Pest Management in Horticultural Ecosystems
Vol. 24, No.1 pp 1-7 (2018)

Isolation and characterization of indigenous *Bacillus thuringiensis* Berliner from animal ordure effective against South American tomato pinworm, *Tuta absoluta* (Meyrick)

GOWTHAM. V[1]*, M. KANNAN[2], M. SENTHILKUMAR[3], and R.P. SOUNDARARAJAN[4]

[1, 2, 4]Department of Agricultural Entomology, Tamil Nadu Agricultural University,
Coimbatore, [3]Department of Agricultural Microbiology, Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu, India 641 003
E-mail: gowtham.v.agri@gmail.com

ABSTRACT: Studies were carried out for identification of effective indigenous *Bacillus thuringiensis* (*Bt*) strain from animal ordure. Eight *Bt* isolate were isolated from five different indigenous animal ordure samples samples *viz.,* rice moth faecal pellet, cow dung, poultry, goat, pig wastes and characterized along with reference *Bacillus thuringiensis* strain (HD1). The bacterial culture colonies were creamy white to milky white in colour, circular or irregular in shape with entire or partially serrated margins. *Bt* isolates were rod-shaped, gram positive with endospore and parasporal body produced at sporulation stage. Further the protein profile analysis revealed that all isolates showed major protein bands around 135, 110, 60-75, 48 and 20-30 kDa. These proteins may belong to Cry1 and Cry2 protoxin. *Bt* isolates showed significant differences in their toxicity to the 2nd instar larvae of *Tuta absoluta* wherein mortality ranged from 70 to 100 per cent as compared to 1.67 per cent in control. Among the isolates, KGS2 isolate was considered as best and caused 65.00±2.31, 90.00±3.37 and 100.00±0.00 per cent mortality on 3rd, 5th and 7th day, respectively compared to other isolates.
Keywords: *Tuta absoluta, Bacillus thuringiensis,* isolates, mortality

INTRODUCTION

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is the key and invasive pest of tomato, *Lycopersicon esculentum* Mill. (Barrientos *et al.,* 1998). In India, it was first reported at Bengaluru and Pune on tomato plants grown in polyhouse and field during October 2014 (Sridhar *et al.,* 2014 Chandrashekar *et al*., 2015). In Tamil Nadu infestation was noticed during March, 2015 at Krishnagiri and Dharmapuri districts (Ballal *et al.*, 2016). It can caused 50 to 100 per cent yield loss in both greenhouse and field conditions. *T. absoluta* damage the plants by direct feeding on leaves, stems, buds, calyces, immature fruits and ripe fruits (EPPO, 2005). Currently, to overcome the pest problem, chemical pesticides are being recommended and it is undesirable because of its known ill effects. However, chemical control may become ineffective due to the rapid development of resistance to insecticides (Lietti *et al.,* 2005), let alone the negative impacts on natural enemies, the environment and human health (Desneux *et al.,* 2010). Considering the facts, the interest in biocontrol approaches has much increased. The present study intended for isolation and characterization of indigenous *Bacillus thuringiensis* with higher efficiency on larval mortality of *T. absoluta* under laboratory conditions.

MATERIALS AND METHODS

Isolation of *Bt* strains was carried out from fresh rice moth faecal pellet, cow dung, poultry, goat and pig wastes (Table 1). 1gm of sample was added to 20 ml of Luria broth buffered with 0.25 M sodium acetate. The mixture was shaken for 4 h at 150 rpm at 30°C. Then the sample was then taken and heat treated at 80°C for 10 min. Serial dilutions were made (10^5) and 1ml of suspension was pore plated on T3 medium and incubated at 30°C for 48 hrs. The *Bt* like colonies (creamy white, flat, dry, matte structure, uneven border) were sub-cultured for four side streaking method on T3 medium (Travers *et al.*, 1987) and incubated for 24 h at 30°C. The colonies were examined for morphological characters after 24 hrs. Acetone precipitated spore-crystal mixture of all the *Bt* strains was prepared by following the methodology adopted by Dulmage *et al.* (1970). All the prepared acetone powder was stored in airtight sterile glass vials at 4°C for further use.

