T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCE AND TECHNOLOGIES

BIOCONTROL STUDIES ON FALL WEBWORM (*Hyphantria cunea* Drury) BY USING THE SPORE-CRY TOXINS OF THE *Bacillus thuringiensis* STRAINS FROM TURKEY

Prepared By
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Supervisor
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Prof. Dr. Semih YILMAZ

PhD Thesis

September 2022
KAYSERİ

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SCIENTIFIC ETHICS CONFORMITY

I hereby declare that all information in this thesis has been composed and presented in accordance with the academic rules and ethical guidelines. I also declare that, as required by these rules and conduct, I have fully and accurately cited and referenced all materials and results that are not original to this work.

Esraa Mahdi SALEH AL-OBAIDI

PhD thesis titled **"Biocontrol studies on fall webworm (*Hyphantria cunea* Drury) by using the spore-Cry toxins of the *Bacillus thuringiensis* strains from Turkey"** has been composed with the Postgraduate Thesis Proposal and Thesis Writing Directions of Erciyes University.

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KAYSERİ, September 2022

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ABSTRACT

This study aimed to determine the effects of local *Bacillus thuringiensis* (*Bt*) strains and their Cry proteins on the larvae of the fall webworm insect, *Hyphantria cunea*. Six strains of *Bt* were used: *Bt* SY49.1, *Bt* SY25.1, *Bt* SY33.3, *Bt* SY56.3, *Bt* SY27.1 and standart strain *Bacillus thuringiensis* var *kurstaki* (*Btk*). The larvae of the insect *Hyphantria cunea* used in this study were collected from the Rize region of Northeastern Turkey. Seven doses were determined for each strains according to the concentrations added: 10 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml, 2250 µg/ml. Larvae of different ages were exposed to *Bt* strains' products. Larval mortality was monitored for ten days, and the number of dead larvae and live larvae were recorded. The results showed that the mortality rate of *Hyphantria cunea* larvae increased with the progression of time and increment of doses in strains of *Bt* that were applied. The results showed that the highest LD_{50} value was obtained from the *Bt* SY56.3 strain, while the lowest value was obtained from *Bt* SY49.1 strain. Moreover, the highest value for LD_{99} was obtained after using *Bt* SY33.3 products, while the lowest value was achieved after *Bt* SY49.1 treatment. Also, *Bt* SY56.3 showed lower mortality rates compared to the other strains, while *Bt* SY27.1 and *Bt* SY49.1 showed higher mortality rates among the strains used. The effect of mixture of spores for the three strains (*Bt* SY49.1, *Bt* SY27.1and *Btk*) also showed the highest mortality rate was *Bt* SY49.1, while *Btk* showed lower mortality rate. On the third stage of the larvae (L3), there was a higher average mortality rate for *Bt* SY27.1 with the crystal and spore-crystal, and a lower average mortality rate for *Btk* with the crystal. In the fourth stage of the larvae (L4), the mean mortality was higher for *Bt* SY49.1 with the spore-crystal mixture, while the mean mortality was lower than *Btk* with the spore-crystal, Cry

proteins and spore-crystal mixtures, it can be concluded that it has potential effects on all larval stages, including early and advanced larval stages of *Hyphantria cunea*. Also, the extracted Cry proteins and spores of *Bt* strains have considerable lethal toxicity on the third (L3) and forth (L4) larvae of *Hyphantria cunea*. The result of this study indicated that spore and Cry proteins of the local *Bt* strains have lethal effects on *Hyphantria cunea* larvae.

Keywords: Biocontrol, *Hyphantria cunea*, *Bacillus thuringiensis*, *Btk*, Cry protein, Sustainable agriculture

AMERİKAN BEYAZ KELEBEĞİNİN (*Hyphantria cunea* Drury) BİYOKONTROLÜ AMACIYLA TÜRKİYE'DEN İZOLE EDİLEN *Bacillus thuringiensis* (Bt) SUŞLARININ SPOR-CRY TOKSİNLERİNİN KULLANIMI ÜZERİNE ARAŞTIRIMALAR

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ÖZET

Bu çalışma yerel *Bacillus thuringiensis* (*Bt*) suşlarının ve bunlardan elde edilen Cry proteinlerinin Amerikan beyaz kelebeği *Hyphantria cunea* üzerine toksik etkilerini belirlemek amacıyla gerçekleştirilmiştir.

Bu çalışmada altı farklı bakteri suşu olan *Bt* SY49.1, *Bt* SY25.1, *Bt* SY33.3, *Bt* SY56.3, *Bt* SY27.1 ve *Bacillus thuringiensis* var *kurstaki* (*Btk*) standart suşu kullanılmıştır. Çalışma Türkiye'nin kuzey doğusunda bulunan Rize bölgesi'den elde edilen *Hyphantria cunea* larvaları üzerinde gerçekleştirilmiştir. Her bir suş için yedi doz olarak 10 µg/ml: 50 µg/ml: 100 µg/ml: 250 µg/ml: 500 µg / ml, 1000 µg/ml ve 2250 µg/ml belirlenmiş ve böcek besiyerine uygulanmıştır. On gün boyunca ölen larvalar izlenmiş ve ölü larva sayısı ve kalan canlı sayısı kaydedilmiştir. Kullanılan tüm *Bt* suşlarında *Hyphantria cunea*'nın ölüm oranının, kullanılan tüm dozlarda gün geçtikçe arttığı görülmüştür. Zaman ilerledikçe ölüm oranı artmış; ayrıca kullanılan doz artışına göre de ölüm oranı daha fazla gerçekleşmiştir. Elde edilen sonuçlara göre; *Bt SY*56.3 proteinlerinin daha yüksek bir LD_{50} değeri, *Bt SY*49.1 proteinleri için ise daha düşük bir değeri olduğunu göstermiştir. Ayrıca, en yüksek LD99 değeri *Bt* SY33.3 ürünlerinde elde edilirken, en düşük değer *Bt* SY49.1 ürünlerinde elde edilmiştir, gosterildigi gibi *Bt* SY56.3 diğer suşlara göre daha düşük ölüm oranları gösterirken, *Bt* SY27.1 ve *Bt* SY49.1 kullanılan suşlar arasında daha yüksek ölüm oranları gösterdi. Üç suş (*Bt* SY49.1, *Bt* SY27.1 ve *Btk*) için spor karışımının etkisi de en yüksek ölüm oranını *Bt* SY49.1 olarak gösterirken, *Btk* daha düşük ölüm oranı gösterdi. Üçüncü evre larvasında (L3) *Bt* SY27.1 kristali ve spor- kristal için daha yüksek bir ortalama ölüm oranı ve *Bkt* kristali için daha düşük bir ortalama ölüm oranı elde edilmiştir. Dördüncü evre larvasında (L4),

kristal ve spor ile *Bt* SY49.1 için daha yüksek ortalama ölüm oranı, *Btk* spor- kristal için daha düşük bir ortalama ölüm oranı elde edilmiştir. Sonuç olarak *Bt*'nin *Hyphantria cunea* larvalarının tüm evreleri, erken ve ileri evreleri üzerinde potansiyel toksisite gösterdiği belirlenmiştir. Ayrıca ekstrakt edilen *Bt* spor-kristal ürünleri *Hyphantria cunea*'nın üçüncü (L3) ve dördüncü (L4) evre larvaları üzerinde anlamlı toksik etki göstermiştir. Çalışma sonuçları lokal *Bt* suşlarından elde edilen spor ve kristal proteinlerin *Hyphantria cunea* larvaları üzerine öldürücü etki gösterdiğini ortaya koymuştur.

Anahtar Kelimeler: Biyokontrol, *Hyphantria cunea*, *Bacillus thuringiensis*, *Btk*, Cry proteinleri, Sürdürülebilir tarım

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ICONS AND ABBREVIATIONS

٪	**:** Percent
µl	**:** Micro liter
µm	**:** Micrometer
APS	**:** Ammonium per sulfate
Bt	**:** *Bacillus thurringiensis*
Btk	**:** *Bacillus thurringiensis kurstaki*
BtsR1	**:** *Bt* small RNA
Cry	**:** Crystal protein
Cyt	**:** Cytolytic protein
g	**:** Gram
GPS	**:** Global positioning system
IPM	**:** Integrated pest management
L1	**:** The First stage of the insects life
L2	**:** The Second stage of the insects life
LB	**:** Luria Bertani
LD 50	**:** 50% lethal dose concentration
LD 99	**:** 99% lethal dose concentration
M	**:** Molar
Mg/ml	**:** Miligram/Mililiter
Ml	**:** Milli liter
mM	**:** Milli molar
ºC	**:** Degrees centigrade
PAGE	**:** Poly Acryl amide Gel Electrophoresis
PMSF	**:** Phenyl Methyl Sulfonyl Fluoride
Rpm	**:** Revolutions per minute
SDS	**:** Sodium dodecyl sulfate
SY	**:** Semih Yılmaz

T3 **:** Liquid sporulation medium

β **:** Beta

δ **:** Delta

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INTRODUCTION

Biological control is a method that is both beneficial to the ecosystem and successful in decreasing or neutralizing the impacts of pests. It refers to any action taken by one species that mitigates the negative impact of another species. In the field of pest management, the term "biological control" most often refers to the use of parasites, predators, or diseases on a pest population in order to bring the number of individuals down to the point at which they would not cause economic damage (Kergunteuil et al., 2016).

Natural enemies of insect pests are predators, competitors, parasites, and parasitoids. They are regarded as biological control agents due to their effectiveness in controlling insect pests. Antagonists are the most common term to describe biological management agents for plant diseases. Predators, herbivores, and plant diseases are examples of weeds' biological control agents (Bale, Van Lenteren, & Bigler, 2008).

There are many studies that have emphasized on the subject of the biological control agents such as predators (Bouvet, Urbaneja, Pérez-Hedo, & Monzó, 2019), parasitoids (Schmidt et al., 2003), pathogens (Lacey et al., 2015), bacteria (Daranas et al., 2019), fungi (Thambugala, Daranagama, Phillips, Kannangara, & Promputtha, 2020), viruses (Di Giallonardo & Holmes, 2015), competitors, and the employment of parasitoids in conjunction with pathogens (Ons, Bylemans, Thevissen, & Cammue, 2020).

Hyphantria cunea is known as fall webworm. It is a species of moth belonging to Erebidae family, most larvae build webbed nests on the hardwood trees throughout the fall and late summer (Q. Chen et al., 2020).

Hyphantria cunea is mostly distributed from Mexico to Canada and also in many parts of the world. It was found in 1949 in Yugoslavia, and nowadays, it has occupied entire Europe and most Asia (Alves, Ikeda, & Kobayashi, 2002).

The fall webworm is a polyphagous insect that may be seen in large groups. The larvae reside in huge webs that they have constructed for themselves on the limbs of trees. These webs facilitate the discovery of mates, the management of temperature, and the protection from predators; it causes the infection and predation of the organisms (Loewy et al., 2013).

Bacillus thuringiensis (*Bt*) is a soil-dwelling bacterium that is the most widely used biological insecticide in the world. The bacteria are Gram-positive. Aside from being found in the guts of numerous moth and butterfly species, it may be found on leaf, ponds, animals, and flour mills, and many other places (Madigan & Martinko, 2006).

Many *Bt* strains create crystal proteins during sporulation, known as delta endotoxins which have insect killing activity. As a consequence of this, they have been used in the production of insecticides, and more recently, in the creation of genetically altered crops carrying *Bt* genes, in order to control insect pests (Cox, 2013).

Toxic mechanisms include Cry proteins binding to particular receptors on the membranes of the midgut of the targeted pests, causing the membranes to rupture and causing death. Similarly, some creatures that do not have the proper receptors in their guts are resistant to the Cry protein and, as a result, are not harmed by *Bt* (Kumar, Sharma, & Malik, 1996).

Crystals of two different types of the delta endotoxins are produced by *Bt* during the sporulation process. These proteinaceous insecticidal delta endotoxins are crystal proteins, which are encoded by *cry* genes. Cyt proteins are expressed by *cyt* genes. It is well known that Cry toxins have specific insecticidal activity against insect species belonging to the orders Lepidoptera, Diptera, Coleoptera, and Hymenoptera, in addition to nematicidal activity (Crickmore et al., 2014).

Aims of the study:

The fall webworm *Hyphantria cunea* is a serious insect species that damages forests and agricultural lands in many countries. Various studies have shown that this insect species feeds on various trees such as hazelnut, mulberry, maple and oak. Synthetic insecticides are currently used to control such pests. However, research has shown that its use can reach dangerous dimensions due to its negative environmental effects on the development of resistance and leaving residues in the environment. It is necessary to use more natural control agents in order to minimize the negative environmental effects and to provide the effective control mechanism. This study aimed to provide an environmetally-friendly approach by using *Bt* strains to provide an effective control of pest insects. In this direction, in order to provide effective control of the lepidopteron insect *Hyphantria cunea*, it was aimed to apply *Bt* first and then to determine at which stage of the insect life cycle it is affected. By this way, it was aimed to contribute to the development of toxin formulations from natural sources for effective control of *Hyphantria cunea*. In general, this is an effort to incorporate all of the most current advancements in the research of *B. thuringiensis* within the framework of biological control of the agriculturally hazardous lepidopteron insect *Hyphantria cunea,* as well as an attempt to determine the various phases of the insect life cycle that are mostly impacted by the *Bt* products.

CHAPTER 1

LITERATURE REVIEW

1.1. The Biological Control

The use of natural enemies as a way of biological control is a method that is both beneficial to the ecosystem and successful in decreasing or neutralizing the impacts of pests. It refers to any action taken by one species that mitigates the negative impact of another species. In the field of pest management, the term "biological control" most often refers to the use of parasites, predators, or diseases on a pest population in order to bring the number of individuals down to the point at which they would cause economic damage (Kergunteuil et al., 2016).

Biological control manages pests (plant diseases, insects, weeds, and mites by using the organisms in the environment as natural enemies). It is based on natural processes like herbivory, parasitism, or predation, but it often includes an all-human role in the management. It is also a critical component of İntegrated Pest Management (IPM) strategies (Sprouffske et al., 2012).

Natural enemies of pests are used for achieving biological control by importation strategies; augmentation strategies are used to administer natural enemies for pest control, and conservation strategies are used to ensure that natural enemies are maintained by the regular establishment of natural enemy’s populations (Gurr & You, 2016).

Natural enemies of insect pests are predators, competitors, microbial diseases, and parasitoids. They are biological control agents because they are effective in controlling insect pests. Antagonists are the most common term to describe biological management

agents plant diseases. Seed predators, herbivores, and plant diseases are examples of weeds' biological control agents (Bale et al., 2008).

It is conceivable for biological control to have negative repercussions for biodiversity by attacking non-target species via any of the processes listed above. This is particularly true when a species is introduced without complete knowledge of the potential ramifications (Peixoto et al., 2018).

1.1.1. History

When Harry Scott Smith used the phrase "biological control" in 1919, it was the first time the phrase had been used. Paul H. DeBach, an entomologist who spent his whole career researching citrus crop pests, was responsible for popularizing the term. On the other hand, the practice has been around for generations (Leppla & Clercq, 2019).

According to the Chinese botanist Ji Han, the first record of an insect being used to control an insect pest which lives on the plants (c.304 AD) (Anderson & Leppla, 2021). Citrus fruits grown in the southern hemisphere may suffer significant pest damage if these ants are not present. The ants that were utilized were known as Chinese ants. Several authors have written on the practice, including (Bigler, 1989).

The first examples of biological control methods appeared in the 1870s., the Entomologist Riley and Lebaron pioneered the practice of the parasitoids to manage agricultural pests in the USA. When Riley shipped the predatory mites to France in 1873, he was credited with the first shipment of an insect used as a control agent for aid in the fight against the grapevine phylloxera France was destroying grapevines. Riley was appointed as Chief of the Division of Entomology of U.S. Department of Agrıculture (USDA) in 1881 (Bellows, 2001).

When the braconid *Cotesia glomerata* was introduced into the United States in 1883-1884, it was doing so to combat the invasive beetles and butterfly, this was the first time a parasiticidal wasp was introduced into the country. The vedalia beetle, *Rodolia cardinalis*, a lady beetle, was transported from Australia to California in 1888-1889 to control the cottony cushion scale, *Icerya purchasi*, which was introduced in the same year. In California, it was significant for the newly expanded citrus sector, but the

cottony cushion scale population was begun to drop by the end of 1889. As a result of this tremendous success, more beneficial insects were introduced into the The United States of America (USA) (De Boer, Kuijper, Heimpel, & Beukeboom, 2012). A large-scale biological control programmer began in 1905 when the USDA sent specialist scientists to Japan and Europe to search for natural enemies of invasive pests such as the gypsy moth and the moth with brown-tail, which were both causing damage to trees and shrubs at the time. Consequently, nine gypsy moth parasitoids (solitary wasps), seven moth parasitoids with brown-tail, and two predators were established in the USA (Kergunteuil et al., 2016). Even though these natural enemies did not completely control the gypsy moth, the frequency, length, and intensity of its outbreaks were decreased, and the programmer was deemed a success. Many ideas and processes for the execution of biocontrol programmers were developed as a result of this effort (Pimentel, 2005). Larvae of the *Cactoblastis cactorum* prey on the *Opuntia prickly* pear cactus, causing it to die. In 1788, the prickly pear cactus were used in Australia's Queensland region as an ornamental plant, and they have since gained widespread acceptance. Eventually, they covered more than 25 million hectares by 1920, with the area rising at a pace of one million hectares per year until they reached their maximum extent in Western Australia. Crushing and burying were all ineffective techniques of removing the bodies. Two control agents are used to manage the spread of the plant: scale insect *Dactylopius* and cactus moth *Cactoblastis cactorum*. *Cactoblastis cactorum* and *Dactylopius coccus* are beneficial in managing the spread of the plant. Cactus moth eggs (10.000) were successfully dispersed across Queensland during in 1926-1931, and by 1932, the bulk of the state's prickly pear trees was eliminated (Babendreier, Bigler, & Kuhlmann, 2005).

For the first time in Canada, it has been reported that a conventional biological control effort was made using the parasitoidal wasp. When William Saunders collected and released individuals in New York State gardens in 1882 to control the invasive currant worm *Nematus ribesii* in Ontario gardens, he was hailed as "the father of modern pest management". The Entomologist James Fletcher continued to use several diseases and parasitoids to manage pests in Canada between 1884 and 1908, when the first Dominion Entomologist retired (Albajes, 2019).

1.1.2. Using Methods of Biological Control

1.1.2.1. Conservation of the Natural Enemies

Pesticide usage should be reduced since natural enemies are very vulnerable to pesticides, and the use of pesticides in the field is a significant constraint to their efficacy in the field. When Integrated Pest management (IPM) was first proposed, it was envisioned to combine chemical and biological control by decreasing pesticide use while using the pesticides that were destroying the biological control agents (Settele & Settle, 2018). The use of cultural approaches and resistant cultivars that minimize pest damage, ways of altering host-finding behaviors and insect mating, in certain situations, that means of control may all help to lessen the need for pesticides. The initial step of defining sampling methodologies and economic criteria for pesticide application has proven difficult for many IPM programmers to complete (Cloyd, 2020).

Pesticide use in significant crops has been evaluated by the USDA and the Environmental Protection Agency, and many studies have shown that the quantity of pesticides used in the USA has either stayed constant or grown since the late 1980s (Karamaouna, Jaques, & Kati, 2021). As reported by the USDA, notwithstanding variances by chemical and crop class, the previously observed decline in pesticide usage, caused by the replacement of pesticides effective at lower doses, has been negated by an increase in the number of crops treated and the number of treatments itself. The stalemate in pest management has prompted some to propose that IPM be re-focused on avoiding pest concerns by addressing pest ecology, boosting the capacity of animals and plants to fight against pests, and expanding populations beneficial species. The phrase "Bio-Intensive Integrated Pest Management" is used to characterize this kind of pest management method (Messelink et al., 2014).

a. Using Pesticides to Reduce the Natural Enemies Effects

When it comes to pesticides and their impact on the natural enemy, the physiological action of the chemical and the method in which the pesticide is administered are essential considerations. The acaricides and insecticides are the most likely to be harmful to natural enemies of insects and mites; herbicides and fungicides may also be poisonous in some circumstances. In order to better understand the effect of pesticides

on good insects, including bees, mites and spiders, a database has been created (Dainese, Schneider, Krauss, & Steffan-Dewenter, 2017).

With this database, you may compare the toxicity of various pesticides, along with the so-called selectivity ratio, the difference between the dosage needed to eliminate half of a pest and the dosage required to eliminate half of the affected natural enemy species. Synthetic pyrethroid pesticides were shown to be among the most harmful to beneficial insects, whereas *Bacillus thuringiensis* and insect growth regulators were found to be among the least toxic to beneficial insects. Generally speaking, systemic insecticides, which need the eating of plant material for exposure, and ingested insecticides, which are toxic only when consumed, have a substantially greater effect on natural enemies than they do on pests (Bommarco, Miranda, Bylund, & Björkman, 2011).

Pesticides may also alter the physiology of natural enemies, but these effects are less evident than direct harm. Many fungicides, including carbendazim and benomyl, have been shown to suppress oviposition by *Phytoseiid mites*, which are voracious predators of plants. Certain herbicides make the soil in vineyards resistant to predacious mites, making the soil in vineyards more productive (Martin, Reineking, Seo, & Steffan-Dewenter, 2013).

The effect of pesticides on natural enemies may be minimized by applying them at the right time and in the right location at the right time to ensure that the beneficial organisms and the pesticide do not come into contact. When used with natural enemy biology, less lasting pesticides may help decrease interaction, particularly when sensitive life stages are avoided. Spot treatments do not harm natural enemies in nearby regions in areas of high pest density or by alternating strips within a field, which are common practices in pest management today. The mobility of the natural enemy and the treated pest may impact the efficacy of confining the regions treated (Luo et al., 2019).

b. Providing Resources for Natural Enemies

Natural enemies are not active in the North East during the winter months, and they are seldom active unless they are active yearly. Overwintering pathogens and parasitoids may occur in the host body in some cases, while others can be found in agricultural wastes, soil, or vegetation in others (Holland et al., 2020). Predacious mites

overwintering in fruit orchards is a famous example of this phenomenon. Ground cover in these orchards offers protection from the elements during the winter, protection from pesticides used on the fruit trees, and a supply of pollen and alternative prey for bees and other pollinators (Landis, Wratten, & Gurr, 2000).

The adults of many predators and parasitoids, especially during the summer, may need or benefit from pollen, nectar, and honeydew (which is generated by aphids). In addition, since many agricultural plants blossom consistently for a limited period, it may be necessary to establish flowering plants around the field's boundaries or inside the field to provide additional pollen and nectar for the crop. However, the variety of plants within a field may also have an adverse effect on the effectiveness of host-finding, which is especially true for parasitoids that are specialists in their area. Although the availability of pollen and alternative prey may help to stabilize the populations of generalist predators, the effectiveness of the predators is still dependent on their ability to respond quickly enough to outbreaks of the target pest, either by aggregation or multiplication, when the target pest is present (Steffan-Dewenter & Schiele, 2008).

In order to effectively manage natural enemies and pests, it is necessary to have a thorough understanding of their behavior and biology. This may be accomplished by plant diversity or other techniques of boosting the diet of natural enemies. The native lady beetle *Coleomegilla maculata* is a potentially significant predator of the Colorado potato beetle (*C. maculata*) eggs and larvae in their early stages of development (Schellhorn, Parry, Macfadyen, Wang, & Zalucki, 2015).

The availability of aphid prey in nearby fields, such as crops of alfalfa, brassicas, cucurbits, and corn, as well as the availability of pollen from maize and numerous weeds, such as dandelion and yellow rocket, determines the size of the population feeding on the potato beetle. This predator does not presently suppress the Colorado potato beetle by itself, but a better understanding of how to manage *C. maculata* numbers in the agricultural setting might make it more successful (Clem & Harmon-Threatt, 2021).

1.1.2.2. Introducing New Natural Enemies

In order to complete this procedure, a significant study must be conducted into the biology of the pest, the biology of its natural enemies, and the risk of unexpected effects (Thomas & Reid, 2007). After appropriate natural enemies have been identified, examined, and collected, they must be subjected to quarantine in order to eradicate any infections or parasites that may have been introduced into the natural enemy population during the search and collection process. Natural enemies are then released in a controlled manner, with careful consideration given to the right time in the enemy and pest life cycles, in an area where the target pest is plentiful and where disturbance of the freshly released enemies is kept to a minimum. Because of its length and complexity, the results may be both remarkable and lasting when this procedure is completed successfully, provided that care is made in production techniques to limit detrimental impacts on the natural enemy's habitat (Hoddle, Warner, Steggall, & Jetter, 2015).

The *Hypera postica* is one of many instances of a pest successfully managed by using new enemies. The *Hypera postica* originates originally from Europe, and it was first discovered in the United States in 1904. It first emerged in the eastern United States about 1951, and by the 1970s, it had become a serious pest across the nation. Larval densities were high enough that most producers were required to spray one or more times each year, on average. Several parasitoids were brought into the United States from Europe to combat this issue. Included among the most effective imports are two species of parasitoids that target the larvae, one species that attacks the adult, and a parasitoid and predator that attacks the eggs. Because of a program that collected and raised enormous numbers of the most effective natural enemies, then released the offspring, some species were able to spread over the globe (Ali et al., 2019).

When used in conjunction with a fungal disease that targets the pupae and larvae, these natural enemies help to maintain alfalfa weevil populations below the level that causes economic damage. According to the study results in this case, cultural techniques, such as timing cuttings to minimize weevil populations and avoid disruption of natural enemies, have boosted the efficiency of biological management. With the introduction of natural enemies of other alfalfa pests and the widespread use of pest-resistant varieties, the need for insecticides against pests like the alfalfa blotch leaf miner and

aphids has been reduced, and thus the disruption of the natural enemies of the alfalfa weevil's enemies has been avoided (Mills, 2018).

1.1.2.3. Natural Enemies

a. Seasonal Releasing

In certain circumstances, a natural adversary is unable to overwinter effectively in the Northeast owing to the weather or a lack of suitable hosts or prey, and this is due to a combination of factors. Others, such as greenhouses, eliminate every viable habitat for the natural enemy after a season or production cycle to ensure that the natural enemy cannot repopulate. As a result, in certain cases, especially in annual crops or other highly disturbed systems, it may be necessary to reintroduce the natural enemy regularly to sustain pest control (Evans, 2016).

It has been shown that the discharge of insect parasitoids and predators throughout the growing season in greenhouses is a very effective technique for biological control in Europe. Producers adopted this method due to the prevalence of pesticide resistance in many greenhouse pests and the growing costs of chemical management in this environment. Originally the program was based on the employment of the parasitoid *Encarsia formosa* against the greenhouse whitefly and the predacious mite *Phytoseiulus persimilis* against the two-spotted spider mite, both of which were shown to be effective. Natural enemies have been introduced to the program throughout the years to manage other pests, including as thrips, leafminers, aphids, caterpillars, and more species of whiteflies, as the need has arisen. In Europe, the costs of utilizing biological control for insect pests are currently much cheaper than the costs of using chemical control for insect pests. Extension consultants, specialist journals, and grower study groups are used to keep growers up to date on the program's implementation specifics and any new discoveries or natural enemies (Lewis, van Lenteren, Phatak, & Tumlinson, 1997) that have been discovered (Peterson, Ode, Oliveira-Hofman, & Harwood, 2016).

The use of *Pediobius foveolatus* against Mexican bean beetles and the use of *Edovum puttleri* against the Colorado potato beetle are two instances of seasonal inoculative release in the field. In the Northeastern United States, none of these parasitoids are able

to survive the winter. However, techniques have been devised for raising them in the laboratory and releasing them once a year, and they reproduce in the field, killing the hosts they come into contact with during the season. A commercially accessible variety of *P. foveolatus* is available, and the New Jersey Department of Agriculture is raising and releasing *E. puttleri* for use in integrated pest management of eggplant (Messelink, Lambion, Janssen, & van Rijn, 2021).

b. Biological Insecticides or Inundative Release

All other biological control tactics are fundamentally different from these two strategies because they do not seek to generate a population of natural enemies that multiplies to the point where it achieves long-term equilibrium with the population of either its hosts or victims. Instead, the goal is to utilize biological agents, such as pesticides, to release them in sufficient numbers to bring the insect population to a crashing halt. The majority of commercially available insect pathogens formulations are applied excessively (Chandler et al., 2011).

The bacterium *B. thuringiensis* is the source of the most well-known biological insecticides, which are those that are based on the bacteria. A *Bt* spray is simply an insecticide that works by paralyzing the digestive system of the insect being sprayed on (depending on the strain used, either caterpillars, Colorado or elm leaf beetle larvae or mosquito or fungus gnat larvae). One of the active ingredients, a protein generated by the bacterium, is responsible for paralyzing the stomach. There are no living bacterial spores present in many formulations, just a concentrated formulation of the active protein. The illness is prevented from spreading further in the insect population as a result (Ko et al., 2015).

The nematodes are live natural enemies which are used on in regular basis. These nematodes may be seen travelling in the soil or on the soil surface, and they are known to fight the insects they invest aggressively. The bacteria release, grow and destroy the host as a result of their presence. The nematodes feed on bacteria and insect tissue before mating and reproducing in a new location (Siegwart et al., 2015).

Young nematodes emerge from the insect carcass after one to two weeks and begin their search for new hosts. Nematodes are very sensitive to desiccation, exposure to UV

radiation, and extremes in temperature, among other conditions. They are especially effective against insects found on or in the soil and other protected areas of the environment (such as tunnelling inside plants). To be successful, the environment must be wet, and the temperature range must be between 53-86 °F (Kupferschmied, Maurhofer, & Keel, 2013).

It is still prohibitively costly to inundate fields with insect and mite natural enemies because of the high expenses of mass breeding, storage, and transportation of live organisms in the large numbers needed for effective control. While research on artificial meals for natural enemies and other elements of commercial production continues, the cost of commercial production continues to decline (Käch, Mathé-Hubert, Dennis, & Vorburger, 2018).

1.1.3. Strategies of the Biological Control

1.1.3.1. Importation

Vedalia beetles, or *Rodolia cardinalis*, were introduced to California from Australia in the 19th century, where they proved effective in eliminating cottony cushion scale. Importation, also known as traditional biological control, refers to the process of moving the natural predators or parasites of a pest to a new region where they would not otherwise be found. In the beginning, introductions were often done on an informal basis and were not based on scientific research. As a result, several imported species later became major pests in their own right (Bale et al., 2008).

A biological control agent must have colonization abilities that enable it to keep up with changes in the pest's environment through time and space in order to be the most successful in controlling the pest. If the agent has temporal persistence, which means that it can sustain its population even when the target species is absent for a short period of time, and if it is an opportunistic forager, which allows it to exploit a pest population quickly, the agent's control potential is maximized (Kergunteuil et al., 2016).

In Australia, *Icerya purchasi* was successfully controlled by a predator *Rodolia cardinalis*, which was one of the initial achievements in this field of study. Californian researchers were able to replicate their achievement by employing a parasitoidal fly,

Cryptochaetum iceryae, as well as the beetle in their experiments. In Texas in the 1960s, *Neodusmetia sangwani* was responsible for the effective protection of *Antonina graminis*, among other accomplishments. Using of the natural enemies decreased the loss by *Hypera postica*, the alfalfa weevil, a severe imported pest of fodder. Twenty years after their first introduction, the number of alfalfa weevils in areas of the USA where alfalfa is grown that are being treated for the pest has remained 75% lower (DiTomaso et al., 2017).

Alligator weed flea beetles were used to manage the invasive plant *Alternanthera philoxeroides* in Florida (United States). In South America, the alligator weed was first imported to USA. This invasive species establishes root in shallow water, causing navigation, irrigation, and flood control systems problems. Florida has seen a significant reduction in the area of land covered by alligator weed with the deployment of the alligator weed flea beetle and two additional biological treatments in 2011. Yet another aquatic plant, the enormous salvinia, is a major nuisance, clogging streams, limiting water flow, and threatening the survival of indigenous species. It is possible to manage salvinia weed using the salvinia weevil and the salvinia stem-borer moth (*Samea multiplicalis*) in warm climes; in Zimbabwe, for example, during a two-year period, 99 % control of the plant was achieved (Chidawanyika, Mudavanhu, & Nyamukondiwa, 2012).

European corn borer control is limited and inconsistent when small parasitoidal wasps (*Trichogramma ostriniae*) are used commercially to treat this important pest. Better results may be obtained by using well formulated *B. thuringiensis* strains. As compared to pesticide treatments, The *O. nubilalis* integrated control, which combines the introduction of *Tricogramma brassicae* and then *Bt subsp. kurstaki*, decreases pest damages as well as or even better than the traditional control method. A typical biological management approach wasn't successful in bringing the population of the Levuana moth, also known as the *Levuana iridescens*, which was a significant coconut pest in Fiji, under control until the 1920s (Syed Ab Rahman, Singh, Pieterse, & Schenk, 2018).

1.1.3.2. Augmentation

In the United States, *Hippodamia convergens*, also known as the convergent lady beetle, are widely available for aphids' biological control. Augmentation is the additional release of natural enemies found in a particular region, intending to increase the naturally present populations in that location. The inoculative release is a technique in which little percentage of control agents are allow them to reproduce in the longer-term control and thus keeping the pest population at a manageable level. It is more often seen as a type of preventive than a treatment for the condition. In contrast, inundative release involves releasing a large number of pests with the intention of rapidly reducing a harmful insect population and thereby addressing an issue that has developed in the field. The goal of inundative release is to address a problem that has already developed in the field. Although it is possible to increase the efficacy of a control agent, doing so is not guaranteed to be successful and is contingent on the particulars of the interactions that take place seen between insect and the controlling operator (Hopper, 2003; Perez-Alvarez, Nault, & Poveda, 2019) .

The inoculative release serves as a representation of what is going on in the field during the development of several crops that are grown in greenhouses for horticultural purposes. The parasitoidal wasp *Encarsia formosa* is released on a regular basis to reduce greenhouse whitefly, and the predatory mite *Phytoseiulus persimilis* is used to suppress two-spotted spider mite populations. Greenhouse whiteflies are managed via the employment of this method. Large numbers of the egg of *Trichogramma* are often released into the environment in order to control potentially dangerous moths. Inundative releases are presently being performed with the use of drones, which is a novel approach to carrying out the task. Egg parasitoids rely on a wide array of indicators to identify the eggs of their preferred host species in order to feed on them. It was revealed that kairomones were located on the scales of a moth. At a manner similar to this, *Bt* and other microbial pesticides are used in high enough concentrations to produce observable results in a short period of time (Michaud, 2018).

The recommended release rates for *Trichogramma* in vegetable or field crops vary from 5.000 to 200.000 per acre (1-50) per square meter-week, depending on the severity of pest infestation in the field crop or vegetable crop. A similar approach is used to manage

soil-dwelling insect pests. Nematodes kill the insects when it used at millions of nematodes per acre (Crowder, 2007).

1.1.3.3. Conservation

Keeping naturally existing predators and parasites of pests in a region is the focus of the third method of biological pest control. When nectar-producing crops are planted in the rice fields, the natural enemies are adapted to the environment. The nectar produced by these flowers is consumed by predators and parasitoids that feed on plant hoppers. They are so effective (reducing the insect densities by 10 to 100 fold), that farmers might use 70% fewer pesticides while seeing a 5% gain in yields. This is because they lower the number of pests by 10 or even 100 fold. It has been revealed that similar aphid predators may be found in the tussock grasses that grow along the field boundary hedges in England; however, it has also been shown that these predators spread slowly to reaching the center of the fields. The efficiency of the control was boosted by planting a strip of tussock grasses in field centers that was one meter wide. This enabled aphid predators to overwinter in those areas (Settle et al., 2015).

In order to entice earwigs to the region, an upside-down flowerpot that's been filled with straw is employed. Habitat modification is a method that may be used to battle pests and illnesses and involves changing cropping patterns in order to promote natural enemies. This tactic is utilized infrequently. Natural predators have the potential to live and breed in a wide range of environments, including beetle banks, shelterbelts, and hedgerows. We can contribute to ensure the continued existence of populations of natural enemies by providing a suitable habitat for predatory wasps and other beneficial insects, such as parasitoids. Leaving a layer of fallen leaves or mulch in place is an example of a simple action that provides these benefits. There is a possibility that wood stacks and compost piles provide a safe habitat for insects and other tiny creatures. Amphibians are more common in environments with tall ponds and grass (Letourneau & Bothwell, 2008).

Insects will make use of the hollow stems of the plants during the winter if the dead stems of annuals and non-hardy plants are not removed in the autumn. In California's grape vineyards, planting prune trees in order to provide a better overwintering refuge for a crucial grape pest parasitoid is an extremely unusual practice. It is also often done,

especially in gardens, to provide artificial shelters in the form of boxes, caskets, in order to make a cropped area more desirable to natural enemies. This is typically done for the sake of pest control. This is particularly important to keep in mind while tending to vegetable crops. In order to entice natural predators like earwigs, for example, one may hang flowerpots upside down and fill them with wood wool from the structures of the tree. This will encourage earwigs to visit your garden. It is possible to increase the population of green lacewings by putting a cardboard roll inside a plastic container with an open bottom and then leaving the container's bottom unobstructed. Insectivorous birds may construct their nests using birdhouses; the most helpful birds may be recruited by building an entrance that is just large enough for the desired species to fly through (He et al., 2021).

As a result of the decreased risk of pesticide exposure associated with the substitution of broad-spectrum insecticides with targeted control techniques such as *Bt* cotton in cotton production, natural enemies of cotton pests may be able to thrive in a more favorable environment. Predators have great potential for controlling the pest insects. However, there are some situations in which lower food quality and quantity coupled with improved control from *Bt* cotton might cause decreases in the population of natural enemies (Karp, Chaplin-Kramer, Meehan, Martin, & Declerck, 2018).

1.1.4. Biological Control Agents

1.1.4.1. Predators

Predatory lacewings are available for purchase through biocontrol distributors. Predators are mostly free-living organisms that devour a huge number of preys directly throughout the course of their whole life spans. Considering that insects are responsible for the majority of agricultural pests, the majority of the predators that are used in biological control are carnivores in their natural environments. Aphids are no match for lady beetles, especially their larvae, which are most active from May through July in the northern hemisphere. Lady bugs are voracious predators of aphids. In addition to other things, they feed on things like mites, insects, and very young caterpillars. The spotted lady beetles a problem in the United States, feeding on the larvae and eggs of the Colorado potato weevil that is different kind of potato beetle (Bouvet et al., 2019).

One hoverfly larva may consume up to 400 aphids in its lifetime, and certain species of hoverflies have larvae that feed nearly solely on aphids. There has been no investigation on their usefulness in the production of commercial crops. A substantial portion of the running crab spider *Philodromus cespitum*'s diet consists of aphids, which it uses as food. Additionally, this species acts as a biological control agent in fruit orchards across the European Union. Wasps belonging to the genus Polistes may be found on cotton plants, where they hunt for and catch bollworms and other types of caterpillars. There are a number of distinct species of entomopathogenic nematodes, and these nematodes may be found in a wide variety of ecosystems. These nematodes are crucial predators of insect and other invertebrate pests. A stage of the parasite that is resistant to the effects of stress is the infective juvenile stage of entomopathogenic nematodes (Choh, Ignacio, Sabelis, & Janssen, 2012).

These pathogens may be found in the soil, where they can infect insects that serve as suitable hosts. As soon as they penetrate the insect, they make their way to the hemolymph, which is where they can recuperate from their state of stalled growth and release the bacteria which they have taken up home. The bacterial symbionts will grow in order to kill the host insect, and they will release toxins that will kill the host insect. The microscopic nematode known as *Phasmarhabditis hermaphrodita* feeds on slugs and is capable of eliminating them from the environment. In the course of its convoluted life cycle, it goes through a number of phases, one of which is a free-living, infectious stage in the soil. During this stage, it mates with a pathogenic bacterium like *Moraxella osloensis*. The bacteria that infect the digestive system of the slug are eventually what kill the nematode after it has invaded the body of the slug via the posterior mantle region, eaten its way through it, and reproduced inside of it. In Europe, there is a type of nematode that is available for commercial purchase; the soil must first be wet before the nematode may be introduced. This may be partially attributed to the fact that they have a limited tolerance for high temperatures and dry conditions. Nematode has a limited shelf life as a result of their low level of tolerance for high temperatures and dry circumstances. There is also the possibility that the kind of soil to which they are applied might limit the effectiveness of the treatments (Wright et al., 2017).

The following is a condensed version of the life cycle of nematodes that cause disease in insects and the bacteria that live in symbiosis with them: A number of different

predatory mites, such as *Phytoseilus persimilis*, *Neoseilus californicus* and *Amblyseius cucumeris*, as well as *Feltiella acarisuga* and the ladybird *Stethorus punctillum*, have been used effectively in the management of spider mite populations. The two-spotted spider mite and *Frankliniella occidentalis* have been demonstrated to be susceptible to the bug *Orius insidiosus*, which has been shown to be effective against both of these pests (Schausberger, Çekin, & Litin, 2021).

When attempting to eradicate invasive plant species, it is possible to make use of many predators, including the *Cactoblastis cactorum* that was reported before as well as other predators. The poison hemlock moth, also known as *Agonopterix alstroemeriana*, is an additional insect that has the potential to be used to control poison hemlock (*Conium maculatum*). The larval stage of the poison hemlock moth is very voracious in its devouring of the poison hemlock plant, which is its host plant. There may be hundreds of larvae on a single host plant, which results in large regions of poison hemlock being eradicated (Bean, Dalin, & Dudley, 2012).

Wasp of the parasitoid genus *Aleiodes indiscretus* are feeding on the larva of the gypsy moth, a pest that causes serious damage to forests. This photograph was provided courtesy of the author.

Even among arthropods, which are often thought of as obligate predators of animals (arthropods), floral food sources are usually important additional sources of nourishment. After it was discovered in one piece of research that adult *Adalia bipunctata* could live on flowers but never finish its life cycle, a meta-analysis was carried out to determine whether there was any evidence of such a general tendency in previously published data. If such a tendency did exist, the purpose of the meta-analysis was to determine whether or not such evidence existed. In some situations, the use of floral resources is necessary. In general, floral resources extend life spans and encourage fecundity, which indicates that the availability of non-prey food may potentially affect the population numbers of predators. As a consequence of this, the survival of the biocontrol population and the efficiency of its operation may be dependent on the flowers that are located nearby (Benvenuto et al., 2012).

