T.R.

NİĞDE ÖMER HALİSDEMİR UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

IMPROVING CONTROL STRATEGIES AGAINST THE SUGAR BEET CYST NEMATODE *(Heterodera schachtii* SCHMIDT 1871*)*, IN SUGAR BEET.

BURHAN EGEMEN ERDAL

SEPTEMBER 2024

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MASTER THESIS

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Supervisor

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The study titled “**Improving control strategies against the sugar beet cyst nematode (Heterodera schachtii Schmidt. 1871), in sugar beet.**” and presented by **Burhan Egemen ERDAL** with the help of supervisor **Prof. Dr. Halil TOKTAY,** has been found as Master thesis by the jury at the **Department of Plant Production and Technologies** of Nigde Ömer Halisdemir University Graduate School of Natural and Applied Sciences

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CONFIRMATION:

This thesis has been found appropriate at the date of …./…./20.... by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated …./…./20.... and numbered ……………

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DIRECTOR

THESIS CERTIFICATION

It is certified that I have written this thesis by myself. I further confirm that all information included in this thesis is scientific and is in accordance with the university rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.

Burhan Egemen ERDAL

SUMMARY

IMPROVING CONTROL STRATEGIES AGAINST THE SUGAR BEET CYST NEMATODE (Heterodera schachtii Schmidt. 1871), IN SUGAR BEET.

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Department of Plant Production

Supervisor : Prof. Dr. Halil TOKTAY

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This reserch was conducted between 2021 and 2023 in two sugar beet cultivation locations in Bor, Niğde, areas infested with *Heterodera schachtii*, and at Niğde Ömer Halisdemir University in both in vivo and in vitro settings. During the field trials, the collected cysts were identified using morphological and molecular methods and the infection rate in the fields was determined to be an average of 45 cysts per 250 grams of soil. The trials tested control, fluopyram soil surface, and in-furrow applications. The study aimed to study the effects of these applications on nematode populations and yield, focusing on reducing chemical use and promoting sustainable agriculture. The results showed no statistical differences in yield or nematode numbers among the applications. In the laboratory, a *Bacillus thuringiensis* strain carrying the Cry6Aa2, Cry55Aa1, and cry5B genes was tested as a biological control agent and its positive impact on plant growth and nematode population suppression was found to be significant. The findings suggest that in-furrow application should become a routine practice for nematode control, and that *B. thuringiensis* holds potential as a biological control agent.

Keywords: *B. thuringiensis,* biological control, *Heterodera schachtii*, management methods, sugar beet

ÖZET

ŞEKER PANCARINDA, ŞEKER PANCARI KIST NEMATODU *(Heterodera schachtii Schmidt. 1871)*'NA KARŞI MÜCADELE STRATEJILERININ GELIŞTIRILMESI

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Danışman : Prof. Dr. Halil TOKTAY

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Bu tez çalışması, 2021-2023 yılları arasında Bor, Niğde'de *Heterodera schachtii* ile bulaşık şeker pancarı yetiştirilen iki lokasyonda, alanlarda ve Niğde Ömer Halisdemir Üniversitesi'nde *in-vitro* olarak yürütülmüştür. Arazi denemeleri sırasında, elde edilen kistler morfolojik ve moleküler yöntemlerle teşhis edilmiş ve tarlalardaki enfeksiyon oranı ortalama 45 kist/250 gr toprak olarak belirlenmiştir. Denemelerde ilaçsız kontrol, fluopyram toprak yüzeyi ve tohum yatağı uygulamaları denenmiştir. Çalışma, bu uygulamaların nematod popülasyonu ve verime etkisini araştırarak, kimyasal kullanımı azaltmayı ve sürdürülebilir tarımı hedeflemiştir. Laboratuvar denemelerinde ise Cry6Aa2, Cry55Aa1 ve cry5B genlerini taşıyan *Bacillus thuringiensis* bir biyolojik mücadele etmmeni olarak denenmiş ve bu etmenin, bitki gelişimine olumlu etkisi ve nematod popülasyonunu baskılama kapasitesi istatistiksel olarak anlamlı bulunmuştur. Elde edilen bilgiler ışığında tohum yatağı uygulamasının nematod mücadelesinde rütin hale gelmesi gerektiğini ve *B. thuringiensis*'in biyolojik mücadele etmeni olarak potansiyel taşıdığını göstermektedir.

Anahtar Sözcükler; Bacillus thuringiensis, biyolojik mücadele, mücadele yöntemleri, *Heterodera schachtii,* şeker pancari

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SYMBOLS AND ABBREVIATIONS

Symbols	Description
%	Percentage
°C	Degrees Celsius
µm	Micrometer
cm	Centimeter
g	Grams
Kg	Kilogram
ml	Milliliter
mm	Millimeter
t	Ton
V	Volt

Abbreviations	Description
ANOVA	Analysis of Variance
CO_2	Carbon Dioxide
COI	Cytochrome Oxidase Subunit 1 Mitochondrial Gen
ddH_2O	Double-distilled Water
FAO	Food and Agriculture Organization
H_2O	Water
ITS	Internal Transcribed Spacer Region of The Ribosomal
O_2	Oxygen
TÜİK	Türkiye Statistical Institute
$ZnCl_2$	Zinc Chloride

CHAPTER I

INTRODUCTION

Sugar beet (*Beta vulgaris var. saccharifera*) belongs to the Amaranthaceae family and is an important crop plant belonging to root and tuber crops group and it can accumulate about 20% of its root fresh weight as sugar. Sugar beet is mainly used as human food, livestock feed and in industry; sugar cane and sugar beet are two main sources of sugar for human consumption. Sugar content of sugar beet is about 25% higher than from sugar cane. Sugar production is derived from sugar beet 20% of the world's, while the remaining 80% comes from sugarcane but 90% of the sugar produced from sugar beet in Türkiye (FAO 2022). Sugar beet industry is a strategic sector that meets a large portion of the sugar, largest employment provider and a significant contributing sector to gross domestic product, imports and exports. In 2023, Türkiye produced 23,500,000 tons of sugar beet, positioning it as the fifth-largest producer in the world, with a cultivation area of 274,524 hectares, following Russia, England, France, and Germany (FAO, 2022). The top five Turkish provinces in sugar beet production are Konya, Eskişehir, Yozgat, Kayseri, and Aksaray. Niğde ranks 34th in sugar beet production, with 12,410 decares of land and a yield of 87,070 tons (TUIK, 2023).

Heterodera schachtii, commonly known as the beet cyst nematodes (BCN), affects not only sugar beet but also a wide range of other plants. Its host spectrum includes mustard, canola, and about 80% of species in the Chenopodiaceae and Cruciferae families (Börner, 1990). Originally from Europe, this nematode has spread globally alongside sugar beet cultivation, primarily through infested planting materials. BCN's are regarded as one of the most significant threats to global sugar beet production. These pests can inflict severe damage on sugar beet crops, leading to yield reductions of up to 25% (Schlang, 1991). The economic impact is substantial, with annual losses in EU countries estimated at 90 million Euro based on world market sugar prices (Müller, 2000). Plants affected by these nematodes exhibit several visible symptoms. Above ground, they display stunted growth and reduced leaf chlorophyll content. Towards the end of the growing season, especially when subjected to heat or water stress, the plants may show signs of wilting. Infected plants often develop compensatory secondary roots, resulting in a characteristic "bearded" appearance of the root system. This abnormal root growth can lead to overall deformity of the beet (Cooke, 1987; Hillnhütter, 2011).

The life cycle of BCN resembles other cyst nematodes. Soil cysts contain up to 600 eggs, typically housing infectious second-stage juveniles (J2) (Börner, 1990). When conditions are suitable, these J2s emerge, seek host roots, and become sedentary parasites. J2s prefer entering roots at elongation zones, wounds, or emerging lateral roots. After penetration, they move through the cortex to the pericycle, where they induce syncytium formation for feeding (Seinhorst, 1986; Holtmann et al., 2000). After three molts, males exit roots to fertilize females. Females continue growing, rupturing the root cortex, with their white, lemon-shaped bodies visible externally. About a month post-penetration, eggs form, the female dies, and becomes a hardened, brown cyst. Development time varies with environmental factors, primarily temperature (Steele, 1986; Seinhorst, 1986). Optimal reproduction occurs at 25-28°C but is possible between 12-28°C (Thomason and Fife, 1962). Multiple generations can occur in favorable conditions (Kerr et al., 1992; Muller, 1979). *BCN's* soil persistence depends on various factors. Without hosts, annual population decline is roughly 50% but varies. High temperatures accelerate decline (D'Addabbo et al., 2005).

