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Effect of Local *Bacillus thuringiensis* Isolates on Life Stages of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricus)

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ABSTRACT: The present investigation aimed to determine the impact of 11 *Bt* isolates native to Vidarbha region on biological parameters of *H. armigera* and *S. litura* larva which survived the dose of 1mg/ml and 2mg/ml respectively of crude *Bt* toxin from the isolates. It was evident from the study that even after surviving the given doses of *Bt,* some of the surviving larvae were unable to grow normally as compare to the larvae without any exposure to *Bt* toxins. It was recorded that local *Bt* isolate SY-4 adversely affects *H. armigera* among all the other isolates including standard *Btk* HD-1 recording maximum of 36.04, 86.67, 33.15 and 93.33 per cent reduction in larval weight, pupation, pupal weight and adult emergence respectively, similarly highest per cent reduction in fecundity and hatchability of 80.38 % and 45.34% respectively was recorded from pair in which both male and female survived the exposure of local isolate SY-4 during larval stage. In case of *S. litura,* local isolate SY-4 was found most effective among all the other isolates recording 29.79, 86.67, 60.48 and 93.33 per cent reduction on larval weight, pupation, pupal weight and adult emergence respectively. However SGd-1 adversely affected fecundity and hatchability of *S. litura* with 64.30 and 44.20 per cent reduction in fecundity and hatchabilty recorded from the pair in which both male and female were exposed to *Bt* during larval stage. It was evident from the present study that, local *Bt* isolates, SY-4 and SGd-1 were more potential not only causing mortality but also adversely affecting the normal biology of test insects which survived the exposure of these isolates.

Keywords: *Bacillus thuringiensis,* Bioassay, endotoxin, *Helicoverpa armigera, Spodoptera litura.*

INTRODUCTION

The gram pod borer *Helicoverpa armigera* and tobacco leaf eating caterpillar *Spodoptera litura* are most commonly occurring insect pests of different agronomical as well as horticultural crops distributed throughout tropical and temperate Asia (Lalitha and Muralikrishna 2012). Both the insect pests are polyphagous, with high fecundity and dispersing ability with wide host range of more than 182 host plants in case of *H. armigera* and more than 120 host in case of *S. litura* (Pawar, 1998; Ramana *et al.,* 1988). The indiscriminate and injudicious use of insecticides may lead to various problems regarding resurgence, resistance and pest out break as well as soil and human health. The nature of insect damage on attacked crops and use of chemical insecticides may result in high costs of control and low productivity (Fathipour and Naseri 2011). Thus use of biological methods for management of insect pest is getting more importance in integrated pest management practices.

Bacillus thuringiensis is one of the most successful microbial agents used as a biopesticide, because it is as effective as chemical pesticides and fits within the Integrated Pest Management approach, which favors non-chemical pesticides for pest management (Matyjaszczyk, 2018). *Bt* serves as the best alternative to overcome the issues such as various health issues and environmental problem with the high specificity and environmental safety (Kumar *et al.,* 2021). *Bt* is gram positive, facultative anaerobic, motile, endospore forming bacteria producing insecticidal crystal protein during stationary growth phase of sporulation

(Sansinenea, 2012). Its proteins have been studied for many years due to its toxicity against insects mainly belonging to the orders Lepidoptera, Diptera and Coleoptera (Mikel *et al.*, 2020). Widely accepted mode of action of *Bt* endotoxins is that it is stomach poison which kills the target insect only after ingestion in the insect midgut where it gets solubilized and proteolytically activated. After activation, the toxin binds to specific receptors in the host cell membrane which leads to oligomerization followed by formation of pores in the cell membrane and ultimately causing death of insect (Jurat-Fuentes and Crickmore 2017).

The present study emphasizes on the effect of *Bt* toxins on the biology of test insects surviving the given doses under laboratory conditions.

MATERIAL AND METHODS

Source of *Bt* isolates. *Bt* strains used in this study were isolated and identified at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India during the year 2020-2022 (Pawar *et al.*, 2022). The standard strain *Btk* HD-1 was procured from National Collection of Industrial Microorganisms (NCIM), Pune.

Rearing of test insects

Helicoverpa armigera. The larvae of *H. armigera* were collected from cotton, tomato okra and pigeon pea fields in Dr. PDKV university campus and collected larvae were reared in the laboratory on the artificial diet (Kranthi, 2005). After pupation, 0.01% Sodium hypochlorite solution was use to sterilize the pupa and then they were kept on sterilized soil beds in adult emergence chambers. The emerged adults were provided with 5% honey solution and paper sheets were provided to facilitate egg laying. The collected eggs were kept for hatching upto 1-2 days. Neonates hatched were maintained on artificial diet and 3rd instar larva of this population were used further for bioassay. Standard rearing conditions with temperature 26 ± 1°C, relative humidity 75 ± 1 % and photoperiod approximately 14:10 light: dark hours were maintained in the rearing room.