1.1.4.2. Parasitoids

Parasitoids lay their eggs on or inside the body of an insect host, which is then used as a source of nutrition for the developing parasitoid larvae as the host insect dies off. The death of the host is unavoidable in the end. The great majority of insect parasitoids are either flies or wasps, and many of them have a rather narrow range of hosts on which they may successfully feed and reproduce. There are important groups of wasps, including the Ichneumonid wasps, which predominately attack caterpillars; that attack other insects, such as aphids; the chalcid wasps (Schmidt et al., 2003).

The parasitoids are part of a category of biological control agents that are among the most widely used all over the world. There are two types of rearing systems that are available for use in commercial settings: short-term daily output systems that produce a large number of parasitoids each day and long-term systems that produce fewer parasitoids each day. Parasitoids are produced in huge numbers on a daily basis by systems with a short-term daily output. The vast majority of the time, the production process will need to be synchronized with the appropriate release dates, which correspond to times when susceptible host species that are at an appropriate stage of development will be available. Smaller production facilities often only operate at certain times of the year, in contrast to larger factories that are typically operational round the clock. It is not uncommon for breeding facilities to be situated at a great distance from the sites where the agents will be used in the field. As a result, moving the parasitoids from their point of production to the sites where they will be put to use may be a challenging undertaking. During transit, parasitoids may be subjected to high temperatures as well as vibrations from aero planes or cars, both of which may be harmful to the organisms (Zepeda-Paulo, Ortiz-Martínez, Figueroa, & Lavandero, 2013).

The whitefly is a sap-feeding bug that causes wilting and black sooty mold in greenhouse vegetable and ornamental crops. This little predatory chalcid wasp is a parasitoid of the whitefly. Whiteflies are the primary prey of the chalcid wasp known as *Encarsia formosa*, which is a predator. As a result of the fact that it offers protection for a considerable length of time, it is most beneficial in terms of efficiency for dealing with infestations that are not very severe. The wasp lays its eggs in the "scales" of young

whiteflies, which become black as the parasite larvae pupate and grow into the next stage of their life cycle. The glassy-winged sharpshooter, *Homalodisca vitripennis*, has been efficiently suppressed in French Polynesia by the *Gonatocerus ashmeadi*, which was brought there to combat the pest (Rossbacher & Vorburger, 2020).

An example of a detrimental insect that may be present in fir and spruce forests all throughout the United States is the eastern spruce budworm. This particular bug feeds on the buds of spruce trees. Birds are one example of the natural biological control strategies that are commonly employed. The parasitic wasp known as *Trichogramma minutum*, on the other hand, has being investigated as a viable alternative to therapies that include more trouble some chemicals (Hajek & van Nouhuys, 2016).

Much recent research has investigated the use of parasitic wasps to manage urban cockroaches to develop long-term, sustainable pest control techniques. Active-hunter wasps are being used to decrease cockroach populations since the vast majority of them stay inside the sewage system and other sheltered regions inaccessible to pesticides (Sivinski & Aluja, 2012).

1.1.4.3. Pathogens

Microorganisms that may cause disease include, but are not limited to, bacteria, fungus, and viruses. The behaviors of these parasites are very host-dependent, and they either kill or incapacitate the animals they feed on. If they are correctly prepared, several microbial insect illnesses that occur naturally have the potential to potentially be used as biological pesticides in agricultural settings. If they occur naturally, these epidemics are density-dependent, which means that the probability of their occurring increases as the number of insects in a given area increases in concentration (Lacey et al., 2015).

1.1.4.4. Bacteria

Bacteria are used for biological control through damaging the digestive systems of the insect. Because of this, these bacteria are effective against pests that have sucking mouth parts, including aphids and scale insects; they are not useful against other types of insects. *Bacillus thuringiensis (Bt)* which is a bacterium that lives in the soil, is thought to be the species of bacteria that is used for biological control the most

frequently. There are at least four sub-species of *Bt* that are used against pests (e.g. in Lepidoptera, Diptera, Coleoptera, Hymenopteraorders). The bacterium is sent to organic farmers in the form of vegetative and/or spores that have been dried up and packaged in sachets. These are then mixed with water and sprayed onto susceptible insect pests in order to prevent the illness from spreading further. Transgenic crops have had some of the toxins that are produced by *Bt* strains incorporated into them. This has caused the plants to manufacture proteins that are comparable to the toxins that are produced by the bacterium. These plants exhibit resilience to pests, hence lowering the amount of pesticide that has to be applied. If pests are able to build up a resistance to the toxins that are present in organic crops, then the bacteria *Bt* will no longer be effective in organic farming. *Paenibacillus popilliae*, the bacterium that is responsible for milky spore disease, has been shown to be useful in the management of the Japanese beetle. This is due to the fact that it eliminates the Japanese beetle larvae. It has a very specific relationship with its host species and, other than that relationship, it does not pose any threat to any other vertebrates or invertebrates. *Bacillus spp., Pseudomonads spp.,* and *Streptomycetes spp.* are all examples of bacteria that may inhibit the growth of pathogens and can be found in soil (Daranas et al., 2019; Köhl, Kolnaar, & Ravensberg, 2019).

1.1.4.5. Fungi

The fungus *Pandora neoaphidis* is to thank for the demise of the green peach aphid, which was not only a nuisance in its own right but also a vector for the spread of plant viruses. The length of the scale bar is 0.3 millimeters. At least 14 different species of entomopathogenic fungi, which are fungi that may cause sickness in insects, have been identified as aphid-eating fungi (Thambugala et al., 2020).

Beauveria bassiana is widely accessible and used to manage a wide variety of insect pests, such as whiteflies, yellow jackets, thrips, aphids, and weevils. It is also used to prevent the spread of insect-borne diseases. White flies, thrips, and aphids are just some of the pests that may be managed with the use of *Lecanicillium* spp. *Metarhizium* spp. is used as a method for the management of a wide variety of pests, including beetles, locusts, and other grasshoppers, Hemiptera, and spider mites, amongst many more. *Paecilomyces fumosoroseus* may be used to combat white flies, thrips, and aphids,

whilst *Purpureocillium lilacinus* is effective in combating root-knot nematodes. Both of these fungi can be found in the soil. 89 Plants infected with some pathogens may benefit from treatment with *Trichoderma* species. The Dutch elm disease has been successfully treated with *Trichoderma viride*. In the treatment of silver leaf, a disease of stone fruits produced by the *Chondrostereum purpureum*, which has been proved to have some influence on the condition, it has shown some promise. Silver leaf is caused by the fungus *Chondrostereum purpureum* (Chet & Inbar, 1994).

Other species of fungi, bacteria, and yeasts, such as *Gliocladium* spp., *Pythium* spp., *Pythium* spp. with mycoparasitic qualities, *Rhizoctonia* spp. with binucleate types, and *Laetisaria* spp., have the potential to restrict the growth of dangerous fungi under specific circumstances. Cordyceps and Metacordyceps are two genus of fungus that are used in the treatment of a broad range of arthropod infections. Entomophaga is a pesticide that is effective against a wide variety of pests, including the green peach aphid and other insects with similar life cycles. Research has been conducted on a number of fungal divisions, including Chytridiomycota and Blastocladiomycota, to investigate their viability as possible biological control agents. In the United States, research on the fungus *Synchytrium solstitiale*, which belongs to the Chytridiomycota division, is being conducted to determine whether or not it might serve as a viable biological control agent for yellow star thistle (Narladkar, Shivpuje, & Harke, 2015).

1.1.4.6. Viruses

Baculoviruses are viruses that are unique to certain insect host species, and they are effective in biological pest management applications. Examples include the application of the *Lymantria dispar* multicapsid nuclear polyhedrosis virus to spray huge sections of forest in North America where gypsy moth larvae are causing significant defoliation. The virus that has infected the moth larvae causes them to die, with the dissolving cadavers releasing virus particles onto the vegetation, where they may be infected by other larvae (Di Giallonardo & Holmes, 2015).

The mammalian virus known as rabbit hemorrhagic disease was introduced into Australia to control the number of European rabbits already living there. After breaking out of the quarantine and spreading throughout the country, it was responsible for the

deaths of a substantial number of rabbits in large numbers. Even the most defenseless creatures were able to live, ultimately leading to the development of a population that is immune to the virus by virtue of the immunity they passed on to their offspring. The use of the Rabbit Haemorrhagic Disease (RHD) in New Zealand in the 1990s was similarly successful initially, but by the time it had been there for a decade, immunity had developed, and the population had returned to levels observed before the virus was introduced. Mycoviruses are infectious illnesses caused by fungi that are prevented by viruses (Holmeset al., 2015).

1.1.4.7. Oomycota

Lagenidium giganteum is a water-borne mold that parasitizes the larval stage of mosquitoes and is responsible for the spread of the disease. The spores avoid inappropriate host species and look for suitable mosquito larval hosts in the water, which helps to reduce the spread of disease. With the benefits of a latent phase that is resistant to desiccation and slow-release qualities that last for many years, this mold is an excellent choice. Unfortunately, it is vulnerable to many pesticides often employed in mosquito abatement programs and should be avoided (De Andrade Lourenço, Branco, & Choupina, 2020; Kamoun, 2003).

1.1.4.8. Competitors

In the countries of Benin and Vietnam, the bean vine *Mucuna pruriens* is used as a biological control for the invasive species of *Imperata cylindrica* grass that is causing problems there. The vine's great strength allows it to dominate its surroundings and suffocate other plants by outcompeting them for space and light. Reports indicate that outside of the habitat where it is cultivated, *Mucuna pruriens* does not behave like an invasive species. *Desmodium uncinatum* may be used in push-pull farming to limit the growth of the parasitic plant witch weed. This kind of farming is called "intercropping".

However, the natural decomposers that occur in Australia are not suitable for feeding cow manure, which is the primary food source for bush flies. As a direct consequence of this, the *Musca vetustissima* fly, sometimes known as the Australian bush fly, is a serious nuisance pest in the nation. As a result, the Australian Dung Beetle Project, which ran from 1965 to 1985 and was directed by George Bornemissza of the

Commonwealth Scientific and Industrial Research Organization, introduced forty-nine different species of dung beetle into the environment in an effort to lessen the quantity of dung and, as a consequence, the number of places where flies could potentially lay their eggs (Janisiewicz, Tworkoski, & Sharer, 2000; Srinivasan, Sevgan, Ekesi, & Tamò, 2019).

1.1.4.9. The Employment of Parasitoids in Conjunction with Pathogens

In circumstances of widespread and severe infestation by invasive pests, several pest management approaches are often utilised in conjunction with one another. Consider the emerald ash borer, or *Agrilus planipennis*, an imported species from China that has devastated tens of millions of ash trees in its introduced habitat in North America. It is the most destructive insect in the world (Ons et al., 2020).

In 2003, in an effort to battle it, researchers from the United States of America and the Chinese Academy of Forestry started hunting for natural adversaries in their native environments. This investigation led to the discovery of many species of parasitoid wasps, such as *Tetrastichus planipennisi*, which is a gregarious larval endoparasitoid, *Oobius agrili*, which is a solitary, parthenogenic egg parasitoid, and *Spathius agrili*, which is a gregarious larval endoparasitoid. The emerald ash borer infestation in the United States of America is now being examined as a possible biological control for these insects, which have been brought into the country and released there. *Tetrastichus planipennisi*, which is now being introduced with *Beauveria bassiana*, a fungal pathogen that has been proved to have insecticidal characteristics (Tan et al., 2016), has produced some first results that are encouraging (Ishibashi & Choi, 1991).

1.1.5. Applications of the biological control

The use of real, living organisms to combat the presence of unwanted pests is referred to as "biological control". Natural enemies, such as parasites, predators, or disease-causing organisms, may be smuggled into the environment in which a pest lives. If a natural enemy already exists in the environment, it can be helped to thrive and become more effective in reducing the number of pest species, also known as the vedalia beetle, on the cottony cushion scale in California; the limiting of the proliferation of the European rabbit in Australia through the introduction of myxoma (Babendreier et al., 2005).

Some methods of biological control, which can be an efficient and environmentally friendly method of controlling pests, have resulted in the introduction of invasive species into new habitats. One example of this is the use of venomous cane toads in Australia in the 1930s from Hawaii in order to reduce the effects of beetle infestations on sugarcane plantations. These toads were brought to Australia from Hawaii in order to combat the effects of beetle infestations. Cane toads have been linked to a variety of unfavorable outcomes, including population drops in amphibian species that compete with them, population losses in natural prey species (such as bees and other small animals), and the poisoning of species that consume them. Any innovative technique of biological control must first undergo exhaustive study and testing in the laboratory before being released into the environment to prevent the spread of disease (Bigler, 2005).

1.2. *Hyphantria Cunea* (Drury)

1.2.1. Definition

The fall webworm is a species of moth (Erebidae family); its larval stage builds distinctive webbed nests on the hardwood trees throughout the fall and late summer. Even though it's considered a pest, it doesn't damage otherwise healthy trees despite its unattractive appearance. Commercial tree services and arbor culturists are fully aware of its existence.

This insect is characterized by its brown-white color. Some males and females have black spots on the upper wings. A zigzag row of black dots extends from the abdomen to the end of the abdomen (Q. Chen et al., 2020). The average length of the adult body is 11-13 mm in males and 15-16 mm in females, with a wingspan of 25-30 mm (Zhang et al., 2017) (Figure1.1).

Figure 1.1. Hyphantria cunea Male and Female

1.2.2. Classification of *Hyphantria cunea*

Scientific classification of *Hyphantria cunea*

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Insecta
- Order: Lepidoptera
- Superfamily: Noctuoidea
- Family: Erebidae
- Subfamily: Arctiinae
- Genus: Hyphantria
- Species: *Hyphantria cunea* (Drury) (C. Chen et al., 2014).

1.2.3. Distribution of *Hyphantria cunea*

The moth lives from Mexico to Canada and has been used on many continents. Introduced to what was formerly Yugoslavia in 1949, it now has occupied its entire

range in Europe from France to the Caspian Sea in the east and penetrated Central Asia: Turkmenistan, Uzbekistan, Kyrgyzstan. It was also brought into Japan in 1945, and the number of generations each year has been altered since its introduction to the country. It has expanded over China, southern Mongolia, Korea, and the southern Primo sky Krai of Russia, and is currently believed to have a holarctic distribution pattern. The fall webworm was first discovered in USA north, but it spread rapidly over the globe as a result of factors like commerce and fast transit (Alves et al., 2002).

1.2.4. Life Cycle of *Hyphantria cunea*

When it comes to the northern hemisphere of North America, there is only one generation every year, with larvae developing in autumn and late summer. It is estimated that there are two or more generations yearly south of latitude of 40th parallel North. Webs develop increasingly sooner as one moves southward. *Hyphantria cunea* insects go through four phases of development: eggs, larvae, pupae, and adults (Gomi, 2007) (Figure 1.2).

Eggs Larvae Pupae Adults

Figure 1.2. Life cycle of *Hyphantria cunea*

The mother moth deposits her eggs on the underside of leaves in clusters of a few hundred eggs that are coated with 'hair'. Eggs hatch in about one week. The color of the larvae is quite diverse, ranging from a light yellow to a dark grey, with yellow dots and both long and short bristles on their wings. Two cream stripes are running down the sides of the larvae.

The two breeds differ, one of which is more frequent in the north and the other more numerous in the south, vary in the hue of their head capsules. The greatest length of a larva is 35 mm at its longest point. The fall webworm's webs grow in size and become messier in appearance with time, although the tent Larvae's webs are concentrated at the

extremities of the branches, and the tent Larvae's webs are mostly located in the unions. Until they reach the late instars, the larvae eat within the tents. Very early larvae eat exclusively on the top surfaces of leaves, but they begin to swallow the whole leaf as they get older. The larvae stage usually lasts between four and six weeks. Larvae are reported to wriggle strongly at regular intervals in rhythm with their mother larvae. It has not yet been determined how they would coordinate their motions, mainly when they are dispersed across a large region. The pupa stage is found in the bark and leaf litter near the base of the trees, where it overwinters. It's roughly 10 mm long and dark brown in color. The thin brown cocoon is constructed of silk, with particles of debris intertwined throughout it for added texture. Adults in North America are primarily white, but in the southern hemisphere, they may have black or brown patches on their forewings, visible in the northern hemisphere. The front legs have brilliant yellow or orange patterns on it, and it has a lot of 'hair.' There will be less marking on the under wings than on the forewings, and the abdomen will frequently be covered in brown hairs (Tang, Zhang, & Zhang, 2012).

1.2.5. Food Plants

The fall webworm's feeding habits affect a wide variety of deciduous trees. Leaves are eaten, branches are defoliated, and the whole tree may become defoliated. It has been documented in 636 different species throughout the world, and it is believed to be one of the most polyphagous insects on the planet. Pecan trees, black walnut trees, American elm trees, hickory trees, fruit trees, and certain maple trees are the favored hosts in the eastern United States; in some regions, persimmon and sweet gum trees are also quickly consumed. Alder, willow, cottonwood, and fruit trees are the most extensively utilized trees in the western United States. Madrone, Mulberry, Ailanthus, American sycamore, and Asian white birch are examples of host plants that are not often used (Jang, Rho, Koh, & Lee, 2015).

The selection of a host plant is influenced by elements such as the amount of sunlight received by the plant, its age, the amount of environmental stress it has endured, its toughness, and the nutritional content of the plant. When it comes to insects, consuming plants that provide a lot of carbohydrates can be beneficial for those who need energy for processes such as dispersal or diapause. When it comes to insects who are producing

eggs, consuming plants that provide a lot of protein can be beneficial for those who are producing eggs (Schowalter & Ring, 2017) (Figure 1.3).

Figure 1.3. Presence of *Hyphantria cunea* Larvae on Trees

1.2.6. Behavior

The fall webworm is a sociable insect that may be seen in large groups. The larvae reside in huge webs that they have constructed for themselves on the limbs of trees. These webs facilitate the discovery of mates, the management of temperature, the rise in growth rate, and the protection from predators, but they also result in greater infection rates and predation among the organisms (Loewy et al., 2013). The larvae of the fall webworm uses a variety of defensive tactics to keep themselves safe from predators. A few instances of defensive behaviors or defense include shaking and jerking together, emitting a repellent aroma, and irritants on the hairs or spines of the animal (Hunter, 2000). The fall webworm demonstrates a type of parental care in that the female will attempt to protect her freshly produced eggs after oviposition by covering them with the hairs on her abdomen (Loewy et al., 2013).

1.2.7. Physiology

Fall webworms go through a process known as behavioral thermoregulation. The self-created web of the fall webworms has the ability to trap heat. As a result, the fall webworm is able to maintain a warm temperature of around 40-50°C, allowing the larvae to grow and develop at a quicker rate. There is a temperature differential inside

the web because the center section of the web tends to have a higher temperature, whilst the rear half of the web tends to have a lower temperature. However, the heat-trapping mechanism of a web is not always stable; the wind may cause the heat-trapping process to be disrupted (Rehnberg, 2002).

There are various components to the fall webworm's gut, including the foregut and the midgut, which are described below. The foregut and midgut of the fall webworm are both alkaline, and Johnson and Felon discovered that the pH of the midgut varied from 8.7 to 11.4 in their study of the fall webworm (Rehnberg, 2006).

1.3. *Bacillus thuringiensis*

1.3.1. Definition of *Bacillus thuringiensis*

B. thuringiensis (*Bt*) is a soil-dwelling bacterium that is the most widely used biological insecticide in the world. It is a Gram-positive bacterium. Besides from the guts of many species of moths and butterflies, *Bt* can be found in the environment on leaf surfaces, ponds, animal excrement, insect-rich settings, and flour mills and grain-storage facilities, among other places. Additionally, it has been discovered to parasitize other species of moths, such as *Cadra calidella*. In laboratory trials conducted on *C. calidella*, many of the moths were ill as a result of this parasite (Madigan & Martinko, 2006). Many *Bt* strains create crystal proteins during sporulation, known as δ (delta) endotoxins, which have insecticidal properties. This has resulted in their usage as pesticides, and more recently, the development of genetically engineered crops containing *Bt* genes, such as *Bt* maize, to combat insect pests. Many *Bt* strains that produce crystals, on the other hand, do not have insecticidal characteristics. The subspecies israelensis is extensively employed for mosquito and fungus gnat control and a variety of other purposes (Cox, 2013).

Toxic mechanisms include Cry proteins binding to particular receptors on the membranes of the midgut of the targeted pests, causing the membranes to rupture and cause death. Similarly, some creatures that do not have the proper receptors in their guts are unable to be impacted by the Cry proteins and, as a result, are not harmed by *Bt* (Kumar et al., 1996).

1.3.2. History of *Bacillus thuringiensis*

Ishiwatari Shigetane was the first to identify *Bacillus thuringiensis* in silkworms, back in 1902. When German scientist Ernst Berliner identified it as the cause of a sickness known as Schlaffsucht in flour moth caterpillars in Thuringia in 1911, he was credited with rediscovering the bacteria. Later, *B. sotto* was reclassified as *Bacillus thuringiensis* var. *sotto,* which means "sotto bacteria". In 1976, Robert A. Zakharyan discovered the existence of a plasmid in a strain of *Bt* and hypothesized that the plasmid was involved in endospore and crystal production in the bacteria. In addition to *Bt*, which is closely related to *Bacillus cereus*, which is a soil bacterium, and *B. anthracis*, the causative agent of anthrax, the three species are distinguished primarily by their plasmids(Hollingsworth, 2014). All three are capable of creating endospores, like the other members of the genus (Hollingsworth, 2014).

It is classified as a member of the *Bacillus* group, which can be divided into two categories: seven closely related species (*Bacillus cereus*, *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus pseudomycoides*, *Bacillus mycoides*, and *Bacillus cytotoxicus*); or six species (*Bacillus cereus sensu lato*) that are members of the *Bacillus* group (*Bacillus cereus sensu lato*: *Bt* is more closely related to *Bacillus* than any other species in this grouping. *Bw*, *Bm*, *Bp*, and *Bcy* are all more distantly linked to *Bw*, *Bm*, and *Bp*. There are several dozen different subspecies of *Bt* that have been identified. *Bt* subspecies *kurstaki* (*Btk*), *Bt* subspecies *israelensis* (*Bti*), and *Bt* subspecies *aizawa* (*Bta*) are most commonly used species as insecticides. Some *Bti* lineages are clonal, whereas others are not (Martignoni, 1963).

1.3.3. Taxonomy of *Bacillus thuringiensis*

Scientific classification of *Bacillus thuringiensis* (Milner, 1994);

- Domain: Bacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales

- Family: Bacillaceae
- Genus: Bacillus
- Species: *Bacillus thuringiensis* (Figure 1.4)

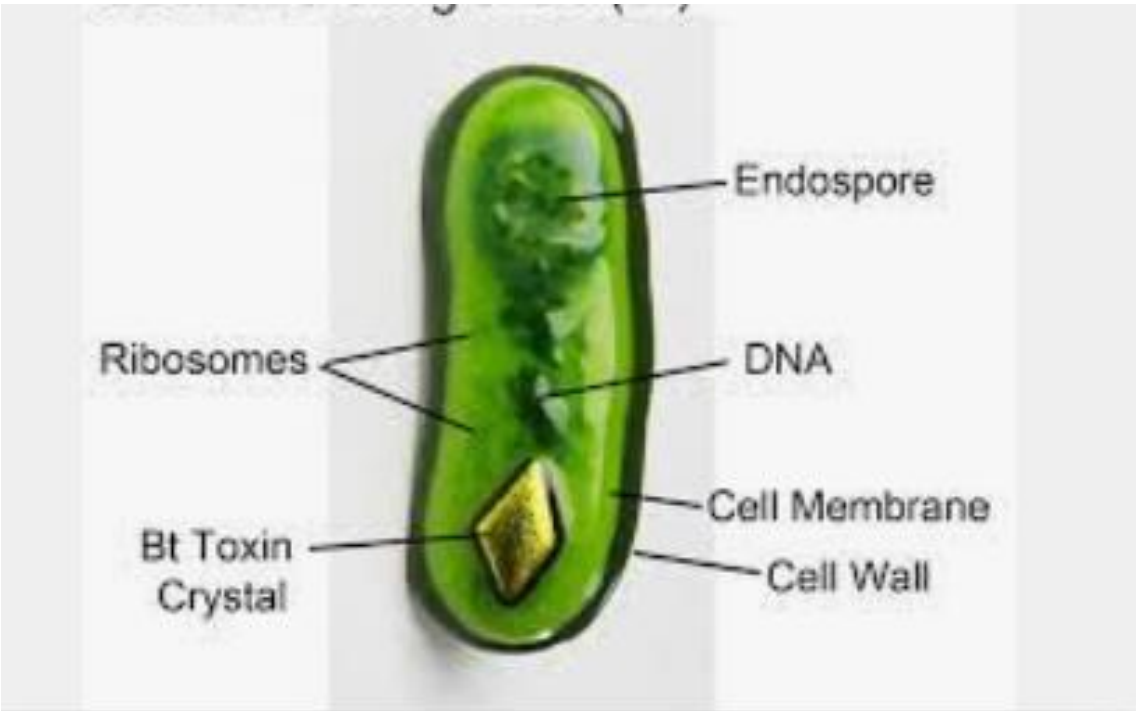

Figure 1.4. General structure of *Bacillus thuringiensis* (Ibrahim, Griko, Junker, & Bulla, 2010a)

While the *Bacillus thuringiensis* genome has nearly 5.4 Mbp long circular chromosome, in addition, they can have many plasmids in the range of mini-, midi-, and mega. Bacteria have been classified so far using serological and biochemical methods for the classification of *Bt* strains. Features are taken into account. Other methods were also used; according to flagella (H), and an crystalline antigens. The classification made according to the data given in (Table1.1) (de Barjac & Frachon, 1990)**.**

Table 1.1. Classification of *Bacillus thuringiensis* strains

H antigen	Serovar	Abbrevia	First Source and First Correct Description
1	Thuringiensis	THU	Berliner, 1915 ; Heimpei & Angus, 1958
2	Finitimus	FIN	Heimpel & Angus, 1958
3a	Alesti	ALE	Toumanoff & Vago, 1951; Heimpel &Angus, 1958
3a3b	Kurstaki	KUR	de Barjac & Lemille, 1970
4a4b	Sotto	SOT	Ishiwata, 1905 ; Heimpel & Angus, 1958
4a4c	Kenyae	KEN	Bonnefoi & de Barjac, 1963
5a5b	Galleriae	GAL	Shvetsova, 1959, de Barjac & Bonnefoi, 1962
5a5c	Canadensis	CAN	De Barjac & Bonnefoi, 1972
6	Entomocidus	ENT	Heimpel & Angus, 1958
7	Aizawai	AIZ	Bonnefoi & de Barjac, 1963
8a8b	Morrisoni	MOR	Bonnefoi & de Barjac, 1963
8a8c	Ostriniae	OST	Gaixin, Ketian, Minghua & Wingmin, 1975
8b8d	Nigeriensis	NIG	De Barjac, Frachon, Rajagopalan & Cosmao, not published
9	Tolworthi	TOL	Norris, 1964 ; de Barjac & Bonnefoi, 1968
10	Darmstadiensis	DAR	Krieg, de Barjac & Bonnefoi, 1968
11a11b	Toumanoffi	TOU	Krieg, 1969
11a11c	Kyushuensis	KYU	Ohba & Aizawa, 1979
12	Thompsoni	THO	De Barjac & Thompson, 1970
13	Pakistani	PAK	De Barjac, Cosmao, Shaik & Viviani, 1977
14	Israelensis	ISR	De Barjac, 1978
15	Dakota	DAK	De Lucca, Simonson & Larson, 1979
16	Indiana	IND	De Lucca, Simonson & Larson, 1979
17	Tohokuensis	TOH	Ohba, Aizawa & Shimizu, 1981
18	Kumamotoensis	KUM	Ohba, Ono, Aizawa & lwanami, 1981
19	Tochigiensis	TOC	Ohba, Ono, Aizawa & lwanami, 1981
20a20b	Yunnanensis	YUN	Wanoyu, Qi-fang, Xue-ping & You-wei, 1979
20a20c	Pondicheriensis	PON	De Barjac, Frachon, Rajagopalan & Cosmao, Not published
21	Colmeri	COL	De Lucca, Palmgren & de Barjac, 1984
22	Shandongiensis	SHA	Ying, Jie & Xichang, 1986
23	Japonensis	JAP	Ohba & Aizawa, 1986
24	Neolenensis	NEO	Dulmage, Tamez-Guerra & Roman Calderon,1988
25	Coreanensis	COR	De Barjac &Lee, not published.
26	Silo	SIL	De Barjac &Lee, not published.
27	Mexicanensis	MEX	Rodriguez-Padilla & Galan-Wong, 1988

1.3.4. Mechanism Action of Insecticidal Proteins

When *B. thuringiensis* sporulates, they produce crystals of proteinaceous insecticidal delta endotoxins: crystal proteins, also known as Cry proteins, which are encoded by *cry* genes, and Cyt proteins, which are expressed by *cyt* genes. It is known that Cry toxins

have particular anti-insect activity against species of the orders Lepidoptera, Diptera, Coleoptera and Hymenoptera as well as anti-nematode activity. In this way, *Bt* is a key source of Cry toxins, which are used in the manufacturing of biological pesticides and insect-resistant genetically engineered crops. When insects swallow crystal toxins, their alkaline digestive tracts denature the insoluble crystals, rendering them soluble and therefore suitable to being cut by proteases present in the insect gut, which liberates the toxin from the crystal and releases it into the environment. Cry toxin is then introduced into the insect gut cell membrane, paralyzing the digestive system and causing it to rupture, creating porous structures. Live *Bt* bacteria may colonise in midgut and cause it to starve to death; but, dead *Bt* sporesmay also colonise the insect and cause it to die. Death may occur within a few hours or as long as many weeks. The midgut bacteria of sensitive larvae may be necessary for the insecticidal action of *Bt* toxins (Crickmore et al., 2014) (Figure 1.5)

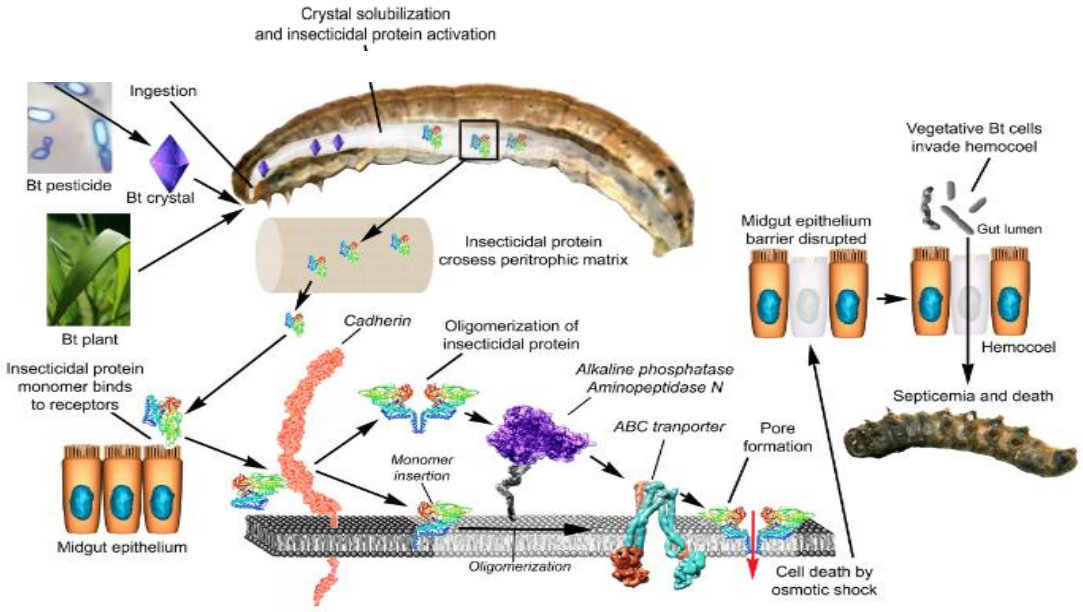

Figure 1.5. Mechanism of Action of Cry Proteins According to the Sequential Binding Model (Fernández-Chapa, Ramírez-Villalobos, & Galán-Wong, 2019)

BtsR1, a short RNA produced by *Bt* that binds to the RBS site of the Cry5Ba toxin transcript, may suppress the production of the Cry5Ba toxin while the nematode is outside the host, allowing the worm to escape behavioral defenses. Following ingestion, the expression of BtsR1 is lowered, leading to the synthesis of Cry5Ba toxin and the

death of the host. It was revealed in 1996 that Unlike Cry proteins, VIP proteins do not share sequence homology with them, and therefore do not compete for the same receptors in most cases. Additionally, certain VIP proteins kill different insects other than Cry proteins (Schnepf et al., 1998).

Bt strains that were not insecticidal were found to have a unique subtype of Cry proteins, termed parasporins, which were identified in 2000. It is described as non-hemolytic but have the ability to kill cancer cells selectively (Wei et al., 2003).

1.3.4.1. Using *Bt* Spore-Crystals in Pest Management

Insect pest management has relied on spores and crystalline insecticidal proteins produced by the bacteria *Bacillus thuringiensis* since 1920s, and these products are often sprayed as liquid sprays. Currently, they are being utilized as particular insecticides under many brand names. As a result of their specificity, these pesticides are considered environmentally friendly, having little or no effect on humans, wildlife, pollinators, and most other beneficial insects; however, the product manuals for these products contain numerous environmental and human health warnings and a 2012 European regulatory peer review of five approved strains found that, while data exists to support some claims of low toxicity to humans and the environment, the data does not support all of them (Bravo, Gill, & Soberón, 2007).

It takes time for new strains of *Bt* to be developed and introduced because insects develop resistance to *Bt*, or because there is a desire to force mutations in organism characteristics, or to use homologous recombinant genetic engineering to improve crystal size and pesticidal activity, or to broaden the host range of *Bt* and obtain more effective formulations, etc. According to the United States Environmental Protection Agency, each new strain is assigned a unique number and registered with the agency, and allowances for genetic modification may be granted based on "its parental strains, the proposed pesticide use pattern, the manner, and extent to which the organism has been genetically modified." The Organic Materials Review Institute (OMRI) maintains a list of *Bt* formulations that have been certified for organic farming in the United States, and some university extension websites provide information on how to utilize *Bt*

spore or protein preparations in organic farming (García-Gutiérrez, Poggy-Varaldo, Esparza-García, Ibarra-Rendón, & Barrera-Cortés, 2011; Schnepf et al., 1998).

1.3.4.2. Use of *Bt* Genes in Genetic Engineering of Plants for Pest Control

By expressing *cry* genes from *Bacillus thuringiensis*, the Belgian business Plant Genetic Systems became the first company to generate genetically engineered crops with insect tolerance. The resultant crops contain delta endotoxin, which was discovered by accident. Despite the fact that *Bt* tobacco was never marketed, tobacco plants are often used to test genetic changes since they are easy to modify genetically and do not form part of the food supply (Xiao & Wu, 2019).

Greater cornstalk borer larvae inflict considerable damage to unprotected peanut leaves (bottom dish), but *Bt* toxins contained in peanut leaves (bottom dish) protect them from this harm (top dish). Potato plants that produce the Cry3A *Bt* toxin were approved as safe by the Environmental Protection Agency in 1985, making it the first human-modified pesticide-producing crop to be approved in the United States, despite the fact that many plants naturally produce pesticides, such as tobacco, coffee plants, cocoa, and black walnut, which were all approved in 1985. This potato was known as the 'New Leaf' and was pulled off the market in 2001 because of a lack of consumer demand (Ibrahim, Griko, Junker, & Bulla, 2010b).

Specifically for the Brazilian market, Monsanto created a soybean that expressed Cry1Ac as well as the glyphosate-resistance gene, which successfully passed the Brazilian regulatory procedure in 2010. *Bt*-modified aspens, notably Populus hybrids, have been produced. However, they have less leaf damage due to insect herbivory. However, not all outcomes have been positive, as shown by the following: One of their main pests continues to feast on the transgenic trees, and on top of that, their leaf litter decomposes differently due to the transgenic toxins, changing the aquatic insect populations in the surrounding area, preventing the expected consequence of enhanced timber production from being realized (Jouzani, Valijanian, & Sharafi, 2017).

1.4. Previous Studies

A *Bacillus thuringiensis* strain was identified from *Hyphantria cunea*larvae that had been infected. The findings of the bioassay revealed that *Bt* has an insecticidal effect against the larvae of *H. cunea*. Furthermore, the Cry9Ea10 protein produced by the *Bt* engineering was shown to be very hazardous to *H. cunea* larvae (Zhao et al., 2016).

By using logand blots and mass spectrometry, the researchers discovered a possible Cry1Ab toxin-binding protein, an APN isoform called HcAPN3, in the midgut of the *H. cunea*. In all larval developmental stages, HcAPN3 was found to be strongly expressed, and it was particularly abundant in the midgut and hindgut tissues. HcAPN3 expression was strongly down-regulated at 6 hours after Cry1Ab toxin treatment and subsequently significantly up-regulated at 12 hours and 24 hours after Cry1Ab toxin treatment. We produced HcAPN3 in insect cells and used logand blot tests to determine if it interacted with the Cry1Ab toxin. Furthermore, RNA interference against HcAPN3 utilizing oral administration and injection of dsRNA resulted in a 61–66 percent drop in transcript levels when compared to the control group. The down-regulation of HcAPN3 expression was shown to be closely linked with the lower sensitivity of *H. cunea* to Cry1Ab in one study. Furthermore, the HcAPN3E fragment peptide produced in *E. coli* increased the toxicity of Cry1Ab against *H. cunea* larvae when administered to the larvae. According to the findings, an APN in *H. cunea* is a potential binding protein that is implicated in Cry1Ab sensitivity in humans (Zhang et al., 2017).

The findings revealed that when *B. thuringiensis* and plant extracts were fed orally to *H. cunea*, they had an effect on the digestive enzyme profiles of the organism. A combination of *B. thuringiensis, A. annua*, and *L. stoechas*extracts on mulberry lowered the activity of digestive enzymes in a dose-dependent way, with the exception of glycosidase and lipase, which remained unchanged. In studies where larvae were exposed to varying doses of the insecticides mentioned above, LDH activity was shown to be boosted by *B. thuringiensis* extracts alone and by *B. thuringiensis* and *L. stoechas* extracts when the two extracts were used in combination. The least amount of activity was seen in the case of *L. stoechas* extracts applied to both hosts individually (Zibaee, Bandani, Sendi, Talaei-Hassanloei, & Kouchaki, 2010).

Entomopathogenic bacteria are capable of producing several protein types that are harmful to a variety of insect, mite, and nematode species when exposed to their toxins. To control insects such as mosquitoes and ants, insecticidal proteins from the Cry and Vip3 families are frequently utilized in prepared sprays and transgenic crops. The advantages of *B. thuringiensis* based products, on the other hand, are jeopardised by the emergence of insect resistance. A large number of studies have shown that mutations in genes encoding surrogate receptors are responsible for giving resistance to *Bt* infection. However, other processes may also contribute to the decline of the efficiency of *Bt* based insecticides in the management of insect pests and even the development of resistance to these pesticides (Pinos, Andrés-Garrido, Ferré, & Hernández-Martínez, 2021).

An increase in the expression of the insect control protein genes of the *Bt* in Populous has been crucial in the production of genetically altered plants with levels of insect resistance that are agronomically acceptable in terms of yield. *Bt* proteins (Cry1Ah1) with high specific toxicity against *Hyphantria cunea* were identified by screening in an indoor bioactivity experiment, and the resulting transgenic poplars were shown to be hyper-resistant. The Cry1Ah1 sequence was then modified and changed to correspond to the optimum codon in poplar using software developed (C. Xu, Wei, Wang, Yin, & Zhuge, 2019). In order to convert poplar NL895, a vector has to be created. Poplar NL895 was transformed with the Cry1Ah1 gene, resulting in the production of six transgenic lines. Cry1Ah1 gene expression and insecticidal activity in transgenic poplar were assessed using PCR, an insecticidal Enzyme-Linked İmmune Sorbent Assay (ELISA), and the specific indoor activity and field insecticidal activity against the pest *H. cunea* were compared with a control. Researchers reported that the insecticidal activity of the transgenic NL895 was much greater against lower instar larvae of *H. cunea* than it was against higher instar larvae, as shown by the results of the study. The mortality and pupation rates of the various instar larvae and between transgenic and non-transgenic poplar were shown to be significantly different (C. Xu et al., 2019).

The effects of three biopesticides, NeemAzal T/S, Laser, and Delphin, on the third larval instar of the fall webworm *H. cunea* were investigated in the laboratory using the fall webworm. There were many different dosages employed in the trial. Laboratory studies revealed that the three dosages of NeemAzal T/S resulted in varying levels of

mortality, with a mortality period ranging from one to seven days for each dose, respectively. The findings of the other biopesticides were similar to those obtained with the first. The highest dosages of all the studied biopesticides resulted in 100 percent death within seven days at the highest concentrations (Saruhan, Akca, & Kushiyev, 2014).

LacZ-HcNPV system was used to generate an insecticidal viral pesticide including the insecticidal protein gene from *Bt* subsp. *kurstaki* HD1. The insecticidal protein gene was obtained from *Bt* subsp. *kurstaki* HD1. Using the HcNPV polyhedrin gene promoter, the ICP gene was put under the control of the HcNPV polyhedrin gene promoter. A polyhedrin-negative virus, designated ICP-HcNPV insecticide, was isolated and characterized. By using Southern hybridization, the researchers were able to determine that the ICP gene had been inserted into the ICP-HcNPV genome. At 5 days after infection with the ICP-HcNPV, a Polyacrylamide Gel Electrophoresis (PAGE) study of *Spodoptera frugiperda* cell extracts revealed that the ICP was expressed in the insect cells as a 130 kDa protein. Aggregates of ICP were found in the cells after they had created it. When extracts from the cells infected with the ICP-HcNPV were given to 20 *Bombyx mori* larvae, the death rate was seen to be as follows: 8 larvae at 1 hour, 10 larvae at 3 hours, and 20 larvae at 12 hours after infection. These findings demonstrate that the baculovirus insecticide induced the *Bt* ICP gene expression in insect cells, resulting in a significant increase in insecticidal activity. We tested the biological activities of the recombinant virus ICP-HcNPV by feeding virus particles and ICP to in According to the findings of the research, The experiment was conducted using six *Bt* strains against *H. cunea* larvae.

