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Bio Efficacy of *Bacillus thuringiensis* Isolates against Diamond Back Moth (*Plutella xylostella* L.) on Cauliflower Plant in Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. Author SST did the investigation, writing original draft and performed the statistical analysis. Author GP did the editing and fund acquisition and author AR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Bacillus thuringiensis (*Bt*) gram positive entomopathogenic bacteria being an eco-friendly biopesticide. In present study the potential of *B. thuringiensis* isolates was studied for the biological control of Diamond back moth (DBM). Fifteen Bt isolates were obtained from the Department of Agricultural Microbiology, TNAU Tamil Nadu. This mainly isolated from the cultivated lands Cotton (*Gossypium hirsutum*), Brinjal (*Solanum melongena*) and Tomato (*Solanum lycopersicum*). All the 15 isolates were identified as *B. thuringiensis* based on the crystalline structure. Four different types of crystalline forms were observed, in which the isolates CC, CB1, BC, TD, BD were produces cuboidal shape crystals. Then, the isolates were characterized based on presence of lepidopteron specific cry gene. Among the 15 *B. thuringiensis* isolates seven of them were found to be positive for lepidopteron specific cry genes include cry 1 & cry 2. Four *Bt* isolates were exhibited presences of both cry 1 and cry 2 genes. The selected 4 isolates further screened for protein profiling by SDS Page. Molecular weights of the protein ranging from 65 to 130 kDa. Toxicity of this four *B. thuringiensis* isolates were evaluated by bioassay using third instar larvae of the diamondback moth (*Plutella xylostella*) and isolate (CC) recorded maximum mortality of 95.33%

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comparable to standard strain HD1 98.31%. In quantitative bioassay, the LC_{50} for third instar larvae of *Plutella xylostella* was found to be least range 197.09 ppm with fiducial limits of 110.28 - 352.21 ppm respectively. Different concentration of the toxic protein (100, 250, 500, 750, 1000, 2000) will reduce the leaf damage and larval growth was (54-8% and 58-25%) observed. This achieved effective control of DBM in cauliflower plant.

Keywords: Bacillus thuringiensis; biopesticide; cry protein; bioassay; Plutella xylostella.

1. INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the most important winter vegetables grown extensively in temperate and tropical areas of the world and in India, with an area of 2,32,800 ha and 2,78,800 ha respectively [1]. The diamond back moth (DBM), *P. xylostella* (Lepidoptera: Plutellidae) is the major destructive pest on cruciferous crops such as cauliflower, cabbage and mustard [2]. The pest is distributed all over India and direct crop losses due to its damage. In India, [3] reported that 52 per cent yield loss on cauliflower due to diamond back moth infestation. The damage caused by *P. xylostella* has been estimated globally to cost US$ 1 billion in direct losses and control costs [4]. The use of synthetic insecticides is the main control strategy [5]. But these chemicals affects the environment, reduce soil fertility and causes health issues [6]. For this reason entomopathogenic bacteria *Bacillus thuringiensis* has become an important alternative for insect control as this pest developed resistance to all major groups of synthetic pesticides. It is distinguished from other *Bacillus* spp. by crystal protein production during sporulation [7]. These crystals are predominantly composed of one or more proteins (Cry and Cyt toxins) called endotoxins [8], which is toxic to many of the insect pests. The insecticidal crystal proteins are divided into five major classes, namely cry 1, cry 2, cry 3, cry 4 and cry 5, with specific insecticidal activity against Lepidoptera, Diptera and Coleoptera [6]. This toxin is ingested by a susceptible host, solubilized and dissolved in the alkaline midgut. The active toxin binds to specific receptor sites on the gut epithelium, leading to slow degradation of the gut lining, results in the leakage of ions in to the haemocoel. Finally death occurs within 1–3 days [9]. These cry proteins are insect specific, harmless to mammals, vertebrates and plants, and are not toxic to the environment [10].

Hence, a novel way of controlling *Putella xylostella* using endophytic *B. thuringiensis* crystal protein, in order to protect cabbage crops and other brassicas from this pest [11] is attempted, so that it could be used as substitute to chemical pesticide for integrated pest management of diamond back moth.