BCN, is a significant threat to sugar beet crops globally (Cooke, 1991). This pest tends to thrive in areas with a long history of beet cultivation and short crop rotation cycles. When BCN populations reach high densities, they can severely impact sugar beet yields and diminish sugar content (Griffin, 1981). To mitigate crop losses, various strategies can be employed. These include cultural practices such as crop rotation, the use of *H. schachtii* resistant trap crops, and improved sanitation measures. Additionally, pesticide applications have been utilized for control. In Europe, sugar beet varieties resistant to *H. schachtii* have been available to growers since 1996 (Schlang, 1999). This difference in available control measures highlights the ongoing challenge of managing BCN across different geographical regions.

In the management of BCN, several chemicals such as: the soil fumigant 1,3-dichloropropene (Telone II), fungicide / nematicide fluopyram and the insecticide / nematicide aldicarb (Temik). However, the use of these pesticides requires careful consideration of economic factors, as well as their potential negative effects on the environment and human health. Reports have shown that both chemicals have contaminated ground and surface water in various states (Griffin, 1987; Thomason, 1987; Kim et al., 2016). Soil fumigation with Telone II represents one of the most significant expenses in sugar beet production (Held, 2003). Given the overproduction of beets in many major growing regions and the resulting low sugar prices (Koo and Taylor, 2004), there may be a shift in focus from increasing yields to reducing input costs. The most effective approach to protecting sugar beets from SBCN damage would likely involve a combination of various management techniques. There is a pressing need for additional cost-effective and environmentally friendly methods to control *BCN*. This need is driven by both economic pressures and growing environmental concerns associated with current chemical control methods.

Bacillus thuringiensis (Bt) is a gram positive, spore forming bacterium. These bacteria produce crystal protein during the sporulation phase (Guo, 2008). The crystal proteins are specifically toxic to insects and some nematodes, mites and protozoa (Sampson, M. N., Gooday, G. W., 1998). When ingested, Cry proteins are activated in the alkaline gut environment of target insects, binding to specific receptors in the insect's midgut epithelium. This process causes cell lysis, leading to gut paralysis and death. Importantly, this mechanism is highly specific to the physiological conditions found in certain insect species, not mammals, birds, or other non-target organisms, including humans.Studies have shown that Bt toxins require specific gut conditions, such as high pH and particular receptor proteins, which are absent in vertebrates, including humans (Schnepf et al., 1998). Therefore, Bt does not pose a risk to human health because its mode of action cannot be replicated in human biology. The U.S. Environmental Protection Agency (EPA) has classified Bt as non-toxic to humans and animals, even when exposure occurs through inhalation, ingestion, or dermal contact. The World Health Organization (WHO) has also endorsed Bt as safe, citing its long history of use without any recorded harmful effects on human health (WHO, 1999). One of the most compelling arguments for the environmental safety of Bt is its selectivity. Unlike chemical pesticides, which often harm a broad spectrum of organisms, Bt specifically targets a narrow range of insects. This selectivity reduces the risk to non-target organisms, including beneficial insects such as bees and natural predators like ladybugs. Importantly, Bt does not persist in the environment; its spores degrade rapidly in sunlight and are easily broken down in soil, reducing the risk of accumulation or contamination (Höfte & Whiteley, 1989).

In their natural habitat, most nematodes reside in soil, where they are susceptible to bacterial and fungal infections. This susceptibility presents an opportunity to utilize soil microorganisms for controlling plant-parasitic nematodes (PPNs) (Tian et al., 2007). The application of beneficial microorganisms has been suggested as a viable, eco-friendly alternative for PPN management (Vos et al., 2013). Recent years have witnessed the identification and characterization of a diverse array of rhizospheric microorganisms exhibiting nematicidal properties. These microorganisms employ various mechanisms to target nematodes. Some act through parasitism, such as *Pasteuria penetrans* (Chen and Dickson, 1998) and *Purpureocillium lilacinus* (Anastasiadis et al., 2008). Others produce nematicidal compounds, like *Bacillus thuringiensis* (Bt) (Wei et al., 2003) and certain *Pseudomonas* species (Ali et al., 2002). Additionally, some microorganisms interfere with nematode behavior (e.g., *Bacillus firmus*, Mendoza et al., 2008), disrupt plant-nematode recognition (e.g., *Pseudomonas fluorescens*, Oostendorp and Sikora, 1990), compete for essential nutrients while promoting plant health (e.g., Serratia marcescens, El-Nagdi and Youssef, 2004), or induce systemic resistance (e.g., *Agrobacterium radiobacter*, *Bacillus sphaericus,* Hasky-Günther et al., 1998, and Rhizobium etli, Reitz et al., 2000). Bt, a common soil bacterium, produces crystal proteins during sporulation (Schnepf et al., 1998). These proteins are highly toxic to target insects but harmless to non-target organisms and the environment. Consequently, they are widely used as an alternative to chemical pesticides or incorporated into crops through genetic engineering for continuous protection (Bravo et al., 2011). In the past decade, several crystal proteins with nematicidal activity have been observed, including Cry5, Cry6, Cry12, Cry13, Cry14, Cry21, and Cry55 (Wei et al., 2003, Guo et al., 2008). Among the documented nematicidal crystal proteins, Cry5B and Cry6A stand out as distinct representatives (Marroquin et al., 2000, Wei et al., 2003). Cry5B shows significant similarity in amino acid sequence to Cry1Ab, a caterpillar-specific Bt toxin (Marroquin et al., 2000), and contains four of the five sequence blocks conserved among most Cry toxins (Höfte and Whiteley, 1989). The resolved structure of Cry5B reveals a conserved three-domain topology like other Bt Cry toxins in the main family, suggesting a common mode of action (Hui et al., 2012). In contrast, Cry6A shares no obvious homology with Cry5B or other members of the main Bt Cry toxin families, lacks the five conserved sequence blocks, and likely differs in structure from other Bt Cry toxins (Feitelson et al., 1992, Crickmore et al., 1998, Schnepf et al., 1998).

The aim is to find new or more environment friendly methods for controlling BCN, a significant pest in sugar beet, by investigate of biological and chemical control methods. In this context, fluopyram will be tested in field conditions using surface application and in-row application methods on field trials, nematode population changes and yield compared from all plots of trial. In laboratory conditions, the effectiveness of Bacillus thuringiensis as a direct biological control agent observed under the in-vitro conditions.

CHAPTER II

LITRETURE REVIEW

2.1 Biology

Taxonomy of *Heterodera schactii* (Subbotin et al.,2010);

Phylum: Nematoda Potts, 1932
Class: Chromadorea Inglis, 1983
Sub-class: Chromadoria Pearse, 1942
Order: Rhabditida Chitwood, 1933
Sub-order: Tylenchina Thorne, 194
Family: Hoplolaimidae Filipjev, 1934
Sub-family: Heteroderinae Filipjev & Schuurmans Stekhoven, 1941
Genus: *Heterodera* Schmidt, 1871
Species: ***Heterodera schachtii***, Schmidt 1871

Gürkan and Erinç (2010) reported that since the cyst nematode has limited mobility, it can be carried by abiotic and biotic factors that enable the transport of soil particles from one field to another. It has been determined that people entering and leaving the field, animals and all kinds of tools-equipment used in the field, as well as irrigation water, flood, drainage waters and dust storms are effective in spreading the cysts.

Steele and Whitehand (1984) compared *H. schachtii* from California with *H. trifolii* from the Netherlands to determine if they could be morphometrically distinguished, they measured second-stage juveniles and eggs from both populations. While *H. schachtii* populations from California and the Netherlands showed no significant differences, *H. trifolii* exhibited notably longer tail lengths, tail widths, hyaline tail lengths, and tail length/tail width ratios compared to *H. schachtii*. Additionally, *H. trifolii* eggs were larger. The study concluded that *H. schachtii* and *H. trifolii* could be easily differentiated based on morphometric characteristics of their eggs and second-stage juveniles, highlighting the importance of detailed morphological analysis in nematode species identification.

