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Study on the Effects of *Bacillus Thuringiensis* H-01-14 on The Growth and Development of *Spodoptera Litura*

Hu J[1,2‡], Zhao Y[1‡], Yu S[1‡], Chen Y[1], Ma R[1], Yang C[1] and Chen H[1*]

[1]College of Agronomy, Sichuan Agricultural University, Chengdu, 611130, China

[2]Technical Center of Wenshan, Yunnan Tobacco Company, Wenshan, 663000, China

[‡]These three authors contributed equally to this work.

***Corresponding author:** Huabao Chen, College of Agronomy, Sichuan Agricultural University, Chengdu, 611130, China

Tel: 13084306538;

Email: chenhuabao@sicau.edu.cn

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Abstract

Bacillus thuringiensis (Bt) is an important microbial resource for pest control. Previous studies have shown that Bt strain H-01-14 exhibits certain insecticidal effects against *Spodoptera litura*. This study systematically investigates the effects of strain H-01-14 on the growth, development, and reproduction of *S.litura*. Histological methods were employed to compare the effects of strain H-01-14 on the testes, ovaries, and midgut of adult *S.litura*. Growth and development indicators demonstrated that strain H-01-14 extended the larval, pupal, adult, and eclosion periods of *S.litura* by 2.47 days, 1.71 days, 1.68 days, respectively. Pupal and eclosion rates decreased by 23.34% and 40.34%, respectively. Furthermore, the fecundity per female decreased by 49.75%, and hatching rate decreased by 68.08%. However, there was no significant effect observed on the growth and development of subsequent generations (F_1) of *S.litura*. Histological observations revealed significant pathological changes in the ovarian germ cells of *S.litura* treated with strain H-01-14, characterized by uneven cytoplasmic structure and vacuolation, while no significant effects were observed in the midgut and testes. RNA-seq analysis identified 279 differentially expressed genes (DEGs), including 198 up-regulated and 81 down-regulated genes. GO and DEGs enrichment analysis indicated that strain H-01-14 mainly affected genes related to chitinase, phospholipase, trypsin, and aldo-keto reductase, thereby influencing the growth, development, immune function, and detoxification metabolism of *S.litura*. The isolation of this strain holds practical significance for the green control and resistance management of *S.litura* and potentially other pests.

Keywords: *Bacillus thuringiensis;* Growth and development; *Spodoptera litura;* Transcriptome sequencing

Introduction

Spodoptera litura (Fabricius) is a significant agricultural pest that causes substantial economic losses to a wide range of crops globally, causing significant economic losses to a variety of crops. Its host range is extensive, including over 300 species across approximately 100 plant families, including both crops and ornamental plants. In recent years, with the continuous adjustment of agricultural planting structures in China and the increase of agricultural replanting index, the severity of *S.litura* damage has been escalating annually [1], especially posing a serious threat to the safe production of vegetables [2]. Historically, chemical control has been the main method for managing *S.litura*, leading to increased resistance and reduced control efficacy. In pursuit of sustainable pest management, biological control has been actively promoted and applied as an effective approach in China.

During the formation of spores, *Bacillus thuringiensis* produces crystalline proteins at one or both ends of its bacterial cells. These crystalline proteins exhibit significant insecticidal effects against various pests such as Lepidoptera and Coleoptera, and they are widely utilized in agricultural and sanitary pest control, thereby significantly reducing the use of chemical insecticides [3]. Studies have shown that different strains of *B. thuringiensis* vary in their toxicity to Lepidoptera larvae. Due to insect avoidance or inhibition of toxin proteins, insects may not ingest sufficient quantities of toxin proteins, hence the lethality of these proteins to insects is not as high as originally thought. However, even small amounts of toxin proteins can sufficiently disrupt normal insect growth and development, leading to abnormalities or even death. Research indicates that *B. thuringiensis* insecticides not only kill target pests but also inhibit, impede, and prolong the growth, development, and reproduction of pests [4,5]. Yu's study [6] found that sublethal concentrations of strain *Bt* 46 adversely affect the growth and development of *Mythimna separata* Walker, particularly reducing their reproductive capacity and the hatchability and survival rates of their offspring, consistent with findings on sublethal concentration treatments of contemporary populations using chemical pesticides [7,8].

Building upon prior research isolating the pathogenic *Bt* strain H-01-14 against *S. litura*, this study extensively investigates its impact

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on the growth, development, and reproduction of *S.litura*. The aim is to enhance the reservoir of wild *B. thuringiensis* strains for biological insecticides, enrich the theoretical foundation of biological control, provide new strategies and methods for the control of *S.litura*, and serve as a reference for biological control to other pests.

Materials and Methods

Materials

S. litura: 2nd instar larvae, bred indoors through artificial rearing; strain H-01-14 isolated and preserved by the Agrochemicals Laboratory of Sichuan Agricultural University.

