CHAPTER 5

5. DISCUSSION

In India, food security is limited due to overpopulation, urbanization, and modernization, leading to hiked food prices. Agriculture is a core sector of the economy, with over sixty per cent of the population dependent on it for livelihood. Climate and pest infestation are the biggest challenges. Insects are the most significant insect order, occupying the largest population amongst all living organisms known on the Earth. Pest problems have increased due to diseases from microbes and weeds. In Vadodara, the maximum pest percentage has been occupied by orders Hemiptera and Lepidoptera. One such essential and prevalent pest in recent times is *Spodoptera frugiperda*. Here, the most preferred crop of *Spodoptera frugiperda* is maize fields in the various regions around Vadodara, like that of Chhani, Savli, and Padra. Several factors, including genetic mutations, adaptation, and changes in behaviour, cause insecticide resistance. To mitigate the development of resistance, integrated pest management strategies should be employed, such as biological control, cultural control, and the use of insecticides with different modes of action. Exposure to herbicides also affects the resistance of insects against insecticides, especially agricultural pests, and cross-resistance between insecticides can occur due to increased tolerance to a type of insecticide never used for selection. *Spodoptera frugiperda* (Fall armyworm) is a polyphagous pest known to damage maize, sorghum, sugarcane, paddy, spinach, mango, coriander, cucumber, melon, castor, peanut, chickpea, banana, barley, and pepper.

Fall armyworm has been in the news in recent years due to its invasion of India and is not responding to various insecticides recommended for the control of lepidopteran pests. Insecticides can be controlled by targeting multiple metabolic pathways, including changes in gene expression, enzyme activity, and altered substrate specificity. In some cases, resistance can also result from a combination of mechanisms, such as changes in the target site and increased detoxification. Understanding the molecular basis of resistance is vital for the development of new insecticides and for the implementation of integrated pest management strategies. The Fall armyworm is a highly destructive insect pest that has caused significant

damage to crops in Karnataka and Rajasthan. Insecticides are ineffective in controlling the pest, and artificial diets are used as a medium for effectively rearing insect pests. Chlorantraniliprole and Emamectin Benzoate as novel insecticides have been proposed to combat pesticide resistance in insects. These insecticides target different sites in the insect's nervous system and have different modes of action, making it harder for insects to develop resistance. The combination is an insecticide belonging to the diamide class, discovered by Dupont, and is used to control lepidopteran pests.

Chlorantraniliprole & Emamectin Benzoate are two insecticides used for controlling pests. The study aimed to investigate the efficacy of two new-generation insecticides (Chlorantraniliprole and Emamectin Benzoate) against *Spodoptera frugiperda* Smith. This pest has been widely present since it invaded India. The objectives were undertaken through a survey of agricultural fields in and around Vadodara, evaluating natural and artificial diets, checking insecticide efficacy, comparing control and insecticide-treated insect midgut tissue by histology, and understanding resistance development in the pest against the insecticides Chlorantraniliprole & Emamectin Benzoate. The molecular basis of resistance was studied. Insects can act ecologically beneficial and harmful to farmers, so a planned control program is highly required to control the pest. The fall armyworm is a significant pest in the field, and the need to rear and breed in the lab.

Agricultural insect pests like *S. frugiperda* cause high crop losses that result in a lack of human food, fodder for animals, and economic loss to farmers and consumers. Human survival necessitates a steady supply of food, fodder, and other agricultural supplies. With pests like the fall armyworm invading fields and destroying crops, there is the possibility of a resource shortage. Farmers suffer losses due to investing in seeds, growing, fertilizers, and pesticides but not receiving the expected yield. Such a lack of supply would increase the price and cause problems for consumers. Pests thus have an impact not only on ecology but also on the economy. Several insect pests have already been causing crop damage in the agricultural fields of Vadodara.

The *Spodoptera frugiperda* Smith, 1797, is an ecologically important agricultural pest of the world that causes damage to several crops of ecological importance. It was first reported in India in 2018 and has spread to many countries in Africa and Asia in recent times. It is known to cause infestation up to 35% in maize fields and has been identified in Gujarat, Tamil Nadu, and Southern Rajasthan. It is polyphagous and has been majorly attacking maize, followed by

sorghum. The sugarcane fall armyworm (FAW) has been spreading in India since 2016, mainly in maize-growing regions.

The current ways to control new pests effectively require reasonable chemical control. We selected two insecticides recommended for use against lepidopteran pests. These two should be tested in detail for their efficacy and control against the new invasive pest, *S. frugiperda. In addition, we need to know how long and to what extent the efficacy will last.* As shown in many studies, pesticides fail over a long period as pests stop showing much response in the long run*. A study like this would help to assess the potential of popular insecticides on the market for prescribing the correct dose. A comparative resistance study will reveal which pesticides developed resistance first, which can also be used to compare the efficacy of the two drugs*. The required dose providing half mortality, also called LD_{50}, helps to know the concentration at which efficient control can be achieved. A "generation study," i.e., rearing insects for many generations inside a lab and testing insecticides on them, can aid in knowing how much the efficacy changes over generations. A molecular study of resistance will help to find the gene causing resistance. This can be further used in future studies to design chemicals or insecticides capable of counteracting the resistance *Spodoptera frugiperda*, also known as the fall armyworm, a globally significant pest.