The results of the bioassay revealed that the toxicity of these strains was different. The findings showed that the mortality rate of *Hyphantria cunea* increased with the days in all the used types of *Bacillus thuringiensis* with all the used doses. As time progresses, the mortality rate increases; also, as the used dose is high, the mortality rate becomes more.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. *Bacillus thuringiensis* strains

The *Bt* strains *Bt* SY49.1, *Bt* SY25.1, *Bt* SY33.3, *Bt* SY56.3, *Bt* SY27.1, and *Bt kurstaki* were kindly supplied by Dr. Semih YILMAZ as stock cultures and used in the present study (Yılmaz, 2010).

2.1.2. Activation of *Bt* strains and obtaining spore-crystal mixtures

Bt strains from the stock culture at -80°C was transferred to a liquid LB medium (pH 6.8±2) and incubated overnight at 37°C at 200 rpm. Then they were cultured in a LB medium a second time to obtain fresh culture and used for sporulation studies.

T3 liquid sporulation medium (pH 6.8±2), containing tryptone (3g), tryptose (2g), yeast extract (1.5g), Manganese chloride ($MnCl_2$, 0.005g), monobasic sodium phosphate (NaH_2PO_4, 6g), and dibasic sodium phosphate (NaH_2PO_4, 7.1g) prepared in 1 liter distilled water were used after autoclaving, to grow and obtain more than 80% sporulation from the bacterial strains. Cultures were incubated at 200 rpm at 37 °C for 4-7 days to achieve at least 80% sporulation.

The sporulated samples were centrifuged at 15.000 rpm at 4ºC for 10 minutes, and the spore-crystal mixture was precipitated and washed twice in 20 mL distilled water and centrifuged again.

2.1.3. The protein extraction

- Bacterial cultures were made in 3 L of T3 medium at 30 °C in a 5 L bioreactor with a volumetric gas transfer coefficient (k_La) of 13.32 h^{-1}. Cells were grown for about 48 h or until approximately complete autolysis had occurred releasing the spores and the toxin crystals in the culture medium.
- The developed purification procedure was first used in an attempt to isolate the spherical crystals of strains.
- The pellet was then suspended in a 50 mL centrifuge tube with a saline solution, in order to enhance the hydrophobic interactions.
- An organic solvent (hexane in this study) was added to a ratio less or equal to 10% (50, 75, or 100 μL/mL of aqueous suspension) to minimize the risk of altering crystals. Finally the pellet was washed twice with cold distilled water (Magnaudet, Toulouse, Mécanique, & Soula, 2012).

Protein quantitation of the products were carried out using the Bradford method (1976). The toxin doses were calculated using the results of this method.

2.1.4. The Insect Pest Used in this Study

In this study, the American white butterfly *Hyphantria cunea* (Drury) larvae were used to determine the effectiveness of previously determined six local *Bacillus thuringiensis* strains toxins and their effect on this pest in different larval stages.

2.1.5. *Hyphantria cunea* Culture

The media for insects was prepared as follows:

Hyphantria cunea larvae were taken from Çayeli, a town located at the Rize Province of the Black Sea coast of Eastern Turkey, 18 km east of the city of Rize. About 4000 larvae of different ages were collected from fields, orchids, hazelnuts and berries. They were placed inside plastic boxes dedicated to collecting larvae, which are sterilized and prepared for storing larvae inside (Figure 2.1).

Figure 2.1. Hyphantria cunea larvae

2.1.6. *Hyphantria cunea* Culture

The insect larvae were kept in the laboratory in boxes designated at 25±2°C, 60±10% of relative humidity, and 12 hours of light and dark photo period. The pupae were transferred to the insect rearing cages containing filter papers and a honey-walter solutions (10%) was provided as food for the emergining adults. The filter papers carrying eggs were put into glas jar and the larvae were reared on the pesticide-free hazelnut leaves (Figure 2.2).

Figure 2.2. Hyphantria cunea larvae in laboratory

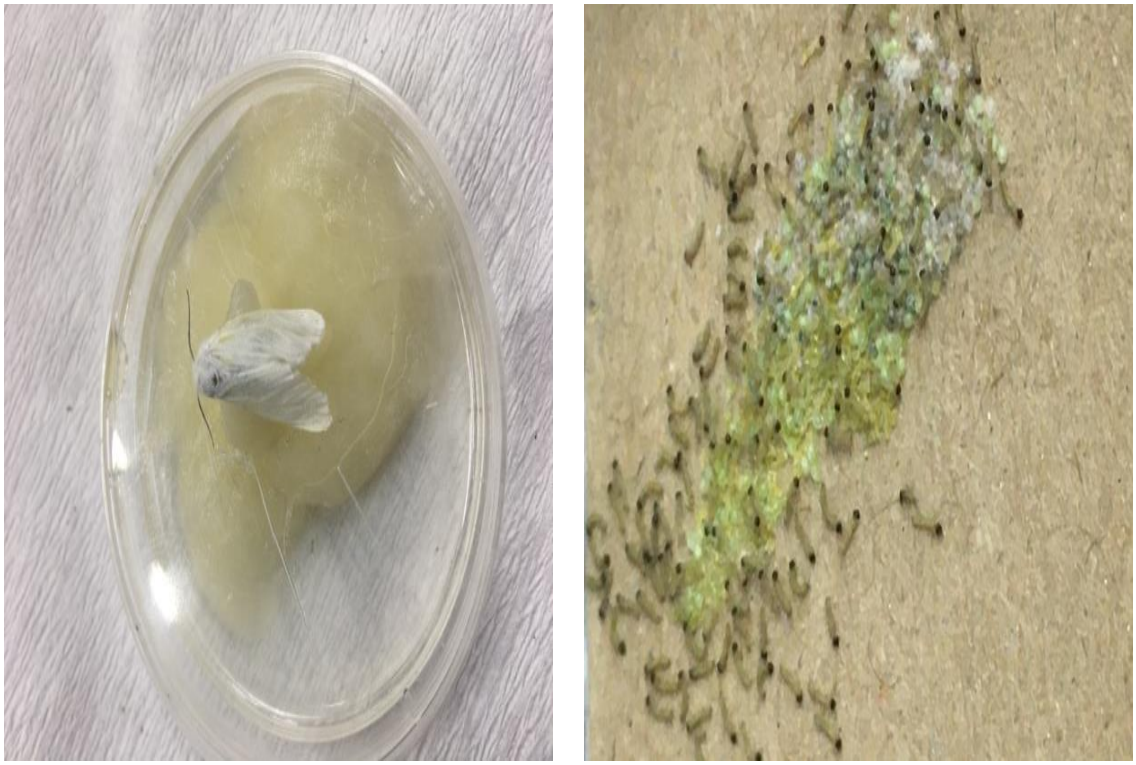

Figure 2.2. Hyphantria cunea larvae in laboratory *(more)*

2.2. Methods

2.2.1. Lethal effect of different strains on *Hyphantria cunea* larvae

The last instar of the larva was used to test the effectiveness of six different strains of *Bt* (*Btk*, *Bt* SY49.1, *Bt* SY25.1, *Bt* SY33.3, *Bt* SY56.3, and *Bt* SY27.1). Toxin complexes of *Bt* strains were applied to the hazelnut leaves at the concentrations of 10, 50, 100, 250, 500, 1000 and 2250 µg/ml. The leaves were placed into Petri dishes (9 cm diameter) containing two filter papers. Then, five last instar larvae were transferred to Petri dishes. The experiment repeated twice on a different date. There were four replication for each treatment (Figure. 2.3). Then Petri dishes were sealed with a parafilm and kept under controlled conditions (25±2°C, 60 ±10% of relative humidity, and 24 hours of light and dark photoperiod) (Figure 2.3)..

Figure 2.3. Hyphantria cunea larvae in petri dishes with toxins

Mortality of the larvae were recorded Daily for ten days. LD_{50} and LD_{99} values were calculated using probit analysis. During the period of conducting the experiment, work was carried out inside the laboratories of the Faculty of Agriculture/Erciyes University and the data were analyzed using SPSS Statistics Software (Version 26) (Figure 2.4).

Figure 2.4. Effects of *Bt* toxin on *Hyphantria cunea* larvae

2.2.2. The lethal effect of a mixture of different strains with spores on *Hyphantria cunea* larvae

The last instar of the larva was used to test the effectiveness of three different strains of *Bt* (*Btk*, *Bt* SY49.1 and *Bt* SY27.1). Toxine complexes of *Bt* strains were applied to the hazelnut leaves at the concentrations of 10, 50, 100, 250, 500, 1000 and 2250 μg/ml. Mixture of spores were sprayed on plant leaves and placed inside dishes containing five larvae with four replications for each dose. Lethal effects were determined and for each dose and were compared with the control. The LD_{50} and LD_{99} were calculated using probit analysis after a ten-day review of the values (Figure 2.5).

Figure 2.5. Application of *Bt* spore mixture of *Hyphantria cunea* larvae

2.2.3. The lethal effect of a mixture of different *Bt* strains with spore-crystal on *Hyphantria cunea* larvae

Three bacterial strains such as (*Btk*, *Bt* SY49.1 and *Bt* SY27.1) were introduced to the third and fourth instar larvae (L3, L4), a mixture of crystals and spores and crystal-only were also introduced to L3 and L4 seperatly. It was applied as follows: 50, 250, 1000 µg/ml on subsequently once and the crystal mixture was added to three doses again for each growth stage mentioned previously. Spores of the mixture were sprayed on the plant leaves and placed in dishes containing ten larvae with three replications for each dose. Lethal effects were determined and compared with the control. LD_{50} and LD_{99} values were evaluated using probit analysis after a ten-day review (Figure 2.6).

Figure 2.6. Application of *Bt* spore crystal mixture on *Hyphantria cunea* larvae

CHAPTER 3

RESULTS

3.1. The Apparent Effects of the Six *Bacillus thuringiensis* Strains on *Hyphantria cunea* Larvae

In general, mortality increased with increasing times in all strains used in this study and this was in comformity with all doses as well. The higher the dose used, the higher the mortality rate.

The strain *Bt* SY25.1 showed that the lowest mortality rate was 5% for the second dose and the highest mortality rate was 80% for the fourth and seventh doses as displayed in Table 3.1. The strain *Bt* SY27.1 showed 100% mortality rate for the fourth and seventh doses. The highest mortality rate recorded for *Bt* SY33.3 was 95% for the seventh dose. *Bt* SY49.1 had 100% mortality rate for the second, third, fourth, fifth, sixth and seventh doses. *Btk* achieved 100% mortality rate for the third dose.

Bt SY56.3 showed lower mortality rates compared to the other strains, while *Bt* SY27.1 and *Bt* SY49.1 showed higher mortality rates among the strains used (Table 3.1).

Table 3.1. Daily mortality rate of *Bt* strains for 10 days and seven different döşe concentrations

Bt Protein	Dose	Days After Treatment									
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Bt SY25.1	10 µg/ml	**10**	**30**	**40**	**40**	**45**	**45**	**45**	**45**	**45**	**45**
	50 µg/ml	**5**	**25**	**50**	**50**	**65**	**65**	**70**	**70**	**70**	**70**
	100 µg/ml	**15**	**40**	**45**	**60**	**70**	**70**	**75**	**75**	**75**	**75**
	250 µg/ml	**40**	**50**	**65**	**70**	**75**	**75**	**80**	**80**	**80**	**80**
	500 µg/ml	**40**	**45**	**45**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
	1000 µg/ml	**25**	**30**	**30**	**60**	**70**	**70**	**70**	**75**	**75**	**75**
	2250 µg/ml	**30**	**65**	**65**	**75**	**75**	**75**	**75**	**75**	**80**	**80**
Bt SY27.1	10 µg/ml	**10**	**25**	**50**	**70**	**85**	**100**	**100**	**100**	**100**	**100**
	50 µg/ml	**0**	**30**	**60**	**90**	**95**	**95**	**95**	**95**	**95**	**95**
	100 µg/ml	**5**	**30**	**50**	**85**	**100**	**100**	**100**	**100**	**100**	**100**
	250 µg/ml	**15**	**25**	**90**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	500 µg/ml	**15**	**25**	**80**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	1000 µg/ml	**20**	**25**	**90**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	2250 µg/ml	**20**	**55**	**80**	**95**	**100**	**100**	**100**	**100**	**100**	**100**
Bt SY33.3	10 µg/ml	**10**	**35**	**45**	**50**	**60**	**60**	**60**	**60**	**60**	**60**
	50 µg/ml	**30**	**30**	**30**	**35**	**40**	**40**	**45**	**45**	**45**	**45**
	100 µg/ml	**5**	**5**	**5**	**30**	**30**	**35**	**35**	**35**	**35**	**35**
	250 µg/ml	**20**	**30**	**30**	**40**	**55**	**55**	**55**	**55**	**55**	**55**
	500 µg/ml	**5**	**30**	**30**	**50**	**50**	**60**	**60**	**60**	**65**	**65**
	1000 µg/ml	**25**	**40**	**50**	**60**	**75**	**75**	**75**	**80**	**80**	**80**
	2250 µg/ml	**0**	**30**	**40**	**60**	**85**	**90**	**90**	**90**	**95**	**95**
Bt SY49.1	10 µg/ml	**0**	**0**	**30**	**35**	**40**	**45**	**50**	**55**	**55**	**55**
	50 µg/ml	**0**	**25**	**70**	**95**	**95**	**95**	**100**	**100**	**100**	**100**
	100 µg/ml	**5**	**40**	**80**	**95**	**100**	**100**	**100**	**100**	**100**	**100**
	250 µg/ml	**5**	**30**	**50**	**95**	**100**	**100**	**100**	**100**	**100**	**100**
	500 µg/ml	**15**	**35**	**80**	**90**	**100**	**100**	**100**	**100**	**100**	**100**
	1000 µg/ml	**15**	**35**	**95**	**95**	**100**	**100**	**100**	**100**	**100**	**100**
	2250 µg/ml	**15**	**25**	**85**	**95**	**95**	**100**	**100**	**100**	**100**	**100**
Bt SY56.3	10 µg/ml	**15**	**30**	**75**	**90**	**95**	**95**	**95**	**100**	**100**	**100**
	50 µg/ml	**0**	**25**	**65**	**80**	**90**	**95**	**95**	**95**	**95**	**95**
	100 µg/ml	**0**	**15**	**80**	**90**	**95**	**95**	**95**	**95**	**95**	**95**
	250 µg/ml	**0**	**5**	**30**	**70**	**80**	**85**	**85**	**90**	**90**	**90**
	500 µg/ml	**0**	**20**	**40**	**55**	**60**	**70**	**70**	**70**	**75**	**80**
	1000 µg/ml	**5**	**15**	**30**	**55**	**60**	**65**	**70**	**70**	**70**	**70**
	2250 µg/ml	**0**	**0**	**25**	**30**	**35**	**40**	**40**	**50**	**55**	**60**
Btk	10 µg/ml	**5**	**10**	**15**	**25**	**30**	**40**	**40**	**45**	**45**	**55**
	50 µg/ml	**0**	**0**	**0**	**0**	**0**	**5**	**10**	**15**	**15**	**35**
	100 µg/ml	**5**	**35**	**40**	**60**	**65**	**95**	**95**	**95**	**95**	**100**
	250 µg/ml	**0**	**0**	**20**	**60**	**60**	**70**	**70**	**70**	**80**	**80**
	500 µg/ml	**0**	**0**	**30**	**50**	**55**	**65**	**65**	**65**	**75**	**75**
	1000 µg/ml	**5**	**20**	**40**	**65**	**75**	**85**	**85**	**85**	**85**	**85**
	2250 µg/ml	**10**	**25**	**45**	**85**	**90**	**90**	**95**	**95**	**95**	**95**

3.2. The Lethal Effect of *Bacillus thuringiensis* Strains on *Hyphantria cunea* Larvae During ten days

On the first day, *Bt* SY25.1 indicated significant increases and differences compared to *Bt* SY27.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk.* The differences were more than LCD = 12.895 except for *Bt* SY33.3. The other strains did not show any statistically significant differences between them because their value was less than LCD = 12.895, where as the lowest mortality rate in *Bt* SY56.3 was in the second, third, fourth, fifth and seventh doses. The highest mortality rate was in *Bt* SY25.1 in fourth and fifth doses, as shown in (Table 3-2, Figure 3-1).

Table 3.2. Percent mortality rates of the Cry proteins at different doses on *H. cunea* larvae on the first day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**10**	**5**	**15**	**40**	**40**	**25**	**30**	**23.57**
***Bt* SY27.1**	**10**	**0**	**5**	**15**	**15**	**20**	**20**	**12.14**
***Bt* SY33.3**	**10**	**30**	**5**	**20**	**5**	**25**	**0**	**13.57**
***Bt* SY49.1**	**0**	**0**	**5**	**5**	**15**	**15**	**15**	**7.86**
***Bt* SY56.3**	**15**	**0**	**0**	**0**	**0**	**5**	**0**	**2.86**
Btk	**5**	**0**	**5**	**0**	**0**	**5**	**10**	**3.57**
Mean Dose	**8.33**	**5.83**	**5.83**	**13.33**	**12.5**	**15.83**	**12.5**	
LSD strains		**4.874**	**LSD** Dose		**5.264**	**LSD** strains*Dose		**12.895**

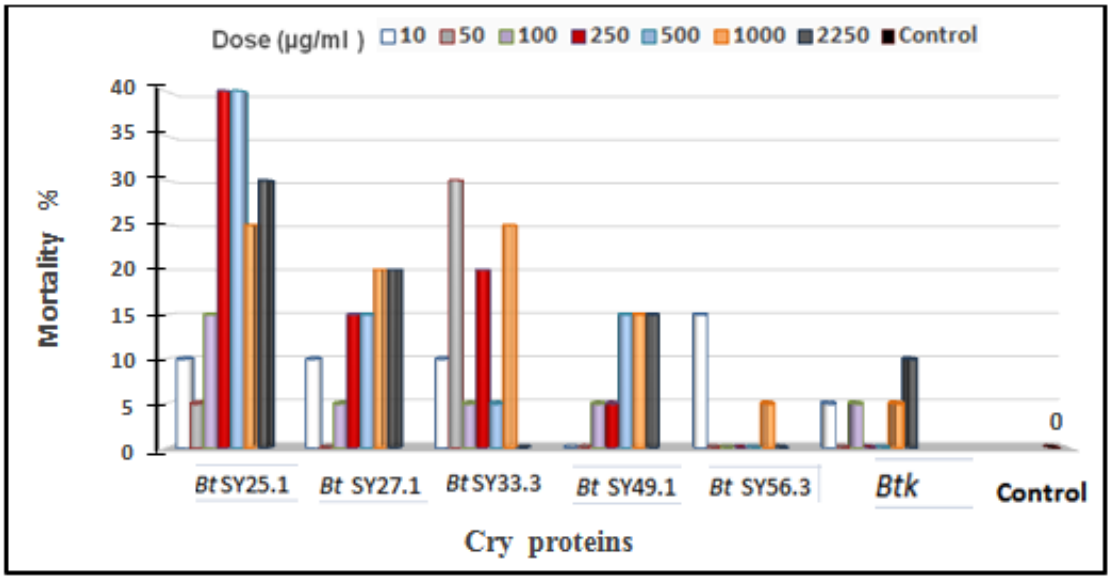

Figure 3.1. Relationship between the doses and mortality rates on *H. cunea* larvae on the first day.

On the second day, *Bt* SY25.1 showed positive significant differences compared to *Bt* SY56.3 and *Btk*. The differences were more than LCD = 18.15 except for *Bt* SY27.1, *Bt* SY33.3, *Bt* SY49.1, while the other strains did not show any significant differences between them because their value was less than LCD = 18.15. Where as the lowest mortality rate was in the *Btk* in the second, fourth and fifth doses, the highest mortality rate was in *Bt* SY25.1 in the seventh dose (Table 3-3, Figure 3-2).

Table 3.3. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the second day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**30**	**25**	**40**	**50**	**45**	**30**	**65**	**40.7**
***Bt* SY27.1**	**25**	**30**	**30**	**25**	**25**	**25**	**55**	**30.7**
***Bt* SY33.3**	**35**	**30**	**5**	**30**	**30**	**40**	**30**	**28.6**
***Bt* SY49.1**	**0**	**25**	**40**	**30**	**35**	**35**	**25**	**27.1**
***Bt* SY56.3**	**30**	**25**	**15**	**5**	**20**	**15**	**0**	**15.7**
Btk	**10**	**0**	**35**	**0**	**0**	**20**	**25**	**12.9**
Mean Dose	**21.7**	**22.5**	**27.5**	**23.3**	**25.8**	**27.5**	**33.3**	
LSD strains		**6.86**	**LSD Dose**		**7.41**	**LSD strains*Dose**		**18.15**

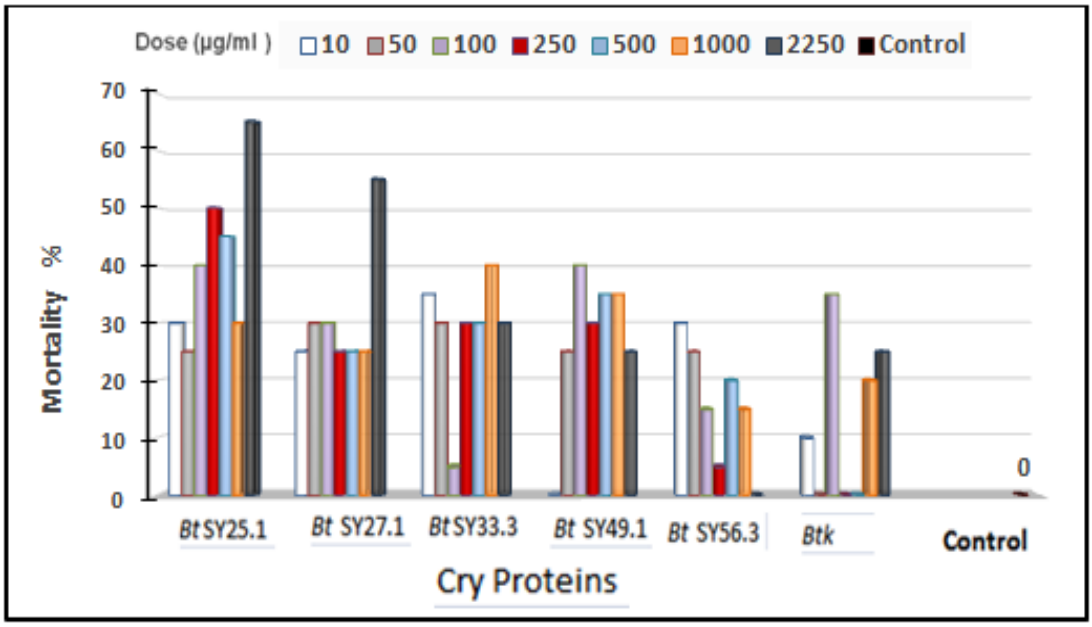

Figure 3.2. Relationship between the doses and mortality rates on *H. cunea* larvae on the second day.

On the third day, *Bt* SY27.1 revealed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk*. The differences were more than LCD = 20.97. The lowest mortality rate was in *Btk* in the second dose, while the highest mortality rate was in *Bt* SY27.1 in fourth, and sixth doses (Table 3-4, Figure 3-3).

Table 3.4. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the third day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**40**	**50**	**45**	**65**	**45**	**30**	**65**	**48.6**
***Bt* SY27.1**	**50**	**60**	**50**	**90**	**80**	**90**	**80**	**71.4**
***Bt* SY33.3**	**45**	**30**	**5**	**30**	**30**	**50**	**40**	**32.9**
***Bt* SY49.1**	**30**	**70**	**80**	**50**	**80**	**95**	**85**	**70**
***Bt* SY56.3**	**75**	**65**	**80**	**30**	**40**	**30**	**25**	**49.3**
Btk	**15**	**0**	**40**	**20**	**30**	**40**	**45**	**27.1**
Mean Dose	**42.5**	**45.8**	**50**	**47.5**	**50.8**	**55.8**	**56.7**	
LSD strains		**7.93**	**LSD $_{Dose}$**		**8.56**	**LSD strains$_{*Dose}$**		**20.97**

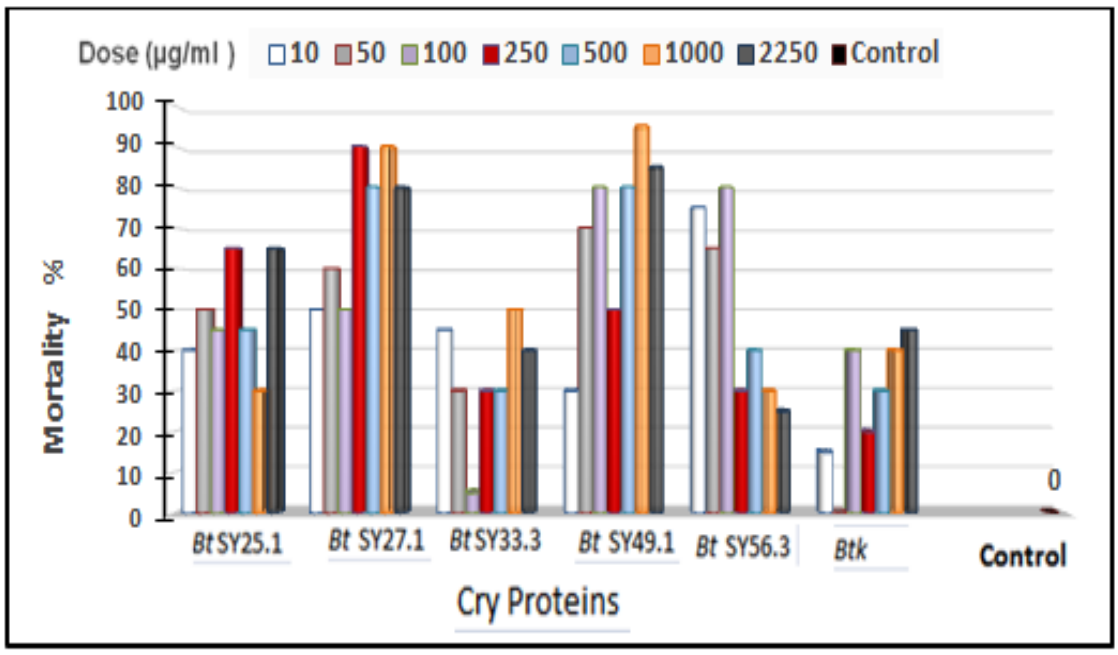

Figure 3.3. Relationship between the doses and mortality rates on *H. cunea* larvae on the third day.

On the fourth day, *Bt* SY27.1 showed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk*. The differences were more than

LCD = 19.99. The lowest mortality rate was recorded in *Bt* SY33.3 in the third dose, while the highest mortality rate was in *Bt* SY27.1 in the fourth, fifth and sixth doses (Table 3-5, Figure 3-4).

Table 3.5. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the fourth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**40**	**50**	**60**	**70**	**50**	**60**	**75**	**57.9**
***Bt* SY27.1**	**70**	**90**	**85**	**100**	**100**	**100**	**95**	**91.4**
***Bt* SY33.3**	**50**	**35**	**30**	**40**	**50**	**60**	**60**	**46.4**
***Bt* SY49.1**	**35**	**95**	**95**	**95**	**90**	**95**	**95**	**85.7**
***Bt* SY56.3**	**90**	**80**	**90**	**70**	**55**	**55**	**30**	**67.1**
Btk	**25**	**0**	**60**	**60**	**50**	**65**	**85**	**49.3**
Mean Dose	**51.7**	**58.3**	**70**	**72.5**	**65.8**	**72.5**	**73.3**	
LSD strains		**7.55**	**LSD $_{Dose}$**		**8.16**	**LSD strains$_{*Dose}$**		**19.99**

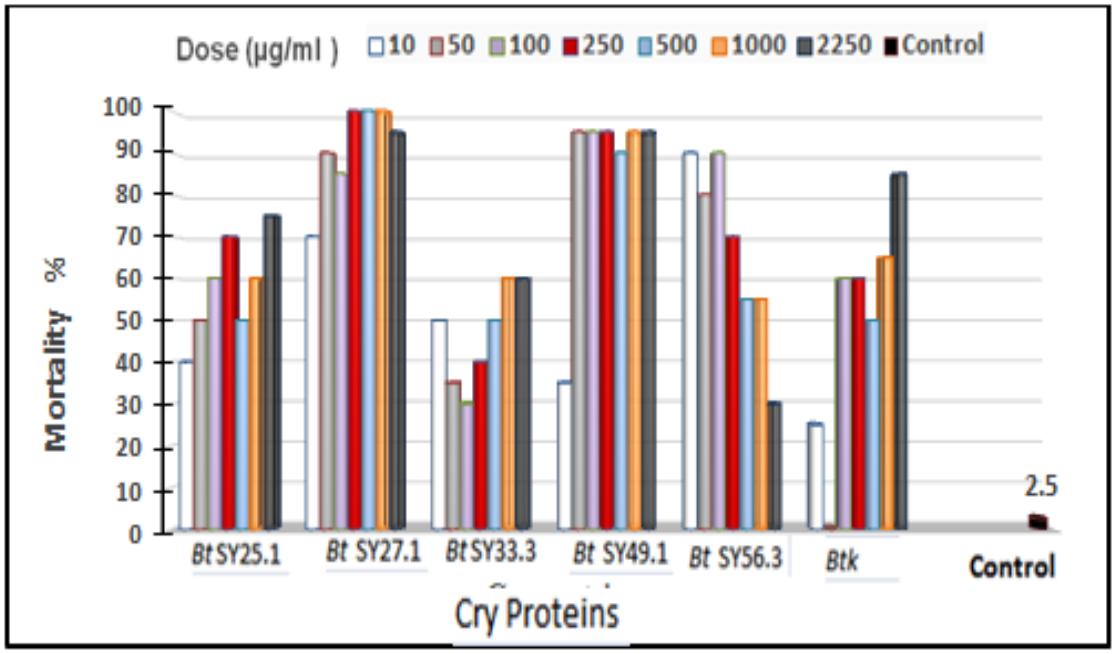

Figure 3.4. Relationship between the doses and mortality rates on *H. cunea* larvae on the fourth day.

On the fifth day, *Bt* SY27.1 showed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk*. The differences were more than LCD = 19.11. Where as the lowest mortality rate was recorded in *Btk* in second dose,

the highest mortality rate was in *Bt* SY27.1 in third, fourth, fifth, sixth and seventh doses (Table 3-6, Figure 3-5).

Table 3.6. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the fifth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**45**	**65**	**70**	**75**	**50**	**70**	**75**	**64.3**
***Bt* SY27.1**	**85**	**95**	**100**	**100**	**100**	**100**	**100**	**97.1**
***Bt* SY33.3**	**60**	**40**	**30**	**55**	**50**	**75**	**85**	**56.4**
***Bt* SY49.1**	**40**	**95**	**100**	**100**	**100**	**100**	**95**	**90**
***Bt* SY56.3**	**95**	**90**	**95**	**80**	**60**	**60**	**35**	**73.6**
Btk	**30**	**0**	**65**	**60**	**55**	**75**	**90**	**53.6**
Mean Dose	**59.2**	**64.2**	**76.7**	**78.3**	**69.2**	**80**	**80**	
LSD strains		**7.22**	**LSD $_{Dose}$**		**7.8**	**LSD strains$_{*Dose}$**		**19.11**

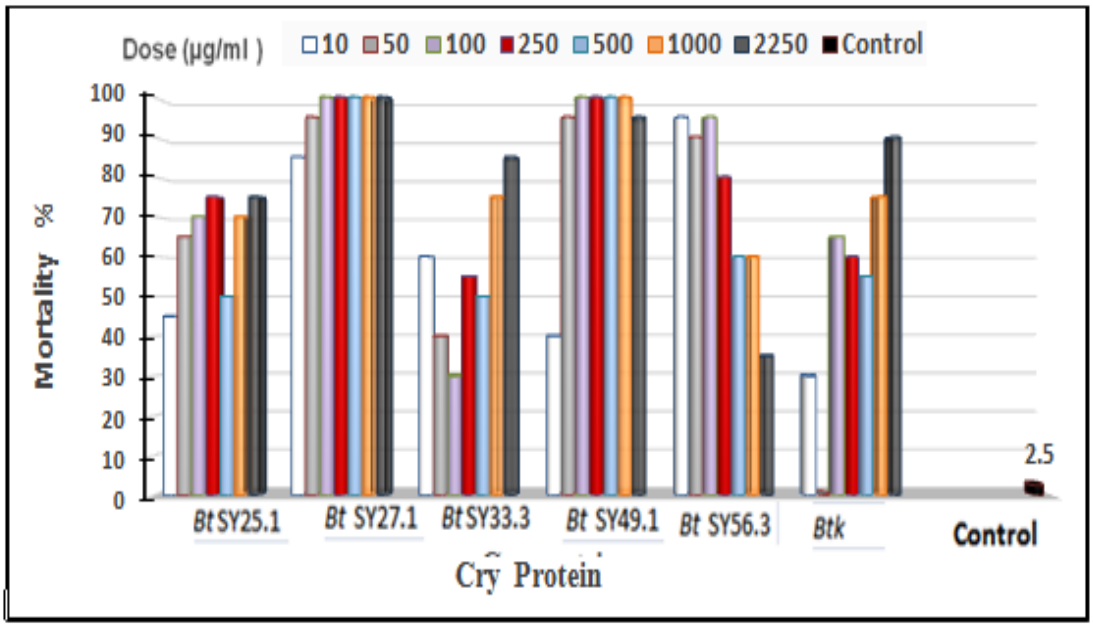

Figure 3.5. Relationship between the doses and mortality rates on *H. cunea* larvae on the fifth day

On the sixth day, *Bt* SY27.1 showed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk* (LCD = 17.849). While the lowest mortality rate was in *Bt* SY3.33 in third dose, the highest mortality rate was in *Bt* SY27.1 in first, third, fourth, fifth, sixth and seventh doses (Table 3-7, Figure 3-6).

Table 3.7. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the sixth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**45**	**65**	**70**	**75**	**50**	**70**	**75**	**64.29**
***Bt* SY27.1**	**100**	**95**	**100**	**100**	**100**	**100**	**100**	**99.29**
***Bt* SY33.3**	**60**	**40**	**35**	**55**	**60**	**75**	**90**	**59.29**
***Bt* SY49.1**	**45**	**95**	**100**	**100**	**100**	**100**	**100**	**91.43**
***Bt* SY56.3**	**95**	**95**	**95**	**85**	**70**	**65**	**40**	**77.86**
Btk	**40**	**5**	**95**	**70**	**65**	**85**	**90**	**64.29**
Mean Dose	**64.17**	**65.83**	**82.5**	**80.83**	**74.17**	**82.5**	**82.5**	
LSD strains		**6.746**	**LSD $_{Dose}$**		**7.287**	**LSD strains$_{*Dose}$**		**17.849**

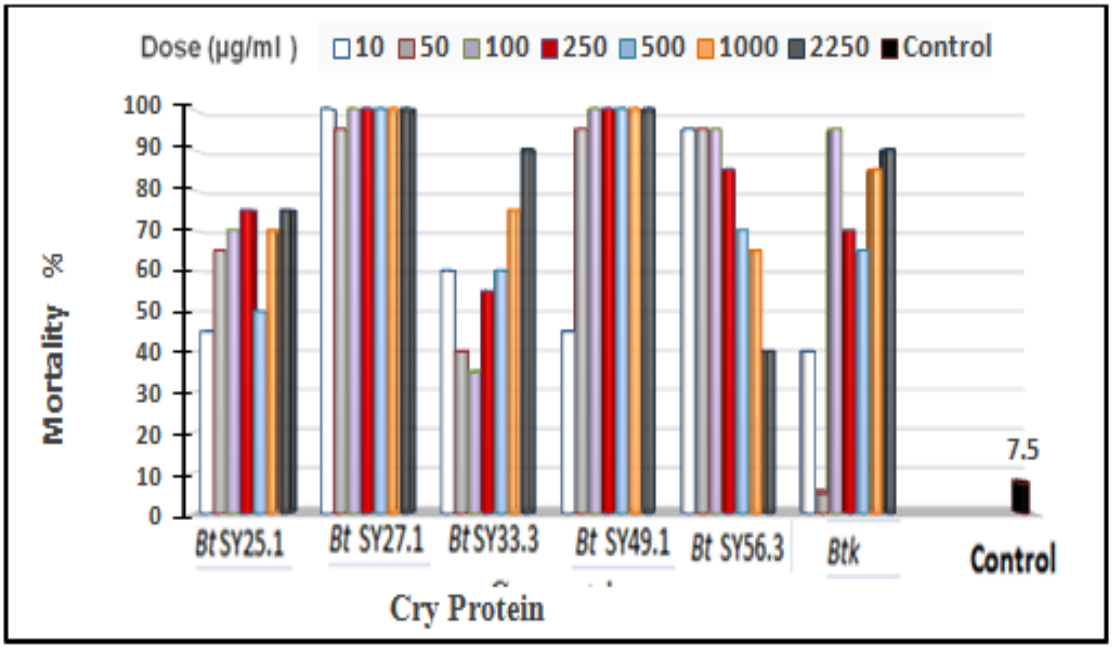

Figure 3.6. Relationship between the doses and mortality rates on *H. cunea* larvae on the sixth day

On the seventh day, *Bt* SY27.1 showed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk* (LCD = 17 .805). The lowest mortality rate was found in *Bt* SY3.33 in third dose, while the highest mortality rate was found in *Bt* SY27.1 in first, third, fourth, fifth, sixth and seventh doses (Table 3-8, Figure 3-7).

Table 3.8. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the seventh day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**45**	**70**	**75**	**80**	**50**	**70**	**75**	**66.43**
***Bt* SY27.1**	**100**	**95**	**100**	**100**	**100**	**100**	**100**	**99.29**
***Bt* SY33.3**	**60**	**45**	**35**	**55**	**60**	**75**	**90**	**60**
***Bt* SY49.1**	**50**	**100**	**100**	**100**	**100**	**100**	**100**	**92.86**
***Bt* SY56.3**	**95**	**95**	**95**	**85**	**70**	**70**	**40**	**78.57**
Btk	**40**	**10**	**95**	**70**	**65**	**85**	**95**	**65.71**
Mean Dose	**65**	**69.17**	**83.33**	**81.67**	**74.17**	**83.33**	**83.33**	
LSD strains		**6.73**	**LSD $_{Dose}$**		**7.269**	**LSD strains$_{*Dose}$**		**17.805**

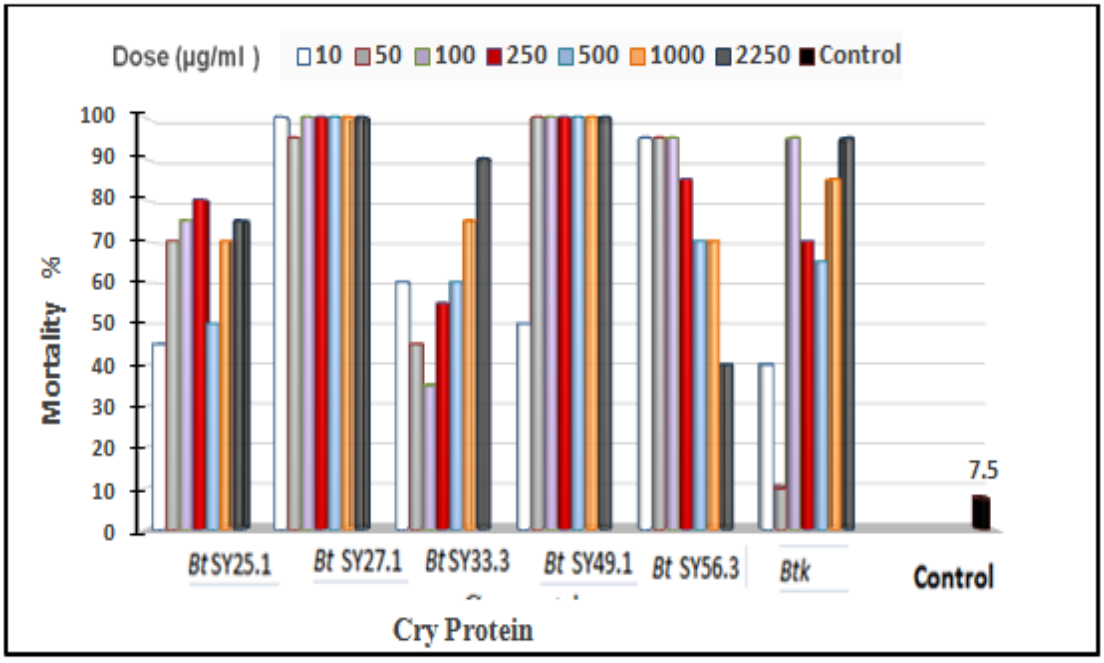

Figure 3.7. Relationship between the doses and mortality rates on *H. cunea* larvae on the seventh day.

On the eighth day, *Bt* SY27.1 displayed positive significant differences compared with *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk* (LCD = 18.108). While the lowest mortality rate was in *Bt* SY3.33 in third dose, the highest mortality rate was in *Bt* SY27.1 in first, third, fourth, fifth, sixth and seventh doses (Table 3-9, Figure 3-8).

