Journal of
Plant Development Sciences
Meerut, India

RESEARCH

BIOASSAY AGAINST *SPODOPTERA FRUGIPERDA* AND BIOSAFETY OF NON TARGETED ORGANISMS

Suvathi B[1], Allwin L*[2], Manivannan. MI[3] and M. Paramasivan[4]

[1]*Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India*
[2]*Department of Agricultural Entomology, AC&RI, Killikulam, Tamil Nadu Agricultural University, Tamil Nadu, India*
[3]*Department of Horticulture, AC&RI, Killikulam, Tamil Nadu Agricultural University, Tamil Nadu, India*
[4]*Department of Soil Science and Agricultural Chemistry, AC&RI, Killikulam, Tamil Nadu Agricultural University, Tamil Nadu, India*
Email: allwin.dr@gmail.com

Received-04.02.2024, Revised-22.02.2024, Accepted-10.03.2024

Abstract: Entomopathogenic potential of nineteen *Bacillius thuringiensis*strains were evaluated against neonate larvae of *S. frugiperda.*KKM 2 and KKM 14 were caused above 60% mortality. KKM 5, KKM 17 and KKM 18 were showed lowest per cent mortality (16.67%). Silkworms were highly susceptible to the selected *B. thuringiensis* isolates. Screened *B. thuringiensis*strains did not cause any adverse effects on beneficial organisms *viz*., honey bees and *Trichogramma chilonis*.

Keywords: *B. thuringiensis*strains, Bioassay, *S. frugiperda*, Biosafety

INTRODUCTION

Indiscriminate use of chemical insecticides leads to the development of resistance in agricultural pests and vectors of human diseases (Georghiou, 1990). It also pollutes the environment and cause adverse effects on non-targeted beneficial organisms, human and animal health. For this reason, biocontrol agents are used over the synthetic insecticides for controlling insect pests in agriculture. Among biocontrol agents, *B. thuringiensis* has been successfully used as microbial pesticide due to its specific activity and ecofriendly nature.

FAO reported that, 20 to 40% of yield loss is caused by the attack of insect pests and pathogenic organisms (Zhou *et al*., 2002). Most of the damaging pests belong to Lepidoptera (Pimentel 2009). Lepidopteran pests causing major economic damage are *Helicoverpa armigera* Hubner on vegetables and pulses, *Spodoptera litura* Fab. on vegetables, cotton and oilseeds, *Pieris brassicae* L. on crucifers, *Maruca vitrata* on pulses particularly in pigeonpea and cowpea (Rathee, 2018). Recently *Spodoptera frugiperda* has been reported in India as an invasive pest. The fall armyworm (*S. frugiperda*) is a major pest of maize, cotton, and rice, sorghum, sugarcane and it attacks more than 80 plant species. Smith and Abbot reported that fall armyworm (*S. frugiperda*) is an important pest of maize in America. In India, *S. frugiperda* (J. E. Smith) is recently reported in Karnataka, Tamil Nadu and Telangana infesting maize crop (Kalleshwaraswamy *et al*., 2018). *S. frugiperda* feed on maize cob or kernel and it reduced the grain yield of maize upto 34%. Currently, synthetic insecticides such as methomyl, carbaryl, and cypermethrin are being used against *S. frugiperda* which adversely affect the environment and non-targeted organisms. But, it has developed resistance to both insecticides and *Bt* toxins (Flagel *et al*., 2018).To combat these problems, there is a need to isolate native strains of *B. thuringiensis* with insecticidal activity.

MATERIALS AND METHODS

Bacillus thuringiensis strains

Nineteen new *B. thuringiensis* strains isolated from the native soil of Tirunelveli and Tuticorin districtsand identified based on the crystal morphology were used for the present study. The reference strain *Bt*-HD1was used for the present study which was obtained from Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology (CPMB & B), Tamil Nadu Agricultural University, Coimbatore.

Mass culturing of *Spodoptera frugiperda* (J.E. Smith)

Different stages of *S. frugiperda* larvaewere collected from maize field at Agricultural College and

*Corresponding Author

Research Institute, Killikulam and cultured on semi synthetic diet (CIMMYT diet) (Table 1). The culture was maintained at 25±1°C with 75±5 % relative humidity (Prasanna *et al*., 2018). The different instars of *S. frugiperda* from laboratory culture were used for conducting bioassays against different *B. thuringiensis* strains (Plate 1).

Table 1. Ingredients for the preparation of semisynthetic diet for *S. frugiperda*

Ingredients	Quantity (g or ml/ Litre)
Fraction A	
Maize leaf powder	25.2 g
Common bean powder	88.4 g
Brewer's yeast	22.7 g
Ascorbic acid	2.5 g
Sorbic acid	1.3 g
Mehthyl-p-hydroxyhenzoate	2.0 g
Vitamin E capsules (200 iu)	2.1 g
Sucrose	35.3 g
Distilled water	403.1 ml
Fraction B	
Agar	12.6 mg
Distilled water	403.1 ml
Fraction C	
Formaldehyde 40%	2.0 ml

All the dry ingredients were mixed thoroughly and then boiled water was added into it. Before adding methyl-p-hydroxybenzoate into the mixture, it was dissolved in 20 ml of absolute ethanol. Entire amount agar from Fraction B was boiled with distilled water and then poured into the prepared mixture. And finally 40% formaldehyde was added and it was mixed thoroughly for 3 minutes. Then prepared diet was poured into sterilized vials and allowed to solidify.

Rearing procedure

A newly hatched first instar larva was introduced into sterilized glass vial along with diet. Then glass vial was tightly closed with a sterilized cotton wool plug, which provided an exchange of air. Before pupation, diet was not allowed to dry. For mass larval development, 25 to 50 vials (one larva/vial) were needed. Individual vials were used to avoid cannibalism. To maintain the pathogen free insect culture larvae were inspected regularly.

Adult emergence

Pupated larvae were taken from capsule vial and placed on a circular piece of blotting paper in the Petridish (100 pupae/ Petridish). The emerged adults were collected carefully and introduced into mating cum oviposition cage.

