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Original Research Article

Entomopathogenic Screening of Indigenous *Bacillus thuringiensis* (Berliner) Isolates against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under Laboratory Condition

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Tomato pinworm, *Tuta absoluta*, *Bacillus thuringiensis*, Indigenous isolates, Insecticidal activity

A B S T R A C T

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is the most destructive pests that infests tomato in many countries. This pest is gaining resistance to a range of pesticides used in tomato fields. Application of *Bacillus thuringiensis* is a better alternative for the existing insecticides in the context of biological pest management approach. The present study investigated the entomopathogenecity of seventy *Bacillus thuringiensis* (Bt) isolates against second instar larvae of *T. absoluta*. Preliminary bioassay started with the preparation of spore crystal lysates further confirmed by tomato leaf dip method. Upon screening 54.29 % of the *B. thuringiensis* isolates exhibited mortality in the range of 26-50 per cent while 14.29 % of the *B. thuringiensis* isolates showed more than 75 per cent mortality after 72 h. Isolate Bt-Oa1 recorded maximum larval mortality of 94.74% after 72h e *et al.* xposure.

Introduction

Tomato pinworm *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a tomato pest in South America and has recently been introduced to India (Shashank *et al.*, 2015). It is a micro-lepidopteran and oligophagous pest native to South America. It was described by E. Meyrick in Peru during 1917 (Shashank *et al.*, 2015). Since 2006, *T. absoluta* has invaded Europe, Africa and Asia, causing major economic losses of 80–100 % under both greenhouse and field conditions (Urbaneja *et al.*, 2013). In India, this pest was initially found in October 2014 in both polyhouse and open field tomatoes in Pune, Maharashtra. 80–100% yield loss is caused by the highest degree of infestation. *T. absoluta* is one of the most destructive tomato pests because it feeds on foliage, buds, fruits and flowers. Larvae infestation can be observed on all stages of the plant growth and development, causing damages that facilitate secondary pathogens infection (Hatice *et al.*, 2017).

The pest species has a high reproductive capacity of 12 generations per year and the female can lay up to 260 eggs per year (Ayalew, 2015). Over the last few decades,

the productivity of tomato has increased worldwide. Heavy dependence on chemical pesticides offers ephemeral benefits, often with adverse side effects that are not viable and in some cases, potentially exacerbate overall pest problems for farmers and this pest has become pesticide resistance (Sandeep *et al.,* 2020a). Therefore, increasing and sustaining crop production with less pesticide usage is the key challenge. Variety of management strategies are used to minimize insect infestation. In order to safeguard the main crop, the first choice is to reduce the population of pests by cultural practices, i.e. deep ploughing and trapping crops. But the most viable approach for the control of pests is chemical management. In order to control insect pests, farmers use large amounts of insecticides; these insects have now established resistance to insecticides (Manivannan *et al.,* 2019).

The failure to control of this pest can have a strong economic impact and the need for studies to establish strategies for its biological control through the use of *Bacillus thuringiensis* (Bt) expressing insecticidal proteins has increased in its recent history of introduction (Gonzalez *et al.,* 2011). The major source of insect pest control is *B. thuringiensis* (Berliner), a species of gram-positive sporulating soil bacteria that forms insecticide crystal (CRY) proteins during the sporulation process of its growth cycle. Crystals contain one or more endotoxins known as cry proteins, which vary from one strain of *B. thuringiensis* to another. Via cloning and sequencing of several cry proteins, *Cry* and *Cyt* genes are named. One or more crystal toxic genes can be produced by each of the *B. thuringiensis* strains and about 323 holotype crystal proteins are documented as toxic to insects of different orders, viz. Coleoptera, Lepidoptera and Diptera (Crickmore, 2017).

