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Pathogenicity of indigenous soil isolate of *Bacillus thuringiensis* to *Helicoverpa armigera* Hübner 1809 (Lepidoptera: Noctuidae)

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Abstract

This study evaluated the pathogenicity of indigenous soil isolate of *Bacillus thuringiensis* (*Bt*) strains, applied without and along with 1.0% $MgCl_2$ salt, to *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Toxicity and effect of *Bt* isolate on larval development (weight) were assessed using in vitro bioassays. Six concentrations of the tested *Bt* with salt (i.e., 1.0×10^7, 0.5×10^5, 1.0×10^5, 1.5×10^5, and 2.0×10^5 cfu/ml), five without salt (i.e., 0.5×10^5, 1.0×10^5, 1.5×10^5, and 2.0×10^5 cfu/ml), and control were bioassayed against third-instar larvae of *H. armigera* under a complete randomized design (CRD), with four replications. Results revealed that both larval mortality and weight changes were significantly affected by time ($F_{5, 19} = 35.98$; $P < 0.001$ and $F_{5, 19} = 11.01$; $P < 0.001$, respectively) and treatments ($F_{5, 19} = 27.45$; $P < 0.001$ and $F_{5, 19} = 25.07$; $P < 0.001$). The highest larval mortality (91.1%) was exhibited by the highest concentration (1.0×10^7 cfu/ml), followed by 2.0×10^5 cfu/ml concentration, without salt (88.9%) and with salt (66.7%). Median lethal concentration (LC_{50}) values of isolated *Bt* strain were 1.7 and 1.27×10^5 cfu/ml, without salt, and 1.80 and 1.13×10^5 cfu/ml, with salt, at 96 and 120 h, respectively. Regarding the impact of *Bt* isolate on larval development, treatment with the highest concentration (1.0×10^7 cfu/ml) had the most significant and negative impact on larval weight change ($R^2 = 0.53$), followed by 2.0×10^5 cfu/ml *Bt* concentration ($R^2 = 0.40$). There was no obvious synergistic or additive effect, but rather an inhibition was observed on the pathogenicity potential or larvicidal effect of *Bt* isolate.

Keywords: *Bacillus thuringiensis*, Microbial insecticides, *Helicoverpa armigera*, Toxicity evaluation

Background

Lepidopterous species are among the most devastating and economically important insect pests causing quantitative and qualitative losses of the yields all over the world (Ndemah et al. 2001; Mazzi and Dorn 2012). *Helicoverpa* spp. and *Spodoptera* spp. are the most damaging and cosmopolitan pest species. These species have well-adapted different agro-ecological zones around the globe from Asia to Africa to Europe (Zhang et al. 2015). They are polyphagous pests with a wide range of host plants including: cotton, maize, gram, sesame, okra, tomato, potato, and many other fruit and vegetable crops (Aggarwal et al. 2006; Nagoshi et al. 2011). Nevertheless, due to blind and extensive use of pesticides, these and other lepidopterous pest species have developed resistance to almost all groups of pesticides including organochlorines, organophosphates, carbamates, and pyrethroids (Kranthi et al. 2001).

Microbial bio-pesticides are eco-friendly and target specific alternates to hazardous synthetic pesticides (Kumar and Singh 2015; Majeed et al. 2017). Worldwide, an annual increase of 10% in the use of bio-pesticides has been estimated and among them, approximately 90% formulations are derived from *Bacillus thuringiensis* (*Bt*) (Kumar and Singh 2015; Osman et al. 2015). However, there is a great potential to look for the indigenous strains and isolates of *Bt* and characterize their pathogenicity and toxicity against insect pest species (Sree and Varma 2015).

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The present study assessed the toxicity of local soil isolates of *Bt* against *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), a notorious pest of cotton, maize, gram, okra, sesame, and other crops in Indo-Pak region (Bibi et al. 2013; Qayyum et al. 2015). Moreover, as the sporulation of *Bt* has been found enhanced in the presence of certain inorganic ions particularly chlorides of Ca and Mg (Tabbene et al. 2009; Bibi et al. 2013), isolates of *Bt* toxins and spores were treated with $MgCl_2$ salt. Apart from determination of lethal concentrations (mortality), the effect of *Bt* strain on the larval development (larval weight) was also assessed.