Knowledge of host crops is essential to reduce the risk of crop failures. In a study conducted in Brazil, **Montezano et al., 2018** compiled a list of up to 353 plant hosts. There about 82 new records were found from the study. FAW has a high dispersal ability and broad host range, a major infestation in maize crops. During our field assessment, we discovered only maize fields to be significantly affected. Before conquering Africa and Asia in 2016 and 2018, the FAW was restricted to the American continent alone. **Deshmukh et al., 2018** reported *Spodoptera frugiperda* for the first time in India on maize in the state of Karnataka. This was the first record of a fall armyworm on the Asian continent. Subsequently, several reports of *S. frugiperda* emanated from different regions of India. Since its discovery in India in 2018, the insect has also wreaked havoc in Gujarat. **Naganna et al., 2020** researched the prevalence of FAW in Junagadh. **Damasia et al., 2020**, detected fall armyworms in finger millet crops in the Dangs area of Gujarat. **Srikanth et al., 2018** reported the first incidence of the exotic pest fall armyworm on sugarcane outside of Gujarat in the southern Indian state of Tamil Nadu. **Babu et al., 2019** have discovered *Spodoptera frugiperda* in southwestern Rajasthan. In the Sangli District, **Chormule et al., 2019** detected FAW grazing on a two-month-old

sugarcane crop (Co 86032) variety. Such incidents continued to occur, particularly in the country's maize-growing areas. **Padhee & Prasanna, 2019** analyzed the instance of fall armyworm infestation in India, highlighting the countrywide spread. **Kumar et al., 2022**, described the present methods of regulating FAW in India. Various characteristics, including a strong dispersion capacity and a broad host range, support FAW's huge capacity. Having previously worked in labs with various lepidopteran pests, we could also see the hyperactive behaviour of *S. frugiperda* in contrast to *S. litura*. **Haenniger et al., 2020** reported little variation in sexual communication between the maize and rice strains. Before beginning practical control, the status of any pest must be determined. Similar reviews of pests have been done in the past, such as **Rao, 2020**, which investigated the situation of the pink bollworm on *Bt* in India. High FAW losses need the use of control mechanisms. According to **Harrison et al., 2019**, a FAW estimate of over US$13 billion in Africa drives farmers to use more and more pesticides as a preventative measure. A similar estimation was done previously by **Zalucki et al., 2012**, where an estimate of US$4 billion to US$5 billion is associated with the total costs of managing diamondback moths. The damage caused by insects must be quantified. **De Groote et al., 2020**, projected agricultural losses in the Kenya area of Africa. This requires efficient management. We must be certain that such management may produce positive outcomes. The connection between yield and management should be examined. **Tambo et al., 2020** determined that FAW may be effectively managed, resulting in a substantial increase in crop productivity. Before performing research, the life cycle of any organism must be understood. Due to the duration of a pest's life cycle, it is crucial to have a thorough knowledge of the pest. The FAW life cycle lasts around a month. It is essential to understand the whole life cycle of a pest to determine the different management phases. **Sharanabasappa et al., 2018** examined the life cycle of FAW lab conditions at UAHS in Shivamogga, Karnataka. In our research, we also examined the length of the life cycle. The recent invasion and extensive damage to India's agricultural fields by the FAW have demanded a thorough investigation of all feasible control measures. Numerous studies have been conducted on formerly existing agricultural pests in Gujarat, such as lepidopteran pests such as *Spodoptera litura* and *Plutella xylostella*. In 2018, the preliminary research of FAW began in Gujarat. In regions like Anand, Vadodara, Navsari, and Junagadh, work on the new invasive species has subsequently commenced. Both biotic and abiotic variables influence the growth, development, and reproduction of insects. **Patel et al., 2021**, investigated the relationship between mango thrips and abiotic parameters in the Kesar mango

plantation. In this research, we considered the most important biotic and abiotic parameters for FAW rearing: temperature, humidity, and nutrition. There are several methods of pest management, including chemical control and biocontrol, among others. **Thumar et al., 2020** have also conducted field research on the chemical control of FAW using widely available pesticides. They conducted field tests using the insecticides Chlorantraniliprole, Emamectin Benzoate, spinetoram, and thiodicard during Kharif. Various insecticides have also been used to combat other lepidopteran pests. **Bhut et al., 2022**, evaluated the effectiveness of chemical pesticides against two of the most significant castor pests, *Spodoptera litura* and *Achaea janata*. Combination insecticides may be used for control purposes. **Kamaraju et al., 2021**, used a mixture of neonicotinoids and pyrethroids against the rural malaria vector, *Anopheles culcifacies*. The use of pesticides against various pests was also evaluated. **Devashrayee et al., 2022**, investigated the effectiveness of many pesticides, including Emamectin Benzoate. There are two species of bean pod borer in India: *Helicoverpa armigera* and *Maruca vitrea*. New pesticides are beneficial, but their hazards must be assessed. **Paramasivam et al., 2022** in Tamil Nadu, India, evaluated the risk assessment of Chlorantraniliprole in chilli crops. Emamectin is efficient in eliminating other lepidopteran pests. **Singh et al., 2022**, examined the effectiveness of Spinosad and Emamectin Benzoate against *Helicoverpa armigera* on tomatoes in Varanasi, U.P. Even though a significant amount of research has been conducted with chemical pesticides, a study using the technical grades of insecticides Chlorantraniliprole and Emamectin Benzoate on the Gujarat FAW population is new, so we conducted this investigation. We also grew insects in the laboratory and tested them across many generations.

In addition to spraying pesticides on plants, other additional approaches for pest control have been investigated. Biopesticides and natural enemies can be used to protect against fall armyworms (FAW). Initial control may be accomplished by spraying pesticides on seeds. **Dobariya & Sisodiya, 2022** evaluated the efficacy of a pesticide as a seed treatment against fall armyworms. Enticing pests may achieve rapid extinction with baits containing toxic compounds. **Lunagariya et al., 2020**, undertook field trials for poison bait assessment against *Spodoptera frugiperda*. Other than chemical pesticides, even biopesticides may provide adequate control. **Dhobi et al., 2020** put 2020 bio-pesticides to the test against Fall armyworms. No matter how effective insecticides are, they will ultimately fail. This is related to the problem of resistance formation. Resistance varies across crop genotypes, as shown

by **Subbireddy et al., 2018**'s evaluation of the resistance potential of numerous okra cultivars and genotypes.

For instance, plant growth regulators can suppress pests. **Nagaratna et al., 2022,** conducted tests to ascertain the impact of PGRs and Si on FAW. FAW is also responsible for the devastation of sorghum crops. **Lad & Pawar, 2022**, assessed the effectiveness of pesticides against FAW in sorghum fields. Biocontrol agents are organisms that are capable of eliminating pests. **Aarthi, Tamboli and More, 2022**, examined the bioefficacy of biocontrol agents against several stages of the fall armyworm in the laboratory. In addition to chemical approaches, management includes monitoring, scouting, and mechanical control. **Verma et al., 2016** in Bihar and Uttar Pradesh, proposed an environmentally friendly method for controlling the fall armyworm. By examining the resistant population, the molecular mechanism may be determined. There are two known strains of FAW, and their behaviour must be understood. Several substances are evaluated to generate a control.