Table 3.9. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the eighth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**45**	**70**	**75**	**80**	**50**	**75**	**75**	**67.14**
***Bt* SY27.1**	**100**	**95**	**100**	**100**	**100**	**100**	**100**	**99.29**
***Bt* SY33.3**	**60**	**45**	**35**	**55**	**60**	**80**	**90**	**60.71**
***Bt* SY49.1**	**55**	**100**	**100**	**100**	**100**	**100**	**100**	**93.57**
***Bt* SY56.3**	**100**	**95**	**95**	**90**	**70**	**70**	**50**	**81.43**
Btk	**45**	**15**	**95**	**70**	**65**	**85**	**95**	**67.14**
Mean Dose	**67.5**	**70**	**83.33**	**82.5**	**74.17**	**85**	**85**	
LSD strains		**6.844**	**LSD $_{Dose}$**		**7.393**	**LSD strains$_{*Dose}$**		**18.108**

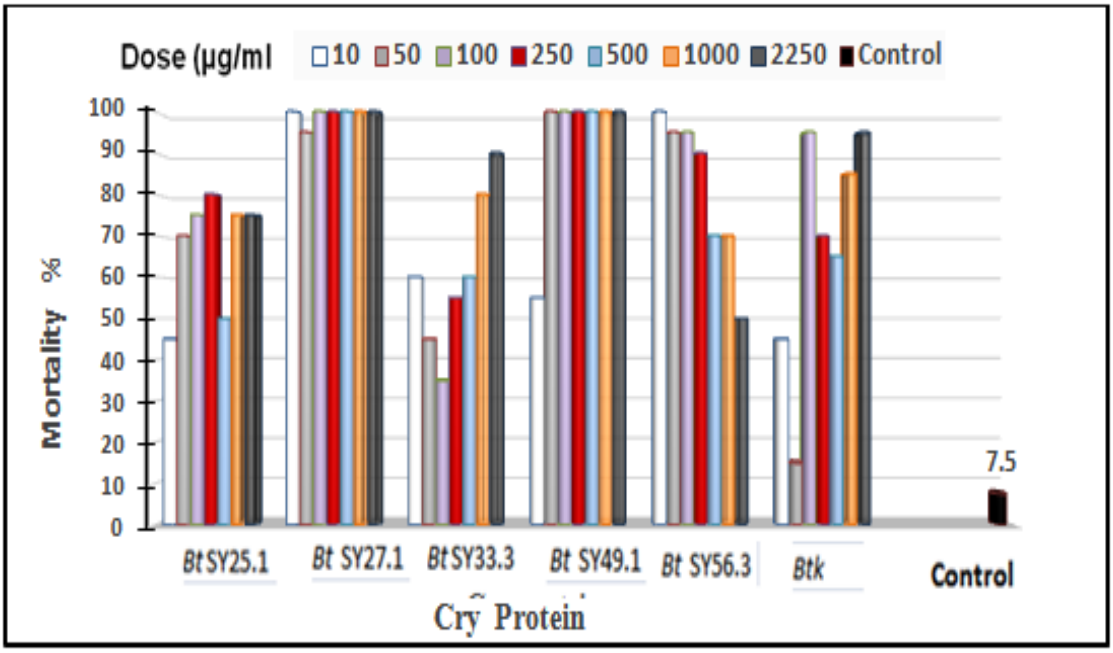

Figure 3.8. Relationship between the doses and mortality rates on *H. cunea* larvae on the eighth day.

On the ninth day, *Bt* SY27.1 showed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk* (LCD = 18.15). While the lowest mortality rate was in *Bt* SY3.33 in the third dose, the highest mortality rate was in *Bt* SY27.1 in the first, third, fourth, fifth, sixth and seventh doses (Table 3-10, Figure 3-9).

Table 3.10. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the ninth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**45**	**70**	**75**	**80**	**50**	**75**	**80**	**67.9**
***Bt* SY27.1**	**100**	**95**	**100**	**100**	**100**	**100**	**100**	**99.3**
***Bt* SY33.3**	**60**	**45**	**35**	**55**	**65**	**80**	**95**	**62.1**
***Bt* SY49.1**	**55**	**100**	**100**	**100**	**100**	**100**	**100**	**93.6**
***Bt* SY56.3**	**100**	**95**	**95**	**90**	**75**	**70**	**55**	**82.9**
Btk	**45**	**15**	**95**	**80**	**75**	**85**	**95**	**70**
Mean Dose	**67.5**	**70**	**83.3**	**84.2**	**77.5**	**85**	**87.5**	
LSD strains		**6.86**	**LSD $_{Dose}$**		**7.41**	**LSD strains$_{*Dose}$**		**18.15**

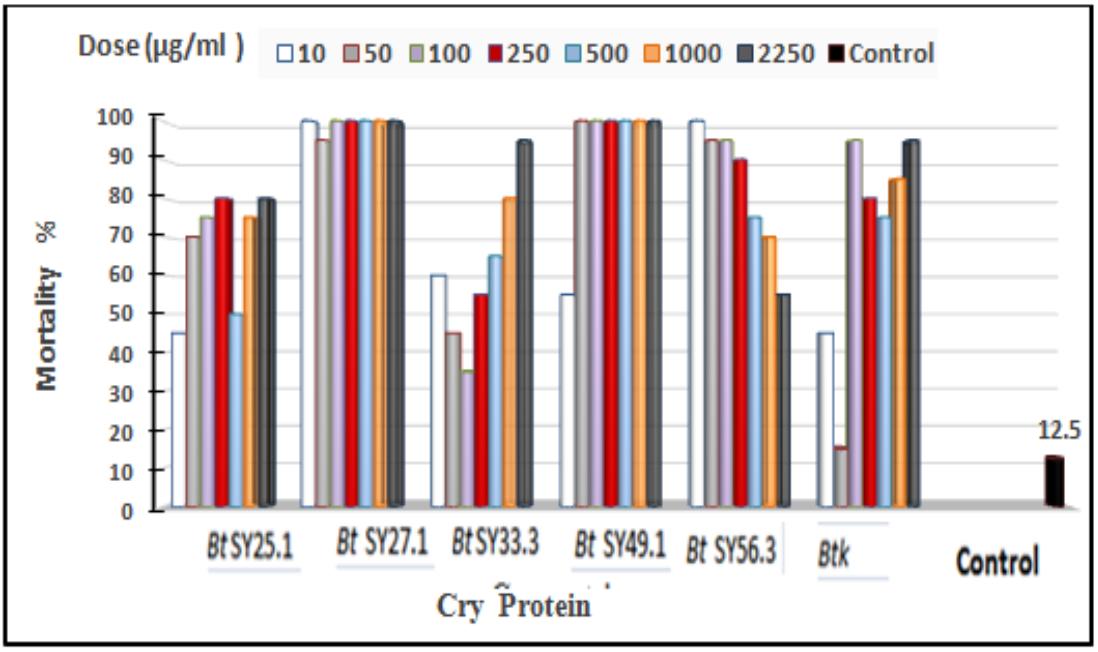

Figure 3.9. Relationship between the doses and mortality rates on *H. cunea* larvae on the ninth day

On the tenth day, *Bt* SY27.1 showed positive significant differences compared to *Bt* SY25.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk* (LCD = 18.45). While the lowest mortality rate was in *Bt* SY3.33 in the third dose, the highest mortality rate was in *Bt* SY27.1 in the first, third, fourth, fifth, sixth and seventh doses (Table 3-11, Figure 3-10).

Table 3.11. Percent mortality rates for the Cry proteins at different doses on *H. cunea* larvae on the tenth day

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY25.1**	**45**	**70**	**75**	**80**	**50**	**75**	**80**	**67.9**
***Bt* SY27.1**	**100**	**95**	**100**	**100**	**100**	**100**	**100**	**99.3**
***Bt* SY33.3**	**60**	**45**	**35**	**55**	**65**	**80**	**95**	**62.1**
***Bt* SY49.1**	**55**	**100**	**100**	**100**	**100**	**100**	**100**	**93.6**
***Bt* SY56.3**	**100**	**95**	**95**	**90**	**80**	**70**	**60**	**84.3**
Btk	**55**	**35**	**100**	**80**	**75**	**85**	**95**	**75**
Mean Dose	**69.2**	**73.3**	**84.2**	**84.2**	**78.3**	**85**	**88.3**	
LSD strains		**6.97**	**LSD $_{Dose}$**		**7.53**	**LSD strains$_{*Dose}$**		**18.45**

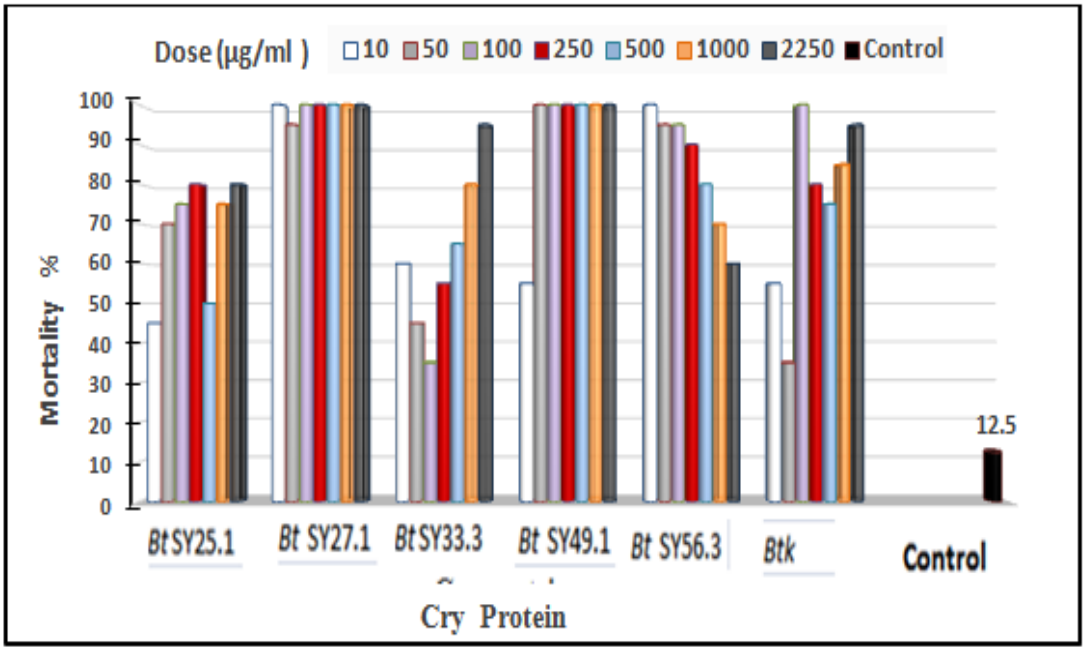

Figure 3.10. Relationship between the doses and mortality rates on *H. cunea* larvae on the tenth day.

The value of LD_{50} for *Bt* SY25.1 was 2.62 µg/ml. The LD_{50} and LD_{99} values for *Bt* SY27.1 were 1.63 µg/ml and 106.56 µg/ml, respectively. The larval mortality rate was very high. The LD_{50} value for *Bt* SY33.3 was 36.18 µg/ml. For the values of LD_{50} and LD_{99} for *Bt* SY49.1, they were 1.17 µg/ml and 5.03 µg/ml, respectively. We note that the values of larval mortality are high, while the values of LD_{50} and LD_{99} for *Bt* SY56.3 were 3991 µg/ml and 15.13 µg/ml. We note that the value of LD_{99} is high, because the larval mortality rate was low, while the values of LD_{50} for *Btk* were 6.51 µg /ml (Table 3.12).

Table 3.12. LD50 and LD99 values for *Bacillus thuringiensis* strains, *Bt* SY25.1, *Bt* SY27.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3, and *Btk* for the tenth day.

***Bt* strains**	**LD50**	**LD99**
***Bt* SY25.1**	**2.62**	**-**
***Bt* SY27.1**	**1.63**	**106.56**
***Bt* SY33.3**	**36.18**	**14004**
***Bt* SY49.1**	**1.17**	**5.03**
***Bt* SY56.3**	**3991**	**15.13**
Btk	**6.51**	**-**

The below charts showed probability plot for the Cry proteins of strains *Bt* SY25.1, *Bt* SY27.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk* for the tenth days. The charts indicates the relationship between the mortality rates and the doses are indicated as curves (Figure 3.11-3.16).

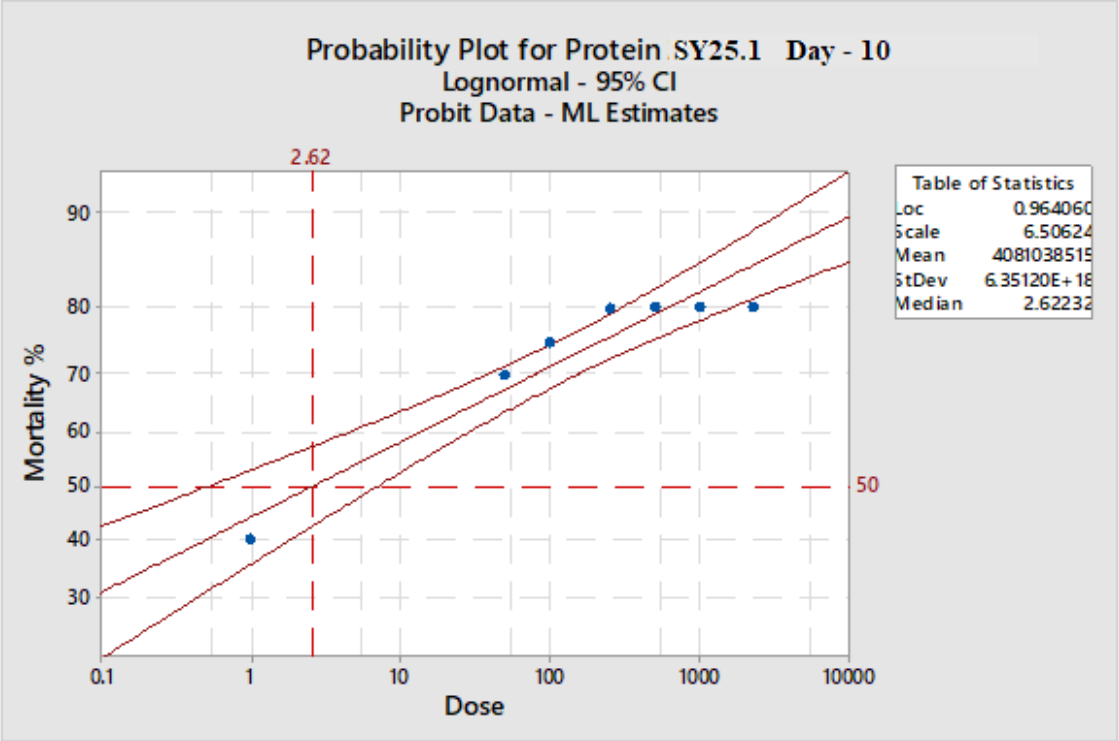

Figure 3.11. Probability plot for Cry proteins of *Bt* SY25.1 on *H. cunea* larvae during ten days.

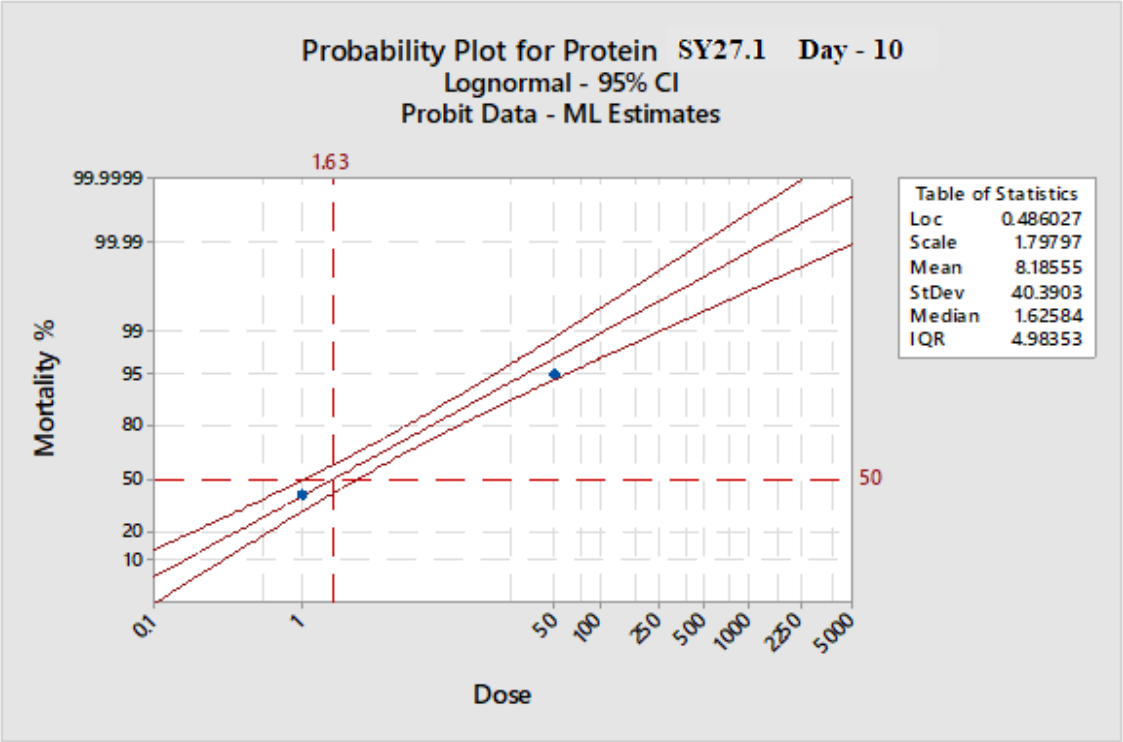

Figure 3.12. Probability plot for Cry proteins of *Bt* SY27.1 on *H. cunea* larvae during ten days.

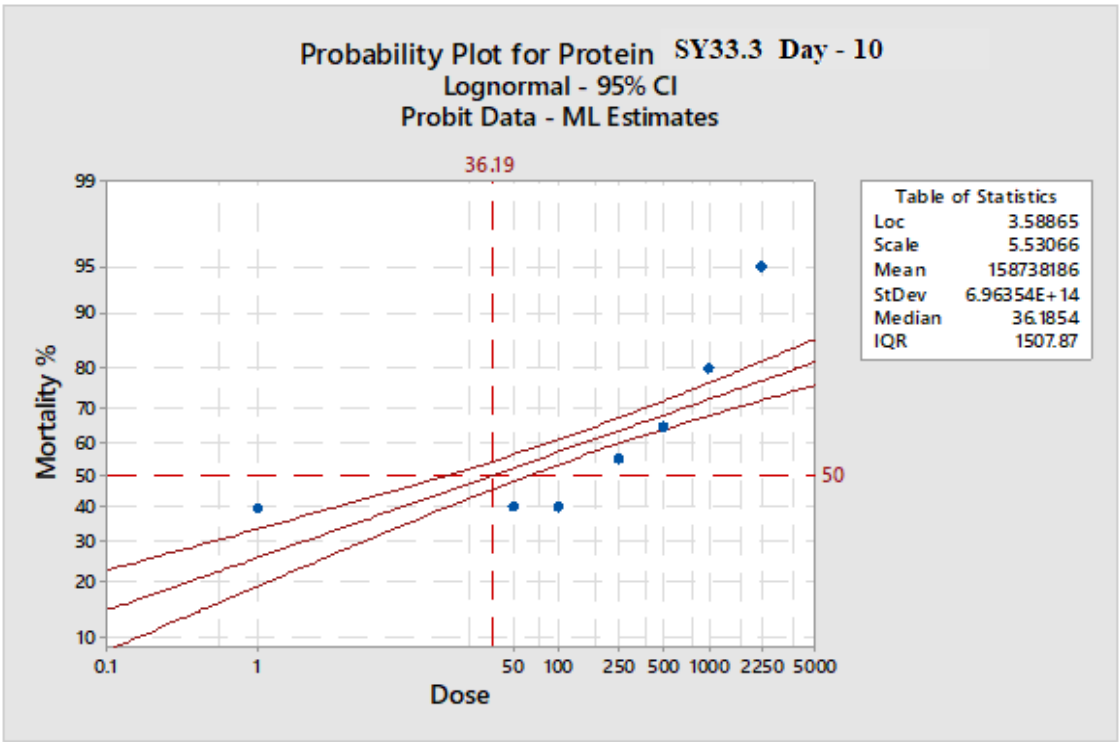

Figure 3.13. Probability plot for Cry proteins of *Bt* SY33.3 on *H. cunea* larvae during ten days.

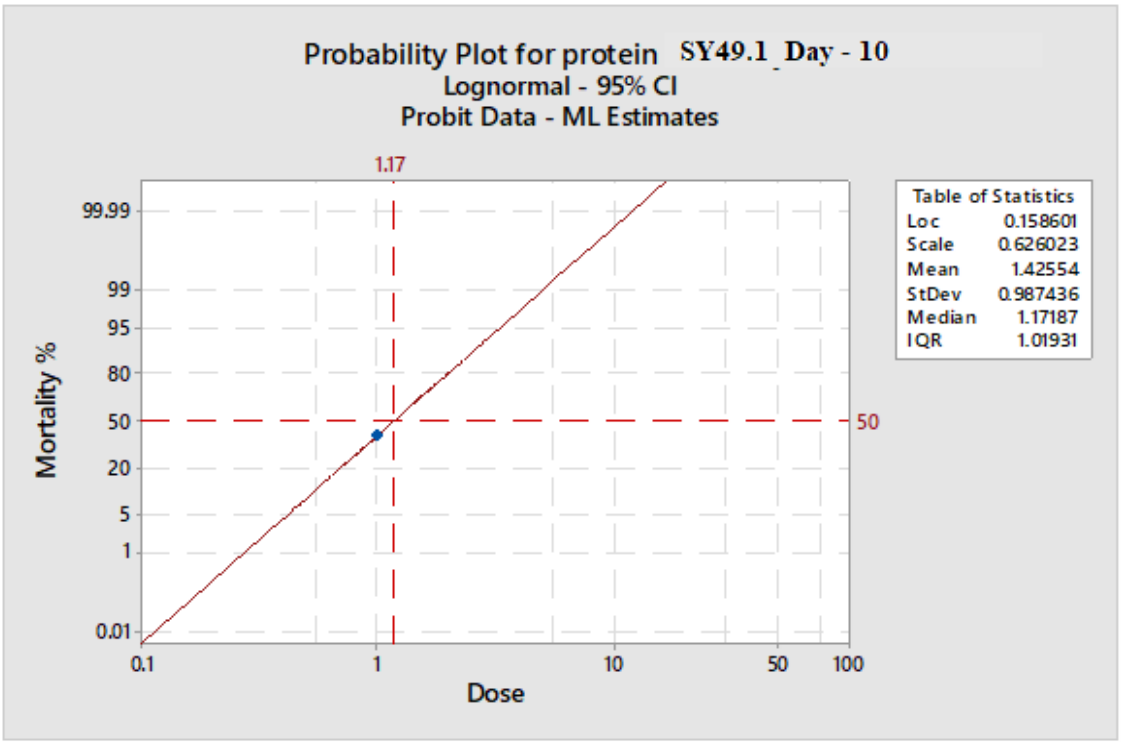

Figure 3.14. Probability plot for Cry proteins of *Bt* SY49.1 on *H. cunea* larvae during ten days.

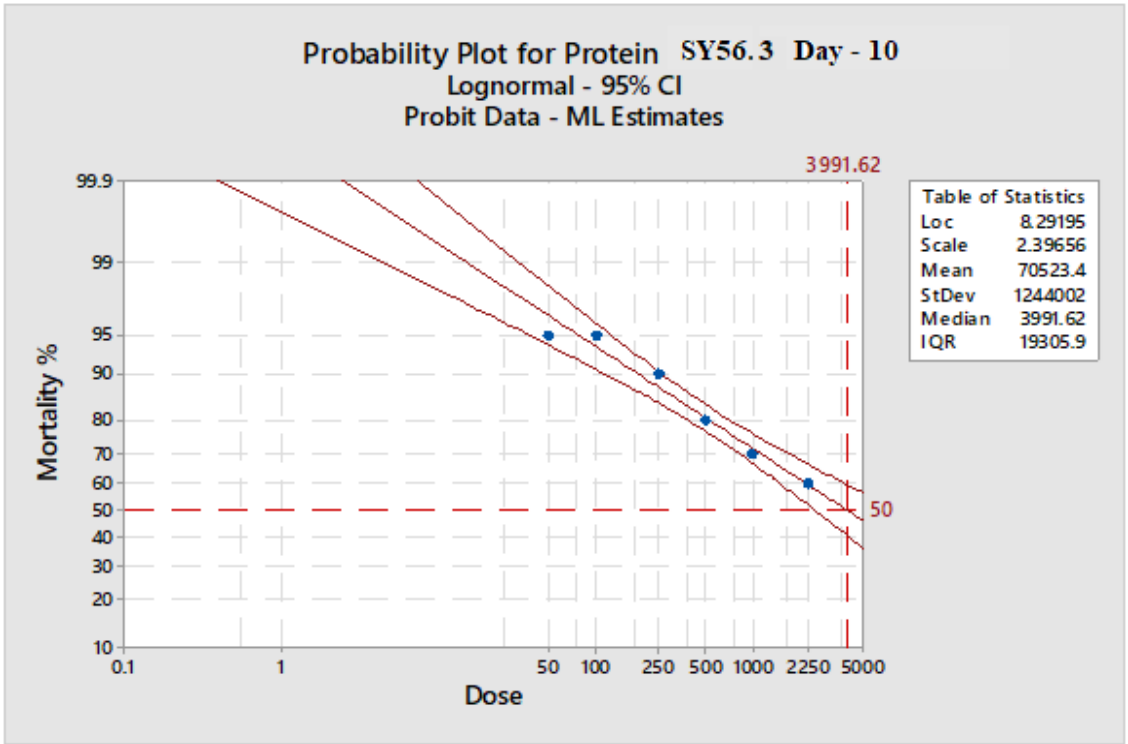

Figure 3.15. Probability plot for Cry proteins of *Bt* SY56.3 on *H. cunea* larvae during ten days

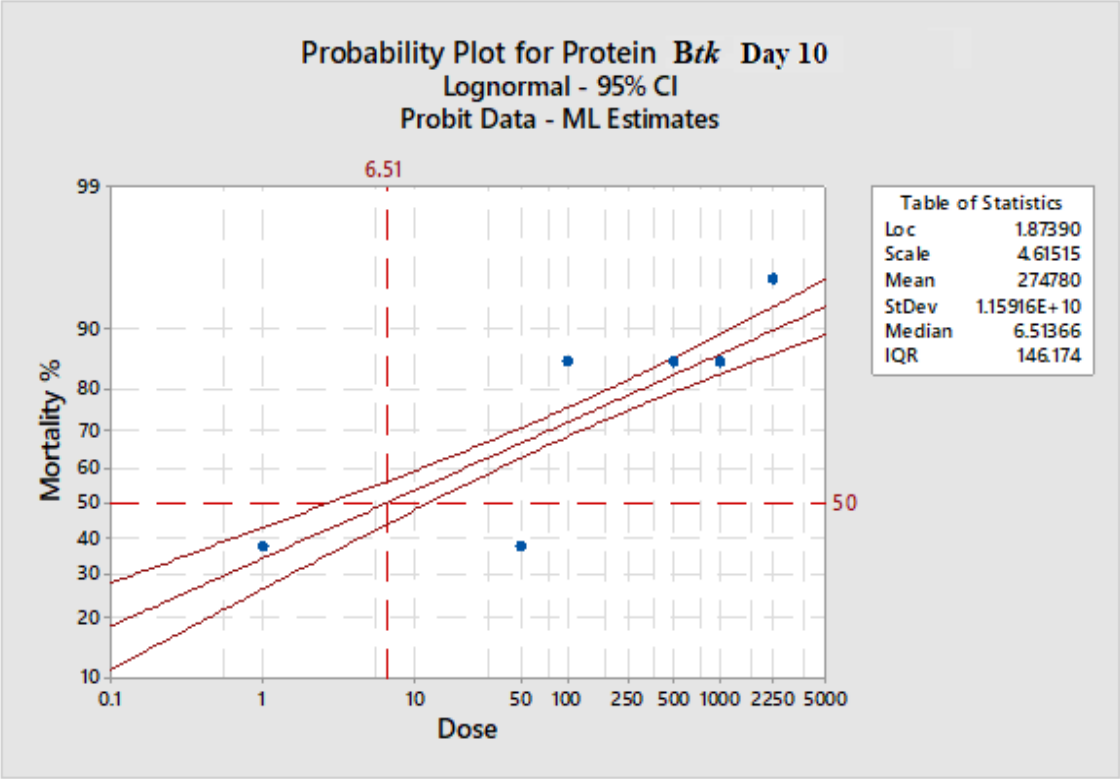

Figure 3.16. Probability plot for Cry proteins of *Btk* on *H. cunea* larvae during ten days

3.3. Lethal Effect of the Spores of *Bt* SY49.1, *Bt* SY27.1, *Btk* on *H. cunea* Larvae

In general, mortality increased with days for the mixture of spores for the three strains *Bt* SY49.1, *Bt* SY27.1, and *Btk* for all doses. *Bt* SY49.1 showed that the lowest mortality was 35% for the first, second and third doses, and the highest mortality was 100% for other doses. *Bt* SY27.1 also showed that the lowest mortality was 20% for the first dose, and the highest mortality was 100% for other doses. *Btk* showed that the lowest mortality was 10% for the third dose, and 100% for other doses (Table 3.13).

Table 3.13. The daily mortalities for the spores of *Bt* SY49.1, *Bt* SY27.1, *Btk* for seven different doses.

***Bt* Cry Protein**	**Dose**	**Days After Treatment**					
		1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
***Bt* SY49.1**	10 µg/ml	35	75	95	95	100	100
	50 µg/ml	35	65	100	100	100	100
	100 µg/ml	35	80	100	100	100	100
	250 µg/ml	65	85	100	100	100	100
	500 µg/ml	85	95	100	100	100	100
	1000 µg/ml	90	100	100	100	100	100
	2250 µg/ml	85	95	100	100	100	100
***Bt* SY27.1**	10 µg/ml	20	55	95	95	95	100
	50 µg/ml	60	70	100	100	100	100
	100 µg/ml	80	85	100	100	100	100
	250 µg/ml	50	85	95	95	100	100
	500 µg/ml	45	60	90	90	95	100
	1000 µg/ml	50	75	100	100	100	100
	2250 µg/ml	65	95	100	100	100	100
Btk	10 µg/ml	15	20	85	90	100	100
	50 µg/ml	20	45	85	90	95	100
	100 µg/ml	10	30	80	85	100	100
	250 µg/ml	15	45	100	100	100	100
	500 µg/ml	30	55	95	95	100	100
	1000 µg/ml	15	60	100	100	100	100
	2250 µg/ml	30	60	100	100	100	100

3.4. The Lethal Effect of the Spores of *Bt* SY49.1, *Bt* SY27.1 and *Btk* on *H. cunea* Larvae During ten days.

On the first day, *Bt* SY49.1 showed positive significant differences compared to *Bt* SY27.1 and *Btk* (LCD = 30.26). *Btk* did not show any significant differences because its value was less than LCD = 30.26, where the lowest mortality rate was in *Btk* in the third dose, while the highest mortality was in *Bt* SY49.1 in the sixth dose as shown in (Table 3-14, Figure 3-17).

Table 3.14. Percent mortality rates for *Bt* spores on *H. cunea* larvae on the first day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY49.1**	**35**	**35**	**35**	**65**	**85**	**90**	**85**	**61.4**
***Bt* SY27.1**	**20**	**60**	**80**	**50**	**45**	**50**	**65**	**52.9**
Btk	**15**	**20**	**10**	**15**	**30**	**15**	**30**	**19.3**
Mean Dose	**23.3**	**38.3**	**41.7**	**43.3**	**53.3**	**51.7**	**60**	
LSD strains		**11.44**	**LSD $_{Dose}$**		**17.47**	**LSD strains$_{*Dose}$**		**30.26**

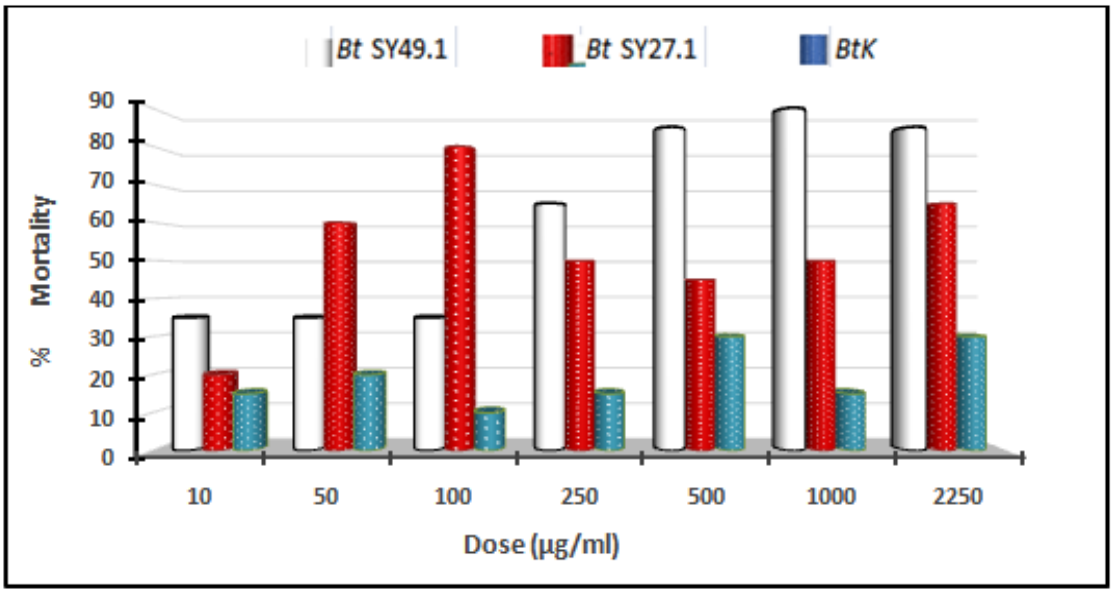

Figure 3.17. Relationship between the doses and mortality rates on *H. cunea* larvae on the first day.

On the second day, *Bt* SY49.1 showed positive significant differences compared to *Bt* SY27.1 and *Btk* (LCD = 28.87). Where the lowest mortality rate was obtained for *Btk* in first dose, while the highest mortality rate was in *Bt* SY49.1 in sixth dose, as shown in (Table 3-15, Figure 3-18).

Table 3.15. Percent mortality rates for *Bt* spores on *H. cunea* larvae on the second day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY49.1**	**75**	**65**	**80**	**85**	**95**	**100**	**95**	**85**
***Bt* SY27.1**	**55**	**70**	**85**	**85**	**60**	**75**	**95**	**75**
Btk	**20**	**45**	**30**	**45**	**55**	**60**	**60**	**45**
Mean Dose	**50**	**60**	**65**	**71.7**	**70**	**78.3**	**83.3**	
LSD strains		**10.91**	**LSD $_{Dose}$**		**16.67**	**LSD strains $_{*Dose}$**		**28.87**

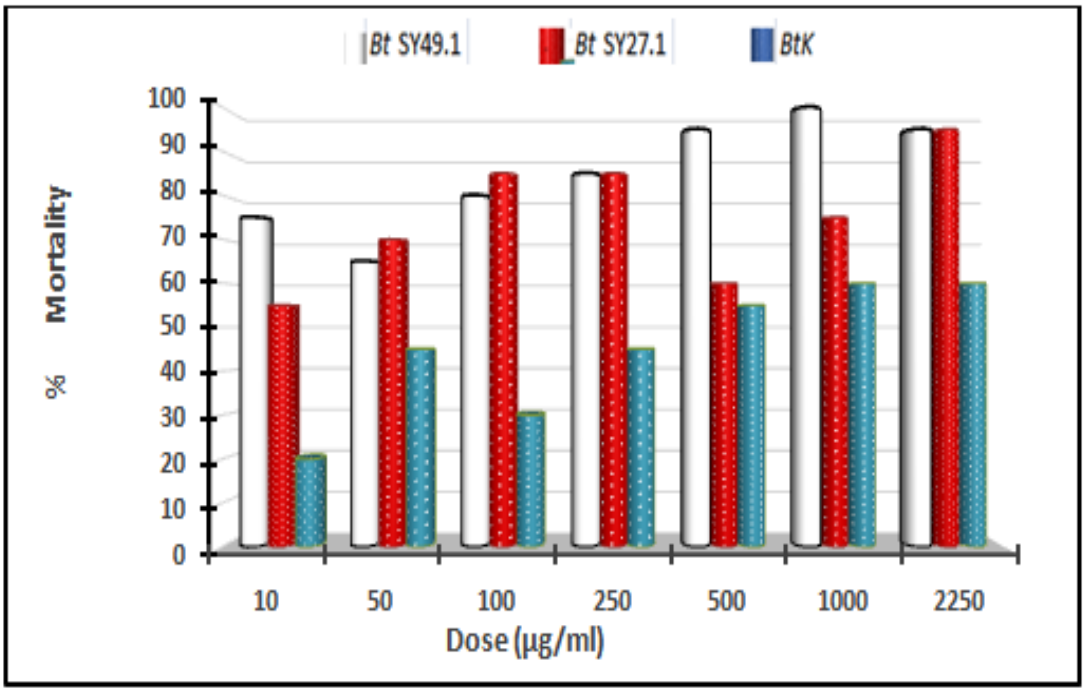

Figure 3.18. Relationship between the doses and mortality rates on *H. cunea* larvae on the second day.

On the third day, *Bt* SY49.1 showed positive significant differences compared to *Bt* SY27.1 and *Btk* (LCD = 9.75). Where the lowest mortality rate for *Btk* was in first, second and third doses, while the highest mortality rate was for *Bt* SY49.1 in second, third, fourth, fifth, sixth and seventh doses, as shown in (Table 3-16, Figure 3-19).

Table 3.16. Percent mortality rates for *Bt* spores on *H. cunea* larvae on the third day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY49.1**	**95**	**100**	**100**	**100**	**100**	**100**	**100**	**99.29**
***Bt* SY27.1**	**95**	**100**	**100**	**95**	**90**	**100**	**100**	**97.14**
Btk	**85**	**85**	**80**	**100**	**95**	**100**	**100**	**92.14**
Mean Dose	**91.67**	**95**	**93.33**	**98.33**	**95**	**100**	**100**	
LSD strains		**3.69**	**LSD Dose**		**5.63**	**LSD strains *Dose**		**9.75**

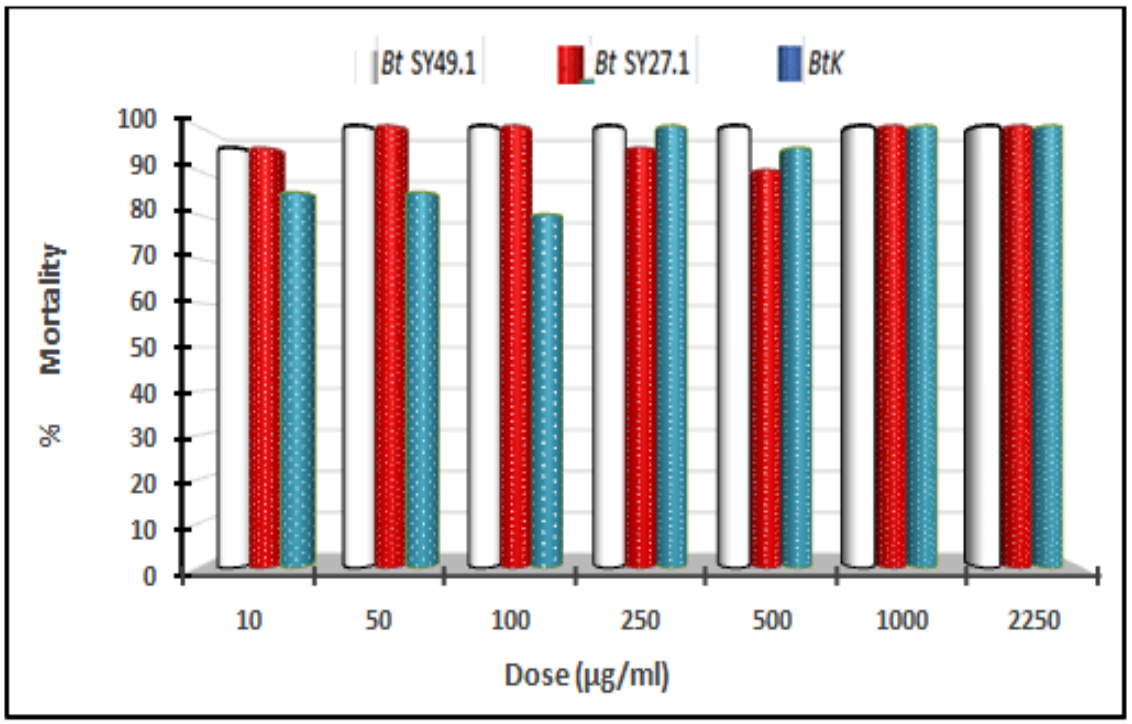

Figure 3.19. Relationship between the doses and mortality rates on *H. cunea* larvae on the third day.

On the fourth day, *Bt* SY49.1 showed positive significant differences compared to *Bt* SY27.1 and *Btk* (LCD = 9.25). Where the lowest mortality rate for *Btk* was in third dose, while the highest mortality rate was for *Bt* SY49.1 in second, third, fourth, fifth, sixth and seventh doses, as shown in (Table 3-17, Figure 3-20).

Table 3.17. Percent mortality rates for *Bt* spores on *H. cunea* larvae on the fourth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY49.1**	**95**	**100**	**100**	**100**	**100**	**100**	**100**	**99.29**
***Bt* SY27.1**	**95**	**100**	**100**	**95**	**90**	**100**	**100**	**97.14**
Btk	**90**	**90**	**85**	**100**	**95**	**100**	**100**	**94.29**
Mean Dose	**93.33**	**96.67**	**95**	**98.33**	**95**	**100**	**100**	
LSD strains		**3.50**	**LSD $_{Dose}$**		**5.34**	**LSD strains $_{*Dose}$**		**9.25**

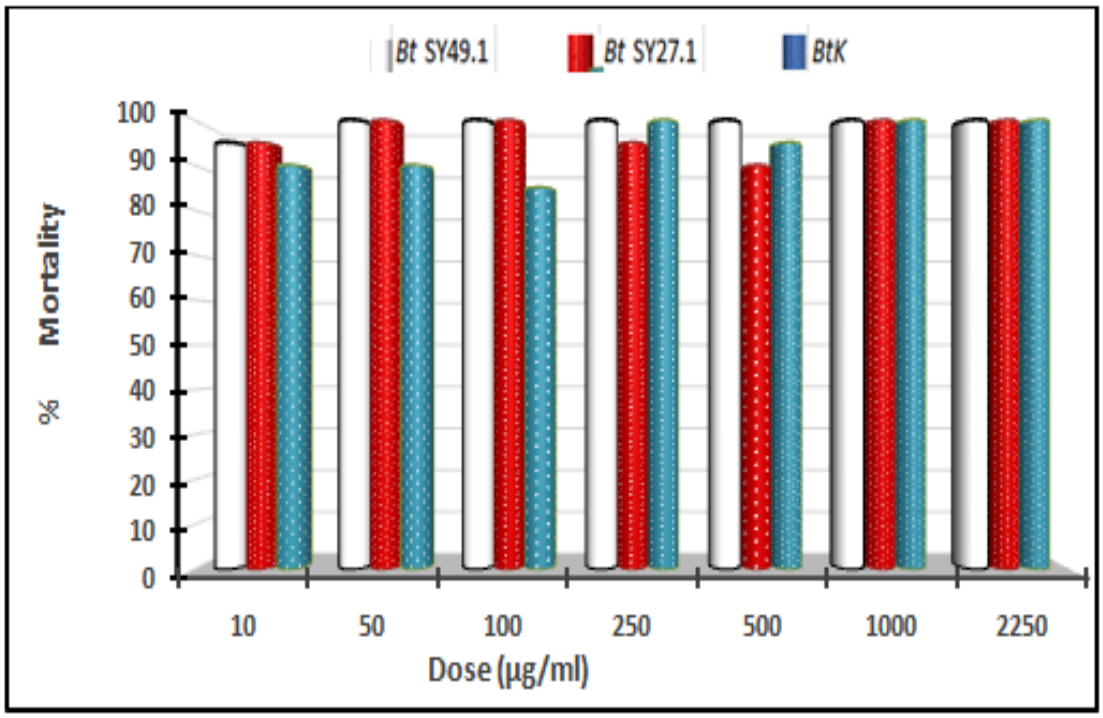

Figure 3.20. Relationship between the doses and mortality rates on *H. cunea* larvae on the fourth day.