Methods

Collection and preparation of samples

Composite soil samples were collected from cultivated and non-cultivated fields from different locations of the district Sargodha (Punjab, Pakistan). Sampling sites were confirmed to have received no application of pesticide including *Bt* formulation. Samples were brought to the laboratory and homogenized. Five grams of aliquot samples was taken from this large composite soil sample for further processing and isolation of *Bt* strains.

Preparation of soil solutions

Five grams of each soil sample was taken and dissolved in 10 ml of sterilized water and was thoroughly homogenized on orbital shaker. One milliliter of this solution was put in 9 ml sterilized water in a sterilized test tube and was homogenized by shaking. From this stock solution, serial dilutions were prepared using double distilled autoclaved water.

Preparation of culture media

Nutrient agar is widely used as a general purpose medium for culturing a large number of microorganisms. Nutrient broth and nutrient agar powder (Merck KGaA, Darmstadt, Germany), 5 g each, was dissolved in 250 ml distilled water and autoclaved at 120 °C for 15–20 min under a pressure of 16–20 psi. Then, this medium was moderately cooled at 28 °C for 20 min and poured in sterilized Petri dishes and left for 20–30 min to be solidified for further utilization in downstream process.

Sample inoculation

In order to get the bacteria present in soil samples, five culture media plates were inoculated with each soil solution by streaking soil sample dilutions starting from lower to higher dilutions. Immediately after inoculation of plates, Petri plates were wrapped with paraffin film and aluminum foil for avoiding foreign contamination and were placed in the incubator for 24 h at 30 °C, after which bacterial colonies were grown on media plates. These bacterial colonies were picked with the help of a wire lope and examined under a compound microscope for confirmation of *Bacillus* spp.

Furthermore, peptone agar medium (Merck) was used for the re-streaking of bacterial colonies already grown on culture media. This medium was poured on sterilized Petri dishes (3 mm layer) and upon solidification at room temperature for 30 min; it was inoculated by the bacterial colonies. Inoculated Petri dishes were wrapped with paraffin film and aluminum foil and incubated at 30 °C. After 36 h, pure colonies (whitish colonial growth) of *Bt* were verified under a microscope and further grown in liquid media for preparation of pure bacterial cultures containing enough bacterial cells to prepare microbial formulation of *Bt*.

Bacterial proliferation in liquid medium

To prepare a liquid medium for bacterial cells multiplication, 100 ml of UG medium (containing 7.5 g peptone and 6.8 g KH_2PO_4; pH 7.4) and 10 ml of stock solution 1 (containing 12.3 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.17 g L^{-1} $MnSO4{\cdot}7H_2O$, and 1.4 g L^{-1} $ZnSO_4{\cdot}7H_2O$), stock solution 2 (containing 2.0 g L^{-1} $Fe_2(SO_4)^3$ and 3 g L^{-1} H_2SO_4), and stock solution 3 (containing 14.7 g L^{-1} $CaCl_2{\cdot}2H_2O$) were added in a sterilized 250-ml conical flask, following inoculation and incubation on orbital shaker (220 rpm) at 30 °C for 48 h.

Preparation of *B. thuringiensis* suspensions

A liquid medium with purified bacterial colonies was centrifuged at 250 rpm for 20 min, and pure whitish colonies of bacteria gathered in the form of pellet in Eppendorf tube base were further suspended in 0.5 M sodium chloride (NaCl) solution and centrifuged again at 250 rpm for 10 min. Whitish colonies collected at the base of Eppendorf tube were re-suspended in double distilled autoclaved water and filtered through filter paper. These filtered out bacterial colonies, containing bacterial toxins, spores, and cells, were used to prepare treatment solutions either directly or by adding 1.0% $MgCl_2$ salt. Five different *Bt* concentrations, i.e., 1.0×10^7, 0.5×10^5, 1.0×10^5, 1.5×10^5, and 2.0×10^5 colony-forming units (cfu) per milliliter, were prepared, using double distilled autoclaved water as solvent.