2. MATERIALS AND METHODS

2.1 Strains

The *Bacillus thuringiensis* cultures were obtained from the Department of Agrl. Microbiology. TNAU, Tamil Nadu, India. Which were isolated from the cultivated soils of Cotton (*Gossypium hirsutum),* Brinjal (*Solanum melongena*), Tomato (*Solanum lycopersicum*) and Kodaikanal Bhendi were characterized phenotypically based on morphological and microscopic observation and named the isolates based on the crops like CA, BA, TA, KBB.

2.2 Identification Based on Crystal Morphology

All the isolates that were Gram positive and rod shape were examined for the presence of parasporal inclusion by phase contrast microscopy. Crystal morphology observed by crystal staining with CBB-G 250 [12] Single colony of *B. thuringiensis* strains was inoculated into 5 mL T3 broth and incubated the isolates for 24 hrs at 30°C at 200 rpm. After the period of incubation 1 per cent (250 µL) inoculum was added to 250 mL flask containing 25 mL of T3 broth and incubated at 30°C in a rotatory shaker and maintained at 200 rpm for 48–60 h. In order to observe the spore crystals suspended in sterile water, wet mounted, heat fix and then examined under Phase Contrast Microscope at a 100X magnification. Colonies were then classified into different groups based on their crystal shape [13].

2.3 Extraction of Spore Crystal Complex from *B. thuringiensis* Isolate

A loopful of *B. thuringiensis* culture from the T3 medium slant was inoculated into 5 ml of T3 broth and incubated in a shaking incubator maintained at 30ºC and 200 rpm. After overnight

growth, 1% inoculum was added to 250 ml flask containing 25 ml of T3 medium and incubated at 30ºC at 200 rpm for nearly 48-60 h. The bacterial sporulation was monitored through bright-field microscope. When more than 90% of cells lysed, the sporulated broth culture was transferred to 4ºC, half an hour before harvesting. The T3 broth containing spore crystal mixture was centrifuged at 10000 rpm 10 min 4ºC. The pellet was resuspended in 25 ml of ice-cold Tris EDTA buffer (Tris 10 mM, EDTA 1 mM, pH 8.0 with 1 mM phenyl methyl sulphonyl fluoride (PMSF) and washed once with 25 ml of ice-cold 0.5 M NaCl centrifuged for 10 min followed by two washes with 25 ml Tris EDTA buffer with 0.5 mM PMSF at the same speed and time. Finally, go for empty wash 5 minutes 10000 rpm at 4°C. Dissolve the final pellet in 500 µl of water containing 10 µl of 100 mM PMSF and homogenize the pellet. Make 100 µl aliquots in the 1.5 ml eppendorff tube and stored at -20ºC [14].

2.4 Protein Profiling by SDS PAGE (Sodium Dodecyl Sulfate–Polya-Crylamide Gel)

Aliquots of spore crystal mixture of the isolates *B. thuringiensis* cultures were analyzed by (SDS-PAGE) using 12% running and 5% stacking gels. The molecular mass of proteins was determined by using a specific protein marker. After the electrophoresis, proteins of different molecular weights were detected by staining with Coomassie Brilliant Blue stain [15].

2.5 Rearing of *Putella xylostella*

Pupae of diamondback moth, *P. xylostella* were collected from cauliflower plants cultivated at Thondamuthur, Coimbatore district Tamil nadu on october 2019. Pupae were sorted out carefully using a forceps and transferred to petridish. Collected samples were kept in 30cm x 30cm x 25cm net cages and provided with absorbent cotton swab soaked in 10 per cent honey solution for feeding, and mustered seedlings for egg laying. Fresh cauliflower leaves were provided to the newly hatched larvae to feed up on. The observations on hatching, larval development, formation of pupae and successful emergence of adults and fecundity of females were recorded periodically conducted at weekly interval. Insect colonies were maintained at 25 ± 1°C and at 65 ± 5 per cent relative humidity (RH) and 16:8 h (L:D) photoperiod in the growth chamber [16].

