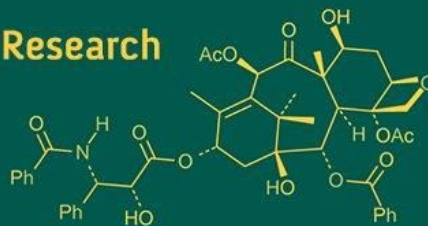
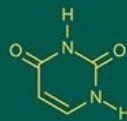
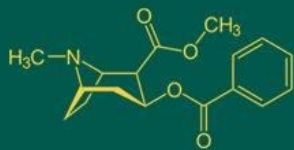


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Evaluation of PDKV *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (J.E. Smith)

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Abstract

The present investigation entitled “Evaluation of PDKV *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (J.E. Smith)” was carried out during the year 2023-2024 in the Department of Entomology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola. The aim of the investigation was to evaluate the toxicity of PDKV *Bt* isolates against *Spodoptera frugiperda*. During the study eleven local *Bt* isolates PDKV-SGd-1, PDKV-SY-4, PDKV-SA-6, PDKV-SAK-6, PDKV-I-3, PDKV-SGn-5, PDKV-SAK-9, PDKV-SBn-2, PDKV-SA-18, PDKV-SGn-4 and PDKV-SA-20 along with *Btk* HD-1 as standard check were used. The endotoxins from these *Bt* strains were isolated following method of acetone co-precipitation and used for conducting the toxicity bioassays against the third instar larvae of *Spodoptera frugiperda*. The lowest median lethal concentration (LC₅₀) was exhibited by PDKV-SA-20 of (172.90 µg/ml), followed by PDKV-SGd-1 (177.61 µg/ml) and PDKV-SY-4 (186.17 µg/ml) and these strains were found more effective than standard *Btk* HD-1 showed the LC₅₀ (191.41 µg/ml). The rest of strains however found at par in toxicity to that of the standard *Btk* HD-1. Further computation of relative toxicities indicated that *Bt* isolate SA-20, SGd-1 and SY-4 were 2.08, 2.03 and 1.93-fold more toxic than lowest toxic *Bt* isolate PDKV-SBn-2.

Keywords: *Bacillus thuringiensis*, bioassay, endotoxin, relative toxicities, *Spodoptera frugiperda*

1. Introduction

Maize is the third most important cereal crop in India after rice and wheat in terms of area and production. Globally it is highly valued for its multifarious use as food, feed, fodder and raw material for large number of industrial products. There are several reasons for the low productivity of maize viz., environmental factors, low mechanization, pest and diseases etc. Of these, insect pests are the major constraints as this attacking maize not only directly as borers, sap suckers, stem and root feeders etc. but also indirectly as vectors of diseases (Wayal Avinash., 2022) [18]. There are four major pests of maize prevalent in India. These are spotted stem borer *Chilo partellus* (Swinhoe), pink stem borer *Sesamia inferens* (Walker), shoot fly *Atherigona* spp. and fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Suby. S.B., 2020) [17]. The fall armyworm, *Spodoptera frugiperda* native to America is one of the important invasive polyphagous pests. It found in Brazil, Argentina and the USA (Porwell *et al.*, 2004; Clark *et al.*, 2007) [14, 5]. This invasive pest was first reported in West Africa in late 2016 (Goergen *et al.*, 2016) [10]. In May 2018, this highly invasive insect pest was noticed for the first time in India on the maize crop in the Shivamogga and Davanagere districts in Karnataka state (Sharanabasappa *et al.*, 2018) [16] and thereafter it was reported to cause major damage to economically important cultivated grasses such as rice, sorghum and sugarcane as well as 23 horticultural crops like cabbage, beet, tomato, potato and onion besides cotton, pasture grasses, peanut, soybean, alfalfa and millets (Pogue, 2002; Chapman *et al.*, 2000; CABI, 2016) [13, 6, 7]. *S. frugiperda*, is a harmful pest, poses a significant threat to tropical crops, potentially causing up to 70% yield losses in corn. Chemical insecticides have failed to manage FAW, leading to environmental implications. Biological control is the only eco-friendly solution for managing FAW populations.