Pathogenicity bioassays

Standard leaf-dip bioassays were conducted, using sterilized glass Petri dishes (9 cm). Test solutions of *Bt* isolate were made in double distilled autoclaved water. Fresh unsprayed gram leaves were cut, washed thoroughly with distilled water, and shade-dried at room temperature (28 °C). These leaves were dipped for 10 s in test solutions and then were placed for 15 min on autoclaved towel paper for drying with their adaxial surface upward. Then, treated leaves were placed in Petri dishes with five laboratory reared third-instar larvae of *H. armigera*. Experimental

design was completely randomized (CRD), with four replications for each treatment including control (water). Mortality of larvae was assessed at 24, 48, 72, 96, and 120 h post treatments. Moribund individuals were considered dead. A separate independent bioassay was carried out to assess the impact of isolated *Bacillus* strain on larval development (body weight), following the same experimental protocol as described above.

Statistical analysis

Statistix® (V8.1 for Windows® (Analytical Software 2005), Statistix, Tallahassee, FL) was used for statistical manipulation of data. Larval mortality data was corrected according to Abbott's procedure (Abbott 1925) before further statistical analyses. Data normality was checked, using Shapiro-Wilk tests at $\alpha = 0.05$, and data were log transformed ($Log_{10}(X + 1)$) in case of abnormally distributed data. Toxicity of *Bacillus* strain was determined by working out median lethal concentrations (LC_{50}) values separately for 72, 96, 120 h post exposure data, using probit analysis with 95% confidence limits (Finey 1971). The effect of *Bt* treatments on larval mortality and weight change was assessed by one-way and two-factor (factorial) analysis of variance, and Tukey's highest significant different (HSD) tests at 5% level of significance were used for comparison of treatment means.

Results and discussion

According to the overall factorial analysis (Tables 1 and 2), when *Bt* strain with salt was tested, there was a significant effect of time ($F_{4,\ 15} = 8.62$; $P < 0.001$) and *Bt* treatments ($F_{4,\ 15} = 8.18$; $P < 0.001$) on larval mortality but not of their interaction ($F_{16,\ 47} = 1.19$; $P < 0.311$), while larval weight change was only affected by time factor ($F_{4,\ 15} = 2.75$; $P < 0.038$) (Table 1). Similarly, both larval mortality and larval weight changes were significantly affected by time ($F_{5,\ 19} = 35.98$; $P < 0.001$ and $F_{5,\ 19} = 11.01$; $P < 0.001$, respectively), *Bt* treatments ($F_{5,\ 19} = 27.45$; $P < 0.001$ and $F_{5,\ 19} = 25.07$; $P < 0.001$), and their interaction ($F_{20,\ 79} = 3.98$; $P < 0.001$ and $F_{20,\ 79} = 2.85$; $P = 0.001$) (Table 2).

Toxicity of isolated *Bt* strain to *H. armigera* larvae

There was no larval mortality at 24-h exposure to all *Bt* concentrations either with or without salt. This is in line with the mode of action of *Bt* strains as their protoxins upon ingestion by the target insect pests take about 48 to 72 h to be converted into toxic protein crystals which further bind with midgut epithelial cells and cause mortality (septicemia) symptoms (Bravo et al. 2017).

However, there was a significant mortality at 48 h ($F_{5,19} = 212$; $P < 0.001$), 72 h ($F_{5,19} = 197$; $P < 0.001$), 96 h ($F_{5,19} = 306$; $P < 0.001$), and 120 h ($F_{5,19} = 478$; $P < 0.001$) post infection without salt. At 48-h exposure, the highest mortality (22.2%) was observed for 1.0×10^7 cfu/ml *Bt* treatment, followed by 2.0×10^5 cfu/ml concentration, while the minimum mortality (0.0%) was recorded for 0.5 and 1.0×10^5 cfu/ml concentrations without significant difference (Fig. 1). At 72 h post exposure, maximum larval mortality (44.4%) was exhibited by 1.0×10^7 and 2.0×10^5 cfu/ml concentrations, while there was no mortality observed for 0.5×10^5 cfu/ml concentration. The highest and significant mortality at 96 h was recorded for 1.0×10^7 cfu/ml (66.7%) and by 2.0×10^5 cfu/ml (55.6%), and both were significantly different from other lower concentrations of *Bt* (Fig. 1). Similarly, at 120 h, minimum larval mortality was recorded for 0.5×10^5 cfu/ml (11.1%), statistically significant from mortalities at 1.0×10^5 cfu/ml (33.3%), and 1.5×10^5 cfu/ml (44.4%). The highest larval mortality was exhibited by 1.0×10^7 and 2.0×10^5 cfu/ml concentration (88.9%), without a significant difference (Fig. 1). Median lethal concentrations (LC_{50}) at 72, 96, and 120 h were 2.2×10^5 cfu/ml, 1.7×10^5 cfu/ml, and 1.27×10^5 cfu/ml, respectively (Table 3).