2.6 Preliminary Bioassay for Insecticidal Activity

Selective bioassays were conducted by leaf dip assay method as described by Tabashnik et al. [17]. Spore crystal mixtures were prepared from *B. thuringiensis* isolates harboring *cry1* and *cry2* proteins that are known to be toxic against lepidopteran insects [18]. Assays were carried out using 10 larvae per dose with five replications. Young and fresh cauliflower leaves of same size were selected and dipped for 5 min in spore crystal mixture. Air dried for 30 min in a sterile environment. Leaves were then placed in a Petri dish with 10 third instar DBM larvae. Five repetitions maintained at 25 ± 1°C, 65 ± 5 per cent RH and at 16:8 h (L:D) photoperiod. Observation the larvae mortality was done at 24 h interval for three days and numbers recorded. After 48 h the rest of the surviving larvae were transferred to a new leaf and the mortality was assessed [19]. The dead larvae are cannot be induced to move, when they are probed with a needle in the siphon or the cervical region. Percentage mortality of each treatment was calculated by using formula reported by Senthil et al. [20].

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \text{X } 100$$

2.7 Quantitative Bioassay

The virulence of the isolates, screened and selected from preliminary toxicity tests, was further studied by dose based assay. Six different concentrations of crude crystal protein (100, 250, 500, 750, 1000 and 2000 ppm) of each isolates, including the reference strain HD1 were prepared by dissolving in sterile distilled water. Fresh cauliflower one leaf discs of 7.5 cm diameter were dipped separately in solutions of different concentrations of crystal protein and air dried and fed to the starved (6 h prior to treatment) third instar larvae of *P. xylostella*. The larvae fed with leaf discs dipped in sterile distilled water served as control. For each isolate, separate plastic containers were used and ten larvae were placed per container. Five replications were maintained for each isolate. The observations on larval mortality were recorded at an interval of 24 hrs for three days. Mortality data was subjected to probit analysis and lethal concentration LC_{50} was determined as described by as described by Finney [21].

2.8 Assessment of Larval Mortality

The larval mortality was assessed [22] at intervals of 24, 48 and 72 hours. Any larva that did not show signs of life after prodding with a needle was counted as dead.

2.9 Leaf Damage Assessment

Leaf damage assessment was conducted 3 days after the placement larvae on the leaves. Ten larvae were introduced for each treatment on fresh cauliflower leaves. They feed on the surface and the upper epidermis fully scraped by the larvae was creating a window like effects. Neves et al. [23]. The number of windows per leaf was analyzed and weight of the leaf were recorded. Leaves and larvae were weighed individually for each treatment and following the 3-days test and percentage of weight loss were calculated.

2.10 Statistical Analysis

The data in each table were the mean of three replicates represented along with standard deviation. Significant differences between the treatments were analyzed using factorial analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level of significance. In bioassay the mortality data were transformed to probits, while different dosages were used to estimated LD50 values. Relative susceptibilities of third instar larvae were compared using LD50 values and slopes of probit lines.

