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BACILLUS THURINGIENSIS ISOLATES AND THEIR *CRY* GENES TOXIC TO CHICKPEA POD BORER *HELICOVERPA ARMIGERA* (HÜBNER) FROM ETHIOPIA

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ABSTRACT

***Helicoverpa armigera* (Hubn) is one of the most destructive insect pests of chickpea in Ethiopia. For sustainable management of insect pests of food crops, *Bacillus thuringiensis (Bt)* is a widely used bioinsecticide. This study was aimed at exploring indigenous *Bt* isolates that harbour *cry* genes to control *H. armigera*. Ten indigenous *Bt* isolates were analyzed for their *cry* genes. Accordingly, all the indigenous *Bt* isolates were observed to harbour two or more *cry* genes. Statistically significant ($p<0.05$) variations were observed among *Bt* species in influencing larval incidence, pod damage (%) and grain yield (t/ ha). Three potential indigenous *Bt* isolates were identified with their respective *cry* genes that included KDL (*cry*2 + *cry*4), AUGHS-1 (*cry*1 + *cry*4), and AUSD-1 (*cry*1 + *cry*2 + *cry*4 + *cry*7, 8 + *cry*9). Indigenous *Bt* isolates exhibited a strong potential in the management of chickpea pod borer. Development of commercial bioinsecticide and other *Bt* technologies using *B. thuringiensis* from Ethiopian sources will be a new avenue to be addressed.**

Key words: Bioinsecticide, *Bt, cry* genes, crystal protein, Ethiopia, *Helicoverpa armigera,* larval mortality, pathogenecity, pod damage, grain yield, toxicity, AUGHS-1, AUSD-1

Soil microbes have been identified as potential candidates for use in biocontrol against insect pests (Riba and Silvy, 1989). For instance, *Bacillus thuringiensis* (*Bt*) is the most important entomopathogenic microbe used to date as an alternative to chemical insecticides against insect pests of agriculture such as chickpea pod borer. *Bt* is a gram-positive, rod shaped, and spore-forming bacterium that produces proteinaceous parasporal crystal proteins encoded as *cry* genes (Bravo et al., 2013; Das et al., 2021). These crystals are composed of *cry* genes that are toxic against a range of insect pests from the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera, and Hemiptera as well as against mites and nematodes (Palma et al., 2014; Das et al., 2021). These toxins are uniquely specific, safe, and completely biodegradable (Rubio-Infante and Moreno-Fierros, 2016). Most of the commercial *B. thuringiensis* formulations used for the control of lepidopteran larvae (e.g., *Helicoverpa armigera*) mainly contain toxins of the *cry*1A and *cry*2A gene families, especially *cry*1Aa, *cry*1Ab, *cry*1Ac, *cry2Aa,* and *cry*2Ac genes (Rubio-Infante and Moreno-Fierros, 2016); *H. armigera* was found susceptible to various *cry* genes (*cry*1Ab, *cry*1Ac, *cry*2Aa, and *cry*2Ab) with complete eradication of the target pest (Liao et al., 2002). The discovery of novel *cry* genes with broad spectra of toxicity is important for the development of new products for the management of insect pests of crops. As a result, the search for new *cry* genes is an on-going effort worldwide with more than 800 *cry* genes discovered so far (Rabha and Das et al., 2023). Aynalem et al. (2021) have made a study on *Tuta absoluta* using indigenous *Bt* isolates and found that lepidopteran active *cry* genes were found in Ethiopian isolates. Therefore, this study was designed to characterize indigenous *Bt* isolates by testing their pathogenecity against chickpea pod borer, *H. armigera* under field conditions.