Spodoptera litura. Rearing of *S. litura* was carried out on natural diet using castor leaves under environmentally controlled conditions in the laboratory at 27 ± 1°C and 70 ± 5 % relative humidity. About 10 neonates were provided with fresh and tender castor leaves per vial until pupation. The pupae were collected and disinfected with 1% sodium hypochlorite solution; air dried and kept in plastic box (25 × 15 cm) until adult emergence. The adults were fed with adult diet of 5% honey solution for effective and uniform egg laying and hatching. Black paper stripes were kept inside the oviposition jars to facilitate egg laying. The egg masses were collected every day and kept in plastic jars marked with oviposition date to get uniform hatched population (Kranthi, 2005)

Bioassay. The spore crystal mixture (SCM) or endotoxin used for bioassay was recovered by acetone co-precipitation method described by Dulmage (1970). The solubalization of dried powder of SCM (endotoxin) obtained through acetone co-precipitation was carried out dissolving the powdered SCM in solubilizing buffer *i.e.* 50 mM Na_2CO_3. Then these samples were sonicated for 2 to 4 seconds, and the cell suspensions were incubated at 37°C for 4 hrs. The supernatants containing solubilized crystal proteins were stored in autoclaved Eppendorf tubes at -20 °C for further bioassay studies Saravanan and Gujar (2005).

Bioassay for *H. armigera* was carried out by diet incorporation method where ten 3rd instar larvae of *H. armigera* from laboratory culture were fed with artificial diet treated with spore crystal mixture (SCM) at a standard dose of 1mg/ml (Bhalla *et al.*, 2005). For *S. litura* leaf dip method was used for bioassay in which ten 2nd instar larvae of *S. litura* from laboratory culture were fed with castor leaf disc treated with spore crystal mixture (SCM) at a standard dose of 2mg/ml (Shelton *et al.*, 1993). Treatment of *Btk* HD1 was used as standard for the bioassay.

Effect of *Bt* isolates on biological parameters of survived test insects. The larvae of both *H. armigera* and *S. litura* which survived the applied dose of *Bt* endotoxin (120 hrs exposure) were selected to study their biological parameters. All the survived larvae were kept separately in plastic vials and regularly provided with fresh normal diets. Observations of larval weight were recorded after 24 hours of last larval molt and weight of pupa was recorded after 48 hours of pupation with the help of weighing balance. Per cent pupation was worked out by recording the number of larvae that underwent complete pupation. The pupae formed were disinfected with 2% sodium hypochlorite solution and were transferred to another container for further observations. To calculate per cent adult emergence, the number of adult emerging from pupa were counted and per cent adult emergence were worked out.

Fecundity. The newly emerged adults were kept in the oviposition chambers in pairs; black chart paper was kept inside oviposition chamber to facilitate egg laying. To calculate the fecundity the numbers of eggs laid per female were counted using magnifying glass. Observations on fecundity were recorded for pair of adults including *Bt* exposed females × non exposed males and exposed males x non exposed females also on pair with both *Bt* exposed female and male.

Per cent egg hatchability. Eggs laid by the female were disinfected using 1% sodium hypochlorite and kept for incubation, after 2-3 days total eggs hatched were calculated by observing eggs under stereozoom microscope and per cent hatchability was worked out. Observations on egg hatchability were also recorded in eggs laid by females under crosses *i.e.* treated females × non treated males, treated males × non treated female also pair with both male and female treated.

RESULTS AND DISCUSSION

Effect of promising *Bt* isolates on biological parameters of *H. armigera* survivors. Average larval and pupal weight recorded under control was 441.93 mg and 265.79 mg respectively. It was revealed that the highest per cent reduction in larval weight *i.e.* 36.04 % over control was recorded due to local *Bt* isolate SY-4 followed by SGd-1, SA-6 and I-3. However, all these treatments were statistically at par with standard *Btk* HD-1. In case of pupal weight maximum per cent reduction over control was recorded due to isolate SY-4 (33.15%) followed by I-3 (28.12%), HD-1 (26.69%), SGd-1 (26.13%), SA-20 (21.63%), SA-18 (17.68%), SGn-4 (14.75), SA-6 (12.73), SGn-5 (12.53%), SAk-9 (10.20%) and SAk-6 (8.61%). Similarly maximum reduction in per cent pupation was observed under treatment, SY-4 (86.67%) and SGd-1 (86.67%) and was found statistically at par with Btk HD-1 (83.33 %). Compared to control, the per cent pupation in other treatments was, however significantly reduced and was found in the range of 13.33 - 36.37 % statistically at par with HD-1 (16.67%) which signifies the efficacy of the local *Bt* isolates. Among all the promising *Bt* isolates, minimum emergence was recorded from HD-1, SGd-1 and SY-4 with 6.67 per cent adult emergence. When the data was statistically analyzed, it was observed that the effect on adult emergence due to these three isolates was statistically at par (Table 1).