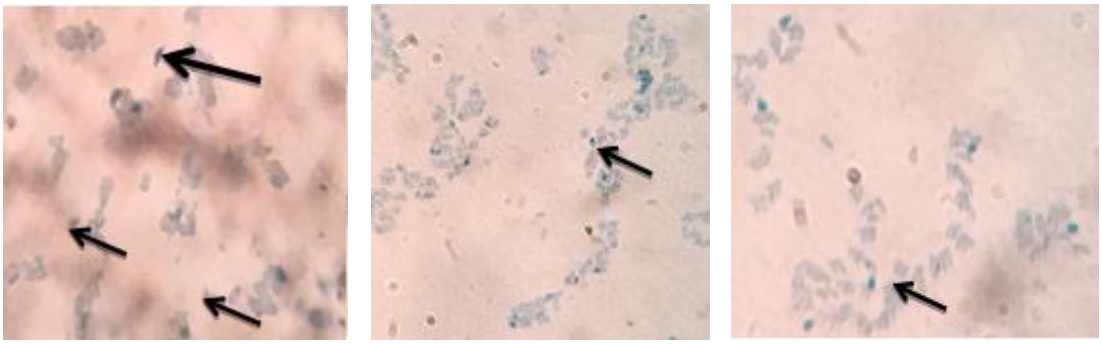
3. RESULTS AND DISCUSSION

3.1 Crystal Morphology of the Isolates

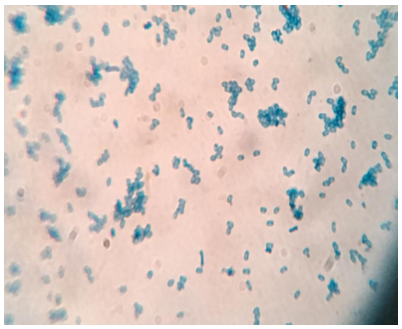
All the 15 *B. thuringiensis* isolates (CA, BP, CB, CC, CB1, TA, TD, BA, BD, BC, KBA, KBB, KBC, TB, TC), were confirmed based on crystal morphology through staining and microscope observation. The shape of the crystalline inclusions varied among the selected *B. thuringiensis* isolates. Strains like CB, BP, TA, TB produced bipyramidal structure, and CC, CB1, BC, TD, BD cuboidal, KBB, CA, KBA showed spherical and KBC, TB, BA produces spores attached with crystals (Fig. 1). Among four types of crystals, many of the isolates produce cuboidal shape followed by bipyramidal crystal and spherical shape. Lowest frequency was observed in cuboidal crystal. Generally initial identification of *B. thuringiensis* was mostly based on the production of crystalline inclusions. Our results are similar to the finding of Geeta et al. [24] reported the selected *B. thuringiensis* isolates from the soil having different crystal shapes, in 44 isolates, 25 had spherical shaped crystals, 13 with irregular type, two with cuboidal type, two with bipyramidal type, one with rectangular type and another one with both cuboidal and bipyramidal shaped crystals. Similarly [25] observed the crystal protein shape and number was varied for each strains isolated from soil and all the isolates showed 3 different crystal proteins bipyramidal, cuboidal, round coleopteran specific crystals.

3.2 Screening of Lepidopteron Specific Cry Gene

The total DNA of reference strains and selected *B. thuringiensis* isolate has been screened for genes that encode the protein against lepidopteran pest. PCR method used to detect the insecticidal genes of the selected isolates. In which lepidopteran active cry genes cry 1, cry 2 were produced by the isolates with expected size at around 277 and 703 bp with specific primers were selected. Among the 15 *B. thuringiensis* isolates 7 (HD1, CB, CC, BP CA, TA, TB, CB1) of them were had *cry1* gene and 7 isolates (HD1, CB, CC, BP, CA, CB1, TB, BD) were amplified for cry 2 gene. Hence, 4 *B. thuringiensis* isolates (CB, CC, CB1, BP) were existing of both *cry1* and *cry2* genes (Fig. 2). The results are concordance with Rashini et al. [26] revealed 21 *B. thuringiensis strains* amplified the characteristic of cry1, cry2, cry4, cry8, cry9, cry10, cry11, vip3A, cyt1 and cyt 2 genes. However dipteran-specific cry10 showed most abundant (76%) of insecticidal gene. Whereas the lepidopteran-specific genes, cry1, cry2 and cry 9 genes were present in 52%, 28% and 71% of the tested strains whereas vip3A gene was present in 42% of the strains. The dipteran-specific cry4/cry11, cyt1 and cyt2 genes showed the frequency of 19%, 23% and 4%, respectively. Of 19% strains concealed the coleopteran active cry 8 gene. It was followed by Showkat et al. [27] reported strains collected from the northern Himalaya had different combinations of cry genes. Among which cry 1 were the most abundant (57.1%) followed by cyt 2 (46.42%), cry 11 (37.5%), cry 2 (28.57%), cry 4 (21.42%), cyt 1 (19.64%), cry 3 (8.9%) and cry 7, 8 (7.14%). Reference strain Bt- HD1 was amplified for three genes viz., cry1, cry 2Aa, cry2Ab.