Bacillus thuringiensis has been highly explored as a microbial biopesticide for the control of insect pests especially in the orders of Lepidopteran, Dipteran and Coleopteran species. It is a ubiquitous gram-positive, rod shape, motile, spore-forming entomopathogenic bacterium

widely used as biocontrol agent. (Fernando *et al.*, 2010) [9]. *B. thuringiensis* products are used in pest management because they are highly specific to the target organism and harmless to non-target organisms and the environment (Palma *et al.*, 2014) [15]. *Bt* produce insecticidal proteins during the sporulation phase as parasporal crystals. These crystals are predominantly comprised of one or more proteins Crystal (Cry) and Cytolytic (Cyt) toxins, also called d-endotoxins. These toxins are highly specific to their target insect, are innocuous to humans, vertebrates and plants, and are completely biodegradable. Therefore, *Bt* is a viable alternative for the control of insect pests in agriculture and of important human disease vectors (Bravo *et al.*, 2005) [3]. Despite of its effectiveness as a biological control of pests, at present it is necessary to identify novel *Bt* isolates with promising Cry proteins for greater toxicity to counter the potential resistance evolved by insects. Hence, the present work attempted at screening of some locally isolated *Bt* strains against *S. frugiperda* and comparing their toxicities with standard HD-1 strain of *Bacillus thuringiensis*.

2. Materials and Methods

2.1 Insect collection and rearing

Fall armyworm (FAW) was collected from an unsprayed maize field in the university campus and brought to a laboratory. To avoid cannibalism the larvae were placed individually in ventilated plastic jars and fed with fresh tender maize leaves till pupation. After pupation, they were placed to a mating jar covered with muslin cloth containing 10% honey solution for adult feeding and folded paper strips for egg laying. After 2-3 days, old egg batches were collected and placed in sterile plastic jars. Eggs were monitored daily for hatching. On hatching, neonates were transferred to natural diet. The third instar larvae from the laboratory culture were used for bioassay experiments. The culture was maintained under controlled laboratory conditions, i.e., at 27 ± 1 °C and $70 \pm 5\%$ relative humidity and a 12L:12D photoperiod.

2.2 Screening of *Bacillus* isolates against larvae of fall armyworm

2.2.1 Preparation of culture media

For this purpose 200 ml of distilled water was measured using glass measuring cylinder and taken in glass bottle. Luria broth (5 gm) and agar powder (3 gm) was added in bottle and heated in microwave for dissolving agar. Then the digested medium was transferred to conical flasks and mouths of the flasks were sealed with tight cotton plugs and autoclaved. After autoclaving the medium was taken into sterile bottles or test tubes. Before and after pouring of media into bottles, the conical flask containing the medium was flamed to prevent contamination. The bottles or tubes were kept open till the media solidified within the laminar hood. The bottles, tubes and petri plates used in culturing were also autoclaved at 15 lbs pressure and 121 °C temperature for 15 minutes. After autoclaving, the material was shifted to the laminar airflow, which was sterilized by U.V. light for 30 minutes. After switching off the U.V. the airflow and illumination (after 10 min.) was switched on and the floor of the cabinet and hands were sterilized with 70% alcohol. The media bottles and other materials were placed in the cabinet. The spirit lamp was lighted and the media was poured into the bottles before it cooled down. The media in the petri plates, bottles and test tubes was allowed to solidify. These media bottles were used for further sub-culturing of various *Bt* strains used in the experiments.

2.2.2 Parasporal preparations

For the bioassay, Spore-crystal mixture was prepared according to the method given by Dulmage *et al.*, (1970) [8]. Spore-crystal mixture was extracted by acetone co-precipitation method. This Spore-crystal mixture was used as a source of endotoxin for bioassay of test insect. The *Bt* isolates mentioned in (Table 1) were inoculated in Luria broth and incubated for 72 hrs. Until sporulation at 30 °C. Then centrifuged and pellet was obtained. The pellet is suspended in 4-6 % lactose solution, until final volume is one tenth of original solution then stirred for 15-30 min. To the suspension 4 volume of acetone is added gradually and filtered to obtain aqueous suspension of spore-crystal mixture with acetone, the process is repeated twice then is dried overnight either in vacuum desiccators or in air room temperature. This was stored at 4 °C and used further for bioassays studies.

2.2.3 Bioassay of selected native isolates of *Bt*

In order to determine the lethal concentrations of selected endotoxins from *Bt* isolates against *Spodoptera frugiperda*, diet incorporation assays were followed as described by Bhalla *et al.*, (2005) [2] for *H. armigera*. The diet for *Spodoptera frugiperda* was prepared by the method described by Burton *et al.*, (1969) [1]. Ten 3rd instar larvae (8 hrs. starvation) were released in each well. A set of 10 such larvae was tested for each concentration of parasporal preparations at one time. Four concentrations, viz., 200, 300, 400 and 500 µg/ml of diet of spore crystal complex of each strain, along with a control, were taken for bioassay under controlled conditions. The plates were covered and kept in the insect culture room at 28 ± 2 °C, $60 \pm 5\%$ R and 14L:10D photoperiod. Mortality was recorded after 3 days. The experiment was replicated three times. The mortality was recorded on the 7th day after the bioassay. LC50 values for bioassays were calculated.