Caswell and Thomason (1991) reported, Egg production of *H. schachtii* starts between 160-270 degree/days (base 8 °C) and the highest production is between 394-480 degree/days. It has been observed that the highest egg production is produced by an average of 420 egg adults, which is equivalent to 680 degree/days. Accordingly, the highest egg production rate is 2.4 eggs degree/days.

Jonaz et al. (2001) reported, the effect of root diameter of cabbage seedlings containing approximately 200 *H. schachtii* larvae on the growth, development of larvae and development of female individuals was studied under greenhouse and laboratory conditions. It was determined that the size of the female individual was related to the root diameter at the location on the host plant and that growth and development in plant parasitic nematodes showed variations in the morphology of the parasite according to the location on the host.

Campagne (2008) reported that cyst nematode is frequently observed in light-textured soils where sugar beet is grown, and depending on soil conditions and climate factors, it can cause up to 50% yield losses in sugar beet crops based on nematode density.

Aytan and Ediz (1978) reported that nematodes require a certain amount of moisture and water to facilitate their movement on the soil surface, as well as for their movements in the plant's under and above ground parts.

Cooke (1991), the symptoms caused by *H. schachtii* in infested fields in England were identified. These symptoms include increased soil tare due to the formation of beard roots as a result of second-stage larvae entering the roots, reduced tuber yield, and with the increase in nematode population, wilting and collapse symptoms observed in the plant's leaves.

2.2 Distribution

Diker (1959) reported that *H. schachtii* Schmidt was first detected in Türkiye in 1958 in Karamesutlu Village of Babaeski.

Tokmakoğlu (1974) reported plant parasitic nematodes in sugar beet were *Ditylenchus dipsaci* (Kühn), *Meloidogyne spp*., and *H. schachtii*, and the types of damage they caused were explained.

In a study conducted by Cooke (1991), the symptoms caused by *H. schachtii* in infested fields in England were identified. Among these symptoms, it was noted that the entry of second-stage larvae into the roots increased soil tare due to the formation of beard roots, reduced tuber yield, and as the nematode population increased, wilting and collapse symptoms were observed in the plant leaves.

Erdal et al. (2001) reported that Erzurum was infested with *H. cruciferae*, while Eskişehir, Adapazarı, and Ankara were infested with *H. schachtii*.

Kaya and Gürkan (2016) determined that an area of 18,444 ha in 137 villages across 18 sugar factories commercial fields in Türkiye was infested with cyst nematodes. They emphasized that in areas infested with sugar beet cyst nematodes, a minimum 4-year crop rotation should be implemented, or tolerant varieties should be planted.

Kabir et al. (2018) is the first to investigate the effects of *H. schachtii* on the reproduction and damage potential in Chinese cabbage (*Brassica rapa pekinensis*) in Beijing. The population density of *H. schachtii* was examined under varying temperatures, and it was reported that a decrease in temperature reduced the populations of *H. schachtii*.

Gedik (2020) collected a total of 300 soil samples from sugar beet fields in 3 districts of Uşak Province and 4 districts of Denizli Province during 2018-2019. As a result of the study, they identified areas infested with *H. schachtii*. In Uşak Province, the highest infestation was determined in Koyunbeyli and Muharremşah villages in the Central district, and Islam village in Banaz, while the lowest infestation was found in Azizler village in the Sivaslı region. In Denizli Province, they identified the highest infestation in Yeniköy in Çivril district and noted that the lowest infestation was in Gelinören village in Çal district.

Genç (2023) collected 32 soil samples from the Turhal district of Tokat province and found that the infestation rate with sugar BCN was 62.5%.

Dababat et al. (2016) reported they have identified *Heterodera schachtii* in Siverek, Karaköprü and Bozova of Şanliurfa. They reported that SBCN cysts were detected in 62% of 130 samples.

Tan and Ökten (2008) investigated the distribution of the sugar beet cyst nematode, *H. schachtii*, in the sugar beet cultivation areas of the Adapazarı Sugar factory commercial fields and identified infested areas. The regions found to be infested include the central areas and some villages of Alifuatpaşa, Kaynarca, and Pamukova. They determined that a total of 10 fields, covering an area of 304 decares, were infested with *H. schachtii*, while other villages growing sugar beets in different regions were found to be clean.

2.3 Identification

Hopper and Cairns (1959) specified the characteristics of the diagnostic features of *H. schachtii*.

Caswell-Chen et al. (1992) identified genomic varieties of *H. schachtii* (Schmidt, 1871) and *H. cruciferae* (Franklin, 1945) samples taken from the same field using RAPD markers.

Amiri et al. (2002) designed primers for the molecular identification of *H. schachtii* using species-specific ITS-rDNA regions.

Amin et al. (2003) morphologically and molecularly compared *Heterodera betae* (Wouts, Rumpenhorst & Sturhan, 2001) and *H. schachtii* populations collected from different countries.

Oro et al. (2020) reported that sugar beet is an important crop in Serbia and in temperate climate zones. Phylogenetic analysis based on the ITS rDNA region showed that the origin of the *H. schachtii* population was the coastal region of the Netherlands and

Belgium. Additionally, it was stated that there is a clear distinction between *H. schachtii* and *H. betae*.

Huston et al. (2022) determined the phylogenetic relationship of *H. schachtii* using not only morphological identification but also molecular methods, utilizing the 18S, ITS, 28S, and coxI gene regions.

Huan et al. (2022) performed morphological identification of *H. schachtii* using second-stage larvae and the vulva cone region of cysts. Molecular identification was carried out using rDNA-ITS, 28S-D2/D3, and mtDNA cytochrome c-oxidase subunit 1 (COI) gene regions. As a result of morphological and molecular identification of samples collected from the Xinjiang region of China, they determined that all of the collected samples were *H. schachtii.*

2.4 Management of *Heterodera schachtii*

Sarrafzadeh et al. (2005) *Bacillus thuringiensis,* a spore-forming bacterium, as a model system for their study. They employed a fed-batch cultivation process, where nutrients were continuously added to the culture medium during the growth phase. The authors introduced the concept of a "permittivity index," a measure that reflects the extent of spore liberation during the sporulation phase. This index was found to correlate with the observed mature spore count. This research provides valuable insights into the dynamics of bacterial growth and sporulation. It demonstrates that on-line dielectric permittivity measurements offer a promising tool for monitoring and understanding the complex processes involved in microbial cultivation.

Guo et al. (2008) should be expanded with more detail on Bt strains that produce Cry5, Cry6, Cry55 proteins and their specific nematicidal activity. This could be tied into how these Cry proteins affect Heterodera schachtii and similar nematodes.

Bravo et al. (2011) explores the molecular action of Cry proteins in nematodes, a fundamental background to understanding how *Bt* functions biologically against *H. schachtii*. The document would benefit from more in-depth discussion on the modes of action at the cellular level.

Huang et al. (2021), investigated the potential of *Bacillus firmus* I-1582 as a biocontrol agent for the cyst nematode *H. schachtii*, a common agricultural pest. They found that *B. firmus* I-1582 is attracted to *Arabidopsis thaliana* root exudates and colonizes the root system in a pH-dependent manner, promoting plant growth. Importantly, their research demonstrated that *B. firmus* I-1582 effectively protected *A. thaliana* from *H. schachtii* infestation over two generations, both by reducing nematode development and reproduction and by decreasing the virulence of subsequent generations of nematodes. This study suggests that *B. firmus* I-1582 is a promising biocontrol agent for integrated pest management strategies in sustainable agriculture.

Wright et al. (2019), investigated the hatching dynamics of the beet cyst nematode, *H. schachtii*, in response to root leachates from various cultivars of sugar beet, white mustard and radish. They found that different cultivars of sugar beet stimulated varying levels of hatching, which may be related to their growth habits and tolerance to *H. schachtii*. Brassica varieties showed contrasting responses, with some inhibiting hatching and others stimulating it, suggesting potential for brassica trap crops in *H. schachtii* control. The age of the plant was also a significant factor in stimulating hatching. These findings provide valuable insights for future research on the use of trap crops and resistant varieties for managing *H. schachtii* infestations.

