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IN VITRO EVALUATION OF NATIVE *BACILLUS THURINGIENSIS* (BERLINER) ISOLATES AGAINST DIFFERENT INSECTS

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ABSTRACT

Evaluation of native strains of *Bacillus thuringiensis* (Berliner) isolates against different orders of insects and characterization of responsible *Cry* genes was carried out. Among the native isolates, BGC-1 showed the least LC_{50} value of 5.24 µg/ ml and was comparable to the reference strain HD1 (2.89 µg/ ml) against tobacco caterpillar *Spodoptera litura* (Fabricius). The isolate RCM-1 recorded least LC_{50} value of 4.69 µg/ ml against the housefly *Musca domestica* Linnaeus. One isolate viz., GHB-1 was found to be potential against both the larvae of *S. litura* and *M. domestica.* The isolate RCM-2 registered the least LC_{50} value of 8.21 µg/ ml against grubs of red flour beetle *Tribolium castaneum* (Herbst). Several isolates had more than one *Cry* gene in them. Among them, 12 isolates (63.15%) were found to contain *Cry*3 genes, 11 isolates harbour *Cry*4 genes, five isolates had *Cry*1, *Cry*2 and *Cry*11 genes each and four isolates contain *Cry*7 genes each. Native isolate, GHB-1 had *Cry*1, *Cry*3, *Cry*4 and *Cry*11 genes.

Key words: *Spodoptera litura*, *Musca domestica*, *Tribolium castaneum*, *Cry* genes, pathogenicity, PCR amplification, specific primers, entomopathogenic bacteria, median lethal concentration, native *Bacillus thuringiensis* isolates, HD-1 strain, LC_{50}, bioassay

In the past half century, control of insect pests has relied exclusively on the use of synthetic organic insecticides. The application of insecticides though contributed to the effective management of insect pests but also led to the development of insecticide resistance, resurgence of sucking pests, contamination of soil, ground water and food materials. Considering severe ill effects associated with the injudicious use of insecticides in agriculture ml/l, there is an urgent need to minimize the use of synthetic chemical insecticides for the management of insect pests. For the increasing public concern over health hazards of synthetic organic pesticides, biopesticides seem to be one of the best alternatives for pest management. Control of insect pests of crops using entomopathogens is an ecologically sound pest management strategy. Among different entomopathogens, bacteria are widely used. Among bacteria, *Bacillus thuringiensis* (Berliner) is a rod shaped, facultative, aerobic, endospore forming bacterial species, which is highly pathogenic to insects. During reproductive phase, it produces spore and one or more crystalline parasporal inclusions known as crystal. These δ-endotoxins are referred as *Cry* proteins and vary among different *B. thuringiensis* strains. Based on *Cry* proteins *B. thuringiensis* was classified into four pathotypes; *Cry*I (Lepidoptera specific toxin), *Cry*II (Lepidoptera and Diptera specific toxins), *Cry*III (Coleoptera specific toxin), *Cry*IV (Diptera specific toxin). *Cry* proteins are named according to their amino acid similarity to established holotype proteins (Crickmore et al., 2021). Strains belonging to one subspecies attack predominantly one family of insects. For example, *B. thuringiensis* sub sp. *kurstaki* infect lepidopteran insect pests, *B. thuringiensis* sub sp. *israelensis* infect dipteran insect pests and *B. thuringiensis* sub sp. *tenebrionis* infect coleopteran insect pests (Malovichko et al., 2019). The use of bio pesticides in India is limited by manufacturing technology and the available commercial products are imported and expensive in comparison to chemicals. The development of *B. thuringiensis* products based on native Indian strains may promote their use in India.

Identification of suitable strains of bacteria against different group of insects would be expected to support their use in integrated pest management programs. Hence an attempt was made to evaluate the native strains of *B. thuringiensis* against insect species viz.,

Spodoptera litura, Tribolium castaneum (Herbst) and *Musca domestica* Linnaeus as a representative of Lepidoptera, Coleoptera and diptera. Further to identify the *Cry* genes in the promising isolates which induce pathogenicity on these insect groups. With this background investigations were carried out with the objectives of evaluation of native strains of *B. thuringiensis* isolates against *S. litura*, *T. castaneum* and *M. domestica* and to characterize the *Cry* genes profile of the selected native *B. thuringiensis* isolates.