MATERIALS AND METHODS

Field experiments were conducted in 2023 at the Kulumsa Agricultural Research Centre, Ethiopian Institute of Agricultural Research (EIAR) and Gonde Basic Seed Farm Center, Ethiopian Agricultural Business Corporation (EABC) both located at 8°01’88’’N and 39°07’10’’E, 8°06’25’’N and 39°07’38’’E; at an elevation of 2210 and 2268 masl, respectively (Abayneh et al., 2003). Morphological characterization and molecular of *Bt* isolates were done at the Holeta Microbial Biotechnology, EIAR, and biochemical characterization was done at Sebeta Animal Health

Institute, Bacteriology laboratory. Treatments of ten indigenous *Bt* isolates (KDL , AUPOS, AUSD-1, AUGHS-1, ZDS, ZDS-3, AUASG-2, GHTSW, GHTSW-1, AUGHS-3) were used at *Bt* concentrations twice the LD_{90} values with distilled water as negative control (Gemmeda et al., 2023). The reference *Bt*. var. *thuringiensis* was produced by Sibbiopharm Ltd. and provided by the Gallica Flower Farm in Menegasha, Ethiopia. A randomized complete block design with three replications were used on plot size of 2 m x 1.5 m, 1.50 m between plots, and 2 m between blocks. Chickpea seeds, Arerti (kabuli type), were sown at recommended seed rate and sowing depth. Each plot received a random set of treatments. The laid eggs and the number of *H. armigera* larvae/ plant were carefully examined and recorded. Applications were coincided with *H. armigera* generation time and economic threshold level (Zahid et al., 2008). Spore-crystal mixture was prepared following standard procedures (Mcfarland, 1907). Each of the bacterial isolates were culturally and morphologically characterized (Zayaitz, 2016; Leboffe et al., 2016; Maza et al., 2020). Some biochemical tests (response to some enzymes and carbohydrates utilization were done (Lehman, 2005; Brink, 2010; Maza et al., 2020; Al-joda et al., 2021).

Bacterial Genomic DNA was prepared and extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Optimization of annealing temperature was carried out using temperature gradient PCR (Ammouneh et al., 2011; Jasmina et al., 2013). Five universal primer pairs *cry*1, *cry*2, *cry*3, *cry*4, *cry*7, 8 and one specific primer *cry*9 (forward and reverse) synthesized by SANGON biotech manufacture (China) were used to analyse the *cry* genes. Their nucleotide primer pairs in chronological order of the above (F1) CAT GAT TCA TGC GGC AGA TAA AC, (R1) TTG TGA CAC TTC TGC TTC CCA TT, (F2) GTT ATT CTT AAT GCA GAT GAA TGGG, (R2) CGG ATA AAA TAA TCT GGG AAA TAGT, (F3) CGT TAT CGC AGA GAG AGA TGA CAT, (R3) CAT CTG TTG TTT CTG GAG GCA AT, (F4) GCA TAT GAT GTA GCG AAA CAA GCC, (R4) GCG TGA CAT ACC CAT TTC CAG GTCC, (F7,8) AAG CAG TGA ATG CCT TGT TTAC, (R7,8) CTT CTA AAC CTT GAC TAC TT, (F9) CAC ATG AGT TTT CTT CCT AT, (R9) AGA TAC GAT GCT TGT TGT AA were used. DNA amplification was performed using twenty microliters of total genomic DNA extracted from *Bt* isolates as template for PCR amplification (da Silva and Valicente, 2013). The presence of specific *cry* genes in the amplicons of all the *Bt* isolates was analyzed by the PCR method using 3% agarose gel mixed with 2 µL loading dye (gel-red) for gel documentation at 100 V/ 45 min/ 1xTBE and the size of the amplicons were estimated based on a 100 bp ladder loaded as size markers (Ben-Dov et al., 1997; da Silva and Valicente, 2013). Data on larval mortality pre and post 1st and 2nd *Bt* sprayed, total number of pods and % pod damage were counted/ plant from ten plants/plot. Data were averaged and means were calculated/ plant and used for ANOVA (Lateef and Reed, 1983). Grain yield was obtained from plot was converted to mt/ ha. SAS statistical software packages, version 9.4 (SAS, 2013) was used. Means were compared and separated using Tukey's Highest Significant Difference test (p=0.05).

