KARADENİZ TECHNICAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

BIOTECHNOLOGY DEPARTMENT

BIOLOGICAL CONTROL OF COTTON PEST *HELICOVERPA ARMIGERA* USING RNA INTERFERENCE (RNAi) TECHNOLOGY

MASTER THESIS

Halime KARATEKE

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PREFACE

This study titled "Biological Control of Cotton Pest *Helicoverpa armigera* Using RNA Interference (RNAi) Technology" prepared as Master thesis at Karadeniz Technical University, Institute of Science and Technology, Department of Molecular Biotechnology.

I am deeply grateful to my dear advisor, Prof. Dr. Remziye NALÇACIOĞLU, for her unwavering support throughout my thesis process. I sincerely thank my esteemed teacher, Prof. Dr. Zihni DEMİRBAĞ, for his valuable assistance in the execution of the thesis. My heartfelt appreciation is extended to the dear jury member, Prof. Dr. Hatice KATI, for honoring me with her participation. I am also thankful to Prof. Dr. Hacer MURATOĞLU and Prof. Dr. İsmail DEMİR for their generous help and guidance. My sincere thanks go to Research Assistant Kübra ZENGİN and Bora ÖKTEM for their support throughout my laboratory work. I also thank my colleagues at the Biotechnology and Microbiology Laboratory of the Faculty of Science, Karadeniz Technical University, for their collaboration and assistance.

My Allah’s mercy and blessings be upon my parents, who have always guided and supported me with their love and prayers; I express my deepest gratitude and respect for their devotion and sacrifices. I dedicate this work to my parents, seeking Allah’s pleasure and mercy.

Halime KARATEKE

Trabzon 2025

THESIS ETHICS STATEMENT

I declare that I have completed this study titled “Biological Control of Cotton Pest *Helicoverpa armigera* with RNA Interference (RNAi) Technology”, which I submitted as a master's thesis, under the responsibility of my advisor Prof. Dr. Remziye NALÇACIOĞLU from the beginning to the end, collected the data/samples myself, conducted the experiments/analyzes in the relevant laboratories, shown the information I received from other sources in the text and references, and acted in accordance with scientific research and ethical rules during the study and accept any legal consequences in case the opposite occurs.
05/11/2025

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SUMMARY

BIOLOGICAL CONTROL OF COTTON PEST *HELICOVERPA ARMIGERA* USING RNA INTERFERENCE (RNAi) TECHNOLOGY

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Karadeniz Technical University
The Graduate School of Natural and Applied Sciences
Molecular Biotechnology Graduate Program
Supervisor: Prof. Dr. Remziye NALÇACIOĞLU
2025, 51 Pages

Helicoverpa armigera (Hübner, 1808) is a member of the lepidopteran order of the Noctuidae family. It is an effective polyphagous species that causes serious damage to agricultural crops in many parts of the world. Control is generally achieved with synthetic insecticides. The development of resistance in insects to synthetic insecticides, coupled with the harm they cause to humans and other non-target organisms, has necessitated the development and use of alternative biological control preparations. RNA interference (RNAi) is an alternative method for controlling pest populations. This method provides a species-specific control method by silencing vital genes in target insects. Chitin, an important biological polymer, is found in all arthropods, including insects. Furthermore, chitin and the enzymes involved in its synthesis are not found in plants or humans.

This study aimed to silence the expression of the *Chitin synthase B (CHSB)* gene, which is involved in chitin synthesis, using double stranded RNA (dsRNA) technology. For this purpose, the CHSB gene from *H. armigera* larvae was amplified by polymerase chain reaction and subsequently cloned into a dsRNA synthesis vector (L4440). dsRNA synthesis was performed in a bacterial system *(E. coli* HT115 (DE3) strain). Subsequently, both the dsRNA-expressing bacterial suspension and the total RNA purified from these cells were mixed with separate feed and fed to *H. armigera* larvae. The effects of dsRNA on insects were investigated through bioassay experiments. Both dsRNA-expressing *E. coli* cells and RNA's isolated from these cells induced significant mortality in third-instar *H. armigera* larvae, reaching 40% and 35%, respectively, whereas control treatments exhibited negligible effects.

Key Words: dsRNA, Chitin Synthase B, RNA interference, *Helicoverpa armigera*

Yüksek Lisans Tezi

ÖZET

RNA İNTERFERANS TEKNOLOJİSİ İLE PAMUK ZARARLISI *HELİCOVERPA ARMİGERA*'NIN BİYOLOJİK KONTROLÜ

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2025, 51 Sayfa

Helicoverpa armigera (Hübner, 1808), Noctuidae familyasına ait lepidopteran takımının bir üyesidir. Dünyanın pek çok yerinde tarımsal ürünlere ciddi zararlar veren etkili bir polifag türdür. Kontrolü genellikle sentetik insektisitlerle yapılmaktadır. Sentetik insektisitlere karşı böceklerin direnç geliştirmeleri, insan ve hedef olmayan başka canlı gruplarının zarar görmeleri bunlara alternatif olan biyolojik kontrol preparatlarının geliştirilip kullanılmasını zorunlu hale getirmiştir. RNA İnterferans (RNAi) yaklaşımı zararlı böcek popülasyonlarının kontrolünde kullanılabilecek alternatif bir yöntemdir. Bu yöntem hedef böceklerde hayati genleri susturarak böcek türlerine özgü bir mücadele yöntemi sağlamaktadır. Önemli bir biyolojik polimer olan kitin böcekler dahil olmak üzere tüm eklem bacaklılarda bulunmaktadır. Kitin polimeri ve bu polimerin senteziyle ilgili enzimler bitki ya da insanlarda bulunmamaktadır.

Bu çalışmada, kitin sentezinde görev alan *Kitin sentaz B* (*CHSB*) geninin ifadesinin çift zincirli RNA (dsRNA) teknolojisi ile susturulması amaçlanmıştır. Bunun için, *H. armigera* larvalarından *CHSB* geni polimeraz zincir reaksiyonu (PCR) ile çoğaltıldı ve ardından dsRNA sentez vektörüne (L4440) klonlandı. dsRNA sentezi bakteriyel sistemde (*E. coli* HT115 (DE3) suşunda) gerçekleştirildi. Daha sonra hem dsRNA ifade eden bakteri süspansiyonu hem de bu hücrelerden saflaştırılan total RNA ayrı ayrı besin ile karıştırılarak *H. armigera* larvalarına yedirildi. dsRNA'ın böcekler üzerine etkisi biyotest denemeleri ile incelendi. Hem dsRNA eksprese eden *E. coli* hücreleri hem de bu hücrelerden izole edilen RNA'lar, üçüncü dönem *H. armigera* larvalarında anlamlı mortaliteye neden olmuş ve sırasıyla %40 ve %35 oranında ölüm gözlenmiş, kontrol gruplarında ise etkiler önemsiz düzeyde kalmıştır.

Anahtar Kelimeler: dsRNA, Kitin sentaz B, RNA interferans, *Helicoverpa armigera*

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ABBREVIATIONS

DNA	: Deoxyribonucleic acid
RNA	: Ribonucleic acid
npcRNAs	: Non-protein coding RNAs
mRNA	: Messenger RNA
rRNA	: Ribosomal RNA
tRNA	: Transfer RNA
RNAi	: RNA interference
PABPC	: Cytoplasmic poly(A)-binding protein
TRBP	: TAR RNA-binding protein
PACT	: Protein kinase R activator
P-body	: Processing body
TEs	: Transposable elements
miRNA	: MicroRNA
piRNA	: Piwi-interacting RNA
pre-miRNAs	: Precursor microRNAs
pri-miRNA	: Primary microRNA
siRNA	: Small interfering RNAs
ssRNA	: Single-stranded RNA
PAZ	: Piwi/Argonaute/Zwille domain
AGO	: Argonaute proteins
RISC	: RNA-induced silencing complex
dsRNA	: Double-stranded RNA
CHSB	: Chitin Synthase B
GlcNAc	: N-acetylglucosamine
UDP-GlcNAc	: UDP-N-acetylglucosamine
bp	: Base pair
M	: Molar
ml	: Milliliter
mM	: Millimolar
µg	: Microgram

µl	: Microliter
µM	: Micromolar
ng	: Nanogram
nm	: Nanometer
dNTP	: Deoxyribonucleotide triphosphate
SDS	: Sodium dodecyl sulfate
EDTA	: Ethylenediaminetetraacetic acid
LB	: Luria-Bertani
dH2O	: Distilled water
OD	: Optical density
PCR	: Polymerase chain reaction
°C	: Celsius
IPTG	: Isopropyl β-D-1-thiogalactopyranoside
DEPC	: Diethyl pyrocarbonate
Amp	: Ampicillin
Tet	: Tetracycline
IPM	: Integrated pest management
PM	: Peritrophic matrix
SIGS	: Spray-induced gene silencing
HIGS	: Host-induced gene silencing
VIGS	: Virus-induced gene silencing

1. GENERAL INFORMATION

1.1. Introduction

Crop losses caused by insect damage and the extensive use of insecticides for pest management result in billions of dollars in economic losses worldwide each year. This ongoing reliance on insecticides not only increases costs but also threatens human health and raises significant concerns about the potential development of insecticide resistance, making pest control a critical area of research (Katoch at al., 2013). Consequently, there has been a growing emphasis on finding alternative methods for managing insect pests.

One promising alternative to conventional chemical insecticides is the use of microorganisms as biopesticides. These microorganisms, including bacteria, fungi, viruses, and nematodes, offer targeted and environmentally friendly options for pest control (Goettel et al., 2005). Microorganisms can infect, kill, or inhibit the growth of pests, often with minimal impact on non-target species and the surrounding ecosystem (Ayilara et al., 2023; Kumar et al., 2021). However, primary limitations of these biological agents include their highly targeted nature against specific diseases and pathogens, which often necessitates the application of several different microbial pesticides, as well as their inconsistent effectiveness resulting from environmental and biological factors that affect these living microorganisms (Bonaterra et al., 2012).

The order Lepidoptera, accounts for roughly 10% of all described animal species on earth includes butterflies, moths, and species known as “skippers,” comprises approximately 160,000 species, making it the second largest group among insects. *H. armigera* (cotton bollworm) and *Plutella xylostella* (diamondback moth) are among the most widespread and resistant agricultural pests in Lepidoptera ordo. Recent advances in high-throughput sequencing technologies have enabled the successful assembly of pest genomes, leading to the identification of key genes involved in insecticide resistance, development, and reproduction. The i5k (5000 Insect Genome Project) initiative, which aims to sequence the genomes of 5,000 arthropod species, has significantly contributed to this progress (Poelchau et al., 2015). Since the sequencing of *Plutella xylostella* in 2013, the number of sequenced Lepidopteran genomes has reached 1,315 as of 2024, although only 599 have achieved

chromosome-level assemblies. This highlights the ongoing challenges of complete genome assembly in species with complex genomic structures (Niu et al., 2024a).

Given the challenges associated with conventional insect control methods, there is an urgent need for alternative strategies that are both sustainable and environmentally benign. RNA interference (RNAi) or post-transcriptional gene silencing (PTGS) has emerged as a revolutionary approach in this regard. RNAi is a natural biological process that involves the degradation of specific mRNA molecules, which in turn inhibits the expression of targeted genes. This method can be harnessed to silence genes essential for the survival, development, or reproduction of insect pests, thereby offering a highly specific and targeted pest control strategy. The precision of RNAi enables the development of interventions that are tailored to disrupt key biological processes within pest species without affecting non-target organisms. Additionally, RNAi-based strategies can be designed to address multiple genes simultaneously, reducing the likelihood of resistance development and enhancing the effectiveness of IPM systems. By integrating RNAi with other pest management practices, it is possible to create a more resilient and ecologically sustainable approach to pest control that aligns with principles of environmental conservation and reduces reliance on chemical insecticides.

1.2. Structure and Function of RNA

Ribonucleic acid (RNA) was first discovered in the mid-20th century and initially considered merely a transient messenger that transfers genetic information from DNA to ribosomes. However, over time, RNA has been recognized not only as a carrier of genetic instructions but also as a central molecule in protein synthesis and gene expression regulation. RNA is a single-stranded polynucleotide in which each nucleotide unit contains a ribose sugar, a phosphate group, and one of four nitrogenous bases (adenine, guanine, cytosine, and uracil). Unlike DNA, RNA contains uracil instead of thymine, and its ribose sugar possesses a hydroxyl (-OH) group at the 2' carbon position, making RNA chemically more reactive and less stable (Minchin & Lodge, 2019). Although RNA usually exists as a single strand, it can fold upon itself through internal base pairing to form double-stranded regions and complex three- dimensional structures (Weeks, 2010). These secondary and tertiary conformations allow RNA to specifically interact with proteins and, in some cases, exhibit catalytic activity (Alonso & Mondragón, 2021). This structural complexity is

particularly notable in RNA types such as transfer RNA (tRNA) and ribosomal RNA (rRNA). Today, RNA molecules are classified into several subtypes based on their functions. Messenger RNA (mRNA) carries the genetic code from DNA to ribosomes to initiate protein synthesis. tRNA recognizes specific amino acids and transports them to the ribosome, facilitating peptide bond formation in the correct sequence. rRNA forms the structural and catalytic core of the ribosome, playing a key role in translation (Cao et al., 2024). Additionally, small regulatory RNAs such as microRNA (miRNA), small interfering RNA (siRNA), and Piwi-interacting RNA (piRNA), discovered more recently, are crucial in the precise post-transcriptional gene silencing of gene expression. To better understand the regulatory roles of these various RNA molecules, it is essential first to examine their molecular structures and functional classifications.

1.2.1. RNA Stability and Post-Transcriptional Gene Regulation

RNA stability refers to the length of time that RNA molecules can persist within the cell without degradation and represents a critical control point in the regulation of gene expression. The lifespan of RNA molecules directly affects the amount and timing of protein synthesis. Cells utilize rapid or slow RNA degradation to adapt to environmental stimuli, developmental stages, or stress conditions. RNA molecules are generally less stable than DNA due to their chemical structure; the presence of the 2'-hydroxyl (-OH) group in the ribose sugar makes RNA more susceptible to cleavage by ribonucleases and more chemically reactive. Cellular ribonucleases degrade RNA either specifically or nonspecifically, thus limiting the half-life of RNA molecules. Among the main factors affecting RNA stability are the RNA type and structure. For example, mRNAs are typically short-lived, allowing the cell to respond rapidly and flexibly; however, some mRNAs may exhibit increased stability. In contrast, structural RNAs such as tRNAs and rRNAs tend to be highly stable, as their continuous cellular function is essential. Additionally, the 5’ cap structure (7-methylguanosine) and the 3’ poly (A) tail, particularly in eukaryotic mRNAs, are important elements that enhance RNA stability (Passmore & Coller, 2022). RNA-binding proteins (RBPs) also play significant roles in modulating RNA stability; these proteins can bind to RNA molecules to either stabilize their structure or promote degradation. For instance, some RBPs protect mRNAs from ribonucleases, while others facilitate the formation of complexes that trigger decay (Hasan et al., 2014). Furthermore, small

regulatory RNAs such as miRNAs and siRNAs bind to target RNAs to increase their degradation or inhibit their translation, a common mechanism in post- transcriptional gene regulation. RNA stability is also influenced by the subcellular localization of RNA; for example, RNA degradation frequently occurs in processing bodies (P-bodies), and accumulation of RNA in these compartments is associated with decay (Blake et al., 2024). Finally, environmental and cellular conditions such as stress, temperature changes, or nutrient deprivation can affect RNA stability by either prolonging or shortening RNA molecule lifespans.

In addition to stability, post-transcriptional gene regulation relies heavily on the actions of non-coding RNAs (ncRNAs). Although protein coding genes represent only about 1.5% of the genome, non-coding RNAs account for nearly 80%, underscoring their importance (Pedrosa da Costa Gomes et al., 2019). Small regulatory RNAs such as miRNAs, siRNAs, and piRNAs are central players in gene silencing, either by promoting transcript degradation or by inhibiting translation. Other ncRNAs also serve critical functions: small nuclear RNAs (snRNAs) are essential for pre-mRNA splicing within the spliceosome, while small nucleolar RNAs (snoRNAs) direct chemical modifications of rRNAs. Recent studies reveal that snoRNAs and snRNAs are not limited to housekeeping roles but also contribute to disease processes, including cancer, by influencing oncogenesis and splicing regulation, suggesting their potential as therapeutic targets (Figure 1).