MATERIALS AND METHODS

Mass rearing of *S. litura* was carried out by collecting larvae from the field and maintained on healthy castor leaves till pupation and adult emergence. Adults were provided with castor leaves for egg laying and culture was maintained. Similarly, mass rearing of *M. domestica* was carried out by collecting insects from UAS, Raichur campus. Adult flies were provided with sugar solution, milk powder and water. Larvae were fed with artificial diet containing wheat bran, sugar, and yeast. Two to three days old maggots were used for bioassay studies. Mass rearing of *T. castaneum* was also established by the adults collected from central warehouse. Adults were reared on disinfested crushed wheat. For bioassay 3rd instar grubs of *T. castaneum* were used. A preliminary bioassay of native and reference strain HD-1 of *B. thuringiensis* was carried out. The cultures were centrifuged, pellet was re-suspended in 1ml sterile distilled water. Artificial diet for rearing of *S. litura* was prepared as suggested by Punia et al. (2021) used for bioassay studies. The procedure for conducting bioassay was followed as suggested by Vimala Devi and Vineela (2014). The diet was poured into multi-cavity trays, bacterial suspension with Tween-80 (0.02%) was overlaid (146 µl) on the diet surface. One pre-starved (4 h) second instar larva of *S. litura* was released in each well. A total of 40 larvae were used for each concentration. Observation was recorded at 1, 2, 3, 4 and 5 days after treatment and mortality was calculated. Similarly, bioassay was conducted against *M. domestica*. The bacterial suspension was poured into each Petri plate @ 1ml and maggot diet of 1gm was poured in each plate and mixed well. Ten, one-day old maggots of *M. domestica* were released in each petriplate. A total of 40 maggots were used for each concentration. The observations on mortality were recorded at 1, 2, 3, 4 and 5 days after treatment. Mortality was calculated as mentioned above. Similarly, bioassay was also conducted against *T. castaneum.* The bacterial suspension @ 5 ml was treated with 10 gm of broken sorghum grains for each treatment and kept in laminar air flow for 30 minutes to absorb the treated *B. thuringiensis* crystal proteins. Ten, 3rd instar grubs of *T. castaneum* were released for each concentration. A total of 40 grubs were used for each concentration. The observations on mortality were recorded at 2, 4, 6, 8 and 10 days after treatment. Mortality was calculated as mentioned in above procedure.

The bacterial isolates showing more than 85.00% mortality against test insects were selected and subjected for lyophilization to establish median lethal concentration (LC_{50}) by conducting bioassay. The procedure followed for preliminary bioassay against all test insects were also used to establish median lethal concentration (LC_{50}). The data generated were subjected to statistical analysis by completely randomized design. After arcsine transformation, data was subjected to duncan's multiple range test. Median lethal concentration (LC_{50}) was calculated by probit analysis.

Characterization and analysis of responsible *Cry* genes from selected native isolates of *B. thuringiensis* was carried out. Total DNA was isolated from 19 *B. thuringiensis* isolates ((HD-1(reference strain), BGC-1, BGC-2, RCM-1, RCM-2, KMF, GHM-1, GHM-2, GBP-1, GBP-2, KMS-1, KMS-2, MDS-1, MDS-2, GHB-1, GHB-2, MDC, MDH, GPP-1, GHP)) by following the standard protocol and purity and yield of the DNA were assessed by nanodrop method. To investigate the presence of different *Cry* genes, the polymerase chain reaction was employed using specific primers which are already published. *Cry* gene profiling was carried out using specific primers. The specific primers used for the PCR amplification of *Cry* genes are *viz.*, *Cry* 1 (Das et al., 2021), *Cry*2, *Cry*3 (Das et al.,2021), *Cry*4 (Hassan et al., 2021), *Cry*7, *Cry*11 (Das et al., 2021). Pair of primers were used each time to analyze the *Cry* profile of the 19 *B. thuringiensis* isolates by PCR amplification. For PCR reaction standard procedure and reaction mixture were used with an annealing temperature of 52°C. Separation of amplicons by agarose gel electrophoresis. *Cry* gene profiling: The amplicons obtained were compared with each other and the amplicons or DNA bands were scored as presence (positive) or absence (negative) of the *Cry* genes among the isolates.