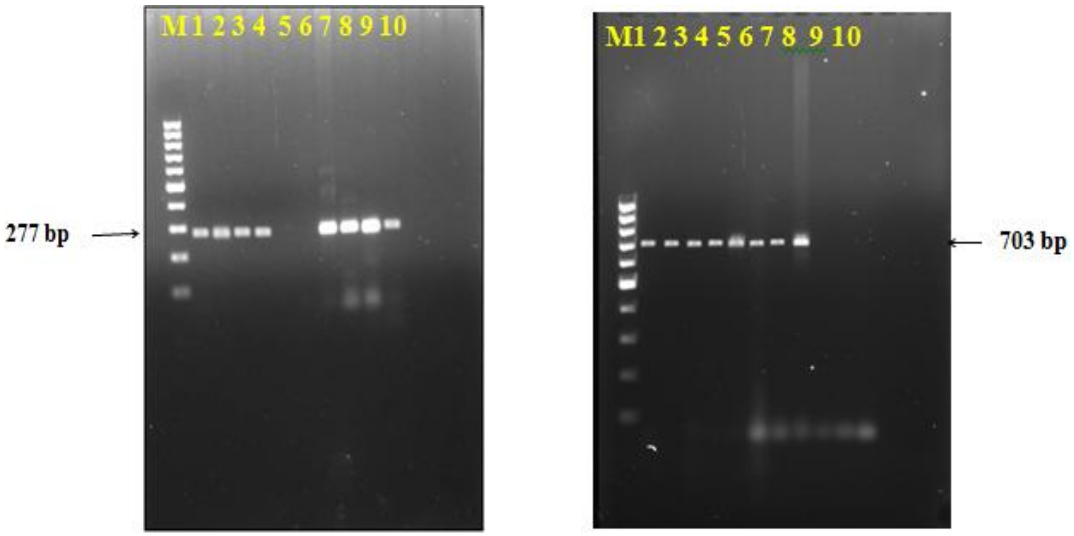


Bipyramidal **Cuboidal** **Spherical**



Spores attached with crystals

Fig. 1. Crystal morphology of the endophytic *Bt* isolates



***B. thuringiensis* strains with the cry1-specific primers**
M - Marker (100 bp); 1-HD1, 2-CB, 3-CC, 4-BP 5-CA, 6-TA, 7- TB, 8- CB1

***B. thuringiensis* strains with the cry 2 - specific primers**
M - Marker (100 bp); 1-HD1; 2- CB, 3-CC, 4- BP, 5-CA, 6- CB1, 7-TB, 8- BD

Fig. 2. Agarose gel (0.8%) electrophoresis of PCR products of cry 1 and cry 2 gene of the *B. thuringiensis* strains

3.3 Cry Protein Estimation by SDS Page

Crystal protein profile of the selected isolates was analyzed by SDS-PAGE. It was evaluated from the extracted spore crystal mixture of the isolates. The molecular weight of the sample revealed that the type of *cry* genes present in the isolates. Among the fifteen isolates (CA, BP, CB, CC, CB1, TA, TD, BA, BD, BC, KBA, KBB, KBC, TB, TC), four (CB, CC, CB1, BP) isolates found to be produce two major polypeptide bands with molecular weights of 135 kDa and 65 kDa (Fig. 3), with reference strain HD1. The prominent bands *viz.,* 65kDa, and 130kDa suggest the presence of cry 1*,* cry *2* gene respectively, which are active against lepidopteran insects mainly, *Plutella xylostella*. Our findings are comparable with results of Mahadeva et al. [28] reported the protein profile of the local isolates while crystal protein production of all the isolates were comparable to each other. All the cry protein profiles of each isolates were identical and showed 2 bands about 130 and 65 kDa, revealed that cry *1* and *cry2* were expressed in all isolates. [29] indicated that selected two isolates BUPM95 and BUPM95 were produced protein band with molecular weight 65 and 130 of lepidopteran active *cry* proteins. Similarly Reyaz et al. [30] investigated the spore–crystal mixtures by SDS-PAGE, and reported that *B. thuringiensis* strains contained proteins of various molecular weights, *viz.,* 150, 135, 130, 105, 98, 95, 91, 90, 70, 65, 45, 42, 34 and 28 kDa.