Together, RNA stability and post-transcriptional regulatory mechanisms form an integrated system that determines RNA lifespan, localization, and translation efficiency. This interconnected regulation allows cells to precisely fine-tune gene expression, ensuring adaptability to developmental cues, environmental stresses, and pathological conditions.

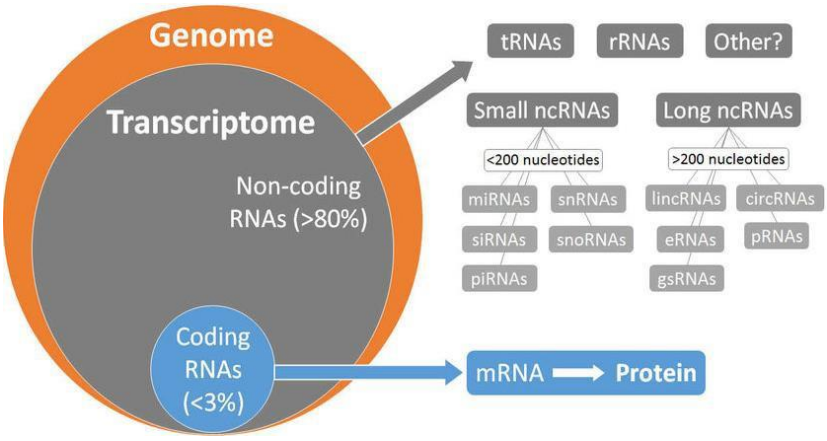

Figure 1. Total RNAs in genome (Pedrosa da Costa Gomes et al., 2019) RNA Inducers

1.3. RNA Inducers

Small regulatory RNAs (RNA inducers), including miRNAs, siRNAs, and interacting piRNAs, are key molecules that modulate gene expression at the post-transcriptional level (Niu et al., 2024). These molecules mediate RNAi by targeting and silencing specific mRNA transcripts, thereby contributing to the precise and dynamic regulation of gene expression. These three RNA types function through distinct biogenetic pathways and molecular mechanisms. miRNAs are typically transcribed from genomic loci by RNA polymerase II as primary transcripts, which are processed by the Drosha and Dicer enzymes into mature miRNAs that are incorporated into the RNA-induced silencing complex (RISC) containing Argonaute proteins. The miRNA-RISC complex binds to the 3′ untranslated region (3′ UTR) of target mRNAs via partial base pairing, leading to translational repression or mRNA destabilization (Khan et al., 2025, Cheng et al., 2022). In contrast, siRNAs are generally derived from exogenous double-stranded RNAs and processed by Dicer into siRNA duplexes, one strand of which is loaded into RISC. The guide strand pairs with high complementarity to its target mRNA, thereby directing the endonuclease activity of Argonaute to cleave the transcript. piRNAs, expressed predominantly in germline cells, are single-stranded RNAs produced independently of Dicer and associate with PIWI proteins to silence transposable elements and maintain genomic stability. "These regulatory mechanisms enable precise post-transcriptional control of gene expression and play essential roles in preserving genomic integrity in multicellular organisms (Niu et al., 2024a). Among these mechanisms, miRNAs, siRNAs, and piRNAs represent key classes of small non-coding RNAs that mediate gene silencing through distinct molecular pathways."

MicroRNAs (miRNAs): miRNAs are short (~22 nucleotides), non-coding RNA molecules that are encoded within eukaryotic genomes. They are typically transcribed from their own promoters or from intronic regions via RNA polymerase II. The primary transcripts, known as primary miRNAs (pri-miRNAs), are characterized by stem-loop structures (Rios et al., 2017). In the nucleus, these structures are processed by the Microprocessor complex composed of Drosha (an RNase III enzyme) and DGCR8 (in humans), generating a precursor miRNA (pre-miRNA) approximately 60–70 nucleotides in length. This pre-miRNA is exported to the cytoplasm via Exportin-5 in a Ran-GTP-dependent manner (Isenmann et al., 2023). Once in the cytoplasm, the Dicer enzyme further processes it into a ~22 nucleotide-long miRNA duplex. One strand of this duplex, usually

the less thermodynamically stable one, is selectively incorporated into the RISC, which includes an Argonaute (AGO) protein. The miRNA-RISC complex binds to complementary sequences, typically located in the 3′ untranslated region (3′ UTR) of target mRNAs, via partial base pairing (Passmore & Coller, 2022). This interaction results in translational repression, poly(A) tail shortening, or destabilization of the target mRNA, as shown in Figure 2 (Khan et al., 2025). In animals, miRNAs mainly exert their effects through translational inhibition, whereas in plants, near-perfect complementarity often leads to direct mRNA cleavage.

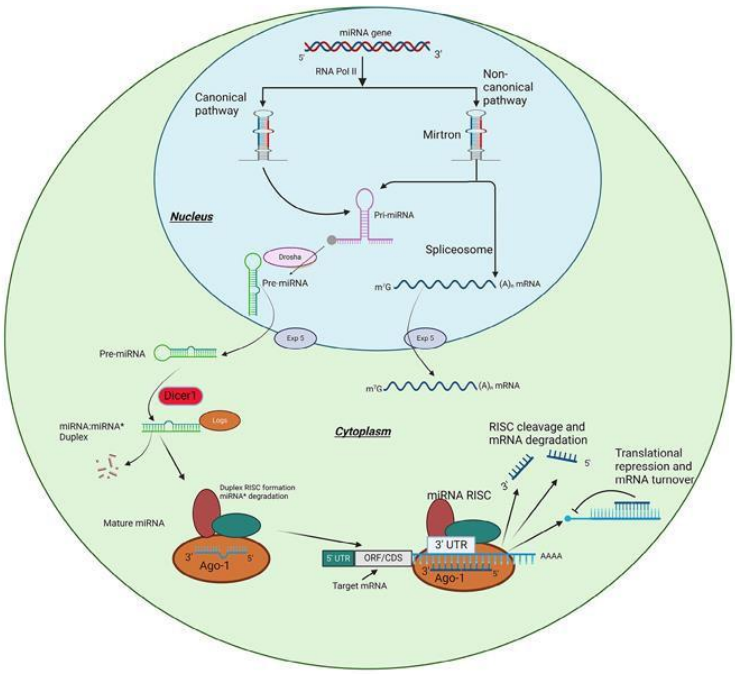

Figure 2. miRNA biogenesis pathways in insects (Khan et al., 2025)

PIWI-interacting RNAs (piRNAs): piRNAs are single-stranded RNA molecules, 24–31 nucleotides in length, predominantly expressed in germline cells. Unlike miRNAs and siRNAs, piRNA biogenesis is Dicer-independent. piRNAs associate with PIWI proteins, a subfamily of Argonaute proteins, to mediate the silencing of transposable elements (TEs) and maintain genomic stability, particularly in the germline. piRNAs are produced from long single-stranded precursor transcripts, and their maturation involves endonucleolytic cleavage by nucleases such as Zucchini. Mature piRNAs are then loaded into PIWI proteins. Through complementary binding to target RNA, they guide PIWI proteins to cleave transposon-derived transcripts. This cleavage event often initiates a secondary amplification

cycle known as the “ping-pong” amplification loop, which is a hallmark of the piRNA pathway. In addition to post- transcriptional repression, piRNAs also contribute to transcriptional gene silencing via DNA methylation and heterochromatin formation. Beyond transposon silencing, piRNAs play crucial roles in germ cell development, sex determination, and spermatogenesis. These multifaceted functions underscore the essential role of the piRNA pathway in preserving genomic integrity across multicellular organisms (Lam et al., 2015).

Small interfering RNAs (siRNAs): siRNAs are short double-stranded RNA molecules, typically 20–23 nucleotides in length, that play a central role in post-transcriptional gene silencing. In insects, perfectly complementary long double-stranded RNAs (dsRNAs) are processed in the cytoplasm by Dicer-2. This Dicer-2-mediated processing generates siRNA duplexes characterized by 5′ phosphate groups and 3′ two-nucleotide overhangs. From these duplexes, one strand is selected as the guide strand while the passenger strand is cleaved and discarded by the slicer activity of Argonaute 2 (AGO2). The selection of the guide strand is influenced by Dicer-2 in combination with cofactors such as R2D2 or the Loqs-PD isoform, which sense the relative stability of the duplex ends. Both strands are initially loaded onto AGO2 in an open conformation; following passenger strand removal, the guide strand is methylated at the 3′ end, and AGO2 adopts a closed conformation. The guide strand then directs sequence-specific recognition and cleavage of complementary target mRNAs, which possess a 5′ cap and 3′ poly(A) tail, marking them for subsequent degradation by exonucleases. In cases where the siRNA and target mRNA contain mismatches, cleavage is inhibited, resulting in translational repression rather than degradation. Dicer-2 contains an amino-terminal helicase domain and a carboxyl-terminal dsRNA-binding domain (C-dsRBD) that mediate substrate recognition and cleavage. AGO2 binds the guide strand within the RNA-induced silencing complex (RISC), with the RISC-loading complex (RLC) assisting strand selection and loading. Additional factors, including the C3PO complex and Hsc70-Hsp90 chaperone complex, support proper RISC assembly and activation (Beltrán Ortolá & Daròs, 2024). AGO2’s functional domains, such as MID, which anchors the 5′ end, and PAZ, which binds the 3′ end of the guide, are essential for target cleavage. The siRNA pathway is widely used in transgenic organisms for gene silencing, antiviral defense, and therapeutic knockdown due to its high sequence specificity and minimal off-target effects. As shown in Figure 3, this pathway integrates double stranded RNA (dsRNA) processing,

guide strand selection, and AGO2-mediated target recognition to achieve efficient post-transcriptional regulation.

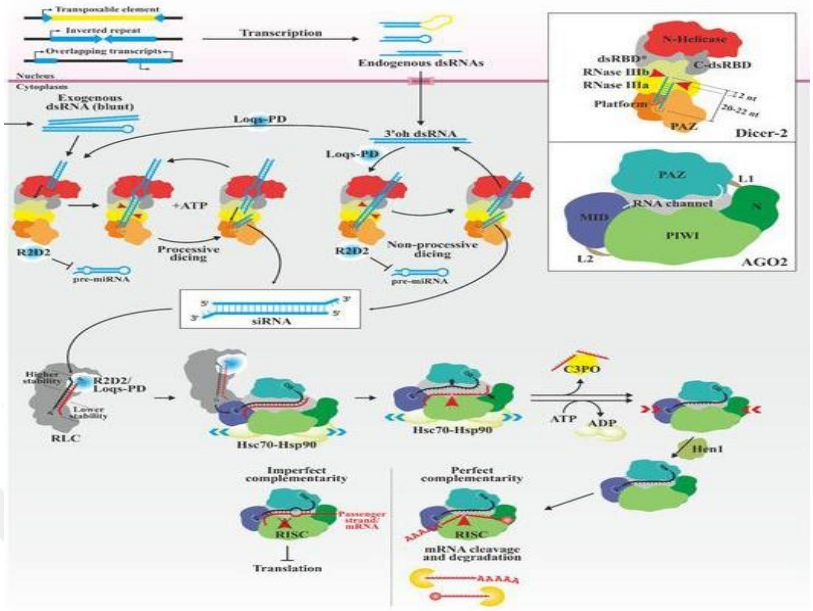

Figure 3. siRNA pathway in insect (Beltrán Ortolá & Daròs, 2024)

1.3.1. Structure and Function of RNAi Pathway Components

Drosha: Drosha is a member of the RNase III enzyme family localized in the nucleus and plays a key role in the microRNA (miRNA) biogenesis pathway within RNA interference (RNAi). It processes long, structurally folded pri-miRNAs, which are transcribed from miRNA genes, into ~70-nucleotide stem-loop precursors called pre-miRNAs. This processing takes place within the "Microprocessor complex", formed by drosha and the RNA-binding protein DGCR8 (DiGeorge syndrome critical region 8). Drosha's substrate specificity enables it to cleave the dsRNA region near the lower stem of the hairpin structure, thereby generating pre- miRNAs suitable for subsequent cytoplasmic processing steps (Khan et al., 2024).

Exportin-5: The pre-miRNA molecule formed after Drosha processing is removed from the nucleus and delivered to the cytoplasm by Exportin-5, which operates with the support of Ran-GTP. Exportin-5 recognizes the structural features of pre-miRNA, including the stem-loop structure and the characteristic 2-nucleotide 3′ overhang. Upon binding, Exportin-5 forms a complex with Ran-GTP and mediates the nuclear export of the pre-miRNA. GTP hydrolysis then facilitates the release of the cargo in cytoplasm. This step is

crucial for the proper and uninterrupted progression of the RNAi pathway (Wilson & Doudna, 2013).

Dicer: Dicer is a type of RNase III endonuclease that processes dsRNA or pre-miRNA into 21–25 nucleotide long siRNAs or miRNAs. It recognizes its substrates via its PAZ (Piwi/Argonaute/Zwille) and RNase III domains. The PAZ domain binds the 3′ overhang of RNA, while the RNase III domain performs symmetric cleavage of both RNA strands (Nowotny & Yang, 2009). Dicer's activity is enhanced by co-factors such as TRBP (TAR RNA- binding protein) or PACT, which support substrate recognition and improve processing efficiency and precision (Carthew & Sontheimer, 2009).

RISC (RNA-induced Silencing Complex): RISC is a multi-protein complex that utilizes one strand of a small RNA duplex (the guide strand) to direct sequence-specific silencing of target mRNAs. Upon Dicer processing, a pre-RISC complex is formed and becomes active when argonaute selects the guide strand. The guide strand typically binds to the 3′ untranslated region (UTR) of target mRNAs via complete or partial complementarity. In the case of siRNAs, perfect complementarity leads to mRNA cleavage by AGO2, whereas miRNAs generally cause translational repression or mRNA destabilization.

Argonaute Proteins (AGO): Argonaute proteins are the core components of the RISC and mediate gene silencing by guiding siRNAs or miRNAs to their complementary target mRNAs. These proteins contain PAZ and PIWI domains; the PAZ domain anchors the 3′ end of the small RNA, while the PIWI domain possesses slicer endonuclease activity that can cleave the target mRNA (Nowotny & Yang, 2009).

TRBP and PACT: The functional activity of Dicer, central to RNA interference, is regulated by several co-factors. Among these, TRBP (TAR RNA-binding protein) and PACT (Protein Activator of PKR) play crucial roles in the processing of dsRNA into functional small RNAs. These proteins assist Dicer in substrate recognition, cleavage, and the delivery of processed small RNAs to the RISC complex. TRBP enhances substrate affinity and stabilizes miRNAs during transfer to RISC, facilitated by its interaction with the helicase domain of Dicer via its C-terminal domain. PACT, on the other hand, modulates Dicer activity under cellular stress conditions, contributing to miRNA biogenesis. Additionally, both TRBP and PACT have been reported to form ~500 kDa complexes with Ago2, supporting the assembly of pre-RISC structures. Loss or dysfunction of these proteins leads to reduced mature miRNA levels and impaired post-transcriptional gene silencing.

Their cooperative function with Dicer is considered vital for the specificity and efficiency of the RNAi machinery (Altayli, 2020).

GW182 and P-Bodies - Post-Silencing Regulatory Sites: GW182 functions as a critical regulator in miRNA-mediated gene silencing by interacting with AGO. After the miRNA-loaded RISC complex binds to target mRNAs, GW182 initiates translational repression and stabilizes the silencing effect. Rather than degrading mRNAs directly, GW182 promotes degradation by facilitating deadenylation and decapping. Another important feature of GW182 is its localization to cytoplasmic granules known as processing bodies (P-bodies). These structures act as sites for storage, decay, or recycling of translationally repressed mRNAs. By directing silenced mRNAs to P-bodies, GW182 contributes to post-transcriptional regulation through mRNA degradation. Loss or downregulation of GW182 not only impairs miRNA function but also disrupts the formation of P-bodies, indicating its central role in stabilizing silencing complexes and timing mRNA decay (Isenmann et al., 2023; Liu et al., 2005).