Similarly, when the *Bt* was tested by 1.0% $MgCl_2$ salt, there was a significant mortality at 48 h ($F_{4,15} = 318$; $P < 0.001$), 72 h ($F_{4,15} = 503$; $P < 0.001$), 96 h ($F_{4,15} = 209$; $P < 0.001$), and 120 h ($F_{4,15} = 597$; $P < 0.001$) of exposure. At 48-h exposure, the highest mortality (22.2%) was observed for 2.0×10^5 cfu/ml *Bt* concentration, followed by 1.5×10^5 cfu/ml

Table 1 Analysis of variance comparison of mortality of the third-instar larvae of *Helicoverpa armigera* Hübner bioassayed with soil isolated strain of *Bacillus thuringiensis* applied with 1.0% $MgCl_2$ salt

Source	DF	Larval mortality (%)			Larval weight change (%)		
		MS	*F* value	*P* value	MS	*F* value	*P* value
Replication	3	103.70			2139.6		
Time	4	2888.89	8.62	< 0.001*	36,772.6	2.75	0.0380*
Treatments	4	2740.74	8.18	< 0.001*	23,774.1	1.78	0.1631
Time × treatments	16	398.15	1.19	0.3113	8266.9	0.62	0.8168
Error	64	335.19			13,373.8		
Total	99						
CV/GM		117.69/15.556			72.00/160.61		

*$P < 0.001$ (highly significant) and $P < 0.05$ (significant); two-way factorial ANOVA at $\alpha = 0.05$

Table 2 Analysis of variance comparison of mortality of the third-instar larvae of *Helicoverpa armigera* Hübner bioassayed with soil isolated strain of *Bacillus thuringiensis* applied without 1.0% $MgCl_2$ salt

Source	DF	Larval mortality (%)			Larval weight change (%)		
		MS	*F* value	*P* value	MS	*F* value	*P* value
Replication	3	345.68			20,280		
Time	4	6462.96	35.98	< 0.001*	46,021	11.01	< 0.001*
Treatments	5	4930.86	27.45	< 0.001*	104,830	25.07	< 0.001*
Time × treatments	20	714.81	3.98	< 0.001*	11,936	2.85	0.0011*
Error	80	179.65			4181		
Total	119						
CV/GM		61.34/21.852			43.66/148.11		

*$P < 0.001$ (highly significant) and $P < 0.05$ (significant); two-way factorial ANOVA at $\alpha = 0.05$

concentration (11.2%), while no mortality was recorded for 0.5×10^5 cfu/ml and 1.0×10^5 cfu/ml *Bt* concentrations (Fig. 1). At 72 h post exposure, maximum larval mortality (33.6%) was exhibited by 2.0×10^5 cfu/ml concentration, and it was significantly different from larval mortalities at 1.5×10^5 cfu/ml and 1.0×10^5 cfu/ml. At 96 h post exposure, the highest mortality was recorded for 2.0×10^5 cfu/ml (44.5%) followed by 1.5×10^5 cfu/ml (33.3%), and both were significantly different from lower concentrations of *Bt* (Fig. 1). Likewise, minimum larval mortality was recorded for 0.5×10^5 cfu/ml (11.1%) at 120-h exposure and was significantly different from mortalities at higher *Bt* concentrations. LC_{50} values for *Bt* with salt were 2.5×10^5 cfu/ml, 1.8×10^5 cfu/ml, and 1.1×10^5 cfu/ml for 72, 96, and 120 h, respectively (Table 3). Estela et al. (2004) and Makhlouf et al. (2016) demonstrated the same trend of larval mortality, i.e., 72 and 85%, respectively, at 120 h of exposure to many soil isolates of *Bt*.