Murillo & Caballero, 2014 reported in 2014, Bt strains expressing Cry1Ac and Cry2Ab toxins are predominantly used against lepidopteran larvae. Studies have demonstrated that effective concentrations typically range from 1×10^8 colony-forming units per milliliter (CFU/mL) to 1,000 International Units per milligram (IU/mg) of active formulation. The recommended dosage for field applications generally falls between 500 to 1,000 grams per hectare (g/ha). These dosages ensure sufficient toxin availability to induce mortality through midgut epithelial cell lysis in the target larvae.

Myers et al. (2020) determined that burrowing nematode (*Radopholus similis*) caused great damage in cut flower production. To control the burrowing nematode, they conducted two location trials and using a commercial pesticide containing fluopyram application for 2 times. And according to their observations, they reported that the nematode population in the roots of the plants decreased by 57%. Based on their studies, they reported that the yield of the plants increased.

Torto et al. (2018) provided a comprehensive overview of the chemical ecology of root-knot nematodes (RKNs) and cyst nematodes (CNs), focusing on their interactions with host plants. They highlight the importance of understanding these interactions for developing sustainable and eco-friendly management strategies. They discuss the complexities of these interactions, including the role of volatile and nonvolatile organic compounds released by plant roots, the influence of factors like plant species, soil composition, and microbial communities, and the discovery of key signaling molecules, such as carbon dioxide, ethylene, and various terpenoids. They also delve into the role of hatching factors, including solanoeclepin A, glycinoeclepin A, B, and C, which stimulate hatching of CNs. Use of trap crops, including sticky nightshade and other Solanaceae species, as well as non-Solanaceae crops like lupine and Oxalis tuberosa, to manage nematode populations. They highlight the importance of understanding the chemical ecology of these trap crops to optimize their effectiveness. They emphasize the need to incorporate the role of rhizosphere microorganisms into future research on nematode-plant interactions, suggesting that studying the complex interplay between plant roots, nematodes, and beneficial microbes will be crucial for developing novel and sustainable management strategies for these economically important pests.

Hauer et al. (2016) reported that integrated control strategies for the sugar beet cyst nematode, *H. schachtii*, in Central Europe. They studied the effects of trap crop cultivation (resistant mustard and a crop mixture), nematicide application before sugar beet sowing, and sugar beet variety choice (susceptible, tolerant, or resistant to *H. schachtii*) on nematode population dynamics and sugar yield. Their field experiments conducted in eight environments across Northern Germany showed that trap crops had limited effect on nematode populations, while nematicide application had no significant effect. However, the sugar beet variety was strongly influential, with the resistant variety significantly reducing the nematode population and resulting in the highest sugar yield. The susceptible variety showed the steepest decline in sugar yield with increasing nematode infestation. Hauer et al. concluded that cultivating the resistant sugar beet variety is the most effective strategy for controlling *H. schachtii* and maximizing sugar yield.

Kim et al. (2016) investigated the efficacy of different nematocidal compounds on the hatching and mortality of *H. schachtii* infective juveniles. They found that fluopyram and imicyafos significantly reduced hatching rates and exhibited the highest mortality, with LC50 values of 0.0543 and 0.0178 ppm, respectively. Terthiophene and Eclipta prostrata extract also showed a degree of nematocidal activity. This study provides evidence that these alternative nematocidal compounds could be used effectively in the control of *H. schachtii*, especially as more environmentally friendly options are needed due to the increased regulatory pressure on traditional nematicides.

Pylypenko et al. (2003) recommended radish cultivation to reduce infection in areas infested with *H. schachtii* in sugar beet production fields. They detected low-density *H. schachtii* populations (1-100 eggs and larvae / 100cm3 soil) in most of the infested areas. The low-density nematode population caused wilting symptoms in sugar beet plants during the hot hours of the day. For medium-density nematode populations (101-300 larvae / 100cm^3 soil), they estimated approximately 12-15% crop loss in infected plants. In high-density nematode populations (300 larvae/100 cm3 soil), stunting was observed in most plants, death of outer leaves occurred, and more than 50% crop loss was estimated.

Wei (2003) reported toxin proteins produced by *Bacillus thuringiensis* are among the most widely used natural insecticides in agriculture. The extent to these proteins may also target nematodes. *Bacillus thuringiensis* crystal toxin proteins can affect the nematodes. They analyzed their toxicity on various free-living nematode species, showing that four of these crystal proteins were active against more than one nematode species and that each nematode species tested was susceptible to at least one toxin.

Scholte & Vos (2020) are mentioned, but additional emphasis could be placed on the benefits of rotating non-host crops to reduce nematode populations. You could also reference *Brassica* trap crops for integrated management.

Boland et al. (2023) could expand your section on the use of resistant sugar beet varieties, explaining recent advancements in breeding for nematode resistance, which can complement chemical and biological treatments.

Oka et al. (2021) has already been included in your thesis, but additional detail could be added on its environmental considerations and alternative application methods to minimize impact while maximizing nematode control.

Greco & Perry (2019) on challenges in applying biological control agents can help you delve into the challenges of implementing IPM strategies in variable environmental conditions. This will further support your discussion on combining *Bt* with other control methods.

CHAPTER III

MATERIALS AND METHODS

3.1 Field Trials

This study was conducted between 2021-2022 at Niğde Ömer Halisdemir University department of Plant Production and Technologies. Susceptible sugar beet variety Turbata, as nematicide fluopyram used on the field trials. These trials will be carried out on 2 locations in Niğde/Bor. These locations preferred as enough infestation with *H. schachtii*. Nematodes in the soil were identified morphologically molecularly.

3.2 Identification of *Heterodera schachtii*

The Fenwick (1940) sieving method was used to extract cyst nematodes (*Heterodera* spp.) from the soil samples. For this, 250 grams of air-dried soil from each sample was placed in a coarse sieve set on top of the apparatus and washed from above using a hose at medium pressure. During this process, the water overflow from the device's trough was passed through sieves with diameters of 250-850 µm to collect the cysts. At the end of the process, the sample retained on the 250 µm sieve was transferred to a counting dish composed of nine interlocking rings with the help of water sprayed from a wash bottle. The water accumulated in the counting dish was drawn off with a Pasteur pipette, and the sample was examined under a binocular microscope.

To prepare permanent slides of the second-stage larvae, a TAF solution was prepared by mixing 7 ml of formalin (40% formaldehyde), 2 ml of triethanolamine, and 91 ml of distilled water. The solution was then heated in a water bath up to 65°C, and 2 ml of the solution was fixed on slides containing larvae. The morphological identification of cyst nematodes was conducted based on the characteristics of sections taken from the vulval area of the cysts and the morphometric characteristics of second-stage larvae. For identification based on vulval features, a section was taken from the ¼ length of the cyst near the vulval region, and the section was left in 15% H_2O_2 until it was decolorized. Afterwards, it was mounted in Canada balsam and the edges of the preparation were sealed with varnish to prepare the slide (Hooper, 1986). For the morphological identification of the fenestra regions of the cysts and the second-stage larvae, a Leica (DM 5500B) LED light microscope was used. Measurements and photography were conducted using a Leica (DFC 450) digital camera and the LAS (Leica Application Suite) software.

According to De Ley and Blaxter (2002), the taxonomic classification and synonyms of the species *Heterodera schachtii*, which belongs to the order Rhabditiae. The species identification was carried out based on female specimens by Prof. Dr. Halil TOKTAY from the Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, at Niğde Ömer Halisdemir University.

3.3 Field Experiments

The necessary soil preparations were undertaken for setting up the trial in the identified fields. The trial area was determined by conducting essential measurements on the land. The boundaries of the plots were marked with stakes. Each plot was designed to be 270 cm wide and 10 meters long. Isolation plots were defined as 1 meter from the outer edges to the surroundings, and 1 meter between the plots and blocks. Within each plot, six rows were planted with 45 cm between rows and 20 cm between plants within a row. Planting was done manually to adhere to the trial layout and plan. Soil samples were taken from each plot to determine the initial populations.