Mating cum oviposition cage

Collected adults were released into an oviposition cage which was enclosed with wax coated paper. Cotton wool pads were soaked in 10 % sucrose solution and placed in a Petri dish. Two Petri dishes were placed inside the oviposition cage to provide food for the ovipositing adults. Deposited eggs were collected daily and kept in plastic containers for incubation and hatching.

Egg incubation

The eggs were incubated at 25±1°C with 75±5% relative humidity in the laboratory. It was hatched within 4 to 6 days. After hatching, larvae were transferred into capsule vials containing diet.

Bioassay

Leaf disc method

Preliminary bioassays were carried out to screen the virulent strains of isolated *B. thuringiensis*.Young maize leaves were collected freshly from the greenhouse, thoroughly washed with distilled water, dried and cut into rectangular leaf discs (approximately 2x1 cm). Each leaf disc was surface coated with 20 µl of crude spore-crystal mixture and allowed to dry in laminar air flow chamber. Ten neonate larvae were released on the leaf disc using camel hair brush without any physical injury. The leaf disc was placed in a Petridisc with a diameter of 9 cm. One larva was released per leaf disc. Ten larvae were used per replication and three replications were maintained for each treatment. *Bt*-HD1 strain was used as a positive control. Larval mortality was observed periodically upto seven days. Per cent mortality in the treatments was corrected by using Abbott's (1925) formula.

$$\text{Per cent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Safety of *B. thuringiensis* strains against non-target organisms

Isolated native strains weretested against non-target organisms like silkworms, honey bees and egg parasitoid, *T. chilonis* to check the biosafety of *B. thuringiensis* strains.

Mulberry Silkworm, *Bombyx mori*

Isolated strains and reference strain *Bt*-HD1 were used for bioassay on fourth instar larvae of silkworm. Mulberry leaf smeared with 2.5 µl of distilled water and after air drying leaves were coated with 2.5 µl suspension spore crystal mixtures of different *B. thuringiensis*strains and then leaves were air dried. Fourth instar larvae were fed with treated mulberry leaves. Ten larvae were used for a replication and three replications per treatment were maintained. Observations were recorded at 12 hours interval upto 72 hours.

Honey bees, *Apis cerana indica*

Bioassays were conducted against newly emerged adults of *Apis cerana indica* to find out the efficacy of isolated *B. thuringiensis*. Adults were fed with sugar syrup which was contaminated with 20 µl spore crystal mixtures of different *B. thuringiensis* strains. Young workers were released into a Petri dish along with cotton wool pads which dipped in 50% sugar syrup contaminated with suspensions of isolated strains. Ten adults per replication and three replications per treatment were maintained and observations were recorded at 12 hours interval upto 72 hours.

Egg parasitoid, *Trichogramma chilonis*

Eggs of *Corcyra cephalonica* were pasted on the egg card @ 100/card. For parasitisation, eggs of *T. chilonis* were transferred to each egg card by using camel hair brush. 20 µl suspensions of different *B. thuringiensis* strain were sprayed on the egg card and allowed to air dry. Three replications per treatment along with untreated control were maintained and observations were recorded based on the emergence of adults from parasitized cards.

Statistical analysis

The data on concentration mortality by different strains was subjected to ANOVA analysis. Per cent mortality in the treatments was corrected by using Abbott's (1925) formula. Mean values of different treatments were separated by least significant difference (LSD) using AGRES ver. (7.01) (Gomez and Gomez, 1984).

RESULTS

Entomopathogenic potential of *B. thuringiensis* strains against *S. frugiperda*

Entomopathogenic potential of nineteen strains was found by conducting preliminary bioassay against neonate larvae of *S. frugiperda*. 20 µl of Crude crystal protein was coated on the maize leaf disc which was fed up by neonate larvae. Among nineteen isolates, KKM 14 (62.33%), KKM 2 (60.67%), KKM 4 (58.67%) and KKM 7 (56.67%) strains were on par with each other with highest per cent mortality. The per cent mortality recorded by *B. thuringienis* strains was inferior to reference strain *Bt*-HD1 (90.00%). KKM 5, KKM 17 and KKM 18 isolates were on par with each other which showed lowest per cent mortality (16.67%) (Table 2, Plate 2).

Safety of *B. thuringiensis* strains against beneficial organisms

PCR positive nineteen *B. thuringiensis*isolates and reference strain *Bt*-HD1 were tested against non-targeted beneficial organisms to evaluate safety nature of the isolates.

Mulberry Silkworm, *Bombyx mori*

Different isolates of *B. thuringiensis* and reference strain *Bt*-HD1 were tested against fourth instar larvae of *B. mori*. Per cent mortality of the isolates was presented in Table 3. The isolate KKM 14 recorded 83.3% mortality followed by KKM 2 (76.67 %). The isolate KKM 3 recorded 36.67 per cent mortality. The mortality recorded by the reference strain was 93.33 %.

Honey bees, *Apis cerana indica*

Different *B. thuringiensis* isolates were tested against honeybees. There was no significant difference between the isolates. Reference strain *Bt*-HD1 recorded 6.00 per cent mortality (Table 4).

Egg parasitoid, *Trichogramma chilonis*

Different isolates of *B. thuringiensis* and also reference strain *Bt*-HD1 were sprayed on Corcyra eggs which were allowed to parasitize by *T. chilonis*. KKM 4 (86.00 %) and KKM 11 (84.00 %) showed highest per cent emergence of *T. chilonis* and reference strain *Bt*-HD1 showed 95.00 % emergence (Table 5).