1.4. Effects of RNA Interference (RNAi) on RNA Stability

RNA interference (RNAi) is a highly conserved biological mechanism that regulates gene expression post-transcriptionally by modulating RNA stability and translation. In RNAi, small regulatory RNAs, such as siRNAs and miRNAs, guide protein complexes to complementary messenger RNAs (mRNAs), leading to their degradation or translational repression. When siRNAs bind perfectly complementary sequences on target mRNAs, they typically induce cleavage and rapid degradation of the mRNA, effectively reducing the transcript's half-life and preventing protein production. This process is mediated by the RISC, which incorporates one strand of the siRNA and cleaves the target mRNA within the cytoplasm. In contrast, miRNAs usually bind with partial complementarity to target mRNAs, primarily at the 3' untranslated region (3' UTR), leading to translational repression and/or deadenylation- mediated mRNA decay. This results in a decrease in RNA stability and reduced protein output without necessarily causing immediate cleavage. Through these mechanisms, RNAi not only controls the levels of specific mRNAs but also fine-tunes gene expression in response to developmental cues, environmental stress, and cellular signaling pathways. Furthermore, RNAi pathways contribute to cellular defense against viral RNAs and transposable elements by targeting their RNA molecules for degradation. Understanding

how RNAi affects RNA stability is crucial in biotechnology and therapeutic contexts, where RNAi-based technologies are used to silence disease-related genes or control pests in agriculture by targeting essential mRNAs. Gaining insight into these effects sheds light on the biological significance and wide-ranging applications of RNA interference. To fully appreciate the impact of RNAi, it is important to examine its historical discovery and the foundational experiments that uncovered this groundbreaking gene regulatory mechanism (Kornienko et al., 2024).

1.4.1. Historical Discovery of RNA Interference (RNAi)

The foundation of RNAi was established in the 1990s when Napoli and colleagues observed an unexpected reduction in flower pigmentation in petunias after introducing additional copies of the chalcone synthase gene (Napoli et al., 1990). This phenomenon was termed "co-suppression"; however, the molecular basis of the process remained unclear at that time. The mechanism of RNAi was elucidated through the groundbreaking experiments conducted by Andrew Fire and Craig Mello in 1998 on *Caenorhabditis elegans*. Their research demonstrated that double-stranded RNA (dsRNA) was far more effective than single-stranded antisense RNA in silencing target mRNA (Fire et al., 1998).

RNA interference (RNAi) is a fundamental biological mechanism that regulates gene expression by suppressing specific target genes. This process is initiated when dsRNA molecules recognize and interact with complementary mRNA sequences, leading to their degradation and preventing protein synthesis. At the molecular level, RNAi is mediated by the RISC and small interfering RNAs (siRNA), which facilitate mRNA binding or cleavage, thereby inhibiting gene expression. Due to its high specificity in targeting only the intended gene, RNAi has become a crucial tool in genetic engineering and biotechnology, offering precise gene regulation strategies for research and applied sciences (Palli, 2023).

RNAi works by silencing specific genes, directly affecting the genetic mechanisms of target pests and inhibiting their development. This disruption of vital functions in harmful insects makes RNAi an attractive alternative for overcoming resistance to Bt toxins, offering a potential solution to the challenges posed by resistant pest populations. Given the increasing prevalence of resistance to conventional pest control methods, it is essential to explore alternative strategies that are both sustainable and environmentally benign.

In this context, RNAi has emerged as a revolutionary approach for pest management. RNAi is a natural biological process that involves the degradation of specific mRNA molecules, thereby inhibiting the expression of targeted genes. By selectively silencing genes that are essential for survival, development, or reproduction, RNAi offers a highly specific and targeted pest control strategy. Unlike chemical insecticides, which often affect a broad range of organisms, RNAi's precision allows interventions to be designed in a way that disrupts key biological processes within pest species while minimizing effects on non-target organisms. Additionally, RNAi-based strategies can be optimized to target multiple genes simultaneously, reducing the likelihood of resistance development and enhancing the effectiveness of IPM systems. When integrated with other pest control practices, RNAi presents a promising approach that aligns with principles of environmental conservation and reduces dependency on chemical insecticides. The foundation of RNA interference as a gene regulation mechanism date back to the 1990s, when Napoli and colleagues observed an unexpected reduction in flower pigmentation in petunias after when extra copies of the chalcone synthase gene were supplied (Napoli et al., 1990). This phenomenon, initially described as 'co-imprinting, pointed to a natural process that could silence certain genes, but its molecular basis remained unclear at the time. Later discoveries revealed that RNAi functions through dsRNA molecules, which recognize and degrade target mRNA within the cell, thereby preventing the translation of genetic information into proteins. This fundamental mechanism has since been harnessed as a powerful tool for targeted gene silencing, particularly in pest management, where it offers new possibilities for controlling insect populations with high specificity and efficiency. This pioneering work by Fire and Mello established RNA as an active participant in genetic regulation and earned them the Nobel Prize in Physiology or Medicine in 2006 (Nobelprize.org, 2006). In subsequent years, the studies by Elbashir and colleagues (2001) contributed to the discovery of small interfering RNAs (siRNA) and enhanced the understanding of RNAi's biological mechanisms. These advancements enabled the application of RNAi in both basic science and practical fields, demonstrating its potential in diverse areas ranging from the treatment of genetic diseases to the development of environmentally friendly technologies pesticides.

1.4.2. Mechanism of RNA Interference (RNAi) Technology

Double-stranded RNA (dsRNA) is made of two matching strands, like DNA structure, and is absorbed by the organism's cells. The effect of RNAi in insects begins when dsRNA structures are acquired from the environment. Upon ingestion, dsRNA structures are transported from the gut lumen to the cytosol of intestinal epithelial cells as shown in Figure 4 (Price and Gatehouse, 2008). The RNAi effect occurs in two stages within the epithelial cells.

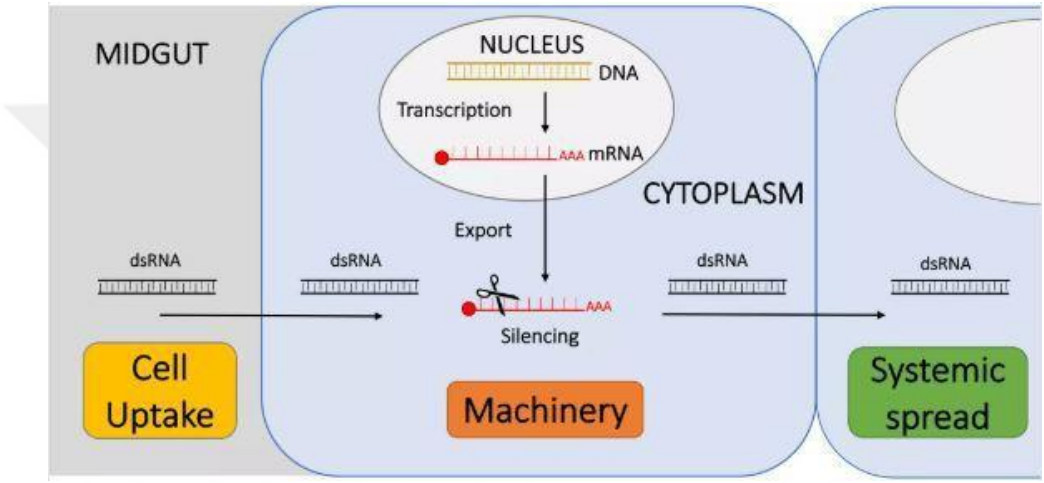

Figure 4. Mechanism of the RNA interference (RNAi) pathway (Price and Gatehouse, 2008)

In the first stage, dsRNA is cleaved into 20–25 bp fragments called RNAs siRNAs by the Dicer enzyme, a ribonuclease III enzyme. In the next stage, the siRNAs are incorporated into a protein complex called the RISC. Here, the siRNAs are unwound into two single-stranded RNAs: the guide strand and the passenger strand. The passenger strand is removed from the complex, while the guide strand directs the RISC to interact with complementary mRNA, preventing its translation and subsequent protein production. The Argonaute-2 protein, a critical component of RISC, plays an essential role in recognizing and degrading the target mRNA. This protein contains two domains, PAZ and PIWI. The PAZ domain protects the guide strand used to capture the target mRNA, while the PIWI domain cleaves the captured mRNA (Darrington et al., 2017).

RNA interferansı (RNAi), böceklerde gen susturma için güçlü bir genetik araçtır ve zararlı kontrolü için umut verici bir yaklaşım olarak ortaya çıkmıştır. Identification and

selection of a suitable target gene is critical for the success of RNAi-based pest management. Genes involved in essential physiological functions, especially those not found in non-target organisms such as humans and plants, are ideal candidates (Li et al., 2013).

1.5. RNAi Targets at Insects

Despite the potential of RNAi to be a targeted and environmentally friendly method of controlling pests, certain physiological and environmental constraints may reduce its effectiveness. Therefore, selecting the most appropriate target genes is of critical importance to ensure that the applied dsRNA achieves maximum effect with minimum dosage (Bera et al., 2025). The success of RNAi largely depends on whether the target gene is involved in vital physiological processes of the pest and whether its silencing results in death, developmental defects, or loss of reproductive capacity. Choosing such genes not only enhances biological efficacy but also contributes to reducing dsRNA production costs, thereby strengthening the economic sustainability of the method for large-scale applications. Consequently, in the development of RNAi-based biopesticides, focusing exclusively on the most effective and specific target genes is indispensable for the success of the strategy. In this context, comprehensive research has shown that, unlike classical pesticide targets, the most effective genes for RNAi are generally involved in fundamental cellular processes. Large-scale RNAi screenings conducted in *Tribolium castaneum* revealed that over 90% of 905 genes associated with protein degradation (proteasome), translation, and ribosomal functions caused mortality. Some of these genes have also been found to be effective through oral applications in leaf beetle species. However, in insect orders such as Lepidoptera, which exhibit higher resistance to RNAi, target gene selection becomes even more critical. In a study performed by Sharif et al., (2022) three vital genes, acetylcholinesterase (AChE), ecdysone receptor (EcR), and the vacuolar ATPase subunit A (v-ATPase-A) were selected as RNAi targets in *Helicoverpa armigera*, an agriculturally significant pest belonging to the order Lepidoptera. The silencing of these genes disrupted fundamental physiological processes such as ATP hydrolysis, developmental gene expression, and neural transmission, thereby increasing larval mortality and demonstrating the potential to control pest populations. In pests of high agricultural importance such as *H. armigera*, the success of RNAi-based biocontrol strategies is directly dependent on the correct selection of target genes. Other studies in this context have identified certain genes that, when targeted by

RNAi, result in high mortality and pronounced phenotypic abnormalities. Among these, the A subunit of the vacuolar-type H^+-ATPase enzyme (v- ATPase-A), acetylcholinesterase (AChE), which is responsible for neural transmission, and chitin synthase B (CHSB), which plays a role in exoskeleton and peritrophic membrane formation, stand out. V-ATPase-A is involved in cellular ion balance and pH regulation (Ioannidis et al., 2022), and its silencing via RNAi leads to irreversible disruptions in larval physiology. Since acetylcholinesterase is involved in synaptic transmission, its suppression results in neural paralysis and death. CHSB, used particularly in this study, is considered a critical target due to its role in the synthesis of intestinal chitin and the exoskeleton during the larval stage; its silencing through RNAi causes molting failure, digestive disorders, and developmental delays. In addition, detoxification genes associated with pesticide resistance such as CYP6AE14 and HMG-CoA reductase, which is involved in juvenile hormone synthesis, are also considered potential targets for RNAi applications (Wang et al., 2013). However, all these studies demonstrate that the highest mortality rates are generally achieved by targeting genes involved in fundamental cellular processes. Therefore, in RNAi-based biopesticide development processes, selecting genes with critical physiological functions and suitable for species-specific RNA design is of great importance for enhancing the strategy's effectiveness and reducing application costs.

1.5.1. Helicoverpa armigera

Helicoverpa armigera (Hübner, 1808) is a lepidopteran species belonging to the Noctuidae family. This insect undergoes complete metamorphosis (holometabolism), progressing through egg, larva, pupa, and adult stages (Figure 5). Due to its high reproductive capacity, broad host range, and strong adaptability, it is considered one of the most significant agricultural pests.

Female moths lay their eggs individually or in clusters on the undersides of leaves, flowers, near fruits, or young shoots. A single female can produce between 1,000 and 3,000 eggs in her lifetime. The eggs are round, dome-shaped, and feature both vertical and horizontal ridges. Initially, they appear pale white, but as they near hatching, their color changes to light yellow or brownish, typically hatching within 2–4 days. Upon hatching, the larvae are creamy or light green in color, with fine body hairs that are not initially prominent. As they grow, their coloration can shift to dark green, brown, reddish, or even black,

influenced by their diet and environmental factors. They possess light-colored lateral stripes and small black tubercles on each body segment. *H. armigera* larvae pass through six developmental stages over 14–22 days, actively feeding throughout this period. Early-stage larvae feed mainly on leaf surfaces, while later stages cause more severe damage by attacking seeds, cotton bolls, buds, and fruit capsules (Vatanparast et al., 2021). Fully grown larvae reach approximately 35–40 mm in length and tend to be darker in color. Once development is complete, larvae burrow 3–10 cm into the soil and enter the pupal stage. Initially light brown, the pupae darken over time. This stage lasts 7– 14 days, though under low temperatures, pupae can undergo diapause, remaining dormant for months or even up to a year. Upon completing metamorphosis, adults emerge from the soil. Adult *H. armigera* moths are of medium size, with a wingspan ranging from 30 to 40 mm. Females are generally larger, with pale brown or yellowish forewings featuring dark wavy patterns. The hindwings are lighter, with a distinct dark brown band along the edge. Males are smaller and paler, with forewings that appear light gray brown. These moths are nocturnal and highly attracted to light. Males detect females through pheromones, and mating typically occurs at night. A few days after mating, females begin laying eggs, continuing the life cycle. Adult moths live between 7 and 20 days (Santos et al., 2018).

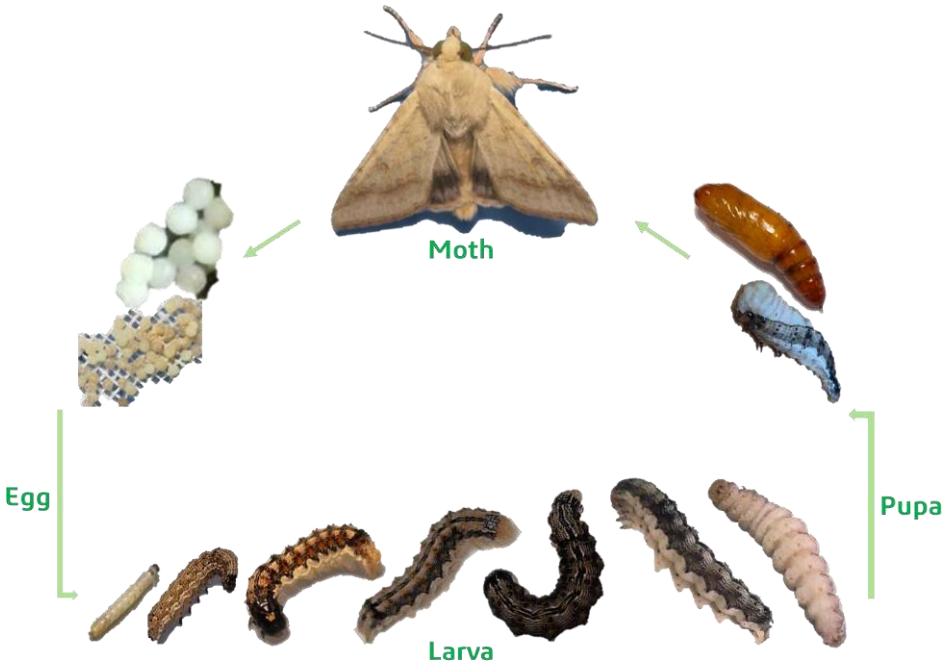

Figure 5. Life cycle of *Helicoverpa armigera* under laboratory conditions

H. armigera exhibits color variation in response to environmental conditions. In the larval stage, their coloration adapts to the plant they feed on, helping them blend into their surroundings and evade predators. Pupae also undergo color changes influenced by temperature and humidity. Under colder conditions, pupae tend to become darker brown or nearly black, which enhances their chances of survival during diapause (Keszthelyi et al., 2024). The development and environmental adaptability of *Helicoverpa armigera* are closely linked to the structural and physiological characteristics of its digestive system. The larval digestive tract is composed of three main regions: the foregut, midgut, and hindgut (Ioannidis et al., 2022). The majority of digestion occurs in the midgut, which is the primary site for enzyme secretion and nutrient absorption. The pH in the midgut is generally alkaline, which facilitates protein degradation and enables larvae to efficiently extract nutrients from various host plants (Nitnavare et al., 2021). Lining the digestive tract is the peritrophic membrane, which serves both as mechanical protection and as a barrier against pathogens. This semi-permeable structure allows effective interaction between digestive enzymes and food particles, while preventing harmful microorganisms from reaching the epithelial cells. One of the main structural components of the peritrophic membrane is chitin, which is synthesized by the enzyme chitin synthase encoded via the CHS (Chitin Synthase) gene. The CHS gene plays a vital role not only in the formation of the intestinal lining but also in the development of the exoskeleton and tracheal system. Therefore, the expression and function of the CHS gene are essential for maintaining both the larva's feeding efficiency and structural integrity.