Table 1: Effect of promising Bt isolates on biological parameters of *H. armigera* survivors.

Sr. No.	*Bt* isolate	Larval weight (mg)*	Per cent reduction in larval weight	Pupation (%)**	Per cent reduction in pupation	Pupal weight (mg)*	Percent reduction in pupal weight	Adult emergence (%)**	Per cent reduction in adult emergence
1.	HD-1	290.91 (17.06)	34.17	16.67 (24.11)	83.33	194.86 (13.96)	26.69	6.67 (14.94)	93.33
2.	SA-6	302.67 (17.40)	31.51	20.00 (26.58)	80.00	231.95 (15.23)	12.73	13.33 (21.38)	86.67
3.	SA-18	373.10 (19.32)	15.57	30.00 (33.23)	70.00	218.80 (14.79)	17.68	23.34 (28.87)	76.67
4.	SA-20	380.49 (19.51)	13.90	23.33 (28.90)	76.67	208.29 (14.43)	21.63	20.00 (26.53)	80.00
5.	SAk-6	421.81 (20.54)	4.55	36.67 (37.29)	63.33	242.90 (15.59)	8.61	33.33 (35.25)	66.67
6.	SAk-9	429.62 (20.73)	2.79	30.00 (33.23)	70.00	238.68 (15.45)	10.20	23.33 (28.85)	76.67
7.	SGd-1	294.44 (17.16)	33.37	13.33 (21.43)	86.67	196.35 (14.01)	26.13	6.67 (14.94)	93.33
8.	SGn-4	370.89 (19.26)	16.07	23.33 (28.90)	76.67	226.60 (15.05)	14.75	16.67 (24.08)	83.33
9.	SGn-5	408.52 (20.21)	7.56	26.67 (31.11)	73.33	232.48 (15.25)	12.53	23.33 (28.87)	76.67
10.	SBn-2	429.70 (20.73)	2.77	33.33 (35.28)	66.67	222.38 (14.91)	16.33	30.00 (33.18)	70.00
11.	I-3	336.25 (18.34)	23.91	23.33 (28.90)	76.67	191.05 (13.82)	28.12	16.67 (24.08)	83.33
12.	SY-4	282.66 (16.81)	36.04	13.33 (21.43)	86.67	177.69 (13.33)	33.15	6.67 (14.94)	93.33
13.	Control	441.93 (21.02)	-	100.00 (90.05)	-	265.79 (16.33)	-	100.00 (90.05)	-
	SE(m)±	0.45	-	1.02	-	0.35	-	0.71	-
	CD	1.36	-	3.00	-	1.04	-	2.10	-
	CV	3.02	-	6.07	-	4.03	-	5.02	-

* Values in parenthesis are square root transformed values, ** Values in parenthesis are arc sin transformed values

Effect on fecundity. The observations on fecundity was recorded from the pair of adult where, female survived the exposure of Bt during larval stage cross with untreated male, adult male survived cross with untreated female and the adult pair in which both male and female survived from *Bt* exposure during their larval stage (Table 2). Maximum fecundity recorded from untreated individuals with, 487.67 average numbers of eggs laid per pair. The minimum fecundity from the pair of male survived after exposure of local *Bt* isolates cross unexposed female, was recorded due to treatment HD-1 with 392.67 average eggs laid per pair. However, the effect on fecundity due to isolates SY-4, SGd-1, SA-6 and I-3 was statistically at par with standard HD-1 with average fecundity per pair 399.67, 395.33, 401.67 and 404.33 respectively. From the pair of female survived *Bt* exposure cross unexposed male, maximum per cent reduction in fecundity was recorded due to local isolate SY-4 (70.77%) followed by SGd-1 (52.24%), HD-1 (47.73%), I-3 (40.87%), SA-6 (31.85%), SA-20 (31.75), SA-18 (27.58%), SBn-2 (19.65%), SGn-5 (15.29%), SAk-6 (14.02%), SAk-9 (9.52%) and SGn-4 (7.72%). The effect on fecundity recorded due to SY-4 with 142.33 average numbers of eggs per pair was statistically at par with effect fecundity due to isolates HD-1, SGd-1 and I-3 with 254.67, 232.67 and 288.35 average number of eggs laid per pair respectively. In case of pair including both female and male survived exposure of local *Bt* isolates cross with each other, maximum per cent reduction in fecundity over control was observed due to SY-4 (80.38%) followed by HD-1 (64.05%), SGd-1 (58.17%), I-3 (53.25%), SA-18 (42.17%), SA-20 (41.90%), SGN-4 (40.40%), SA-6 (35.47%), SAk-6 (27.07%), SGn-5 (22.42%), SBn-2 (19.96%) and SAk-9 (15.04%). The fecundity was recorded due to SY-4 with 95.67 average eggs per pair followed by HD-1 with average 175.33 eggs per pair, whereas fecundity under treatment with isolate SGd-1 with 204.00 average eggs per pair was statistically at par with HD-1.