RESULTS AND DISCUSSION

Based on the results of colony morphology two colony textures (Brittle and Viscous), five colony surfaces (Dull, Smooth, Rough, Veined, and Glistening), three colours (creamy white, white to off white), three forms (circular, wavy, and oval), two types of elevations (flat and raised), and two types of margins (curled and entire) were identified (data not shown). All *Bt* isolates had rod shaped with five cell arrangements (Table 1). It was shown that from the gram stain and motility test results, all the tested *Bt* isolates were gram positive and motile. Results of endospore and crystal protein staining revealed that all the *Bt* isolates were positive for endospore test (Table 1, Fig. 1a). All the *Bt* isolates possessed crystal proteins (Fig. 1b) and the catalase test was also positive (Table 1, Fig. 1c). The findings were in agreement with almost all the *Bt* studies conducted previously (Karen, 2010; Smith and Hussey, 2005, 2013; Ghosh et al., 2017). All the *Bt* isolates were utilized glucose, maltose, and citrate. However, all the isolates showed negative reactions to urease and indole tests similar to the previous investigations (Abirami et al., 2016; Jyothi and Priya, 2018; Gholamveisi et al., 2018).

All the *Bt* isolates harboured combinations of multiple *cry* genes (Fig. 2). Of which *cry*4 gene was detected 100% followed by *cry*2 gene 90%, and *cry*1 gene 50%. Aynalem et al. (2021) in their study on Ethiopian *Bt* isolates against tomato leaf miner, *Tuta absoluta*, found that *cry*2 and *cry*9 genes were more frequently detected genes than *cry*1 gene. On the contrary, Hassan et al. (2021) detected *cry*1 gene (100%) compared to *cry*2 gene but similar result was obtained regarding *cry*4 gene with 84.61% detection

Table 1. Biochemical characters of indigenous *Bt* isolates

Biochemical characteristics	*Bt* isolates										
	KDL	AUPOS	AUSD-1	AUGHS-1	ZDS	ZDS-3	AUASG-2	GHTSW	GHTSW-1	AUGHS-3	*Bt* .var. *thuringiensis*
Gram Stain	+	+	+	+	+	+	+	+	+	+	+
Endospore stain	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	-	+	+	+	+	+	+	+	+
Terhalose	-	N	+	+	+	-	-	N	+	N	+
Maltose	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-
TSI	R/Y	-	R/Y	-	-	R/Y	R/Y	-	-	N	R/Y
Indole	-	-	-	-	-	-	-	-	-	-	-

(+) positive response; (-) negative response; (N) no response; (R/Y) red or yellow