DISCUSSION

S. frugiperda has been reported as a serious invasive pest in India and 9.0 to 62.5 % of incidence was observed (Kalleshwaraswamy *et al*., 2018). PCR screened *Bt* isolates were selected for preliminary bioassay against *S. frugiperda* and insecticidal activity of these isolates were also assessed (Fig. 1). Several authors studied the susceptibility of *S. frugiperda* for different *B. thuringiensis*strains (Bernardi *et al*., 2016 and Burtet *et al*., 2017). The results of the present investigation revealed that KKM 14 (62.33%) and KKM 2 (60.6%) were caused more than 60 per cent mortality whereas KKM 5, KKM 17 and KKM 18 were shown lowest per cent mortality (16.67%). Bohorova *et al*. (1997) studied the performance of *cry1*gene on S. *frugiperda* larvae and found that *cry1D* and *cry1F* gene had highest insecticidal activitiy. Jara *et al*. (2006) reported that sixty-five per cent of the isolates obtained from phylloplane and twelve per cent of the isolates from soil which were showed toxicity towards *S. frugiperda*. Monnerat *et al*. (2006) reported that susceptibility of *S. frugiperda* populations differs

with the different *B. thuringiensis* strains. CIBCM-166, S811, IB412, and LBIT27 and 147-5501 were the most toxic strains against *S. frugiperda*.

dos Santos *et al.* (2009) have screened seven *B. thuringiensis* strains out of 100 strains which showed above 70% mortality against *S. eridania, S. cosmioides* and *S. frugiperda*. BR9, BR37, BR45 S608 and S1905 were showed toxicity towards *S. frugiperda*. Cerqueira *et al*., 2016 reported that four strains(SUFT01, SUFT02,SUFT03 and SUFT04) out of 52 *B. thuringiensis* strains were showed highest (more than 80%) toxicity against *S. frugiperda*. Among four strains, SUFT01 had highest toxicity with an LC_{50} of 44.5 ng / cm^2. del Valle Loto *et al.* (2019) studied the efficacy of native *Bt* RT, *Btk* HD1 on third instar larvae of *S. frugiperda* biotypes and native *Bt* RT strain showed higher toxicity on *S. frugiperda*.

Different *B. thuringiensis* isolates and reference strain *Bt*-HD1 were tested against the fourth instar larvae of *B. mori*. The isolate KKM 14 recorded 83.3% mortality followed by KKM 2 (76.67%). The isolate KKM 3 recorded lowest 36.67 per cent mortality. The mortality recorded by the reference strain was 93.33% (Fig. 2). Similar results also reported by Ihran *et al*. (1993) and Saha (1994).

Nethravathi *et al.* (2010) reported that two *B. thuringiensis*strains (1620b and 1598d) out of 35 strains strain showed highest mortality *i.e.,* 93.33 and 90.00 per cent, respectively. Niu Lin *et al.* (2013) tested the effect of pollen from *Bt* cotton cultivars against *B. mori* larvae and observed no lethal effect in the survival and development. Rai *et al*. (2015) reported that commercial formulations of *B. thuringiensis* showed highest toxicity (95%) in third instar larvae and lowest toxicity in second instar of *B. mori*.

Safety of newly emerged adults of *Apis cerana indica*was evaluated by treating different isolates of *B. thuringiensis* with sugar syrup. There was no significant difference between the isolates. Reference strain *Bt*-HD1 recorded 6.00 per cent mortality. Mommaerts *et al*., (2010) reported that*B. thuringiensis*subsp. *aizawai* and *kurstaki*formulationssprayed on bumble bees and treated with pollen. They found that no siginificant difference was observed in population and reproduction capacity. Dai *et al*. (2012) reported the effects of Cry1Ah toxin on *A. mellifera, A. ligustica* and *A. cerana cerana* and found that it was not caused any harmful effects. Similar kinds of results were obtained by several authors (Malone *et al*., 1999; Malone and Pham-Delègue 2001; Ramirez-Romero *et al*., 2005; Rose *et al*., 2007; Duan *et al*., 2008) in their studies.

Salord *et al.* (2014) tested the effects of *B. thuringiensis* subsp.*kurstaki* on honey bees by aerial application. The results showed that colony performance and brood development of honeybees was not affected. Durso *et al.* (2016) reported that workers of *Apis mellifera* were exposed to *Bt* based biopesticides and observed the changes occurred in midgut of honeybees. They found that lesser concentration of *Bt* based biopesticides were not harmful to honeybees. Jia *et al*. (2017) studied the effect of different concentrations of Cry1Ie toxin on *Apis cerana cerana* and they did not found any effect.

Different isolates of *B. thuringiensis* and also reference strain *Bt*-HD1 were sprayed on Corcyra eggs which were allowed to parasitize by *T. chilonis*. The per cent emergence of *T. chilonis* from *Bt* treated Corcyra eggs were determined for the safety of *T. chilonis*. KKM 4 (86.00%) and KKM 11 (84.00%) showed highest per cent emergence of *T. chilonis* and reference strain *Bt*-HD1 showed 95.00% emergence (Fig. 3). Azizoglu *et al*. (2015) found the effect of *B. thuringiensis var kurstaki* HD1 on female egg parasitoid *T. evanescens* Westwood and it did not cause any effect on parasitism and longevity of *T. evanescens* adults. They suggested that it can be used together for pest management. Nascimento *et al*. (2018) reported that *Bt* suspensions sprayed on the eggs of *H. zea* did not affect the parasitism of *T. pretiosum*Riley. Thilagam (2003) tested the bio-safety of the formulations of *B. thuringiensis* subsp. *kurstaki* at different doses on the predator, *C. carnea* and recorded no adverse effects on its biology and also observed no harmful effects at lower doses on the parasitoid, *T. chilonis* regarding parasitisation, parasitoid emergence and adult longevity.Oluwafemi *et al*. (2009) studied the combined effects of *B. thuringiensis*.