Table 1. Survey and collection sample for isolation of indigenous *Bacillus thuringiensis* isolates from animal ordure

Place	Latitude (°N)	Longitude (°E)	Source	Cfu/g of soil	No. of isolates	Isolate name
Mohanur	11.0599	78.1422	Pig dung	11	1	KGS 1
Vedasanthur	10.5315	77.9482	Goat dung	23	2	KGS 2,3
Kalavai	12.7675	79.4169	Rice moth faecal pellet	19	1	KGS 4
AC & RI,TNAU	11.0123	76.9355	Poultry waste	5	1	KGS 5
Tindivanam	12.2369	79.6500	Cow dung	21	3	KGS 6,7,8

Table 2. Morphological Characterization of indigenous *Bacillus thuringiensis* isolates

Isolate name	Colour of the colony	Shape of the colony	Margin of the colony	Elevation of the colony	Gram reaction	Cell shape	Sporulation	Crystal protein
HD1	Creamy white	Irregular	Undulated	Raised	+	Rod	+	+
KGS 1	Milky white	Circular	Serrate	Flat	+	Rod	+	+
KGS 2	Milky white	Circular	Entire	Raised	+	Rod	+	-
KGS 3	Milky white	Circular	Undulated	Raised	+	Rod	+	-
KGS 4	Milky white	Circular	Entire	Raised	+	Rod	+	+
KGS 5	Milky white	Circular	Entire	Raised	+	Rod	+	+
KGS 6	Milky white	Circular	Entire	Raised	+	Rod	+	+
KGS 7	Milky white	Circular	Serrate	Flat	+	Rod	+	+
KGS 8	Milky white	Circular	Entire	Raised	+	Rod	+	+

Table 3. Biochemical characterization of indigenous *Bacillus thurengiensis* isolates

Isolate name	Mal onate	Voges-Proskauer's	Citrate	ONPG	Nitrate Reduction	Catalase	Argi nine	Sucrose	Man nitol	Glucose	Arab inose	Terhalose
HD1	-	+	+	-	+	+	+	-	-	+	-	+
KGS 1	-	+	+	-	+	+	+	-	-	+	-	+
KGS 2	-	+	+	-	+	+	+	-	-	+	-	+
KGS 3	-	+	+	-	+	+	+	-	-	+	-	+
KGS 4	-	+	+	-	+	+	+	+	-	+	-	+
KGS 5	-	+	+	-	+	+	+	-	-	+	-	+
KGS 6	-	+	+	-	+	+	+	-	-	+	-	+
KGS 7	-	+	+	-	+	+	+	-	-	+	-	+
KGS 8	-	+	+	-	+	+	+	-	-	+	-	+

Gram staining

One day old *Bt* like culture was taken and smeared on a clean microscope glass slide. Allowed to air dry and fixed with gentle heat. The slide was flood with crystal violet solution for one minute and briefly washed with tap water. Gently down poured the smear with Gram's Iodine solution allowed for one minute. The slide was decolorized with alcohol until the blue dye no longer flows from the smear and briefly washed with tap water. Counter stained with safranin for one minute and briefly washed with tab water. Allowed the slide to air dry and examined under oil immersion phase contrast microscope (Muniady *et al.,* 2011).

Spore and crystal staining with Coomassie brilliant blue G 250

Coomassie brilliant blue G 250 staining was done at two phases, one in vegetative phase (after 24 hrs) and another one in sporulation phase (after 48 hrs). *Bt* like culture was taken and smeared on a clean microscope glass slide. The slide was allowed to air dry and fixed by gentle heat. The slide was flooded with CBB stain (0.133 per cent Coomassie brilliant blue stain in 50 per cent acetic acid) for one minute; then washed with tap water safely and dried it. After drying, the slides were viewed under a phase contrast microscope with oil immersion (Rampersad *et al.,* 2002).