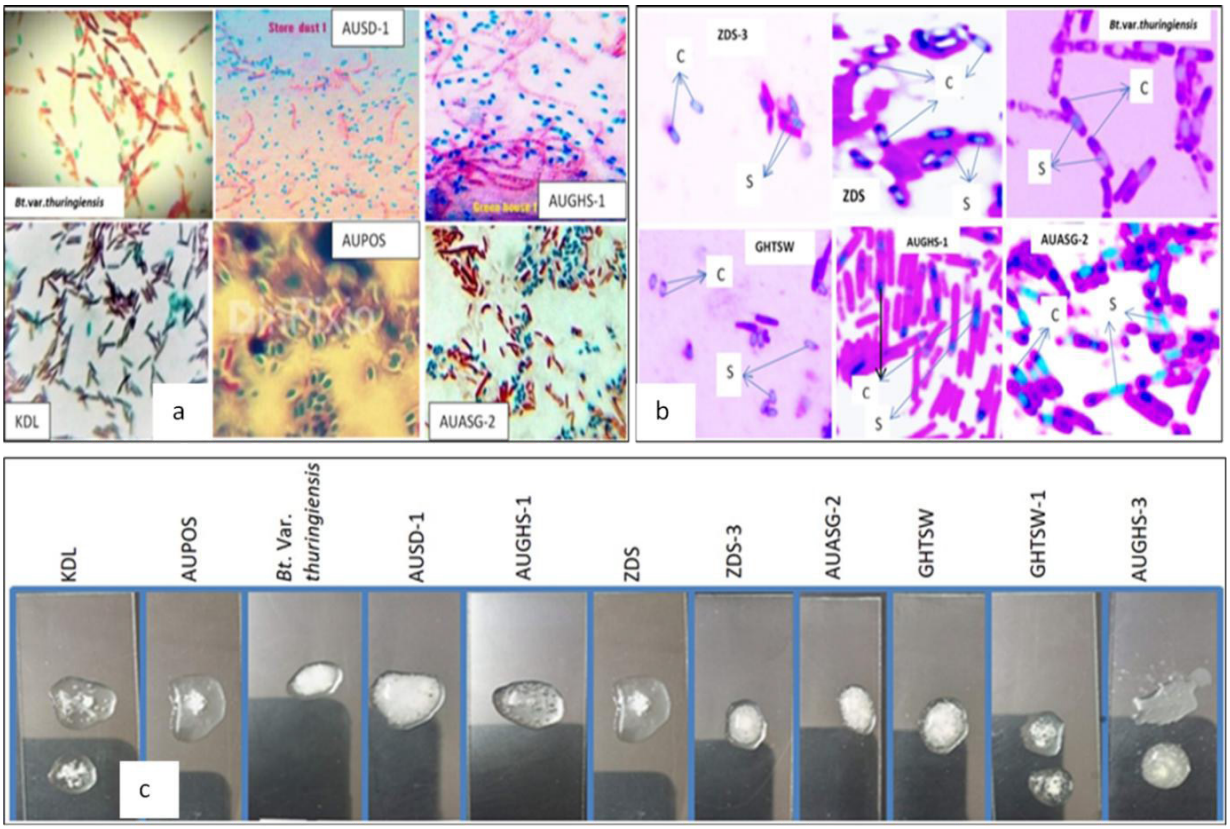

Fig. 1. Biochemical characteristics (a) Gram and endospore stain (b) Insecticidal crystal protein stain (c) Catalase test

rate. Likewise, Baig et al. (2010) made a study on *cry* genes profiling and toxicity evaluation of *Bacillus thuringiensis* against *H. armigera.* The authors' findings show that out of 50 *cry* gene positive *Bt* isolates, 6% isolates were positive for *cry*2 gene and 22% were positive for *cry*4 gene. Moreover, considering isolates with a single *cry* gene 18% and 65% of the isolates were only positive for *cry*2 and *cry*4 genes, respectively. With regards to the combinations of *cry* genes, *Bt* isolates KDL, AUPOS, GHTSW and GHTSW-1 harboured (*cry*2 + *cry*4), AUGHS-1 (*cry*1 + *cry*4), ZDS (*cry*2 + *cry*4 + *cry*9), AUGHS-3 (*cry*1+ *cry*2+ *cry*4), ZDS-3 and AUASG-2 (*cry*1 + *cry*2 + *cry*4 + *cry*9) and AUSD-1 (*cry*1 + *cry*2 + *cry*4 + *cry*7, 8 + *cry*9) (Fig. 2). None of the isolates amplify *cry*3 genes. This result is in agreement with previous investigations (Khojand et al., 2013; Hassan et al., 2021) since most *Bt* studies on *cry* genes show the presence of combinations.