As crystalline protein, are sequestered in bacteria, as crystalline inclusion mediating particular pathogenicity against insects. *B. thuringiensis* strains are highly effective against all *T. absoluta* larval stages. Cry proteins are highly specific and very effective against the tomato pinworm (Sandeep *et al.,* 2020b and Dakshina and Gary, 2003) and narrow specific to lepidopterans (Hernandez *et al.,* 2011 and Muhammad *et al.,* 2019).

A very effective, environmentally safe biopesticide specific to insects has been discovered (Palma *et al.,* 2014). In this context, the current study was carried out to screening the native *B. thuringiensis* against *T. absoluta* under laboratory.

Materials and Methods

Laboratory studies were carried out during 2018 to 2019 at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Hebbal, Bengaluru. Indigenous seventy *B. thuringensis* (*Bt*) isolates such as Bt-15, Bt-21, Bt-23, Bt-26, Bt-27, Bt-28, Bt-30, Bt-31, Bt-34, Bt-35, Bt-37, Bt-38, Bt-39, Bt-40, Bt-41, Bt-43, Bt-44, Bt-47, Bt-54, Bt-72, Bt-83, Bt-88, Bt-90, Bt-92, Bt-103, Bt-104, Bt-105, Bt-106, Bt-107, Bt-108, Bt-109, Bt-110, Bt-111, Bt-113, Bt-115, Bt-117, Bt-118, Bt-122, Bt-126, Bt-129, Bt-132, Bt-138, Bt- 139, Bt-142, Bt-146, Bt-147, Bt-151, Bt-190, Bt-201, Bt-202, Bt-212, Bt-216, Bt-237, Bt-238, Bt-240, Bt -246, Bt-247, Bt-248, Bt-251, Bt-256, Bt-257, Bt-258, Bt-259, Bt-265, Bt-267, Bt-268, Bt-272, Bt-275, Bt-278 and Bt-Oa1 were maintained at ICAR-NBAIR were used to screen their entomopathogenicity against second instar larvae of tomato pin worm. *B. thuringiensis* subsp. *kurstaki* HD-1, obtained from BGSC, Colombus, USA was used as a standard check.

Preparation of spore-crystal formulations of *B. thuringiensis* isolates

Crude protein extraction from *B. thuringiensis* was conducted by following the Dulmage (1970) process. Each *B. thuringiensis* native isolate was grown for 72 h at 37 ^{o}C in 250 ml of LB broth. The pH of the culture broth of each isolate was reduced to 7.0 with 1 N HCl (from 8.4-8.7 in different isolates) and centrifuged for 10 minutes at 8000 rpm at 4 ^{0}C. The supernatant was discarded and the pellet was dissolved in 6.0 per cent lactose (1/10th volume of the initial broth). The suspension was stirred over a magnetic stirrer for 30 minutes and four volumes of cold acetone were added slowly, followed by another 30 minutes of stirring. The content was kept at 4^{o}C for another 2 hours to get complete precipitation of spores and crystal bodies and filtered through Whatman No.1 filter paper under suction in a vacuum pump. The filtrate was discarded and the precipitate containing spores and crystals was allowed to dry in a vacuum desiccator at 25 ^{o}C overnight. The white crystalline powder obtained after drying was used to test bioefficacy against the larvae of *T. absoluta*

Insect rearing and bioassays

The laboratory reared iso-female colony (NBAII-MP-GEL-02a) of tomato pinworm maintained on tomato variety Shivam since Dec 2014 at ICAR-NBAIR was used in the present investigation. Fresh tomato seedlings were provided for oviposition when moths began to emerge. The adults were provided with 10% honey solution fortified with vitamin E as food. Freshly molted 2nd instar larvae were used for bioassay studies.