Biochemical characterization of *Bt* isolates

Biochemical characterization was done based on sugar fermentation pattern in Brain Heart infusion Broth as per method provided by RAPID HiBacillus™ Identification Kit KB013 and KB009 HiCarbohydrate™ Kit (Hi-Media, India). The tests were based on the principle of pH change, substrate utilization and other biochemical reactions exhibiting colour change. Twelve different tests including malonate, Voges Proskauer's, citrate, o-nitro phenyl ß-galactose (ONPG), nitrate reduction, catalase, arginine, sucrose, mannitol, glucose, arabinose and trehalose were carried out as per manufactures instruction (Gorashi *et al.,* 2014).

Isolation of spore-crystal toxins and cry protein solubilization from *Bt* isolates

Spore-crystal toxins were isolated from each *Bt* isolates. A single colony was inoculated in 20 ml T3 broth and incubated in 30° C for 72 hrs at 200 rpm and the cell lysis was monitored under phase contrast microscope. When more than 90 per cent of cells had lysed, the sporulated broth culture was transferred to 4°C, at least half-an-hour before harvesting. The T3 broth containing spore-crystal mixture was centrifuged for 10 min at10,000 rpm at 4°C. The pellet was washed once with 20 ml of ice-cold Tris–EDTA buffer [Tris 10 mM, EDTA 1 mM, pH 8.0 with 1 mM phenyl methyl sulphonyl fluoride (PMSF)], once with 20 ml of ice-cold 0.5 M NaCl followed by two more washes with 20 ml of Tris–EDTA buffer with 0.5 mM PMSF by centrifuging at the same speed and time (Ramalakshmi *et al.,* 2010). Solubilize the final pellet in solubilizing buffer (50 mM Na_2CO_3, pH 10.5 mM dithiothretol) at 37°C for 4 hrs with shaking and then centrifuge the content at 10,000 rpm, 4°C for 15 min. The supernatant containing solubilized protoxin was removed and store at -20° C for future use. Aliquots of spore-crystal mixture of *Bt* isolates were analyzed by SDS-PAGE for identification of cry protein profile.

Cry protein electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method given by Laemmli (1970) using 10 per cent separating and 5 per cent stacking gels. The gels were stained with 0.1 g Coomassie brilliant blue R-250 was dissolved in 100ml of methanol-acetic acid-water (40-10-50 ratio). The molecular mass of proteins was determined using a higher range protein molecular weight marker obtained from BioLit™, Chennai, India.

Bioassay method

Leaf-dip bioassay method was adopted to evaluate the efficacy of *Bt* isolates against *Tuta absoluta* (Tabashnik *et al*., 1991). The tomato leaves were first washed with distilled water containing 0.1 per cent Triton X-100 thoroughly and air-dried. Take the leaflet and dipped in *Bt* toxin of different isolates. Each leaflet was dipped for 5-10 seconds and allowed to air-dry for a period of one hour. After complete evaporation, the leaves were transferred to clean bioassay containers over a moistened filter paper. The leaflets were placed slantingly to rest on side of the container so that larvae can move on either side. 2rd instar larvae were released in each dish and three replicates were maintained per treatment. A treatment without *Bt* protein served as control. Larval mortality was recorded every 24 h, consecutively for seven days. All the experiments were carried out in laboratory with a photoperiod of 12:12 (L: D) and experiments with control mortality more than 20 per cent were discarded and repeated. The data obtained from the lab experiments were arcsine transformed and subjected to ANOVA analysis.