3.4 Bioassay for Insecticidal Activity *B. thuringiensis* against DBM

Protein profiling of the isolates conferred that our *B. thuringiensis* had different Cry protein which could be toxic to lepidopteran insects. In present study concentrated spore-crystal suspensions were used for initial toxicity screening. A different level of toxicity was observed among the isolates with mortality ranging from 0% to 98%. At 24 h, the mean percent mortality ranged between 0 to 44%. After 48 h exposure ranged between 44 to 69.6% and 72 h 95% mortality was observed. In bioassay all four isolates (HD1, CC, CB, BP, CB1) tested, HD1 showed highest mortality 98.43 followed by CC 95.33 and isolates BP, CB1 showed 86.90, 84.67 against the *P. xylostella* larvae (Fig. 4). Hence preliminary screening revealed levels of toxicity ranging from 40% to 95% mortality of DBM larvae. Similarly Katiane et al. [31] Stated among the 300 *Bacillus thuringiensis* isolates tested only 12 *Bacillus thuringiensis* isolates having toxicity against the *Aedes aegypti* larvae, they reported isolates BtMA 104, BtMA 401, BtMA 560 were showed 100% mortality in 24 hrs. Whereas 90% mortality was observed in isolates BtMA 251, BtMA 410, BtMA 413, BtMA 450 after 48hrs. While BtMA 25, BtMA 451 showed 80% mortality and isolates BtMA 64, BtMA 131 reported 76.6% mortality and BtMA 194 causes 66.6% mortality. Similarly Praca et al. [32] tested three strains S1905, S2122 and S2124 against *Putella xylostella* by selective bioassay with cabbage leaf, among which 2 isolates S1905 and S2122 caused 100% of mortality after 48 hrs, while S2124 caused of 98.33% mortality after five days interval. Our findings are concordance with Kahrizeh et al. [33] screened the toxicity of the nine isolates against *P. xylostella* in cabbage leaf. Among which three of *B. thuringiensis* (Bt-IE, Bt-IP, Bt-U3a) induced mortality above 95% against second instar larvae of *Plutella xylostella*.

3.5 Quantitative Bioassay

In dose based mortality response, different concentration (100, 250, 500, 750, 1000 and 2000 ppm) of spore crystal mixture were prepared and (LC50) were calculated. The crude protein of selected *B. thuringiensis* isolates showed differences in their insecticidal activity against third instar larvae of *Putella xylostella* larvae. Mortality was assessed and LC_{50} was calculated by probit analysis (Table 1 and Fig. 5). Among isolates HD1 showed the least LC50 value of 106.82 ppm with fiducial limits ranging from 198.74 to 369.75 ppm followed by the isolates CC, CB and CB1with LC50 values of 197.08, 211.31 and 230.54 ppm respectively. The isolate BP exhibited the maximum LC50 value of 337.76 ppm with fiducial limits ranging from 205.49 to 498.16 ppm. The findings are comparable with Yılmaz et al. [34] mentioned LC_{50} values of the spore crystal mixture of SY49.1 for *Ephestia kuehniella* (Mediterranean flour moth, Pyralidae)*, Plodia interpunctella* (Indian meal moth, Pyralidae) and *Tribolium castaneum* (Red flour beetle Tenebrionidae family) were 195.47, 147.33 and 7597.62 µg g^{-1} respectively. Similarly Ebrahimi and Ahad [35] reported the toxicity level of *B. thuringiensis* (var. *kurstaki*, serotype H-3a3b, strain Z-52) was used against DBM larvae and estimated LC_{50} value for protein concentration was 210 ppm. However, more than 80 per cent mortality was recorded at 450 ppm concentration. Mortality varied with different concentration of bacterial isolate and also time of exposure. The wide range of

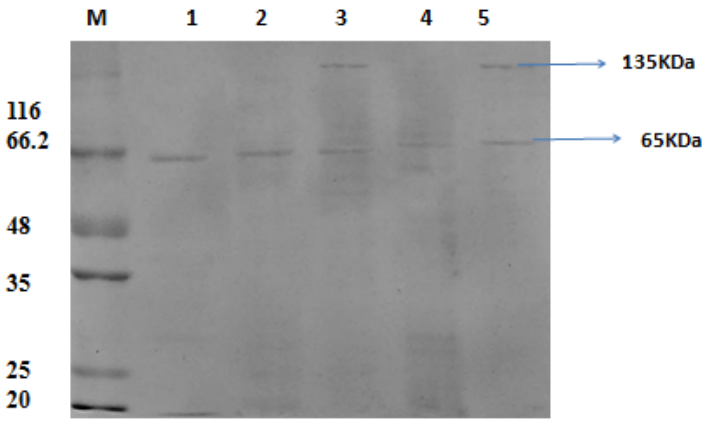


Fig. 3. SDS PAGE analysis of spore–crystal from *B. thuringiensis* isolates and reference strain
M - Marker; 1-CB; 2 - CB1; 3 - CC; 4 - BP; 5 - Reference strain HD1