1.5.2. Biological Significance and Biosynthetic of Chitin in Insects

Arthropods constitute the largest group of organisms in the animal kingdom and being the only invertebrate group capable of flight, account for more than 80% of species (Yu et al., 2024). In this group of organisms, chitin is an important structural component and is a linear polysaccharide consisting of GlcNAc units linked via β-(1,4) glycosidic bonds (Yu et al., 2024). The first successful cloning of a chitin synthase gene was reported in *Lucilia cuprina* in 2000. Following this, the chitin synthase gene sequences of various insect species, including *Tribolium castaneum*, *Drosophila melanogaster* and *Spodoptera frugiperda*, were subsequently identified and characterized (Chang et al., 2022). In insects, chitin is found in ectodermal tissues, where it forms chito-protein cuticles, and in the gut, where it forms a

Peritrophic Matrix (PM) (De Giorgio et al., 2023). It is a major component of structures such as the insect exoskeleton and the peritrophic matrix (Merzendorfer, 2013). Chitin is present in high concentrations in these structures as part of chito-protein complex, providing mechanical strength, structural integrity, and protection against external factors (Zhao et al., 2019). In insect genomes, there are two CHS genes that play a catalytic role in chitin formation: CHS1 (CHSA) and CHS2 (CHSB) (Chen et al., 2023a). CHS1 is generally responsible for chitin synthesis in soft tissues such as the cuticle and trachea, while CHS2 (CHSB) is mainly responsible for chitin synthesis in the larval midgut peritrophic matrix (Wang et al., 2023). The CHSB enzyme is highly expressed in midgut epithelial cells, which are particularly active during the larval stage (Ali et al., 2020). This high expression level ensures the accumulation of chitin derivatives ingested with food or synthesized endogenously in the peritrophic matrix structure. Thus, it provides structural support to the digestive system and acts as a physical barrier against pathogenic microorganisms (Zhu et al., 2023). Although chitin is present in arthropods, including insects and fungi, it is absent in vertebrates and plants, making chitin synthesis an ideal target for insect growth regulators (Rana et al., 2020; Van Leeuwen et al., 2012; Chen et al., 2023b). Besides these, in yeast species such as Saccharomyces cerevisiae, chitin synthesis is regulated by the CHS1, CHS2, and CHS3 genes; simultaneous loss of function of these genes is lethal to the cell (Chen et al., 2023b). In addition, in phloem-feeding Hemiptera species lacking a peritrophic matrix, only the CHS1 gene responsible for chitin biosynthesis is present. The insect chitin biosynthesis pathway is a multi-step enzymatic process involving intermediate products related to glucose metabolism. The process begins with the degradation of trehalose, continues with the synthesis of GlcNAc derivatives and their activation to UDP form, and concludes with the formation of long chitin chains by membrane-bound chitin synthase (CHS). Enzymes involved in this pathway include trehalase, hexokinase, phosphohexose isomerase, glutamine: fructose-6-phosphate amidotransferase (GFAT), glucosamine-6-phosphate N-acetyltransferase (GNA), phosphoacetylglucosamine mutase, UDP-N-acetylglucosamine pyrophosphorylase (UAP), and chitin synthaseThe chitin biosynthesis process begins with the breakdown of trehalose. Trehalose, the main sugar in insect hemolymph, is hydrolyzed by the enzyme *trehalase* into two glucose molecules (Liu et al., 2022a). Each of the resulting glucose molecules is first converted into glucose-6-phosphate by *hexokinase*. Then, the enzyme *phosphohexose isomerase* transforms glucose-6-phosphate into fructose-6-phosphate. These steps occur in insect peripheral tissues or intestinal cells.

Fructose-6-phosphate is converted into *glucosamine-6-phosphate* (GlcN-6-P) by *glutamine: fructose-6-phosphate amidotransferase* (GFAT; EC 2.6.1.16), using the amino group of glutamine. This reaction produces the first amine-containing intermediate, glucosamine-6-phosphate. The resulting GlcN-6-P is then transformed into *N-acetylglucosamine-6-phosphate* (GlcNAc-6-P) by the enzyme *N-acetylglucosamine-6-phosphate N-acetyltransferase* (GNA; EC 2.3.1.4), which transfers an acetyl group from acetyl-CoA. GlcNAc-6-P is the monomer precursor of chitin (Liu et al., 2013). GlcNAc-6-P is subsequently converted to *GlcNAc-1- phosphate* by *phosphoacetylglucosamine mutase*. Next, the enzyme UDP-N-acetylglucosamine pyrophosphorylase (UAP; EC 2.7.7.23) catalyzes the reaction between GlcNAc-1-P and UTP to generate UDP-N-acetylglucosamine (UDP-GlcNAc). This compound, UDP-GlcNAc, represents the active form of GlcNAc and serves as the final metabolite preceding chitin Synthesis. In this step, the UAP enzyme adds a uridine diphosphate group from UTP to GlcNAc-1-P, resulting in the formation of UDP-GlcNAc, which is ready for chitin polymerization. In the final step, UDP-GlcNAc is polymerized into long chitin chains via β-(1,4)-linkages by *chitin synthase* (CHS), a multi-transmembrane glycosyltransferase located in the cell membrane (Niu et al., 2024b).

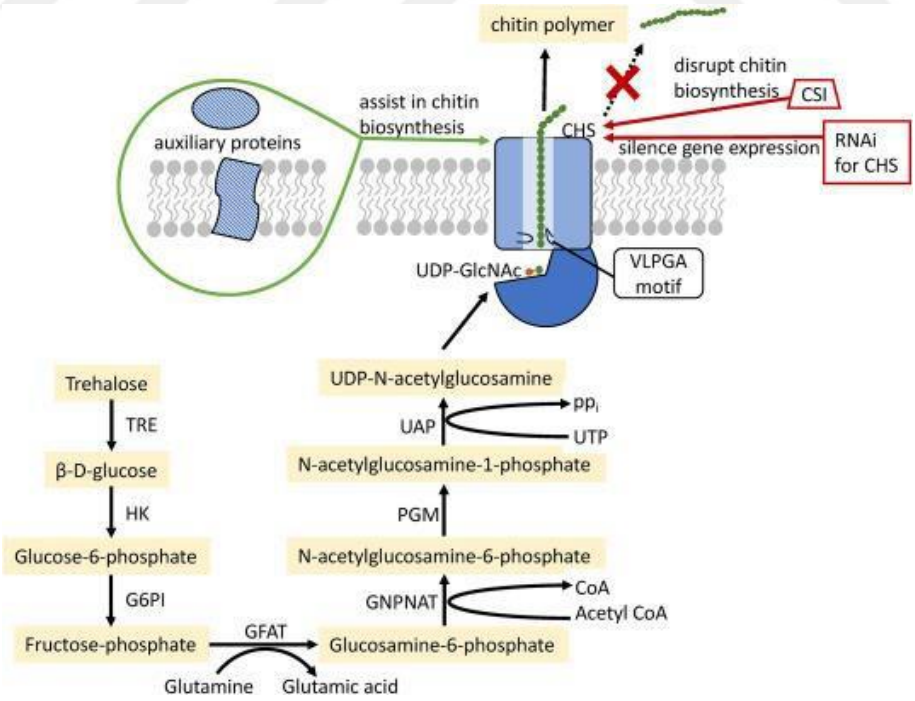

Figure 6. Chitin biosynthetic pathway (Yu et al., 2024)

The process initiates with trehalose and proceeds through sequential enzymatic reactions leading to the formation of UDP-N-acetylglucosamine (UDP-GlcNAc), followed by chitin polymerization catalyzed by the membrane-integrated chitin synthase (CHS) enzyme. Key enzymes and intermediate metabolites involved in the pathway are indicated as shown in Figure 6 (Yu et al., 2024).

1.5.3. Chitin Synthase (CHS) Genes and CHS-B

CHS is the main enzyme that links UDP-GlcNAc to the chitin polymer chain. In insects, two types of CHS exist: CHS-A (CHS1) and CHS-B (CHS2). CHS-A is generally active in the cuticle and the tracheal walls of the respiratory system, while CHS-B is mainly responsible for chitin synthesis in the peritrophic matrix of the midgut. CHS-B enzymes are highly expressed especially in the epithelial cells of the larval midgut. Therefore, a reduction or inhibition of CHS-B activity leads to disruption of the peritrophic matrix structure, impairing nutrient digestion and protective barrier functions. For example, genetic silencing of CHS-B significantly reduces chitin production in the larval midgut and causes severe developmental defects in the insect (Chen et al., 2023b). In a study conducted by Shi et al. (2016), on *Leptinotarsa decemlineata*, silencing of both genes via dsRNA led to developmental abnormalities in larvae, a reduction in chitin content, and high mortality rates. Additionally, silencing of the CHSB gene resulted in decreased feeding behavior, whereas silencing of CHSA inhibited adult emergence and reduced reproductive capacity. These findings indicate that both CHSA and CHSB are potential targets for RNAi-based biological pest control strategies (Shi et al., 2016). “Current research generally focuses on model insect species, and in terms of gene types, significantly fewer studies have been conducted on CHS2 compared to CHS1(Chang et al., 2022, Zhang et al., 2023). In this context, Liu et al. (2022b) reported that RNAi-mediated silencing of the *SfCHSB* gene in *Spodoptera frugiperda* larvae, achieved by microinjection of dsRNA, had significant effects on larval development. A decrease in the target gene’s mRNA levels corresponded with disrupted chitin synthesis, leading to developmental abnormalities. These findings indicate that the *SfCHSB* gene plays a critical role in chitin biosynthesis and larval growth processes. In another study, Wan et al. (2021) successfully silenced the SfCHSB gene expression in *Spodoptera frugiperda* using a bacterium-mediated RNAi system. Specifically, the knockdown of the CHSB gene by dsRNA caused significant negative effects on larval

development, including reduced larval body weight, prolonged developmental duration, and decreased survival rate. These results support the critical role of chitin synthase B enzyme in insect development and the maintenance of the midgut peritrophic membrane. In another study conducted, silencing of the *HvChsb* gene in *Heortia vitessoides* during the third larval stage via dsRNA injection resulted in a 40% reduction in gene expression at 12 hours post-treatment (Chen et al., 2023). This gene, which is highly expressed in midgut, plays a key role in peritrophic membrane formation; its suppression disrupted feeding behavior and hindered larval development. Treated larvae exhibited reduced growth rates, shortened body length, yellowing of the body, and significantly decreased pupation success, with only 43.33% transitioning to the pupal stage. These outcomes point to the critical role of *Chsb* in larval feeding and energy metabolism and support the potential of RNAi-based approaches in pest control strategies.

In summary, the CHS enzyme ensures the entire chitin polymerization process, and CHS2/CHS-B plays a vital role in chitin synthesis within the insect gut structures. All enzymatic steps and intermediates involved in insect chitin biosynthesis described above are shown in Figure 6. In this pathway, all stages from UDP-GlcNAc production to the formation of chitin chains by CHS are tightly regulated, with each enzyme catalyzing a specific transformation. Chitin synthesis is a fundamental process for insect development and exoskeleton integrity, and UDP-GlcNAc synthesis along with the CHS-B enzyme form key points in this pathway (Liu et al., 2013). CHS is an ideal target gene for the development of new generation insecticides and has high research value. Induction of RNA interference (RNAi) via dsRNA specific to the CHS gene enables effective silencing of CHS expression, leading to disruption in chitin synthesis, developmental defects, and increased mortality. Therefore, CHS stands out as a critical target for molecular-based pest control strategies (Shi et al., 2016).

1.6. The Potential and Challenges of dsRNA-Based RNAi in Pest Control

1.6.1. The Potential of dsRNA in Insect Pest Management

The double-stranded RNA (dsRNA) approach holds substantial potential in the control of insect pest populations. Several key features contribute to this potential: (i) the risk of resistance development against gene silencing is considered moderate, especially when

compared to conventional chemical insecticides (ii) dsRNA molecules can be designed to target specific messenger RNAs (mRNAs) with high precision, minimizing effects on non-target organisms (iii) dsRNA can be prepared in sprayable products that allow for selective targeting of pest species in semi-field applications and (iv) dsRNA-based RNAi has been shown to be effective in both chewing and piercing-sucking insect orders, making it a broadly applicable strategy across multiple taxa (Lu et al., 2023).

Despite these advantages, several challenges currently limit the application of RNAi in pest management. dsRNA may be degraded before it can be taken up by the insect, either in the environment or within the insect gut. Additionally, variability in RNAi efficacy between insect species, inefficient uptake across the gut epithelium, and the absence of dsRNA amplification mechanisms in most insects all contribute to these limitations (Ghosh & Gundersen-Rindal, 2017). Nevertheless, advances in formulation technologies, such as encapsulation or nanoparticle carriers, have shown promise in improving dsRNA stability and uptake, thereby enhancing RNAi efficiency (Bera et al., 2025)

Among the different application methods such as HIGS, VIGS, and SIGS the oral delivery of dsRNA via feeding (SIGS) is the most preferred approach for pest control purposes. This method does not involve any physical damage to the insect body and is non-invasive. Orally delivered dsRNA expressed in bacterial systems has been reported to be highly effective in triggering RNAi responses in certain insect species (Baum et al., 2007; Khajuria et al., 2015).

However, inconsistent results have been observed in other insect orders, highlighting the importance of evaluating dsRNA efficacy on a case-by-case basis for different target pests.

1.6.2. Factors Affecting Insect Responses to dsRNA

The feasibility of using RNAi for insect pest control is largely determined by the insect's ability to ingest and respond to dsRNA through oral delivery. Insect responses to dsRNA are highly variable across different taxa. In this context, RNAi studies such as Whyard et al. (2009) have shown that orally delivered dsRNA can induce gene silencing and species-specific effects in insects, and that coleopteran species often display relatively high sensitivity to dietary dsRNA when compared to other orders. In contrast, other insect orders

such as Lepidoptera, Diptera, and Hemiptera exhibit variable and often reduced sensitivity to dsRNA- mediated gene silencing (Christiaens et al., 2020).

The effectiveness of RNAi in different insect species is influenced by several factors:

(i) the stability of dsRNA before and after ingestion; (ii) insufficient dsRNA internalization across gut epithelial cells; (iii) weak activation or incomplete functioning of the RNAi machinery; (iv) poor systemic spread of the silencing signal; and (v) genetic or physiological resistance of the target genes (Silver et al., 2021). Additionally, sensitivity to dsRNA may vary not only between insect orders or species, but also within species among different developmental stages, tissues, or target genes (Christiaens et al., 2020).

Therefore, for dsRNA to be effectively employed in pest management strategies, it is crucial to investigate the RNAi responsiveness of the target insect species, including the identification of susceptible genes and appropriate developmental stages for intervention. Such targeted research can significantly enhance the success of RNAi-based biocontrol programs.

1.7. Aim of the Study

In Turkey, four species of *Helicoverpa* (greenworm) exist, representing a highly effective group of polyphagous insects that cause significant damage to various agricultural crops. Chitin synthase B (CHSB) is a key regulatory enzyme in insects responsible for chitin synthesis and secretion, and essential for cuticle and peritrophic membrane formation in insects. By using RNAi technology, the expression of the CHSB gene, which is specific to greenworms, can be reduced. This reduction is expected to impair the normal growth, development, and viability of the pest, thereby preventing the damage it causes. This approach has significant potential for the development of insect-specific and environmentally friendly control methods. In this study, *Helicoverpa armigera* a major lepidopteran pest with a wide host range and significant resistance development against conventional control strategies, was selected as the insect model due to its economic impact on cotton and other agricultural. We silenced the expression of the *H. armigera CHSB* gene with dsRNA technology. By that way we aimed to develop an environmentally friendly, reliable, and organic alternative to chemical pesticides by targeting the CHSB gene specific to *H. armigera* using RNAi technology. We synthesized two dsRNA fragments of different

lengths to silence the target gene, thereby enabling an assessment of the effect of dsRNA length on gene silencing efficiency.