On the fifth day, *Bt* SY49.1 showed positive significant differences compared to *Bt* SY27.1 and *Btk* (LCD = 5.341). Where the lowest mortality rate was for *Bt* SY27.1 in first and fifth doses, while the highest mortality rate was for *Bt* SY49.1 for all doses, as shown in (Table 3-18, Figure 3-21).

Table 3.18. Percent mortality rates for *Bt* spores on *H. cunea* larvae on the fifth day.

***Bt* strains**	**Dose (µg/ml)**							**Mean**
	10	**50**	**100**	**250**	**500**	**1000**	**2250**	
***Bt* SY49.1**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
***Bt* SY27.1**	**95**	**100**	**100**	**100**	**95**	**100**	**100**	**98.57**
Btk	**100**	**95**	**100**	**100**	**100**	**100**	**100**	**99.29**
Mean Dose	**98.33**	**98.33**	**100**	**100**	**98.33**	**100**	**100**	
LSD strains		**2.019**	**LSD $_{Dose}$**		**3.084**	**LSD strains $_{*Dose}$**		**5.341**

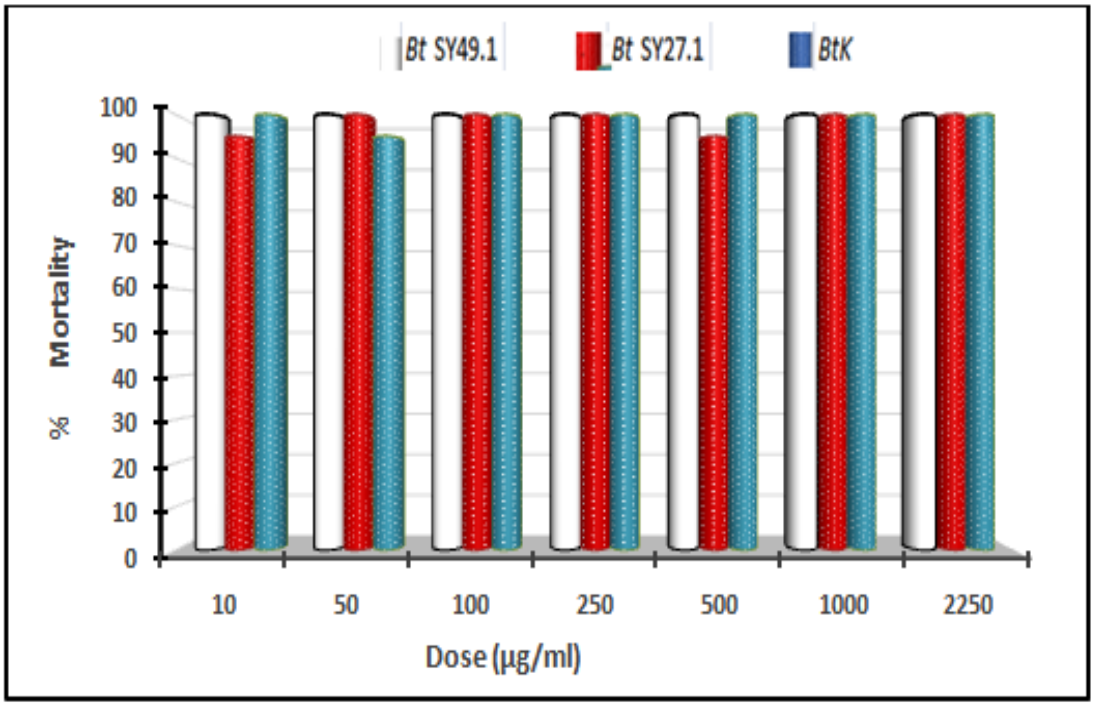

Figure 3.21. Relationship between the doses and mortality rates on *H. cunea* larvae on the fifth day.

On the sixth day, *Bt* SY49.1, *Bt* SY27.1 and *Btk* showed mortality rates of 100%, thus no significant difference was observed among the strains as shown in (Table 3-19, Figure 3-22).

Table 3.19. Percent mortality rates for *Bt* spores on *H. cunea* larvae on the sixth day.

Bt strains	Dose (µg/ml)							Mean
	10	50	100	250	500	1000	2250	
***Bt* SY49.1**	100	100	100	100	100	100	100	100
***Bt* SY27.1**	100	100	100	100	100	100	100	100
Btk	100	100	100	100	100	100	100	100
Mean Dose	100	100	100	100	100	100	100	
LSD strains		*	**LSD** $_{Dose}$		*	**LSD strains** $_{*Dose}$		*

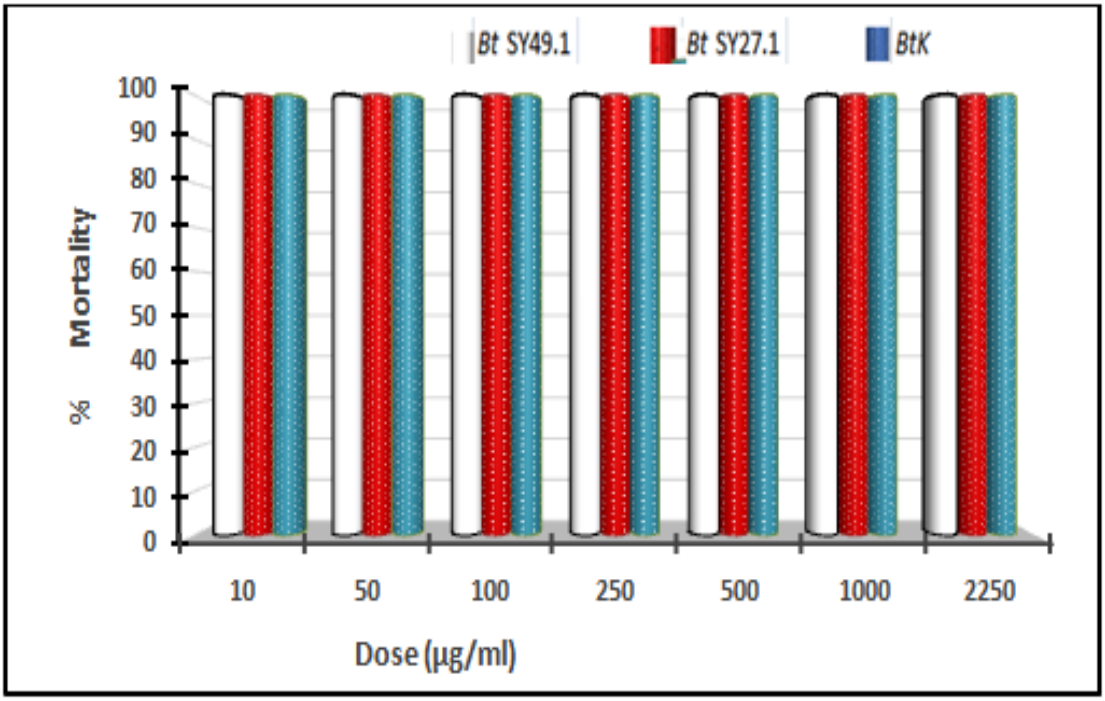

Figure 3.22. Relationship between the doses and mortality rates on *H. cunea* larvae on the sixth day.

According to the results, the LD_{50} value for *Bt* SY49.1 was 35.7, 0.085, 0.228, and 0.228 µg/ml for the first five days. It was noted that the larval mortality rate on the first day was low and it increased with time. The LD_{50} value of *Bt* SY27.1 was 76.81, and 0.255 µg/ml for the first five days. The larval mortality rate increases with the progression of time, so the larval mortality rate for the last day was nearly complete. The LD_{50} value for *Btk* was 372.24, 0.006, and 0.0002 µg/ml for the first five days. On the first day, the mortality rate of larvae was very low, then it began to rise, and on the fifth day, the mortality rate was nearly complete, as shown in (Table 3-20).

Table 3.20. LD50 and LD99 values for *Bt* SY49.1, *Bt* SY27.1 and *Btk* for the spores for five days.

***Bt* strains**	**LD50**	**LD99**
***Bt* SY49.1 – 1st**	**35.70**	**-**
***Bt* SY49.1 – 2nd**	**0.085**	**-**
***Bt* SY49.1 – 3rd**	**0.228**	1.84331
***Bt* SY49.1 – 4th**	**0.228**	1.84331
***Bt* SY49.1 – 5th**	**0**	0
***Bt* SY27.1 – 1st**	**76.81**	1.7837
***Bt* SY27.1 – 2nd**	**0.255**	-
***Bt* SY27.1 – 3rd**	**0**	-
***Bt* SY27.1 – 4th**	**0**	-
***Bt* SY27.1 – 5th**	**0**	259.043
***Btk* – 1st**	**1257247643**	3.5172
***Btk* – 2nd**	**372.24**	-
***Btk* – 3rd**	**0.006**	-
***Btk* – 4th**	**0.0002**	-
***Btk* – 5th**	**0**	14.14

The below charts showed probability plot for the spore mixtures of *Bt* SY49.1, *Bt* SY27.1 and *Btk* during 5 days. The charts indicates the relationship between the mortality rates and the doses are indicated as curves (Figures 3.23-3.35).

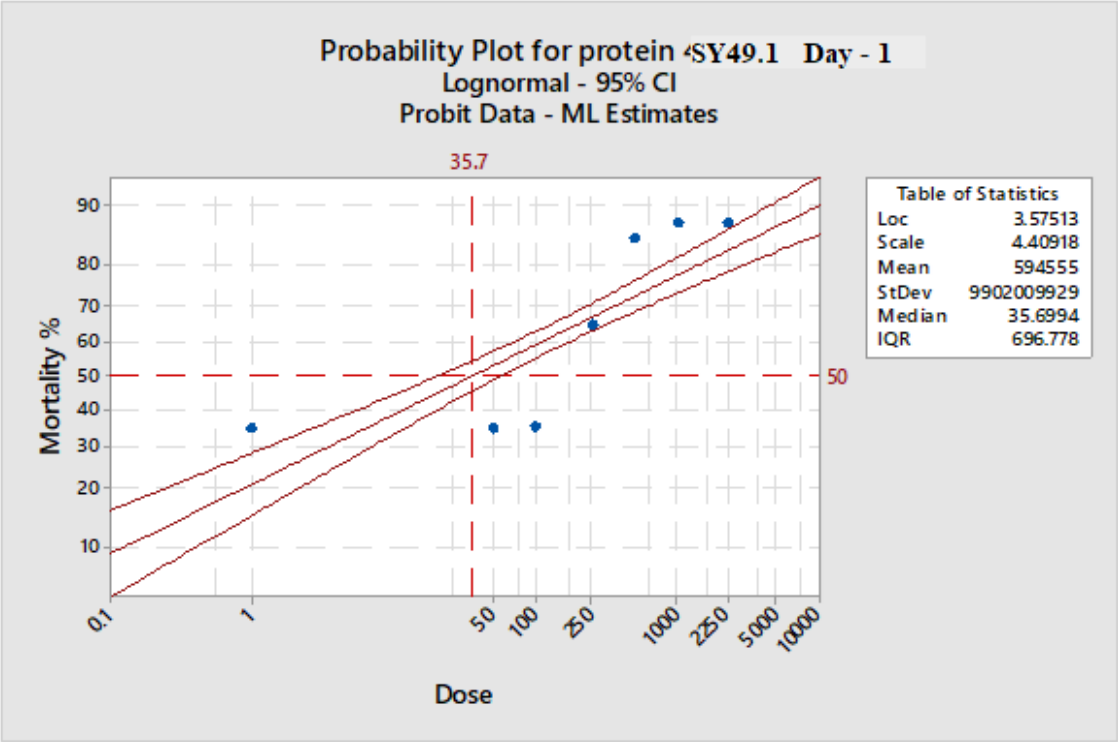

Figure 3.23. Prospective plot for *Bt* SY49.1 spores on *H. cunea* larvae during the first day.

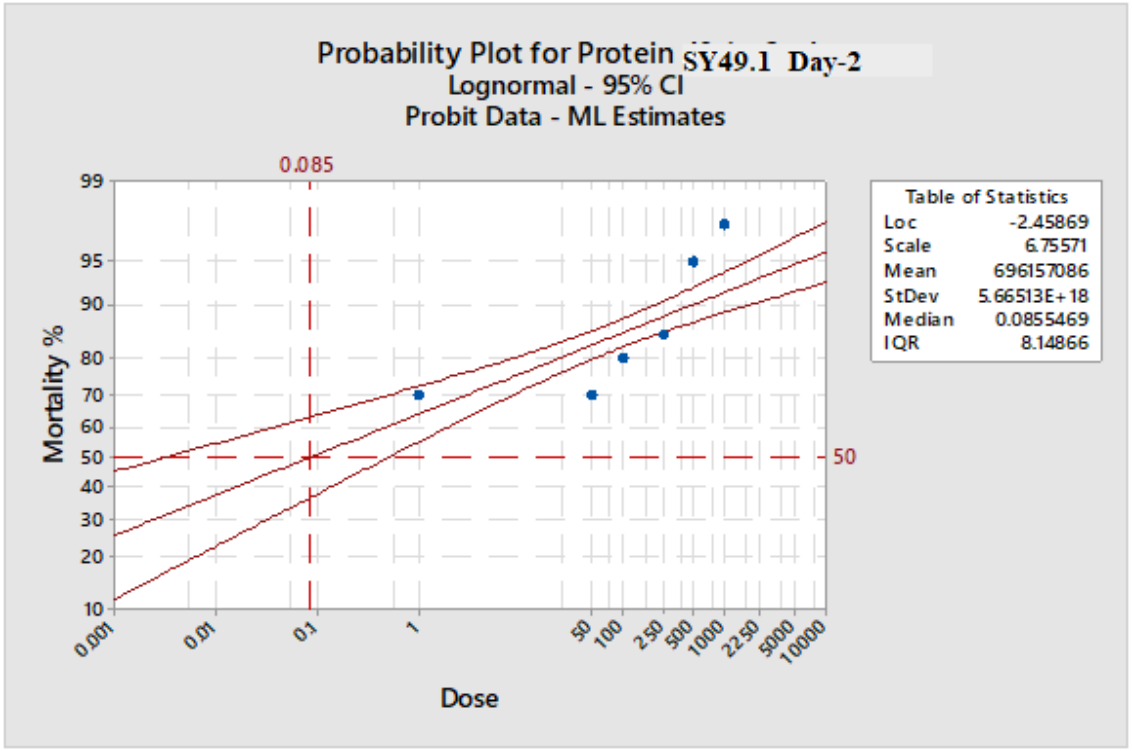

Figure 3.24. Prospective plot for spores of *Bt* SY49.1 on *H. cunea* larvae during the second day.

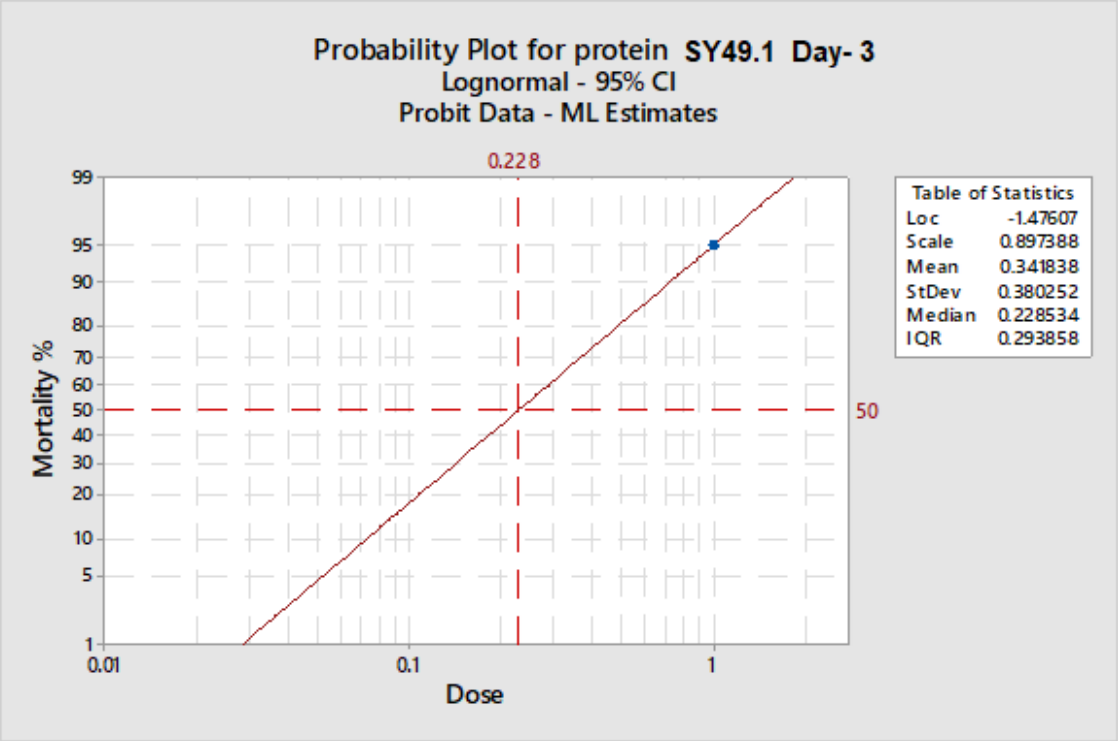

Figure 3.25. Prospective plot for spores of *Bt* SY49.1 on *H. cunea* larvae during the third day.

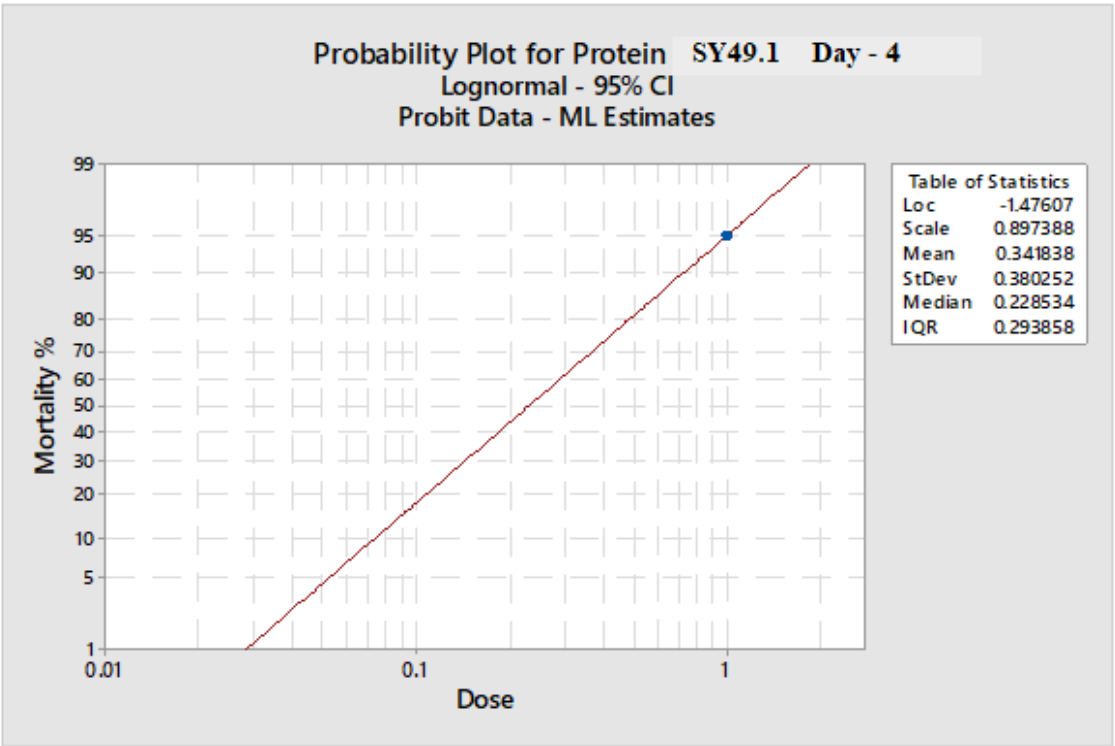

Figure 3.26. Prospective plot for spores of *Bt* SY49.1 on *H. cunea* larvae during the fourth day.

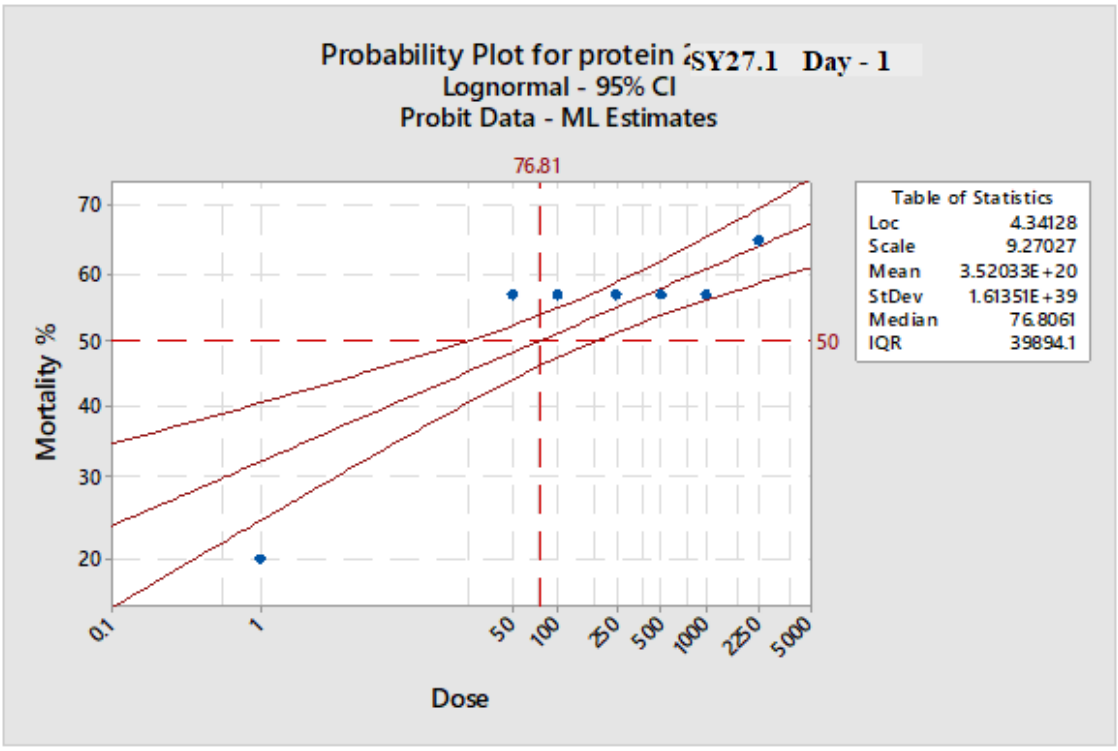

Figure 3.27. Prospective plot for spores of *Bt* SY27.1 on *H. cunea* larvae during the first day.

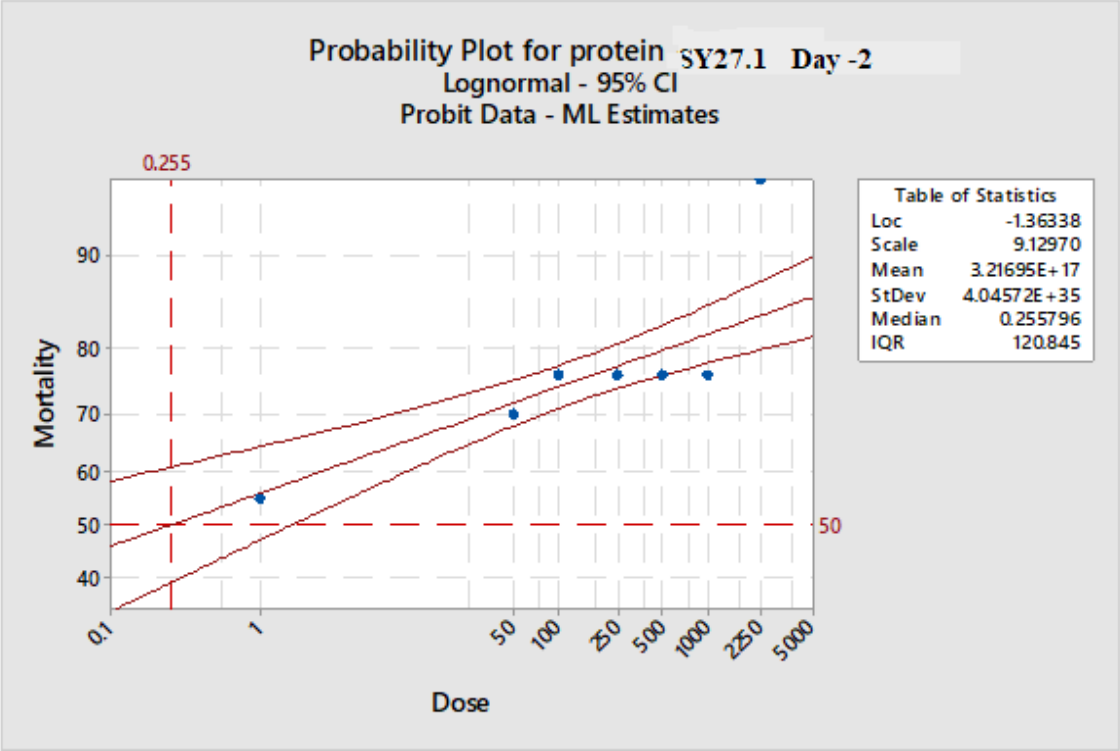

Figure 3.28. Prospective plot for spores of *Bt* SY27.1 on *H. cunea* larvae during the second day.

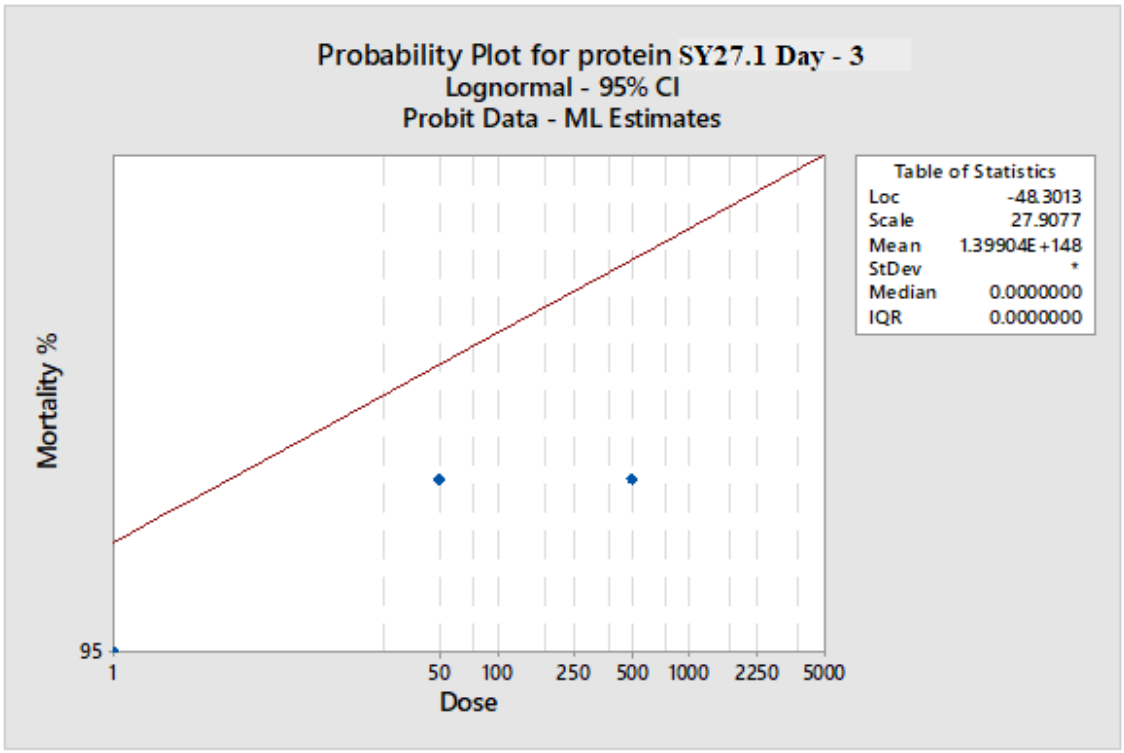

Figure 3.29. Prospective plot for spores of *Bt* SY27.1 on *H. cunea* larvae during the third day.

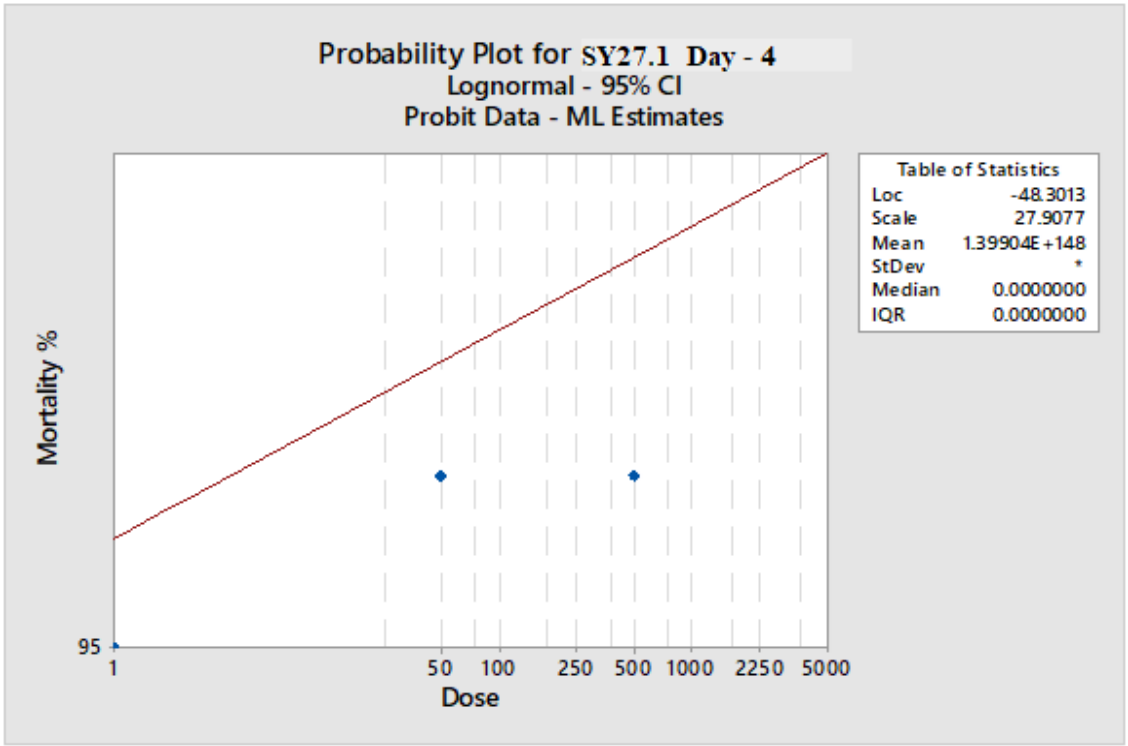

Figure 3.30. Prospective plot for spores of *Bt* SY27.1 on *H. cunea* larvae during the fourth day.

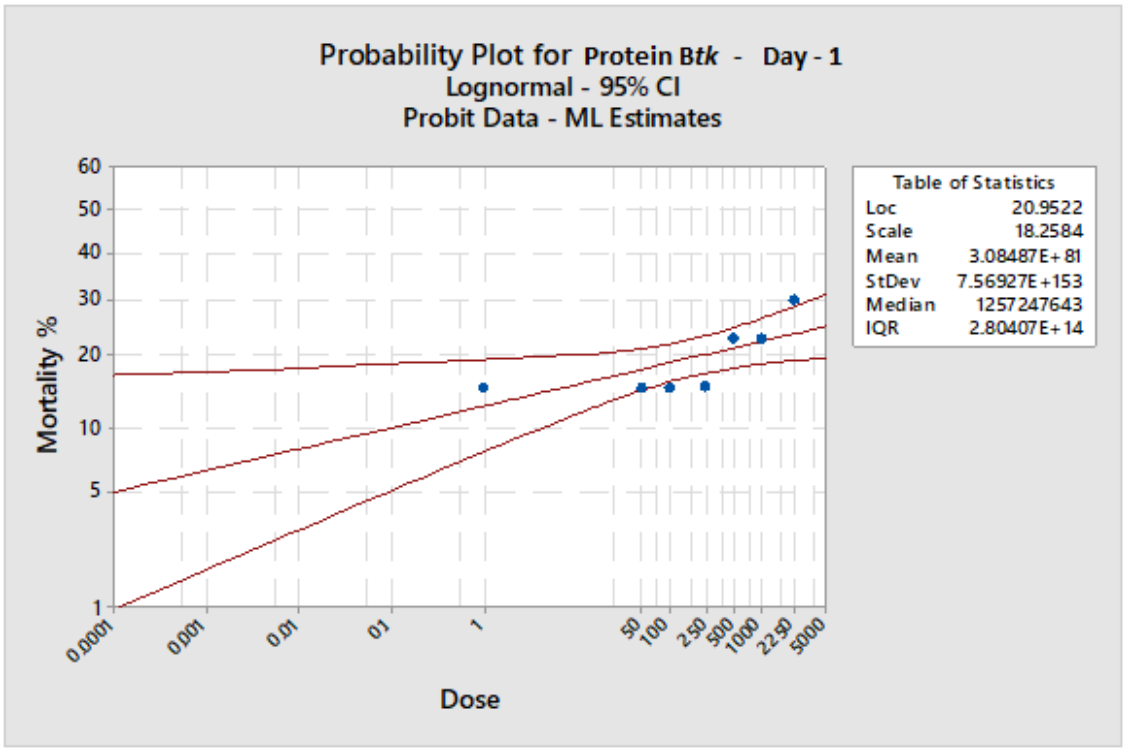

Figure 3.31. Prospective plot for spores of *Btk* on *H. cunea* larvae during the first day.

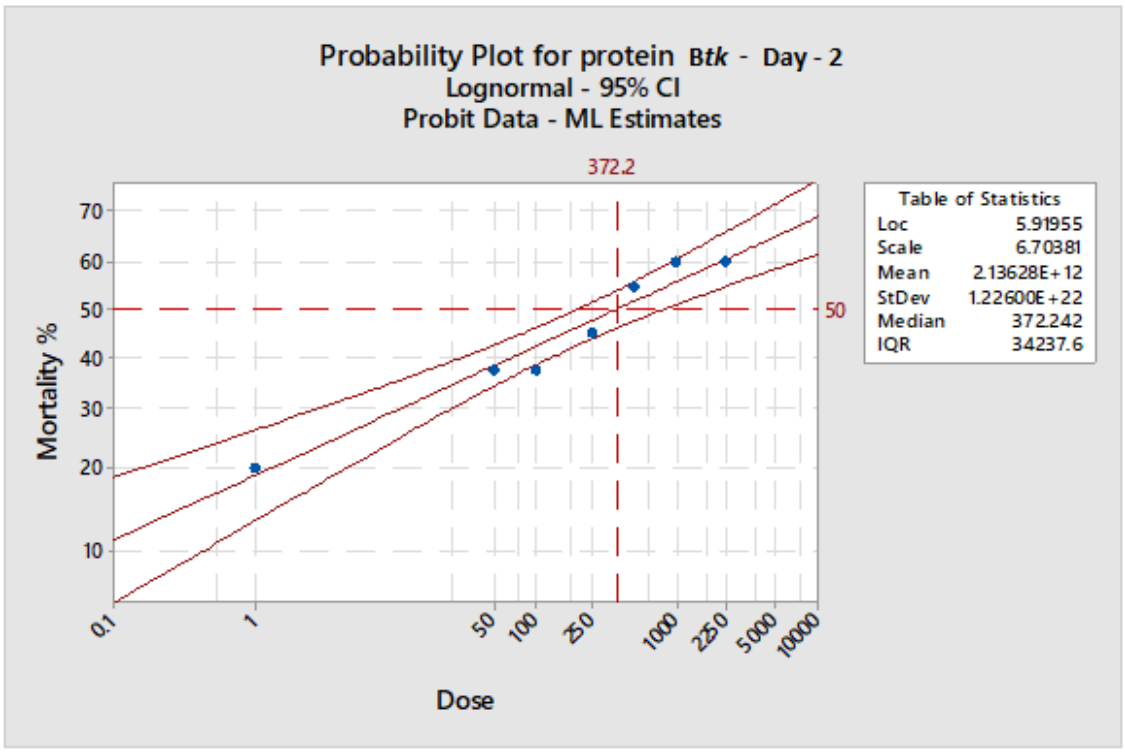

Figure 3.32. Prospective plot for spores of *Btk* on *H. cunea* larvae during the second day.

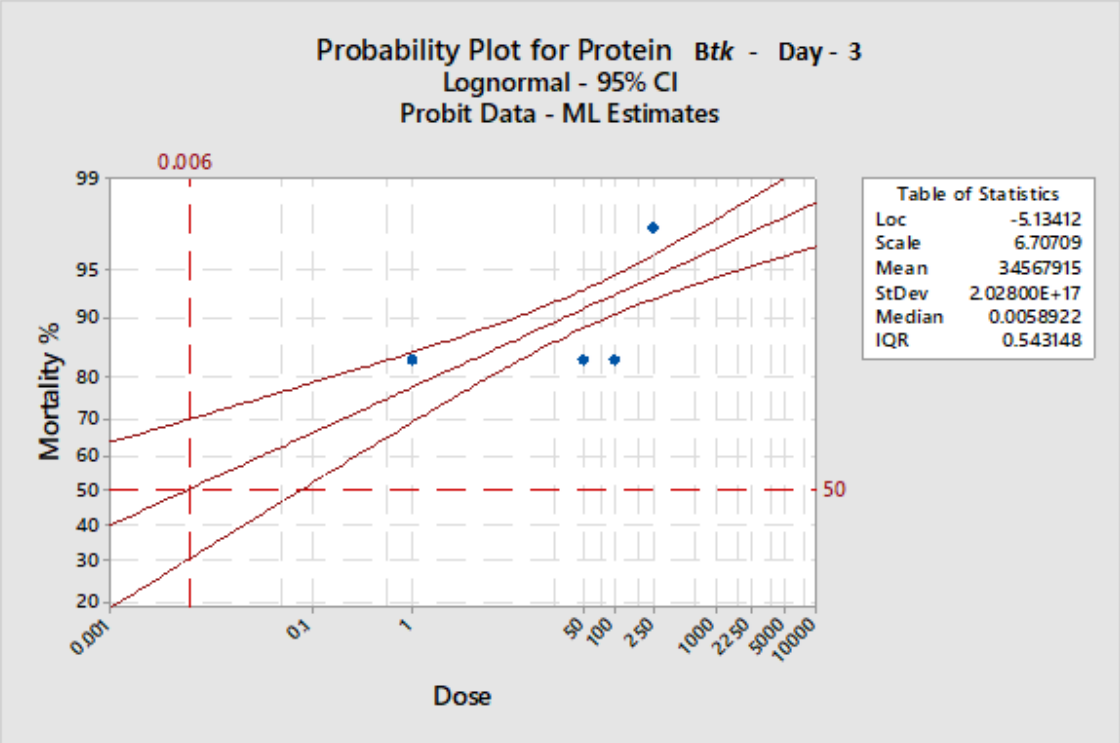

Figure 3.33. Prospective plot for spores of *Btk* on *H. cunea* larvae during the third day.

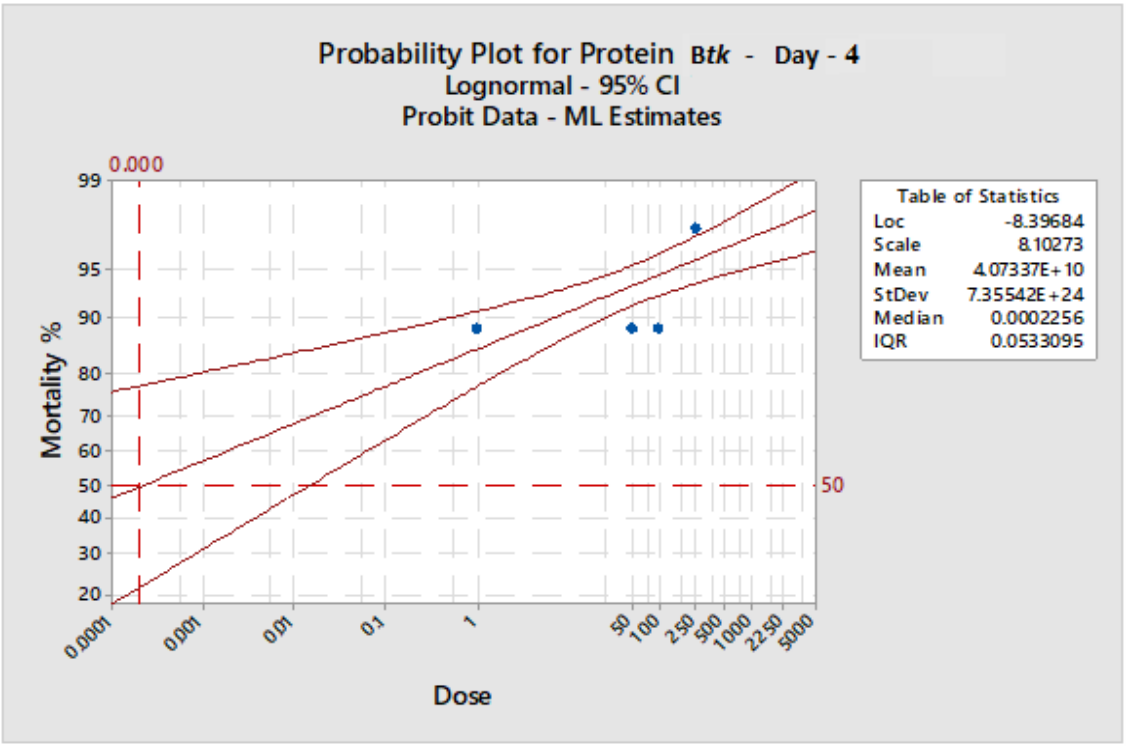

Figure 3.34. Prospective plot for spores of *Btk* on *H. cunea* larvae during the fourth day.