2.2.3.1 Data analysis

The concentration response data was analysed using probit analysis with OPSTSAT software to determine the LC50 values, standard errors, slope and 95% fiducial limits (FL). From which relative toxicities of different *Bt* isolates was also determined as below:

$$\text{Relative Toxicity (RT)} = \frac{\text{LC}_{50} \text{ of more toxic } Bt \text{ isolates}}{\text{LC}_{50} \text{ of less toxic } Bt \text{ isolates}}$$

Table 1: *Bt* endotoxins used

Sr. No.	<i>Bt</i> isolate	Accession no.	Source
1	HD- 1	5118	NCIM
2	PDKV SA-20	ON331908.1	Department of Entomology (Dr. PDKV AKOLA)
3	PDKV SY-4	ON331906.1	
4	PDKV SGd-1	ON331905.1	
5	PDKV SA-6	ON331907.1	
6	PDKV SA-18	OP209984	
7	PDKV SBn-2	OP209989	
8	PDKV SAK-6	OP209985	
9	PDKV SAK-9	OP209987	
10	PDKV I -3	OP209990	
11	PDKV SGn-4	OP209987	
12	PDKV SGn-5	OP209988	

3. Results and Discussion

3.1 Bioassay studies of potential *Bt* isolates against *Spodoptera frugiperda* (J.E. Smith)

Eleven PDKV *Bt* strains for toxicity studies viz. SA-6, SA-20, SAK-6, SAK-9, SGd-1, SBn-2, SGn-4, SGn-5, SY-4, SA-18 and I- 3 were used for conducting bioassay to determine the LC₅₀ values against the test insect i.e. *Spodoptera frugiperda* (J.E. Smith).

3.1.1 LC₅₀ of PDKV *Bt* isolates against *S. frugiperda*

Data presented in the (Table 2) depicted in Fig 1. indicate that the local *Bt* isolates PDKV-SA-20, PDKV-SGd-1 and PDKV-SY-4 recorded lower LC₅₀ values of 172.90 µg/ml, 177.61 µg/ml and 186.17 µg/ml, respectively and found relatively more toxic than standard *Btk* HD-1 strain which reported LC₅₀ of 191.41 µg/ml. The LC₅₀ values of rest of the other *Bt* isolates PDKV-SAK-6, PDKV-SAK-9, PDKV-SGn-4, PDKV-I-3, PDKV-SA-6, PDKV-SA-18, PDKV-SGn-5 were seen lying within the range of fiducial limits of the standard *Btk* HD-1 strain, hence all these local *Bt* isolates said to have equal toxicity potential to that of standard *Btk* HD-1 strain against *S. frugiperda*. As local SA-20 isolate seen to exceeds the fiducial limits of the standard *Btk* HD-1 strain it can be referred to as the most promising strain against *Spodoptera frugiperda*.

Similar study regarding LC₅₀ was reported by M. Maheesha *et al.*, (2021) ^[12], estimated LC₅₀ of the isolate T350 and positive control HD-1 produced 100% mortality against the neonates of *S. frugiperda*. In probit analysis the isolate T350 recorded LC₅₀ value of 2.04 µg/ml as against 1.99 µg/ml in positive control HD-1 which in accordance with the LC₅₀ range obtained in present study.

Likewise, Karupaiyan *et al.*, (2022) ^[11] worked on characterization and evaluation of indigenous *Bt* isolate T352 against *S. frugiperda*. Bioassay studies revealed that 100% larval mortality was exhibited in both T352 and standard strain HD-1, were LC₅₀ value of isolate T352 recorded 1.927 µg/ml whereas HD-1 exhibited 0.421 µg/ml of LC₅₀.

Similarly, Harika *et al.*, (2021) ^[4] recorded toxicity of six native *Bt* isolates viz., isolate 49, 51, 52, 55, 493 and reference strain HD-1. The LC₅₀ values ranged from (1.34×10¹¹ to 2.64×10⁷ CFU^{ml-1}). Among native *Bt* isolates lowest LC₅₀ value recorded by the isolate-493 with 2.03×10⁸ CFU^{ml-1} followed by isolate-51, isolate-55 and isolate-49 with LC₅₀ values of 1.51×10¹⁰, 4.71×10¹⁰ and 5.38×10¹⁰ respectively. The highest LC₅₀ value was obtained with the isolate-52 (1.34×10¹¹ CFU^{ml-1}) and thus found to be the least effective out of all the native isolates tested.