The insecticidal activities of native *B. thuringiensis* isolates were carried out as per the procedure given by Navon (2000). Tomato leaf dip method was followed to test the isolates at 50 ppm concentration. The fully matured tomato leaves were washed with sterile water containing 0.01% Triton X-100. The leaves were dipped for 5 sec in the respective dilutions and shade dried. The treated leaves were placed in bioassay containers. 60 freshly molted second instar larvae of tomato *T. absoluta* were released in three replication of each isolate and closed with perforated lid. The larvae fed with leaf dipped in sterile distilled water served as control. Each treatment was replicated three times and the experiment was repeated on alternate days. The observations on larval mortality were recorded after 24, 48 and 72 hours intervals. The mortality data were subjected to Abbott's correction. The corrected percent mortality was subjected to analysis of variance to find out the most effective *B. thuringienisis* isolates.

Results and Discussions

Screening of toxicity of *B. thuringiensis* isolates against the larvae of *T. absoluta*

Preliminary screening of the *B. thuringiensis* native isolates was carried against the freshly molted second instar larvae of *T. absoluta* and the findings are presented in Table 1.

In preliminary screening assays, the per cent mortality after 24 h of exposure to second-instar larvae of *T. absoluta* ranged from 0.01 to 24.43 %. The highest larval mortality of 37.0 % was reported by the reference strain Btk HD-1. Bt-247 recorded the highest mortality of 24.36 % among the native *B. thuringiensis* isolates, followed by Bt-247 and Bt-257 isolates (21.50%), which were on par with each other. Bt-142 and Bt-268 isolates reported 18.42 % mortality, followed by Bt-54, Bt-115 and Bt-212 (15.79% mortality). Bt-21, Bt-31, Bt-40, Bt-108, Bt-122, Bt-248, Bt-275 and Bt-147 isolates were on par with each other in terms of toxicity

with a larval mortality rate of 13.16 %. No larval mortality was observed for the isolates Bt-90, Bt-138, Bt-190, Bt-201 and Bt-246 isolates at the end of 24 hours of exposure.

The larval mortality ranged from 5.26 and 42.11 per cent after 48 h of exposure. The mortality of the reference strain Btk HD-1was 44.74 per cent and was significantly superior than all the other isolates tested. Among the isolates, the highest mortality (42.11%) was reported by Bt-Oa1 followed by Bt-257 and Bt-247 (39.47%).

Isolates Bt-142 and Bt-147 exhibited 34.21 % mortality. Larval mortality in the treatments with Bt-122, Bt-115 (31.58%) and Bt-21, Bt-40 and Bt-54 (28.95%) was on par with each other. Isolate Bt-92 recorded the significant lowest mortality of 3.25 per cent.

The larval mortality after 72 h of exposure varied from 21.05 to 94.74 per cent. Bt-Oa1 isolate exhibited the highest mortality of 94.74 per cent against 99.99 per cent mortality in Btk HD-1 reference strain. Bt-275 and Bt-247 isolates recorded 92.11 per cent mortality, followed by Bt-142 and Bt-247 (89.47%), which were on par with the Bt-21 (84.21%) and Bt-108 (81.58%) in terms of toxicity.

The insecticidal activities of Bt-27 (76.32%), Bt-212 (76.32%), Bt-115 (73.68%), Bt-54 (71.05%), Bt-122 (71.05%), Bt-31, Bt-40, Bt-147 (68.42%), Bt-256 and Bt-265 (60.53%) were all on par with each other.

The remaining isolates showed less than 60 per cent mortality, the lowest being with Bt-26 (21.05%). To summarize, 54.29 per cent (38/70) of the *B. thuringiensis* isolates exhibited mortality in the range of 26-50 per cent while 14.29 per cent (10/70) of the *B. thuringiensis* isolates showed more than 75 per cent mortality after 72 h (Table 1).

To summarize, 54.29 per cent (38/70) of the *B. thuringiensis* isolates exhibited mortality in the range of 26-50 % while 14.29 % (10/70) of the *B. thuringiensis* isolates showed more than 75 % mortality after 72 h (Table 2).

The differences in the efficacy of various *B. thuringiensis* isolates have been suggested to be due to the difference in domain II carbohydrate affinity resulting in variable binding specificity with the insect larvae of brush boundary membrane receptors, causing a difference in cry protein toxicity (Burton *et al.,* 1999).