Table 2: Effect of local *Bt* isolates on fecundity of survived adult *H. armigera*.

Sr. No.	*Bt* isolate	Fecundity (No. of eggs/pair)			Reduction in fecundity (%)		
		***Bt* exposed male × normal female**	***Bt* exposed female × normal male**	***Bt* exposed female × male**	***Bt* exposed male × normal female**	***Bt* exposed female × normal male**	***Bt* exposed female × male**
1.	HD-1	392.67 (19.84)	254.67 (15.97)	175.33 (13.26)	19.48	47.73	64.05
2.	SA-6	401.67 (20.07)	332.33 (18.24)	314.67 (17.75)	17.64	31.85	35.47
3.	SA-18	426.33 (20.67)	353.00 (18.80)	282.00 (16.81)	12.58	27.58	42.17
4.	SA-20	412.33 (20.33)	332.33 (18.24)	283.33 (16.85)	15.45	31.75	41.90
5.	SAk-6	438.33 (20.96)	419.33 (20.49)	355.67 (18.87)	10.12	14.02	27.07
6.	SAk-9	426.33 (20.67)	441.33 (21.02)	414.33 (20.37)	12.58	9.52	15.04
7.	SGd-1	395.33 (19.91)	232.67 (15.27)	204.00 (14.30)	18.93	52.24	58.17
8.	SGn-4	431.33 (20.79)	452.00 (21.27)	290.67 (17.06)	11.55	7.12	40.40
9.	SGn-5	466.33 (21.62)	413.33 (20.34)	378.33 (19.46)	4.38	15.29	22.42
10.	SBn-2	459.00 (21.45)	391.67 (19.80)	390.33 (19.77)	5.88	19.65	19.96
11.	I-3	404.33 (20.13)	288.35 (17.00)	228.00 (15.12)	17.09	40.87	53.25
12.	SY-4	399.67 (20.02)	142.33 (11.95)	95.67 (9.81)	18.05	70.77	80.38
13.	Control	487.67 (22.11)	487.67 (22.11)	487.67 (22.09)	-	-	-
	SE(m)±	0.15	0.58	0.54	-	-	-
	CD	0.45	1.69	1.57	-	-	-
	CV	4.28	5.39	5.42	-	-	-

Values in parenthesis are square root transformed values

Effect on egg Hatchability. The data on per cent hatchability and per cent reduction in hatchability over control was recorded in Table 3. The observations hatchability were recorded from eggs laid by pair including exposed male cross with unexposed female, exposed female cross with unexposed male and from exposed male and female. The average hatchability per cent from eggs laid by unexposed pair of adult was observed 96.90 per cent. The least average hatchability per cent from the pairs of exposed male and unexposed female, was observed under treatment SY-4 (84.22%), where the effect on percent hatchability due to isolates *Btk* HD-1 (85.31%), SGd-1 (86.68%) and SA-6 (86.75%) was found statistically at par with SY-4. Similarly, mean per cent hatchability of eggs laid by the pairs in which female was exposed and unexposed male was recorded lowest due to SY-4 (55.30%) with which SGd-1 was statistically at par with 58.07 per cent mean hatchability whereas, per cent hatchability under treatment HD-1 (72.97%) was at par with SA-6 (75.90%). Thus it can be recorded that the effect due to isolates SY-4 and SGd-1 was found superior than standard strain HD-1. From the eggs laid by pairs of adult male and female both exposed to *Bt* isolates during larval period lowest mean per cent hatchability was recorded due to SY-4 (52.96%) was found significantly superior than per cent hatchability under treatment HD-1 (74.33%) whereas, SGd-1 with 77.94 per cent hatchability was statistically at par with HD-1.

Table 3: Effect of local Bt isolates on hatchability of survived adult *H. armigera*.