RESULTS AND DISCUSSION

The results of the preliminary screening against

S. litura indicated that five days after treatment, highest mortality was recorded with 100.00% in reference strain HD-1. Among the native isolates, the significantly highest mortality of 96.66% was recorded in isolate BGC-1 followed by the isolates GBP-2 and GHB-1 (Table 1). The results are in line with the reports of Lalitha et al. (2012) who reported that the native *B. thuringiensis* strains caused mortality ranged from 16.67% to 94.44% after 98 h of feeding. Similarly, Saroja, (2017) revealed that the twenty *B. thuringiensis* strains were toxic to second instar larvae of *H. armigera* and caused the mortality ranging from 36.66% to 100.00% after 120 h of feeding. Results of the preliminary screening against *M. domestica* indicated that, 5 days after exposure, the native isolates, GHB-1 and MDC isolates were recorded significantly highest mortality of 96.66% followed by the isolate RCM-1. The results are in agreement with reports of Lonc et al. (1997), who evaluated crude suspension of *B. thuringiensis* spores and *Cry*stals against *M. domestica, Drosophila melanogaster* and cockroach species. Generally, all strains showed low (<50 % mortality) toxic effects against fruit fly larvae and nymph of cockroaches, in contrast with high toxic effects (up to 90% mortality) against housefly larvae. The susceptibility of larvae of *D. melanogaster* varied between 0 to 18%, nymphal stages of *B. germanica, B. orientalis and P. americana* seemed to be more susceptible (0 to 44.9%) and increased toxicity of all tested isolates was observed in the experiments with larvae of *M. domestica*. Preliminary screening was also conducted against *T. castaneum*, significantly highest

Table 1. Preliminary evaluation of native isolates of *B. thuringiensis* against *S. litura*, *M. domestica* and *T. castaneum* in the laboratory

Sl. No.	Isolates	% mortality of *S. litura*	% mortality of *M. domestica*	% mortality of *T. castaneum*
		5 DAT	5 DAT	10 DAT
1	HD-1(ref.)	100 (71.80) [a]	0.00 (0.00) [h]	0.00 (0.00) [f]
2	BGC-1	96.66 (68.08) [b]	53.33 (42.89) [bcdef]	73.33 (52.10) [bc]
3	BGC-2	83.33 (56.99) [abc]	50.00 (41.39) [cdefg]	83.33 (56.99) [ab]
4	RCM-1	50.00 (41.39) [e]	90.00 (62.53) [a]	83.33 (56.99) [ab]
5	RCM-2	76.66 (53.60) [bcd]	60.00 (45.95) [bcd]	93.33 (66.26) [a]
6	KMF	60.00 (45.95) [def]	40.00 (36.61) [fg]	56.66 (44.46) [d]
7	GHM-1	50.00 (41.39) [e]	36.66 (35.04) [g]	73.33 (52.10) [bc]
8	GHM-2	43.33 (38.32) [e]	66.66 (48.95) [b]	86.66 (58.81) [a]
9	GBP-1	76.66 (53.60) [bcd]	56.66 (44.46) [bcde]	63.33 (47.45) [cd]
10	GBP-2	93.33 (66.26) [ab]	50.00 (41.39) [cdefg]	66.66 (48.95) [cd]
11	KMS-1	53.33 (42.89) [ef]	63.33 (47.45) [bc]	63.33 (47.45) [cd]
12	KMS-2	73.33 (52.10) [cd]	60.00 (45.95) [bcd]	60.00 (45.95) [d]
13	MDS-1	53.33 (42.89) [ef]	43.33 (38.32) [efg]	60.00 (45.95) [d]
14	MDS-2	60.00 (45.95) [def]	43.33 (38.32) [efg]	73.33 (52.10) [bc]
15	GHB-1	86.66 (58.81) [abc]	96.66 (68.08) [a]	73.33 (52.10) [bc]
16	GHB-2	50.00 (41.39) [e]	83.33 (56.99) [a]	73.33 (52.10) [bc]
17	MDC	46.66 (39.89) [e]	96.66 (68.08) [a]	66.66 (48.95) [cd]
18	MDH	73.33 (52.10) [cd]	46.66 (39.89) [defg]	83.33 (56.99) [ab]
19	GPP-1	70.00 (50.52) [cde]	56.66 (44.46) [bcde]	33.33 (35.27) [e]
20	GHP	76.66 (53.60) [bcd]	43.33 (38.32) [efg]	73.33 (52.10) [bc]
21	Control	0.00 (0.00) [g]	0.00 (0.00) [h]	0.00 (0.00) [f]
S. Em ±		1.09	1.02	1.26
CD@1% (P=0.01%)		2.63	2.99	2.74
CV (%)		4.22	4.32	4.16