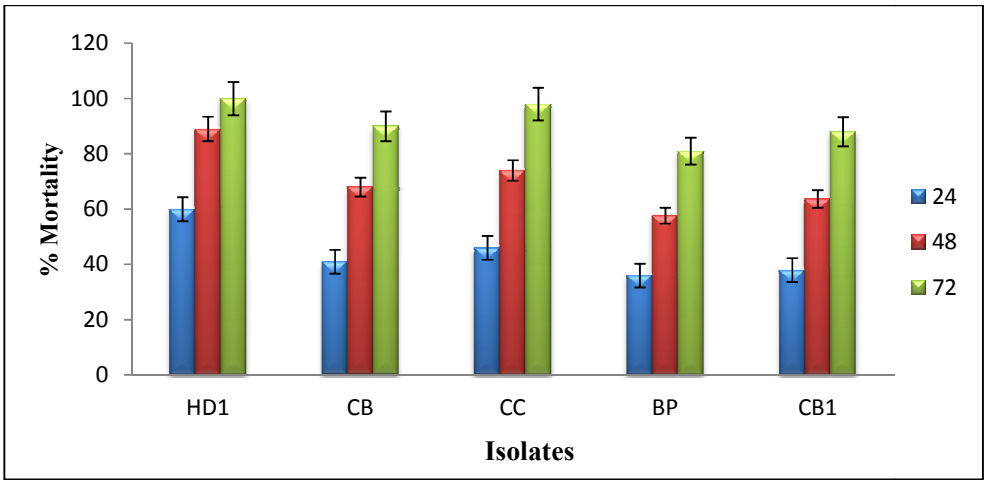


Fig. 4. Bioefficacy of *Bacillus thuringiensis* isolates against third instar larvae of Diamond back moth

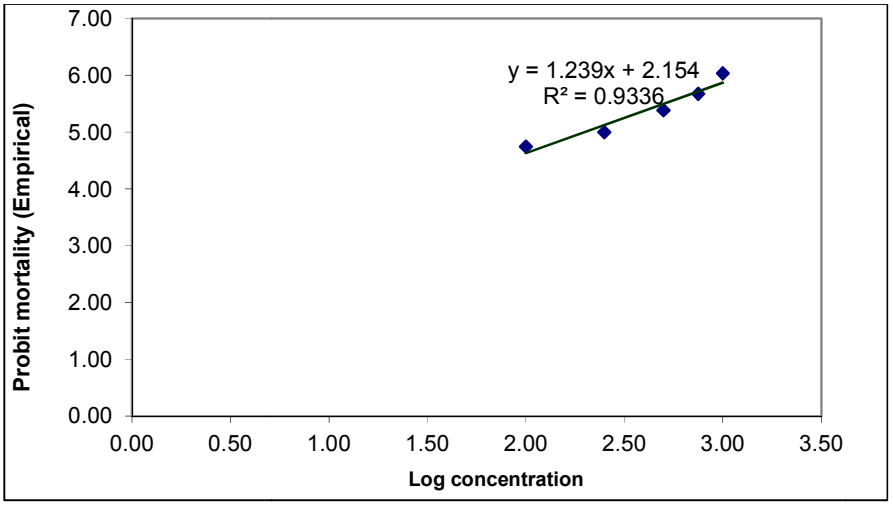


Fig. 5. Probit analysis for mortality of DBM larvae after application *B. thuringiensis* at 72 hours

Table 1. Lethal concentration (LC_{50}) of *B. thuringiensis* isolates against 3rd instar larvae of DBM (*Putella xylostella*)

Strain tested	Slope ± SE	LC_{50}(ppm)	95% Fiducial limits (ppm)	Chi-square
HD1	1.23±0.07	106.82	198.74 -369.75	2.67
CB	1.46±0.06	211.31	109.86- 406.45	1.58
CC	1.52±0.05	197.08	110.28-352.21	2.28
CB1	1.41±0.08	230.54	126.67- 419.59	1.72
BP	1.39±0.08	337.76	205.49-498.16	1.58