Table 1. Per cent mortality caused by *B. thuringiensis*strains against neonate larvae of *S. frugiperda*

S.No.	Strains	Per cent Mortality
1	KKM 1	33.33 $(35.22)^{def}$
2	KKM 2	60.67 $(51.16)^{b}$
3	KKM 3	26.67 $(31.00)^{fgh}$
4	KKM 4	58.67 $(50.00)^{b}$
5	KKM 5	16.67 $(23.86)^{i}$

6	KKM 6	36.67 (37.22)^{de}
7	KKM 7	56.67 (48.83)^{b}
8	KKM 8	20.00 (26.57)^{hi}
9	KKM 9	23.33 (28.78)^{ghi}
10	KKM 10	30.00 (33.21)^{efg}
11	KKM 11	43.33 (41.15)^{c}
12	KKM 12	33.33 (35.22)^{def}
13	KKM 13	40.00 (39.23)^{cd}
14	KKM 14	62.33 (52.15)^{b}
15	KKM 15	33.33 (35.22)^{def}
16	KKM 16	23.33 (28.78)^{ghi}
17	KKM 17	16.67 (23.86)^{i}
18	KKM 18	16.67 (23.86)^{i}
19	KKM 19	43.33 (41.15)^{c}
20	HD-1	90.00 (71.57)^{a}
21	Control	0.00 (1.65)^{j}

SEd = 2.46 CD(.05) = 4.97

*Mean of three replications;

Means in parentheses are arc sine transformed values. In a column, means superscripted with common alphabets are not significantly different by LSD ($p < 0.05$)

Table 2. Senstivity of different strains of *B. thuringiensis* to fourth instar larvae of *B. mori*

S.No.	Isolates	Per cent Mortality
1	KKM 1	60.00 (50.85)^{def}
2	KKM 2	76.67 (61.22)^{bc}
3	KKM 3	36.67 (37.22)^{h}
4	KKM 4	66.67 (54.78)^{cde}
5	KKM 5	43.33 (41.15)^{h}
6	KKM 6	56.67 (48.85)^{efg}
7	KKM 7	70.00 (57.00)^{cde}
8	KKM 8	40.00 (39.15)^{h}
9	KKM 9	56.67 (48.93)^{efg}
10	KKM 10	63.33 (52.78)^{cdef}
11	KKM 11	73.33

		(59.00)^{bcd}
12	KKM 12	70.00 (56.79)^{cde}
13	KKM 13	63.33 (53.07)^{cdef}
14	KKM 14	83.33 (66.14)^{b}
15	KKM 15	56.67 (48.85)^{efg}
16	KKM 16	50.00 (45.00)^{fgh}
17	KKM 17	50.00 (45.00)^{fgh}
18	KKM 18	43.33 (41.15)^{gh}
19	KKM 19	70.00 (56.79)^{cde}
20	HD-1	93.33 (77.71)^{a}
21	Control	0.00 (1.65)^{i}

SEd = 4.1771 CD(.05)= 8.4424
*Mean of three replications
Means in parentheses are arc sine transformed values

Table 3. Safety of different strains of *B. thuringienis* to *Apis cerana indica*

S.No.	Isolates	Per cent Mortality
1	KKM 1	3.33 (6.14)
2	KKM 2	6.67 (12.29)
3	KKM 3	3.33 (6.14)
4	KKM 4	6.67 (12.29)
5	KKM 5	3.33 (6.14)
6	KKM 6	3.33 (6.14)
7	KKM 7	3.33 (6.14)
8	KKM 8	6.67 (12.29)
9	KKM 9	3.33 (6.14)
10	KKM 10	3.33 (6.14)
11	KKM 11	3.33 (6.14)
12	KKM 12	3.33 (6.14)
13	KKM 13	3.33 (6.14)
14	KKM 14	3.33 (6.14)
15	KKM 15	3.33 (6.14)
16	KKM 16	3.33 (6.14)

17	KKM 17	3.33 (6.14)
18	KKM 18	3.33 (6.14)
19	KKM 19	3.33 (6.14)
20	HD-1	3.33 (6.14)
21	Control	0.00 (1.65)

Table 4. Safety of different strains of *B. thuringienis*to *T. chilonis*

S.No.	Isolates	Emergence Per cent
1	KKM 1	74.67 (59.83)^{abcd}
2	KKM 2	82.67 (65.43)^{g}
3	KKM 3	72.00 (58.05)^{g}
4	KKM 4	86.00 (68.04)^{abcde}
5	KKM 5	79.00 (62.75)^{bcdef}
6	KKM 6	78.67 (62.53)^{abcd}
7	KKM 7	83.33 (66.02)^{abcd}
8	KKM 8	78.00 (62.03)^{def}
9	KKM 9	71.00 (57.42)^{abc}
10	KKM 10	77.00 (61.35)^{abcd}
11	KKM 11	84.00 (66.50)^{fg}
12	KKM 12	73.67 (59.13)^{abcd}
13	KKM 13	77.33 (61.60)^{bcde}
14	KKM 14	82.67 (65.41)^{ab}
15	KKM 15	77.00 (61.35)^{abcd}
16	KKM 16	78.00 (62.03)^{def}
17	KKM 17	78.00 (62.03)^{abcd}
18	KKM 18	79.67 (62.03)^{cdef}
19	KKM 19	74.33 (59.57)^{abcde}
20	HD-1	86.00 (68.05)^{bcde}
21	Untreated Control	95.00 (77.08)^{a}

SEd = 5.08 CD (.05) = 10. 25

*Mean of three replications

Means in parentheses are arc sine transformed values

In a column, means superscripted with common alphabets are not significantly different by LSD