*Cry genes detected through PCR amplification in all the native Bt isolates; Cry 1: Isolates GBP-2, BGC-1, GHP, GHB-1 and GHB-2 showed positive (26.31% of total Bt isolates); Cry 2 : Isolates BGC-2, KMS-1, GHP, MDH and GPP-1 showed positive (26.31% of total Bt isolates); *Cry*3 : Isolates BGC-2, GHM-2, GHM-1, KMF, GHB-2, GHB-1,MDH, MDS-2, GBP-2, RCM-2, RCM-1 and GHP showed positive (63.15% of total Bt isolates); *Cry*4: Isolates GHB-1, GHB-2, RCM-1, MDS-1, MDS-2, MDC, MDH,GHM-1, GHM-2, GBP-1 and GPP-1 showed positive (57.89% of total Bt isolates); *Cry7:* Isolates BGC-2, RCM-2, GHM-2 and MDH showed positive (21.05% of total Bt isolates); *Cry11:* Isolates HB-1, GHB-2, MDC, RCM-1 and GHM-2 showed positive (26.31% of total Bt isolates)

mortality of 93.33% and 86.66% were recorded in native isolates namely, RCM-2 and GHM-2 respectively ten days of treatment (Table 1). However, native strains which recorded more than 85.00% mortality were further selected to establish LC_{50} values against different insects. The native strains viz., BGC-1, GBP-2 and GHB-1,RCM-1,GHB-1 and MDC, RCM-2 and GHM-2 were used against *S. litura*, *M. domestica* and *T. castaneum* respectively.

Median lethal concentration (LC_{50}) of *B. thuringiensis* isolates against *S. litura, M. domestica* and *T. castaneum* was established. The LC_{50} value of reference strain HD-1 was found to be lowest 2.89 µg/ ml with fiducial limit ranging from 1.01 to 8.28 values, which was comparable with the BGC-1 isolate, was 5.24 µg/ ml with fiducial limit ranging from 1.82 to 15.03 value (Table 2). The results are in line with the reports of Punia et al. (2021), who established LC_{50} values against *S. litura*, indicated that mortality increases as a progressive increase in the dose required to cause 50.00% reported. Among three native isolates (RCM-1, GHB-1 and MDC) of *B. thuringiensis*, the isolate RCM-1 was more virulent against *M. domestica* with lowest LC_{50} values of 4.69 µg/ ml with fiducial limit ranging from 1.91 to 11.51 value. As the concentration of isolates increases mortality also increases (Table 2). The results of our present findings are corroborated with the results of Zimmer et al. (2013) evaluated *B. thuringiensis* var. *israelensis, B. thuringiensis* var. *kurstaki and Brevibacillus laterosporous* against *M. domestica* at the concentration of $1x10^7$, $1x10^8$ and $1x10^9$ CFU/ ml recorded the mortality of 53.00%, 40.00% and 34.00% respectively. Similarly, LC_{50} values were also calculated against *T. castaneum*. Among native isolates *B. thuringiensis* (RCM-2 and GHM-2), RCM-2 isolate was found more virulent with the lowest LC_{50} value of 8.21 µg/ ml with fiducial limit ranging from 3.06 to 22.00 values (Table 2). The dose mortality response of *T. castaneum* to the different concentrations of *B. thuringiensis* indicated that slight variation in mode of action. Such differences in the insecticidal activity of different native isolates of *B. thuringiensis* might be ascribed to the difference in the carbohydrate affinity of the domain II of the *Cry* proteins resulting in variable binding specificity with the receptors at the brush border membrane of the insect larvae leading to differences in toxicity of the *Cry* protein. Also, may be due to the presence of acidic pH in the midgut of grubs of *T. castaneum* may registered with differential toxicity at the dosages which were used the current study against *T. castaneum*. The present findings are in conformity with the results of Sabbour and Moharam (2014), reported the LC_{50} of the different bacterial strains recorded 79, 67, 66, 44 and 74 µg/ ml after *T. confusum* treated with different concentrations of *B. thuringiensis J, B.thuringiensis* 0900, *B. thuringiensis* NRRL 2172, *B. thuringiensis* IP *thuricide* and *B. thuringiensis* HD112, respectively. The corresponding LC_{50} of *T. castaneum* are 77, 65, 65, 39 and 44 µg/ ml, respectively.