Stomach Poison Activity Assay

The biological assay method for *S. litura* utilized a feed mixing technique: Healthy 2nd instar larvae of uniform size were selected as test insects. Artificial feed was prepared according to a specified formula and preparation method. A mixture of 200 mg/mL spore crystal suspension was added, mixed thoroughly, allowed to solidify, and cut into suitable pieces, which were then placed into bioassay trays. Each treatment involved 10 larvae per replicate, with 3 replicates per sample, and larvae were reared at 28 °C, 80% humidity, and 12 h light/12 h dark cycle conditions. The control group was treated with sterile water under similar conditions. After 72 hours of cultivation, dead larvae were recorded to calculate corrected mortality rates [9].

$$\text{Corrected Mortality Rate (\%)} = \frac{\text{Mortality Rate of Treatment Group - Mortality Rate of Control Group}}{\text{Mortality Rate of Control Group}} \times 100$$

Morphological Observation and 16S rDNA Sequence Determination

Morphological identification of colony and cell morphology of bacterial strains followed the the methodology described by Ammons et al. (2002) [10].

Total DNA of the tested strains was extracted using a bacterial genomic DNA kit. PCR amplification was performed using universal bacterial primers 27F-1492R: 1 μl cDNA, 1 μl upstream primer F, 1 μl downstream primer R, 12.5 μl 2×Taq Master Mix, and 9.5 μl ddH2O. The PCR reaction program included initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The quality of PCR products was checked by 2% agarose gel electrophoresis. PCR products were sequenced by Sangon Biotech (Shanghai, China). Sequences were compared with NCBI database for homology and a phylogenetic tree was constructed using MEGA 7.0 software to determine their taxonomic status.

Effects of H-01-14 on Growth and Development of *S. litura*

A mixture of 200 mg/mL spore crystal suspension of strain H-01-14 was added to artificial feed. The control group received artificial feed containing an equivalent volume of sterile water. Each treatment involved 100 larvae with 3 replicates per treatment. After 5 days of feeding, larvae were observed daily for mortality and developmental stage changes until pupation and eclosion. Adults were provided with 10% honey water post-eclosion. Male and female individuals were paired in a 1:1 ratio and placed in rectangular disposable boxes (10.0 cm×10.0 cm×5.0 cm) with 10-20 biological replicates per treatment. Nutrition was supplemented with cotton balls dipped in 10% honey water, with daily changes of cotton balls and oviposition papers. Eggs were collected until adult death. Eggs collected were placed in an artificial climate incubator, and eggs from the F_0 generation (*S. litura* fed for 5 days) were transferred to the next generation (F_1) to investigate whether strain H-01-14 affects the growth and development of *S. litura*. Finally, statistics were conducted on larval period, pupal period, pupation period, adult period, eclosion period, as well as the number of F_1 and F_2 larvae hatched and unhatched egg masses to calculate hatchability.

Histological Observation of the effect of H-01-14 Strain on *S. litura* Ovary, Oocyte, and Midgut

The method for handling *S. litura* larvae was the same as above. Within 0-12 hours post-eclosion, 10 male and 10 female *S. litura* adults from generations F_0 and F_1 were respectively selected. The reproductive organs (testes, ovaries) of treatment and control groups of *S. litura* adults (F_0, F_1) and midgut tissues of fifth instar larvae were dissected using dissecting scissors. Paraffin sections were prepared according to modified Lee's methods [11], including embedding, sectioning, staining, and observation using an optical microscope. Morphological changes in reproductive organs of *S. litura* adults after treatment with strain H-01-14 were observed with reference to related literature [12-15].

Transcriptome Analysis of Stomach Poison Action of *S. litura* after Treatment with H-01-14 Strain

Sample collection: Samples were collected when *S. litura* reached the 6th instar after stomach poison treatment. Samples from control and experimental groups were frozen rapidly in liquid nitrogen and stored at -80°C until further experiments for RNA extraction and sequencing conducted by Shanghai Ouiyi Biotechnology Co., Ltd.

RNA Extraction, cDNA Library Construction and Sequencing

During the library construction phase, total RNA of *S. litura* male and female adults was extracted using the Trizol method. RNA sample purity, concentration, and integrity were assessed to obtain high-quality RNA for constructing cDNA libraries. Subsequently, mRNA was enriched from eukaryotic mRNAs, which were fragmented using ultrasound. cDNA synthesis involved first-strand cDNA synthesis using fragmented mRNA and random hexamers, followed by second-strand cDNA synthesis using RNAseH and dNTPs under DNA polymerase I system. Purified cDNA was subjected to end repair and A-tailing, followed by adapter ligation. cDNA fragments of approximately 200-300 bp were selected using AMPure XP beads, PCR amplified, and purified again using AMPure XP beads to evaluate library quality and make sure the cDNA fragments are of moderate length and the library content is sufficient. Finally, sequencing was performed on the Illumina Hiseq Nova 6000 platform by Shanghai Ouiyi Biotechnology Co., Ltd.