RESULTS AND DISCUSSION

Totally eight *B. thuringiensis* strains, from five

Table 4. Carbohydrate utilization by different indigenous *Bacillus thuringiensis* isolates

Isolate	Lac tose	Xylose	Mal tose	Fru ctose	Dex trose	Galac tose	Raffi nose	Treh alose	Melib\ iose	Sucrose	L-Arabinose	Mannose
HD1	-	-	+	+	+	-	-	+	-	-	-	-
KGS 1	-	-	+	+	+	-	-	+	-	-	-	-
KGS 2	-	-	+	+	+	-	-	+	-	-	-	-
KGS 3	-	-	+	+	+	-	-	+	-	-	-	-
KGS 4	-	-	+	+	+	-	-	+	-	+	-	-
KGS 5	-	-	+	+	+	-	-	+	-	-	-	-
KGS 6	-	-	+	+	+	-	-	+	-	-	-	-
KGS 7	-	-	+	+	+	-	-	+	-	-	-	-
KGS 8	-	-	+	+	+	-	-	+	-	-	-	-

Table 5. Characterization of size and shape of *Bt* crystal protein

Isolate	Protein size (kDa)	Crystal Shape
HD-1	135, 110, 80, 65, 45, 37, 20, 15	Bipyramidal
KGS 1	80, 76, 66, 48, 32	Spherical
KGS 2	116, 88, 65, 50, 45, 35, 22, 15	Bipyramidal
KGS 3	100, 88, 65, 50, 45, 40, 22, 15	Bipyramidal
KGS 4	110, 70, 53, 35, 25	Bipyramidal
KGS 5	75, 40	Bipyramidal
KGS 6	82, 48	Bipyramidal
KGS 7	88, 80, 64, 48, 45, 35, 25, 18	Bipyramidal
KGS 8	75, 53, 45	Spherical

Table 6. Entomopathogenic potential of *Bt* isolates from animal ordure against *T. absoluta*

	Larval mortality (%)		
Isolate	**3rd day**	**5th day**	**7th day**
HD-1	15.00±0.51 (22.78)^{e}	50.00±1.76 (45.00)^{d}	95±2.52 (79.24)^{b}
KGS 1	10.00±0.20 (18.43)^{f}	25.00±1.16 (29.99)^{f}	75±2.03 (60.04)^{d}
KGS 2	65.00±2.31 (53.75)^{a}	90.00±3.37 (72.04)^{a}	100±0.00 (89.48)^{a}
KGS 3	50.00±1.70 (45.00)^{c}	85.00±2.57 (67.38)^{a}	100±0.00 (89.48)^{a}

Isolate	Larval mortality (%)		
	3rd day	5th day	7th day
HD-1	15.00±0.51 (22.78)^{e}	50.00±1.76 (45.00)^{d}	95±2.52 (79.24)^{b}
KGS 4	35.00±0.24 (36.27)^{d}	60.00±2.82 (50.79)^{c}	75±1.12 (60.01)^{d}
KGS 5	55.00±2.09 (47.88)^{b}	75.00±3.80 (60.12)^{b}	100±0.00 (89.48)^{a}
KGS 6	15.00±0.39 (22.78)^{e}	45.00±0.87 (42.13)^{d}	90±1.99 (71.73)^{c}
KGS 7	10.00±0.11 (18.44)^{f}	30.00±1.54 (33.20)^{f}	70±0.20 (56.79)^{d}
KGS 8	5.00±0.14 (0.52)^{g}	40.00±0.10 (39.23)^{e}	100±0.00 (89.48)^{a}
Control	0.00±0.00 (0.52)^{h}	0.00±0.00 (0.52)^{g}	1.67±1.67 (4.66)^{e}
SE d	0.9669	2.2974	3.1397
CD (0.05)	2.0170	4.7923	6.5493

Means followed by the same letter in column do not differ statistically by Least Significant Difference (LSD) test ($P \leq 0.05$).