Trial 1 location: Niğde/Bor Bağdüz Villige

Trial 2 location: Niğde/Bor Acıgöl Neighborhood

The plots were mentioned as follows: 0: Control, 1: Fluopyram in-furrow application, 6: Fluopyram soil surface application.

Figure 3.1. Images of the experimental setup established in Bor district of Niğde province to investigate the efficiency of different application methods against *Heterodera schachtii*

Table 3.1. The planting and harvest dates of the trials established in Bor district of Niğde province to test of application methods of fluopyram against *Heterodera schachtii*

	Trial 1	**Trial 2**
Planting Date	23.04.2021	14.10.2021
Harvest Date	25.04.2021	17.10.2021

		16 m							
		1. Blok		**2. Blok**		**3. Blok**		**4. Blok**	
GİRİŞ		3 m	1m	3 m	1m	3 m	1m	3 m	1m
	10 m	**0**		**6**		**1**		**0**	
	1 m								
	10 m	**1**		**0**		**6**		**1**	
	1 m								
	10 m	**6**		**1**		**0**		**6**	

Figure 3.2. The plans of trials established for investigating the biological efficacy of application methods against *Heterodera schachtii* in the Bor district of Niğde. Trial 1 and 2 plans 0: Contol, 1: Fluopyram in-furrow application, 2: Fluopyram soil surface application

After the experiment is established, it will be checked by going to the field on the 56th days after emergence. On the 56th day, 5 plants will be selected randomly from each plot and the height measurement will be made.

The harvest date will be determined by considering the sugar beet harvest dates and the regional harvest dates. Plants of each parcel will be weighed and recorded separately. Yield differences between treated and untreated characters will be compared. The effect of the applications on the yield will be observed. Soil samples will be taken to determine the final population for each plot.

3.4 *In-vivo* Experiment

Bacillus thuringiensis (genes containing of Cry6Aa2, Cry55Aa1, and cry5B) bacteria filtered disks was provided by Bacillus Genetic Stock Center (BSGC) from the USA. Bacteria reproduce for in vitro experiments and configurate the density of bacteria culture used by thoma slide and spectrophotometer. After that, the same densities of bacteria cultures applied in 10%, 30% and 100% applied for plots.

Experiment application dosage mentioned below:

1. Low Dose: $3.7x10^8$ *Bacillus thuringiensis* concentration 1.25 lt/da (10% application) with 1200 2nd stage juvenile inoculated.
2. Medium Dose: $3.7x10^8$ *Bacillus thuringiensis* concentration 3.75 lt/da (30% application) with 1200 2nd stage juvenile inoculated.
3. High Dose: $3.7x10^8$ *Bacillus thuringiensis* concentration 12.5 lt/da (100% application) with 1200 2nd stage juvenile inoculated.
4. Biological Contral: $4.7x10^{10}$ *Purpureocillium lilacinus* 75 ml/da with 1200 2nd stage juvenile inoculated.
5. Chemical Control: Fluopyram 120 ml/da with 1200 2nd stage juvenile inoculated.
6. Bacteria Control: $3.7x10^8$ *Bacillus thuringiensis* concentration 12.5 lt/da without inoculum.
7. Control: Without any application and inoculum.
8. Plant Nematode Control: 1200 2nd stage juvenile inoculated.

3.4.1 Bacterial reproduction

The proceeding protocol was followed for bacteria culture preparation and reproduction:

1. Prepare nutrient agar plates and nutrient broth stock according to the need, the instructions are usually on the container. For Nutrient agar, usually 28 gr per 1 L, for Nutrient broth 14 gr per 1 liter.

2. Preparation of 50% glycerol stock. Check the concentration of the glycerol that is available and then in a falcon tube adjust the concentration of glycerol to 50% by adding the appropriate amount of autoclaved distilled water. As an extra step, filter the glycerol in the falcon tube before adding autoclaved distilled water.
3. For culturing bacteria, in a ventilation hood, about 10 – 15 ml nutrient broth is added to a falcon tube. Then about 8 – 10 µl of bacteria preserved in glycerol stock is added. Then the tube is shifted to a water bath at 28 – 32 °C and left overnight.
4. In a ventilation hood, After the bacteria has grown, 200 – 300 microliters of the newly grown bacteria are placed on the nutrient agar plate and spread. Then the plates are shifted to a laboratory cabinet incubator and left overnight at 28 °C, for faster growth they can be incubated at 37 °C.
5. in a ventilation hood, single bacteria colonies are selected, and Step 4 will be repeated.
6. Glycerol bacteria stock is prepared by adding bacteria to a prepared 50% glycerol stock in a 2 mL Eppendorf tube with the ratio of 1:1 (Bacteria:50% Glycerol). Then the tubes are shifted to a -80 freezer and preserved for later use (Sanders, 2012).

3.4.2 Preparing bacterial concentration

For find the optimum concentration of bacteria these flowing steps carried:

- 1 mL of the bacterial culture broth was pipetted onto a Thoma counting slide. The sample was evenly distributed across the slide and prepared for microscopic examination.ml of bacteria culture broth media added to thoma slide and counted under the light microscope (Figure 3.4)
- The microscope was first focused on the grid lines using low magnification to identify the correct positioning of the grid. Afterward, the magnification was increased to 100x to observe the bacterial cells in greater detail and facilitate accurate counting.
- Several large squares from different regions of the grid were selected for counting to ensure a representative sample of the bacterial population. Typically, counts were performed on at least five large squares.

- The number of bacteria present within each selected square was counted. To maintain consistency and accuracy in the counting process, bacteria were only counted if they were located entirely within the square or touching the top and left boundaries of the square. Bacteria touching the right and bottom boundaries were excluded from the count to avoid duplication.
- The total number of bacteria counted across the selected squares was summed, and the average number of bacteria per square was calculated.
- The concentration of bacteria in the culture broth was calculated

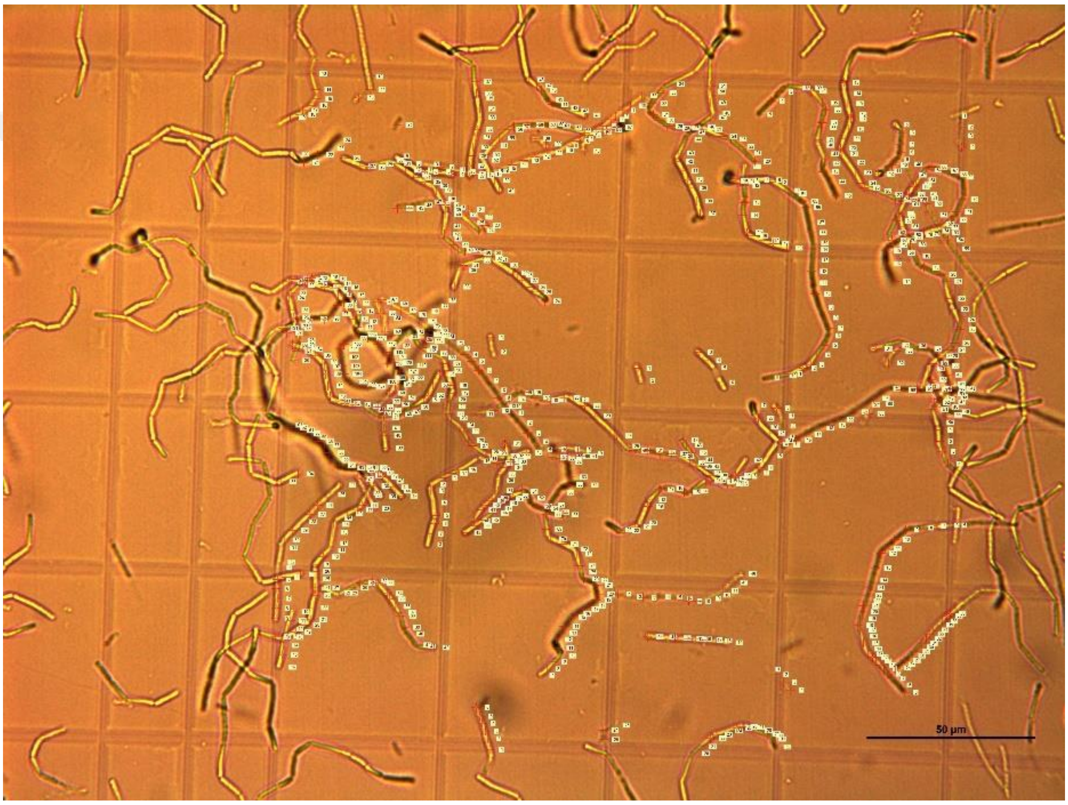

Figure 3.3. Concentration determination study using a Thoma slide to investigate the biological efficacy of *Bacillus thuringiensis* (Bt) against *Heterodera schachtii* under *in-vitro* conditions