In the field experiment, *Bt* sprayed plots revealed statistically significant ($p<0/05$) differences with regards to the larval incidence post 1st, pre 2nd, and post 2nd, pod damage and grain yield. At Gonde post 2nd *Bt* sprayed plot, the lowest number (0.97± 0.17 and 0.97± 0.20) of larvae/ plant was recorded from indigenous *Bt* isolates of AUSD-1 and AUGHS-1, but the highest (3.50±0.23) was obtained from the control plot. Similarly, the findings significantly ($p<0.05$) differed from the reference *Bt.*var. *thuringiensis,* which resulted in 1.70± 0.60 larvae/ plant. Within the indigenous *Bt* isolates, AUSD-1 and AUGHS-1 also significantly ($p<0.05$) differed from AUPOS, GHTSW, and GHTSW-1. Pre and post 2nd *Bt* sprayed larval population counted/ plant showed a significant ($p<0.05$) difference only between *Bt* isolates and the control plots. The values ranged from 0.87± 0.25 to 3.91± 0.31 and 0.84± 0.32 to 2.82± 0.14 both pre and post 2nd *Bt* sprayed, respectively. Whereas at Kulumsa, the lowest 0.99± 0.17 and 1.00± 0.21 both post 1st and pre 2nd *Bt* sprayed obtained from indigenous *Bt* isolate of AUGHS-1, and 0.73± 0.17 post 2nd *Bt* sprayed was from indigenous *Bt* isolate of AUSD-1. Nevertheless, the highest 3.50± 0.23, 3.91± 0.31, and 2.82± 0.14 larval populations recorded/ plant post 1st, pre 2nd, and post 2nd *Bt* sprayed from the control (Table 2, 3). There was no significant ($p>0.05$) difference among the indigenous *Bt* isolates and the reference *Bt*. var. *thuringiensis* in pod damage at both locations (Table 4, 5). However, significant ($p<0.05$) variations recorded from the control plots.

Pod damage ranged from 10.42± 6.63 to 49.35± 15.02 and 8.63± 2.57 to 52.25± 9.72. Similarly, grain yields ranged from 0.75± 0.16 to 1.95± 0.48 and from 1.07± 0.19 to 2.42± 0.23 mt/ ha both at Gonde and Kulumsa, respectively. The results of the current study are in agreement with the study made by Kumar et al. (2016) using liquid formulation of *Bt* strain PDBC

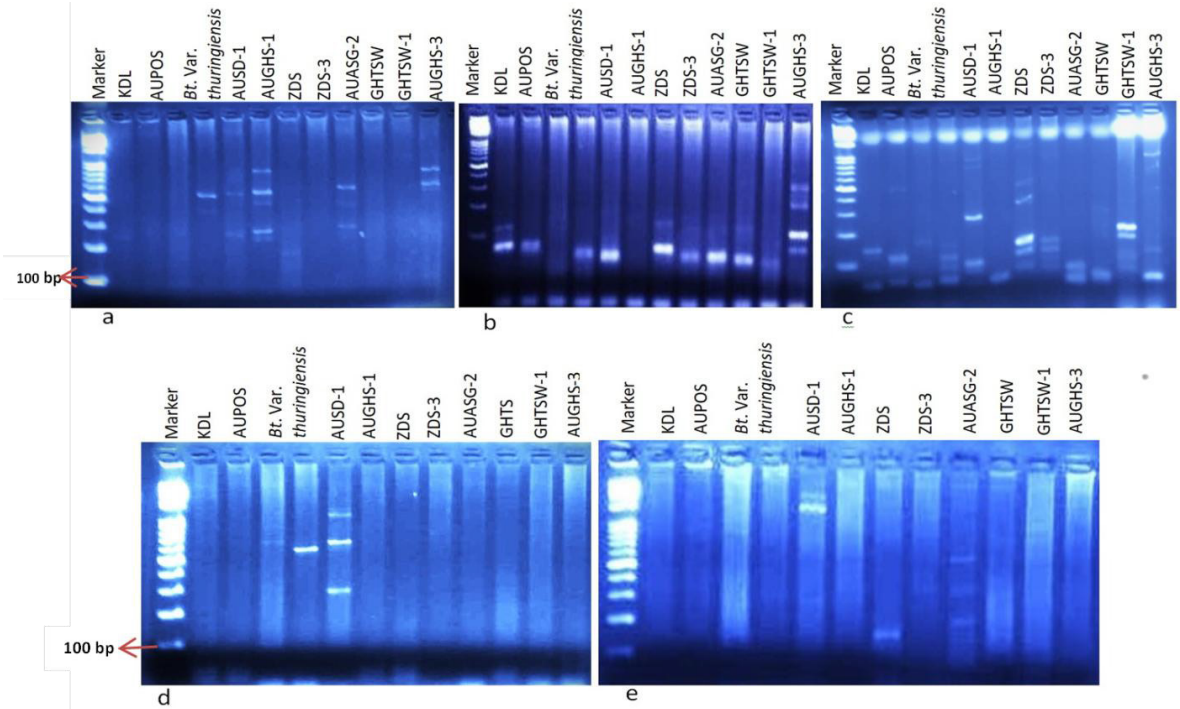

Fig. 2. PCR analysis of cry gene profile of indigenous Bt isolates and their respective band size reading(bp) (a) cry1, (b) cry2, (c) cry4, (d) cry7, 8, (e) cry9