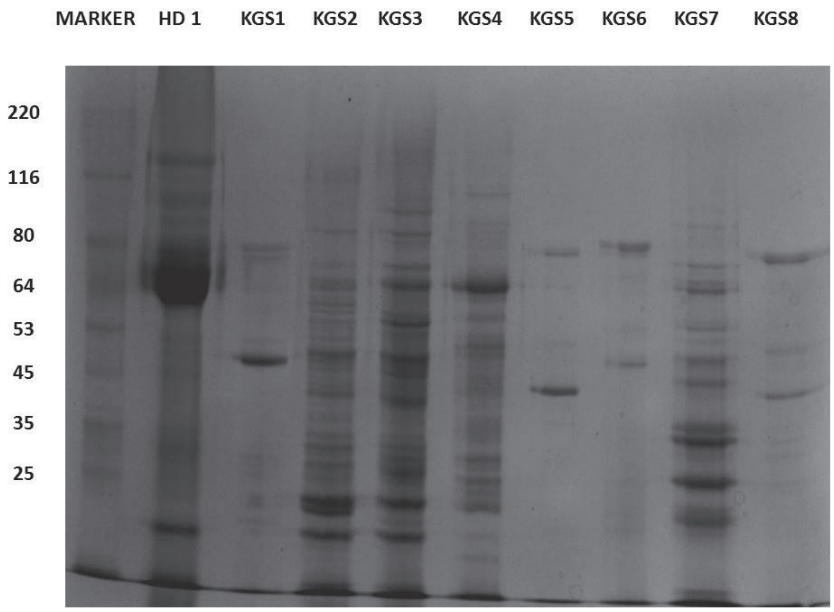

Plate 1. Crystal protein profiles of *Bacillus thuringiensis* isolates

different indigenous samples (rice moth faecal pellet, cow dung, poultry, goat and pig wastes) were isolated and sub cultured in T3 agar medium plate.

Morphologically, all the eight isolates were creamy white to milky white colonies with circular or irregular in shape with entire or serrate or undulated margins and most of cultures showed raised appearance in the T3 agar medium plate (Table 2). Further morphological characterization was done though gram staining and Coomassie brilliant blue staining. Gram staining revealed that all the nine isolates were rod-shaped, gram positive bacteria, which were appeared to be blue or violet in colour under the light microscope. Also the Coomassie brilliant blue staining confirmed the presence of endospore and parasporal body in the cells of eight isolates. The shape of parasporal crystals of isolates reference strain HD-1, KGS2, KGS3, KGS4, KGS5, KGS6 and KGS7 was bipyramidal whereas it was spherical in isolates KGS1 and KGS8 (Table 5). The results of the study are in agreement with the results of Yilmaz *et al.*(2017) who identified 88 *Bt* isolates from 120 samples by the presence of bipyramidal, cubic, spherical, rhomboidal, irregular shaped spherical parasporal inclusions. Chatterjee *et al.*(2017) also found similar variation in the morphological characteristics of *Bt* isolates on nutrient agar medium and reported circular, white, flat, and undulate colonies. Further our findings were similar to the findings of Asokan (2007), Patel *et al.* (2011), Lenina *et al.* (2014).

Microorganisms are extremely specific in their biochemical characteristics which provide tool for selective identification of the organism. Eight indigenous *Bacillus* isolates and one reference strain *Btk* HD-1 were screened for biochemical test using KB013 HiBacillus identification kit and KB009 HiCarbohydrate™ Kit (Tables 3 and 4). The results indicated that all strains were showed similar positive reaction to nitrate reduction by converting nitrate to nitrite. Catalase test was positive for all strains by generating oxygen when treated with H_2O_2. Regarding utilization of citrate as carbon source all the strains effectively utilized citrate and showed good growth. All isolates showed similar positive reaction to aregnine, voges-proskauers, glucose, maltose, fructose, dextrose and trehalose when compared with reference strain HD1. All the nine strains showed negative reaction with ONPG, malonate, sucrose, lactose, xylose, mannitol, arabinose, galactose, raffinose, melibiose, L-arabinose and mannose. However, KGS4 alone showed positive response to sucrose utilization. The results of the present study are in harmony with the findings of Rajashekhar *et al.* (2017), who reported that *Bt* isolates (VKK-AC1and VKK-SL1) were positive to catalase, nitrate, citrate, glucose, argenine and negative reaction with Voges-proskauers. Tripathi *et al.* (2016) concluded that *Bt* strains collected from high altitude mountains, forests, horticultural plantations also showed same positive reaction to above biochemical testings. The findings of Gorashi *et al.*(2014) also supported the results of the present study that all *Bt* isolates showed positive reaction for catalase and nitrate reduction; and negative test for malonate.