3.4.3 Cyst collection and *in-vitro* experiments

Heterodera schachtii population collecting from sugar beet field in Niğde/Bor province. To set up the experiment, cysts of the sugar beet cyst nematode were collected under a binocular microscope. The sugar beet cyst nematode is classified in the third group due to its need for root exudates and a significant amount of water for egg hatching (Masler and Perry, 2018). To facilitate the hatching of second-stage larvae, the cysts were placed in a mixture of 3mM $ZnCl_2$ and tap water in a graduated cylinder, then agitated at 25°C and 500 rpm for 24 hours, achieving 50% hatching. Later, after 48 hours, 70% hatching was achieved, and by 52 hours, 100% hatching occurred, resulting in an inoculum. After finalizing the hatching 2nd stage juveniles collected by 500 mesh sieve and scrim and then transferred to 50 ml falcon tubes.

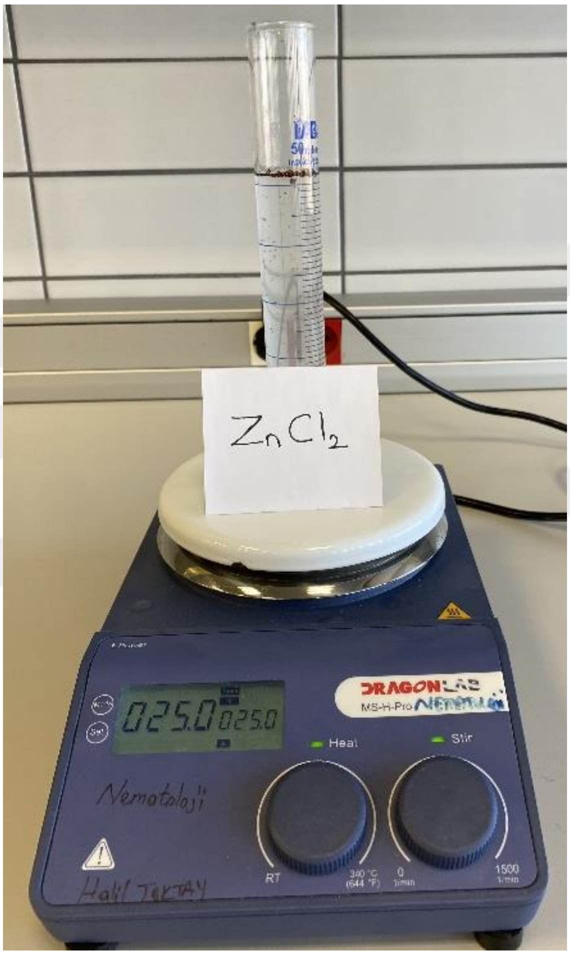

Figure 3.4. Hatching of *Heterodera schachtii* eggs in $ZnCl_2$ solution for inoculum preparation in an *in-vitro* trial on sugar beet

After preparing the inoculum source, a sterile soil mix containing silver sand, clay powder, and hydroculture granules (in a ratio of 4:0.7:1) was prepared for use in the in-vivo experiment with pots. To this mixture, Steiner Plant Solution was added uniformly (20 liters per 1000 kg of soil mixture). One kilogram of the prepared mixture was placed in 1.1-liter pots (140 x 125 mm) lined with strong paper at the bottom. After preparing the soil, 1 ml sub-samples were taken from the suspension and counted under a microscope to adjust the concentration of the suspension. The varieties used in the study were obtained from relevant institutions and organizations. Once the seedlings reached the 3-4 leaf stage, an inoculum containing 1200 larvae + eggs (equivalent to an average of 5 cysts) was introduced into the soil in the pots through 8 holes. The experiment was set up in a randomized block design, with each pot containing one plant in 1.2 kg pots, replicated five times. In the experiment 3 different bacteria culture dose with infested plots (30%, 50%, 100%), control, 100% bacteria culture dose without inoculum and inoculum without bacteria and also for the comparison fluopyram and *Purpureocillium lilacinus* ($4.7x10^{10}$).

After 18-20 weeks from the establishment of the trials, following the maturation of the cysts, each pot was harvested individually. The soil was washed, and the cysts present in the soil were counted under a binocular microscope. The differences between the initial population (Pi) and the final population (P) were evaluated.

3.5 Statistical Analysis

To determine the reproduction factor values of the *Heterodera schachtii* population on sugar beet plants under eight different control treatments, a one-way analysis of variance (ANOVA) was applied. To identify differences between characteristics and rank these differences according to their significance levels, the Duncan test was used (SPSS 25).

CHAPTER IV

RESULTS

4.1 Identification

Morphological Identification and Measurements of *Heterodera schachtii*

The initial diagnosis of samples taken from sugar beet fields was performed morphologically. The morphological diagnosis was based on the second-stage juveniles and the morphological shapes of the cysts, while the morphometric diagnosis was completed by measurements (Siddiqi, 2000). The taxonomic position of *Heterodera schachtii* was determined according to De Ley and Blaxter (2002) (Decraemer and Hunt, 2013).

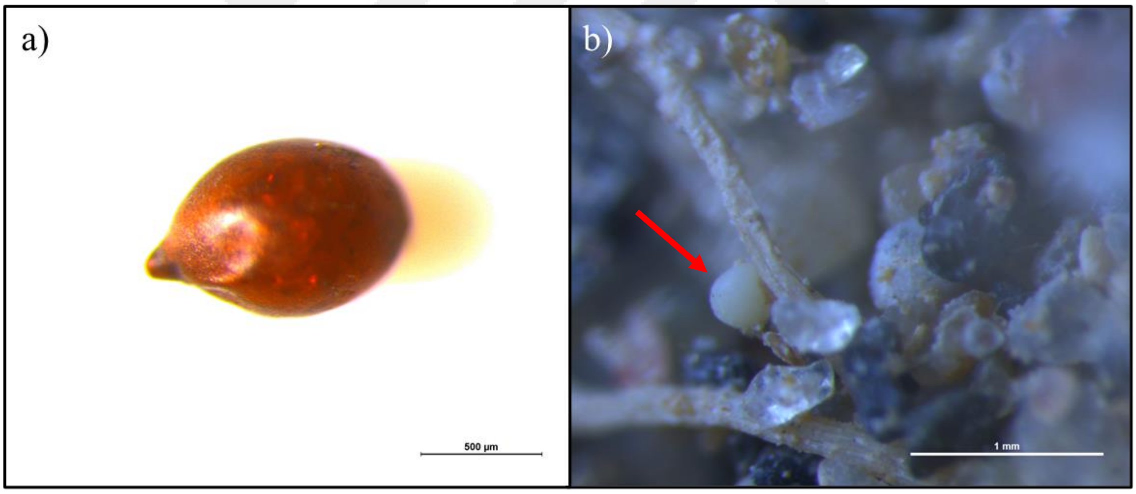

Figure 4.1 Binocular Microscope images of *Heterodera schachtii* cysts obtained from in-vitro trial. a) Cyst form of *H. schactii.* b) Female form of *H. schactii* showed with red arrow on the root system

The typical cyst shape of *Heterodera schachtii* is lemon-shaped as seen as Figure 4.1, which differentiates it from other nematode species. The cysts are symmetrical with a broad mid-section tapering towards both ends, where the vulval cone and neck are located. The female is white and flask-shaped, with a short neck embedded in the host root, while the swollen body remains on the root surface (Perry & Moens, 2006). Notably, there is no yellow phase in its development (Luc, Sikora & Bridge, 2005). A thin, white, waxy material known as the "sub-crystalline layer" covers the surface of the female body (Jones, Gheysen & Fenoll, 2011) **(Figure 4.1 b)**. The vulva is located terminally on a vulval cone, which is covered by a gelatinous matrix that forms an egg sac containing the eggs (Perry & Moens, 2006).