Table 2. Effect of *Bt* applications on *H. armigera* larval incidence (Gonde Basic Seed Farm Center, EABC, 2023)

Treatments	Concentrations LD_{90} (mℓ/ 10g)	1st spray		2nd spray	
		[a]PrSC plant^{-1}	[b]PoSC plant^{-1}	[c]PrSC plant^{-1}	[d]PoSC plant^{-1}
Bt. vr. *thuringiensis*	3.32	2.40± 1.00^{a}	1.70± 0.60^{bc}	0.97± 0.76^{b}	0.95± 0.19^{b}
KDL	3.08	1.43± 0.75^{a}	1.40± 0.26^{bcd}	0.95± 0.24^{b}	0.95± 0.15^{b}
AUPOS	3.70	2.05± 0.65^{a}	1.50± 0.25^{bc}	1.13± 0.05^{b}	0.92± 0.27^{b}
AUSD-1	3.52	1.86± 1.38^{a}	0.97± 0.17^{d}	0.95± 0.20^{b}	0.89± 0.09^{b}
AUGHS-1	3.58	1.03± 0.58^{a}	0.97± 0.20^{d}	0.87± 0.25^{b}	0.84± 0.32^{b}
ZDS	3.96	1.40± 0.98^{a}	1.20± 0.06^{bcd}	0.90± 0.32^{b}	0.85± 0.03^{b}
ZDS-3	3.54	2.10± 0.80^{a}	1.10± 0.15^{bcd}	1.05± 0.17^{b}	0.87± 0.29^{b}
AUASG-2	3.26	1.93± 1.24^{a}	1.03± 0.30^{bcd}	0.99± 0.28^{b}	0.86± 0.25^{b}
GHTSW	3.32	1.95± 0.65^{a}	1.75± 0.10^{b}	1.17± 0.18^{b}	1.17± 0.09^{b}
GHTSW-1	3.52	1.75± 0.65^{a}	1.60± 0.00^{bc}	1.08± 0.16^{b}	0.91± 0.01^{b}
AUGHS-3	3.78	1.95± 1.00^{a}	1.40± 0.10^{bcd}	1.04± 0.24^{b}	1.00± 0.03^{b}
control	-	1.50± 1.38^{a}	3.50± 0.23^{a}	3.91± 0.31^{a}	2.82± 0.14^{a}

Means with the same letters within the same columns not significantly different. Data means ± SD, means separated using Tukey's highest Significant Difference (HSD) test. [a]PrSC, pre first sprayed larval counted (pr<0.1107); [b]PoSC, post first sprayed counted, (*** pr<0.0001); [c]pre second sprayed larval counted (***Pr<0.0001); [d]PoSc, post second sprayed larval counted (***Pr<0.0001).

Table 3. Effect of *Bt* applications on *H. armigera* larval incidence (Kulumsa Agricultural Research Center, EIAR, 2023)