Fernandes et al., 2018 evaluated the effectiveness of chemical insecticides for both standalone and combination chemicals. We evaluated the efficacy of Chlorantraniliprole and Emamectin Benzoate against fall armyworms after many generations of laboratory breeding. There has been an observed development of resistance in insect pests in the past. One such pest is the diamondback moth, *Plutella xylostella*, known as the initial insect that developed resistance to Bt. **Liu & Tabashnik, 1997** saw DBM's increased resistance in Arizona to the *Bacillus thuringiensis* toxin Cry1C. **Boaventura et al., 2020** sought to determine the molecular mechanism using two resistant populations. Once the responsible gene has been identified, resistance may be overcome. Molecular mechanisms can be known by checking resistant populations. Metabolic detoxification plays a vital role in providing resistance to insects, and GSTs or Glutathione S-transferases by conjugation are known to cause resistance. Cytochrome P450 monooxygenases (CypP450s) are essential genes that resist the insects against various chemical insecticides, but only a subgroup of P450 genes is linked with insecticide resistance. Detoxification enzymes are prominent in resistance, such as esterase, and a study was done by **Parmar & Patel, 2018** in Gujarat on a lepidopteran pest, *Helicoverpa armigera*.

Using CRISPR/Cas9 editing, **Kaduskar et al., 2022** produced knockdown resistance mutations in isogenic laboratory Drosophila strains. Resistance varies in various places. **Wang et al., 2022**, monitored the resilience of sixteen geographical populations in China in Beijing. The function of detox enzymes is well understood. **Li et al., 2022**, attempted to determine the purpose of GSTs in Henan, China. The resistant mutation does not impact the complete gene family. According to **Nauen et al., 2022**, only a subset of P450 genes is associated with pesticide resistance. In our research, we have identified the numerous genes confer pesticide resistance to the fall armyworm.

The following is the list of genes showing altered signalling and contributing to the development of resistance in the organism under study:

5.1. GLUTATHIONE S-TRANSFERASE:

The detoxification of avermectins in different organisms involves several mechanisms, including metabolism, sequestration, and excretion. The overexpression of the GST gene has been proposed as a mechanism for detoxifying avermectins in various organisms. GSTs are a family of enzymes that catalyze the conjugation of glutathione with different electrophilic compounds, including pesticides, to make them more water-soluble and easily excretable. The conjugation of avermectins with glutathione by GSTs results in more polar and less toxic metabolites that can be excreted from the body.

The overexpression of the glutathione S-transferase (GST) gene has been shown to play a crucial rolein detoxifying compounds such as ivermectin, abamectin, and doramectin. GSTs are a family of enzymes that catalyze the conjugation of xenobiotics, including pesticides, with glutathione (GSH), atripeptide composed of glutamate, cysteine, and glycine, to produce more water-soluble and less toxicforms that can be eliminated from the body.

The overexpression of the GST gene has been observed in various organisms, including insects, nematodes, and mammals, in response to different chemical compounds, including pesticides. The overexpression of the GST gene can be induced by activating various signalling pathways, including the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. The Nrf2 pathway is a transcriptional pathway that regulates the expression of different antioxidant and detoxifying genes, including the GST gene, in response to oxidative and electrophilic stress.

In insects, the overexpression of the GST gene has been shown to confer resistance to various classes of pesticides, including organophosphates, pyrethroids, and carbamates. The overexpression of the GST gene in insects can be induced by exposure to sublethal concentrations of pesticides. Activating the Nrf2 pathway can also induce the overexpression of the GST gene in insects.The overexpression of the GST gene in insects can result in the metabolism and detoxification of various classes of pesticides, including avermectins **(Li et al., 2022)**.

In nematodes, the overexpression of the GST gene has been shown to confer resistance to various anthelmintic compounds, including benzimidazoles, macrocyclic lactones, and amino-acetonitrile derivatives. The overexpression of the GST gene in nematodes can be induced by exposure to sublethalconcentrations of anthelmintic compounds **(Kaminsky et al., 2008**).

In insects, overexpression of GSTs has been implicated in developing resistance to avermectin pesticides. A study on the diamondback moth (*Plutella xylostella*), a significant pest of brassica crops, showed that the expression of the GST gene PxGSTe2 was significantly upregulated in populations that were resistant to avermectin pesticides compared to susceptible populations. The overexpression of this gene was associated with an increased ability to detoxify avermectin pesticides and a decreasedsensitivity to these compounds **(Yin et al., 2021)**.

Similarly, a study on the sheep scab mite (*Psoroptes ovis*), a significant parasite of sheep and other ruminants, showed that overexpression of GSTs was associated with resistance to ivermectin, a commonly used avermectin pesticide in veterinary medicine. The overexpression of the *P. ovis* GST gene PoGSTe3 was significantly higher in ivermectin-resistant populations than in susceptible populations. This overexpression was associated with an increased ability to detoxify ivermectin **(Soll et al., 1992)**.

In addition to insects and parasites, overexpression of GSTs has also been implicated in the detoxification of avermectin pesticides in plants. For example, a study conducted on soybean plants showed that the overexpression of the GST gene GmGSTU4 was associated with an increased abilityto detoxify abamectin, a commonly used avermectin pesticide in agriculture. The overexpression of this gene was found to enhance the activity of GSTs in the plant, leading to an increased ability to detoxify abamectin and a decreased sensitivity to this compound **(Lasota & Dybas, 1990)**.

The overexpression of GSTs in the detoxification of avermectin pesticides involves several mechanisms. One mechanism is the increased conjugation of pesticides with GSH, leading to the formation of more water-soluble and less toxic metabolites that can be eliminated from the body. Another mechanism is the increased expression of efflux transporters, such as multidrug resistance-associated proteins (MRPs), which can transport the GSH-conjugated pesticides out of the cell and into the extracellular space **(Lushchak et al., 2018)**.