Characterization and analysis of responsible *Cry* genes profile from selected native isolates of *B. thuringiensis* was carried out. *Cry*1 primer (290 bp) : Among 19 *B. thuringiensis* isolates *viz*., GBP-2 (93.33%), BGC-1 (96.66%), GHP (76.66%), GHB-1 (86.66%) and GHB-2 (50.00%) were amplified with *Cry*1 genes, they shown more than 50% mortality and

Table 2. Concentration mortality response (LC_{50}) of *S. litura, M. domestica, T. castaneum* to lyophilized native isolates of *B. thuringiensis*

Sl. No.	Isolates	LC_{50} (µg/ml)	Fiducial limit		Regression equation	χ^2 value
			Lower limit	Upper limit		
			S. litura			
1	HD-1	2.89	1.01	8.28	Y = 4.790206 + 0.4590316x	7.95
2	BGC-1	5.24	1.82	15.03	Y = 4.680234 + 0.4443457x	4.82
3	GBP-2	8.65	2.83	26.40	Y = 4.60274119 + 0.4239566x	2.06
4	GHB-1	21.31	7.20	63.10	Y = 4.3896317 + 0.4593534x	3.69
			M. domestica			
1	RCM-1	4.69	1.91	11.51	Y = 4.6332188 + 0.5460917x	1.10
2	GHB-1	10.86	3.45	34.13	Y = 4.5692062 + 0.4158031x	2,33
3	MDC	21.93	7.75	62.05	Y = 4.345763 + 0.4877944x	3.09
			T. castaneum			
1	RCM-2	8.21	3.06	22.00	Y = 4.5509096 + 0.4910087x	1.68
2	GHM-2	20.11	6.64	60.85	Y = 4.417649 + 0.4467752x	2.94

reference strain HD-1 shown mortality of 100% against III instar larvae of *S. litura* (Table 1). Only 26.31% (5 isolates) of the isolates showed PCR amplification for *Cry*1 primer. Our results were in concurrence with the findings of Ohba and Aizawa (1986), who reported only 10% of 186 *B. thuringiensis* isolates showed more than 50 % mortality against *Bombyx mori*, which contains *Cry*1 genes to be the reason for activation of crystal proteins. *Cry*2 primer (1300 bp): Among 19 *B. thuringiensis* isolates, five native isolates (26.31%), namely BGC-2, KMS-1, GHP, MDH and GPP-1 amplified band of 1300 base pairs for *Cry*2 gene (Table 1), which caused the mortality of 83.33, 53.33, 76.66, 73.33 and 70.00% respectively against III instar larvae of *S. litura* (Table 1). Reference strain HD-1 recorded mortality of 100.00 % (Table 1).

Cry 4 primer (500 bp): Out of 19 *B. thuringiensis* isolates, 11 native isolates (57.89%) viz., GHB-1, GHB-2, RCM-1, MDS-1, MDS-2, MDC, MDH, GHM-1, GHM-2, GBP-1 and GPP-1 were amplified band of 500bp (Table 1), recorded mortality of more than 50 % in isolates namely, GHB-1 (96.66%), GHB-2 (83.33%), RCM-1 (90.00%), MDC (96.66%), GHM-2 (66.66%), GBP-1 (56.66%) and GPP-1 (56.66%) against *M. domestica* (Table 1). Mortality data against *M. domestica* was in correlation with *Cry* gene profile of the isolates, confirming the presence of *Cry* 4 protein. Reference strain HD-1 was not amplified for *Cry*4 primer and it does not showed any mortality against *M. domestica*.

*Cry*11 primer (376 bp): Among 19 *B. thuringiensis* isolates, 5 isolates (26.31%) namely, GHB-1, GHB-2, MDC, RCM-1 and GHM-2 were showed amplification of band size 376 bp (Table 1), caused 96.66, 83.33, 96.66, 90.00 and 66.66% mortality, respectively (Table 1). Reference strain HD-1 was not amplified with *Cry*11 primer shown mortality of 0.0% against first instar maggots of *M. domestica* (Table 1). In the present study, *B. thuringiensis* isolates such as BGC-1, BGC-2, RCM-2, GBP-2, KMS-1 and KMS-2 were not amplified for both the dipteran specific primers like *Cry*4 and *Cry*11 but they recorded more than 50% mortality. It might be due to the result of the production of active crystal proteins, i.e., a gene could be under the control of an efficient promotor (Porcar and Juarez-perez, 2003). Also, may be due to the presence and expression of other dipteran specific *Cry* proteins other than *Cry*4 and *Cry*11 crystal proteins resulting in the larval mortality of *M. domestica.*