Table 1: Median lethal concentration (LC₅₀) of PDKV *Bt* strains against *S. frugiperda*

Sr. No.	Isolates	Regression Equation	Chi-Square	LC ₅₀ (µg/ml)	Fiducial limit		LC ₉₀ (µg/ml)	Fiducial limit	
					Lower	Upper		Lower	Upper
1	HD- 1	Y=-5.605+2.456x	0.900	191.41	115.95	315.98	636.38	385.50	1050.53
2	PDKV-SA-6	Y=-6.173+2.614x	0.760	229.97	145.35	363.87	711.13	449.44	1125.17
3	PDKV-SA-18	Y=-5.919+2.447x	0.941	262.70	162.63	424.36	877.60	543.29	1417.62
4	PDKV-SA-20	Y=-5.235+2.339x	0.979	172.90	101.37	294.89	610.36	357.87	1040.99
5	PDKV-SAK-6	Y=-5.317+2.310x	0.938	200.30	118.63	338.18	718.53	425.57	1213.14
6	PDKV-SAK-9	Y=-5.160+2.228x	0.809	207.12	120.96	354.669	778.41	454.84	1333.63
7	PDKV- SGd-1	Y=-5.028+2.235x	0.839	177.61	102.35	308.23	665.09	383.25	1154.19
8	PDKV-SGn-4	Y=-4.612+1.971x	0.703	218.60	120.30	397.21	976.89	537.63	1775.04
9	PDKV-SGn-5	Y=-6.131+2.504x	0.927	281.17	176.29	448.44	913.83	572.97	1457.47
10	PDKV-SBn-2	Y=-4.443+1.737x	0.990	361.05	186.45	699.182	1973.4	1019.0	3821.5
11	PDKV-I-3	Y=-5.534+2.358x	0.963	222.30	134.04	368.68	777.13	468.58	1288.84
12	PDKV-SY-4	Y=-5.011+2.208x	0.989	186.17	107.20	323.32	708.61	408.03	1230.62

*y=a+bx; where y=probit; x= concentration (µg/ml); a= intercept; b=slope

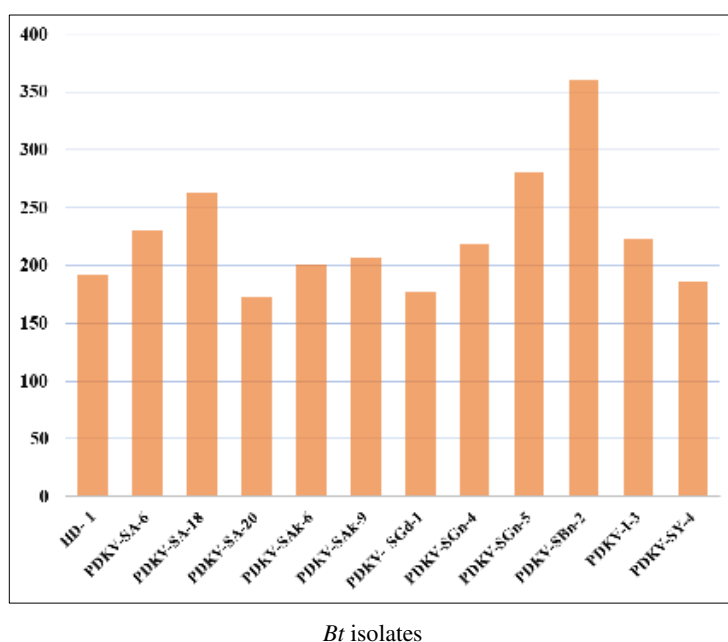


Fig 1: Median lethal concentration of local *Bt* isolates against *S. frugiperda*