Treatments	Concentrations LD_{90} (mℓ/ 10g)	1st spray		2nd spray	
		[a]PrSC plant^{-1}	[b]PoSC plant^{-1}	[c]PrSC plant^{-1}	[d]PoSC plant^{-1}
Bt. var. *thuringiensis*	3.32	1.75± 0.06^{a}	1.32± 0.14^{b}	1.48± 0.70^{b}	0.73± 0.50^{b}
KDL	3.08	1.20± 0.10^{a}	1.16± 0.21^{b}	1.28± 0.07^{b}	0.76± 0.20^{b}
AUPOS	3.70	1.63± 0.40^{a}	1.21± 0.08^{b}	1.35± 0.22^{b}	0.80± 0.55^{b}
AUSD-1	3.52	1.37± 0.15^{a}	1.05± 0.17^{b}	1.08± 0.08^{b}	0.73± 0.17^{b}
AUGHS-1	3.58	1.83± 0.46^{a}	0.99± 0.17^{b}	1.00± 0.21^{b}	0.76± 0.40^{b}
ZDS	3.96	1.83± 0.20^{a}	1.07± 0.16^{b}	1.21± 0.08^{b}	0.80± 0.15^{b}
ZDS-3	3.54	1.47± 0.80^{a}	1.13± 0.36^{b}	1.22± 0.21^{b}	0.80± 0.10^{b}
AUASG-2	3.26	1.73± 0.26^{a}	1.15± 0.17^{b}	1.22± 0.11^{b}	0.83± 0.23^{b}
GHTSW	3.32	1.77± 0.15^{a}	1.45± 0.36^{b}	1.59± 0.02^{b}	0.80± 0.15^{b}
GHTSW-1	3.52	1.87± 0.10^{a}	1.19± 0.31^{b}	1.33± 0.05^{b}	0.80± 0.10^{b}
AUGHS-3	3.78	1.77± 0.15^{a}	1.14± 0.16^{b}	1.25± 0.04^{b}	0.83± 0.00^{b}
control	-	1.62± 0.42^{a}	2.66± 0.12^{a}	2.69± 0.20^{a}	2.70± 0.40^{a}

Means with the same letters within the same columns not significantly different. Data means ±SD, means separated using Tukey's highest Significant Difference (HSD) test. [a]PrSC, pre first sprayed larval counted (pr<0.1794); [b]PoSC, post first sprayed counted (***pr<0.0001); [c]PrSC, pre second sprayed larval counted (***pr<0.0001); [d]PoSC, post second sprayed larval counted (*** Pr<0.0001).

*Bt*1 and commercial *Bt* k (Halt 5% WP) against *H. armigera* of pigeon pea. Ahmed et al. (2015) have made investigation for two years on bio-pesticide DiPel 2x alone and with mixtures of milk powder, molasses and K_2CO_3 to control chickpea pod borer that significantly lowered pod damage with higher grain yield. The lowest (10.42± 6.63 and 8.63± 2.57) pod damage at both locations but the highest grain yield in at Gonde obtained from indigenous *Bt* isolate AUGHS-1. Highest grain yield was recorded from KDL at Kulumsa (Table 4, 5). Alike the current study, Singh and Dhkal (2019) have made two years study on *H.*

Table 4. Effect of *Bt* applications on pod damage (%) and grain yield (t/ ha) (Gonde Basic Seed Farm Center, EABC, 2023

Treatments	Concentrations LD_{90} (mℓ/ 10g)	Parameters		
		[a]TPDS plant^{-1}	[b]PD (%) plant^{-1}	[c]GY (t/ ha)
Bt. var. *thuringiensis*	3.32	96.50± 12.58[a]	10.58± 3.48[b]	1.89± 0.05[ab]
KDL	3.08	111.0± 27.60[a]	10.99± 5.09[b]	1.95± 0.27[ab]
AUPOS	3.70	73.33± 37.20[a]	11.24± 3.90[b]	1.75± 0.23[ab]
AUSD-1	3.52	94.67± 33.30[a]	10.57± 1.89[b]	1.89± 0.52[ab]
AUGHS-1	3.58	76.83± 17.60[a]	10.42± 6.63[b]	1.95± 0.48[ab]
ZDS	3.96	76.00± 25.00[a]	11.52± 3.26[b]	1.58± 0.31[ab]
ZDS-3	3.54	67.83± 32.90[a]	11.35± 3.36[b]	1.73± 0.15[ab]
AUASG-2	3.26	85.33± 21.10[a]	14.70± 4.09[b]	1.78± 0.16[ab]
GHTSW	3.32	103.67± 24.0[a]	13.91± 4.63[b]	1.50± 0.15[ab]
GHTSW-1	3.52	77.17± 28.50[a]	13.32± 3.35[b]	1.68± 0.16[ab]
AUGHS-3	3.78	77.83± 56.70[a]	17.81± 9.38[b]	1.33± 0.77[b]
Control	-	104.25± 15.75[a]	49.35± 15.02[a]	0.75± 0.16[c]

