuringiensis* from the state of Amazonas, in Brazil, and screening against *Aedes aegypti* (Diptera, Culicidae). *Rev. Bras. Entomol.,* **59**, 1–6 (2015).

Srinivasan, R., K. Muthusamy and M. Raja: Effects of *Earias vittella* on okra plant growth and yield. *Int. J. Pest Manag.,* **64**, 234-245 (2018).

Tukey, J.: Comparing individual means in the analysis of variance. *Biometrics,* **5**, 99-114 (1949).

Vennila, S., G. Rajasri and J. Singh: Comparative Morphological Study of *Earias vittella* and *Earias insulana* on cotton and okra. *Indian J. Entomol.,* **80**, 270-275 (2018).

Wu, D. and F. Chang: Synergism in the mosquitocidal activity of 26 and 65 kDa proteins from *Bacillus thuringiensis* subsp. *fukuoka ensis* crystal proteins. *Appl. Environ. Microbiol.,* **57**, 1075-1081 (1985).