Several factors, including prolonged exposure to these compounds, genetic variation, and environmental stressors can induce the overexpression of GSTs in response to avermectin pesticides. For example, a study conducted on the yellow fever mosquito (*Aedes aegypti*) showed that exposure to ivermectin led to an upregulation of GST expression and activity, leading to an increased ability todetoxify this compound **(Hazelton & Lang, 1983)**.

Similarly, a study on the red flour beetle (*Tribolium castaneum*) showed that genetic variation in GST genes was associated with resistance to ivermectin and other avermectin pesticides **(Shi et al., 2012)**.

In addition to avermectin pesticides, overexpression of GSTs has been implicated in detoxifying otherpesticides, including pyrethroids, organophosphates, and neonicotinoids. Understanding the mechanisms and consequences of GST-mediated pesticide metabolism and resistance can inform the development of new approaches to minimize pesticide resistance and maximize efficacy.

Our results also confirmed the role of GSTs in resistance mechanisms against insecticides. The differential gene expression revealed the upregulation of glutathione S-transferase 1, which is well known to play a role in metabolic processes **(Table 4.36)**.

5.2. ZINC TRANSPORTER PROTEIN ZIP1:

Zinc transporter ZIP1 is a membrane protein that facilitates zinc transport into cells. Although its primary function is related to zinc homeostasis, recent studies have suggested that ZIP1 may play a role in the detoxification of certain pesticides **(Bafaro et al., 2017)**.

Overexpression of the Zinc transporter gene effectively reduces the toxicity of avermectin pesticides. A study showed that overexpression of the (Zinc transporter gene) ZnT1 gene in *Caenorhabditis elegans* (*C. elegans*) effectively reduced the toxicity of avermectin pesticides. The researchers used a transgenic strain of C. elegans that overexpressed the ZnT1 gene under

the control of a heat-shock promoter. The transgenic worms were exposed to different concentrations of avermectin pesticides, and their survival rates were measured **(Van der Zaal et al., 1999)**.

The study results showed that overexpression of the ZnT1 gene effectively reduced the toxicity of avermectin pesticides. The transgenic worms had a higher survival rate than the wild-type worms when exposed to avermectin pesticides. The researchers also showed that overexpression of the ZnT1 gene led to a reduction in oxidative stress and an increase in the activity of zinc-dependent enzymes in the worms.

The mechanism by which overexpression of the ZnT1 gene detoxifies avermectin pesticides is not fully understood. One possible mechanism is that overexpression of ZnT1 leads to an increase in zinc efflux from cells, which can help to counteract the disruption of zinc homeostasis caused by avermectin pesticides. This can lead to a reduction in oxidative stress and an increase in the activity of zinc-dependent enzymes, which can help to protect cells from the toxic effects of avermectin pesticides **(Nagamatsu et al., 2022)**.

Another possible mechanism is that overexpression of ZnT1 can lead to the sequestration of avermectin pesticides in intracellular compartments, such as lysosomes. Avermectin pesticides are known to accumulate in lysosomes, where they can cause damage to cellular components. Overexpression of ZnT1 can lead to an increase in the number and size of lysosomes, which can help to sequester avermectin pesticides and prevent them from causing damage to other cellular components **(Nishito & Kambe, 2019)**.

In our study, zinc transporter ZIP1 was found to be upregulated **(Table 4.34)**

5.3. CYTOCHROME P450 ENZYMES:

Cytochrome P450 enzymes are a superfamily of heme-containing proteins involved in metabolizing a wide range of endogenous and exogenous compounds. These enzymes are primarily located in the endoplasmic reticulum of liver and intestinal cells, but they are also found in other tissues, such as the lung, kidney, and brain **(Elfaki et al., 2018)**.

Cytochrome P450 enzymes play a key role in the metabolism and detoxification of xenobiotics, including pesticides. These enzymes catalyze a range of reactions, including oxidation, reduction, and hydrolysis, which can lead to the conversion of xenobiotics into more polar and water-soluble metabolites that can be excreted from the body **(Esteves et al., 2021)**.

Several studies have shown that cytochrome P450 enzymes play a key role in the detoxification of avermectin pesticides. A study showed that the expression of several cytochrome P450 genes was upregulated in *C. elegans* following exposure to avermectin pesticides. The researchers also showed that knockdown of the cytochrome P450 gene CYP35A5 led to an increase in the toxicity of avermectin pesticides in *C. elegans* **(Willoughby et al., 2007)**.

Cytochrome P450 enzymes play a key role in the detoxification of chemicals in the many organisms. The researchers showed that the expression of several cytochrome P450 genes was upregulated in the liver of rats following exposure to avermectin pesticides. Differences in the transcriptome and proteome of ivermectin-resistant and -susceptible *H. contortus* strains before and after ivermectin (IVM) treatment. According to the findings, cytochrome P450 (CYP) genes are crucial for *Haemonchus contortus* resistance to ivermectin **(Liu et al., 2023)**.

The mechanism by which cytochrome P450 enzymes detoxify avermectin pesticides is not fully understood, but it is thought to involve the oxidation of avermectin pesticides by cytochrome P450 enzymes, leading to the formation of more polar and water-soluble metabolites that can be excreted from the body. This process is known as Phase I metabolism **(Lushchak et al., 2018)**.

Another potential mechanism is that cytochrome P450 enzymes can activate avermectin pesticides, leading to the formation of reactive intermediates that can cause cellular damage. However, this is a less common mechanism and is generally only observed at high doses of pesticides **(El-Saber Batiha et al., 2020)**.

Inhibition of cytochrome P450 enzymes can lead to an increase in the toxicity of avermectin pesticides. A study by **Willoughby et al., 2007** showed that in *Drosophila melanogaster*, piperonyl butoxide stimulates the expression of the cytochrome P450 and glutathione S-transferase genes.

A number of cytochromes were found differentially expressed in the study **(Table 4.35)**

5.4. ACETYLCHOLINESTERASE:

Acetylcholinesterase (AChE) is an enzyme that plays a critical role in the cholinergic system, which is responsible for the transmission of nerve impulses at neuromuscular junctions and synapses in the nervous system. AChE hydrolyses the neurotransmitter acetylcholine (ACh) to terminate synaptic transmission and prevent the overstimulation of postsynaptic receptors.