Means with the same letters within the same columns not significantly different. Data means ±SD, means separated using Tukey's highest Significant Difference (HSD) test. [a]TPDS, total number of pods/ plant (pr<0.7898); [b]PD, % pod damage/ plant, (**pr<0.0082); [c]GY, grain yield mt/ ha (**pr<0.0063).

Table 5. Effects of *Bt* applications on pod damage (%) and grain yield (t/ ha) (Kulumsa Agricultural Research Center, EIAR, 2023)

Treatments	Concentrations LD_{90} (mℓ/10g)	Parameters		
		[a]TPDS plant^{-1}	[b]PD (%) plant^{-1}	[c]GY (t/ha)
Bt. vr. *thuringiensis*	3.32	96.92± 13.07[a]	8.65± 2.49[b]	2.34± 0.02[ab]
KDL	3.08	112.25± 10.5[a]	8.72± 4.34[b]	2.42± 0.23[ab]
AUPOS	3.70	90.42± 15.09[a]	10.31± 0.82[b]	1.80± 0.71[ab]
AUSD-1	3.52	91.33± 15.87[a]	8.63± 2.57[b]	2.36± 0.41[ab]
AUGHS-1	3.58	97.17± 27.71[a]	9.13± 3.18[b]	2.19± 0.19[ab]
ZDS	3.96	87.83± 9.64[a]	17.13± 4.24[b]	1.55± 0.57[ab]
ZDS-3	3.54	99.17± 15.39[a]	15.87± 5.76[b]	1.57± 0.73[ab]
AUASG-2	3.26	89.42± 6.24[a]	11.42± 2.40[b]	1.87± 0.23[ab]
GHTSW	3.32	106.0± 12.49[a]	8.99± 2.75[b]	2.01± 0.36[ab]
GHTSW-1	3.52	85.42± 24.98[a]	12.81± 2.02[b]	1.62± 0.19[ab]
AUGHS-3	3.78	89.92± 15.09[a]	12.34± 4.74[b]	1.58± 0.98[ab]
Control	-	93.83± 6.00[a]	52.25± 9.72[a]	1.07± 0.19[b]

Means with the same letters within the same columns not significantly different. Data means ±SD, means separated using Tukey's highest Significant Difference (HSD) test. [a]TPDS, total number of pods/ plant (pr<0.9208); [b]PD, % pod damage/ plant (***pr<0.0001); [c]GY, grain yield mt/ ha (*pr<0.0314).

armigera on chickpea using commercial *Bt* product *Bt*. var. *kurstaki* (WP) (DOR *Bt*-1) that reduced pod damage with increased chickpea grain yield. *Cry*1 and *cry*2 genes from Ethiopian *Bt* isolates expressed insecticidal crystal proteins to lepidopteran larvae. Toxicity of the indigenous *Bt* isolates KDL, AUGHS-1 and AUSD-1 were superior to the rest *Bt* species tested. This implied that the presence of potent *Bt* isolates harbouring *cry* genes active against lepidopteran larvae. Therefore, the potent *Bt* isolates identified as potential candidates to control *H. armigera* in chickpea and associated host crops. Thus, continuous screening for specific *cry* gene families for commercialization and development of *Bt* technologies have paramount importance in sustainable production of chickpea.

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AUTHOR CONTRIBUTION STATEMENT

LG, EG and DM perceived and carefully designed the experiment**,** LG prepared the materials, conducted the experiment, collected and analysed the data, prepared first draft manuscript, EG supervised the work, interpreted results and figures, edited and revised the manuscript, DM provided guidance, interpreted results and figures, edited and revised the manuscript. All the authors read and approved the final manuscript submission.

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CONFLICT OF INTEREST

No conflict of interest

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