Protein profile of *B. thuringiensis* isolates were studied using Sodium dedocyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Plate 1). The results of the protein profile analysis revealed that all isolates showed major protein bands around 135, 110, 60-75, 48 and 20-30 kDa. (Table 5). Which may belong to Cry1 and Cry2 protoxin and their activation products. The protein of 15 kDa may correspond to the cytolytic (Cyt) toxin. Cry1 class proteins have a molecular weight of approximately 130-140 kDa, whereas proteins of the Cry2 group, which are active against Lepidoptera and Coleoptera, have values of 65-70 kDa. The results are in concurrence with the findings of Gorashi *et al.*(2014) and Rajashekhar *et al.* (2017).

The toxicity potential of crude crystal proteins of different *Bt* isolates from animal ordure against *T. absoluta* was evaluated through bioassays (Table 6). The results revealed that the *Bt* isolates showed significant differences in their toxicity to the 2nd instar larvae of *T. absoluta* wherein mortality ranged from 70 to 100 per cent as compared to 1.67 per cent in control. The mortality of *T. absoluta* gradually increased in all the *Bt* isolates. Among the *Bt* isolates KGS8 caused least mortality of 10.00±0.11, 30.00±1.54 and 70±0.20 per cent on 3rd, 5th and 7th day respectively. Whereas, KGS 2 (65.00±2.31, 90.00±3.37 and 100±0.00 on 3rd, 5th and 7th day, respectively), KGS 3 (50.00±1.70, 85.00±2.57 and 100±0.00 on 3rd, 5th and 7th day, respectively), KGS 5 (55.00±2.09, 75.00±3.80 and 100±0.00 on 3rd, 5th and 7th day, respectively) and KGS 8 (5.00±0.14, 40.00±0.10 and 100±0.00 on 3rd, 5th and 7th day, respectively) showed 100 per cent mortality. Based on the pattern of mortality, KGS2 considered as best isolate and caused 65.00±2.31 per cent mortality on 3rd day itself compared to than other isolates. The results of the present study are in accordance with the findings of Gorashi *et al.* (2014). They confirmed the variation in toxicity of indigenous *Bt* isolates against the larvae of *H. armigera* wherein mortality ranged from 6.6 per cent (St-5) to 70 per cent (Wh-1) as compared to 0 per cent in control on the 4th day of treatment. Further, Rajashekhar *et al.* (2017) found that the new isolate VKK-AC1 was significantly different and effective than reference strain Btk HD-1 and Bs VKK-SL1 in both solubilized as well as trypsinized form and against *H. armigera* larvae with 70 per cent mortality.

REFERENCES

Asokan, R. 2007. Isolation and characterization of *Bacillus thuringiensis* Berliner from soil, leaf, seed dust and insect cadaver. *Journal of Biological Control,* **21**(1): 83-90.

Ballal, C. R., Gupta, A., Mohan, M., Lalitha, Y. and Verghese. A. 2016. The new invasive pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India and its natural enemies along with evaluation of Trichogrammatids for its biological control. *Current Science*, **110** (11): 2155-2159.

Barrientos, Z.R., Apablaza, H.J., Norero, S.A. and Estay, P.P. 1998. Threshold temperature and thermal constant for development of the South American tomato moth, *Tuta absoluta* (Lepidoptera, Gelechiidae), *Cienciae Investigacion Agraria,* **25**: 133-137

Chandrashekar, K., Shashank, P. R., Naresh, M. and Sreedevi, K. 2015. Occurrence of *Tuta absoluta* (Lepidoptera: Gelechiidae): An invasive pest from India. *Indian Journal of Entomology*, **77**(4): 323 – 329.

Chatterjee, S., Bhattacharya, T., Dangar, T. K. and Chandra, G. 2007. Ecology and diversity of *Bacillus thuringiensis* in soil environment. *African Journal of Biotechnology,* **6**(13).