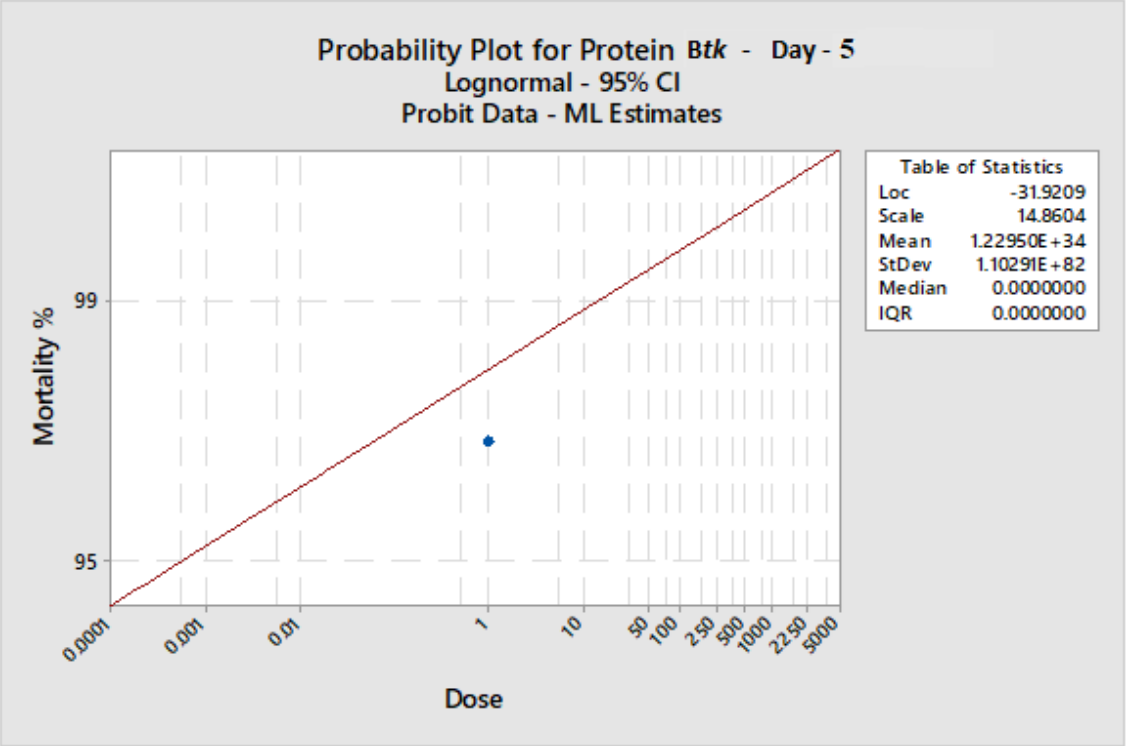

Figure 3.35. Prospective plot for spores of *Btk* on *H. cunea* larvae during the fifth day.

3.5. The Lethal Effect of Spore-Crystal Mixture of *Bt* SY49.1, *Bt* SY27.1, *Btk* on the third Instar Larval Stage (L3) of *H. cunea* Larvae.

In the third stage of life of the larva (L3) in general, mortality increased with increasing days for the mixture of spore-crystal mixtures for *Bt* SY49.1, *Bt* SY27.1, and *Btk* for all doses.

On the first day, *Bt* SY49.1 showed that the lowest mortality was 30% for the fourth dose with spore-crystal and the highest mortality was 56.7% for the sixth dose with crystal, and was 56.7% for the second and sixth doses with spore-crystal. *Bt* SY27.1 had the lowest mortality rate of 13.3% for the second dose with spore-crystals, while highest mortality was 86.7% for the sixth dose with crystal. *Btk* showed that the lowest mortality was 30% for the fourth dose with spore-crystal, while highest mortality was 76.7% for the sixth dose with spore-crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD = 5.44), as shown in (Table 3-21, Figure 3-36).

Table 3.21. Percent mortality rates *Bt* spores–crystals mixtures on *H. cunea* larvae on the first day.

***Bt* strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**Crystal**	**40.0**	**43.3**	**56.7**
	Spore- crystal	**56.7**	**30.0**	**56.7**
***Bt* SY27.1**	**Crystal**	**70.0**	**86.7**	**50.0**
	Spore- crystal	**13.3**	**43.3**	**60.0**
Btk	**Crystal**	**33.3**	**53.3**	**43.3**
	Spore- crystal	**40.0**	**30.0**	**76.7**

***Bt* Strains * Dose**				
***Bt* Stains**	**50 µg/ml**	**250 µg/ml**	**1000 µg/ml**	**Mean *Bt* Strains**
***Bt* SY49.1**	**48.3**	**36.7**	**56.7**	**47.2**
***Bt* SY27.1**	**41.7**	**65.0**	**55.0**	**53.9**
Btk	**36.7**	**41.7**	**60.0**	**46.1**
LSD strains *dose	**9.43**			**LSD strains** **5.44**

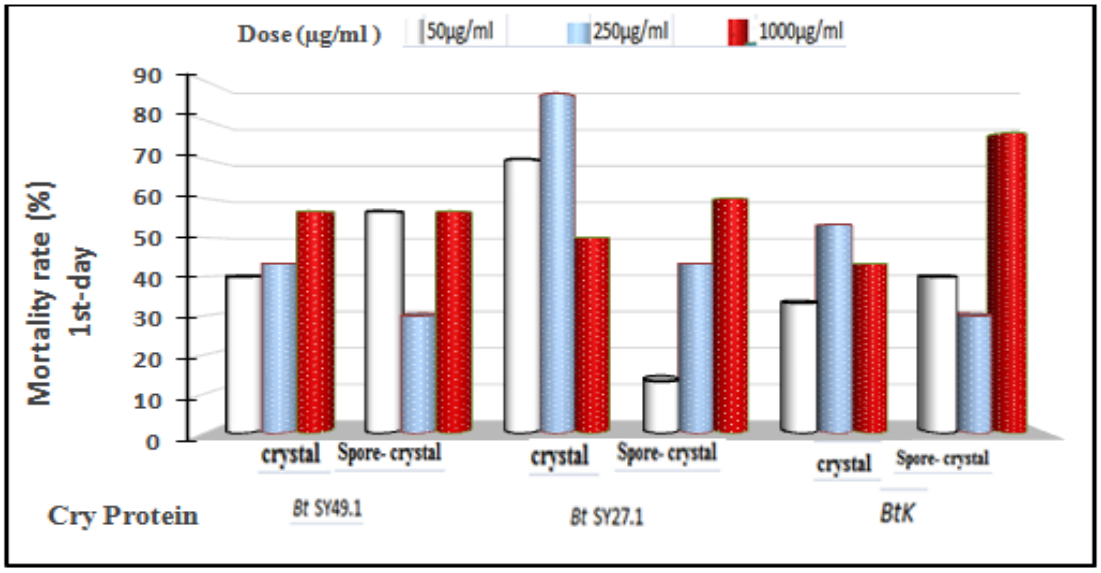

Figure 3.36. Relationship between the doses and mortality rates for spore-crystal mixtures of strains on *H. cunea* larvae on the first day.

On the second day, *Bt* SY49.1 showed that the lowest mortality was 70% for the fourth dose with crystal while the highest mortality was 90% for the sixth dose with spore-crystal mixture. *Bt* SY27.1 showed that the lowest mortality was 53.3% for the second dose with spore-crystal, while highest mortality was 96.7% for the second and fourth doses with crystal. *Btk* showed that the lowest mortality was 53.3% for the sixth dose with crystal, while highest mortality was 93.3% for the sixth dose with spore-crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD = 3.79), as shown in (Table 3-22, Figure 3-37).

Table 3.22. Percent mortality rates for spore- crystal mixtures on *H. cunea* larvae on the second day.

***Bt* strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**73.3**	**70.0**	**80.0**
	Spore- crystal	**86.7**	**86.7**	**90.0**
***Bt* SY27.1**	**crystal**	**96.7**	**96.7**	**83.3**
	Spore- crystal	**53.3**	**80.0**	**90.0**
Btk	**crystal**	**80.0**	**70.0**	**53.3**
	Spore- crystal	**73.3**	**63.3**	**93.3**

***Bt* strains * Dose**					
***Bt* strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean *Bt* strains**	
***Bt* SY49.1**	**80.0**	**78.3**	**85.0**	**81.1**	
***Bt* SY27.1**	**75.0**	**88.3**	**86.7**	**83.3**	
Btk	**76.7**	**66.7**	**73.3**	**72.2**	
LSD strains *dose	**6.57**			**LSD strains**	**3.79**

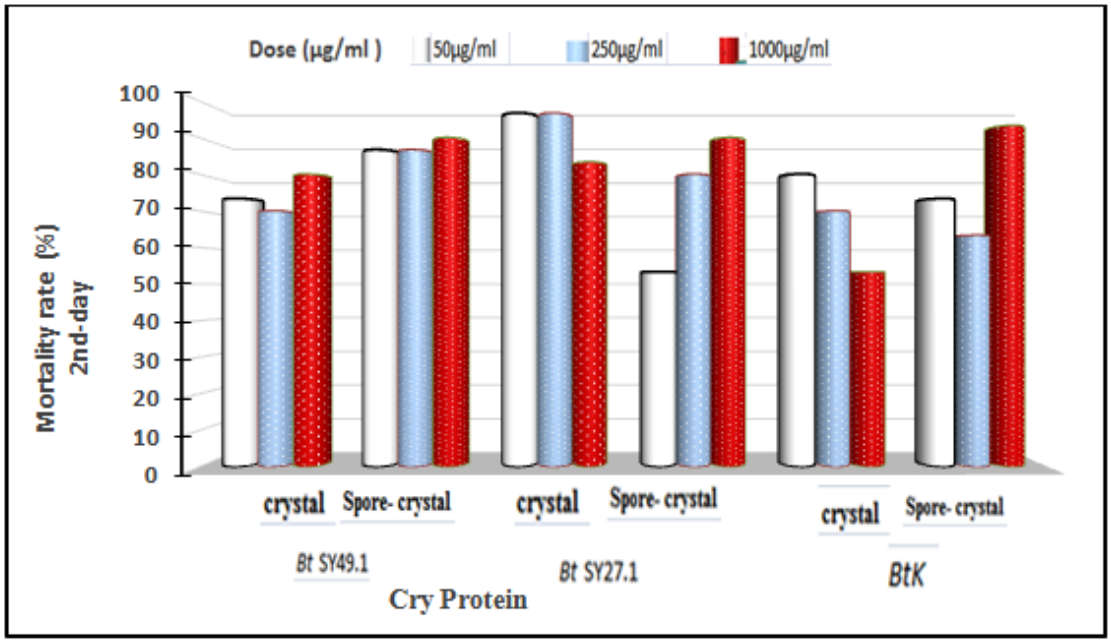

Figure 3.37. Relationship between the doses and mortality rates for spore- crystal mixtures on *H. cunea* larvae on the second day.

On the third day, *Bt* SY49.1 showed that the lowest mortality was 83% for the sixth dose with crystal while the highest mortality was 100% for the fourth dose with crystal and 100% for the sixth dose with spore-crystal mixture. *Bt* SY27.1 showed that the lowest mortality was 93% for the sixth dose with crystal, while highest mortality was 100% for the second dose with crystal and 100% for the second dose with spore-crystal. *Btk* showed that the lowest mortality was 83% for the sixth dose with crystal, while highest mortality was 96% for the second dose with crystal and 96.7% for the

second and sixth doses with spore-crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD = 3.3), as shown in (Table 3-23, Figure 3-38).

Table 3.23. Percent mortality rates for spore- crystal mixtures on *H. cunea* larvae on the third day

***Bt* strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**93.3**	**100**	**83.3**
	Spore- crystal	**93.3**	**96.7**	**100**
***Bt* SY27.1**	**crystal**	**100**	**96.7**	**93.3**
	Spore- crystal	**100**	**96.7**	**96.7**
Btk	**crystal**	**96.7**	**86.7**	**83.3**
	Spore- crystal	**90.0**	**96.7**	**96.7**

***Bt* strains * Dose**					
***Bt* strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean**	
***Bt* SY49.1**	**93.3**	**98.3**	**91.7**	**94.4**	
***Bt* SY27.1**	**100.0**	**96.7**	**95.0**	**97.2**	
Btk	**93.3**	**91.7**	**90.0**	**91.7**	
LSD strains *dose	**5.7**			**LSD strains**	**3.3**

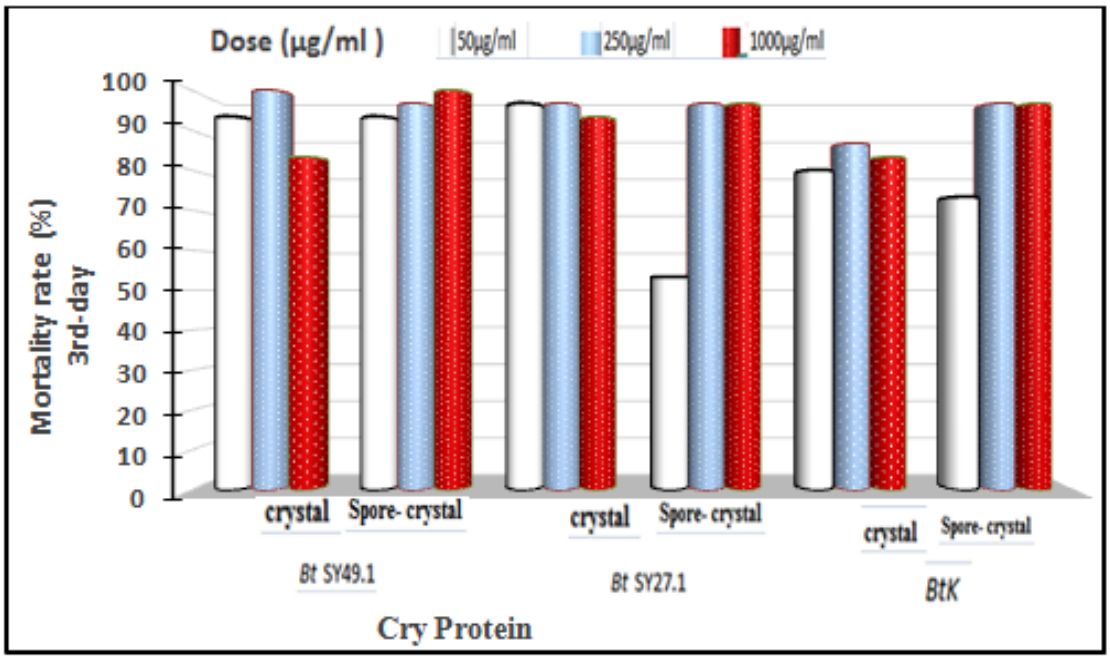

Figure 3.38. Relationship between the doses and mortality rates for spore-crystal mixture on *H. cunea* larvae on the third day.

On the fourth day, *Bt* SY49.1 showed that the lowest mortality was 96.7% for the fourth dose with spore–crystal, while the highest mortality was 100% for the second, fourth and sixth doses with crystal and 100% for the second and sixth doses with spore-

crystal. *Bt* SY27.1 showed that the larval mortality rate was 100% in crystal and spore-crystal. *Btk* showed that the lowest mortality was 96.7% for the second and sixth doses with crystal, while highest mortality was 100% for the fourth dose with crystal and 100% for the second, fourth and sixth doses with spore-crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD = 1.6), as shown in (Table 3-24, Figure 3-39).

Table 3.24. Percent mortality rates for spore-crystal mixtures *H. cunea* larvae on the fourth day.

***Bt* strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**96.7**	**100**
***Bt* SY27.1**	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**
Btk	**crystal**	**96.7**	**100**	**96.7**
	Spore- crystal	**100**	**100**	**100**

***Bt* strains * Dose**					
***Bt* strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean**	
***Bt* SY49.1**	**100**	**98.3**	**100**	**99.4**	
***Bt* SY27.1**	**100**	**100**	**100**	**100**	
Btk	**98.3**	**100**	**98.3**	**98.9**	
LSD strains *dose	**2.8**			**LSD strains**	**1.6**

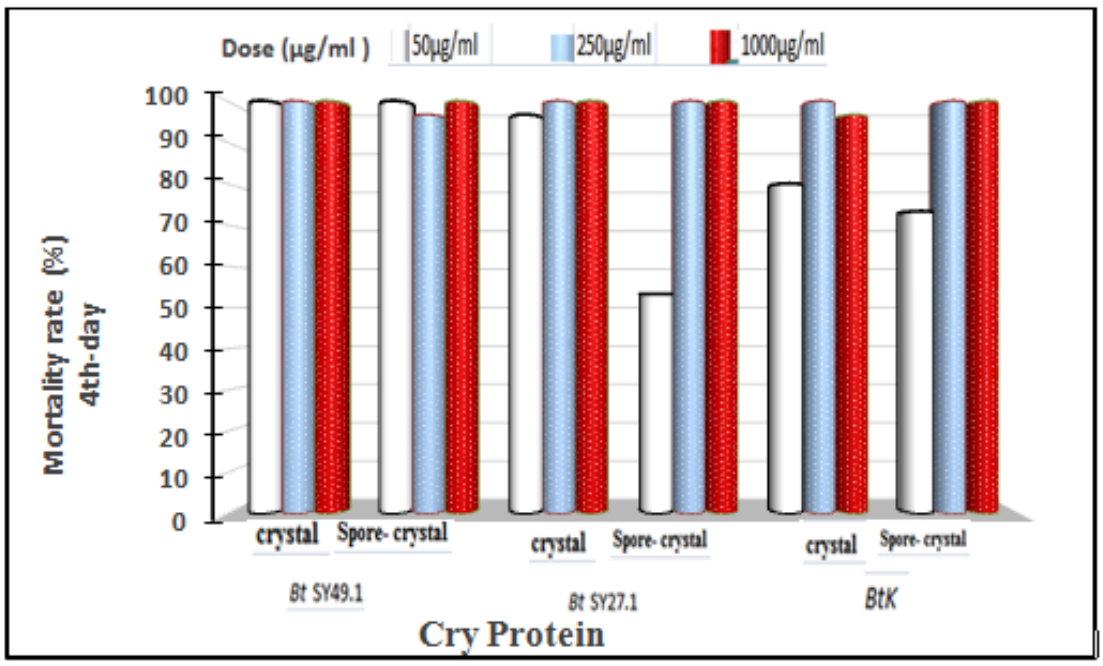

Figure 3.39. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the fourth day

On the fifth day, *Bt* SY49.1, *Bt* SY27.1 and *Btk* showed that the rate of larval mortality was 100% for all doses with crystal and spore–crystal. The Significant differences were positive for the strains *Bt* SY49.1, *Bt* SY27.1 and *Btk* (LCD = 0). Therefore, the larval mortality rate for these strains was 100% for all doses, as shown in (Table 3-25, Figure 3-40).

Table 3.25. Percent mortality rates for spore- crystal mixtures on *H. cunea* larvae on the fifth day.

***Bt* strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**
***Bt* SY27.1**	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**
Btk	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**

***Bt* strains * Dose**					
***Bt* strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean *Bt* strains**	
***Bt* SY49.1**	**100**	**100**	**100**	**100**	
***Bt* SY27.1**	**100**	**100**	**100**	**100**	
Btk	**100**	**100**	**100**	**100**	
LSD strains *dose	*			**LSD strains**	*

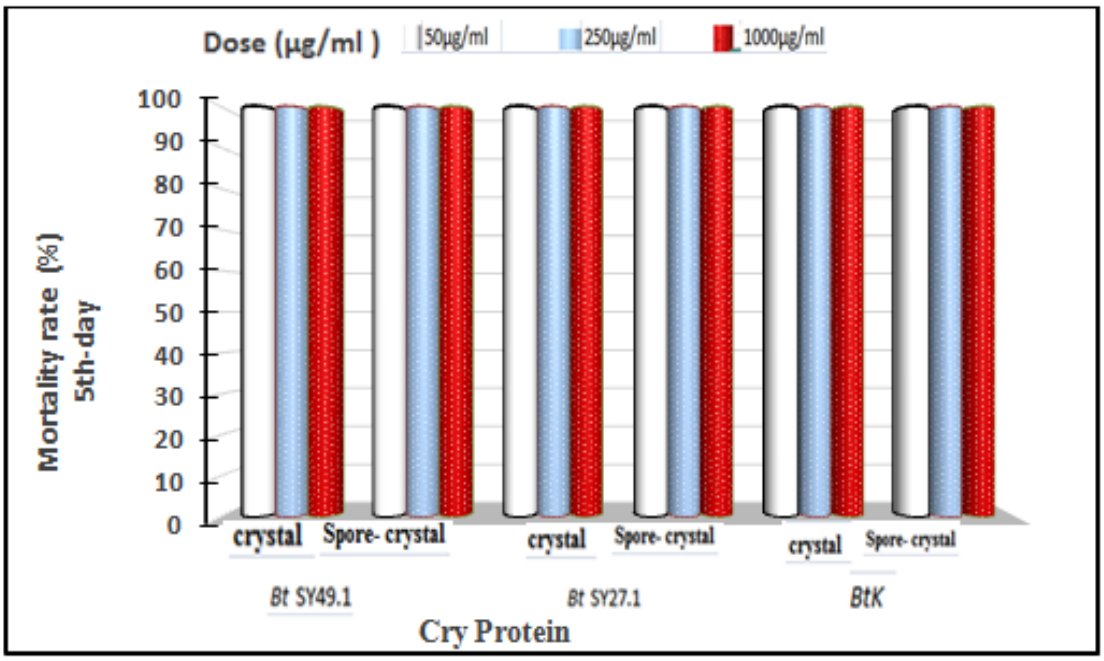

Figure 3.40. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the fifth day.

According to our results, the LD50 value of *Bt* SY49.1 was 4.27, 0.07, 0.098, and 1.42 µg/ml for the first four days and the values of LD99 were 530, 2552, 17.72, and 2.09 µg/ml for the first four days. We note that the larval mortality rate on the first day is low and the larval mortality rate increased with time. LD50 value for *Bt* SY27.1 was 2.99, 0.47, and 0.25 µg/ml for the first three days, and the values of LD99 were 247, 61.3, and 5.22 µg/ml for the first three days. The larval mortality rate increased with the progression of time. LD50 values for *Btk* were 4.4, 0.40, 0.001, and 0.036 µg/ml for the first four days, and the values of LD99 were 475, 2245, 731, and 4.04 µg/ml for the first four days. The larval mortality rate increased with the progression of time, as shown in (Table 3.26).

Table 3.26. LD50 and LD99 values for spore-crystal mixtures of the strains *Bt* SY49.1, *Bt* SY27.1 and *Btk* on fourth larval stage of *H. cunea* for the four days.

***Bt* strains**	**LD50**	**LD99**
***Bt* SY27.1– 1st**	**2.99**	**247**
***Bt* SY27.1– 2nd**	**0.47**	**61.3**
***Bt* SY27.1– 3rd**	**0.25**	**5.22**
***Bt* SY27.1– 4th**	**0**	**0**
***Bt* SY49.1– 1st**	**4.27**	**530**
***Bt* SY49.1– 2nd**	**0.07**	**2552**
***Bt* SY49.1– 3rd**	**0.098**	**17.22**
***Bt* SY49.1– 4th**	**1.42**	**2.09**
***Btk* – 1st**	**4.40**	**475**
***Btk* – 2nd**	**0.40**	**2245**
***Btk*– 3rd**	**0.000**	**731**
***Btk* – 4th**	**0.037**	**4.04**

The graphs below shows a mixture probability plot of spore-crystals for the strains *Bt* SY49.1, *Bt* SY27.1 and *Btk* for the first four days. Graphs indicate the relationship between mortality rates and doses are indicated as curves (Figures 3.41-3.63).

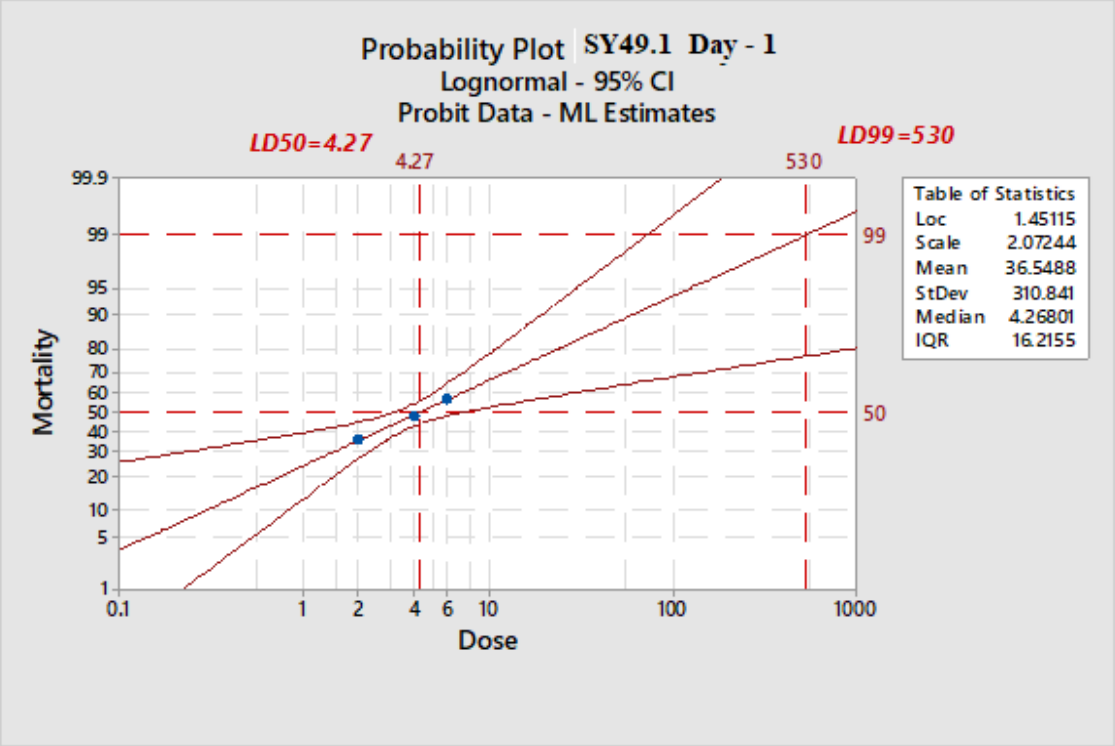

Figure 3.41. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the first day.

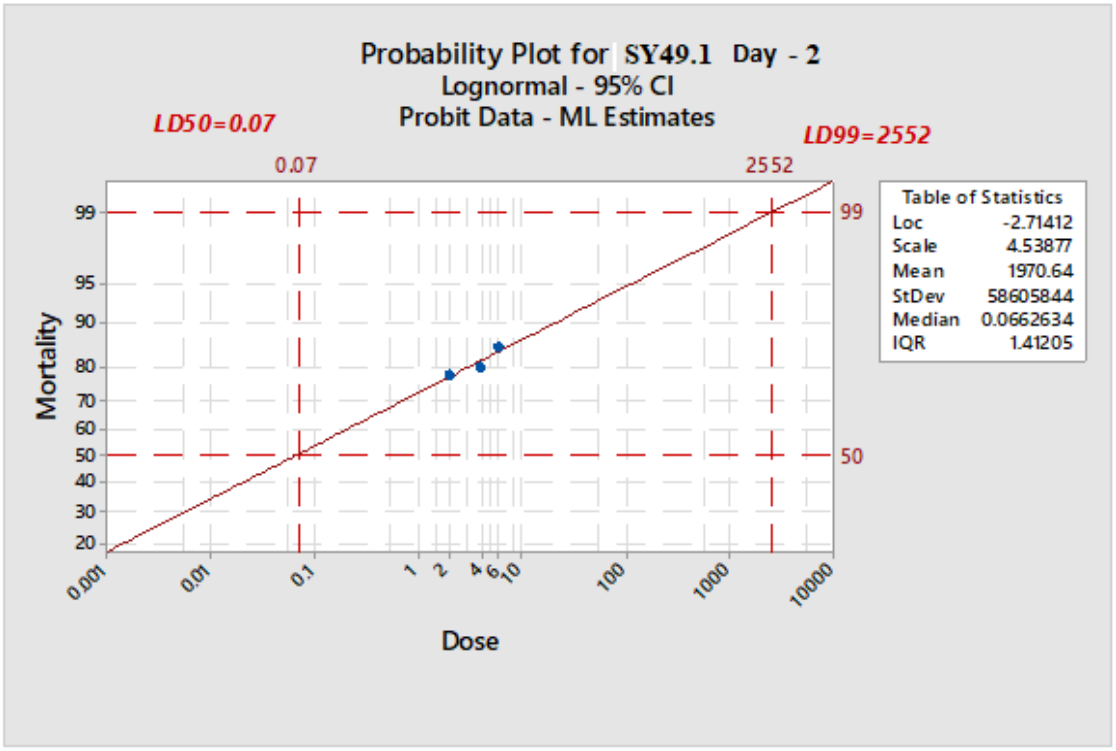

Figure 3.42. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the second day.

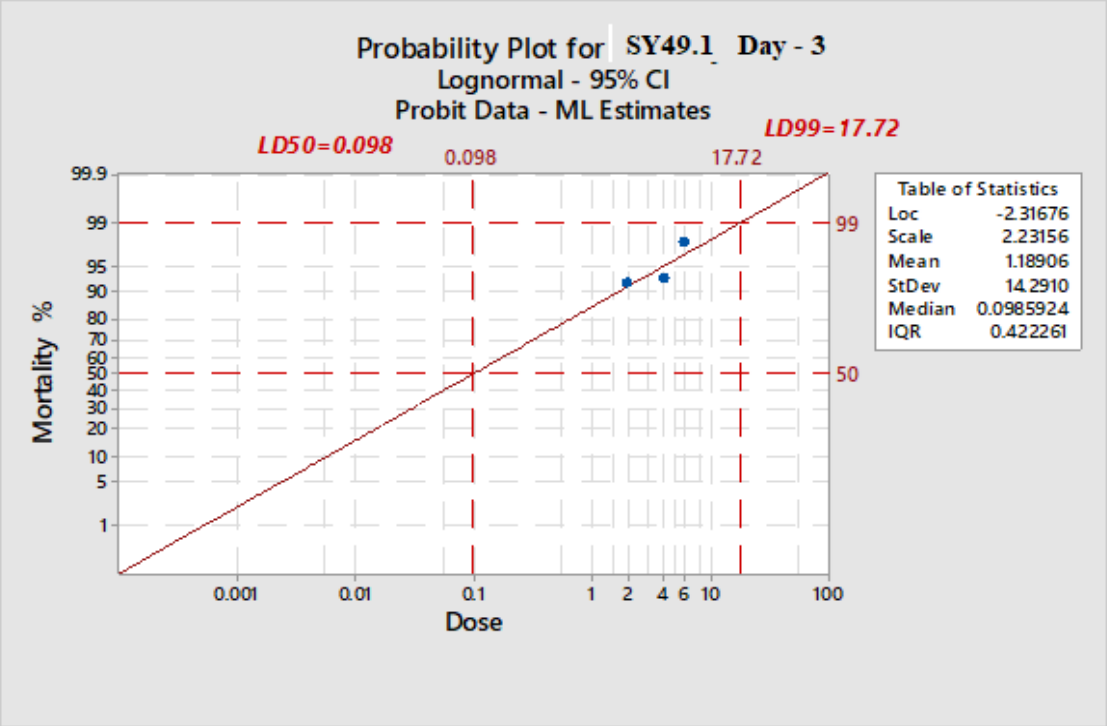

Figure 3.43. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the third day.

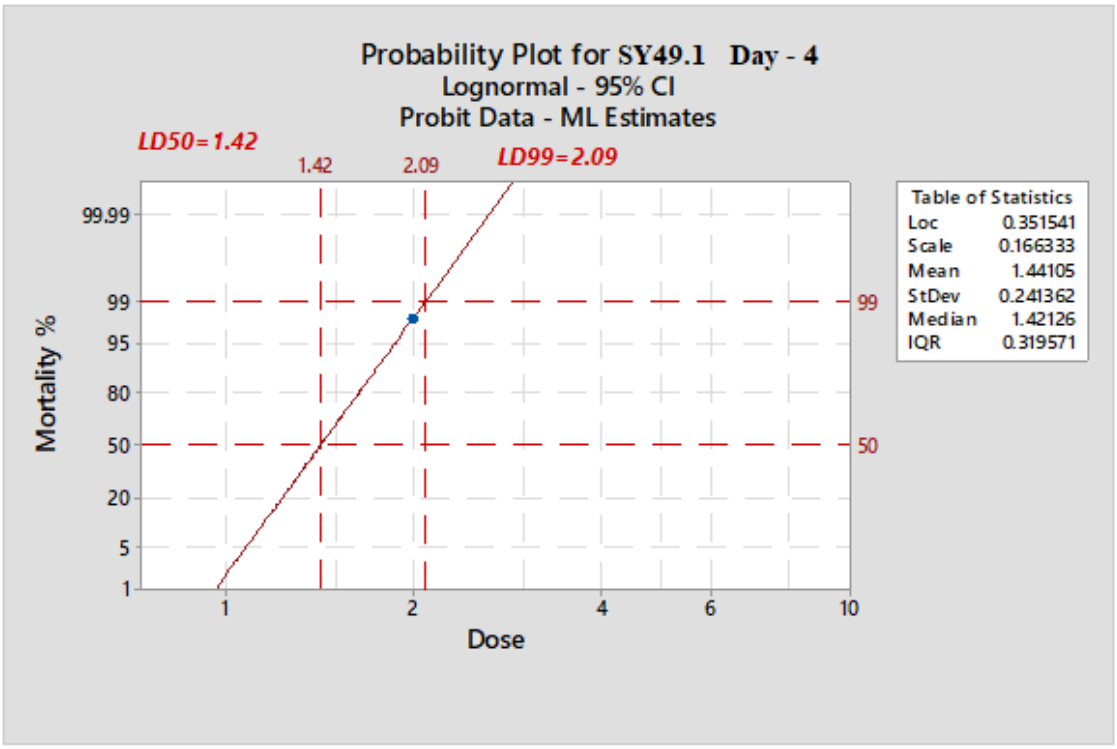

Figure 3.44. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the fourth day.

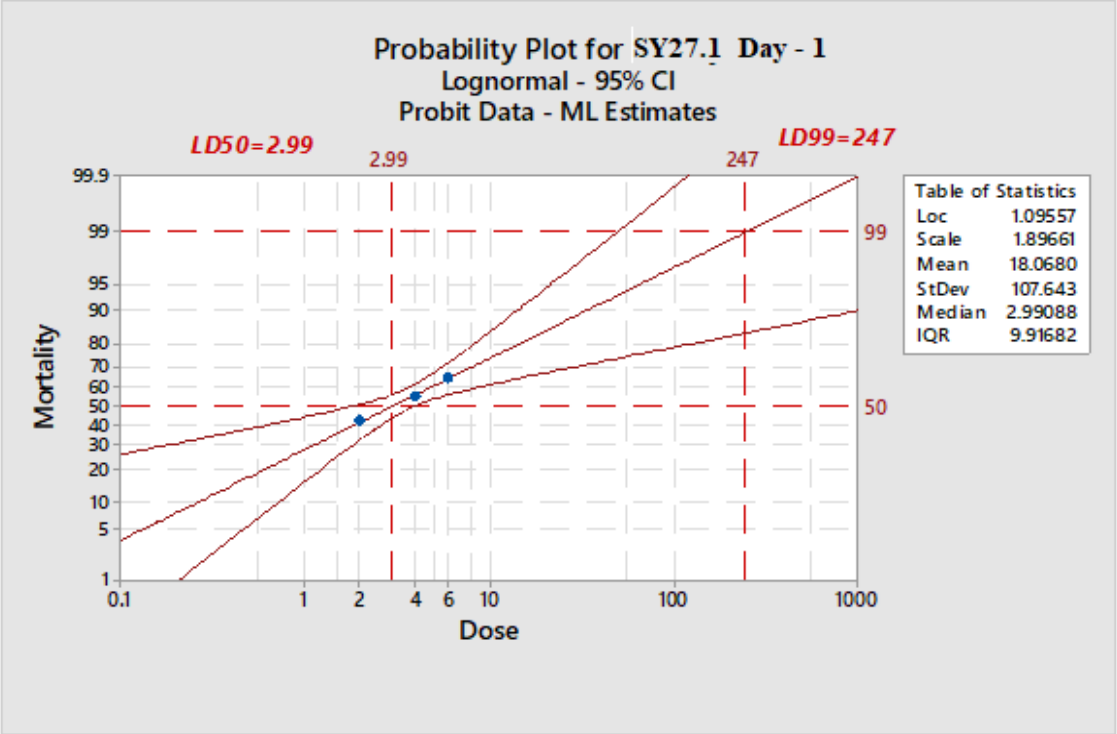

Figure 3.45. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the first day.

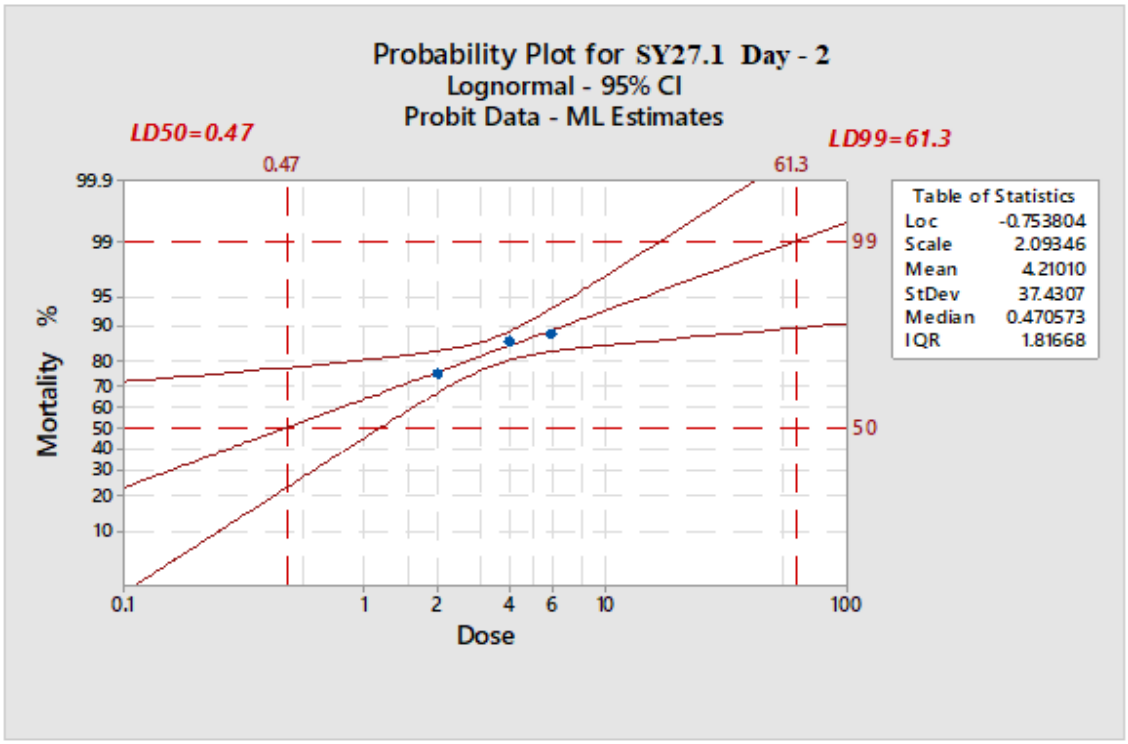

Figure 3.46. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the second day.

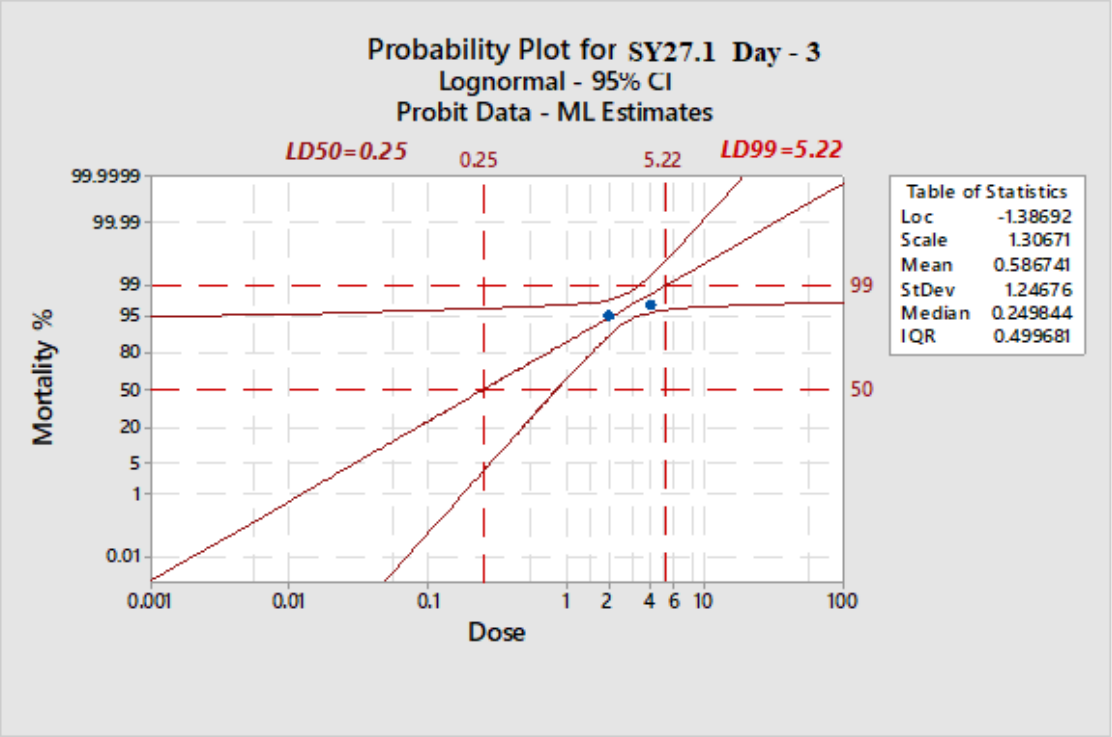

Figure 3.47. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the third day.