Sr. No.	*Bt* isolate	Hatchability (%)			Reduction in Hatchability (%)		
		***Bt* exposed male × normal female**	***Bt* exposed female × normal male**	***Bt* exposed female × male**	***Bt* exposed male × normal female**	***Bt* exposed female × normal male**	***Bt* exposed female × male**
1.	HD-1	85.31 (67.74)	72.97 (58.65)	74.33 (59.58)	11.60	24.70	23.29
2.	SA-6	86.75 (68.17)	75.90 (60.58)	85.70 (67.86)	11.06	21.67	11.56
3.	SA-18	91.54 (73.84)	84.99 (67.29)	84.04 (66.45)	4.92	12.29	13.27
4.	SA-20	90.29 (72.23)	84.80 (67.09)	83.12 (65.80)	6.39	12.49	14.22
5.	SAk-6	91.28 (72.02)	87.94 (69.84)	87.35 (69.18)	6.61	9.25	9.86
6.	SAk-9	90.88 (72.60)	88.48 (70.14)	89.14 (70.74)	6.00	8.69	8.01
7.	SGd-1	86.68 (68.57)	58.07 (49.63)	77.94 (61.97)	10.55	40.08	19.57
8.	SGn-4	90.42 (71.20)	88.96 (70.68)	84.52 (67.00)	7.49	8.19	12.78
9.	SGn-5	87.12 (68.74)	83.21 (65.80)	88.10 (69.83)	10.37	14.13	9.08
10.	SBn-2	93.81 (74.84)	84.41 (66.74)	88.47 (70.21)	3.86	12.89	8.70
11.	I-3	89.56 (72.52)	75.79 (60.50)	80.26 (63.60)	6.09	21.79	17.17
12.	SY-4	84.22 (66.08)	55.30 (48.12)	52.96 (46.68)	13.73	42.93	45.34
13.	Control	96.90 (79.85)	96.90 (79.85)	96.90 (79.85)	-	-	-
	SE(m) ±	0.86	1.66	1.97	-	-	-
	CD	2.58	4.93	4.00	-	-	-
	CV	4.63	4.86	3.58	-	-	-

Values in parenthesis are arc sin transformed values

The results recorded in present study were found in accordance with previous studies including, Cui and Xia *et al.* (1999) studied the effects of BT toxins from BT transgenic cotton on development and reproduction of *Helicoverpa armigera* and observed that rate of pupation and emergence decreased by 48.2-87.5 per cent and 66.7-100 per cent, respectively. The previous studies regarding effect of Bt toxin on the biology of *H. armigera* recorded similar results as the present study. Feeroza and Ahemad (2003) reported 5-6 per cent reduction in per cent pupation at 60.00 µg ml^{-1} dose of Bt toxin and also reported that Bt toxin from strains HD-695 and HD-1-S-1980 affects rate of adult emergence with only 49 and 70 per cent adult emergence respectively after larval exposure to toxins. Effect on adult emergence was also observed with 20 to 24 per cent reduction in adult emergence over control. Arshad *et al.* (2009) also reported the significant reduction in pupal weight from the pupa formed by larvae feeding on Bt transgenic cotton as compared to non Bt cotton which support the results in the present study that Bt toxins causes significant effect on pupal weight.

Zhang *et al.* (2013) reported 26.1 to 57.5 per cent and 13.2 to 57.5 per cent reduction in fecundity after the exposure of female *H. armigera* at different concentration of Cry1Ac and Cry1Ca toxin respectively, in contrast a little effect on fecundity was observed when *H. armigera* males were exposed to either of Bt toxins and mated with untreated females, which also supports the findings in present study. Abedi *et al.* (2014) reported sublethal effects due to Bt causes 18.4 % reduction in fecundity of *H. armigera* when with sublethal doses of LC$_{30}$ of *Bt* was used against 3rd instar larvae along with reduction in the larval and pupal weight and increased larval and pupal duration of *H. armigera* was also reported due to sublethal effect of Bt.

Effect of promising *Bt* isolates on biological parameters of survived *S. litura.* Average larval and pupal weight recorded under control was 1354.65 mg and 494.88 mg respectively. Maximum per cent reduction in larval and pupal weight over control observed due treatment with isolate SY-4 with 29.79% reduction in larval weight and 60.48% reduction in pupal weight. The minimum average weight of larva were recorded under treatment with local isolate SY-4 (951.06 mg) which was found significantly less than larval weight recorded under treatment with standard HD-1 (1129.84 mg) however, effect on weight due to isolates SGd-1 with average weight 1090.99 mg, I-3 with 1145.73 mg and SA-6 with 1156.02 mg was found statistically at par with standard HD-1. In case of effect on pupal weight, maximum reduction in pupal weight over control was observed under isolate SY-4 (60.48%) followed by HD-1 (58.23%), SA-6 (56.14), SGd-1 (54.98%), I-3 (52.18), SA-20 (51.95%), SBn-2 (50.21%), SA-18 (50.08%), SAk-6 (46.18%), SGn-5 (45.96%), SAk-9 (41.85%) and SGn-4 (41.09%).Minimum average weight of pupa recorded under treatment with local isolate SY-4 (195.59 mg) whereas effect on pupal weight due to isolates HD-1 (206.71 mg), SA-6 (217.07 mg) and SGd-1 (222.79 mg) was observed statistically at par with SY-4. Least average pupation was observed under treatment by isolates SY-4 (13.33%) %) followed by SGd-1 (16.67%), HD-1 (16.67%), SA-6 (20.00%), I-3 (23.33%), SGn-5 (23.33%), I-3 (23.33%), SA-18 (23.33%), SAk-6 (26.67%), SA-20 (30.00%), SGn-4 (33.33%), SBn-2 (36.67%) and SAk-9 (40.00%). However, effect on per cent pupation due to isolates SY-4, SGd-1, and HD-1, was found statistically at par. Among all the promising Bt isolates, minimum emergence of *S. litura* was recorded from HD-1 and SY-4 with 6.67 per cent adult emergence (Table 4).