2. MATERIALS AND METHODS

2.1. Insect Rearing

The *Helicoverpa armigera* eggs used in this study were obtained from the Wuhan Institute of Virology, Chinese Academy of Sciences (Wuhan, China) through Prof. Dr. Zhihong Hu. These eggs were used to establish a laboratory culture and were reared under standard conditions at the Microbiology Laboratory of Karadeniz Technical University, as shown in Figure 7 An incubator providing 26 ± 2°C, a 16 h light/8 h dark photoperiod, and a relative humidity 70 ± 5% was used for larval and adult development. These controlled environmental conditions provided a suitable environment for the healthy completion of all biological stages of *H. armigera* and for the establishment of a stable laboratory culture for experimental use.

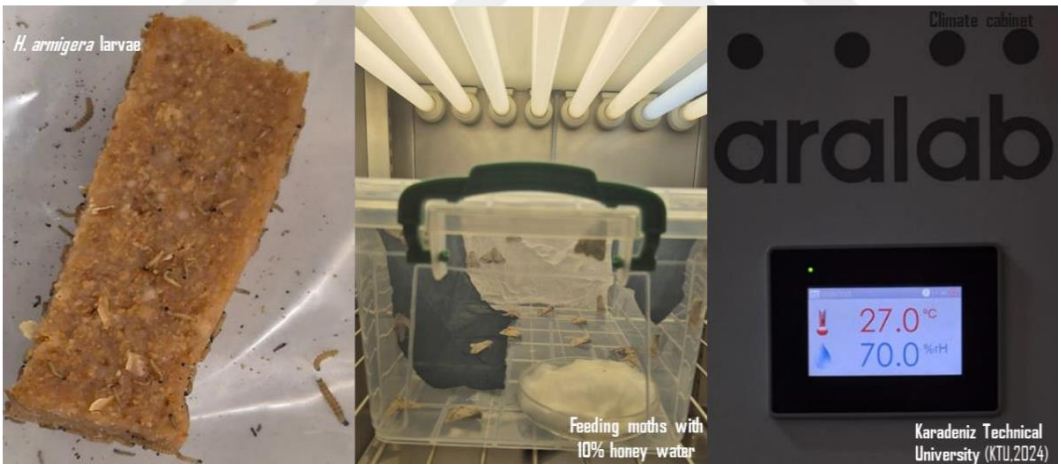

Figure 7. *Helicoverpa armigera*, reared in the climate chamberat

2.2. Primer Design and Polymerase Chain Reaction (PCR)

To amplify and clone the Chitin Synthase B (CHSB) gene of *H. armigera* for further generating CHSB specific dsRNAs, two primer pairs were designed. One primer pair was targeting a 360-nucleotide fragment and the other amplifying a 527-nucleotide region of the CHSB gene. The aim of producing dsRNA of two different lengths was to investigate not only the gene silencing effect but also the influence of dsRNA length on silencing efficiency.

The primer sets were designed using the Primer3 online platform (Untergasser et al., 2012), synthesized by Sentebiolab (https://sentebiolab.com.tr/), with the sequences of the designed primers shown in Table 1.

Table 1. Primers used in the amplification of *Helicoverpa armigera* Chitin Synthase B (CHSB) gene

		Primer Sequences	
No	**Size**	**Forward**	**Reverse**
P1	360CHSB	5'- TGGGCATTGTCGATCCTG-3'	5'-CAGCGATAACCTCCTTGC-3'
P2	550CHSB	5'-AAGATCAGAGCGCTCGAAGG-3'	5'-GAGCCCACAGGATGGATACG-3'

2.3. Total RNA Isolation

CHSB gene of *H. armigera* was amplified from the cDNA that was produced from insect total RNA. For this process first total RNA was isolated. Total RNA isolation was performed using TRIzol reagent. First, *H. armigera* larvae were surface sterilized by washing with DEPC- treated ddH_2O, then treated with 70% ethanol and transferred back to sterile water. The sterilized larvae were crushed in a mortar and pestle with liquid nitrogen and transferred to a 1.5 mL eppendorf tube. 1 mL of TRIzol (Sigma) was then added and mixed via vigorous shaking. Incubation was continued for 5 minutes at room temperature, followed by the addition of 200 µL of chloroform, and the sample was mixed rapidly (do not vortex). Centrifugation was carried out at 12,000 rpm at 4°C for 15 minutes. The supernatant was carefully transferred to a new sterile tube, 500 µL of cold isopropanol was added, and incubated at room temperature for 10 minutes. The pellet was then centrifuged at 12,000 rpm for 10 minutes at 4°C. The resulting pellet was washed with 75% ethanol, air-dried for 10 minutes, and dissolved in 30–50 µL of DEPC-treated sterile ddH_2O. RNA samples were incubated in a heat block at 55°C for 10 minutes. Total RNA concentration was determined using a Nanodrop spectrophotometer, and the quality was assessed on a 1% agarose gel under UV light. The sample was stored at -80°C for molecular analyses.

2.4. cDNA Synthesis and PCR Amplification

For cDNA synthesis, the "Thermo Fisher RevertAid First Strand cDNA Synthesis Kit" was used. A mixture containing RNA (1 µg), specific primers (1 µL each of forward and reverse, 20 pmol), and DEPC-treated water (12 µL) was prepared. The mixture was then

incubated at 70°C for five minutes and cooled on ice. Subsequently, 4 µL of reaction buffer (5X), 2 µL of dNTP mix (10 mM), and 1 µL of RNase inhibitor (20 U/µL) were added and incubated at 37°C for five minutes. In the final step, 1 µL of reverse transcriptase (200 U/µL) was added, and the mixture was incubated at 42°C for 60 minutes. Finally, a heat shock at 70°C for 10 minutes was applied to terminate the reaction. The prepared cDNA was used for amplification of the target gene fragments (CHSB).

PCR Reaction (50 µl volume)

2x Buffer	5 µl
dNTP	1 µl (200 µM)
cDNA	0.75 µl (20ng/µl)
Forward primer	1 µl (50 pmol)
Reverse primer	1 µl (50 pmol)
Taq DNA plymerase (Thermo Scientific)	0.25 µl
dH2O	41 µl

The CHSB gene fragments amplified by PCR were subjected to electrophoresis on a 1% agarose gel, and the target bands were excised and purified using the GeneJET Gel Extraction Kit (Thermo Fisher, USA). The purified PCR products were then ligated into the intermediate vector pUCM-T (Biobasic) at a 3:1 (insert/vector) ratio using T4 DNA Ligase (Promega, USA).

2.5. Preparation of Competent Cells

Compotent *Escherichia coli (E. coli)* DH10β cells were prepared using the $CaCl_2$ method. A single colony of cells was inoculated into 3 mL of Luria Bertani (LB) broth supplemented with ampicillin (100 µg/mL) and incubated overnight at 37°C with continuous shaking at 200 rpm. The following day, the overnight culture was diluted 1/10 with sterile dH_2O, and its optical density (OD) at 600 nm was measured using a spectrophotometer. An appropriate volume of the overnight culture was transferred into 30 mL of LB medium, adjusting the OD600 to 0.1. The culture was then incubated at 37°C with shaking at 200 rpm until it reached an OD_{600} of 0.4–0.5, which took approximately 1.5 hours. Once the desired OD was achieved, the culture was transferred to a sterile 50 mL falcon tube and kept on ice for 5–10 minutes. Following this, centrifugation was carried out at 4500 rpm for 5 minutes, and the supernatant was carefully discarded. The resulting bacterial pellet was gently resuspended in 10 mL of ice-cold 100 mM $CaCl_2$ and incubated on ice for 30 minutes.

Subsequently, the suspension was centrifuged again under the same conditions, and the supernatant was removed. Finally, the pellet was resuspended in 2 mL of ice-cold $CaCl_2$. The DH10β competent cells were stored at +4°C and remained viable for use within 2–3 days.

Competent *Escherichia coli* HT115(DE3) cells were also prepared using $CaCl_2$ method as modified by Kamath & Ahringer (2003). The *E. coli* HT115 (DE3) cells was incubated overnight at 37°C in a shaking incubator at 225 rpm in 5 mL of liquid LB medium supplemented with tetracycline (5 μg/mL). Subsequently, 500 μL of this overnight culture was inoculated into 50 mL of fresh LB medium containing tetracycline and incubated in a sterile 250 mL erlenmeyer flask. The culture was grown at 37°C with shaking at 220 rpm until it reached an optical density (OD600) of approximately 0.4, which typically took around 5 hours. Following this, the bacterial cells were harvested by centrifugation at 1000 xg for 10 minutes at 4°C and collected into a falcon tube. The resulting pellet was resuspended in 50 mL of sterile 10 mL 100 mM $CaCl_2$ and kept on ice for 30 min. After a second centrifugation step under the same conditions, the supernatant was discarded, and the pellet was resuspended in 2 mL, $CaCl_2$. 80% glycerol was added to this suspension. Competent cells were then aliquoted into 100 μL aliquots and stored at -80°C for future use.

2.6. Transformation

A 100 μL aliquot of previously prepared competent cells was taken, and 5 μL of the ligation product was added. The mixture was incubated on ice for 30 minutes to facilitate DNA uptake. Following this, a heat shock was applied by incubating the sample at 42°C for 2 minutes**,** after which it was immediately transferred back onto ice for 1 minute to stabilize the cells. Subsequently, 1 mL of LB medium was added to the transformed cells, and the tube was gently inverted before incubation at 37°C for approximately 1 hour to allow bacterial recovery and initial expression of antibiotic resistance genes. After incubation, the culture was centrifuged at 6000 × g for 3 minutes**,** and the supernatant was carefully removed, leaving approximately 50 μL of the medium**.** The bacterial pellet was gently resuspended, ensuring even distribution of cells. Following resuspension, the transformed culture was plated onto LB agar containing ampicillin (100 μg/mL) and incubated at 37°C for 12–16 hours to allow colony formation.

After incubation, bacterial colonies were screened by plasmid isolation using GeneJET Plasmid Miniprep Kit (Thermo Fisher) and its protocol, followed by restriction enzyme digestion for verification. The KpnI-XbaI enzyme pair was used to digest the 550 bp CHSB fragment, while the XhoI-NcoI enzyme pair was used for the 360 bp fragment**.** Results were visualized on a 1% agar gel to confirm the presence of the expected fragments.

2.7. Construction of dsRNA Expression Vector

To prepare dsRNA expression vector, the gene fragments were excised from pUCM-T vector and cloned into the L4440 plasmid (Addgene). For this process, first L4440 plasmid, stored in the laboratory stock within the DH10β bacterial strain, was isolated using plasmid miniprep kit. The plasmid concentration and purity were verified using a Nanodrop spectrophotometer, and the plasmid was further analyzed by agarose gel electrophoresis and visualized under UV light for quality control. The CHSB550bp and CHSB360bp gene fragments, excised from the pUCM-T vector using the KpnI-XbaI and KpnI-NcoI restriction enzyme pairs, respectively.

These gene fragments were subcloned into L4440 plasmid, digested with the same restriction enzyme pairs. The ligation reaction was prepared as indicated above. After ligation, the product was transformed into HT115 cells as decribed above.

2.8. Biosynthesis of Bacterial dsRNA's

To use at bioassays both dsRNA synthesizing recombinant bacteria and purified total RNAs including specific dsRNAs were prepared. Overnight cultures of recombinant HT115 (DE3)

E. coli cells were prepared. Next day, 100 mL of LB medium was inoculated with 1ml of the overnight culture to initiate bacterial growth. Cultures were incubated at 37 °C until they reached an OD600 of 0.4. At this point, RNA expression was induced via adding 0.4 mM IPTG (final concentration of 50 μM). Following induction, cultures were incubated for an additional 4 hours at 37 °C by shaking at 200 rpm. After incubation, cultures were transferred to sterile 50 mL Falcon tubes and centrifuged at 1000 rpm for 10 minutes at 4 °C. The resulting bacterial pellets were collected. The pellet in one tube was dissolved in PBS to achieve a bacterial concentration of 10^8 CFU/mL. The other pellet was used for total RNA isolation using the TRIzol reagent. These extractions were performed for each groups

separately. The extracted RNA was stored at -80 °C for subsequent use in bioassay experiments.

2.9. Biotest

Biotest were performed by feeding the insects both by dsRNA expressing recombinant bacteria (10^8 CFU/mL) and purified total RNAs including specific dsRNAs. Third instar *H. armigera* larvae were used in the bioassays and starved for 24 hours prior to the experiment. Groups include the larvae fed with, (i) *E. coli* cells expressing 550 bp CHSB gene specific dsRNA, (ii) *E. coli* cells expressing 360 bp CHSB specific dsRNA, (iii) total RNA including 550 bp CHSB specific dsRNA, (iv) total RNA including 350 bp CHSB gene specific dsRNA, (v) *E. coli* cells carrying the empty L4440 plasmid, and 6-water treated food. Thirty larvae were used for each treatment, and each treatment repeated three times.

The bacteria and RNAs were dropped onto chickpea-sized diet pellets and left to dry in the sterile cabinet. The treated food was placed into individual containers, and the starved 3th instar larvae were placed onto the food as shown below. The larvae with the treated foods were placed at 27 °C, under a 16:8 h light/dark photoperiod and 70% relative humidity. The bioassays continued for 10 days, during which daily larval mortality was systematically recorded. Dead and non-feeding larvae were collected in tubes and stored at –80 °C for use in further molecular analyses. The ones that produced developmental abnormalities were photographed.

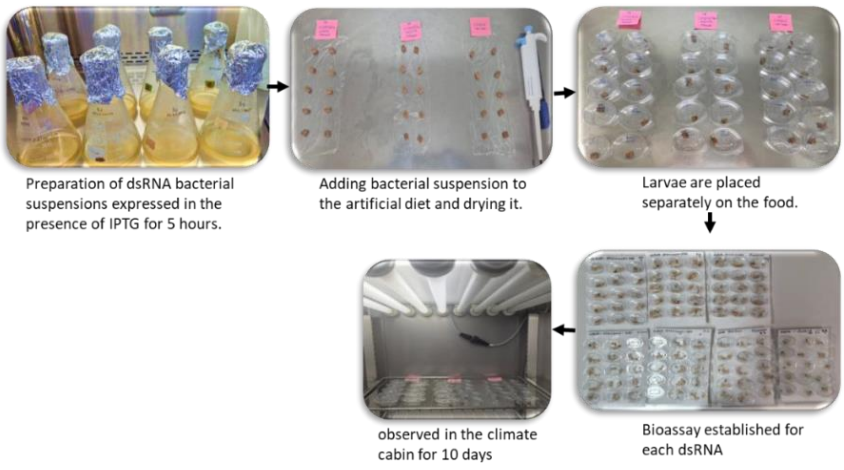

Figure 8. Bioassay with dsRNA and bacterial suspension

3. RESULTS

3.1. Total RNA Isolation

Total RNA was isolated by using TRIzol (sigma) reagent. The isolated RNA was electrophoresed in 1% agar gel to evaluate its purity and integrity. RNA samples formed clear and dense bands on the gel. The sharpness and intensity of the 28S and 18S ribosomal RNA bands indicate that the RNA has good quality. This result showed that the RNA samples are intact and free from contamination (Figure 9).

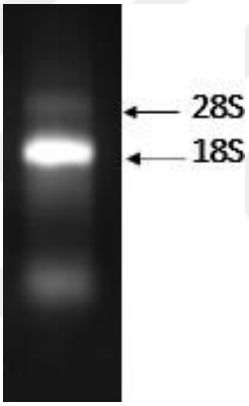

Figure 9. Agarose gel image of eukaryotic total RNA from *Helicoverpa armigera*

3.1.1. cDNA Synthesis and PCR Amplification of CHSB Gene Fragments

The isolated total RNA samples were used for cDNA synthesis. Amplification was performed by PCR using primers specific to the CHSB gene. The products obtained after PCR amplification were analyzed via 1.5% agarose gel electrophoresis. In Figure 10, two separate CHSB gene fragments of the expected sizes (550 bp and 360 bp) were shown.