Transcriptome Splicing, Assembly, and Annotation of Gene Function

Raw reads were processed using fastp for quality control to remove adapter sequences and low-quality reads, resulting in clean reads. Trinity software was used for transcriptome assembly to obtain a high-quality unigene library of *S. litura* adults. The assembled

unigene sequences were functionally annotated using BLAST software (E-value ≤ 10-5) against seven databases: Nr (https://blast.ncbi.nlm.nih.gov/Blast.cgi), Nt (https://blast.ncbi.nlm.nih.gov/Blast.cgi), Swiss-Prot (https://www.uniprot.org), GO (http://geneontology.org), KO (https://www.genome.jp/kegg), KOG/COG (https://www.ncbi.nlm.nih.gov/COG), and Pfam (http://pfam.xfam.org) [16-18].

Results and Analysis

Insecticidal Activity Assay Results

Using indoor-reared fall armyworms as test insects, the biological activity of the parasporal crystal mixture from 143 strains of *Bacillus thuringiensis* was assessed. The results are shown in Table 1. Strains H-02-05, K-03-02, P-11, M-02-04, and H-01-14 exhibited insecticidal effects against fall armyworm larvae, with significant toxicity observed for strain H-01-14, achieving a corrected mortality rate of 46.67% at 72 hours.

Strain Identification

Strain H-01-14 was morphologically characterized under a microscope (Figure 1a), exhibiting irregular circular, flattened white colonies with slight elevation in the center, dry surface, and incomplete edges; parasporal crystal morphology appeared circular and rhomboid (Figure 1b).

The 16S rRNA PCR product sequence of strain H-01-14 was compared for homology with sequences in the GenBank nucleotide database, confirming its identity as *Bacillus thuringiensis* subsp. kurstaki H-01-14, as depicted in the phylogenetic tree constructed using MEGA7.0 software (Figure 2).

Table 1: Biological activity screening of partial isolates.

Strains	Number of Tested Insects	Corrected Mortality Rate (%)
K-03-02	30	16.67±0.71a
H-01-14	30	46.67±0.19cd
P-11	30	20.00±0.75b
H-02-05	30	40.00±1.15c
M-02-04	30	20.00±0.15b

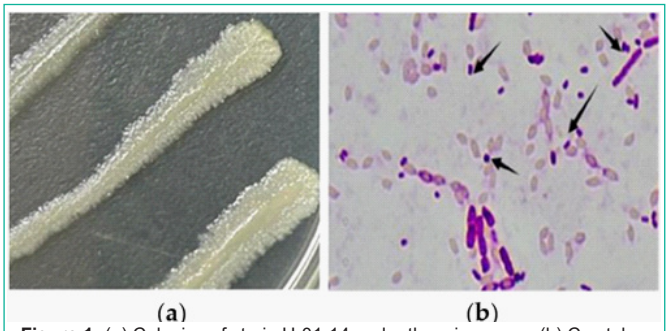


Figure 1: (a) Colonies of strain H-01-14 under the microscope (b) Crystals of strain H-01-14 under the microscope.

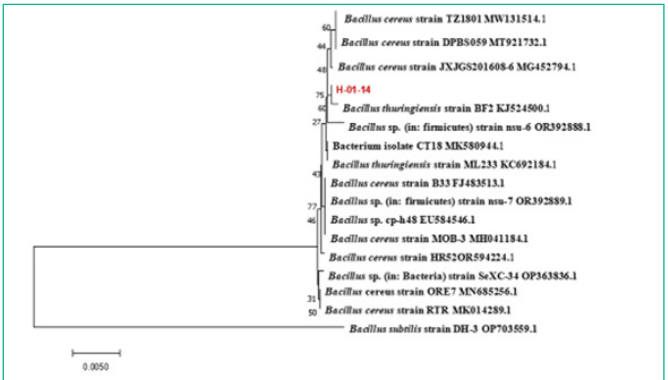


Figure 2: Constructing a phylogenetic tree based on 16S r DNA sequences.