Table 4: Effect promising *Bt* isolates on biological parameters of *S. litura* survivors.

Sr. No.	*Bt* isolate	Larval weight (mg)*	Per cent reduction in larval weight	Pupation (%)**	Per cent reduction in pupation	Pupal weight (mg)*	Per cent reduction in pupal weight	Adult emergence (%)**	Per cent reduction in adult emergence
1.	HD-1	1129.84 (33.62)	16.60	16.67 (24.06)	83.33	206.71 (14.39)	58.23	6.67 (14.94)	93.33
2.	SA-6	1156.02 (33.73)	14.66	20.00 (26.53)	80.00	217.07 (14.75)	56.14	16.67 (24.06)	83.33
3.	SA-18	1241.30 (34.59)	8.37	23.33 (28.83)	76.67	247.02 (15.85)	50.08	13.33 (21.37)	86.67
4.	SA-20	1182.39 (35.24)	12.72	30.00 (33.19)	70.00	237.77 (15.56)	51.95	20.00 (26.55)	80.00
5.	SAk-6	1314.88 (34.40)	2.94	26.67 (31.08)	73.33	266.32 (15.66)	46.18	23.33 (28.87)	76.67
6.	SAk-9	1296.31 (35.72)	4.31	40.00 (39.21)	60.00	287.76 (16.49)	41.85	36.67 (37.25)	63.33
7.	SGd-1	1090.99 (33.04)	19.46	16.67 (24.06)	83.33	222.79 (14.94)	54.98	10.00 (18.41)	90.00
8.	SGn-4	1266.21 (36.00)	6.53	33.33 (35.24)	66.67	291.53 (16.14)	41.09	26.67 (31.07)	73.33
9.	SGn-5	1272.64 (35.68)	6.05	23.33 (28.87)	76.67	267.41 (16.28)	45.96	20.00 (26.55)	80.00
10.	SBn-2	1279.26 (34.81)	5.57	36.67 (37.25)	63.33	246.41 (15.79)	50.21	33.33 (35.24)	66.67
11.	I-3	1145.73 (33.86)	15.42	23.33 (28.87)	76.67	236.67 (15.40)	52.18	16.67 (24.08)	83.33
12.	SY-4	951.06 (30.85)	29.79	13.33 (21.38)	86.67	195.59 (14.18)	60.48	6.67 (14.94)	93.33
13.	Control	1354.65 (35.78)	-	100.00 (90.05)	-	494.88 (18.09)	-	100.00 (90.05)	-
	SE(m)±	0.36	-	0.78	-	0.30	-	0.69	-
	CD	1.04	-	2.29	-	0.88	-	2.03	-
	CV	1.77	-	4.53	-	3.32	-	4.68	-

* Values in parenthesis are square root transformed values; ** Values in parenthesis are arc sin transformed values

Adult fecundity. Maximum fecundity was observed from untreated individuals with, 1232.33 average numbers of eggs laid per pair. Minimum fecundity among all the sets of treatment was recorded due local *Bt* isolate SGd-1 (440.00 average eggs per female) from pair of adult in which both male and female were exposed to *Bt* during larval stage. From the pair of treated female cross with normal male minimum fecundity was recorded due isolate lowest fecundity was observed under SGd-1 and SY-4 with 494.00 and 593.67 average numbers of eggs per pair respectively, whereas effect on fecundity due to these isolate SGd-1 was found significantly superior over SY-4 and standard HD-1 (827.67 per pair). The effect on fecundity due to isolate SA-6 (926.67 eggs per pair) was statistically at par with HD-1. However, the effect on fecundity due to isolates SY-4, SGd-1, HD-1, SA-6 and SA-20 was statistically at par with average fecundity of 1027.33, 1048.67, 1060.67, 1092.00 and 1103.67 respectively per female from the pair in which male was exposed and female was unexposed (Table 5).