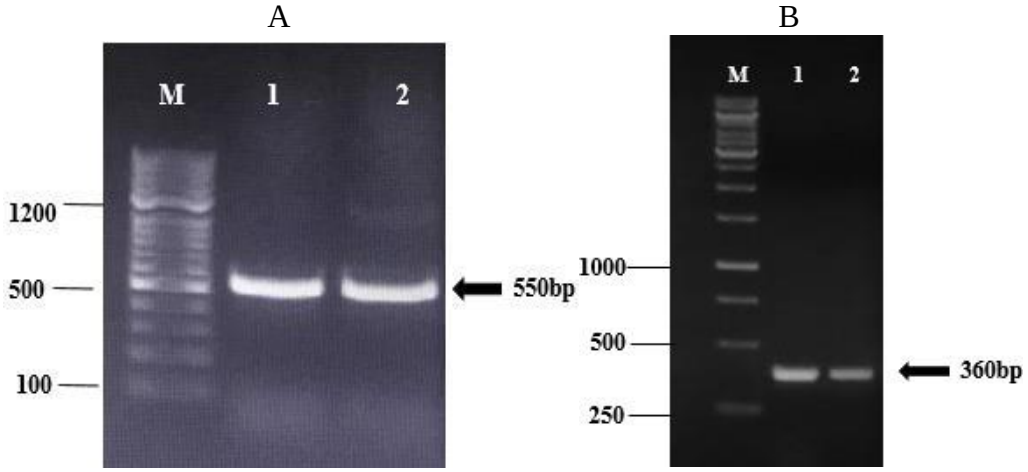

Figure 10. PCR amplified CHSB gene fragments of *Helicoverpa armigera*. A: 550 bp, B: 360 bp)

3.1.2. Transformation of CHSB Genes Into pUCM-T Intermediate Vector

Two different fragments (550 bp and 360 bp) of the *CHSB* (Chitin Synthase B) cDNA's were cloned into the intermediate cloning vector pUCM-T. In Figure 11, a schematic representation of the pUCM-T (2773) vector used in the cloning process is shown, including restriction endonuclease sites and the insertion regions of the relevant gene fragments. The recombinant pUCM-T vectors were subsequently verified by restriction enzyme digestions (Figure 12-13).

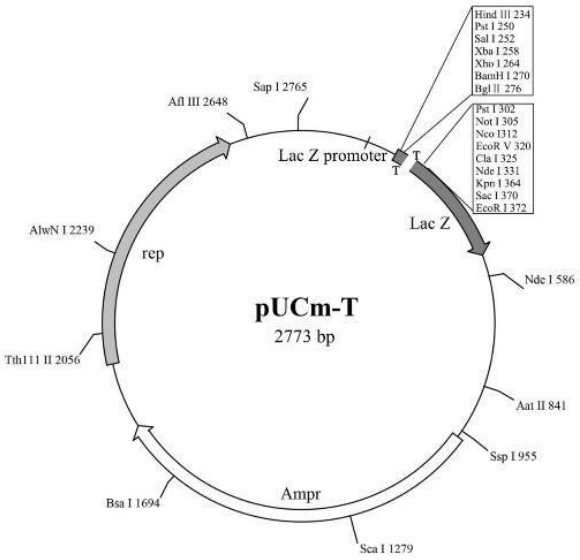

Figure 11. Map of the pUCM-T vector (Biobasic)

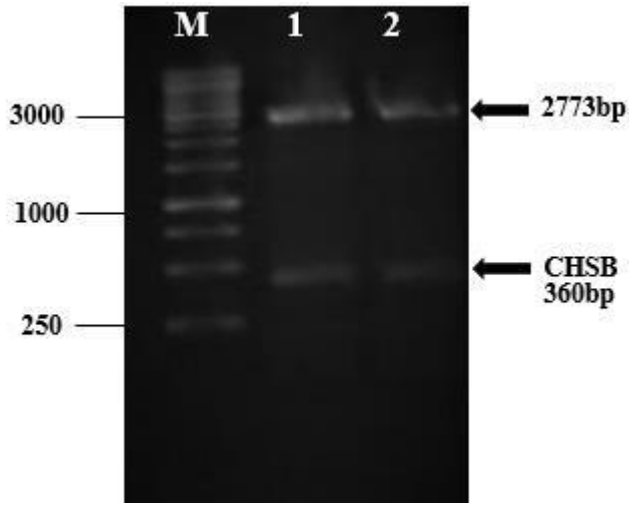

Figure 12. Restriction enzyme analysis of the pUCM-T::360 (CHSB) clone. pUCM-T::360 (CHSB) clones digested with XhoI-NcoI restriction enzymes (1-2). M: 1 Kb marker (GeneRuler).

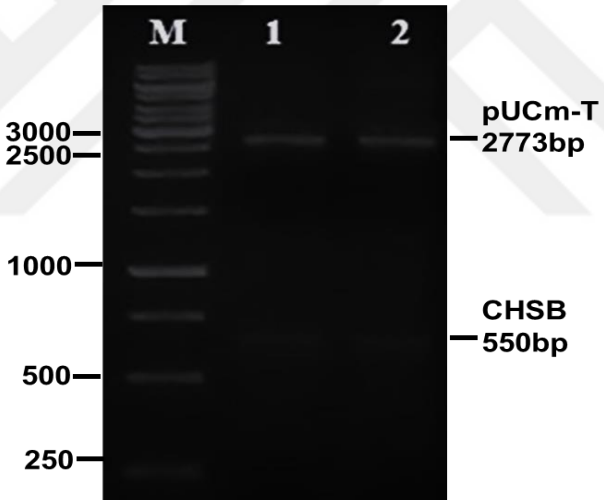

Figure 13. Restriction enzyme analysis of the pUCM-T::550 (CHSB) clone. pUCM-T::550 (CHSB) clones digested with KpnI-XbaI restriction enzymes (1-2).
M: 1 Kb marker (GeneRuler)

3.2. Preparation of dsRNA Expression Vectors

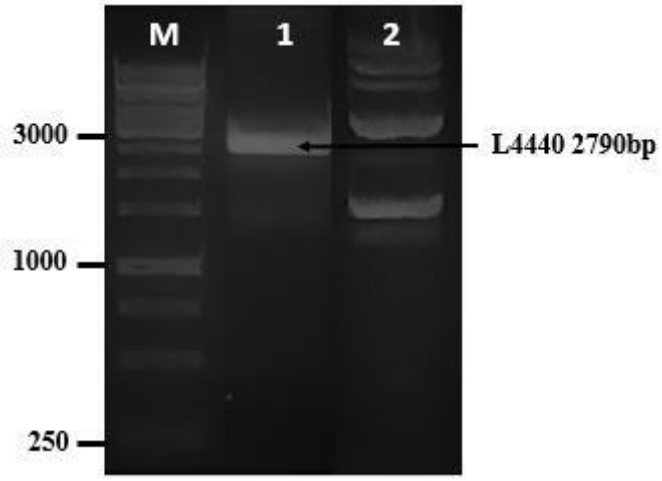

Figure 14. Agarose gel image of the L4440 dsRNA expression vectors. The L4440 plasmid digested with KpnI-XbaI enzymes (1), undigested L4440 plasmid (2), M: 1kb DNA Marker (GeneRuler)

The 360 and 550 bp CHSB gene fragments in the pUCM-T vector was excised and subcloned into the L4440 (2790bp) vector for dsRNA production. A schematic map of the L4440 vector and the relevant restriction sites is provided in Figure 15.

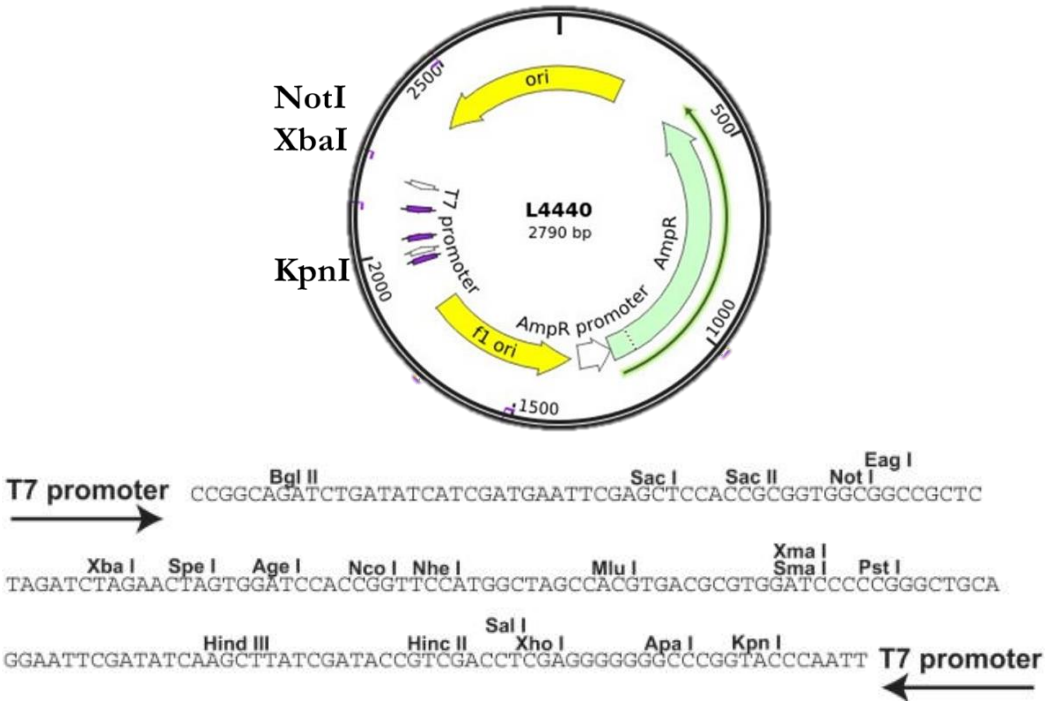

Figure 15. Map of the L4440 cloning vector and multiple cloning site

The 360 bp fragment was inserted between the KpnI and NotI sites of the L4440 vector, whereas the 550 bp fragment was inserted between the KpnI and XbaI sites. In both cases, the gene fragments were successfully oriented between the two T7 promoter regions of the L4440 vector, ensuring their proper expression of dsRNAs. The recombinant L4440 plasmids were confirmed by colony PCR and restriction enzyme analysis (Figure 16-17).

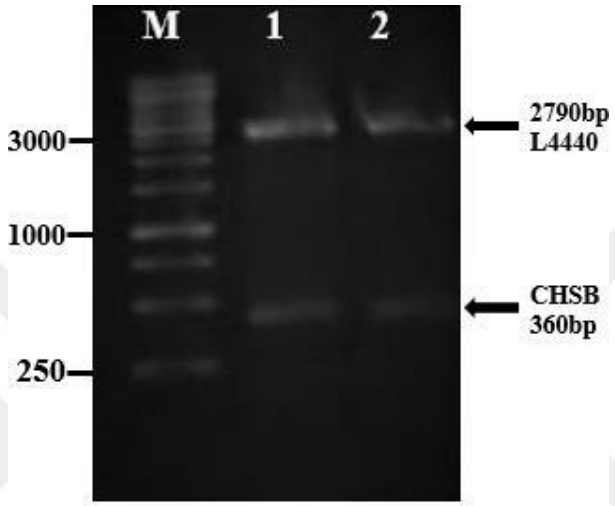

Figure 16. Cloning of CHSB360 genes into L4440 vector. The L4440::360 clones digested with KpnI-NotI restriction enzymes (1-2). M: 1kb DNA Marker (GeneRuler)

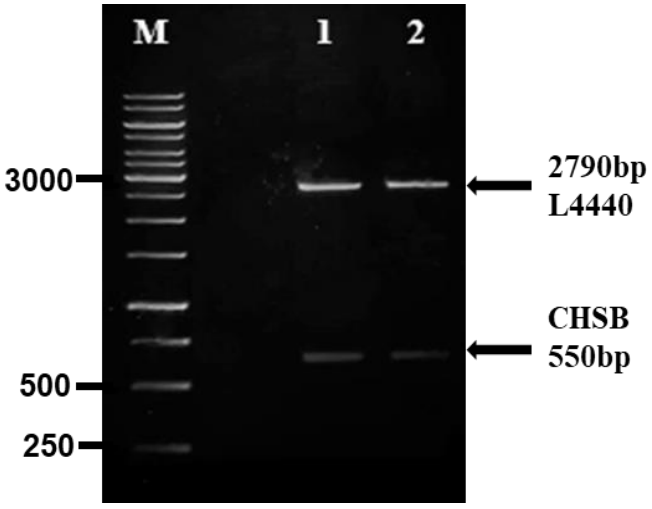

Figure 17. Cloning of CHSB550f genes into L4440 vector. The L4440::550 clones digested with KpnI-XbaI restriction enzymes (1-2). M: 1kb DNA Marker (GeneRuler)

3.3. Biosynthesis of Bacterial dsRNAs

Total RNAs were isolated using Trizol reagent from HT115 (DE3) *E. coli* cells containing 550 bp and 360 bp long CHSB gene fragments in L4440 plasmids (Figures 18, 19).

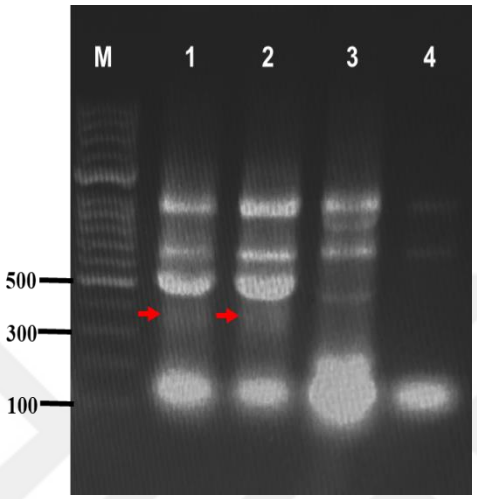

Figure 18. Biosynthesis of bacterial dsRNA (CHSB360). Total RNAs isolated from HT115 (DE3) *E. coli* cells containing the L4440::360 clones (1-2), and total RNA isolated from HT115(DE3) *E. coli* cells containing the empty L4440 plasmid (3).
M: 1 kb DNA Marker (GeneRuler).

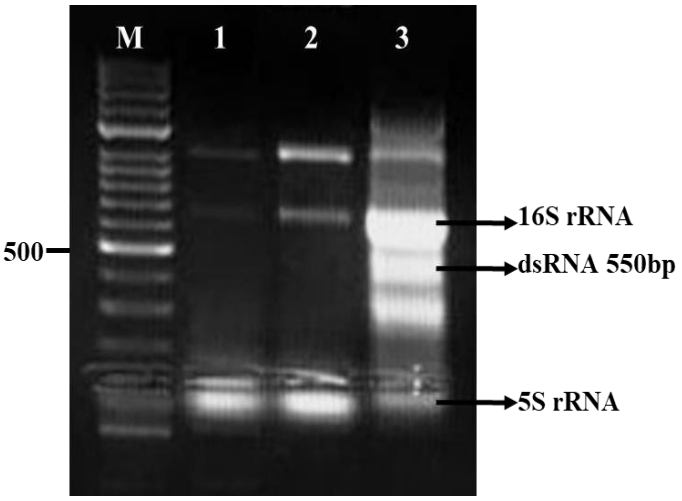

Figure 19. Biosynthesis of bacterial dsRNA (CHSB550). Total RNA isolated from HT115 empty cells (1), total RNA isolated from HT115 cells containing the empty L4440 plasmid (2), total RNA isolated from HT115 cells containing the L4440:550 clone (3).
M: 100 bp DNA marker (Genesta).

3.4. *Helicoverpa armigera* Mortality Tests

The effects of dsRNA were tested on third-instar H. armigera larvae. Larvae were fed with a diet treated with HT115 Escherichia coli cells carrying the empty L4440 plasmid and larvae fed only with water were used as control groups**.** The biotest was monitored for 10 days**,** and larval mortality was recorded daily**.** The results showed that *E. coli* cells expressing 360 base pairs of dsRNA caused the highest mortality, with a rate of 40% (Figure 20). The control groups consisted of larvae fed with dsRNA isolated from HT115 *E. coli* cells carrying the empty L4440 plasmid and larvae fed only with water. Furthermore, dsRNA of 360 base pairs isolated and purified from the same bacterial cells was found to be effective, causing 35% mortality in larvae (Figure 21).