*Cry*3 primer (800 bp): Out of 19 *B. thuringiensis* isolates, 12 native isolates (63.15%) viz., BGC-2, GHM-2, GHM-1, KMF, GHB-2, GHB-1, MDH, MDS-2, GBP-2, RCM-2, RCM-1 and GHP were amplified band size of 800 bp and found positive for *Cry*3 primer (Table 1). In present study, *B. thuringiensis* isolates *viz*., BGC-1 (83.33%), GHM-2 (86.66%), GHM-1 (73.33%), KMF(56.66%), GHB-2 (73.33%), GHB-1 (73.33%), MDH (83.33%), MDS-2 (73.33%), GBP-2 (66.66%), RCM-2 (93.33%) and GHP (73.33%) were amplified with *Cry*3 gene shown more than 50% mortality against III instar grubs of *T. castaneum* (Table 1). The high mortality may be due to the presence of *Cry*3 genes or the insecticidal activity of other coleopteran specific crystal proteins. They produced crystals and showed insecticidal activity of more than 50 %, possibly as a result of the production of active crystal proteins.

*Cry*7 primer (700 bp): Among 19 *B. thuringiensis* isolates, 4 isolates (21.05%) namely, BGC-2, RCM-2, GHM-2, were observed amplification products band size of 700 bp for *Cry*7 (Table 1). In the present study, *B. thuringiensis* isolates *viz*., BGC-2 (83.33%), RCM-2 (93.33%), GHM-2 (86.66%) and MDH (83.33%) were amplified with *Cry*7 gene registered more than 50.00% mortality against III instar grubs of *T. castaneum.* This may due to presence of *Cry*7 genes or may be other coleopteran specific crystal proteins responsible for the mortality. The reference strain HD-1 was not showed amplification for *Cry*7 primer because it does not harbor the coleopteran specific *Cry* genes and showed zero % mortality against *T. castaneum,* since midgut of grubs has acidic pH so that *Cry* proteins of HD-1 strain cannot solubilize in to activated protoxin forms.

The reference strain HD-1 positive for *Cry*1 and *Cry*2 genes and most of the isolates had more than one *Cry* genes, among the isolates studied, GHB-1 and GHB-2 were harbored more than one *Cry* genes like *Cry*1, *Cry*3, *Cry*4 and *Cry*11. Isolate MDH contains *Cry*2, *Cry*3, *Cry*4 and *Cry*7 genes. Isolate, BGC-2 contains *Cry*2, *Cry*3 and *Cry*7 genes, isolate, RCM-1 harbors *Cry*3, *Cry*4 and *Cry*11 genes, GHM-2 isolate harbors *Cry*3, *Cry*4 and *Cry*7 genes. Similarly, GHP was positive for *Cry*1, *Cry*2 and *Cry*3 genes, isolates, GHM-1 and MDS-2 were found to be positive for *Cry*3 and *Cry*4 genes each. Isolate RCM-2 was positive for *Cry*3 and *Cry*7 genes, GBP-2 with *Cry*1 and *Cry*3 genes, MDC harbors *Cry*4 and *Cry*11 genes. Similarly, native isolates BGC-1, KMF, GBP-1, KMS-1, MDS-1 and GPP-1 were found to harbors *Cry*1, *Cry*3, *Cry*4, *Cry*2, *Cry*4, *Cry*2 genes respectively. Only one isolate called KMS-2 was not amplified and showed the presence

of none of the *Cry* genes (primers that were used to identify *Cry* genes in the current study) but it may harbor other than screened *Cry* genes which are responsible for insecticidal activity against test insects used in the current study (Table 1). It is known that *B. thuringiensis* isolates are conjugate in nature and *B. thuringiensis* isolates have long been known to contain plasmids of variable number and size in different isolates. Most of the *Cry* genes are found on plasmids and horizontal transfer may results in the creation of new strains or isolates with a novel complement of *Cry* genes (Arsov et al., 2023). *B. thuringiensis* isolates may lose its plasmids, coding crystal toxins genes, spontaneously or by growing at elevated temperatures (Naveenarani, et al., 2022). Thus, *B. thuringiensis* isolates are potential to have varied number and size of plasmids. So that *B. thuringiensis* isolates can have a variable number of *Cry* genes responsible for the synthesis of different endotoxins. That is the reason why most *B. thuringiensis* isolates can able to form more than one type of crystalline inclusion bodies.