The insecticidal activity of a given *B. thuringiensis* strain depends on various factors such as the insect species, subspecies of *B. thuringiensis*, number of *cry* genes and other toxins, gene copy number, expression level and differences in amino acid sequences of the same toxins *etc.* (Gorashi *et al.,* 2014). These studies confirmed the variation in toxicity of indigenous *B. thuringiensis* isolates against the larvae of *H. armigera*, wherein mortality ranged from 6.6 % (St-5) to 70 % (Wh-1). Rajashekhar *et al.,* (2017) found that a new isolate VKK-AC1 was significantly more toxic than the reference strain Btk HD-1 against *H. armigera* larvae with 70 % mortality.

Earlier studies by Gowtham *et al.,* (2018) reported that KGS2, KGS5 and KGS8 which were isolated from animal ordure showed a 100 % mortality rate in the 2nd stage of *T. absoluta* on the 7th day after treatment compared to normal reference strain Btk HD-1 (95 %) among all the tested *B. thuringiensis* isolates. Theoduloz *et al.,* (1997) reported that the larvae of *T. absoluta* was highly susceptible to native *B. thuringiensis* strains (121e, 66b, 72a, 104a) of Chile with LC_{50} values of 6.1, 18.5, 39.6, 16.4 and 19.2 µg per larva respectively.

Table.1 Entomopathogenicity of *B. thuringiensis* isolates against 2nd instar larvae of tomato pinworm, *T. absoluta*

Sl No	*B. thuringiensis* isolates	Corrected per cent mortality after		
		24h	48h	72h
1	Bt - 15	2.63 (9.34)^{cd}	10.53 (18.93)^{de}	31.58 (34.19)^{h}
2	Bt - 21	13.16 (21.27)^{b}	28.95 (32.55)^{bc}	84.21 (66.59)^{cd}
3	Bt - 23	5.26 (13.26)^{c}	13.16 (21.27)^{de}	26.32 (30.86)^{h}
4	Bt - 26	7.89 (16.32)^{b}	18.42 (25.42)^{c}	21.05 (27.31)^{h}
5	Bt - 27	10.53 (18.93)^{b}	23.68 (29.12)^{bc}	76.32 (60.88)^{e}
6	Bt - 28	7.89 (16.32)^{b}	13.16 (21.27)^{de}	23.68 (29.12)^{h}
7	Bt - 30	5.26 (13.26)^{c}	15.79 (23.41)^{de}	34.21 (35.8)^{g}
8	Bt - 31	13.16 (21.27)^{b}	26.32 (30.86)^{bc}	68.42 (55.81)^{e}
9	Bt - 34	2.63 (9.34)^{cd}	7.89 (16.32)^{e}	26.32 (30.86)^{h}
10	Bt - 35	7.89 (16.32)^{b}	15.79 (23.41)^{de}	23.68 (29.12)^{h}
11	Bt - 37	2.63 (9.34)^{cd}	18.42 (25.42)^{d}	26.32 (30.86)^{h}
12	Bt - 38	5.26 (13.26)^{c}	15.79 (23.41)^{de}	47.37 (43.49)^{g}
13	Bt - 39	7.89 (16.32)^{b}	18.42 (25.42)^{c}	31.58 (34.19)^{h}
14	Bt - 40	13.16 (21.27)^{b}	28.95 (32.55)^{bc}	68.42 (55.81)^{e}
15	Bt - 41	7.89 (16.32)^{b}	15.79 (23.41)^{de}	31.58 (34.19)^{h}
16	Bt - 43	5.26 (13.26)^{c}	13.16 (21.27)^{de}	39.47 (38.92)^{g}
17	Bt - 44	2.63 (9.34)^{cd}	10.53 (18.93)^{de}	31.58 (34.19)^{h}
18	Bt - 47	2.63 (9.34)^{cd}	13.16 (21.27)^{de}	52.63 (46.51)^{f}
19	Bt - 54	15.79 (23.41)^{b}	28.95 (32.55)^{bc}	71.05 (57.45)^{e}
20	Bt - 72	5.26 (13.26)^{c}	15.79 (23.41)^{de}	28.95 (32.55)^{h}
21	Bt - 83	7.89 (16.32)^{b}	13.16 (21.27)^{de}	39.47 (38.92)^{g}