Effect of Strain H-01-14 Treatment on *S. litura* Growth and Development

Starting from second-instar larvae, *S. litura* were fed with strain H-01-14 for 5 days, and the developmental stages from F0 to F1 pupation and eclosion were recorded (Table 2 and Figure 3). Results indicated that compared to the control, strain H-01-14 treatment significantly prolonged the larval, pupal, adult, and eclosion periods of F0 generation *S. litura* by 2.47 d, 1.71 d, 1.68 d, and shortened adult lifespan significantly by 4.51 d, with no significant effect on pupation period. For the F1 generation under continuous culture, strain H-01-14 prolonged eclosion and pupal periods by 0.67 d and

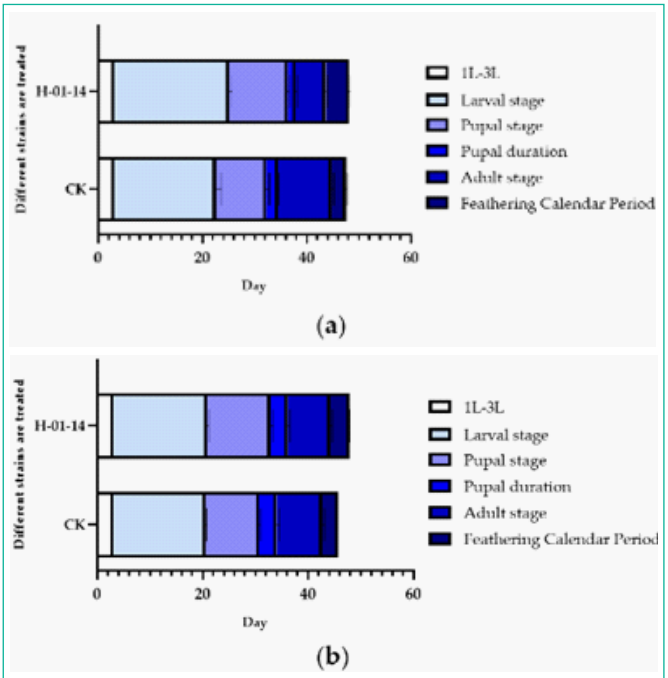


Figure 3: (a) Effects of different strains on the development duration of F_0; (b) Effects of different strains on the development duration of *S. litura*.

Table 2: Effects of different strains on the growth and development of F0 and F1 of *S. litura*.

Generation	Strains	Larval Period (d)	Pupal Periods (d)	Pupation Period (d)	Adult Period (d)	Eclosion Period (d)	Pupal Rate (%)	Eclosion Rate (%)
F_0	CK	19.57±1.05a	9.63±0.59a	3.17±0.21a	10.29±0.49b	2.89±0.19a	97.67±0.11b	89.67±0.03b
	H-01-14	22.04±0.66b	11.35±0.24b	4.82±0.72b	5.77±0.25a	4.57±0.11b	74.33±0.02a	49.33±0.29a
F_1	CK	17.58±0.15a	10.06±0.31a	3.41±0.54a	8.62±0.47b	3.00±0.00a	94.00±0.05b	98.00±0.01b
	H-01-14	17.79±0.34a	12.06±0.54b	3.29±0.38a	6.72±0.31a	3.61±0.09b	75.00±0.15a	97.00±0.00a

Note: The data in the table represents mean±SE and the followed letter indicate significant level of difference (p<0.05).

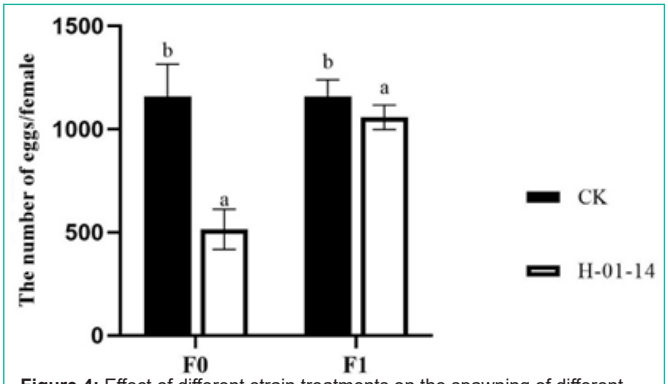


Figure 4: Effect of different strain treatments on the spawning of different *S. litura* generations.

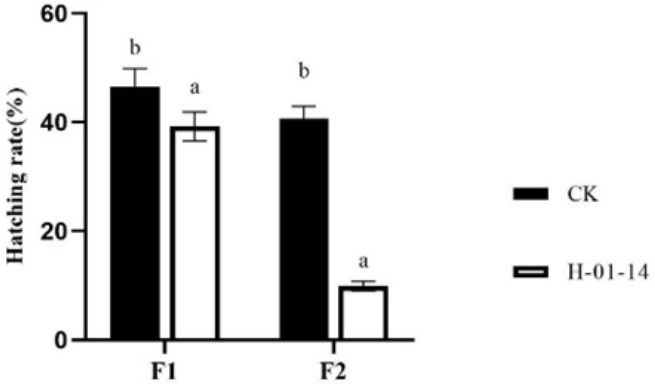


Figure 5: Effect of different strain treatments on hatching rate of different *S. litura* generation.