In results indicated that *B. thuringiensis* isolates can have variable number of *Cry* genes responsible for the synthesis of different endotoxins. That is the reason why most *B. thuringiensis* isolates can form more than one type of crystalline inclusion. Despite the variability of *Cry* proteins and the range of susceptible organism, a significant number of insects that cause great losses on crop production are not sensitive to the commercially available *Bt* toxins. Besides, resistance has been reported in some insects to *B. thuringiensis* based toxins (Tabashnik, 2020). Thus, the identification and characterization of natural strains of bacteria could help in discovering proteins showing a wider range of insecticidal activity. Our results were corroborated with the study of *Cry* gene profile of selected eleven isolates with lepidopteran specific primers by Prabhakar (2011). *Cry*1 was amplified in Tx-201, Tx-202, Tx-29, Tx-232, AIM-63(2) and AIM-213(1) isolates. *Cry*2 was observed in Tx-202, AIM-63(2), Tx-232, AIM-72(2) isolates. *Cry*8 was found in Tx-379, AIM63 (2) isolates. Whereas *Cry*9 was amplified in 531/a and AIM-72(2) isolates which were lepidopteran toxic but *Cry*20 gene was not found in any of the isolates which was Lepidopteran and Dipteran specific (De Maagd et al., 2001). In a similar study, Valtierra-de-Luiset al.,(2020) reported that 1707/5 possess *Cry*20, which is toxic to both lepidopteran and dipteran larvae. Yadav (2007) reported that *Cry*2 gene was the most common (48.11%) from three different locations. Gislayne et al. (2004) found that *Cry*1 gene was the most abundant (48.0%). According to Kayam et al. (2020) PCR analysis revealed dominance of *Cry*1 and *Cry*2 genes in the alluvial soil of Mahi River basin isolates.

The present study concludes that, among 19 *B. thuringiensis* isolates BGC-1, GBP-2 and GHB-1caused mortality ranged from 85 to 100 percent against second instar larvae of *S. litura* with the least LC_{50} value of 5.24µg/ ml (BGC-1). The isolates RCM-1, GHB-1 and MDC found promising against *M. domestica* with minimum LC_{50} value of 4.69µg/ ml (RCM-1). Similarly, RCM-2 and GHM-2 caused highest mortality against *T. castaneum* with least LC_{50} value of 8.21µg/ ml in RCM-2. Among 19 *B.t.* isolates, 12 isolates (63.15%) were found to contain *Cry*3 genes, 11 isolates (57.89%) were found to harbour *Cry*4 genes, five isolates (26.31%) had *Cry*1, *Cry*2 and*Cry*11 genes each and four isolates (21.05%) were found to contain *Cry*7 genes each. Native isolate, GHB-1 had *Cry*1, *Cry*3, *Cry*4 and *Cry*11 genes and was found potential to both *S. litura* and *M. domestica.*

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AUTHOR CONTRIBUTION STATEMENT

Basavaraj S Kalmath conceptualized and framed the research proposal. Hareesh Shiralli conducted the experiment, curated data and prepared original draft. Prabhuraj Aralimarad and Ayyanagouda Patilhad given the technical suggestions. Arunkumar Hosamani contributed to the samples, analyzed the results and corrected draft. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Arsov A, Gerginova M, Paunova-Krasteva T, Petrov K, Petrova P. 2023. Multiple cry genes in *Bacillus thuringiensis* strain BTG suggest a broad-spectrum insecticidal activity. International Journal of Molecular Sciences 24(13): 11137.

Crickmore N, Berry C, Panneerselvam S, Mishra R, Connor T R, Bonning B C. 2021. A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. Journal of Invertebrate Pathology 186: 107438.

Das S K, Pradhan S K, Samal K C, Singh N R. 2021. Structural, functional, and evolutionary analysis of Cry toxins of *Bacillus thuringiensis*: an in-silico study. Egyptian Journal of Biological Pest Control 31: 1-14.

De Maagd R A, Bravo A, Crickmor N. 2001. How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends in Genetics 17(4): 193199.

Gislayne T, Vilas-Boas, Manoel V, Franco L. 2004. Diversity of *Cry* genes and genetic characterization of *Bacillus thuringiensis* isolated from Brazil. Canadian Journal of Microbiology 50: 605-613.