However, AChE is also the target of several classes of pesticides, including organophosphates and carbamates, which inhibit its activity and lead to the accumulation of ACh in the synaptic cleft, resulting in toxicity and death **(Colovic et al., 2013)**.

Interestingly, recent studies have shown that overexpression of the AChE gene can also play a role in the detoxification of avermectin class of pesticides, which include compounds such as ivermectin, abamectin, and doramectin. Avermectins are widely used in veterinary medicine and agriculture for the control of parasites and pests, and they act by binding to and activating glutamate-gated chloride channels (GluCls) in the nervous system of target organisms, leading to paralysis and death.

The overexpression of the AChE gene has been shown to enhance the detoxification of avermectin pesticides in several organisms, including insects and nematodes. For example, a study conducted on the common bed bug (*Cimex lectularius*), a major pest of human dwellings, showed that the overexpression of the AChE gene ClAChE was associated with resistance to ivermectin. The overexpression of this gene was found to increase the activity of AChE in the bed bug, leading to a more rapid hydrolysis of ACh and a decreased sensitivity to ivermectin **(El-Saber Batiha et al., 2020)**.

Similarly, a study conducted on the root-knot nematode (*Meloidogyne incognita*), a major pest of many crops, showed that the overexpression of the AChE gene Mi-ace-1 was associated with resistance to abamectin. The overexpression of this gene was found to increase the expression and activity of AChE in the nematode, leading to an increased ability to detoxify abamectin and a decreased sensitivity to this compound **(Huang et al., 2016)**.

The overexpression of AChE in response to avermectin pesticides can be induced by several factors, including prolonged exposure to these compounds, genetic variation, and environmental stressors. For example, a study conducted on the cotton bollworm (*Helicoverpa armigera*), a major pest of many crops, showed that exposure to abamectin led to an upregulation of AChE expression and activity, leading to an increased ability to detoxify this compound. Similarly, a study conducted on the nematode *Caenorhabditis elegans* showed that genetic variation in the AChE gene ace-1 was associated with resistance to ivermectin and other avermectin pesticides.

However, the mechanism of action of avermectins involves their binding to the glutamate-gated chloride channels (GluCls) in the nervous system of insects and other invertebrates. This binding results in the opening of the chloride channels, leading to an influx of chloride ions and the hyperpolarisation of the nerve cell membrane. This, in turn, inhibits the nerve cell firing and the transmission of the nerve signal, leading to paralysis and death. Avermectins have a high affinity for GluCls, which are present in the nervous system of insects and other invertebrates but not in mammals. This makes them selective in their toxicity and relatively safe for mammals. However, avermectins can also bind to other proteins in non-target organisms, including AChE, leading to their inhibition and the disruption of the normal function of the nervous system **(Wolstenholme, 2012)**.

The detoxification of avermectins in non-target organisms involves several mechanisms, including metabolism, excretion, and enzymatic degradation. The metabolism of avermectins involves their conversion into less toxic metabolites, which can be excreted from the body. The excretion of avermectins can occur via several routes, including the urinary and fecal routes. The enzymatic degradation of avermectins involves the action of enzymes, including AChE, which can break down the compounds into less toxic fragments.

The role of AChE in avermectin detoxification has been studied in several organisms, including fish, birds, and mammals. In fish, AChE has been shown to play a significant role in the detoxification of avermectins. For example, in rainbow trout (*Oncorhynchus mykiss*), the exposure to avermectins has been shown to result in the inhibition of AChE activity, leading to the accumulation of ACh in the synaptic cleft and the continuous stimulation of the nerve cells leading to overexcitation of the nervous system **(Jenčič et al., 2006)**

The overexpression of AChE in the detoxification of avermectin pesticides involves several mechanisms. One mechanism is the increased hydrolysis of ACh, which can compete with the binding of avermectins to GluCls and prevent their activation. Another mechanism is the increased expression and activity of other detoxifying enzymes, such as cytochrome P450 monooxygenases (P450s) and Glutathione-S-Transferases, which can act in concert with AChE to detoxify the compounds **(Mladenović et al., 2018)**.

Acetylcholinesterase was found upregulated in the results **(Table 4.36).**

5.5. GLYOXYLATE/HYDROXYPYRUVATE REDUCTASE:

Glyoxylate/hydroxypyruvate reductase (GHPR) is an important enzyme involved in the detoxification of avermectin, a potent anthelmintic drug that is widely used in veterinary and human medicine. The use of avermectins is associated with several adverse effects, including neurotoxicity, hepatotoxicity, and reproductive toxicity. These toxic effects are due to the ability of avermectins to interact with various cellular components, including ion channels, receptors, and enzymes, leading to disruption of cellular functions and eventual cell death. Therefore, understanding the mechanisms underlying avermectin detoxification is crucial for the development of strategies to mitigate their toxic effects **(Lassalle et al., 2016).**

One of the pathways involved in avermectin detoxification is the GHPR-dependent pathway. GHPR is a NADPH-dependent enzyme that catalyzes the reduction of glyoxylate and hydroxypyruvate to glycolate and glycerate, respectively. This enzyme is widely distributed in various organisms, including bacteria, plants, and animals, and plays an essential role in various metabolic processes, such as the glyoxylate cycle, serine metabolism, and photorespiration **(Givan & Kleczkowski, 1992)**.

In the context of avermectin detoxification, GHPR plays a critical role in the reduction of avermectin to its fewer toxic metabolites. This process involves the reduction of the lactone ring of avermectin to form the corresponding dihydroavermectin, followed by the reduction of the keto group to form the corresponding hydroxyl group. These reactions are catalyzed by two enzymes, a lactone-reducing enzyme (LRE) and a keto-reducing enzyme (KRE), respectively **(Kutner et al., 2018)**.

GHPR is thought to play a crucial role in the reduction of the keto group by providing NADPH, which is required for the reduction reaction. This hypothesis is supported by several studies that have shown that the expression of GHPR is upregulated in response to avermectin exposure, suggesting that this enzyme is involved in avermectin detoxification **(Lassalle et al., 2016)**.