2.00 d, respectively, with no significant effects on larval and pupation periods. Strain H-01-14 also significantly inhibited pupation rate, reducing pupation by 23.34% and eclosion rate by 40.34% for F0 generation *S. litura*.

Effect of Strains H-02-05 and H-01-14 on *S. litura* Oviposition and Hatchability

S. litura second-instar larvae were treated with strain H-01-14, and oviposition of F0 and F1 generations was recorded (Figure 4). Compared to the control, strain H-01-14 significantly reduced egg production by single-headed females in F0 by 49.75% and F1 by 2.84%.

Hatchability rates of F1 and F2 generations were also assessed following treatment with strain H-01-14 (Figure 5), revealing significant reductions of 13.86% and 68.08%, respectively, compared to controls.

The effect of H-01-14 strain on the *spermatozonal* and ovarian tissues of *S. litura*

To further understand the effects of strain H-01-14 on the reproductive system of *S. litura*, this experiment conducted paraffin section observations of the testes, ovaries, and midgut. Ten slides per organ were examined, selecting fields with a reproducibility rate exceeding 80% for photographic analysis, as shown in Figure 6.

Observations of testis sections revealed no significant pathological changes in the testes of *S. litura* F_0 and F_1 compared to the control following treatment with strain H-01-14 (Figure 6 a-c).

Table 3: Statistical table of differentially expressed genes related to insect growth and development.

Gene id	Regulation	Log_2FoldChange	Description
LOC111351884	Up	4.299908861	probable chitinase 10
LOC111360476	Up	4.608821585	phospholipase A2-like
LOC111349968	Up	7.808258842	trypsin inhibitor-like
LOC111352379	Up	3.688287007	trypsin alpha-3-like
LOC111353053	Down	-2.513289559	trypsin, alkaline B-like
LOC111353057	Down	-1.30484442	trypsin CFT-1-like
LOC111353061	Down	-2.122893266	trypsin CFT-1-like
LOC111353068	Down	-1.294820307	trypsin CFT-1-like
LOC111355079	Up	1.454905756	trypsin, alkaline B-like
LOC111356620	Down	-1.37208869	chymotrypsin-1-like
LOC111356621	Down	-1.043310978	chymotrypsin-1-like
LOC111350094	Up	4.267998005	aldo-keto reductase AKR2E4-like

Examination of ovary sections showed marked pathological changes in the ovarian tissue of adult *S. litura* F_0 treated with strain H-01-14, characterized by uneven cytoplasmic structure and the presence of vacuoles. Follicular cell rupture and irregular structure were observed, whereas no significant pathological changes were noted in the F_1 generation (Figure 6).

Observation of midgut sections revealed regular epithelial cell arrangement with numerous microvilli in the normal midgut of *S.litura* larvae. Treatment with strain H-01-14 did not induce significant pathological changes in the midgut of fifth-instar larvae compared to the control (Figure 7).

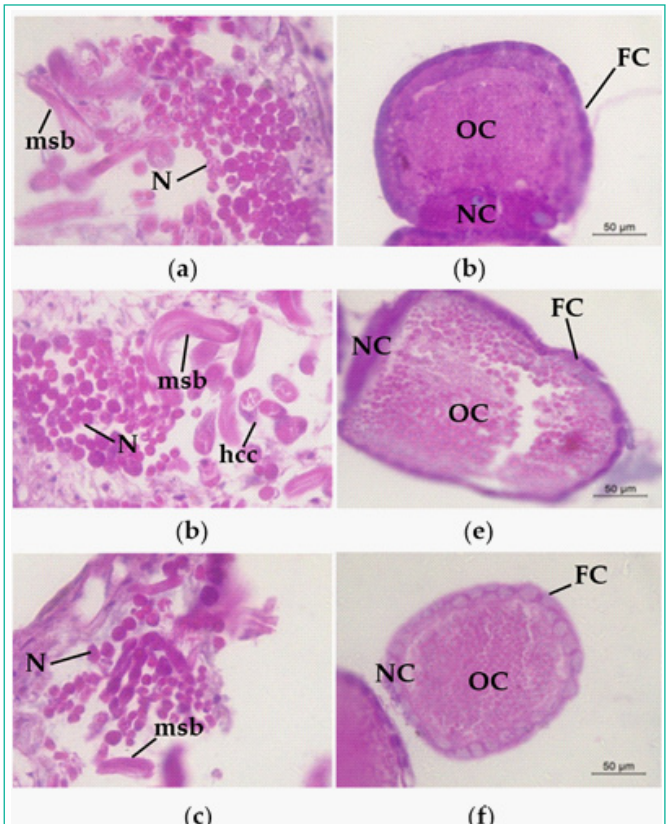
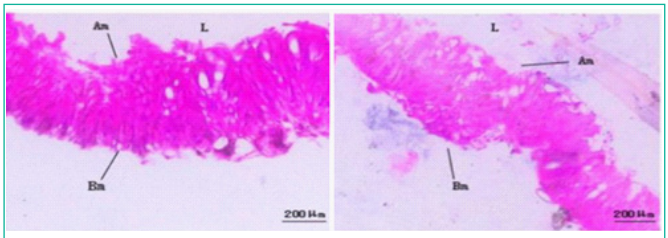