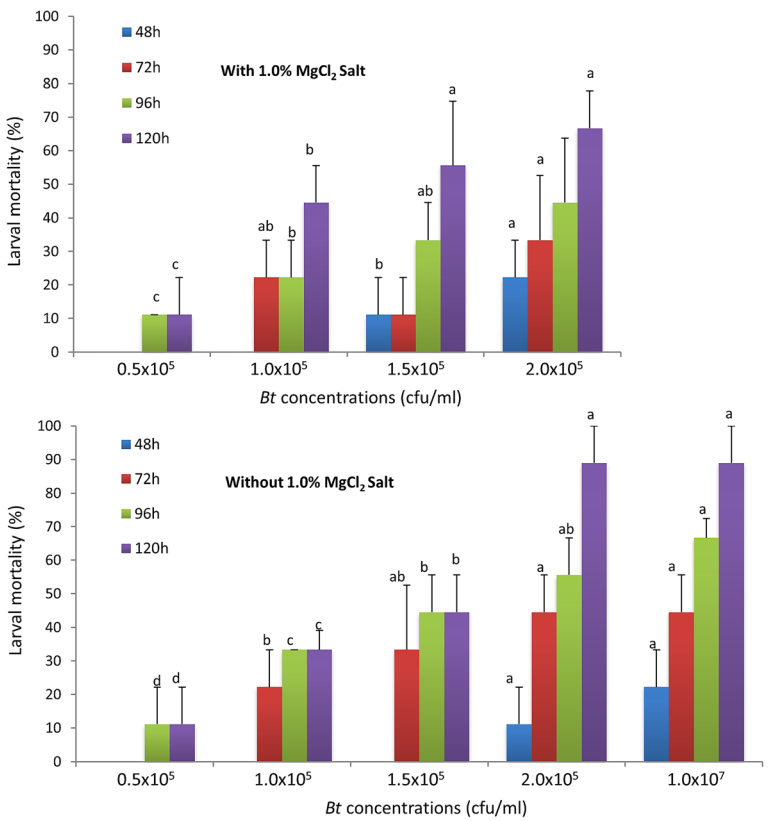

Fig. 1 Mean mortality of the third-instar larvae of *Helicoverpa armigera* Hübner exposed to different concentrations of *Bacillus thuringiensis* isolate with or without 1.0% MgCl2 salt. Columns represent mean percent mortality ± standard error ($n = 4$). For each time interval, bars with similar alphabetic letters are not significantly different (ANOVA; $P \leq 0.05$)

Table 3 Median lethal concentration (LC_{50}) values of *Bacillus thuringiensis* strain isolated from soil bioassayed against the third-instar larvae of *Helicoverpa armigera* Hübner

Treatments	Observation time (h)	LC_{50} ($\times 10^5$ cfu/ml)	95% FL	Slope	χ^2 (df = 10)	*P* value
Without salt	72	2.204	1.762–4.644	4.005 ± 0.343	169.43	0.615
	96	1.699	1.225–4.280	2.218 ± 0.188	132.77	0.472
	120	1.270	0.903–1.912	3.571 ± 0.206	243.69	0.403
With salt	72	2.529	–	1.804 ± 0.192	152.28	0.858
	96	1.797	1.325–4.118	2.397 ± 0.194	192.47	0.626
	120	1.132	0.793–1.561	3.633 ± 0.203	226.39	0.349

LC_{50} lethal concentration ($\times 10^5$ cfu/ml) of tested *Bt* treatment that killed 50% of exposed larvae, *FL* 95% Fiducial (confidence) limits, *df* degree of freedom, – incalculable

Effect on larval development (body weight) of *H. armigera*

Weight of *H. armigera* third-instar larvae was also determined at each treatment during the entire *Bt* bioassay and is represented in Fig. 2. Average weight of third-instar larva of *H. armigera* was 72.5 ± 8.7 Mg. At 48 h post exposure, average larval weight increased almost three times for all treatments except for treatment with the highest *Bt* concentration (1.0×10^7 cfu/ml) for which only 1.5 time increase was observed but without significant difference (Fig. 2). However, a significant difference was observed in average larval weight change after exposure of 72 h and upwards. At 96-h exposure, a significant reduction in average larval weight was recorded for 1.0×10^7 and 2.0×10^5 cfu/ml *Bt* concentration treatments, while the maximum weight gain was exhibited by control (363%) and at 0.5×10^5 cfu/ml *Bt* concentration (264%). The same trend was recorded for larval weight change at 120-h exposure (Fig. 2). Regarding individual treatment impact, 1.0×10^7 cfu/ml *Bt* concentration had the most significant and negative impact on average larval weight change ($R^2 = 0.53$), followed by 2.0×10^5 cfu/ml ($R^2 = 0.40$), while the most significant and positive effect was exhibited by the control ($R^2 = 0.88$), followed by 0.5×10^5 cfu/ml *Bt* concentration ($R^2 = 0.61$) (Fig. 2).