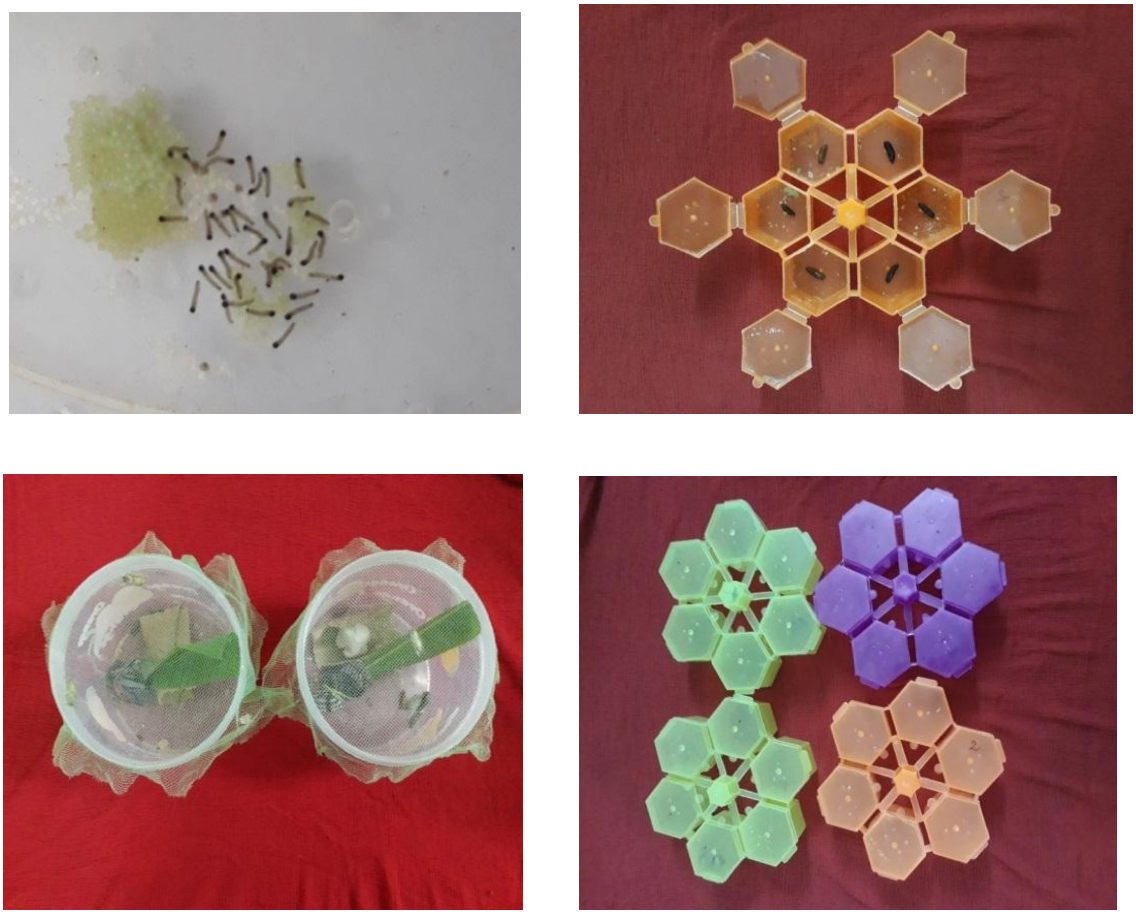

Plate 1. Mass rearing of *S. frugiperda*

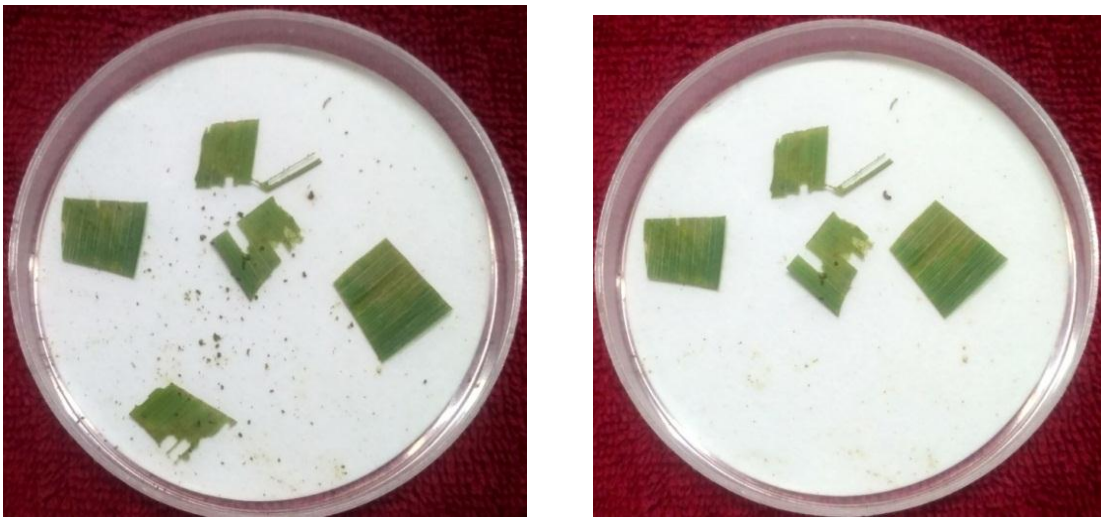

Plate 2. Preliminary bioassay against *S. frugiperda*

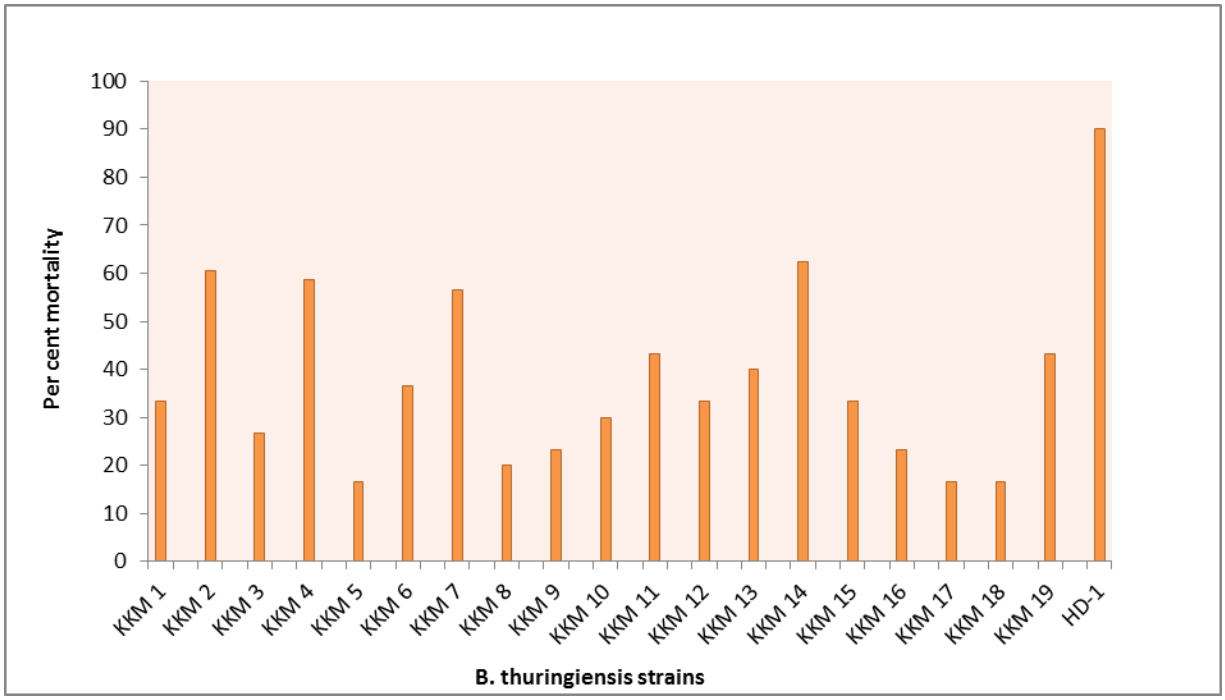