Figure 6: Pathological changes of H-01-14 strain on the spermatozonal and ovarian tissues of *S. litura*. (a) nest of CK; (b) fine nest of F_0 generation; (c) nest of F_1 generation. (d) ovaries of CK; (e) ovaries of F_0 generation. (f) ovaries of F_1 generation. MSB: mature sperm bundle; N: nucleus; hcc: apical sac cell; OC: oocyte; NC: trophoblast; FC: follicular cells.

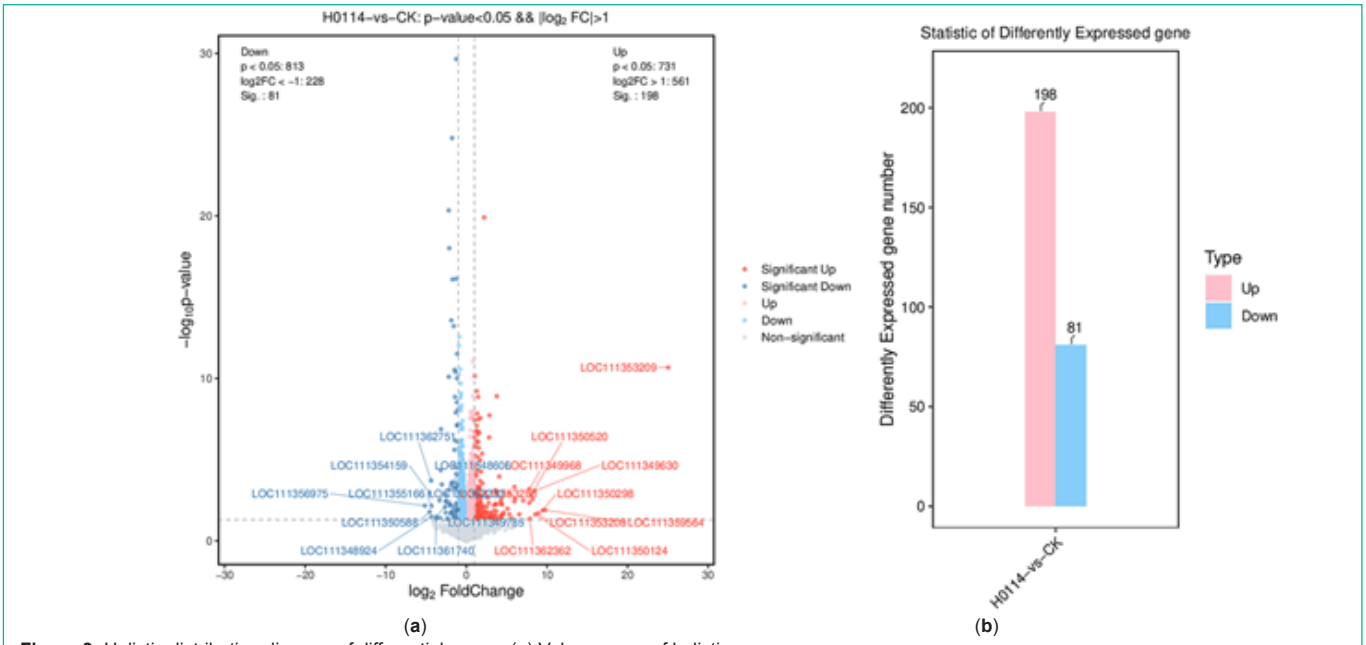


(**a**) (**b**)

Figure 7: Pathological changes of strain H-01-14 on midgut tissue of *S. litura*. (a) CK midgut; (b) H-01-14 midgut; Am: apical membrane; Bm: basement membrane; L: lumen.

Differentially Expressed Genes Related to Insect Growth and Development

Chitinases, phospholipases, aldo-keto reductases, and serine proteases play significant roles in the overall growth and development of insects. This study identified 12 relevant genes from the transcriptomes of *S. litura* treated with gastric toxins from strain H-01-14 and the control group, including 1 chitinase gene (chitinase 10), 1 phospholipase gene (phospholipase A2-like), 1 trypsin inhibitor-like gene, 4 trypsin genes (trypsin alpha-3-like, trypsin CFT-1-like), 2 chymotrypsin genes (chymotrypsin-1-like), and 1 aldo-keto reductase gene (aldo-keto reductase AKR2E4-like), as shown in Table 3 and Figure 10.