Desneux, N., Wajnberg, E., Wyckhuys, K. A., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A. and Frandon, J. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of pest science,* **83**(3): 197-215.

Dulmage, H. T., Correa, J. A. and Martinex, A. J. 1970 Co-precipitation with lactose as a means of recovering the spore-crystal complex of *Bacillus thuringiensis*. *Journal of Invertebrate Pathology,* **15**: 15-20.

EPPO. 2005. EPPO datasheets on quarantine pests: *Tuta absoluta*. *PPO Bulletin* 35:434-435. Accessed December 18, 2009.

Gorashi, N., Tripathi, M., Kalia, V. and Gujar, G. 2014. Identification and characterization of the Sudanese *Bacillus thuringiensis* and related bacterial strains for their efficacy against Helicoverpa armigera and Tribolium castaneum. *Indian journal of experimental biology,* **52:** 637-649.

Laemmli, U. K. 1970. Cleavage of structural proteins during theassembly of the head of Bacteriophage T4. *Nature,* **227**: 680–685.

Lenina, N., Naveenkumar, A., Sozhavendan, A., Balakrishnan, N., Balasubramani, V. and Udayasuriyan, V. 2014. Characterization of parasporin gene harboring Indian isolates of *Bacillus thuringiensis*. *3 Biotech,* **4**(5): 545-551.

Lietti, M.M.M., Botto, E. and. Alzogaray, R. A. 2005. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop. Entomol.*, **34**: 113–119.

Muniady, S., Rathinam, X. and Subramaniam, S. 2011. Quick isolation and characterization for the confirmation of a novel *Bacillus thuringiensis* strains from chicken manure samples. *African Journal of Microbiology Research,* **5**(20): 3131-3137.

Patel, K. D., Chudasama, C. J. and Ingle, S. S. 2012. Molecular characterization of *Bacillus thuringiensis* isolated from diverse habitats of India. *Journal of Basic Microbiology,* **52**(4): 437-445.

Rajashekhar, M., Shahanaz. And Kalia, V. K. 2017. Biochemical and molecular characterization of Bacillus spp. isolated from insects. *Journal of Entomology and Zoology Studies,* **5**(5): 581-588.

Ramalakshmi, A. and Udayasuriyan, V. 2010. Diversity of *Bacillus thuringiensis* isolated from western ghats of Tamil Nadu state, India. *Current microbiology,* **61**(1): 13-18.

Rampersad, J., Khan, A. and Ammons, D. 2002. Usefulness of staining parasporal bodies when screening for *Bacillus thuringiensis*. *Journal of invertebrate pathology,* **79**(3): 203-204.

Sridhar, V., Chakravarthy, A. K., Asokan, R., Vinesh, L. S., Rebijith, K. B. and Vennila, S. 2014. New record of the invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India. *Pest Management in Horticultural Ecosystems,* **20**: 148-154.

Tabashnik, B. E., Finson, N. and Johnson, M. W. 1991. Managing resistance to *Bacillus thuringiensis*: Lessons from the diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.*, **84**: 49-55.

Travers, R. S., Martin, P. A. and Reichelderfer, C. F. 1987. Selective process for efficient isolation of soil *Bacillus* spp. *Applied and environmental microbiology,* **53**(6): 1263-1266.

Tripathi, M., Kumar, A., Kalia, V., Saxena, A. and Gujar, G. 2016. Isolation and characterization of Lepidoptera specific *Bacillus thuringiensis* strains predominantly from north-eastern states of India. *Indian journal of experimental biology,* **5**(7): 431-452.

Yilmaz, S., Ayvaz, A. and Azizoglu, U. 2017. Diversity and distribution of cry genes in native *Bacillus thuringiensis* strains isolated from wild ecological areas of East-Mediterranean region of Turkey. *Tropical Ecology,* **58**(3): 605-610.

MS Received : 9 April 2018

MS Accepted : 12 May 2018