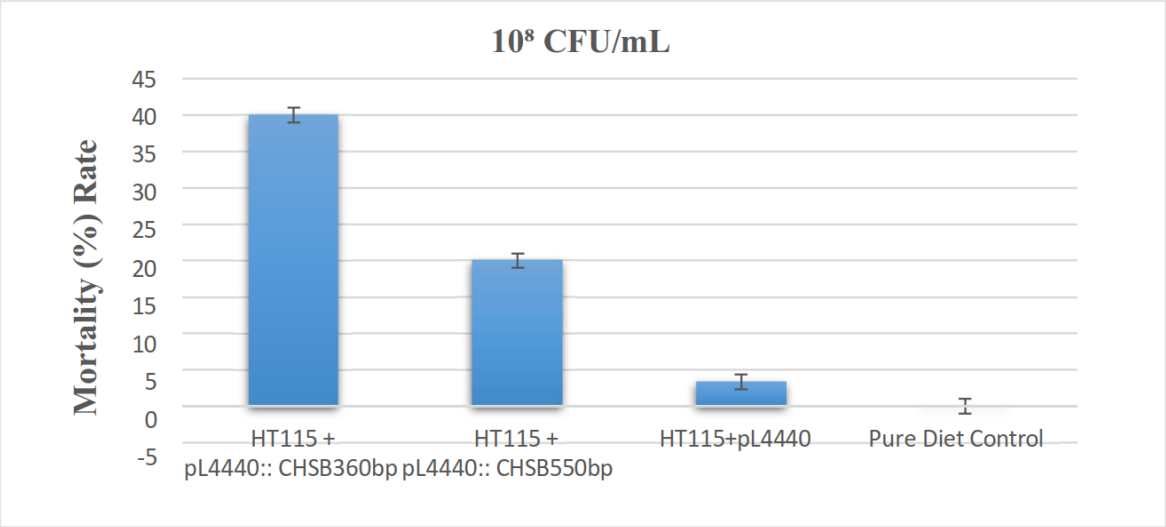

Figure 20. Insecticidal effects of *E. coli* cells containing clones

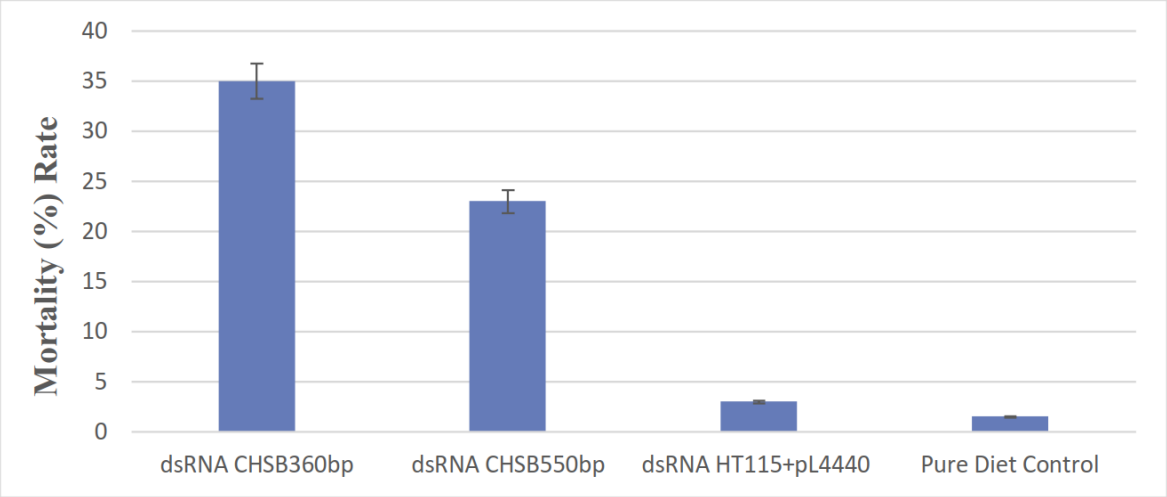

Figure 21. Insecticidal effects of dsRNAs

3.5. Morphological Changes in dsRNA Treated *Helicoverpa armigera*

In our study, RNAi-mediated silencing of the CHSB gene in *H. armigera* larvae not only caused partial mortality but also led to significant developmental abnormalities (Figure 22). Larvae in the control group exhibited normal growth and successfully developed into healthy larva, pupae and adults (Figures A, B, and C), whereas RNAi-treated larvae showed delayed growth and pupation defects, and in some cases failed to enter the pupal stage (Figure D). The resulting adults displayed wing deformities and reduced body size (Figure E). These findings indicate that the CHSB gene plays a vital role in insect growth and metamorphic development, and that its targeting via RNAi can significantly disrupt both larval development and pupal and adult morphology. Therefore, CHSB represents a promising target for RNAi-based, species- specific, and environmentally friendly pest management strategies against *H. armigera*.

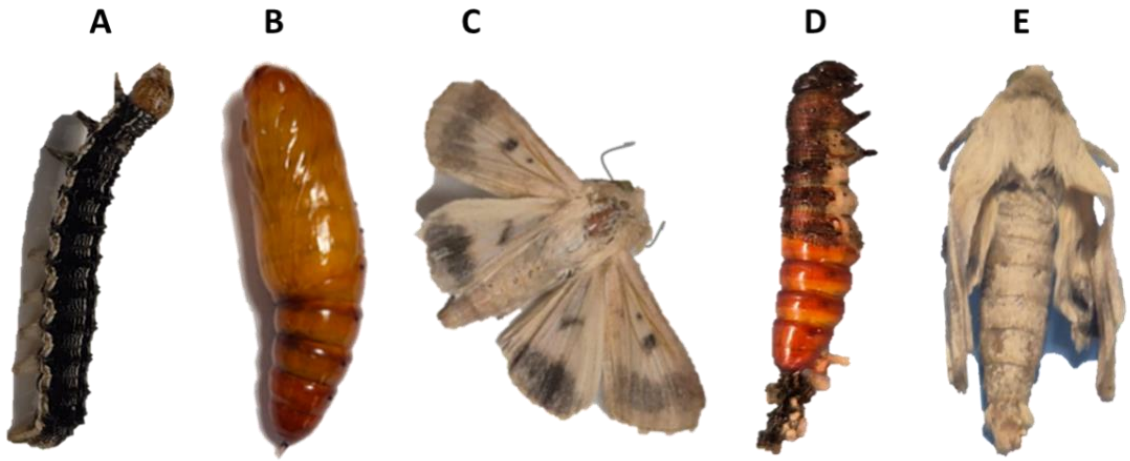

Figure 22. Morphological conditions observed in *Helicoverpa armigera.* (A) Healthy larva in the control group, (B) healthy pupa in the control group, (C) healthy adult in the control group, (D) abnormal larva and pupa that failed to pupate in the RNAi-treated group, (E) wingless and deformed adult in the RNAi-treated group.

4. DISCUSSION

Helicoverpa armigera (Hübner, 1808) is a highly polyphagous species that causes serious damage to a wide range of agricultural crops worldwide; the larval stage is the primary damage stage, feeding voraciously on leaves, buds, flowers and fruits. Traditional control strategies have relied on chemical insecticides such as pyrethroids, quinolphos, monocrotophos, and endosulfan; however, their effectiveness has declined due to resistance development in pest populations (Tatchell, 1997). Microbial strategies, particularly using *Bacillus thuringiensis* (Bt), have also been widely applied, yet resistance to Bt strains has been reported in *H. armigera* populations (Cherry et al., 2003; Luttrell & Jackson, 2012; Yang et al., 2013). Intensive pesticide use has additionally harmed non-target organisms and caused environmental degradation, emphasizing the urgent need for environmentally friendly and species-specific alternatives. In this context, RNAi has emerged as a promising tool for pest management, offering sequence-specific gene silencing via dsRNA, first demonstrated in *Caenorhabditis elegans* (Fire & Mello, 1998) and further developed through oral dsRNA feeding techniques (Timmons & Fire, 1998; Timmons et al., 2001). Among potential targets, chitin, a biopolymer found in insect exoskeletons and digestive systems but absent in plants and vertebrates, is particularly attractive. Chitin synthase genes CHSA (CHS1) and CHSB (CHS2) are responsible for chitin biosynthesis in different tissues, with CHSB being specific to the midgut peritrophic matrix, essential for nutrient absorption and pathogen defense, making it an ideal target for RNAi-based control.

In this study, the effects of two dsRNA fragments (360 bp and 550 bp) derived from the CHSB gene were evaluated by administering recombinant *E. coli* expressing dsRNA and total RNAs purified from these bacteria to larvae with artificial diet. Both fragments effectively silenced CHSB and disrupted larval development, with the 360 bp fragment inducing higher mortality of about 40%, causing developmental abnormalities such as failed pupation, premature pupation, and wingless adult emergence. These results support previous findings that oral dsRNA uptake is most efficient within 200–500 bp (Vogel et al., 2019) and that fragment length critically influences RNAi efficacy (He et al., 2020).

Our results are consistent with studies performed by Chen et al. (2023a). They reported that CHSB knockdown in *Heortia vitessoides* caused severe larval defects and ~43.33%

survival from larva to pupa. Additionally, bacterially mediated RNAi, targeting Sf-CHSB in *Spodoptera frugiperda,* reduced larval weight and survival (Wan et al., 2021).

Continuous dsRNA exposure has been shown to improve efficiency in Lepidoptera like in the *Spodoptera litura*, where single bacterial feeding yielded <30% knockdown, whereas continuous feeding from the third instar achieved ~83% silencing (Wan et al., 2021). Bacteria mediated delivery offers practical advantages, including sustained dsRNA production and partial protection from degradation, though gut-associated degradation still limits mortality (~40% in our study), consistent with observations in *Spodoptera exigua* where sonication of dsRNA-expressing bacteria enhanced RNAi efficiency (Vatanparast & Kim, 2017). Our results are also in consistent with literature since the bacteria expressing dsRNA feeded larvae produced more moratlity.

Studies targeting other essential genes in *H. armigera* further support our conclusions. Sharif et al. (2022) reported that silencing of EcR, v-ATPase-A, and AChE through bacterial or plant-mediated dsRNA delivery induced significant mortality in early instars, with qRT-PCR analysis confirming transcript reductions of up to 93%. Similarly, in *Leptinotarsa decemlineata,* bacterially expressed dsRNA against LdChSB caused severe larval malformations, reduced pupal and adult size, and ~50% adult emergence (Shi et al., 2016). Emerging technologies, such as nanoparticle carriers, cell-penetrating peptides, plant-mediated expression, and spray-induced gene silencing (SIGS), can further protect dsRNA and improve uptake. Transplastomic plants expressing dsRNA targeting CHSB have shown strong protection against chewing pests by providing continuous dsRNA exposure (Bally et al., 2016; He et al., 2020).

The influence of dsRNA length on silencing efficiency has been documented in multiple studies. Vogel et al. (2019) suggested that dsRNA fragments between 200–500 bp generally achieve maximal uptake and siRNA generation in insects, whereas fragments shorter than 60 bp are typically ineffective (He et al., 2020). Supporting this, our results show that the 360 bp fragment was more effective than the 550 bp fragment, both in bacterial and purified dsRNA treatments. This aligns with previous observations in *Tribolium castaneum* and *Leptinotarsa decemlineata*, where moderate-length dsRNAs provided stronger knockdown than longer constructs (Alatorre et al., 2025; Shi et al., 2016). Thus, our data reinforce the concept that fragment length must be empirically optimized for each target gene and insect species.

Delivery method also significantly impacts RNAi efficacy. Sharif et al. (2022) demonstrated that bacterially expressed dsRNA targeting essential genes in *H. armigera* (e.g., EcR, v-ATPase-A, and AChE) caused dose-dependent mortality in early instars, with survival decreasing to 40% at the highest dsRNA dose. In our study, recombinant *E. coli* expressing the 360 bp CHSB dsRNA induced comparable mortality (~40%), whereas purified total RNA applied orally produced slightly lower mortality. These results suggest that bacterial delivery can provide sustained dsRNA exposure and partial protection from midgut nucleases, as previously reported (Christiaens et al., 2020). However, the modest overall mortality observed in *H. armigera* relative to coleopteran insects highlights species-specific barriers, such as gut- associated dsRNases and limited systemic uptake in Lepidoptera.

Larval stage also modulates RNAi effectiveness. Sharif et al. (2022) observed high mortality in early instars (L1–L3) but limited effects in later instars (L4–L5), likely due to elevated RNase activity in older larvae. Consistently, our study revealed that fifth-instar larvae exhibited developmental abnormalities, such as pupation failure and wingless adults, rather than immediate lethality. This suggests that while RNAi can disrupt growth and metamorphosis in older larvae, early instars remain the most susceptible stage for inducing mortality, corroborating findings from other lepidopteran studies (Vatanparast & Kim, 2017).

Comparisons across insect orders further contextualize our results. Shi et al. (2016) showed that bacterially expressed CHSB dsRNA in *L. decemlineata* larvae caused severe malformations and approximately 50% adult emergence, reflecting both developmental disruption and mortality. Similar trends were observed in our study, emphasizing that CHSB knockdown universally impairs larval development, although the absolute mortality varies according to species, dsRNA delivery method, fragment size, and larval stage.

From a practical standpoint, the modest mortality observed in older larvae highlights the need for optimizing dsRNA delivery for field applications. Techniques such as bacterial cell disruption, formulation with protective carriers, or incorporation into transgenic plants could enhance uptake and stability (Wan et al., 2021; He et al., 2020). For instance, *Spodoptera exigua* larvae fed with sonicated dsRNA-expressing bacteria exhibited significantly higher knockdown and mortality, indicating that physical release of dsRNA improves efficiency (Vatanparast & Kim, 2017). Similarly, nanomaterial-based carriers and transplastomic plants have been reported to sustain dsRNA exposure and overcome degradation in the

insect gut (Bally et al.,2016). Integrating such delivery strategies with optimized dsRNA fragment design could increase mortality rates in refractory species like *H. armigera*.

In summary, our results confirm that CHSB is a potent RNAi target in *H. armigera*, with the 360 bp fragment outperforming the 550 bp fragment in both bacterial and purified dsRNA applications. The observed larval abnormalities and partial mortality are consistent with previous lepidopteran studies, emphasizing species-specific considerations such as larval stage, dsRNA length, and gut-associated degradation. The concordance between our findings and literature supports the feasibility of developing CHSB-targeted, species-specific RNAi biopesticides. Future research should focus on refining dsRNA delivery methods, evaluating field-level efficacy, and optimizing fragment selection to achieve more consistent and higher mortality, thereby enabling practical, environmentally safe control of *H. armigera*.

5. CONCLUSION

1. Moderate-length dsRNA (~360 bp) targeting CHSB achieves more effective gene silencing and higher mortality than longer fragments
2. Bacterial delivery improves dsRNA stability and ensures continuous exposure, resulting in mortality levels comparable to or greater than purified dsRNA
3. Larval stage strongly influences RNAi efficacy, with early instars being more susceptible.

These results align with previous studies in Lepidoptera and Coleoptera, highlighting both the robustness and the species-specific considerations necessary for RNAi-based pest management.

6. RECOMMENDATION

The findings of this thesis indicate that silencing the CHSB gene through RNA interference (RNAi) using medium-length double-stranded RNA (dsRNA) of approximately 360 base pairs results in a significantly higher mortality rate compared to longer dsRNA fragments. In light of these results, several considerations are recommended to enhance the efficacy and specificity of RNAi-based pest management strategies.

1. First, the use of appropriate delivery systems is critical for maximizing the cellular uptake and stability of dsRNA molecules. Carriers such as fluorescent nanoparticles, lipid-based nanoparticles, and liposomes have been demonstrated to facilitate efficient dsRNA delivery and protect the nucleic acid from degradation. Incorporating such delivery platforms may therefore improve the overall effectiveness of RNAi treatments in target insect populations.

2. First and second, the developmental stage of the insect plays a crucial role in determining the efficiency of RNAi-mediated gene silencing. Larvae at the second instar stage appear to be more responsive to dsRNA exposure, suggesting that targeting this stage could enhance mortality outcomes and overall pest control efficacy. Accordingly, careful consideration of the insect's life cycle is essential when designing RNAi-based interventions.

3. Finally, accurate assessment of gene knockdown is indispensable for validating the success of RNAi treatments. Following bioassays, the expression levels of the CHSB gene should be quantified using real-time polymerase chain reaction (RT-PCR) after the isolation of total RNA from treated larvae. This molecular analysis not only confirms the degree of gene silencing but also provides valuable insights for optimizing dsRNA design, dosage, and delivery strategies for future applications.