(**a**) (**b**)

Figure 8: Holistic distribution diagram of differential genes. (a) Volcano map of holistic distribution diagram of differential genes; (b) bar plot of holistic distribution diagram of differential genes.

Transcriptome Analysis of *S. litura* after Treatment with Strain H-01-14

Number of Differentially Expressed Genes: DEseq2 method was used to analyze differentially expressed genes (DEGs) obtained from transcriptome data. The results indicated a total of 279 DEGs between control and treated samples, with 198 upregulated genes and 81 downregulated genes. The overall distribution of DEGs is shown in Figure 8.

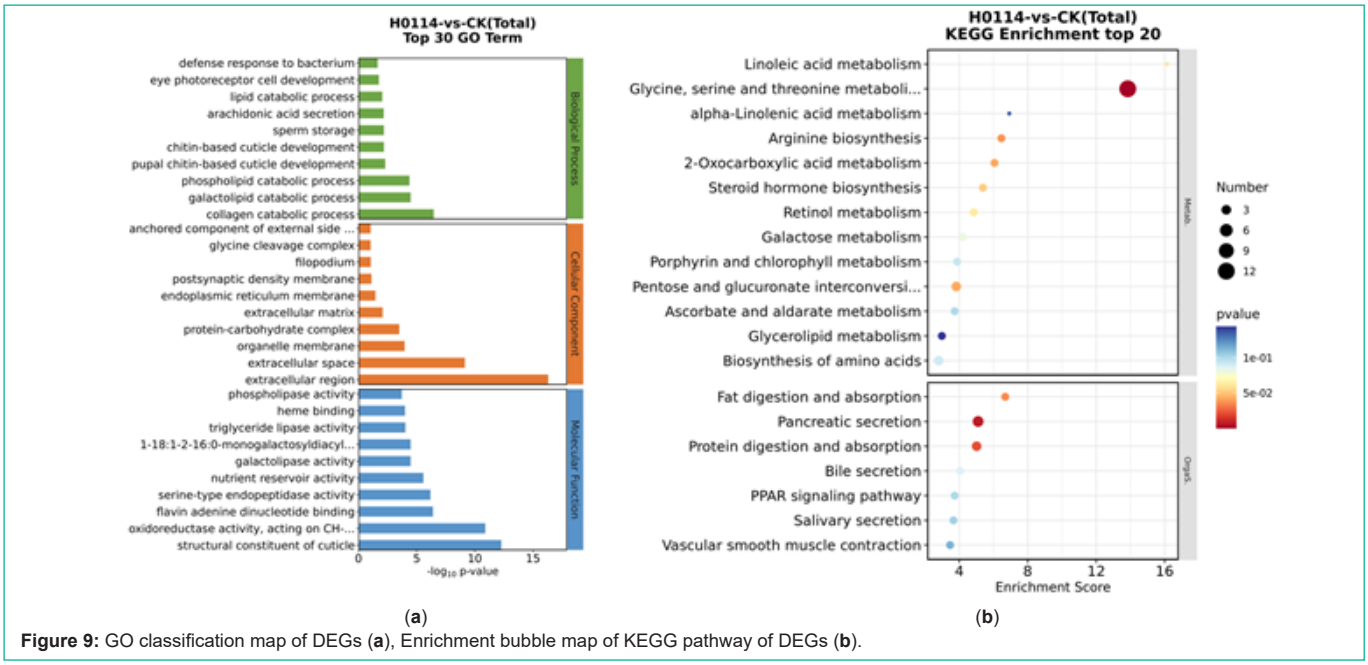
GO and KEGG Enrichment Analysis of Differentially Expressed Genes

Analysis of differentially expressed genes in *S. litura* after treatment with strain H-01-14 revealed enrichment in GO pathways primarily associated with serine-type endopeptidase activity, phospholipases, and chitin-based cuticle development, as depicted in Figure 9**a**. The top 20 pathways with the smallest p-values were selected for significant enrichment analysis in bubble plots. KEGG pathway enrichment analysis (Figure 9**b**) indicated significant enrichment in glycine, serine, and threonine metabolism, pancreatic secretion, and protein digestion and absorption pathways.

Summary and Discussion

In this study, we found that *Bacillus thuringiensis* strain H-01-14 significantly prolonged the larval, pupal, and eclosion periods of *S. litura*, while also shortening their adult lifespan. It also significantly affected oviposition and offspring hatchability, and disrupted ovarian tissue of *S. litura*. Transcriptomic sequencing revealed that strain H-01-14 primarily influences the expression of genes related to chitinase, phospholipase, trypsin, and aldo-keto reductase, thereby affecting the growth, development, immune function, and detoxification metabolism of *S. litura*.