Figure 1. Per cent mortality caused by *B. thuringiensis* strains against neonate larvae of *S. frugiperda*

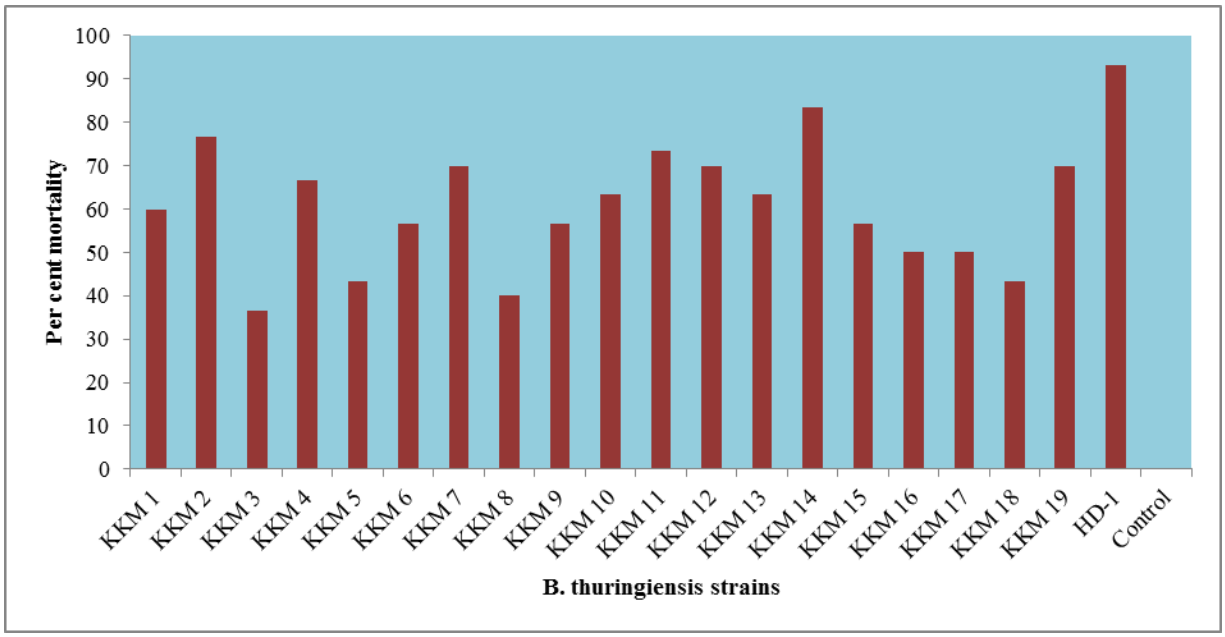

Figure 2. Sensitivity of fourth instar larvae of *B. mori* to different strains of *B. thuringiensis*