7. REFERENCES

Ali, M. W., Khan, M. M., Song, F., Wu, L., He, L., Wang, Z., Zhang, Z.-Y., Zhang, H., & Jiang, Y. (2020). RNA Interference-Based Silencing of the Chitin Synthase 1 Gene for Reproductive and Developmental Disruptions in *Panonychus citri*. *Insects*, *11*(11), 786.

Alonso, D., & Mondragón, A. (2021). Mechanisms of catalytic RNA molecules. *Biochemical Society Transactions, 49*(4), 1529–1535.

Altayli, E. (2020). Regulator non-coding RNAs: miRNA, siRNA, piRNA, lncRNA, circRNA. *Journal of Clinical Medicine of Kazakhstan, 6*(60), 29–39.

Artika, I. M., Dewi, Y. P., Nainggolan, I. M., Siregar, J. E., & Antonjaya, U. (2022). Real-time polymerase chain reaction: Current techniques, applications, and role in COVID-19 diagnosis. *Genes*, *13*(12), 2387.

Ayilara, M. S., Adeleke, B. S., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, L. A., Omole, R. K., Johnson, R. M., Uthman, Q. O., & Babalola, O. O. (2023). Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Frontiers in Microbiology*, *14*, 1040901.

Baum, J. A., Bogaert, T., Clinton, W., Heck, G. R., Feldmann, P., Ilagan, O., ... & Roberts, J. (2007). Control of coleopteran insect pests through RNA interference. Nature biotechnology, 25(11), 1322-1326.

Beltrán Ortolá, & Daròs, J.-A. (2024). *RNA interference in insects: From a natural mechanism of gene expression regulation to a biotechnological crop protection promise*. Biology, 13, 137.

Bera, P., Suby, S. B., Vadassery, J., Dixit, S., Vijayan, V., Kumar, N., & Sekhar, J. C. (2025). Identification of novel target genes for RNAi mediated management of the pest, Fall Armyworm (*Spodoptera frugiperda*, J. E. Smith). *Crop Protection, 187*, 106972.

Bonaterra, A., Badosa, E., Cabrefiga, J., Francés, J., & Montesinos, E. (2012). Prospects and limitations of microbial pesticides for control of bacterial and fungal pomefruit tree diseases. *Trees*, 26(1), 215–226.

Blake, L. A., Watkins, L., Liu, Y., Inoue, T., & Wu, B. (2024). A rapid inducible RNA decay system reveals fast mRNA decay in P-bodies. *Nature Communications.*

Cao, X., Zhang, Y., Ding, Y., & Wan, Y. (2024). Identification of RNA structures and their roles in RNA functions. *Nature Reviews Molecular Cell Biology*.

Carthew, R. W., & Sontheimer, E. J. (2009). Origins and mechanisms of miRNAs and siRNAs. *Cell, 136*(4), 642–655.

Chang, Y.-W., Wang, Y.-C., Yan, Y.-Q., Xie, H.-F., Yuan, D.-R., & Du, Y.-Z. (2022). RNA interference of chitin synthase 2 gene in *Liriomyza trifolii* through immersion in double- stranded RNA. *Insects, 13*(9), 832.

Chen, Q., Sun, M., Wang, H., Liang, X., Yin, M., & Lin, T. (2023a). Characterization of Chitin Synthase B Gene (HvChsb) and the Effects on Feeding Behavior in *Heortia vitessoides* Moore. *Insects*, *14*(7), 608.

Chen, D.-D., Wang, Z.-B., Wang, L.-X., Zhao, P., Yun, C.-H., & Bai, L. (2023b). Structure, catalysis, chitin transport, and selective inhibition of chitin synthase. *Nature Communications*, *14*, Article 4786.

Cheng, Y., Lu, T., Guo, J., Lin, Z., Jin, Q., Zhang, X., & Zou, Z. (2022). *Helicoverpa armigera miR-2055 regulates lipid metabolism via fatty acid synthase expression*. Open Biology, 12, 210307.

Christiaens, O., Whyard, S., Vélez, A. M., & Smagghe, G. (2020). Double-stranded RNA technology to control insect pests: current status and challenges. Frontiers in plant science, 11, 451.

Darrington, M., Dalmay, T., Morrison, N. I., & Chapman, T. (2017). Implementing the sterile insect technique with RNA interference–a review. Entomologia experimentalis et applicata, 164(3), 155-175.

De Giorgio, E., Giannios, P., Espinàs, M. L., & Llimargas, M. (2023). A dynamic interplay between chitin synthase and the proteins Expansion/Rebuf reveals that chitin polymerisation and translocation are uncoupled in *Drosophila*. *PLoS Biology*, *21*(1), e3001978.

Elbashir, S. M., Martinez, J., Patkaniowska, A., Lendeckel, W., & Tuschl, T. (2001). Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. The EMBO Journal, 20(23), 6877–6888.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, 391(6669), 806–811.

Ghosh, S. K. B., & Gundersen-Rindal, D. E. (2017). Double strand RNA-mediated RNA interference through feeding in larval gypsy moth, *Lymantria dispar* (Lepidoptera: Erebidae). *European Journal of Entomology*, *114*, 170–178.

Goettel, M. S., Eilenberg, J., & Glare, T. (2005). Entomopathogenic fungi and their role in regulating insect populations. *Insect Pathology*, 361-406.

Hasan, A., Cotobal, C., Duncan, C. D. S., & Mata, J. (2014). *Systematic analysis of the role of RNA-binding proteins in the regulation of RNA stability*. PLoS Genetics, 10 (11), e1004684.

Ioannidis, P., Buer, B., Ilias, A., Kaforou, S., Aivaliotis, M., Orfanoudaki, G., Douris, V., Geibel, S., Vontas, J., & Denecke, S. (2022). A spatiotemporal atlas of the

lepidopteran pest *Helicoverpa armigera* midgut provides insights into nutrient processing and pH regulation. *BMC Genomics, 22*, 717.

Isenmann, M., Stoddart, M. J., Schmelzeisen, R., Gross, C., Della Bella, E., & Rothweiler, R. M. (2023). Basic principles of RNA interference: Nucleic acid types and in vitro intracellular delivery methods. *Micromachines, 14*(7), 1321.

Katoch, R., Sethi, A., Thakur, N., & Murdock, L. L. (2013). RNAi for insect control: Current perspective and future challenges. *Applied Biochemistry and Biotechnology*, *171*(4), 847–873.

Keszthelyi, S., Orsi-Gibicsár, S., Pál-Fám, F., Somfalvi-Tóth, K., & Balog, A. (2024). Colour polymorphism of cotton bollworm larvae as a function of the type of host plant providing its development. *Frontiers in Ecology and Evolution, 12*, 1376435.

Kumar, J., Ramlal, A., Mallick, D., & Mishra, V. (2021). An overview of some biopesticides and their importance in plant protection for commercial acceptance. *Plants*, *10*(6), 1185.

Khajuria, C., Vélez, A. M., Rangasamy, M., Wang, H., Fishilevich, E., Frey, M. L., ... & Siegfried, B. D. (2015). Parental RNA interference of genes involved in embryonic development of the western corn rootworm, Diabrotica virgifera virgifera LeConte. Insect biochemistry and molecular biology, 63, 54-62.

Khan, A., Smagghe, G., Li, S., Yang, G., & Ahmed, N. (2025). Insect metamorphosis and chitin metabolism under miRNA regulation: A review with current advances. *Pest Management Science*. Advance online publication.

Kornienko, I. V., Aramova, O. Y., Tishchenko, A. A., Chikindas, M. L., & Rudoy, D. V. (2024). RNA stability: A review of the role of structural features and environmental conditions. *Molecules*, *29*(24), 5978.

Lam, J. K. W., Chow, M. Y. T., Zhang, Y., & Leung, S. W. S. (2015). *siRNA versus miRNA as therapeutics for gene silencing*. Molecular Therapy—Nucleic Acids, 4, e252.

Li, H., Jiang, W., Zhang, Z., Xing, Y., & Li, F. (2013). Transcriptome analysis and screening for potential target genes for RNAi-mediated pest control of the beet armyworm, *Spodoptera exigua*. *PLOS ONE*, 8(6), e65931.

Liu, X., Li, F., Li, D., Ma, E., Zhang, W., Zhu, K. Y., & Zhang, J. (2013). Molecular and functional analysis of UDP-N-acetylglucosamine pyrophosphorylases from the migratory locust, *Locusta migratoria*. *PLoS ONE, 8*(8), e71970.

Liu, J., Rivas, F. V., Wohlschlegel, J., Yates, J. R., III, Parker, R., & Hannon, G. J. (2005). A role for the P-body component, GW182, in microRNA function. *Nature Cell Biology, 7*(12), 1261–1266.

Liu, X.-Y., Wang, S.-S., Zhong, F., Zhou, M., Jiang, X.-Y., Cheng, Y.-S., Dan, Y.-H., Hu, G., Li, C., Tang, B., & Wu, Y. (2022a). Chitinase (CHI) of *Spodoptera frugiperda*

affects molting development by regulating the metabolism of chitin and trehalose. *Frontiers in Physiology, 13*, 1034926.

Liu, X., Wang, S., Yu, Y., Cheng, Y., Hu, C., Zhou, M., Li, C., Tang, B., & Wu, Y. (2022b). *Effects of inhibiting the expression of chitin synthase gene SfCHSB on the metabolism of trehalose and chitin in Spodoptera frugiperda larvae*. Agriculture, 12(12), 2019.

Lu, Y., Deng, X., Zhu, Q., Wu, D., Zhong, J., Wen, L., & Yu, X. (2023). The dsRNA delivery, targeting and application in pest control. *Agronomy, 13*(714).

Merzendorfer, H. (2013). Chitin synthesis inhibitors: old molecules and new developments. *Insect Science, 20*(2), 121–138.

Minchin, S., & Lodge, J. (2019). Understanding biochemistry: Structure and function of nucleic acids. *Essays in Biochemistry, 63*(4), 433–456.

Napoli, C., Lemieux, C., & Jorgensen, R. (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *The Plant Cell*, 2(4), 279–289.

Niu, D., Zhao, Q., Xu, L., & Lin, K. (2024a). Physiological and molecular mechanisms of lepidopteran insects: Genomic insights and applications of genome editing for future research. *International Journal of Molecular Sciences, 25*(22), 12360.

Niu, S., Qi, L., Zhang, X., He, D., Li, P., Wang, H., & Bi, Y. (2024b). Chitin translocation is functionally coupled with synthesis in chitin synthase. *International Journal of Molecular Sciences, 25*(21), 11667.

Nowotny, M., & Yang, W. (2009). Structural and functional modules in RNA interference. *Current Opinion in Structural Biology*, 19(3), 286–293.

Palli, S. R. (2023). RNAi turns 25: Contributions and challenges in insect science. *Frontiers in Insect Science, 3*, 1209478.

Passmore, L. A., & Coller, J. (2022). Roles of mRNA poly(A) tails in regulation of eukaryotic gene expression. *Nature Reviews Molecular Cell Biology, 23*(2), 93–106.

Pedrosa da Costa Gomes, C., Ágg, B., Andova, A., Arslan, S., Baker, A., Barteková, M., … Devaux, Y. (2019)**.** Catalyzing transcriptomics research in cardiovascular disease: The CardioRNA COST Action CA17129. Non-coding RNA, 5(2), 31.

Poelchau, M., Childers, C., Moore, G., Tsavatapalli, V., Evans, J., Lee, C.-Y., Lin, H., Lin, J.- W., & Hackett, K. (2015). The i5k Workspace@NAL—Enabling genomic data access, visualization and curation of arthropod genomes. *Nucleic Acids Research, 43*(D1), D714–D719.

Price, D. R. G., & Gatehouse, J. A. (2008). RNAi-mediated crop protection against insects. *Trends in Biotechnology, 26*(8), 393–400.

Rana, S., Rajurkar, A. B., Kumar, K. K., & Mohankumar, S. (2020). Comparative analysis of chitin synthase A dsRNA mediated RNA interference for management of crop pests of different families of Lepidoptera. *Frontiers in Plant Science, 11*, 427.

Rios, C., Warren, D., Olson, B., & Abbott, A. L. (2017). Functional analysis of microRNA pathway genes in the somatic gonad and germ cells during ovulation in *C. elegans*. *Developmental Biology*, *429*(2), 207–217.Shapiro-Ilan, D. I., Gouge, D. H., Piggott, S. J., & Fife, J. P. (2006). Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control, 38*(1), 124–133.

Silver, K., Cooper, A. M. W., & Zhu, K. Y. (2021). *Strategies for enhancing the efficiency of RNA interference in insects*. Pest Management Science, **77**(5), 2646–2658.

Sharif, M. N., Iqbal, M. S., Alam, R., Awan, M. F., Tariq, M., Ali, Q., & Nasir, I. A. (2022). Silencing of multiple target genes via ingestion of dsRNA and PMRi affects development and survival in *Helicoverpa armigera*. *Scientific Reports, 12*(1), 10405.

Shi, J.-F., Mu, L.-L., Chen, X., Guo, W.-C., & Li, G.-Q. (2016). RNA interference of chitin synthase genes inhibits chitin biosynthesis and affects larval performance in *Leptinotarsa decemlineata* (Say). *International Journal of Biological Sciences, 12*(11), 1319–1331.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3 new capabilities and interfaces. *Nucleic Acids Research*, 40(15), e115.

Wan, X.-S., Shi, M.-R., Xu, J., Liu, J.-H., & Ye, H. (2021). Interference efficiency and effects of bacterium-mediated RNAi in the fall armyworm (Lepidoptera: Noctuidae). *Journal of Insect Science, 21*(5), 8, 1–12.

Wang, Z., Long, G.-Y., Zhou, C., Jin, D.-C., Yang, H., & Yang, W.-J. (2022). Molecular characterization of UDP-N-acetylglucosamine pyrophosphorylase and its role in the growth and development of the white-backed planthopper *Sogatella furcifera* (Hemiptera: Delphacidae). *Genes, 13*(8), 1340.

Wang, Z., Dong, Y., Desneux, N., & Niu, C. (2013). RNAi silencing of the *HaHMG-CoA reductase* gene inhibits oviposition in the *Helicoverpa armigera* cotton bollworm. *PLoS ONE, 8*(7), e67732.

Weeks, K. M. (2010). Advances in RNA secondary and tertiary structure analysis by chemical probing. *Current Opinion in Structural Biology, 20*(3), 295–304.

Whyard, S., Singh, A. D., & Wong, S. (2009). *Ingested double-stranded RNAs can act as species-specific insecticides*. Insect Biochemistry and Molecular Biology, 39(11), 824–832.

Wilson R, Doudna JA. Molecular mechanisms of RNA interference. *Annu Rev Biophys*. 2013;42:217-239.

Yu, A., Beck, M., Merzendorfer, H., & Yang, Q. (2024). Advances in understanding insect chitin biosynthesis. *Insect Biochemistry and Molecular Biology, 164*, 104058.

Van Leeuwen, T., Demaeght, P., Osborne, E. J., Dermauw, W., Gohlke, S., Nauen, R., Grbić, M., Tirry, L., Merzendorfer, H., & Clark, R. M. (2012). Population bulk segregant mapping uncovers resistance mutations and the mode of action of a chitin synthesis inhibitor in arthropods. *Proceedings of the National Academy of Sciences of the United States of America (PNAS), 109*(12), 4407–4412.

Vatanparast, M., Kazzazi, M., Sajjadian, S. M., & Park, Y. (2021). Knockdown of *Helicoverpa armigera* protease genes affects its growth and mortality via RNA interference. *Archives of Insect Biochemistry and Physiology, 108*(3), e21840.

Vatanparast, M., & Kim, Y. (2017). Optimization of recombinant bacteria expressing dsRNA to enhance insecticidal activity against a lepidopteran insect, *Spodoptera exigua*. *PLOS ONE, 12*(8), e0183054.

Zhao, X., Zhang, J., & Zhu, K. Y. (2019). Chito-protein matrices in arthropod exoskeletons and peritrophic matrices. In E. Cohen & H. Merzendorfer (Eds.), *Extracellular sugar-based biopolymers matrices* (Biologically-Inspired Systems, Vol. 12, pp. 1–25). Springer Nature Switzerland AG.

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