Table.1 continued

Sl No	*B. thuringiensis* isolates	Corrected per cent mortality after		
		24h	48h	72h
22	Bt - 88	2.63 (9.34)^{cd}	10.53 (18.93)^{de}	36.84 (37.37)^{g}
23	Bt - 90	0.01 (0.57)^{d}	5.26 (13.26)^{f}	26.32 (30.86)^{h}
24	Bt - 92	2.63 (9.34)^{cd}	15.79 (23.41)^{de}	47.37 (43.49)^{g}
25	Bt - 103	5.26 (13.26)^{bc}	13.16 (21.27)^{de}	36.84 (37.37)^{g}
26	Bt - 104	7.89 (16.32)^{b}	15.79 (23.41)^{de}	39.47 (38.92)^{g}
27	Bt - 105	5.26 (13.26)^{c}	18.42 (25.42)^{d}	52.63 (46.51)^{f}
28	Bt - 106	7.89 (16.32)^{b}	15.79 (23.41)^{de}	57.89 (49.54)^{f}
29	Bt - 107	10.53 (18.93)^{b}	21.05 (27.31)^{bc}	52.63 (46.51)^{f}
30	Bt - 108	13.16 (21.27)^{b}	26.32 (30.86)^{bc}	81.58 (64.58)^{d}
31	Bt - 109	7.89 (16.32)^{b}	10.53 (18.93)^{de}	55.26 (48.02)^{f}
32	Bt - 110	2.63 (9.34)^{cd}	10.53 (18.93)^{de}	47.37 (43.49)^{g}
33	Bt - 111	2.63 (9.34)^{cd}	13.16 (21.27)^{de}	39.47 (38.92)^{g}
34	Bt - 113	7.89 (16.32)^{b}	18.42 (25.42)^{c}	42.11 (40.46)^{g}
35	Bt - 115	15.79 (23.41)^{b}	31.58 (34.19)^{b}	73.68 (59.14)^{e}
36	Bt - 117	7.89 (16.32)^{b}	15.79 (23.41)^{de}	47.37 (43.49)^{g}
37	Bt - 118	2.63 (9.34)^{b}	7.89 (16.32)^{e}	36.84 (37.37)^{g}
38	Bt - 122	13.16 (21.27)^{b}	31.58 (34.19)^{b}	71.05 (57.45)^{e}
39	Bt - 126	5.26 (13.26)^{c}	13.16 (21.27)^{de}	42.11 (40.46)^{g}
40	Bt - 129	5.26 (13.26)^{c}	15.79 (23.41)^{de}	39.47 (38.92)^{g}
41	Bt - 132	7.89 (16.32)^{b}	10.53 (18.93)^{de}	34.21 (35.8)^{g}
42	Bt - 138	0.01 (0.57)^{d}	7.89 (16.32)^{e}	26.32 (30.86)^{h}