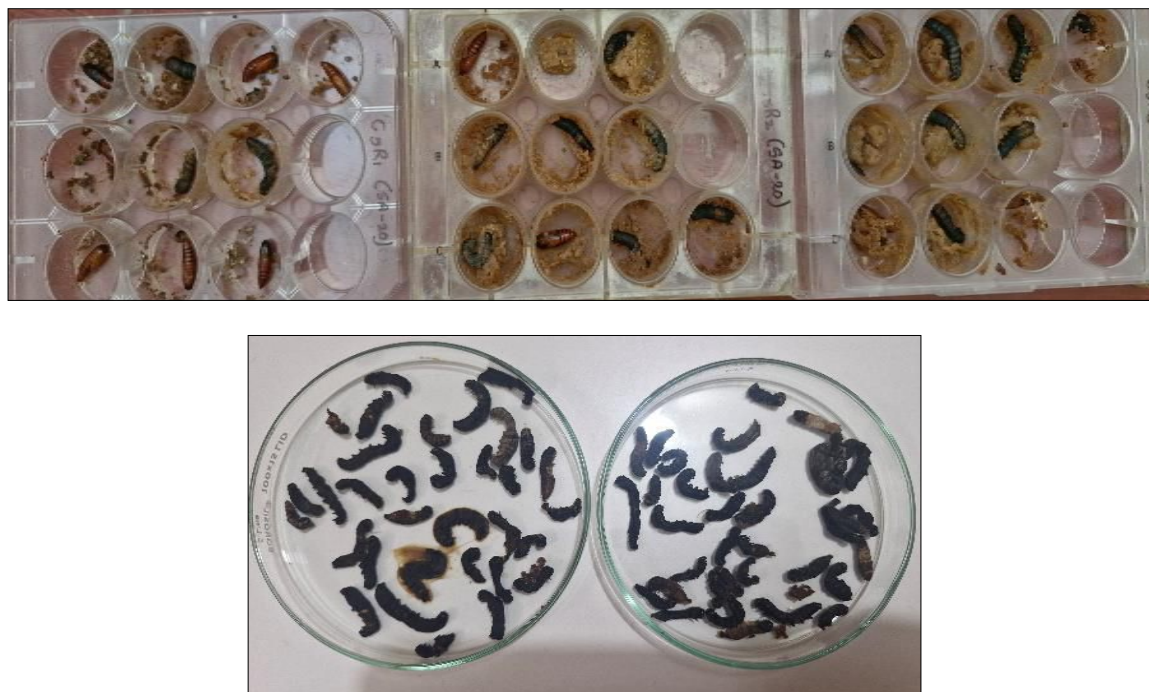


Fig 2: Mortality of *S. frugiperda* larvae treated with *B. thuringiensis* strains

3.2 Relative toxicity of *Bt* isolates to *Spodoptera frugiperda*

The data on relative toxicity were calculated and presented in (table 2) indicates that relative toxicity is greater in more toxic substance. It was found that SBn-2 (361.05 µg/ml) recorded highest LC₅₀ value, other LC₅₀ values were compared with SBn-2 and relative toxicity was calculated. The *Bt* isolate SA-20 and SGd-1 and SY-4 found 2.08-, 2.03- and 1.93-fold times more toxic than the lowest toxic *Bt* isolate i.e. SBn-2 followed by *Btk* HD-1 (1.88), SAK-6 (1.80-fold), SAK-9 (1.74-fold), SGn-4 (1.65-fold), I-3 (1.62-fold), SA-6 (1.56-fold), SA-18 (1.37-fold), SGn-5 (1.28-fold) and SBn-2 (1.00-fold).

Table 2: Relative toxicity of *Bt* isolates to *Spodoptera frugiperda*

Sr. No.	<i>Bt</i> isolates	LC ₅₀ (µg/ml)	Relative toxicity at LC ₅₀	Relative toxicity rank
1.	HD-1	191.41	1.88	4
2.	PDKV SA-6	229.97	1.56	9
3.	PDKV SA-18	262.70	1.37	10
4.	PDKV SA-20	172.90	2.08	1
5.	PDKV SAK-6	200.30	1.80	5
6.	PDKV SAK-9	207.12	1.74	6
7.	PDKV SGd-1	177.61	2.03	2
8.	PDKV SGn-4	218.60	1.65	7
9.	PDKV SGn-5	281.17	1.28	11
10.	PDKV SBn-2	361.05	1.00	12
11.	PDKV I-3	222.30	1.62	8
12.	PDKV SY-4	186.17	1.93	3

4. Conclusion

The bioassay studies of different *Bt* strains against *Spodoptera frugiperda* revealed that PDKV *Bt* strains SA-20, SGd-1 and SY-4 have higher toxicity than the standard *Btk* HD-1 strain. The three strains were found 2.08, 2.03 and 1.93-fold, respectively more toxic to *Spodoptera frugiperda* than lowest toxic strain i.e. SBn-2. PDKV *Bt* strains SAK-6, SAK-9, SGn-4, I-3, SA-6, SA-18, SGn-5 were found at par in toxicity with standard *Btk* HD-1 strain as the LC₅₀ values of these *Bt* isolates were lying within the range of fiducial limits of the standard *Btk* HD-1 strain. Hence, all these local

Bt isolates said to have equal toxicity potential to that of standard strain against *Spodoptera frugiperda* which can be used for further formulation studies and novel characterization of *Bt* isolates can be used for development of transgenic plants against FAW and other lepidopteran insects.

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