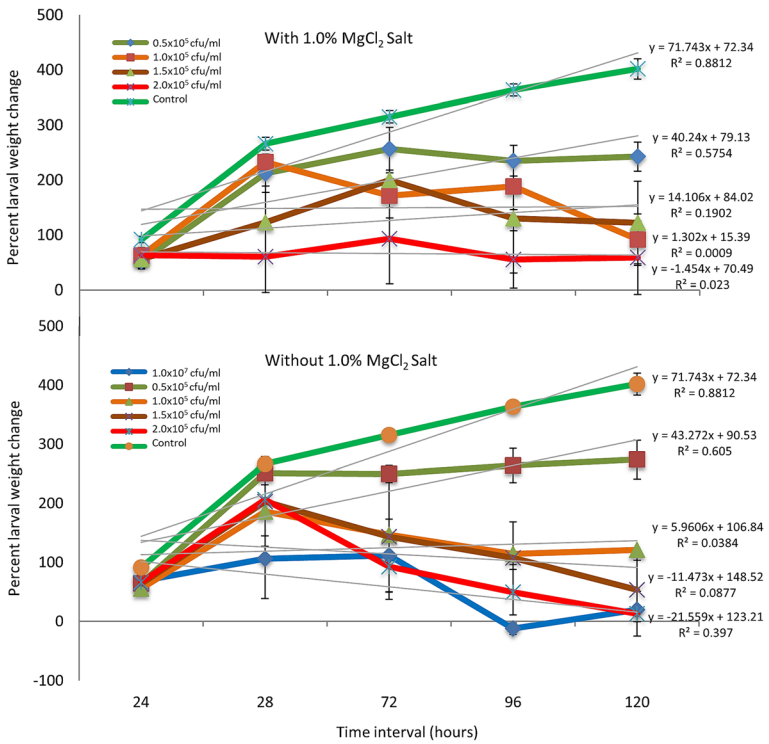

Fig. 2 Mean weight change of the third-instar larvae of *Helicoverpa armigera* Hübner exposed to different concentrations of *Bacillus thuringiensis* isolate with or without 1.0% MgCl2 salt. Data points represent mean percent weight change ± standard error ($n = 4$)

A similar trend was observed for bioassay of *Bt* with $MgCl_2$ salt (Fig. 2). Obtained results are in agreement with those of Huang et al. (2005) and Sellami et al. (2013) who revealed a significant loss of body weight in the third-instar larvae of *Ostrinia nubilalis*, *Spodoptera littoralis*, and *Ephestia kuehniella* after 3 to 4 days post exposure to *Bt* Cry1Ac andVip3 toxins.

Impact of $MgCl_2$ mineral salt on pathogenicity of *Bacillus* spp. against *H. armigera*

Regarding the effect of mineral salt ($MgCl_2$) on the pathogenicity potential of *Bt* isolate, results showed no obvious synergistic or additive effect but there was an antagonistic effect on the larval mortality and weight change (Figs. 1 and 2). The highest mortalities of larvae (55 and 65%) were observed at 96 and 120 h post exposure, respectively. One reason of this negative or antagonistic effect of $MgCl_2$ salt on toxicity of *Bt* isolate would be very high amount of salt resulted in delayed toxicity of *Bt* toxins as evidenced by Estela et al. (2004).

Conclusions

In conclusion, the results of present study corroborate the biocontrol potential of different entomopathogenic agents such as *B. thuringiensis* found in indigenous soils for the non-chemical and eco-friendly management of economically important insect pests such as *H. armigera* and manifest the significance of taking into consideration the pathogenic characterization of native soil strains of B. thuringiensis against exotic insect pests as emphasized by different studies (Bravo et al., 2017).

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Availability of data and materials

All datasets on which conclusions of the study have been drawn are presented in the main manuscript.