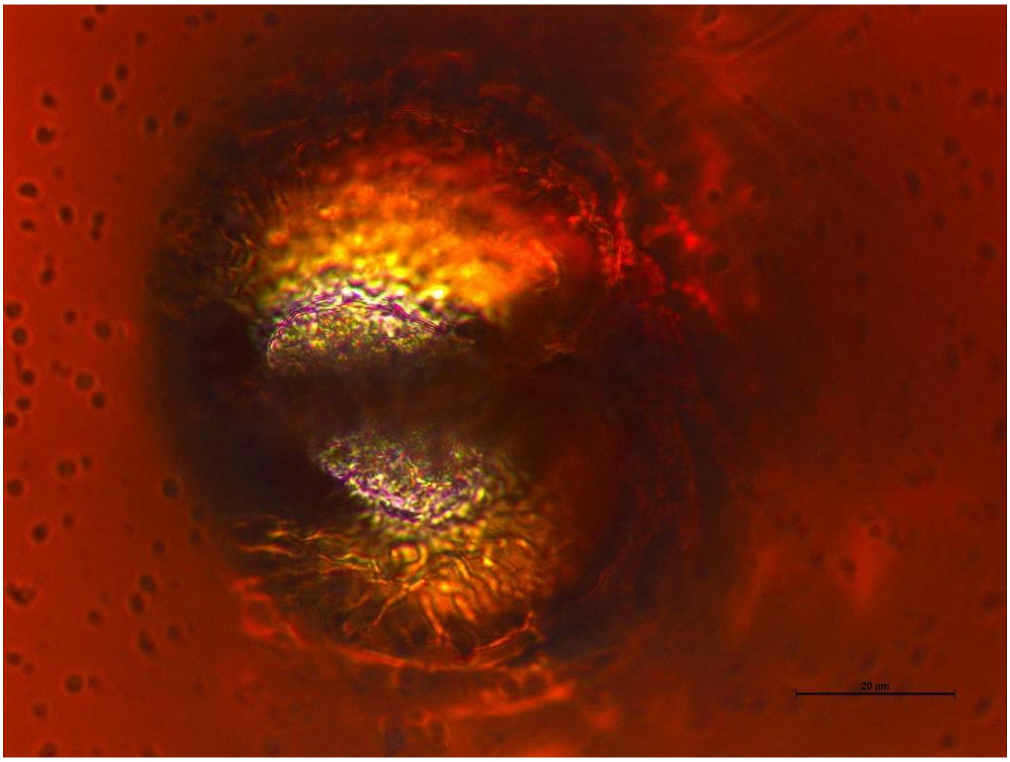

Figure 4.2. Fenestral opening image of a permanent slide prepared for the morphological and morphometric diagnosis of *Heterodera schachtii*

Fenestra a thin, semi-transparent area within the vulval cone, which is essential for species identification. In *Heterodera schachtii*, the fenestra is relatively small compared to other species, such as *Heterodera glycines*. This size difference in fenestra length is a primary differentiating characteristic.

Figure 4.3 2nd stage juvenile images of a permanent slide prepared for the morphological and morphometric diagnosis of *Heterodera schachtii*. a) The whole body, b) head and c) tail region of J2s

H. schachtii juveniles possess a well-defined stylet which has distinct basal knobs (Figure 4.3 a,b). The head region is rounded and offset from the body by a slight constriction (Figure 4.3 a). The esophagus is divided into a procorpus, metacorpus, and basal bulb, characteristic of Heterodera species (Baldwin & Mundo-Ocampo, 1991). The tail of the J2 is conically shaped, tapering to a finely pointed tip, which is crucial for movement and infection of host roots (Figure 4.3 c) (Luc, Sikora & Bridge, 2005).

Table 4.1. Comparison of morphometric cyst measurements performed to identified *Heterodera schachtii* populations comparison with earlier studies

Cyst Measurements	Measurements (Avr. ± Std. Er.)	Xijiang, China (Peng Huan et al. 2022)	Jeongseon, Korea (Mwamula et al. 2018)	Bozova, Türkiye (Cui et al. 2016)
Cyst Length	929.3±21.18 (836-1143)	752±87.2 (622.0–867.4)	795.8±97.1 (639.5–882.6)	700.3±80.1 (610–783)
Cyst Wide	624.2±18.54 (514-736)	485.2±55.3 (391.1–594.1)	510.8±75.1 (378.5–600.4)	459.8±52.2 (395–505)
Fenestra Lenght	32.71±0.6 (27.9-38.3)	37.2±5.5 (32.5–43.5)	42.2±3.6 (36.1–45.3)	-
Vulval Slit Lenght	33.62±0.65 (30.7-39.8)	40.2±4.4 (37.2–48.9)	37.4±5.2 (30.3–42.2)	43.9±1.9 (41–45)
Under Bridge Lenght	102.85± 5.4 (67.9-142.1)	105.4±9.9 (88.1–116.7)	102.3±10.8 (93.7–120.8)	113.3±10.4 (98–120)

For the cyst measurements, the average cyst length in this study was 929.3 ± 21.18 µm (Table 4.1), which is larger than the reported lengths from China (Peng Huan et al., 2022), Korea (Mwamula et al., 2018), and Türkiye (Cui et al., 2016). The cyst width was 635.8 ± 20.77 in Table 4.1 µm, again larger than those reported in other regions. The length of the fenestra and vulval slit measured 32.19 ± 0.6 µm and 32.77 ± 0.68 µm, respectively in Table 4.1. These values were consistent with previous reports, though slightly lower compared to populations from Korea and China.

Table 4.2. Comparison of some morphometric 2nd stage juvenile measurements of identified *Heterodera schachtii* populations comparison with earlier studies

2nd Stage Juvenile Measurements	Measurements (Avr. ± Std. Er.)	Xijiang, China (Peng Huan et al.2022)	Jeongseon, Korea (Mwamula et al. 2018)	Bozova, Türkiye (Cui et al. 2016)
Body Length	452.7 ±8.79 (410.2-511.8)	442.1±13.7 (429.2–512.3)	484.2±11.1 (473.0–504.2)	-
DGO	3.91±0.04 (3.8-4.1)	4.4±0.5 (3.5–5.1)	4.0±0.3 (3.5–4.4)	-
Stylet Lenght	27.79±0.16 (26.6-28.7)	24.2±0.5 (22.3–26.5)	22.1±0.9 (21.3–24.3)	24.3±0.5 (24–25)
Hylain	25.21±0.47 (22-28.9)	27.8±2.7 (24.7–30.9)	29.2±2.1 (26.7–34.3)	24.0±0.6 (23–25)
Tail	46.89±0.59 (44-51.6)	50.8±2.1 (47.5–52.7)	49.9±1.8 (48.2–53.2)	47.0±1.4 (45–49)
Distance of the Body from the Excretory Pore	95.2 ± 1.76 (83 - 109)	73.1±0.4 (65.1–78.4)	76.4±0.4 (75.8–77)	-
a	23.71 ± 0.46 (19.6 - 26.7)	23.7±1.1 (22.2–24.8)	23.8±0.9 (22.9–25.1)	-
b	4.84 ± 0.12 (4.1 - 5.9)	4.5±0.3 (4.1–4.7)	4.2±0.1 (3.7–4.6)	-
c	9.8.1 ± 0.2 (8.5 - 11.3)	9.8±0.6 (8.9 –10.1)	9.6±0.4 (9.4–10.0)	-

The body length of J2 measured 452.7 ± 8.79 µm, which is similar to the body lengths observed in China but smaller than those reported from Korea and Bozova, Türkiye. The stylet length was measured at 27.79 ± 0.16 µm, which is greater than values reported from other regions. The distance of the excretory pore from the anterior end was also greater in this study (95.2 ± 1.76 µm) compared to other studies.