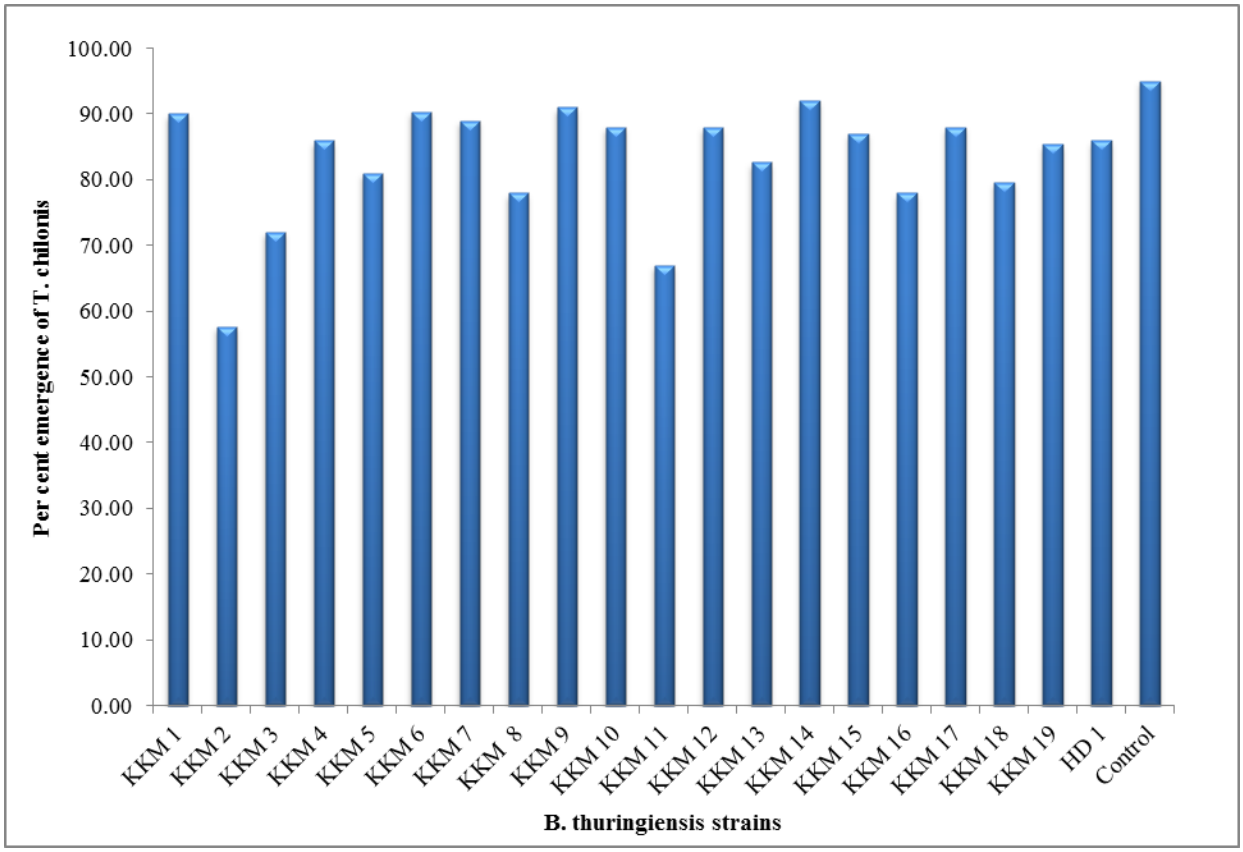

Figure 3. Per cent emergence of *T. chilonis*

REFERENCES

Abbott, Walter S. (1925). "A method of computing the effectiveness of an insecticide." *Journal of Economic Entomology*, **18** (2): 265-267.

Google Scholar

Azizoglu, Ugur, Semih Yılmaz, Abdurrahman Ayvaz and Salih Karabörklü (2015). "Effects of *Bacillus thuringiensis subsp. kurstaki* HD1 spore-crystal mixture on the adults of egg parasitoid *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae)." *Biotechnology & Biotechnological Equipment*, **29** (4): 653-658.

Google Scholar

Bernardi, Daniel, Oderlei Bernardi, Renato Jun Horikoshi, Eloisa Salmeron, Daniela Miyuki Okuma and Celso Omoto (2016). "Biological activity of *Bt* proteins expressed in different structures of transgenic corn against *Spodoptera frugiperda*." *Ciência Rural*, **46** (6): 1019-1024.

Google Scholar

Bohorova, N., M. Cabrera, C. Abarca, R. Quintero, A. M. Maciel, R. M. Brito, D. Hoisington and A. Bravo (1997). "Susceptibility of four tropical lepidopteran maize pests to *Bacillus thuringiensis* CryI-type insecticidal toxins." *Journal of Economic Entomology*, **90** (2): 412-415.

Google Scholar

Burtet, Leonardo M., Oderlei Bernardi, Adriano A. Melo, Maiquel P. Pes, Thiago T. Strahl and Jerson VC Guedes (2017). "Managing fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), with *Bt* maize and insecticides in southern Brazil." *Pest management science*, **73** (12): 2569-2577.

Google Scholar

Cerqueira, Fernando Barnabe, Giselly Batista Alves, Roberto Franco Teixeira Corrêa, Érica Soares Martins, Luiz Carlos Bertucci Barbosa, Ildon Rodrigues do Nascimento, Rose Gomes Monnerat, Bergmann Morais Ribeiro and RW de S. Aguiar (2016). "Selection and characterization of *Bacillus thuringiensis* isolates with a high insecticidal activity against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)." *Embrapa Recursos Genéticos e Biotecnologia-Artigo em periódico indexado (ALICE)*.

Google Scholar

Dai, Ping-Li, Wei Zhou, Jie Zhang, Wei-Yu Jiang, Qiang Wang, Hong-Juan Cui, Ji-Hu Sun, Yan-Yan Wu and Ting Zhou (2012). "The effects of *Bt* Cry1Ah toxin on worker honeybees (*Apis mellifera ligustica* and *Apis cerana cerana*)." *Apidologie*, **43** (4): 384-391.

Google Scholar

del Valle Loto, Flavia, Alfonso Emanuel Carrizo, Cintia Mariana Romero, Mario Domingo Baigorí and Licia María Pera (2019). "*Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from northern Argentina: esterases, profiles, and susceptibility to *Bacillus thuringiensis* (Bacillales:

Bacillaceae)." *Florida Entomologist*, **102** (2): 347-352.
Google Scholar

dos Santos, Karen Bianchi, Pedro Neves, Ana Maria Meneguim, Rachel Bianchi dos Santos, Walter Jorge dos Santos, Gislaine Villas Boas and Vinicius Dumas *et al.* (2009). "Selection and characterization of the *Bacillus thuringiensis* strains toxic to *Spodoptera eridania* (Cramer), *Spodoptera cosmioides* (Walker) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)." *Biological Control*, **50** (2): 157-163.
Google Scholar

Duan, Jian J., Michelle Marvier, Joseph Huesing, Galen Dively and Zachary Y. Huang (2008). "A meta-analysis of effects of *Bt* crops on honey bees (Hymenoptera: Apidae)." *PloS one*, **3** (1): e1415.
Google Scholar

D'urso, Vera, Gaetana Mazzeo, Valerio Vaccalluzzo, Giorgio Sabella, Fabio Bucchieri, Renata Viscuso and Danilo GM Vitale (2017). "Observations on midgut of *Apis mellifera* workers (Hymenoptera: Apoidea) under controlled acute exposures to a *Bacillus thuringiensis*-based biopesticide." *Apidologie*, **48** (1): 51-62.
Google Scholar

Flagel, Lex, Young Wha Lee, Humphrey Wanjugi, Shilpa Swarup, Alana Brown, Jinling Wang and Edward Kraft *et al.* (2018). "Mutational disruption of the *ABCC2* gene in fall armyworm, *Spodoptera frugiperda*, confers resistance to the Cry1Fa and Cry1A. 105 insecticidal proteins." *Scientific reports*, **8** (1): 7255.
Google Scholar

Georghiou, George P. (1990). "Overview of insecticide resistance." In.: ACS Publications.
Google Scholar