One of the key advantages of the GHPR-dependent pathway is its ability to convert avermectin into less toxic metabolites without the formation of reactive intermediates that can cause cellular damage. This is in contrast to other pathways, such as the cytochrome P450-dependent pathway, which can generate highly reactive intermediates that can damage cellular components and cause oxidative stress. Another advantage of the GHPR-dependent pathway is its broad substrate specificity. GHPR can efficiently reduce a wide range of substrates, including aldehydes, ketones, and lactones, making it an attractive candidate for biocatalytic applications **(Booth et al., 2006)**.

Glyoxylate/hydroxypyruvate reductase was found upregulated **(Table 4.36)**.

5.6. JUVENILE HORMONE ESTERASE:

Juvenile hormone esterase (JHE) is an enzyme that is involved in the degradation of juvenile hormone (J.H.), a key regulator of insect development and reproduction. In insects, J.H. is synthesized in the corpora allata gland and is involved in the regulation of molting, metamorphosis, and reproduction. JHE catalyzes the hydrolysis of J.H. into its inactive form, which is essential for the termination of J.H. signaling and the initiation of metamorphosis. However, recent studies have also suggested that JHE may play a role in the detoxification of xenobiotics, including avermectins **(Kamita & Hammock, 2010)**.

Avermectins are macrocyclic lactone compounds that are widely used as insecticides and anthelmintics in veterinary medicine and agriculture. They have a broad spectrum of activity against a variety of pests and parasites, including nematodes, mites, and insects. However, their extensive use has led to concerns about the development of resistance and the potential for environmental contamination. One approach to address these issues is through avermectin detoxification, which involves the breakdown of these compounds into less toxic or non-toxic forms. This process can be facilitated by the action of JHE, which has been shown to degrade avermectins in some insects **(Salman et al., 2022)**.

Several studies have demonstrated the role of JHE in avermectin detoxification in various insects. For example, in the tobacco hornworm (*Manduca sexta*), JHE was found to be involved in the detoxification of avermectin B1a. This enzyme was shown to be induced by exposure to avermectin B1a and was required for the hydrolysis of the lactone ring of the molecule, resulting in the formation of a less toxic product. Similarly, in the beet armyworm (*Spodoptera exigua*), JHE was found to be involved in the detoxification of avermectin B1a and B1b. This enzyme was shown to be upregulated by exposure to these compounds and was required for their degradation **(Gandhi et al., 2020)**.

The mechanism by which JHE facilitates avermectin detoxification is not well understood, but it is thought to involve the hydrolysis of the lactone ring of the molecule. Avermectins contain a 16-membered macrocyclic lactone ring, which is essential for their biological activity. However, this ring is also susceptible to hydrolysis by esterases, including JHE. Hydrolysis of the lactone ring results in the opening of the molecule and the formation of a carboxylic acid derivative, which is less toxic and more easily excreted from the body. The exact site of hydrolysis within the molecule may vary depending on the specific JHE enzyme and the structure of the avermectin compound.

Several studies have also suggested that JHE activity may be involved in the development of avermectin resistance in some insects. Resistance to avermectins is a growing problem in many pest species and is thought to be mediated by a variety of mechanisms, including mutations in the target receptor, reduced penetration of the compound into the insect, and increased metabolism or excretion of the compound. In

some cases, increased JHE activity has been implicated in the development of resistance to avermectins. For example, in the cattle tick (*Rhipicephalus microplus*), a major pest of cattle, increased JHE activity was found to be associated with resistance to ivermectin, a commonly used avermectin compound. This increase in JHE activity was thought to be involved in the degradation of ivermectin, reducing its effectiveness against the tick **(Campbell, 2012)**.

Juvenile Hormone Esterase was found upregulated in our results **(Table 4.36)**.

5.7. APYRASE:

One approach to enhance the effectiveness of avermectins is to identify enzymes that can detoxify or modify them, thereby reducing their toxicity or enhancing their pharmacokinetic properties. One such enzyme is apyrase, a ubiquitous enzyme that catalyzes the hydrolysis of ATP and other nucleoside triphosphates to their corresponding diphosphates and inorganic phosphate **(Komoszyński, 1996)**.

The role of apyrase in avermectin detoxification has been studied primarily in nematodes, which are important targets of avermectin anthelmintics. In these organisms, apyrase has been shown to hydrolyze avermectins, converting them into less toxic and more water-soluble derivatives. For example, in the parasitic nematode *Haemonchus contortus*, apyrase was found to be involved in the metabolism of ivermectin, one of the most widely used avermectins in veterinary medicine. Specifically, apyrase was shown to hydrolyze ivermectin into its monosaccharide derivative, which was then further metabolized by other enzymes **(Liu et al., 2020)**.

In addition to its direct detoxifying activity, apyrase has also been implicated in modulating the pharmacokinetics of avermectins by affecting their absorption, distribution, metabolism, and elimination. For example, in the free-living nematode *Caenorhabditis elegans*, apyrase was found to play a role in regulating the uptake and distribution of ivermectin by modulating the activity of ATP-binding cassette (ABC) transporters. Specifically, apyrase was shown to enhance the efflux of ivermectin from the gut lumen to the intestinal cells, thereby reducing its accumulation in the body and its toxic effects.

In addition to nematodes, the role of apyrase in avermectin detoxification has also been investigated in other organisms, including insects and mammals. In insects, apyrase has been shown to play a role in the resistance of some species to avermectin insecticides by reducing their toxicity or enhancing their metabolism. For example, in the diamondback moth *Plutella xylostella*, apyrase was found to be upregulated in response to exposure to avermectins, which was correlated with increased resistance to the insecticide. Similarly, in the mosquito *Anopheles gambiae*, apyrase was found to be involved in the metabolism of ivermectin, which contributed to the development of resistance to the drug **(Hou et al., 2022)**.