Table.1 continued

Sl No	*B. thuringiensis* isolates	Corrected per cent mortality after		
		24h	48h	72h
43	Bt - 139	2.63 (9.34)^{cd}	5.26 (13.26)^{f}	28.95 (32.55)^{h}
44	Bt - 142	18.42 (25.42)^{a}	34.21 (35.8)^{a}	89.47 (71.07)^{cd}
45	Bt - 146	5.26 (13.26)^{c}	15.79 (23.41)^{de}	36.84 (37.37)^{g}
46	Bt - 147	13.16 (21.27)^{b}	34.21 (35.8)^{a}	68.42 (55.81)^{e}
47	Bt - 151	2.63 (9.34)^{cd}	10.53 (18.93)^{de}	39.47 (38.92)^{g}
48	Bt - 190	0.01 (0.57)^{d}	5.26 (13.26)^{f}	26.32 (30.86)^{h}
49	Bt - 201	0.01 (0.57)^{d}	7.89 (16.32)^{e}	28.95 (32.55)^{g}
50	Bt - 202	5.26 (13.26)^{c}	13.16 (21.27)^{de}	34.21 (35.8)^{g}
51	Bt - 212	15.79 (23.41)^{b}	26.32 (30.86)^{bc}	76.32 (60.88)^{e}
52	Bt - 216	2.63 (9.34)^{cd}	7.89 (16.32)^{e}	36.84 (37.37)^{g}
53	Bt - 237	5.26 (13.26)^{c}	15.79 (23.41)^{de}	47.37 (43.49)^{g}
54	Bt - 238	7.89 (16.32)^{b}	18.42 (25.42)^{c}	42.11 (40.46)^{g}
55	Bt - 240	2.63 (9.34)^{cd}	15.79 (23.41)^{de}	21.05 (27.31)^{h}
56	Bt - 246	0.01 (0.57)^{d}	13.16 (21.27)^{de}	42.11 (40.46)^{g}
57	Bt - 247	21.05 (27.31)^{a}	39.47 (38.92)^{a}	89.47 (71.07)^{cd}
58	Bt - 248	13.16 (21.27)^{b}	26.32 (30.86)^{bc}	52.63 (46.51)^{f}
59	Bt - 251	10.53 (18.93)^{b}	23.68 (29.12)^{bc}	57.89 (49.54)^{f}
60	Bt - 256	7.89 (16.32)^{b}	21.05 (27.31)^{bc}	60.53 (51.08)^{f}
61	Bt - 257	21.05 (27.31)^{a}	39.47 (38.92)^{a}	92.11 (73.68)^{c}
62	Bt - 258	5.26 (13.26)^{c}	26.32 (30.86)^{bc}	42.11 (40.46)^{g}
63	Bt - 259	7.89 (16.32)^{b}	23.68 (29.12)^{bc}	57.89 49.54)^{f}

Table.1 continued

Sl No	Isolates	Corrected per cent mortality after		
		24h	**48h**	**72h**
64	Bt - 265	10.53 (18.93)^{b}	26.32 (30.86)^{bc}	60.53 (51.08)^{f}
65	Bt - 267	7.89 (16.32)^{b}	21.05 (27.31)^{bc}	52.63 (46.51)^{f}
66	Bt - 272	5.26 (13.26)^{c}	18.42 (25.42)^{c}	47.37 (43.49)^{g}
67	Bt - 275	13.16 (21.27)^{b}	28.95 (32.55)^{bc}	92.11 (73.68)^{c}
68	Bt - 278	18.42 (25.42)^{b}	18.42 (25.42)^{d}	55.26 (48.02)^{f}
69	Bt - Oa1	24.68 (29.12)^{a}	42.11 (40.46)^{a}	94.74 (76.74)^{b}
70	HD 1	26.32 (30.86)^{a}	44.74 (41.98)^{a}	99.99 (89.41)^{a}
F-test		*	*	*
SEm ±		**0.65**	**0.43**	**0.60**
CD @ 1%		**2.39**	**1.59**	**2.24**

Values in the parentheses are arcsin transformed values. The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications.