Table 5: Effect of local *Bt* isolates on fecundity of survived *S. litura.*

Sr. No.	*Bt* isolate	Fecundity (Number of eggs/pair)			Reduction in fecundity (%)		
		Bt exposed male × normal female	*Bt* exposed female × normal male	*Bt* exposed female × male	*Bt* exposed male × normal female	*Bt* exposed female × normal male	*Bt* exposed female × male
1.	HD-1	1060.67 (32.58)	872.67 (29.56)	755.33 (27.49)	13.93	29.19	38.71
2.	SA-6	1092.00 (33.05)	926.67 (30.46)	866.00 (29.43)	11.39	24.80	29.73
3.	SA-18	1154.33 (33.99)	1165.33 (34.15)	1133.33 (33.68)	6.33	5.44	8.03
4.	SA-20	1103.67 (33.23)	1116.33 (33.42)	1087.67 (32.99)	10.44	9.41	11.74
5.	SAk-6	1173.33 (34.27)	1193.00 (34.55)	1139.00 (33.76)	4.79	3.19	7.57
6.	SAk-9	1190.67 (34.52)	1065.67 (32.65)	1011.67 (31.82)	3.38	13.52	17.91
7.	SGd-1	1048.67 (32.39)	494.00 (22.23)	440.00 (20.95)	14.90	59.91	64.30
8.	SGn-4	1137.33 (33.74)	1089.67 (33.02)	1035.67 (32.19)	7.71	11.58	15.96
9.	SGn-5	1175.33 (34.29)	1155.00 (34.00)	1101.00 (33.19)	4.63	6.28	10.66
10.	SBn-2	1185.33 (34.44)	1064.33 (32.64)	1010.33 (31.79)	3.81	13.63	18.01
11.	I-3	1105.67 (33.36)	982.33 (31.35)	928.33 (30.47)	10.28	20.29	24.67
12.	SY-4	1027.33 (32.06)	593.67 (24.39)	539.67 (23.24)	16.63	51.83	56.21
13.	Control	1232.33 (35.11)	1232.33 (35.11)	1232.33 (35.11)	-	-	-
	SE(m)±	0.42	0.39	0.85	-	-	-
	CD	1.21	1.15	2.59	-	-	-
	CV	2.14	2.17	3.10	-	-	-

*Values in parenthesis are square root transformed values

Egg Hatchability. After recording the observations regarding fecundity, the data was recorded on per cent hatchability from exposed male cross with unexposed female, exposed female cross with unexposed male and from exposed both male and female along with per cent reduction in hatchability over control. The average hatchability per cent of eggs laid by unexposed pair of adult was observed 97.75 per cent. From the pairs of exposed male and unexposed female, the least average hatchability per cent was observed under treatment SGd-1 (81.29%), followed by HD-1 (87.91%), SY-4 (87.96%), SA-6 (88.61%), I-3 (89.92%), SA-20 (89.99%), SGn-4 (90.08%), SA-18 (90.53%), SAk-9 (91.06%), SAk-6 (91.78%), SBn-2 (92.14%) and SGn-5 (95.46%). Effect due to isolate SGd-1 was found significantly superior to standard HD-1 whereas isolates SY-4, SA-6 and I-3 was at par with HD-1. In case of pairs in including exposed female and unexposed male, least per cent hatchability was recorded due to isolate SGd-1 (44.80%) followed by SY-4 (60.02%), HD-1 (77.31%), SA-6 (82.27%), SA-20 (85.07%), SA-18 (85.76%), I-3 (86.73%), SAk-6 (87.00%), SAk-9 (87.86%), SGn-5 (89.21%), SGn-4 (89.54%) and SBn-2 (91.79%). However effect due to isolate SGd-1 was found significantly superior to SY-4 and standard HD-1, whereas effect due to isolate SA-6 was found at par with HD-1. Similarly least per cent hatchability of eggs laid by pairs of adult male and female both exposed to *Bt* isolates was recorded due to SGd-1 (54.55%) followed by SY-4 (56.39%), HD-1 (74.62%), SA-6 (76.83%), SA-20 (78.39%), I-3 (79.62%), SA-18 (81.62%), SAk-6 (82.32%), SAk-9 (82.97%), SGn-5 (84.77%), SGn-4 (84.84%) and SBn-2 (87.10%). When compared statistically it was observed that effect due to isolates SGd-1 and SY-4 was at par with each other and significantly superior to standard strain HD-1 whereas, isolates SA-6 and I-3 was at par with HD-1.