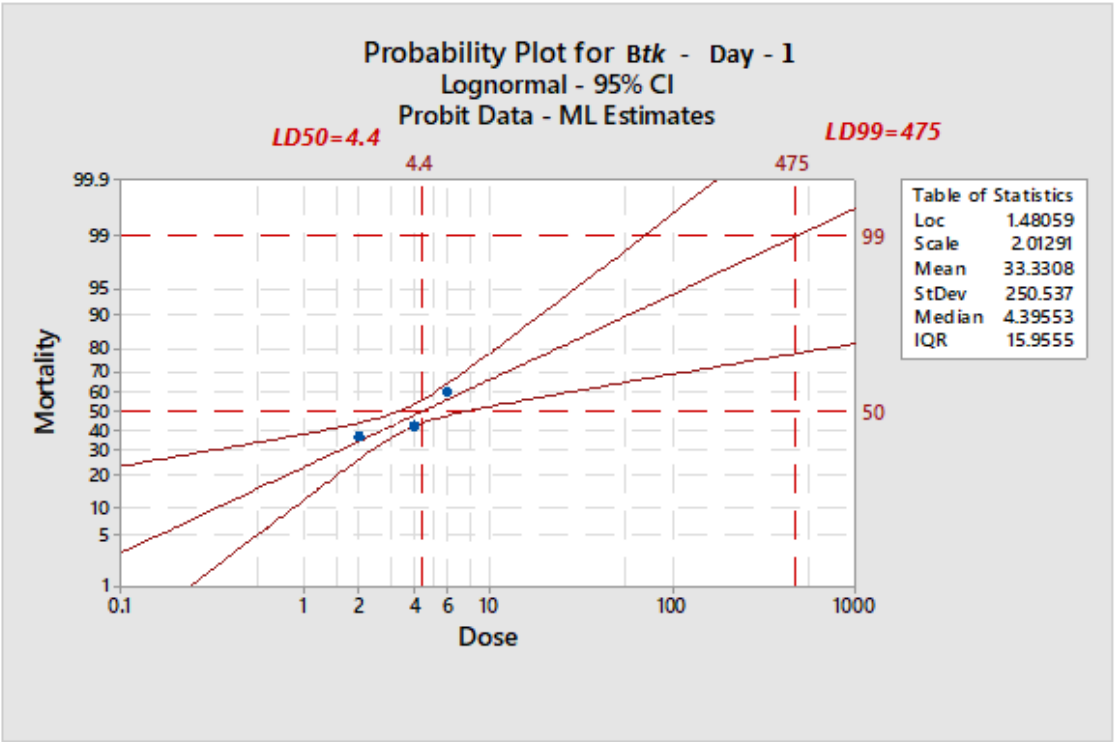

Figure 3.48. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the first day.

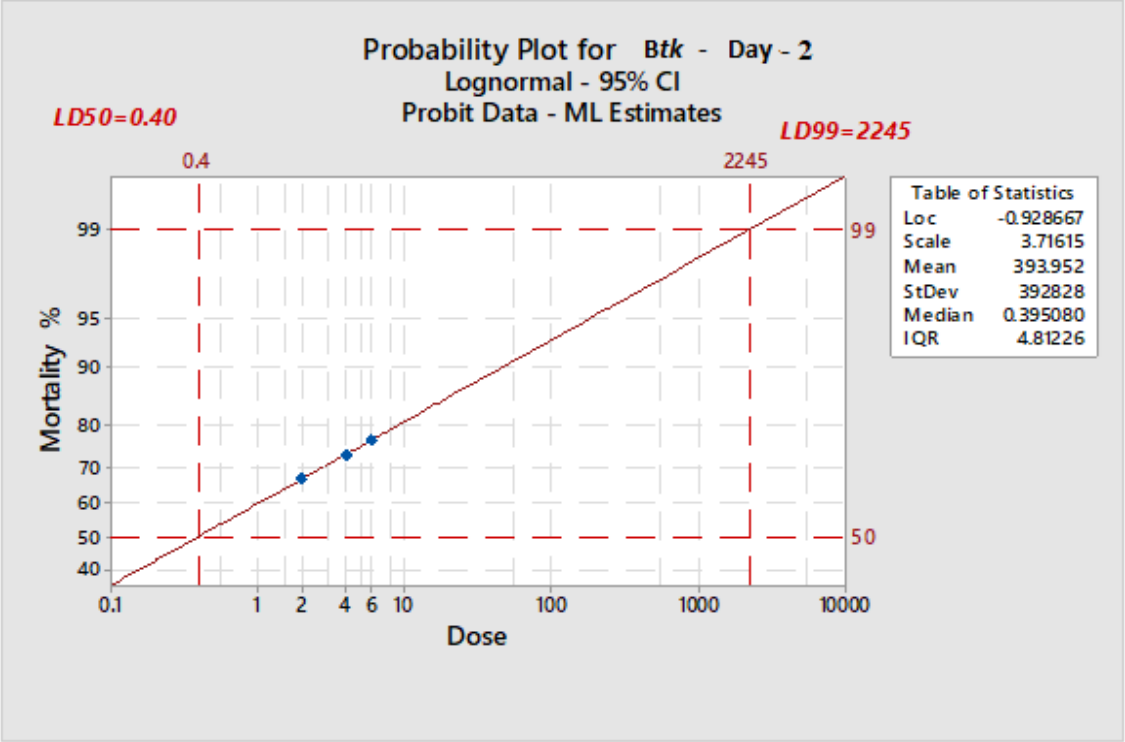

Figure 3.49. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the second day.

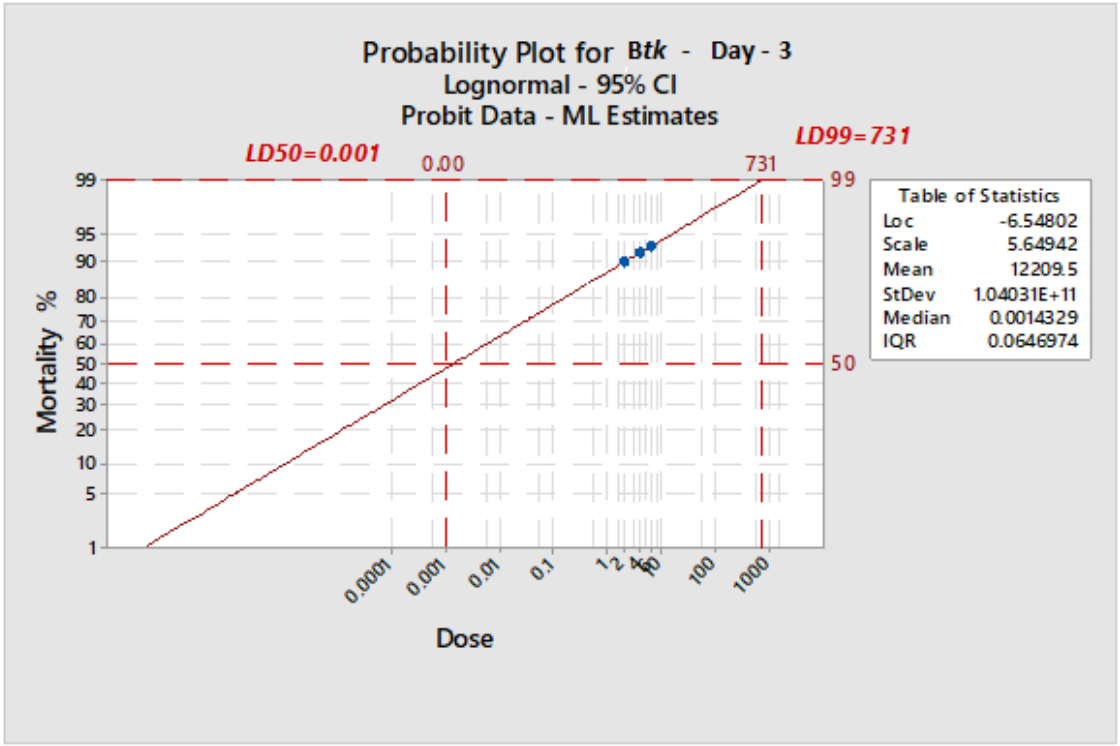

Figure 3.50. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the third day.

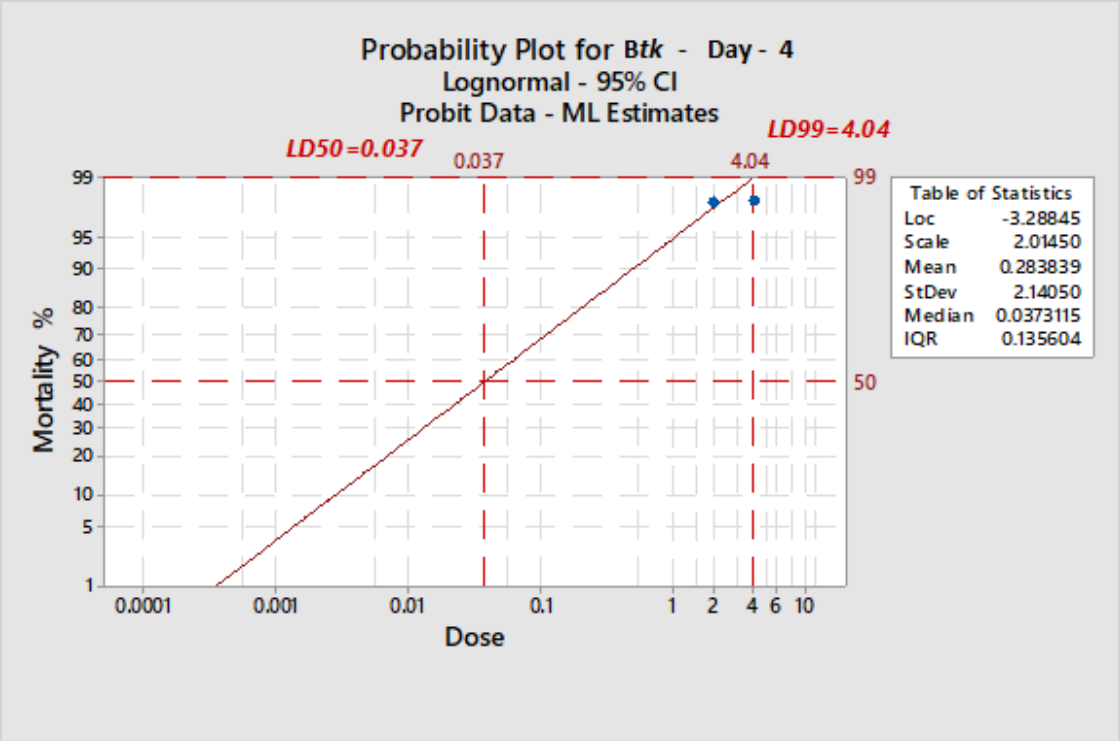

Figure 3.51. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the fourth day.

3.6. Lethal Effect of the Spore-Crystal Mixtures of *Bt* SY49.1, *Bt* SY27.1, and *Btk* on the Fourth Stage of Life (L4) of *Hyphantria cunea.*

In the fourth larval stage, mortality increased with days for the mixture of spores-crystal for the strains *Bt* SY49.1, *Bt* SY27.1, and *Btk* in all doses.

On the first day, *Bt* SY49.1 showed that the lowest mortality was 70% for the fourth dose with crystal while the highest mortality was 90% for the sixth dose with spore-crystal. *Bt* SY27.1 showed that the lowest mortality was 53.3% for the second dose with spore-crystal, while highest mortality was 96.7% for the second and sixth doses with crystal. *Btk* showed that the lowest mortality was 53.3% for the sixth dose with crystal, while highest mortality was 93.3% for the sixth dose with spore-crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD = 5.4), as shown in (Table 3.27, Figure 3-52).

Table 3.27. Percent mortality rates for spore-crystal mixtures on *H. cunea* larvae on the first day.

Bt Strains	Addition	Dose (µg/ml)		
		50µg/ml	250µg/ml	1000 µg/ml
Bt SY49.1	crystal	73.3	70	80
	Spore- crystal	86.7	86.7	90
Bt SY27.1	crystal	96.7	96.7	83.3
	Spore- crystal	53.3	80	90
Btk	crystal	80	70	53.3
	Spore- crystal	73.3	63.3	93.3

Bt Strains * Dose					
Bt Strains	50µg/ml	250µg/ml	1000 µg/ml	Mean	
Bt SY49.1	80.0	78.3	85.0	81.1	
Bt SY27.1	75.0	88.3	86.7	83.3	
Btk	76.7	66.7	73.3	72.2	
LSD Strains *dose	6.6			LSD Strains	5.4

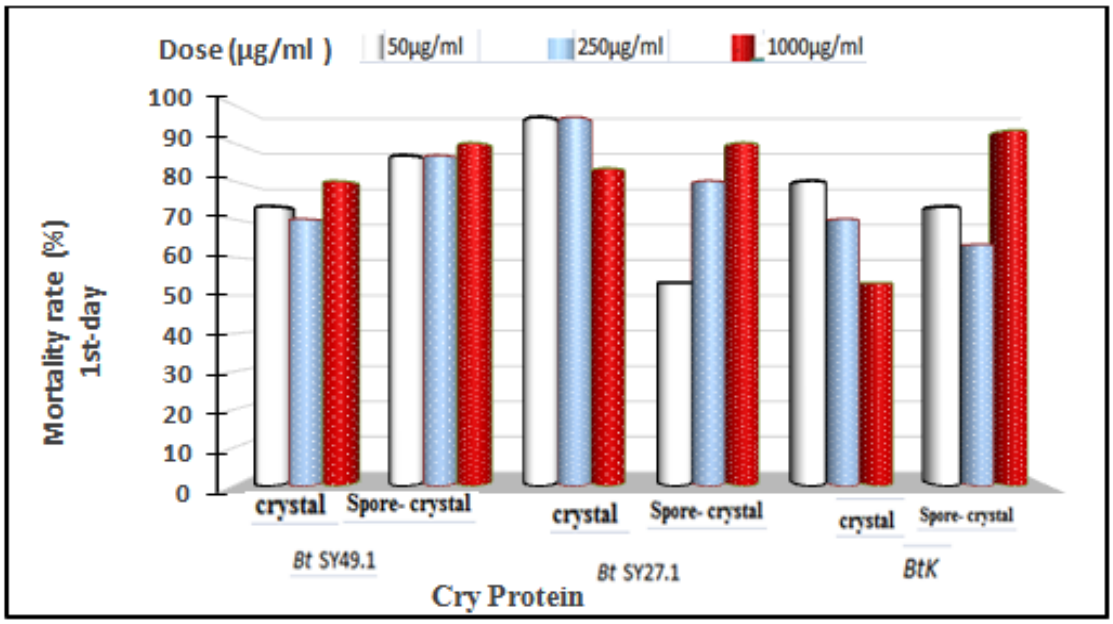

Figure 3.52. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the first day.

On the second day, *Bt* SY49.1 showed that the lowest mortality was 76.7% for the second, fourth and sixth doses with spore-crystal while the highest mortality was 90% for the sixth dose with crystal. *Bt* SY27.1 showed that the lowest mortality was 70% for the fourth dose with spore-crystal, while highest mortality was 96.7% for the second dose with crystal. *Btk* showed that the lowest mortality was 53.3% for the second and sixth doses with crystal, while highest mortality was 70% for the sixth dose with crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD = 5.0), as shown in (Table 3.28, Figure 3-53).

Table 3.28. Percent mortality rates for spore-crystal mixtures on *H. cunea* larvae on the second day.

***Bt* Strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**83.3**	**86.7**	**90**
	Spore- crystal	**76.7**	**76.7**	**76.7**
***Bt* SY27.1**	**crystal**	**96.7**	**90**	**86.7**
	Spore- crystal	**80**	**70**	**80**
Btk	**crystal**	**53.3**	**53.3**	**70**
	Spore- crystal	**60**	**63.3**	**56.7**

***Bt* Strains * Dose**					
***Bt* Strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean**	
***Bt* SY49.1**	**80**	**81.7**	**83.3**	**81.7**	
***Bt* SY27.1**	**88.3**	**80**	**83.3**	**83.9**	
Btk	**56.7**	**58.3**	**63.3**	**59.4**	
LSD Strains *dose	**8.6**			**LSD Strains**	**5.0**

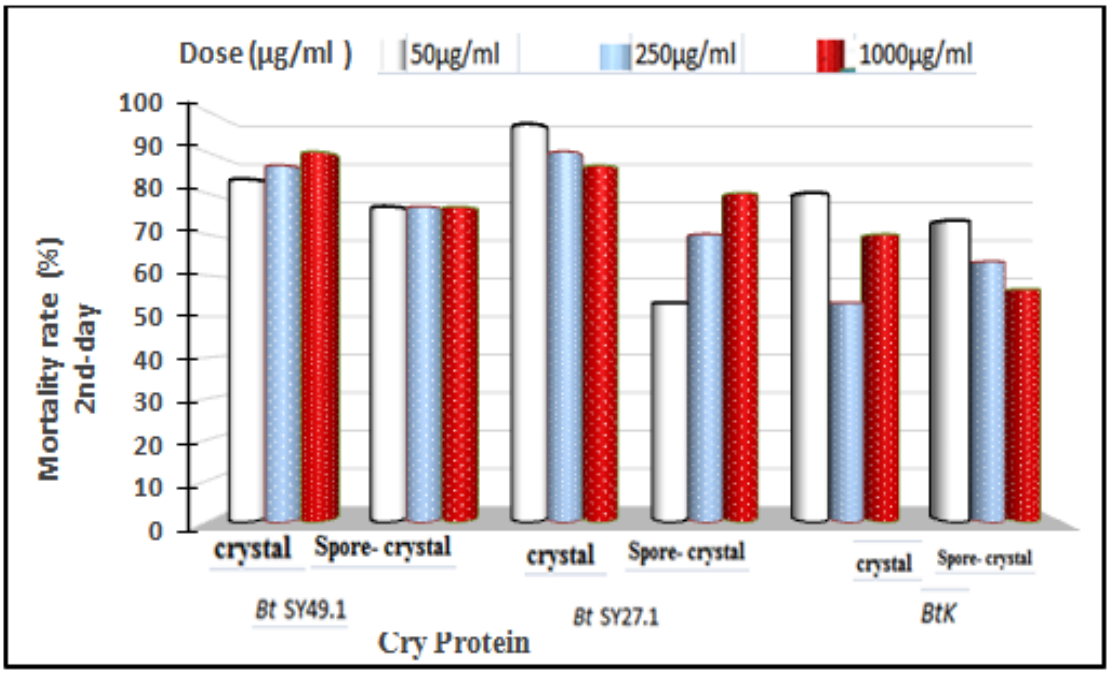

Figure 3.53. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the second day.

On the third day, *Bt* SY49.1 showed that the lowest mortality rate was 90% for the fourth dose with spore-crystal while the highest mortality rate was 96.7% for the fourth and sixth doses with crystal and 96.7% for the sixth dose with spore-crystal. *Bt* SY27.1 showed that the lowest mortality rate was 90% for the second dose with spore- crystal, while highest mortality was 100% for the second dose with crystal, The *Btk* showed that the lowest mortality was 73.3% for the second dose with crystal, while highest mortality

was 96.7% for the sixth dose with crystal and 96.7% for the sixth dose with spore-crystal. The *Bt* SY27.1 and *Bt* SY49.1 showed positive significant differences compared to *Btk* (LCD =3.9), as shown in (Table 3.29, Figure 3-54).

Table 3.29. Percent mortality rates for spore-crystal mixtures on *H. cunea* larvae on the third day

***Bt* Strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**93.3**	**96.7**	**96.7**
	Spore- crystal	**93.3**	**90**	**96.7**
***Bt* SY27**	**crystal**	**100**	**93.3**	**93.3**
	Spore- crystal	**90**	**96.7**	**93.3**
Btk	**crystal**	**73.3**	**83.3**	**96.7**
	Spore- crystal	**76.7**	**93.3**	**96.7**

***Bt* Strains * Dose**					
***Bt* Strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean**	
***Bt* SY49.1**	**93.3**	**93.3**	**96.7**	**94.4**	
***Bt* SY27.1**	**95**	**95**	**93.3**	**94.4**	
Btk	**75**	**88.3**	**96.7**	**86.7**	
LSD Strains *dose	**6.8**			**LSD Strains**	**3.9**

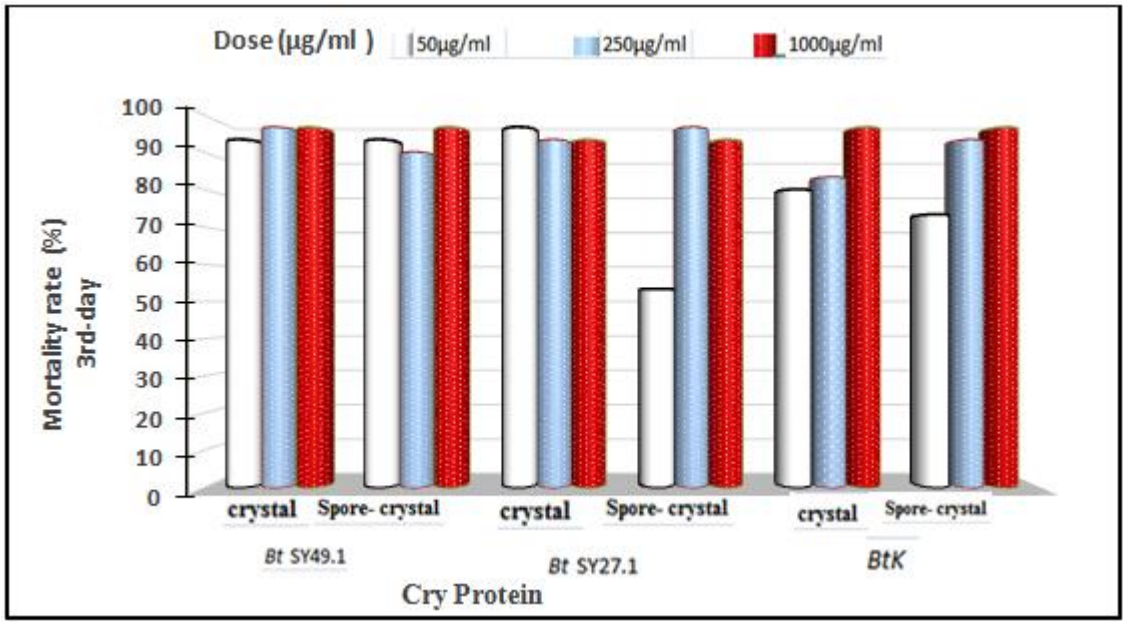

Figure 3.54. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the third day.

On the fourth day, *Bt* SY49.1 showed that the lowest mortality was 96.7% for the sixth dose with crystal while the highest mortality was 100% for the second and fourth doses with crystal, and 100% for the second, fourth and sixth doses with spore-crystal. *Bt* SY27.1 showed that the lowest mortality was 96.7% for the second dose with crystal, and

96.7% for the second and fourth doses with spore–crystal, while highest mortality was 100% for the second and sixth doses with crystal, and 100% for the sixth dose with spore–crystal. *Btk* showed that the lowest mortality was 93% for the second and fourth doses with spore–crystal, while highest mortality was 100% for the second and sixth doses with crystal, and 100% for the sixth dose with spore–crystal. *Bt* SY27.1 showed positive significant differences compared to *Bt* SY49.1 and *Btk* (LCD =2.4), as shown in (Table 3.30, Figure 3-55).

Table 3.30. Percent mortality rates for spore-crystal mixtures on *H. cunea* larvae on the fourth day.

***Bt* Strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**100**	**100**	**96.7**
	Spore- crystal	**100**	**100**	**100**
***Bt* SY27**	**crystal**	**100**	**96.7**	**100**
	Spore- crystal	**96.7**	**96.7**	**100**
Btk	**crystal**	**100**	**96.7**	**100**
	Spore- crystal	**93.3**	**93.3**	**100**

***Bt* Strains * Dose**					
***Bt* Strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean**	
***Bt* SY49.1**	**80**	**78.3**	**85**	**81.1**	
***Bt* SY27.1**	**75**	**88.3**	**86.7**	**83.3**	
Btk	**76.7**	**66.7**	**73.3**	**72.2**	
LSD Strains *dose	**4.2**			**LSD Strains**	**2.4**

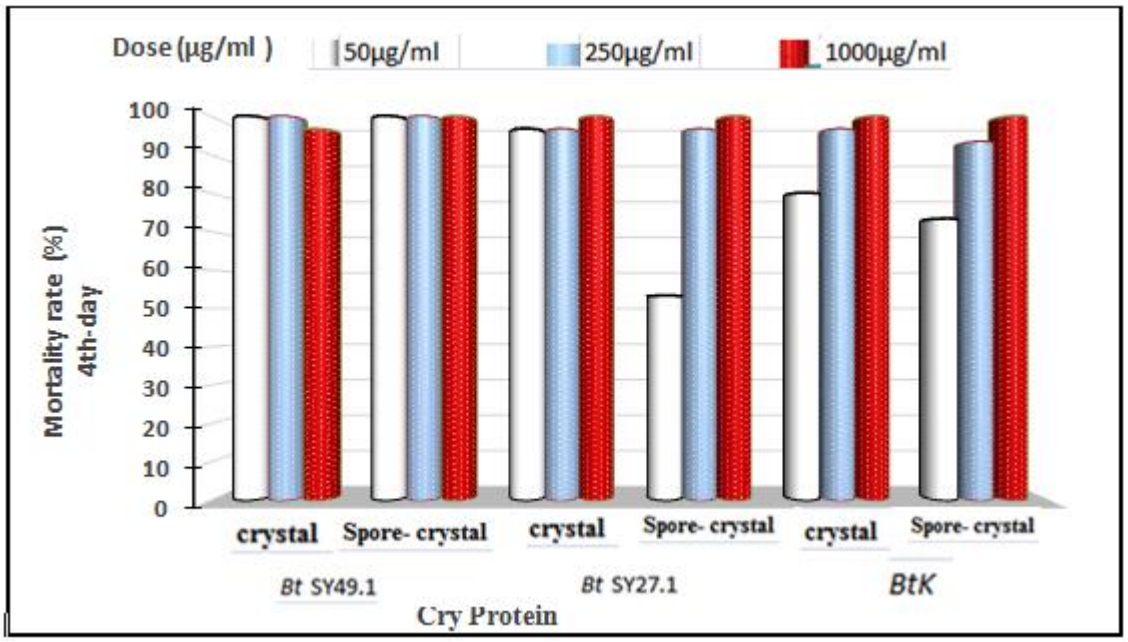

Figure 3.55. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the fourth day.

On the fifth day, it was found that the larval mortality rate for *Bt* SY49.1, *Bt* SY27.1 and *Btk* was nearly 100% in all doses used with crystal and spore–crystal as shown in (Table 3.31, Figure 3-56).

Table 3.31. Percent mortality rates for spore-crystal mixtures on *H. cunea* larvae on the fifth day.

***Bt* Strains**	**Addition**	**Dose (µg/ml)**		
		50µg/ml	**250µg/ml**	**1000 µg/ml**
***Bt* SY49.1**	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**
***Bt* SY27.1**	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**
Btk	**crystal**	**100**	**100**	**100**
	Spore- crystal	**100**	**100**	**100**

***Bt* Strains * Dose**					
***Bt* Strains**	**50µg/ml**	**250µg/ml**	**1000 µg/ml**	**Mean**	
***Bt* SY49.1**	**100**	**100**	**100**	**100**	
***Bt* SY27.1**	**100**	**100**	**100**	**100**	
Btk	**100**	**100**	**100**	**100**	
LSD Strains *dose	*			**LSD Strains**	*

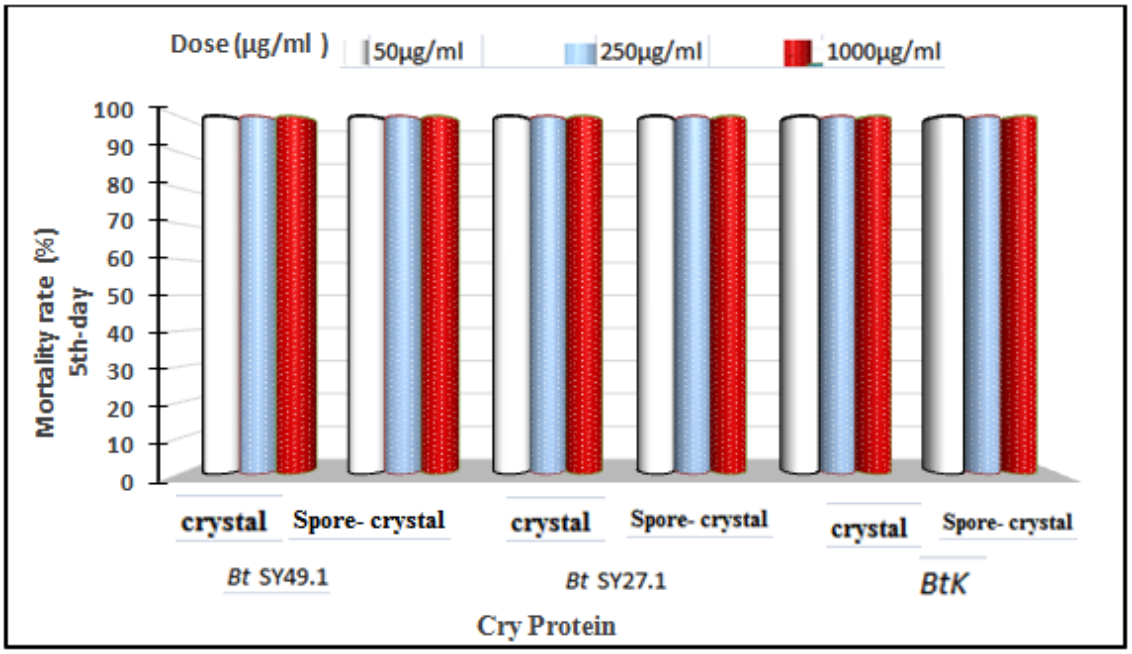

Figure 3.56. Relationship between the doses and mortality rates for spore-crystal mixtures on *H. cunea* larvae on the fifth day.

According to the results, the LD50 values of *Bt* SY49.1 were 0.066, 0.005, 0.02, and 1.42 µg/ml for the first four days. We noted that the larval mortality rate on the first day is low and over time the larval mortality increased. The LD50 values of *Bt* SY27.1 were 0.48, 0.108, and 0.13 µg/ml for the first four days, and also the values of LD99 were 6.58, 423.4, 8805, and 4.22 µg/ml for the first four days. The larval mortality rate increased with the progression of time. LD50 values of *Btk* were 0.40, 0.59, 1.03, and 0.13 µg/ml for the first four days. The larval mortality rate increased with the progression of time, as shown in Table (3.32).

Table 3.32. LD50 and LD99 values for spore-crystal mixtures of *Bt* SY49.1, *Bt* SY27.1 and *Btk* on fourth larval stage of *H. cunea* for four days.

***Bt* strains**	**LD50**	**LD99**
***Bt* SY27.1– 1st**	**0.48**	**6.58**
***Bt* SY27.1– 2nd**	**0.108**	**423.4**
***Bt* SY27.1– 3rd**	**0.00**	**8805**
***Bt* SY27.1– 4th**	**0.13**	**4.22**
***Bt* SY49.1– 1st**	**0.066**	**2552**
***Bt* SY49.1– 2nd**	**0.005**	**-**
***Bt* SY49.1– 3rd**	**0.02**	**33.6**
***Bt* SY49.1– 4th**	**1.42**	**2.09**
***Btk* – 1st**	**0.40**	**2244**
***Btk* – 2nd**	**0.59**	**-**
***Btk*– 3rd**	**1.03**	**11.40**
***Btk*– 4th**	**0.13**	**4.22**

The graphs below show probability plot of spore-crystal mixtyres for *Bt* SY49.1, *Bt* SY27.1 and *Btk*, over four days. Graphs indicate the relationship between mortality rates and doses are designated as curves (Figures 3.57-3.68),

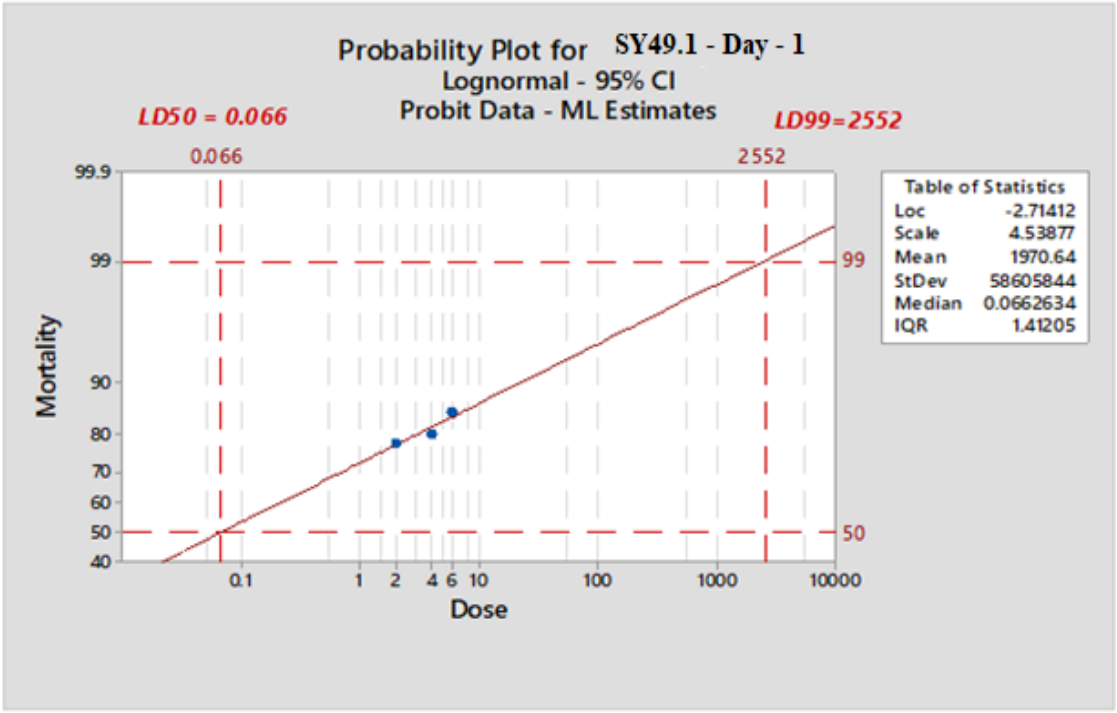

Figure 3.57. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the first day.

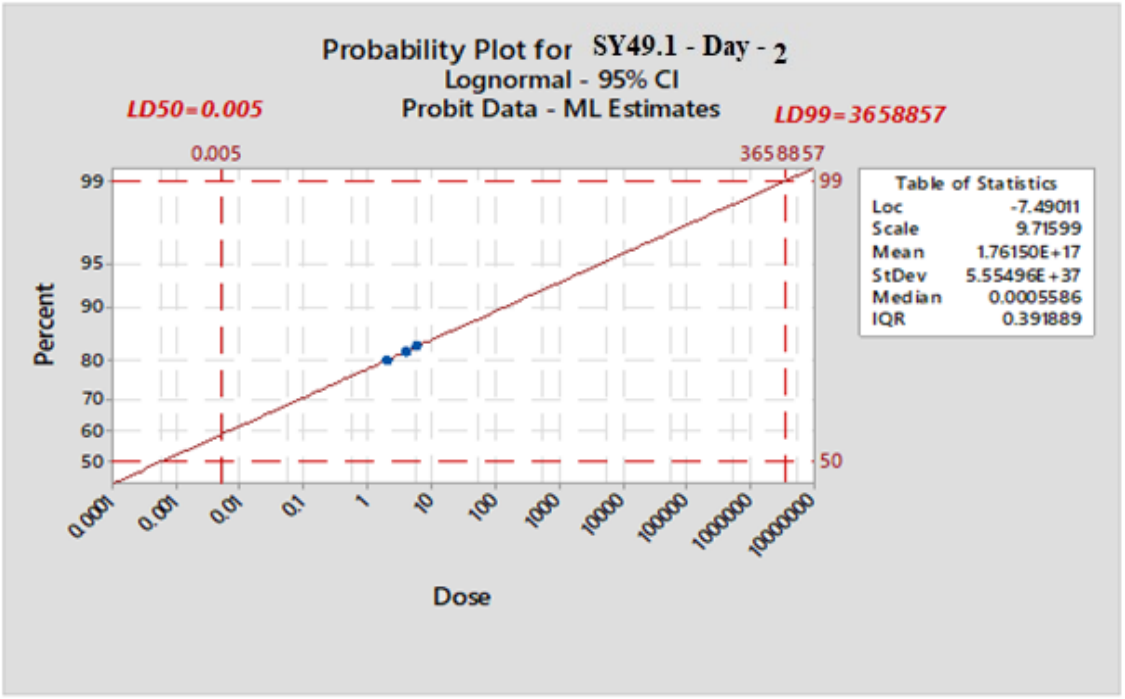

Figure 3.58. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the second day.

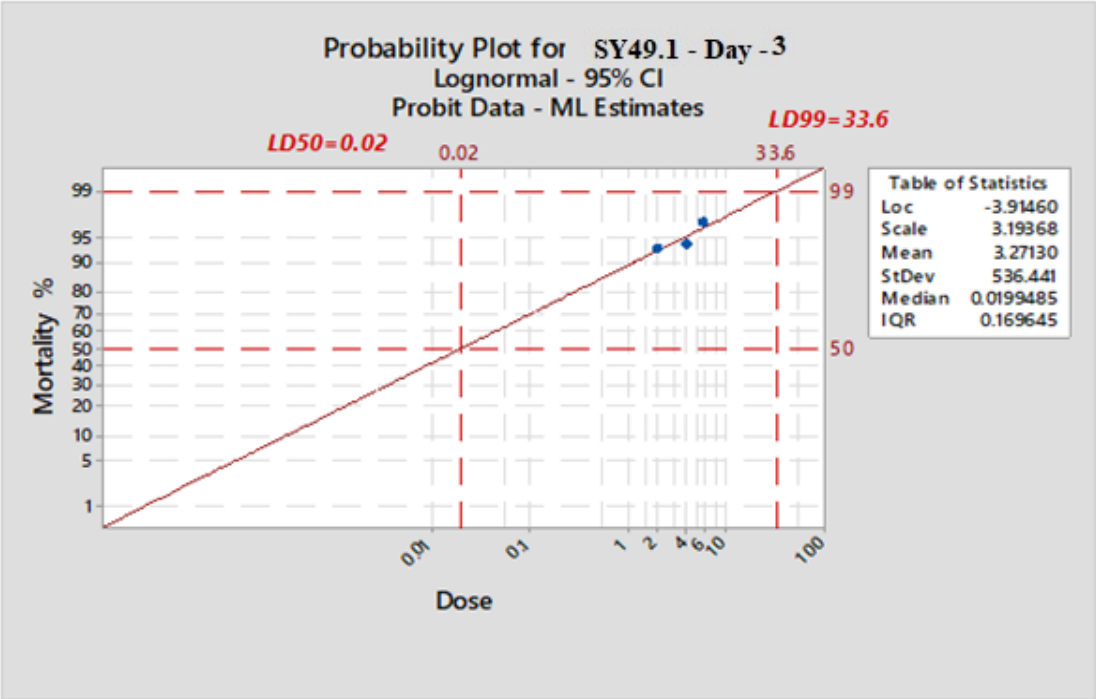

Figure 3.59. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the third day.

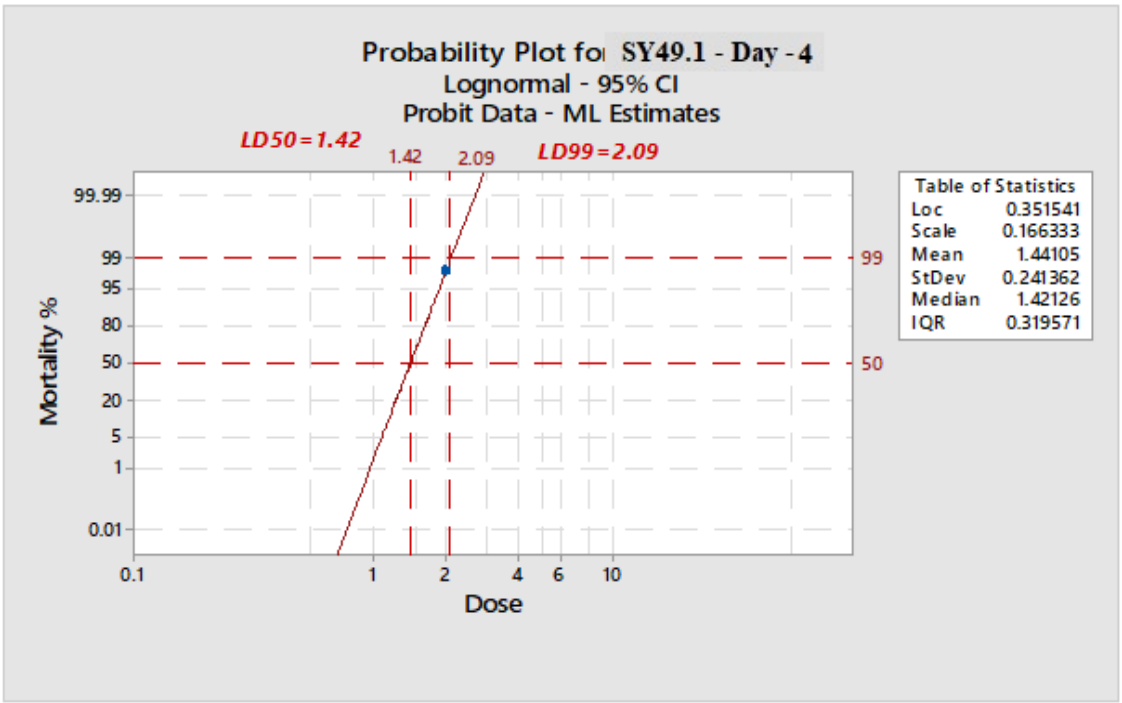

Figure 3.60. Prospective plot for spore-crystal mixtures of *Bt* SY49.1 on *H. cunea* larvae during the fourth day.