The study demonstrates that *Bt* insecticides not only kill target pests but also inhibit, hinder, and prolong pest growth, development, and reproduction [19]. We found that extending the developmental period of surviving larvae can correspondingly reduce the number of pest generations, thereby mitigating crop damage and preventing unnecessary losses [5]. Song [20] observed pathological changes in the midgut tissues of third-instar *S. litura* fed with Vip3Aa protein for 24 hours, showing epithelial swelling, cytoplasmic vacuolation, and shortened, deformed, and detached microvilli. GO enrichment analysis of transcriptomic results revealed that genes differentially expressed in *S. litura* larvae treated with strain H-01-14 were mainly enriched in pathways such as serine-type endopeptidase activity,



(**a**) (**b**)

Figure 9: GO classification map of DEGs (**a**), Enrichment bubble map of KEGG pathway of DEGs (**b**).

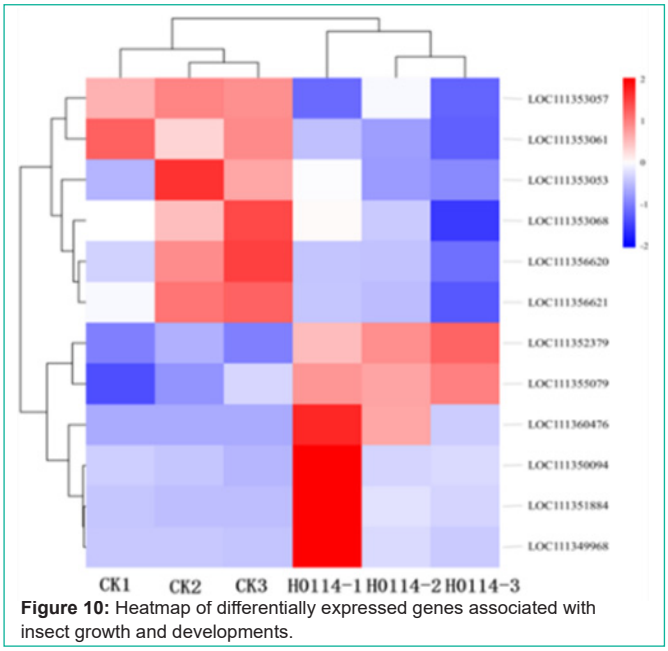


Figure 10: Heatmap of differentially expressed genes associated with insect growth and developments.

phospholipase, and chitin-based cuticle development. The significant upregulation of the differential genes, including chitinase 2 and phospholipase A2, aligns with previous studies on their impact on fall armyworm growth and development [21]. In earlier reports [20,22], it was found that the chitinase 2 gene plays a crucial role in insect growth, development, and molting processes, regulating growth and reproduction. The significant upregulation of this differential gene in our study is consistent with previous research on its impact on fall armyworm growth and development. Phospholipase is an important enzyme present in organisms, playing a significant role in cell membrane structure formation, maintenance, and breakdown, as well as in phospholipid metabolism, hormone signal transduction, and biological membrane stability. The gene significantly associated with this experiment is phospholipase A2. This enzyme has been studied in several insects, including Drosophila melanogaster, red flour beetle, and tobacco budworm [23-26]. Research has shown that targeted inhibition of sPLA2 weakens the immune response of various lepidopteran insects [27]. KEGG pathway analysis in our study revealed that differentially expressed genes were mainly enriched in pathways such as pentose and glucuronate interconversions, galactose metabolism, glycerolipid metabolism, pancreatic secretion, protein digestion, and absorption. Serine proteases in lepidopteran insect intestines are involved in *Bt* protoxin activation and toxin degradation processes. Resistance of insects to *Bt* may depend on changes in the expression levels or types of serine proteases (e.g., trypsin and chymotrypsin) in the insect gut [28-31], Rodriguez [32] identified a crucial molecular gene called serine protease-like serine protease, which encodes a protein that confers resistance to *Bt* Cry1Ca1 protoxin in the fall armyworm *Spodoptera frugiperda*. Further studies have shown that RNA interference technology can significantly reduce the sensitivity of *S. frugiperda* to Bt Cry1Ca1 protoxin by silencing this gene. Aldo-keto reductase family genes participate in the ecdysteroid metabolism of lepidopteran insects. Yamamoto et al. [33] discovered another aldo-keto reductase AKR2E8 in silkworm *Bombyx mori*, which is expressed in the testes, ovaries, and fat bodies of *B. mori*, with the highest expression in the midgut.

Author Contributions

J.H., C.Y. and H.C. conceived and designed the research. Y.Z. and S.Y. conducted the experiments. Y.Z., Y.C., R.M. analyzed the data. Y.Z. and S.Y wrote the manuscript. All authors read and approved the manuscript.

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Data Availability Statement: Data are contained within the article.

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