Authors' contributions

MZM, MAR, and SA conceived and designed the experimental protocols. MAK and MAR performed the experiments. CSM and MAR provided the technical assistance in the experimentation. MZM and SA performed the statistical analyses. MZM, MAR, and MAK prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Aggarwal N, Holaschke M, Basedow T (2006) Evaluation of bio-rational insecticides to control *Helicoverpa armigera* (Hübner) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) fed on *Viciafaba* L. J Appl Entomol 15:245–250

Bibi A, Ahmed K, Ayub N, Alam S (2013) Production of low cost *Bacillus thuringiensis* based-biopesticide for management of chickpea pod-borer *Helicoverpa armigera* (Hübner) in Pakistan. Nat Sci 5(11):1139

Bravo A, Pacheco S, Gómez I, Garcia-Gómez B, Onofre J, Soberón M (2017) Insecticidal proteins from *Bacillus thuringiensis* and their mechanism of action. In: *Bacillus thuringiensis* and *Lysinibacillus sphaericus*. Springer International Publishing, Gewerbestrasse pp 53–66

Estela A, Escriche B, Ferré J (2004) Interaction of *Bacillus thuringiensis* toxins with larval midgut binding sites of *Helicoverpa armigera* (Lepidoptera: Noctuidae). Appl Environ Microbiol 70(3):1378–1384

Finey DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge, UK, p 303

Huang F, Buschman LL, Higgins RA (2005) Larval survival and development of susceptible and resistant *Ostrinia nubilalis* (Lepidoptera: Pyralidae) on diet containing *Bacillus thuringiensis*. Agric For Entomol 7(1):45–52

Kranthi KR, Jadhav DR, Wanjari RR, Ali SS, Russell D (2001) Carbamate and organophosphate resistance in cotton pests in India, 1995 to 1999. Bull Entomol Res 91(1):37–46

Kumar S, Singh A (2015) Biopesticides: present status and the future prospects. J Biofert Biopest 6:e129

Majeed MZ, Fiaz M, Ma CS, Afzal M (2017) Entomopathogenicity of three muscardine fungi, *Beauveria bassiana*, *Isaria fumosorosea* and *Metarhizium anisopliae*, against the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Egypt J Biol Pest Control 27(2):211–215

Makhlouf A, El-Kemany AA, Riad A, Abou-Youssef YA (2016) Isolation, toxicity and molecular characterization of native *Bacillus thuringiensis* isolates from Egyptian soil. Egypt J Genet Cytol 44(2):11–19

Mazzi D, Dorn S (2012) Movement of insect pests in agricultural landscapes. Ann Appl Biol 160(2):97–113

Nagoshi RN, Brambila J, Meagher RL (2011) Use of DNA barcodes to identify invasive armyworm *Spodoptera* species in Florida. J Insect Sci 11(154):1–11

Ndemah R, Schulthess F, Korie S, Borgemeister C, Cardwell KF (2001) Distribution, relative importance and effect of lepidopterous borers on maize yields in the forest zone and mid-altitude of Cameroon. J Econ Entomol 94(6):1434–1444

Osman GEH, Already R, Assaeedi ASA, Organji SR, El-Ghareeb D, Abulreesh HH, Althubiani AS (2015) Bioinsecticide *Bacillus thuringiensis* a comprehensive review. Egypt J Biol Pest Control 25(1):271–288

Qayyum MA, Wakil W, Arif MJ, Sahi ST, Saeed NA, Russell DA (2015) Multiple resistances against formulated organophosphates, pyrethroids, and newer-chemistry insecticides in populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Pakistan. J Econ Entomol 108(1):286–293

Sellami S, Zghal T, Cherif M, Zalila-Kolsi I, Jaoua S, Jamoussi K (2013) Screening and identification of a *Bacillus thuringiensis* strain S1/4 with large and efficient insecticidal activities. J Basic Microbiol 53(6):539–548

Sree KS, Varma A (eds) (2015) Biocontrol of lepidopteran pests: use of soil microbes and their metabolites, vol 43. Springer International Publishing, Switzerland ISBN: 978-3-319-14499-3

Tabbene O, Slimene IB, Djebali K, Mangoni ML, Urdaci MC, Limam F (2009) Optimization of medium composition for the production of antimicrobial activity by *Bacillus subtilis* B38. Biotechnol Prog 25(5):1267–1274

Zhang W, Ma L, Zhong F, Wang Y, Guo Y, Lu Y, Liang G (2015) Fitness costs of reproductive capacity and ovarian development in a *Bt*-resistant strain of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pest Manag Sci 71(6):870–877