Table 2. Effect of *B. thuringiensis* concentrations on cauliflower leaf damage

Concentration of the toxin (ppm)	RGR of the larva	Leaf loss (%)
100	58 (±0.01) [a]	54 (±0.01) [a]
250	47 (±0.03) [b]	43 (±0.01) [b]
500	41 (±0.01) [c]	21 (±0.01) [c]
750	35 (±0.04) [d]	15 (±0.01) [d]
1000	30 (±0.04) [e]	11 (±0.01) [e]
2000	25 (±0.02) [f]	8 (±0.01) [f]
S.Ed	8.9355	0.8619
CD (0.05)	1.9468**	1.8780**

*Means within each columns followed by the same letters are not significantly different at (p=0.05) and the numbers are mean of three replications. *Significant ** Highly Significant*

variation showed the effectiveness of *B. thuringiensis* isolates against target insects [36].

Fig. 6. Effect of *B. thuringiensis* concentrations on cauliflower leaf damage and larval growth

Similarly Torres et al. [37] selected 17 *B. thuringiensis* strains identified as highly effective even at low protein concentration (10 ng/µL) which producing mortality rates above 70%. Among 17 effective strains, 12 were isolated from dead insects belonging to Aphididae, 2 were from Psyllidae, 2 were from Coccidae, and 1 from Cercopidae. Toxicity level against the *Myzus persicae* was vary for each strains, which is most likely related to the expression of different proteins. However in another study [38] mentioned the selected *B. thuringiensis* isolates BR58 are the most effective against *Hypothenemus hampei with lowest* LC_{50} value ranged from 0.037×10^9 to 0.956×10^9 spores/mL. Whereas which has no differ significantly with standard IPS-82.

3.6 Leaf Damage Assessment

The increased concentration spore crystal mixture will reduce the damage of leaves. Hence the percentage of damaged leaves was assessed in 3 days at different concentration (100-2000 PPM). The consumption rate decreased with different concentration of *B. thuringiensis* toxin. Initially 54% leaf damage was observed at 100 ppm concentration followed by 43%, 21%, 15%, 11% with the concentration of 250, 500, 750, 1000 ppm. Finally 8% leaf damage occurs at 2000 ppm (Table 2). When the concentration increased the percentage of the leaf damage decreased accordingly (Fig. 6). In case of larval growth, *P. xylostella* were fed the leaf containing spore crystal of *B. thuringiensis* at the concentrations of 100 to 1000 ppm which effects on larval survival. The weight of larvae reduced day by day and larval period was extended when exposed to the *B.thuringiensis* toxin. The weight of larval fed the *B.thuringiensis* treated leaf was 58% after 100 ppm concentration and at concentration of 250, 500, 750, 1000 the weight was 47%, 41% 35% and 30%. Whereas 25% was observed at 2000 ppm concentration. The results of Legwailal et al. [39]

revealed that the effect of different insecticide concentrations on the intensity of cabbage leaf damage per plant. The damage was 14.3%, 12.7% and 3.3% per plant that received 6, 8 and 10 gl^{-1} of *B. thuringiensis kurstaki* as treatment respectively, while damage was 81.3% in untreated plants. Chandrasekaran et al. [40] reported that the damage to cauliflowerplant was less at higher concentrations of crystal toxin.

4. CONCLUSION

Identified and characterized native strains of *B. thuringiensis* from the cultivated field soils of Coimbatore, Tamilnadu, India. Selected isolates are positive for cry 1 and cry 2 gene and they produced different crystal proteins. SDS PAGE represented the lepidopteran specific proteins with molecular weight of 65KDa and 130KDa. Then isolates were evaluated for insecticidal activity against the third instar larvae of *Putella xylostella* by bioassay. Among the all isolates CC and CB had higher insecticidal activities with LC50 of 1.52, thus comparable to the reference strain used in the study. Highest mortality (96%) was observed at 72 hr. Leaf damage was found reduced with increased concentration of 2000 ppm. Finally the selected virulent strains can be used as a biopesticide against lepidoperan insects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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