Table.2 Categorization of native *B. thuringiensis* strains according to their toxicity against 2nd instar larvae of *T. absoluta*

Sl No	Corrected per cent of mortality (72 h)	No. of *B. thuringiensis* isolates	% of *B. thuringiensis* isolates
1	0-25%	4.0	5.71
2	26-50%	38.0	54.29
3	51-75%	18.0	25.71
4	76-100%	10.0	14.29

Narmen and Hassan (2013) recorded a mortality rate of 80 to 93.3 % of 4th instar larvae produced by *B. thuringiensis* strains (B1, B2, B3 and B4) compared to 13.3 % mortality by B12 isolate and the highest mortality was shown by a commercial *B. thuringiensis* formulation (Protecto®) at 2 g/l concentration from 96.7 to 100 per cent. The present findings are in agreement with the findings of Higuchi *et al.*, (2000), who assessed the ability of *B. thuringiensis* strains (Btk HD-1, 84-F-51-46, 93-Y-18-1, 84-F26-3 and 94-F(M)633-2) against the larvae of *P. xylostella*, where LC_{50} values were 14.56, 17.24, 18.20, 20.15 and 21.75 ppm , respectively. Similarly, Sabbour (2014) assessed the efficacy of Diprel®, *B. thuringiensis* subsp. *kurstaki* HD-73 and *B. thuringiensis* subsp. *kurstaki* HD-234 against *T. absoluta*. They found LC_{50} ranged between 140, 109 and 90 µg/ml for Diprel®, Bt *kurstaki* HD 73 and *B. thuringiensis* subsp. *kurstaki* 234, respectively. Commercially affordable *B. thuringiensis* strains formulations are widely used for the control of a variety of economically significant pests, especially the larval stage of many lepidopteran pests. In addition to the simplicity of its mass production and formulation and its abundance in nature, the specificity of this agent makes it an excellent choice for use in combination with other biological control agents for the management of *T. absoluta.*

References

Ayalew, G., 2015, Efficacy of selected insecticides against the South American tomato moth, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on tomato in the Central Rift Valley of Ethiopia. *Afric Ent.*, 23: 410–417

Burton, S. L., Ellar, D. J., LI, J. and Derbyshire, D. J., 1999, N-Acetylgalactosamine on the putative insect receptor aminopeptidase N is recognised by a site on the domain III lectin-like fold of a *Bacillus thuringiensis* insecticidal toxin. *J. Mol. Biol.,* 287: 1011- 022.

Crickmore, N., BAUM, J., Bravo, A., Lereclus, D., Narva, K., Sampson, K., Schnepf, E., Sun, M. and Zeigler, D. R., 2017, *Bacillus thuringiensis* toxin nomenclature. http://www. btnomenclature.info/.

Dakshina, R. S., Gary, L. L., 2003, Toxicity of *Bacillus thuringiensis* CRY1-Type insecticidal toxin to geographically distant populations of tomato pinworm. *Flor. Entomol.,* 86(2): 222-224.

Dulmage, H. T., 1970, Insecticidal activity of HD-1, a new isolate of *B. thuringiensis* var. *alesti*. *J. Invertebr. Pathol.,* 15: 232-239.

Gonzalez-Cabrera, J., Molla, O., Monton, H. and Urbaneja, A., 2011, Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biol Control,* 56: 71–80.

Gorashi, N. E, Thripathi, M., KALIA, V. and Gujar G. T., 2014, Identification and characterization of Sundanese Bt and related bacterial strains for their efficacy against *Helicoverpa armigera* and *Tribolium castaneum. Indian J. Exp. Bio.*, 52: 637-649.

Gowtham, V., Kannan, M., Senthi KUMAR, M., Soundararajan, R. P., 2018, Isolation and characterization of indigenous *Bacillus thuringiensis,* Berliner from animal ordure effective against South American Tomato pinworm, *Tuta absoluta* (Meyrick). *Pest. Manag. Hort. Eco.,* 24(1): 1-7.

Hatice, S., Fathih, G., Nedim, M., Sami, D. and Anne, F., 2017, Cry1Ac-mediated resistance to tomato leaf miner (*Tuta*

absoluta) in tomato. *Plant. Cell. Tissue Org. Cult.*, 131(1):65–73.

Hernandez-Fernandez, J., Ramrez, L., Ramrez, N., Fuentes, L. S. and Jimenez, J., 2011, Molecular and biological characterization of native *Bacillus thuringiensis* strains for controlling tomato leafminer (*Tuta absoluta*) (Meyrick) (Lepidoptera: Gelechiidae) in Colombia. *Wor. J. Micribiol. Biotech.*, 27: 579–590.