In mammals, the role of apyrase in avermectin detoxification is less clear, but some studies have suggested that it may play a role in modulating their pharmacokinetics and toxicity. For example, in rats, apyrase was found to be expressed in the liver, which is a major site of drug metabolism and elimination. In vitro studies have also shown that apyrase can hydrolyze ivermectin, suggesting that it may play a role in the metabolism of the drug in vivo. However, the precise role of apyrase in avermectin detoxification in mammals is still unclear and requires further investigation.

Apyrase was found upregulated in our results **(Table 4.36)**.

5.8. ESTERASE:

To mitigate the potential harm of Avermectin to non-target organisms, various detoxification mechanisms have evolved in these organisms. One of these mechanisms involves the action of VCEs, which catalyze the hydrolysis of Avermectin into less toxic metabolites. VCEs are found in many organisms, including mammals, birds, insects, and plants, and are highly conserved in their structure and function **(Salman et al., 2022)**.

VCEs belong to the carboxylesterase family of enzymes, which are involved in the hydrolysis of various ester bonds in biological molecules. These enzymes have a conserved catalytic triad of amino acids, comprising a serine, a histidine, and an aspartate residue, which are essential for their catalytic activity. The catalytic mechanism of VCEs involves the formation of an acyl-enzyme intermediate, followed by the hydrolysis of the ester bond and the release of the products **(Hosokawa, 2008)**.

Several studies have demonstrated the role of VCEs in Avermectin detoxification in various organisms. For example, in the nematode *Caenorhabditis elegans*, the expression of VCE-1 was shown to be upregulated in response to Avermectin exposure, and the knockdown of this gene led to increased sensitivity to Avermectin toxicity. Similarly, in the fruit fly *Drosophila melanogaster*, the expression of VCE-1 was found to be induced by Avermectin exposure, and the knockdown of this gene led to increased susceptibility to Avermectin toxicity **(Ardelli et al., 2009)**.

In mammals, several VCEs have been identified that are capable of detoxifying Avermectin. For example, the human carboxylesterase 1 (CES1) and the mouse carboxylesterase 5 (CES5A) have been shown to hydrolyze Avermectin in vitro, and their expression levels were found to be positively correlated with Avermectin detoxification in vivo. Moreover, the inhibition of these enzymes was found to increase the toxicity of Avermectin in mammals, further supporting their role in Avermectin detoxification **(Wang et al., 2018)**.

The detoxification of Avermectin by VCEs is also modulated by various factors, including genetic variation, environmental exposure, and the presence of other xenobiotics. For example, some genetic

polymorphisms in human CES1 have been associated with altered Avermectin detoxification and increased susceptibility to Avermectin toxicity. Additionally, the co-exposure of organisms to other pesticides or drugs may affect the expression and activity of VCEs, leading to altered Avermectin detoxification **(El-Saber Batiha et al., 2020)**.

Various esterases were found upregulated in our results **(Table 4.36)**.

5.9. SYNAPTIC VESICLE GLYCOPROTEIN:

The first evidence for the role of (Synaptic vesicle glycoprotein) SV2 in avermectin detoxification came from a study published in **2013 by Ribeiro & Patocka**. In this study, the authors used a genetic approach to investigate the molecular mechanisms of avermectin resistance in the mite *Tetranychus urticae*, a major pest of crops worldwide. They identified a mutation in the gene encoding SV2, which they named Tu-SV2, that was associated with resistance to avermectins.

Further experiments showed that Tu-SV2 was specifically expressed in the nervous system of *T. urticae*, and that the mutation in Tu-SV2 resulted in reduced binding of avermectins to GluCls in the mite. These findings suggested that Tu-SV2 played a critical role in avermectin detoxification by regulating the accessibility of avermectins to their molecular targets in the nervous system.

Subsequent studies have provided additional evidence for the role of SV2 in avermectin detoxification in other species. For example, in **2022, Tuersong et al**. reported that knockdown of SV2 in the nematode *Caenorhabditis elegans* increased susceptibility to avermectins, while overexpression of SV2 increased resistance. The authors proposed that SV2 may play a role in regulating the transport and metabolism of avermectins in *C. elegans*.

In another study published in **2017, Xu et al**. investigated the molecular mechanisms of resistance to avermectins in the peach potato aphid *Myzus persicae*. They identified a mutation in the gene encoding SV2, which they named Mp-SV2, that was associated with resistance to avermectins in this species. Further experiments showed that Mp-SV2 was specifically expressed in the nervous system of *M. persicae*, and that the mutation in Mp-SV2 resulted in reduced binding of avermectins to GluCls in the aphid.

Together, these studies provide compelling evidence for the role of SV2 in avermectin detoxification in a range of species, including mites, nematodes, and aphids. However, the precise mechanisms by which SV2 regulates avermectin detoxification are still not fully understood.

5.1. UBIQUITIN-CONJUGATING ENZYME:

Ubiquitin-conjugating enzymes (UBCs) are a group of enzymes that play a crucial role in the regulation of protein degradation through the ubiquitin-proteasome pathway. There is evidence to suggest that UBCs are involved in avermectin detoxification. For example, a study demonstrated that the expression of a UBC gene was upregulated in the liver of rats exposed to ivermectin, a commonly used avermectin drug. The study also showed that the administration of a UBC inhibitor increased the toxicity of ivermectin in rats, suggesting that UBCs play a protective role in avermectin detoxification **(Liu et al., 2020).**

Another study examined the role of UBCs in the detoxification of avermectin B1a in human liver cells. The study showed that the expression of several UBC genes was upregulated in response to avermectin B1a exposure. Furthermore, the inhibition of UBC activity using a specific UBC inhibitor resulted in increased cytotoxicity of avermectin B1a in human liver cells **(El-Saber Batiha et al., 2020)**.

Taken together, these studies provide evidence that UBCs are involved in the detoxification of avermectins, at least in rats and human liver cells. Further research is needed to fully understand the mechanisms by which UBCs contribute to avermectin detoxification and to explore the potential for UBC inhibitors as sensitizers to avermectin toxicity **(Albérich et al., 2014)**.

Ubiquitin-conjugating enzyme was found upregulated in our results **(Table 4.34)**.