Groot A T, Dicke M. 2002. Insect resistant transgenic plants in a multi tropic content. Journal of Plant Protection Research 31: 387-406.

Juarez -Perez V M, Ferrandis M D, Frutox R. 1997. PCR based approach for detection of novel *Bacillus thuringiensis Cry* genes. Journal of Applied and Environmental Microbiology 63: 2997-3002.

Kayam D, Tirupati M K, Karanam H P. 2020. Diversity of *Bacillus thuringiensis Cry* genes in soils of Andhra Pradesh, India. Indian Journal of Biochemistry and Biophysics 57(4): 471-480.

Lalitha C, Muralikrishna T, Sravani S, Devaki K. 2012. Laboratory evaluation of native *Bacillus thuringiensis* isolates against second and third instar *Helicoverpa armigera* (Hubner) larvae. Journal of Biopesticide 5(1): 4-9.

Lonc E, Lecadet M, Lachowicz T M, Panek E. 1997. Description of *Bacillus thuringiensis* subsp.*wratislaviensis* (H-47), a new serotype originating from Wroclaw (Poland) and other *Bt* soil isolates from the same area. Letters in Applied Microbiology 24: 467-473.

Malovichko Y V, Nizhnikov A A, Antonets K S. 2019. Repertoire of the *Bacillus thuringiensis* virulence factors unrelated to major classes of protein toxins and its role in specificity of host-pathogen interactions. Toxins 11(6): 347.

Manuel P, Victor J P. 2002. PCR-based identification of *Bacillus thuringiensis* pesticidal crystal genes. FEMS Microbiology 24: 419-432.

Naveenarani M, Suresha G S, Srikanth J, Hari K, Sankaranarayanan C, Mahesh P, Singaravelu B. 2022. Whole genome analysis and functional characterization of a novel *Bacillus thuringiensis* (Bt 62) isolate against sugarcane white grub *Holotrichia serrata* (F). Genomics 114(1): 185-195.

Ohba M, Aizawa K. 1986. Insect toxicity of *Bacillus thuringiensis* isolated from soils of Japan. Journal of Invertebrate Pathology 47(1): 12-20.

Porcar M, Juarez-Perez V. 2003. PCR based identification of *Bacillus thuringiensis* pesticidal crystal genes. FEMS Microbiology Reviews 26(5): 419-432.

Prabhakar. 2011. Bioefficacy and molecular characterization of native *Bacillus thuringiensis* (Berliner) isolates against lepidopteran pests of cabbage. MSc (Ag) Thesis Agriculture Entomology UAS Dharwad pp: 79.

Punia A, Chauhan N S, Singh D, Kesavan A K, Kaur S, Sohal S K. 2021. Effect of gallic acid on the larvae of *Spodoptera litura* and its parasitoid *Bracon hebetor*. Scientific Reports 11(1): 531.

Sabbour M, Moharam M E. 2014. Evaluation of *Bacillus* species against red flour beetle, *Tribolium castaneum* and confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) under laboratory and stored condition. European Journal of Academic Essays 1(10): 36-41.

Saroja. 2017. Development and evaluation of native *Bacillus thuringiensis* (Berliner) formulations against *Helicoverpa armigera* (Hubner). M.Sc. agric Thesis UAS Raichur pp: 135.

Tabashnik B E, Carrière Y. 2020. Evaluating cross-resistance between vip and cry toxins of *Bacillus thuringiensis*. Journal of Economic Entomology 113(2): 553-61.

Valtierra-de-Luis D, Villanueva M, Berry C, Caballero P. 2020. Potential for *Bacillus thuringiensis* and other bacterial toxins as biological control agents to combat dipteran pests of medical and agronomic importance. Toxins 12(12): 773.

Vimala Devi P S, Vineela V. 2014. Suspension concentrates formulation of *Bacillus thuringiensis* var. *kurstaki* for effective management of *Helicoverpa armigera* on sunflower (*Helianthus annuus*). Bioscience and Technology 25(3): 329-336.

Yadav B P. 2007. Molecular characterization of *Cry*/*Vip* genes and efficacy of native *Bacillus thuringiensis* isolates. M. Sc (Agri) Thesis Plant Biotechnology UAS Dharwad pp: 129.

Zimmer C R, Castro L D, Pires S M, Menezes A D, Ribeiro P B, Leite F L. 2013. Efficacy of entomopathogenic bacteria for control of *Musca domestica*. Journal of Invertebrate Pathology 114(3): 241-244.

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