Zhang *et al.* (2013) reported reduction in fecundity of *Spodoptera exigua* females due to exposure of toxin Cry1Ac and Cry1Ca with 6.3-35.4 and 4.1-45.8 per cent reduction of egg masses laid by successful mated females whereas, very less effect on fecundity was observed when expose male was mated with unexposed female. Obeidat (2008) also reported that Females of drosophila were more susceptible than male to local Bt isolates from Jordan which supports the result in present study In which effect on adult female as compare to male. Huang *et al.* (2018) recorded effects of native Bt isolate, Bt CAB109 from South Korea on the growth, development, and generation mortality of lepidopteran insect, *Spodoptera exigua* and recorded that affected not only the larvae, pupae, and adults, but also the eggs laid by the adult. Same strain Bt CAB109 was previously studied and it was reported that though amount of Bt toxin was insufficient to cause the death of insects *Spodoptera exigua*, but it was enough to affect their normal growth and development (Da-yong and Yong-man 2013). From the results recorded on per cent reduction in hatchability of exposed individuals, it can be confirmed that Bt isolates used under present study are found to have effectivity against offspring of exposed *S. litura* which was found in accordance with Souza *et al.* (2018) reported the transfer of Cry1F toxin from adult *S. frugiperda* to their offspring when exposed to Bt during larval stage, provided that one or both sexes were exposed, and more amount of Bt toxins was transferred when both parents were exposed.

Table 6: Effect of local *Bt* isolates on hatchability of survived *S. litura.*

Sr. No.	*Bt* isolate	Hatchability (%)			Reduction in Hatchability (%)		
		Bt exposed male × normal female	*Bt* exposed female × normal male	*Bt* exposed female × male	*Bt* exposed male × normal female	*Bt* exposed female × normal male	*Bt* exposed female × male
1.	HD-1	87.91 (69.70)	77.31 (61.55)	74.62 (59.93)	10.06	20.91	23.66
2.	SA-6	88.61 (70.33)	82.27 (65.13)	76.83 (61.52)	9.35	15.84	21.40
3.	SA-18	90.53 (72.23)	85.76 (67.84)	81.62 (65.08)	7.39	12.27	16.51
4.	SA-20	89.99 (72.83)	85.07 (67.28)	78.39 (63.37)	7.93	12.97	19.80
5.	SAk-6	91.78 (73.65)	87.00 (69.59)	82.32 (65.12)	6.11	10.99	15.78
6.	SAk-9	91.06 (72.91)	87.86 (69.79)	82.97 (65.74)	6.85	10.11	15.12
7.	SGd-1	81.29 (64.37)	44.80 (42.21)	54.55 (47.43)	16.84	54.16	44.20
8.	SGn-4	90.08 (71.68)	89.54 (71.59)	84.84 (67.37)	7.85	8.40	13.21
9.	SGn-5	95.46 (78.38)	89.21 (70.84)	84.77 (67.17)	2.34	8.74	13.28
10.	SBn-2	92.14 (74.03)	91.79 (73.70)	87.10 (69.13)	5.73	6.09	10.90
11.	I-3	89.92 (71.51)	86.73 (68.62)	79.62 (63.18)	8.01	11.28	18.55
12.	SY-4	87.96 (69.69)	60.02 (50.79)	56.39 (48.74)	10.01	38.60	42.31
13.	Control	97.75 (82.74)	97.75 (82.74)	97.75 (82.74)	-	-	-
	SE(m)±	0.76	1.49	1.14	-	-	-
	CD	2.28	4.47	3.39	-	-	-
	CV	3.51	4.89	5.03	-	-	-

*Values in parenthesis are arc sin transformed values

CONCLUSION

It was evident from the present investigations that all the 11 local *Bt* isolates have produced significant adverse effects such as reduction in larval and pupal weights, per cent pupation, adult emergence, fecundity and egg hatchability in larvae exposed to treated diet as compared to normal diet. From the data recorded under present study it can be concluded that, the larva of *H. armigera* and *S. litura*, surviving the *Bt* exposed diet, were further unable to grow normally and sustain. In case of *H. armigera* the isolate SY-4 has an equivalent effect as Btk HD-1 on adult emergence and it has more adverse effects on fecundity and hatchability of *H. armigera* than Btk HD-1. In case of *S. litura*, the isolate SY-4 was found equally effective as standard Btk HD-1 in terms of reduction in adult emergence However, the isolate SGd-1 was found to be more potent than the standard strain Btk HD-1 in terms of reduced fecundity and hatchability of *S. litura*. It was evident from the studies that exposure of larva to Bt during larval stage also have pronounced effects on reproductive capabilities of both male and female members. However the effects were more predominant in female members of both *H. armigera* and *S. litura*.

FUTURE SCOPE

The present investigation has paved the way to explore effect of *Bt* isolates on next generation insects surviving the exposure of Bt toxins. Further it is also important to study the effect of *Bt* on insect pest belonging to different insect orders including Diptera, Coleoptera and Hymenoptera.

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