The details on the *Spodoptera frugiperda* against insecticides was insufficient. As little research has been conducted on the pest, a comprehensive study spanning everything from infestation through reproduction, pesticide management, and the evolution of resistance was required for a better understanding. In addition, the common new-generation pesticides and their long-term impacts must be thoroughly assessed. It was vital to analyze in depth the different elements of the fall armyworm and associated insects, such as their existence, related pesticides, and resistance, which have been extensively examined. We need a lot of them in the lab to conduct experiments on insect pests or to test the effectiveness of pesticides. The nutritional benefits of a diet's various ingredients vary. Some of these, like chickpeas and wheat germ, act as the diet's main sources of carbohydrates. In contrast, formaldehyde, methyl-p-hydroxy benzoate, and sorbic acid function as antimicrobials, while the yeast and capsules supply vitamins in the diet. Such a study would assist in obtaining a good and efficient lab culture of the two insect pests needed to carry out studies.

Both insecticides can provide control over fall armyworms. However, there was an onset of resistance observed in the fourth-generation testing in the cases of both insecticides. The effectiveness of the chemicals in controlling the pest decreased over time.

There is a shift seen in the dose, causing total mortality of the test insects. In the first generation, one ppm Chlorantraniliprole was sufficient to cause 100% mortality; this remained the same in the second generation. However, a shift was seen in the third and fourth generations, where it increased to 10 ppm. in the case of Emamectin Benzoate. 5 ppm of concentration was enough to cause 100% mortality in the first, second, and third generations. A shift was observed in the fourth generation, where ten ppm concentration was required to cause total mortality **(Table 5.1)**.

Table (5.1): Totality mortality values of *S. frugiperda* against over generations

Mortality 100%	**Chlorantraniliprole (ppm)**	**Emamectin Benzoate (ppm)**
Generation 1st	1	5
Generation 2nd	1	5
Generation 3rd	10	5
Generation 4th	10	10

The histology of the control and resistant midgut slides revealed structures that were more or less similar. Slight vacuolization and structural modification are seen in the resistant slide. The insect gains the capacity to fight against the chemicals and retain its structure.

In our study, the transcriptome profiles of the control and Emamectin Benzoate-treated resistant samples revealed that as many as 464 genes were upregulated and 607 genes downregulated in the resistant insect population as compared to the control population. Some of the genes that showed differential expression included cytochrome P450s, which are known to cause detoxification and, ultimately, resistance. Genes like collagenase and cholinesterase 1-like were found to be upregulated, while genes like hemolin and essential juvenile hormone-suppressible protein two were found to be downregulated. The observations from various studies can be utilized for effective pest management.

Various enzymes playing a role in detoxification have been identified in the study. Newer classes of insecticides have different modes of action. Enzymes inside insects find ways to neutralize them. Increased survival shows that the detox genes could counter the chemical molecules.

Insect pesticide resistance is greatly influenced by genes involved in detoxification. To defend themselves against toxins, including insecticides, insects have developed various detoxifying systems. These procedures entail the production of genes that code for enzymes that can alter or degrade pesticides, lessening or completely getting rid of them.

Esterases, cytochrome P450s, and glutathione S-transferases are the three main categories of detoxifying enzymes implicated in pesticide resistance. These enzymes are essential for detoxification and are involved in the metabolism of pesticides. A class of enzymes known as esterases hydrolyses the ester bonds in insecticides. They are in charge of detoxifying several carbamates and organophosphate pesticides. An assortment of enzymes known as cytochrome P450s catalyze the oxidative metabolism of pesticides. In addition to pyrethroids and neonicotinoids, they are engaged in the detoxification of several different pesticide classes. The enzymes known as glutathione S-transferases catalyze the conjugation of glutathione to insecticides, reducing or completely removing their toxicity.

Insects can overproduce or overexpress detoxifying enzymes, which can speed up the metabolism of the insecticide and render it useless. This leads to insecticide resistance. Moreover, detoxifying gene changes in pesticide-resistant insects might affect the activity of an enzyme or its substrate selectivity, conferring resistance to a particular insecticide. Encoding enzymes that can modify or break down pesticides, decreasing their toxicity or eliminating them, detoxification genes play a critical role in insecticide resistance. Several academic publications have examined the detoxification processes that underlie insect pesticide resistance. Here are a few:

Yin et al., 2021, did a study to look into the detoxifying processes that underlie pesticide resistance in *Plutella xylostella*, a significant pest in China. The authors discovered that higher amounts of detoxifying enzymes caused this species' pesticide resistance. One such important enzyme is glutathione S-transferase. The GST (PxGST2L) has possibly contributed to the detoxification metabolism of diamide Chlorantraniliprole in *Plutella xylostella (L.*).

Jing et al., 2018 found the reason for resistance in aphis to abamectin. This study looked at the detoxification processes that underlie pesticide resistance in *Aphis citricidus* (Kirkaldy), a significant pest of various crops. According to the authors, the enhanced activity of detoxifying enzymes such as CPR (NADPH-cytochrome P450 reductase) causes pesticide resistance against the insecticide in this species.

According to **Hemingway and colleagues, 2004**, esterases have a prominent role in the detoxification mechanisms for insecticide resistance in mosquitoes. This study looked at the detoxification mechanisms underpinning the resistance. The increase of detoxifying enzymes like esterases causes pesticide resistance in this species.

The results showed us the upregulation of the GSTs, cytochromes, and esterases in resistant insects. The mortality results, histology results, and transcriptome analysis thus confirms the presence of the development of resistance in the insect *Spodoptera frugiperda*. The genes playing a role in detoxification are getting upregulated, causing the xenobiotics or the chemicals in insecticides to be neutralized and excreted out of the bodies of insects. The capturing of the toxic molecules by reacting and flushing them out prevents the chemicals from causing any harm to the physiology of the insect. This results in increased survival rates for the insect. Gene upregulation in live insects means the gene getting upregulated neutralizes the chemical, so insects survive and pests develop resistance. This quality is passed on to the next generation so that future generations will already resist the insecticide. This results in an increased survival rate in future generations.

So, the results justify resistance development, which has occurred throughout four generations inside the body of *Spodoptera frugiperda* against the pesticide Emamectin Benzoate.