Gomez, Kwanchai A. and Arturo A. Gomez (1984). *Statistical procedures for agricultural research*: John Wiley & Sons.
Google Scholar

Ihara, Hideshi, Emi Kuroda, Akira Wadano and Michio Himeno (1993). "Specific toxicity of δ-endotoxins from *Bacillus thuringiensis* to *Bombyx mori*." *Bioscience, biotechnology, and biochemistry*, **57** (2): 200-204.
Google Scholar

Jara, S., P. Maduell and S. Orduz (2006). "Diversity of *Bacillus thuringiensis* strains in the maize and bean phylloplane and their respective soils in Colombia." *Journal of applied microbiology*, **101** (1): 117-124.
Google Scholar

Jia, Hui-Ru, Ping-Li Dai, Li-Li Geng, Cameron J. Jack, Yun-He Li, Yan-Yan Wu, Qing-Yun Diao and James D. Ellis (2017). "No effect of *Bt* Cry1Ie toxin on bacterial diversity in the midgut of the Chinese honey bees, *Apis cerana cerana* (Hymenoptera, Apidae)." *Scientific reports*, **7**: 41688.
Google Scholar

Kalleshwaraswamy, CM, MS Maruthi and HB Pavithra (2018). "Biology of invasive fall army worm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) on maize." *Indian journal of entomology*, **80** (3): 540-543.
Google Scholar

Malone, Louise Anne, Elisabeth Phyllis June Burgess and Dragana Stefanovic (1999). "Effects of a *Bacillus thuringiensis* toxin, two *Bacillus thuringiensis* biopesticide formulations, and a soybean trypsin inhibitor on honey bee (*Apis mellifera* L.) survival and food consumption." *Apidologie*, **30** (6): 465-473.
Google Scholar

Malone, Louise A. and Minh-Hà Pham-Delègue (2001). "Effects of transgene products on honey bees (*Apis mellifera*) and bumble bees (*Bombus* sp.)." *Apidologie*, **32** (4): 287-304.
Google Scholar

Mommaerts, Veerle, Kris Jans and Guy Smagghe (2010). "Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (*Bombus terrestris*)." *Pest Management Science: formerly Pesticide Science*, **66** (5): 520-525.
Google Scholar

Monnerat, Rose, Erica Martins, Paulo Queiroz, Sergio Ordúz, Gabriela Jaramillo, Graciela Benintende and Jorge Cozzi *et al.* (2006). "Genetic variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) populations from Latin America is associated with variations in susceptibility to *Bacillus thuringiensis* Cry toxins." *Applied and Environmental Microbiology*, **72** (11): 7029-7035.
Google Scholar

Nascimento, Priscilla T, Marcos AM Fadini, Fernando H Valicente and Paulo EA Ribeiro (2018). "Does *Bacillus thuringiensis* have adverse effects on the host egg location by parasitoid wasps?" *Revista Brasileira de entomologia*, **62** (4): 260-266.
Google Scholar

Nethravathi, C. J., P. S. Hugar, P. U. Krishnaraj, A. S. Vastrad and J. S. Awaknavar (2010). "Bioefficacy of native Sikkim *Bacillus thuringiensis* Berliner isolates against lepidopteran insects." *Journal of Biopesticides*, **3** (2): 448.
Google Scholar

Niu, Lin, Yan Ma, Amani Mannakkara, Yao Zhao, Weihua Ma, Chaoliang Lei and Lizhen Chen (2013). "Impact of single and stacked insect-resistant *Bt*-cotton on the honey bee and silkworm." *PLoS One*, **8** (9): e72988.
Google Scholar

Oluwafemi, Akinkurolere Rotimi, Qiong Rao, Xi-Qiao Wang and Hong-Yu Zhang (2009). "Effect of *Bacillus thuringiensis* on *Habrobracon hebetor* during combined biological control of *Plodia interpunctella*." *Insect science*, **16** (5): 409-416.
Google Scholar

Pimentel, David (2009). "Pesticides and pest control." In *Integrated pest management: innovation-development process*, 83-87. Springer.

Google Scholar

Prasanna, B. M., J. E. Huesing, R. Eddy and V.M. Peschke (2018). "Fall armyworm in Africa: a guide for integrated pest management." vii-109.

Google Scholar

Ramirez-Romero, Ricardo, Josette Chaufaux and Minh-Hà Pham-Delègue (2005). "Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach." *Apidologie*, **36** (4): 601-611.

Google Scholar

Rathee, Mandeep and Pradeep Dalal (2018). "Emerging insect pests in Indian agriculture." *Indian Journal of Entomology*, **80** (2): 267-281.

Google Scholar

Rose, Robyn, Galen P. Dively and Jeff Pettis (2007). "Effects of *Bt* corn pollen on honey bees: emphasis on protocol development." *Apidologie*, **38** (4): 368-377.

Google Scholar

Saha, B., Khan, A.R. and Faruki, S.T. (1994). "Changes in lipid and water content of *Bombyx mori* ingested with *B. thuringiensis var kurstaki* ." *Indian Journal of Sericulture*, **33**: 55-67.

Google Scholar

Salord, Maria del Mar Leza, Gregori Llado, Ana Belén Petro Balaguer and J. Ana Alemany (2014). "First field assessment of *Bacillus thuringiensis subsp. kurstaki* aerial application on the colony performance of *Apis mellifera* L. (Hymenoptera: Apidae." *Spanish Journal of Agricultural Research*, **2**: 405-408.

Google Scholar

Thilagam, P. (2003). Evaluation of *Bacillus thuringiensis var. kurstaki Berliner* (Spic Bio) against pod borer complex of pigeon pea and tomato fruit borer. Unpublished M.Sc.,(Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, India. 126 P P .

Google Scholar

Zhou, Xuguo, Michael E Scharf, Srinivas Parimi, Lance J Meinke, Robert J Wright, Laurence D Chandler and Blair D Siegfried (2002). "Diagnostic assays based on esterase-mediated resistance mechanisms in western corn rootworms (Coleoptera: Chrysomelidae)." *Journal of Economic Entomology*, **95** (6): 1261-1266.

Google Scholar