Higuchi, K., Saitoh. H., Mizuki, E., Ichimatsu, T. and Ohba, M., 2000, Larval susceptibility of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), to *Bacillus thuringiensis* H serovars isolated in Japan. *Microbiol. Res.*, 155: 23-29.

Manivannan, A., Kumar, K. K., Arul, L., Varanavasiappan, S., Kalayarasan, P., Manimegalai, S., Poornima, K., Devrajan, C., Sudhakar, D. and Balasubramani, V., 2019, Toxicity of Bt crystal protein Cry55Aa against pest of tomato and model nematode, *Caenorhabditis elegans*. *J. Ento.* and *Zoo. Stu.*, 7(6): 67–70.

Muhammad, I. S., Muhammad, A., Mansoor, U. H. and Muhammad, A. K., 2019, Efficacy of Cry1Ac toxin from *Bacillus thuringiensis* against the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). *Egypt J. Biol. Pest Cont.*, 29: 55-63.

Narmen, A. Y. and Hassan, G. M., 2013, Bioinsecticide activity of *Bacillus thuringiensis* isolates on tomato borer, *Tuta absoluta* (Meyrick) and their molecular identification. *African J. Biotech.*, 12(23): 3699-3709.

Navon, A. 2000, Bioassays of *Bacillus thuringiensis* products used against agricultural pests, bioassays of Entamopathogenic microbes and nematodes *CABI publishing*, Wallingford, U.K. pp. 1-24.

Palma, Leopoldo, Munoz, D., Berry, C., Murillo, J. and Caballero, P., 2014, "*Bacillus thuringiensis* toxins: An overview of their biocidal activity. *Toxins*, 6(12): 3296-3325.

Rajashekhar, M., Mittal, A., Dharavath, V. and Kalia, V., 2017, Characterization of potential native *Bacillus thuringiensis* strains isolated from insect cadavers against cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae). *Indian J. Entomol.*, 5(5): 214-219.

SABBOUR, M. M., 2014, Biocontrol of the tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. *J. Agric. Res.*, 3(3): 499-503.

Sandeep Kumar, J., Jayaraj, J., Shanthi, M., Theradimani, M., Balasubramani, V., Irulandi, S, and Prabhu, S., 2020a, Toxicity of insecticides to tomato pinworm, *Tuta absoluta* (Meyrick) populations from Tamil Nadu. *Ind J. of Agri. Res*., e-5443.

Sandeep Kumar, J., Jayaraj, J., Shanthi, M., Theradimani, M., Venkatasamy, B., Irulandi, S. and Prabhu, S., 2020b, Potential of standard strains of *Bacillus thuringiensis* against the tomato pinworm, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera). *Egyt. J. Bio. Pest Control*, 30: 123-130.

Shashank, P. R., Chandrasekhar, K. and Meshram, S. K., 2015, Occurrence of *Tuta absoluta* (Lepidoptera: Gelechiidae) an invasive pest in India. *Ind. J. Entomol.*, 77(4): 323–329.

Theoduloz, C., Roman, P., Bravo, J., Padilla, C., Vasquez, C., Meza-Zepeda, L. and Meza-Basso, L., 1997, Relative toxicity of native Chilean *Bacillus thuringiensis* strains against *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae). *J. Appl. Microbiol*., 82:462-468.

Urbaneja, A., Desneux, N., Gabarra. R., Arno. J., Gonzalez Cabrera, J., Mafra-Neto, A., Stoltman, L., PINTO ADA. and Parra, J. R. P., 2013, Biology, ecology and management of the South American tomato pinworm, *Tuta absoluta*, 98–125. In: PENA, J (Ed.). Potential invasive pests of agricultural crops. CAB International, Oxford shire, UK, 464 pp.