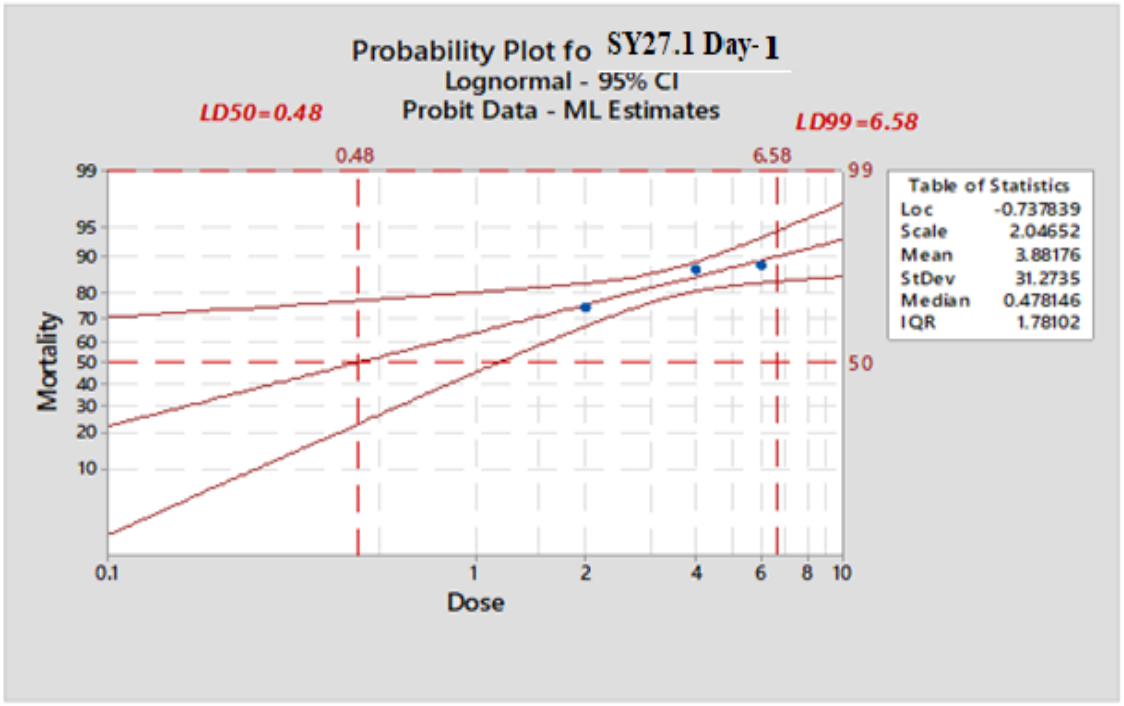

Figure 3.61. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the first day.

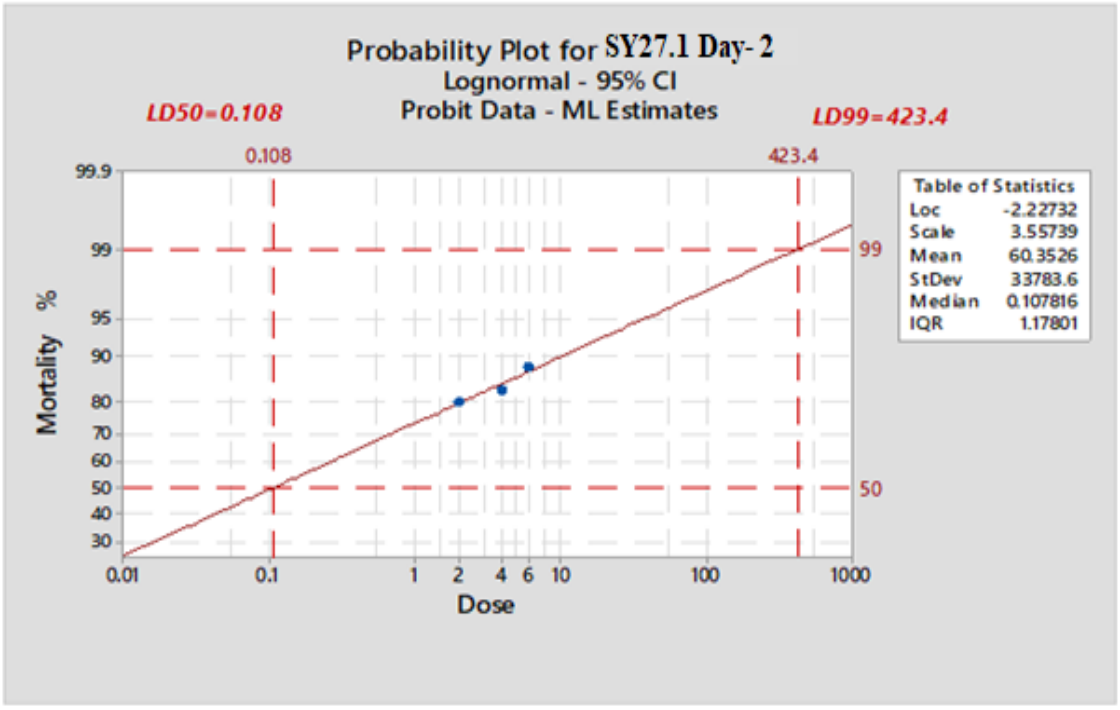

Figure 3.62. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the second day**.**

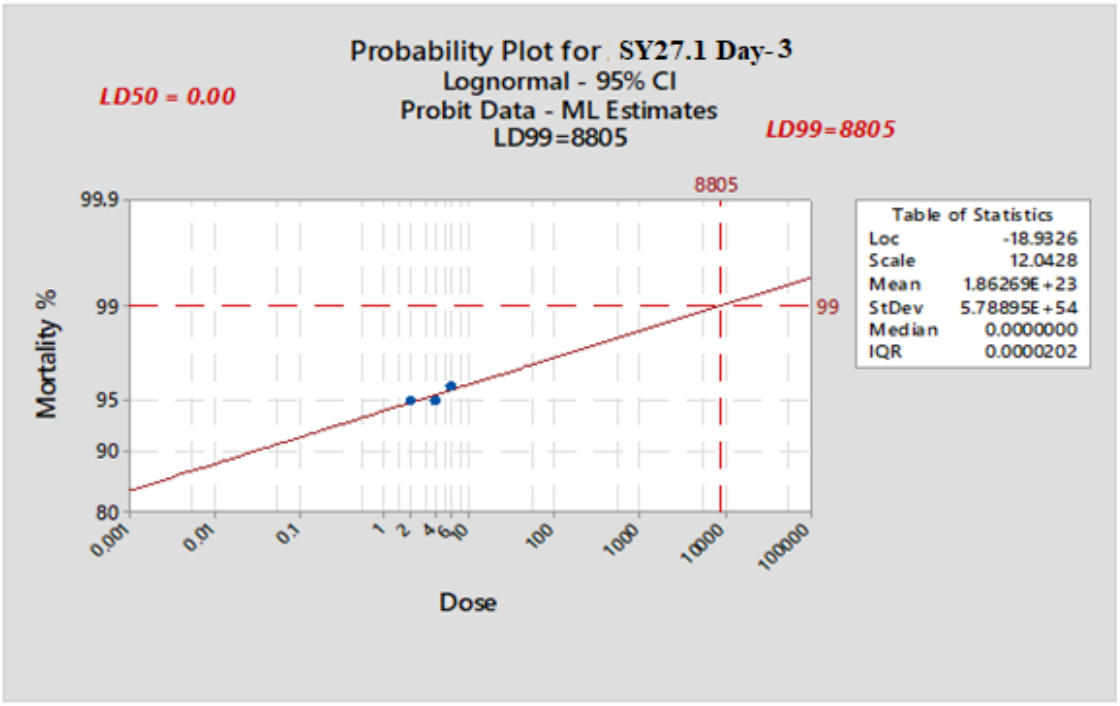

Figure 3.63. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the third day**.**

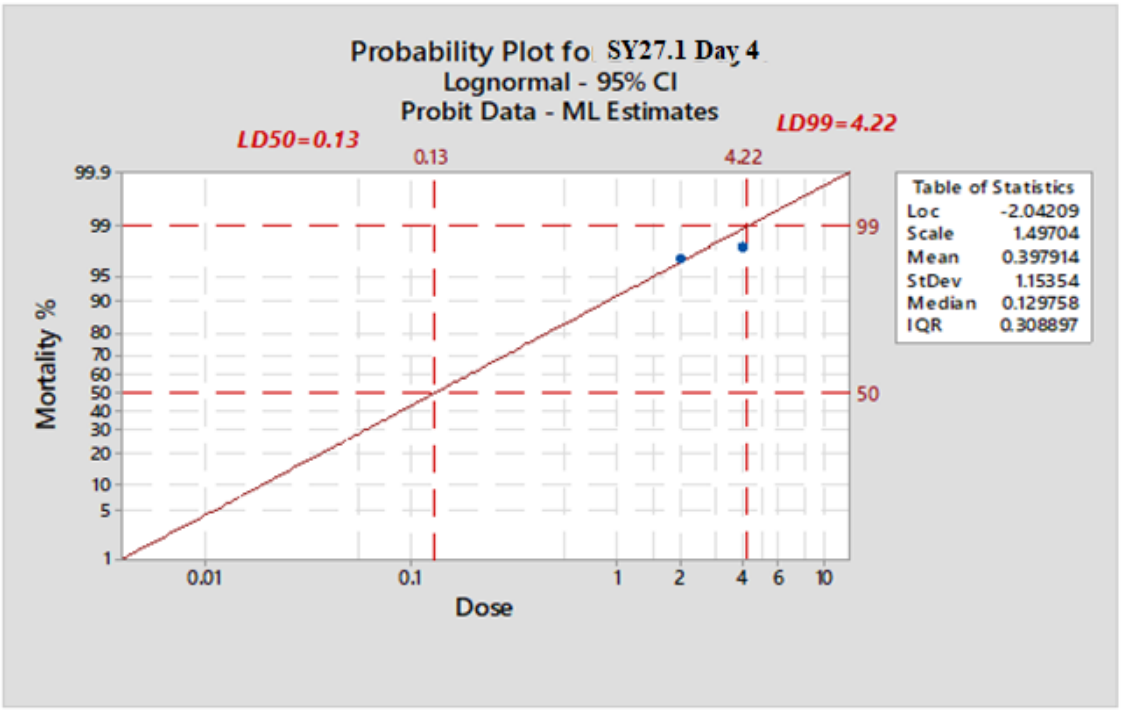

Figure 3.64. Prospective plot for spore-crystal mixtures of *Bt* SY27.1 on *H. cunea* larvae during the fourth day**.**

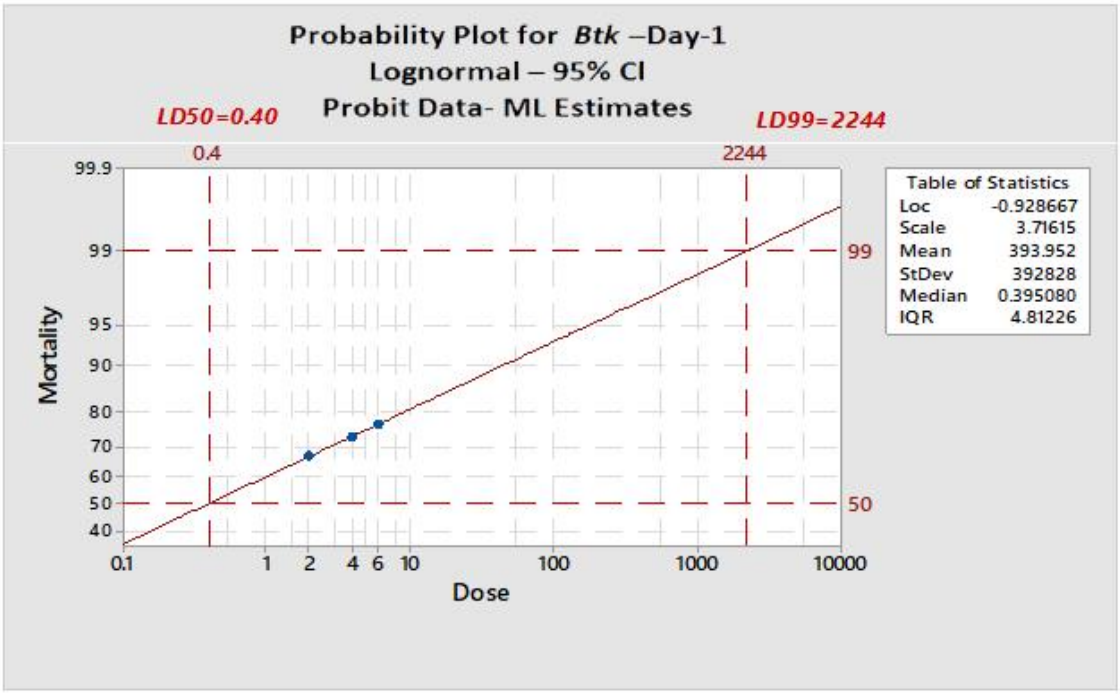

Figure 3.65. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the first day.

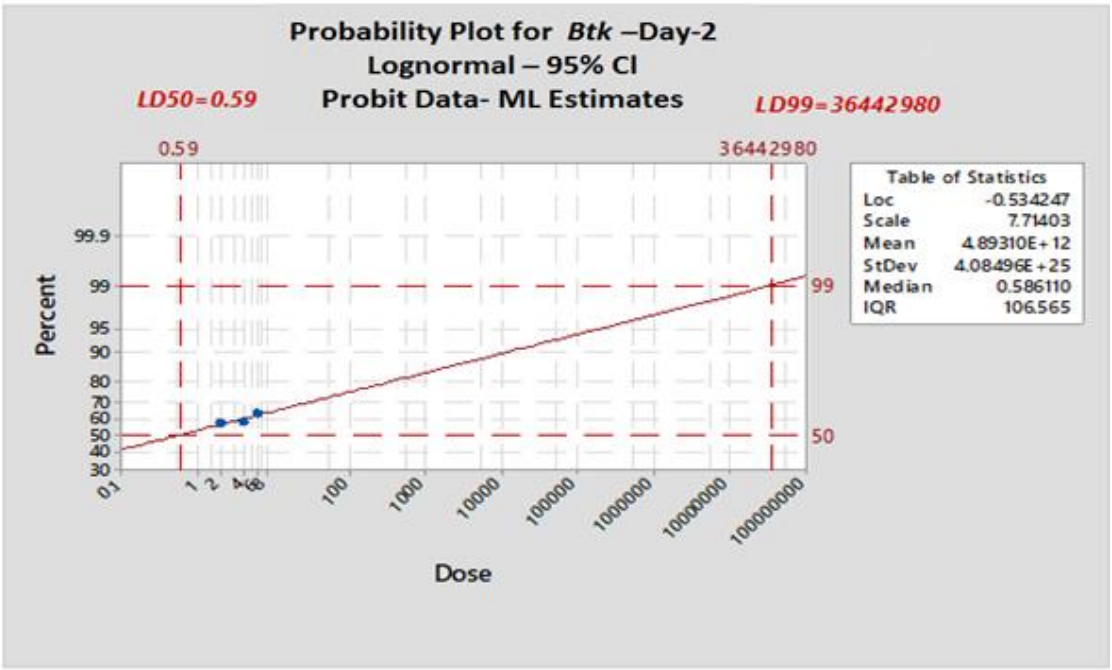

Figure 3.66. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the second day

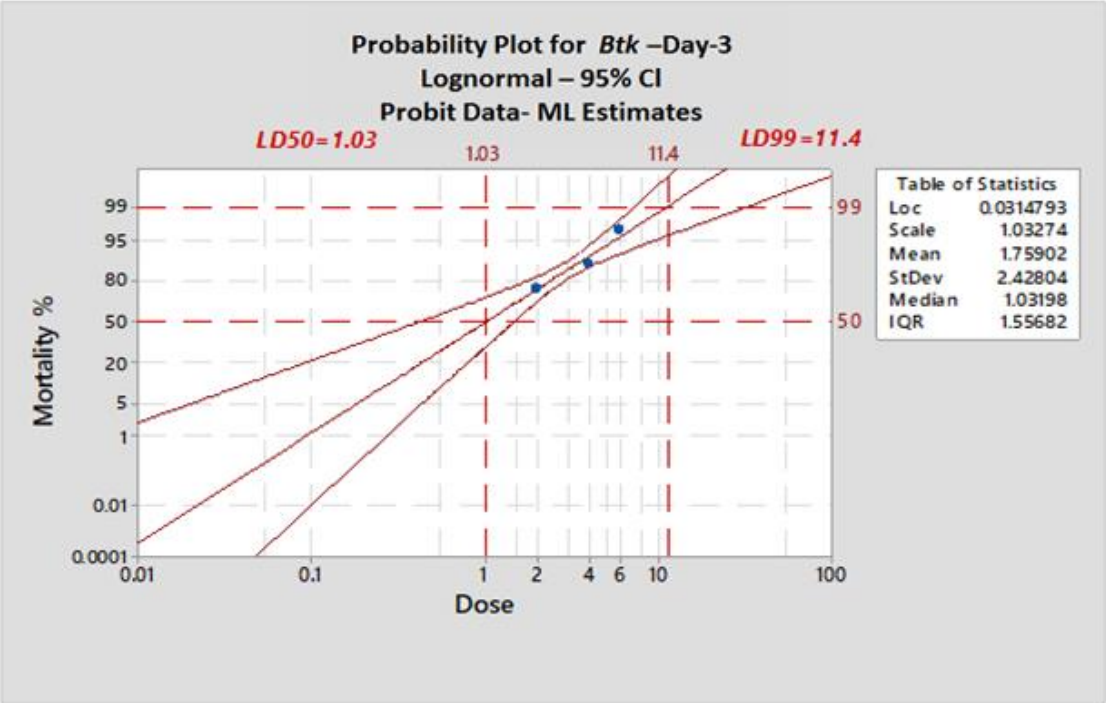

Figure 3.67. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the third day

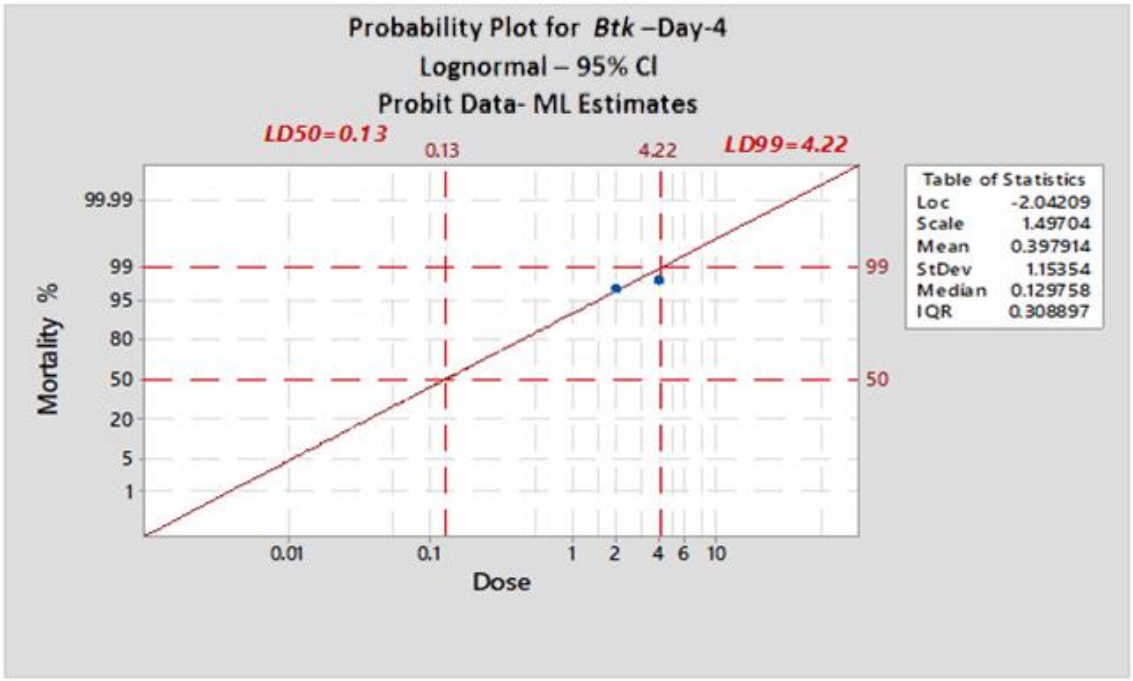

Figure 3.68. Prospective plot for spore-crystal mixtures of *Btk* on *H. cunea* larvae during the fourth day

CHAPTER 4

DISCUSSION

In present study, The mortality effect of *B. thuringiensis* (*Bt*), a particular kind of soil dwelling bacteria was tried on *H. cunea* larvae. *Bt* produces insecticidal proteins that, when consumed by some insects, have a harmful effect on those insects, but not on others beneficial insects. Beneficial organism don't have receptors for Cry proteins in their digestive tract and therefore the proteins remain inactive and do not pose any threat. *Bt* is not harmful to wildlife that is not its intended target ("The Pesticide manual: world compendium," 2013)

There are many different kinds of *Bt* subspecies and strains. There are a variety of insect families that are susceptible to these strains. For example the family of beetles, the family of flies including mosquitoes, and the family of butterflies are the insects that are targeted and susceptible to some *Bt* toxins (El-Bendary, 2006)

The fall webworm known as *H. cunea* is a moth of the family Erebidae. It is best known for the larval stage of its life cycle, which is responsible for building distinctive bare nests in late summer and dropping to tree limbs(Tang et al., 2012)

Generally, the mortality rate of *H. cunea* increased with days with all *Bt* strains and alldoses used in this study. As time progress, the mortality rate was increased, also, as the dose increased, the mortality rate increased.

The long residual action and toxicity of chemical insecticides have contributed to the development of serious environmental issues. These issues include the spread of insecticide resistance in many different species of vectors, the toxicity of mammalian organisms, and the aggregation of the pesticide in the foods and food products. The existence of all these issues has brought to light the need to providing alternative bio-

control agents. *Bt* and *B. sphaericus* are two majör examples of effective but risk-free biological control agents.

The long residual action and toxicity of chemical insecticides have contributed to the development of serious environmental issues. These issues include the spreading of insecticide resistance in many different species of vectors, and the aggregation of the pesticide in the food. The existence of all these issues has brought to light the need of providing alternative bio-control agents. *Bt* and *B. sphaericus* are two examples of effective but risk-free biological control agents.

These organisms have garnered a lot of attention as potential alternatives to the chemical pesticides that are now on the market. Although microbial pesticides that are based on *Bt* and *B. sphaericus* are available for use, large-scale use of these products in under developed nations is impractical due to the high cost of these products. The economic production of these two microorganisms by submerged fermentation and solid-state fermentation utilizing agro-industrial by-products and other wastes(Argolo-Filho & Loguercio, 2013)

There have been several efforts made by researchers to discover insecticides that are both safe and successful in the fight against economic pests. Pathogens have only been used to manage insect populations to a limited extent. Some of these agents have shown a great deal of promise and provide an outstanding alternative to insecticides made from chemicals. *Bt* products, which is an insecticide based on a bacterial pathogen, is now the most widely used kind of microbial pesticide (Argolo-Filho & Loguercio, 2013).

Bt has shown to be an efficient bio pesticide due to the fact that it produces the proteins *Cry* and *Cyt*, both of which may be very lethal to insects in certain environments. *Bt* protects plants from the infection caused by pathogens. As a biological inhibitor that could stop plant disease, *B. thuringiensis* was used in certain research studies. In tomato plants, the presence of wilt symptoms may be prevented by the bacterium *B. thuringiensis* (Betz, Hammond, & Fuchs, 2000).

Despite the prevalence of many plant diseases, its agricultural impact on a wide range of crops, especially the dipteran order, is of paramount importance to mankind. *B. thuringiensis* is a pesticide bacterium that produces proteins that accrue in crystals

with insecticidal and is widely used throughout the biological control of insect pests such as Lepidoptera order. *Bt* is responsible for the formation of crystals with insecticidal effect(Schmitges et al., 2016)

When given orally, *B. thuringiensis* var. *kurstaki* has detrimental effect on the digestive enzyme of *H. cunea* and lowered the activity of enzymes of the digestive system in a dose-related manner, with the exception of beta-glycosidase and lipase. After treatment of the larvae, a rise in the pest's LDH level was observed (Zibaee et al., 2010).

Researchers in Korea were able to extract *Bt* from the grain dust of soybeans. Three proteins with different apparent molecular weights made up the δ-endotoxins crystal that was produced by strain *Bt*-209. At 72 hours, *Bt*-209 demonstrated a high level of lethality against *Hyphantria cunea* larvae, with a mortality rate of between 70% and 87% (Jung, Kim, Son, Lee, & Song-Hae, 1995).

In *Hyphantria cunea* larvae, infected with *Bacillus thuringiensis* subsp. *kurstaki*, which exhibited the greatest gallotannin quantity and phenolic compounds had an influence on the host defense and antioxidant enzyme activities. In addition, a decline was seen in the levels of the flavonoids catechin and rutin (Yanar, Topkara, Mercan, Demir, & Bayramoglu, 2022).

Insect mortality and bacterial growth in the gut and in the hemolymph were studied in *Hyphantria cunea* to a commercial preparation of *Bacillus thuringiensis* and showed that *Hyphantria cunea* were killed rapidly by relatively low do sages (Shaikh & Morrison, 1966).

Upon internal autopsy of the dead larvae, it was observed that *Hyphantria cunea* larvae died as a result of infection with *B. thuringiensis*, and the number of viable cells of these bacteria increased 15-66 times(M. Xu, Xu, & Wu, 2017)

Bt is a spore-forming microorganism that is widely used as a biocontrol agent in agriculture. The insecticidal proteins that are secreted by *B. thuringiensis* include toxins that are formed during the vegetative growth phase, parasporal crystalline δ-endotoxins that are produced during the vegetative stationary phase, and δ-endotoxins. There has been a vast variety of Cry proteins discovered up to this point, and the majority of them

have been classified as three-domain-Cry toxins, Bin-like toxins, or Etx Mtx2-like toxins (Chattopadhyay & Banerjee, 2018).

Bacillus thuringiensis wasefficient against leaf-chewing insects of Lepidoptera. The activity of pathogens against the larva of *Hyphantria cunea* of early instars was around 74 (7-100%). The strains of *Bt* were protective against leaf chewing insects (Kuznetsova & Konoplyova, 2007).

Bt crystals have various forms (bipyramidal, cuboidal, flat rhomboid, or a composite with two or more crystal types). The crystal toxins (δ-endotoxin) are belonging to two structurally different groups. The first:

- Cry family, with specific cytolytic activity as Cry1Aa1, Cry1Ba1, Cry2Aa1.
- The second: Cyt family, which is a nonspecific cytolytic and hemolytic as Cyt1Aa1, Cyt2Aa1 (Calamari et al., 1999).

Bt is a bacterium that is found naturally in soils throughout the world. To reproduce, *Bt* makes spores that grow into new vegetative cell. *Bt* spores have proteins that are toxic to insect larvae when eaten. Because *Bt* comes from a natural source, it is called a biopesticide. In general, bio-pesticides tend to pose fewer risks than typical human-made pesticides (Dakhel, Jaronski, & Schell, 2020).

The pathogenicity of these bacteria has been attributed to the expression of toxic proteins (10–30% dry weight of the bacteria) that are produced in crystalline form. Ingestion of these toxic proteins causes the destruction of the larval gut epithelial cellcausing the death of the larvae (Evans, 2016).

A number of studies (Smith, Cámara-Artigas, Brune, & Allen, 2005) revealed that the action of the crystal toxin on susceptible larvae involved the following series of steps:

- The larvae of the target insect ingest crystal proteins from water.
- The crystal proteins are solubilized and activated under the combination of alkaline pH and proteins of the larval mid-gut.

- Active toxins bind to apical microvilli of midgut cells. They bind to specific membrane receptors (Phospholipids, phosphatidylcholine, and sphingomyelin, for *Bt* toxins and a 60 kDa -glucosidase for *Bt* toxins, attached to the apical midgut membrane by a glycosyl phosphatidylinositol anchor).

- After binding of the toxin to the receptor site, a part of the toxin inserts into the membrane lipid bilayer forming an ionic-selective channel or pore, which leads to the entry of water in to the cell and exit of ions and other larger components, leading to swelling and lyses of the cell by a colloid-osmotic lyses mechanism.

- There is another mechanism for pore formation called the theory of oligomeric pore formation, which proposed that the crystal toxins form oligomers, which are necessary for pore formation.

- The most drastic cytological changes caused by *Bt* toxin consist of swelling of midgut epithelial cells and lyses, while *Bt* toxin causes large vacuoles in the midgut cells and mitochondrial swelling. Late damage of neural tissues and skeletal muscles has also been reported.

In a study by Smith et al: 2018 on crystals and spores produced by *B. thuringiensis* for purification, each component—crystals, spores, and a mixture of crystals and spores was put through its steps against *H. cunea* larvae. On larvae of the second and third instar (L2 and L3) instars of *H. cunea*, the insecticidal activity of crystals, spores and crystalline spore mixtures were found to be 37.5, 25 and 62.5%, respectively. However, the mixture of crystalline spores has 6.5% more insecticidal effect than the plant cells of *Bacillus thuringiensis* (Qi, Aiuchi, Tani, Asano, & Koike, 2016) Insecticidal crystal protein gene of *Bacillus thuringiensis* subsp *kurstaki* HD-73 by using the biotinylated DNA probes (pC34) showed significant effects on *Hyphantria cunea* (Mclinden, Sabourin, & Clark, 1985). *Bt* can secrete ellipsoidal spore and protein crystal (δ-endotoxin) which have toxic effects to a variety of insects, such as protozoan and nematodes (Feitelson, 1993).

Bacillus thuringiensis has been used as an effective bioinsecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations.

However, recently, *B. thuringiensis* was used as a biological control agent that can suppress plant disease (Qi et al., 2016).

A novel chitin deacetylase gene (hccda5) was identified from *Hyphantria cunea* larvae with *Bacillus thuringiensis* Cry1A protein induced its down-regulation, which provided potential protection against all stages of *H. cunea* larvae (Shaoya et al., 2017).

The mortality and pupation rates differed among the various instar larvae and between transgenic and non-transgenic poplar (C. Xu et al., 2019). *H. cunea* was affected by (*cry*) proteins which produced by *Bacillus thuringiensis*. In the midgut of *H. cunea*, it was discovered that there is a possible Cry1Ab toxin-binding protein. This protein is an APN isoform classified as HcAPN3. The level of expression of HcAPN3 was high throughout the whole larval developmental stages, and it was abundant in both the midgut and the hindgut tissues. After treatment with Cry1Ab toxin, there was a large down-regulation of HcAPN3 for the first 6 hours, followed by a considerable up-regulation over the next 12-24 hours. There was a correlation between the reduction in the expression of HcAPN3 and the decreased vulnerability of *H. cunea* to Cry1Ab (C. Xu et al., 2019).

Plants with insect resistance were produced as a result of an increase of the control protein contained inside *Bacillus thuringiensis. B. thuringiensis* (Cry1Ah1) proteins exhibit toxicity that is quite particular when it comes to *Hyphantria cunea*. The Cry1Ah1 sequence was improved and changed such that it corresponds to the poplar tree's optimum codon. When compared with a control group, the Cry1Ah1 gene had a significant contribution to increasing the degree of insecticidal action against *H. cunea* (Siegwart et al., 2015; C. Xu et al., 2019).

The first instars of the fall webworm, *Hyphantria cunea*, were not adversely affected by the soluble protein that was isolated from *Bacillus thuringiensis* HD-290-1. The toxin that had been processed by trypsin was somewhat more poisonous to forest tent and cottonwood leaf beetles than protoxin that had not been digested. As cottonwood trees aged, their susceptibility to attack by cottonwood leaf beetles diminished. When exposed to the greatest dosage of toxin that was tested (100 g/ml for 96 hours), the

death rate of adult cottonwood leaf beetles did not surpass 30 percent (Ramachandran et al., 1993).

Contrasting level of effect of spores and protein among the reports during vegetative growth, the transition phase, and sporulation phase is attributed into several factors which related to growth and toxin production by *Bt*. It is included carbon level, nitrogen level, potassium, metal ions, pH, temperature, and effect of aeration (Içgen, Içgen, & Ozcengiz, 2002; Karim, Lucas, Osborne, & Rogers, 1993; Mercan, Caglar, Aslim, & Beyatli, 2003; Ozkan, Dilek, Yetis, & Ozcengiz, 2003).

Our results showed by adding Cry proteins to the six strains (*Bt* SY25.1, *Bt* SY27.1, *Bt* SY33.3, *Bt* SY49.1, *Bt* SY56.3 and *Btk*), a higher value of LD_{50} was obtained by *Bt* SY56.3, while that of a lower value for isolation is *Bt* SY49.1. Moreover, the higher value for LD_{99} was achieved by *Bt* SY25.1, while the lower value for LD_{99} was achieved by *Bt* SY49.1.

B. thuringiensis has the ability to become a significant agent for the control of pests. There were substantial changes in the LD_{50} for northern masked chafers in the native soil. The LD_{99} and LD_{50} values of *Bt* toxin and spores that had LD_{50} values of 3.93 autoclaved native soil were one of the factors that caused these discrepancies(Zouridis et al., 2015)

The LD_{50} and LD_{99} for *Bt* against first instar larvae at (L3) larvae were estimated 219 and 105, respectively. The LD_{50} and LD_{99} of adding *Bt* with treated larvae reported 11.86 and 5.18, respectively. The use of the *Bt* had an additive interaction and can be highly effective in integrated *Hyphantria cunea* management(Saruhan et al., 2014)

In the third stage instar larval (L3), there was a higher mean rate of mortality for *Bt* SY27.1 with crystal and spor-crystal, while there was a lower mean mortality rate for *Btk* with crystal.

On the first day, there was a higher mean mortality rate of *Bt* SY27.1 with crystal, while lower mean morality rate for *Btk* with crystal was recorded. *Bt* SY49.1 showed a higher mean mortality rate as compared with the other proteins that were used. The crystal caused a higher mortality rate than crystal-spore mixture. On the second day, higher

mean mortality rate of *Bt* SY27.1 with crystal was observed, while a lower mean mortality rate for *Btk* with crystal was documented. *Bt* SY49.1 showed a higher mean mortality rate as compared with the other strains. The crystal-spore mixture caused a higher mortality than the crystal alone. On the third day, the was a higher mean mortality rate for *Bt* SY27.1 with crystal-spore, while lower mean mortality rate for *Bt* SY49.1 with crystal was observed. *Bt* SY49.1 showed a higher mean mortality rate as compared with the used other strains. The crystal -spore together cause a mortality rate more than the crystal alone. On the fourth day, generally, most of the used isolate showed a high mortality rate. *Bt* SY27.1 showed a higher mortality rate as compared with the others used strains. The crystal alone showed a higher mortality rate as compared with crystal-spore. On the fifth day, all larvae died with the crystal alone and with crystal-spore together.

The larvae of *H. cunea* are killed by the insecticidal of *B. thuringensis*, which also has a poisonous effect. The GM-10 strain is a major Mexican strain and is of the aizawai type, was chosen for the formulation because it provided an LD_{50} of 0.007 in an artificial diet and an LD_{50} of 80.83. Ash was chosen as the phago-stimulant to use in the formulations because it attracted 61.4% of the larvae during the food preference tests that were performed with leaves of walnut, ash, blackberry, loquat, and walnut shell powder along with a commercial phage-stimulant. These tests were performed in order to prepare the formulations. Guar gum was one of the components put through its paces during the process of narrowing down the candidates for the role of adhesion addition. After being subjected to microencapsulation, the poisonous activity of the examined formulations was still present, with an LD_{50} of less than 0.05 of *B. thuringensis*. Whether or whether it is possible to create an active substance that is effective against the immature stages of the first instar larval (L1) of *H. cunea* by employing the spray drying technique(Disler et al., 2019)

In field testing conducted in Hungary, the application of Thuricide HP, which is a preparation of *Bacillus thuringiensis* subsp. *kurstaki*, at a rate of 0.5 kg/ha from a helicopter proved effective stages of the first and second instar larval (L1) , (L2) of *Hyphantria cunea*. The older larvae needed higher rates in order to be eradicated completely. The larvae that had been treated for seven days and had survived had slowed down physiological processes; eighty percent of them had stopped eating, and

their mortality rate was increased to 12–14 days(Aksoy, Saruhan, Kaya, & Ozturk, 2018).

In the fourth stage instar larval (L4), higher mean rate of mortality for *Bt* SY49.1 with crystal and spore-crystal, while there is a lower mean mortality rate for *Btk* with spore-crystal. According to the results, on the first day, higher mean rate of mortality rate for *Bt* SY49.1 with spore-crystal, while a there was a lower mean mortality rate for *Btk* with crystal. *Bt* SY27.1 showed a higher mean mortality rate as compared with the other strains. On the second day, higher mean rate of mortality rate for *Bt* SY27.1 strain with crystal, while a lower mean mortality rate for *Btk* with crystal. *Bt* SY27.1 showed a higher mean mortality rate as compared with the used other strains. The crystal causes a mortality rate more than spore-crystal together. On the third day, higher mean rate of mortality rate for *Bt* SY49.1 with crystal, while a lower mean mortality rate for *Btk* with crystal. *Bt* SY49.1 and *Bt* SY27.1 showed a higher mean mortality rate as compared with *Btk*. The crystal and spore-crystal showed the same molarity rate. On the fourth day, higher mean rate of mortality rate for *Bt* SY49.1 with spore-crystal, while lower mean mortality rate for *Btk* with spore-crystal. *Bt* SY49. showed a higher mean mortality rate as compared with the used other strains. On the fifth day, the mortality rate was nearly 100% in all the used strains, and all the used doses, with crystal only and with spore-crystal together.

After using the spray of *Bacillus thuringiensis kurstaki* sampling for three days, the biological compounds were successful in controlling the fall webworm in the second stage instar larval (L2), and in many experiments they ranked athigh level with high effect (65-97%)(Pirouz, Damavandian, Besheli, Science, & Sciences, 2017).

Researchers stated that *B. thuringiensis* and *Streptococcus sp*. had 56 and 38% effects, respectively on (L2) and (L3) larvae of *H. cunea* (Schnepf et al., 1998)[. *Bacillus megaterium* caused abnormalities in wings shapes and without hair on the thorax and abdomen of *Hyphantria cunea* (Aksoy et al., 2018).

Bacillus thrungiensis are used against different instars of *Hyphantria cunea* under laboratory and field conditions. *Bt kurstaki* caused 95% mortality in the second and third stage of life of the larva (L2) (L3) and 51% mortality in the fourth and fifth stage

of life of the larva (L4) (L5). The older instars were less susceptible, which may be attributable to the phenomenon known as “maturation immunity”. *Bacillus thuringiensis kurstaki* has already been recommended for the management of this pest. In Saruhan, I.; et, al study used three biopesticides, Delphin (32000 IU/mg *Bt*) were studied in the third stage (L3) larval instar of the fall webworm *Hyphantria cunea* under laboratory conditions with different doses (200, 100, 50 and 25) and were demonstrated that the three doses of dolphin gave 100% mortality during period ranged between 1-7 days. The seventh day showed a higher mortality rate as compared with the rest days (Saruhan et al., 2014).

In studies in Japan, spore-crystal of *Bacillus thuringiensis* was at least 25 times as toxic as other products to larvae of *Hyphantria cunea*. Spores of strains had low toxicity in the fourth stage of life of the larva (L4) larvae when they were used alone, but crystals of strains were highly toxic (Su et al., 2008). The insecticidal activity of the transgenic NL895 was better against in the first and second stage (L1) (L2) of larvae of *H. cunea* than in the third and fourth larval stages (C. Xu et al., 2019)(Emami Bistgani, Siadat, Bakhshandeh, Ghasemi Pirbalouti, & Hashemi, 2017).

Finally, our results revealed that the local *Bacillus thuringiensis* strains have direct and potential effects on larval stages of *Hyphantria cunea.* The reports and studies mentioned above were in agreement with our findings, and support our results.

CONCLUSION

In this study, *Bt* strains previously isolated and characterized from Adana region were used to determine their toxicity against the larvae of *Hyphantria cunea,* which were collected from the Rize region. The strains used were *Bt* SY49.1, *Bt* SY25.1, *Bt* SY33.3, *Bt* SY56.3, *Bt* SY27.1 ans standard strain *Btk*. The strains were grown in T3 medium for 4-7 days for sporulation and then the doses were adjusted after protein quantitation. The doses 10, 50, 100, 250, 500, 1000 and 2250 µg/ml were used for the trials. Different ages were taken from the larvae and they were exposed to these six types of strains' products. It was monitored for ten days, and the number of dead and live larvae were recorded. The findings showed that the mortality rate of *Hyphantria cunea* larvae was creased with the days in all the used *Bt* strains with all the used doses. As time progresses, the mortality rate increased; also, as the used dose is high, the mortality rate becomes more. Our results showed that a higher value of LD_{50} was achieved by *Bt* SY56.3, while a lower value was obtained after *Bt* SY49.1 application. Furthermore, the higher value of LD_{99} was obtainedby *Bt* SY33.3, while the lower value of LD_{99} was obtained by *Bt* SY49.1. Also, *Bt* SY56.3 showed lower mortality rates compared to the other strains, while *Bt* SY27.1 and *Bt* SY49.1 showed higher mortality rates among the strains used.

In the third stage instar larvae (L3), higher mean rate of mortality for *Bt* SY27.1 with crystal and spore-crystal, while there is a lower mean mortality rate for *Btk* with crystal. In the fourth stage larvae (L4), higher mean rate of mortality was obtained for *Bt* SY49.1 with crystal and spor-crystal, while there is a lower mean mortality rate for *Btk* with spor-crystal. We can conclude that local *Bacillus thuringiensis* strains had potential effects on *Hyphantria cunea* larvae. Local *Bt* strains' products can safely be used aginst *H. cunea* larvae for controlling them in agricultural fields via limiting the use of chemical insecticides.

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