The results show regional variation in both cyst and larval measurements, indicating potential differences in environmental or host conditions that may influence morphological characteristics. Despite these variations, the overall morphological and morphometric features remain consistent with the descriptions for Heterodera schachtii, confirming the species identification.

4.2 Field Experiments

The field trials were conducted in two distinct plots in Niğde Province, specifically in the Bor district, where *Heterodera schachtii* populations were naturally established. The purpose of these trials was to assess the efficacy of fluopyram, a nematicide, applied using different techniques on sugar beet plant growth and yield performance. The treatments included in-furrow applications and surface soil applications of fluopyram, compared to untreated control plots.

Table 4.3 Effect of different nematicide application techniques on plant development against sugar beet cyst nematode (*Heterodera schachtii*) in two different infested fields in the Bor district of Niğde province on day after emergence 28

	Trial 1		**Trial 2**	
	Average Plant Count ± Std Er.	**Vigor (%) ± Std Er.**	**Average Plant Count ± Std Er.**	**Vigor (%) ± Std Er.**
Control	80,75±30,71	33,75±9,43	67,25±10,80	41,25±15,86
Fluopyram in-furrow	85,5±32,46	40±12,416	71,75± 8,61	57,50± 17,72
Fluopyram soil surf. app.	85,75±23,10	40±10,80	87,75±11,98	57,50±6,291

Table 4.3 provides the average plant count and vigor index at day 28 after emergence (DAE 28). Notably, soil surface application of fluopyram showed the highest plant counts (avr. 86,75), indicating a potentially favorable establishment phase. The vigor index, while not significantly different, trended higher in the in-furrow application compared to surface application. Statistical analyses using ANOVA revealed no significant differences ($p > 0.05$), although data shows fluopyram treatments might promote early plant growth depends of the DAE 28 data. The lack of significant differences could be attributed to the short time frame (DAE 28) in which nematode pressure may not yet have fully impacted plant vigor.

Table 4.4 Effect of different nematicide application techniques effects on canopy closure, plant height and vigor against sugar beet cyst nematode (*Heterodera schachtii*) in two different infested fields in the Bor district of Niğde province on DAE 56

	Trial 1			Trial 2		
Treatment	**Canopy (cm) ± Std. Er.**	**Plant Height (cm) ± Std Er.**	**Vigor (%) ± Std Er.**	**Canopy (Cm) ± Std. Er.**	**Plant Height (cm) ± Std Er.**	**Vigor (%) ± Std Er.**
Control	53,2±4,07	36,1±2,62	58,75±6,61	60,85±3,91	42,3±3,87	61,25±9,66
Fluopyram in-furrow app.	53,9±5,19	37,25±5,94	62,50±7,74	62,9±3,78	46,45±3,32	71,25±9,66
Fluopyram soil surf. app.	53,8±5,29	41,7±8,24	60,00±14,72	63,45±6,12	46,55±3,45	68,75±12,14

By DAE 56, canopy closure and plant height metrics were assessed. In-furrow and soil surface applications again showed increased canopy width and plant height, compared to the control (Table 4.4). However, similar to earlier results, statistical significance was not achieved ($p > 0.05$). The observed increases in plant height and canopy closure suggest that fluopyram.

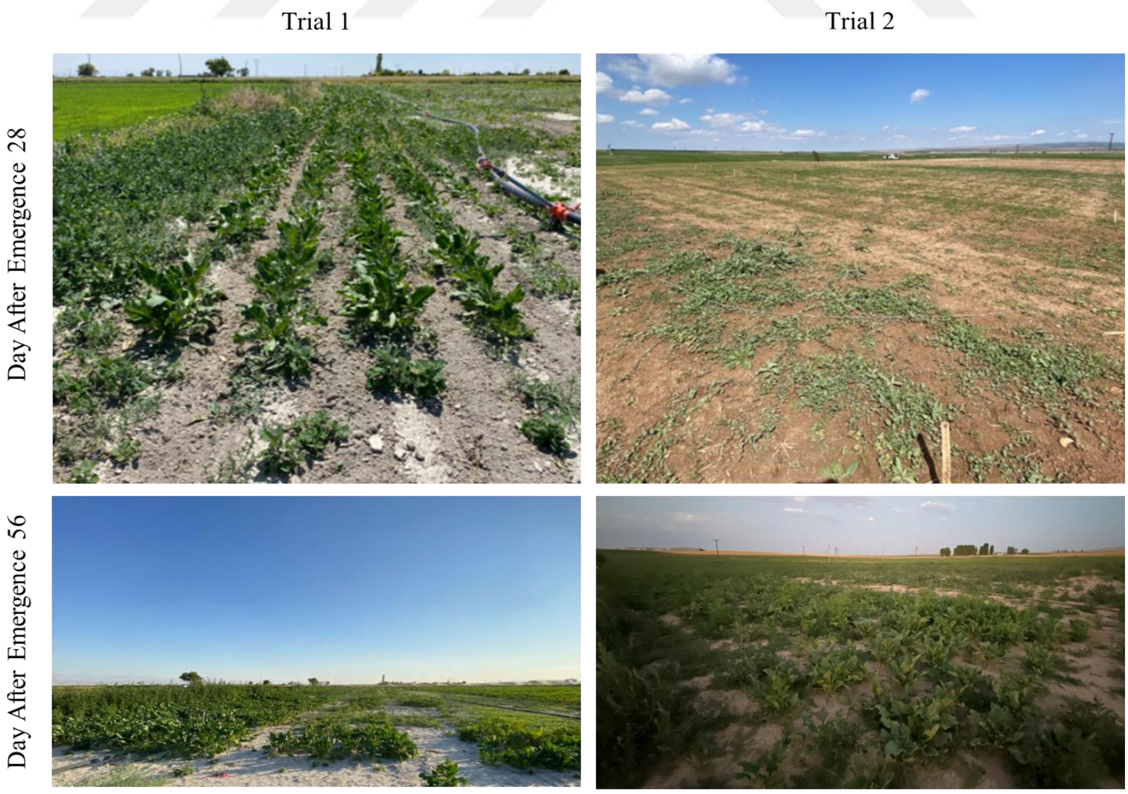

Figure 4.4. Figures depict the plant development at different stages (DAE 28 and DAE 56), showing the effects of the various nematicide application methods in Niğde/Bor

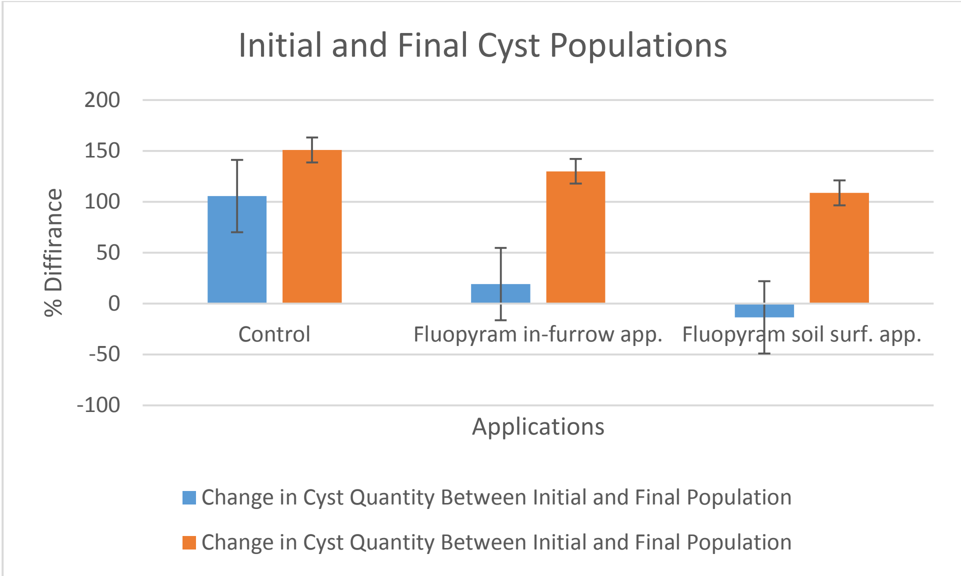

Figure 4.5. Effect of different nematicide application techniques on sugar beet cyst nematode population difference on sugar beet fields in the Bor district of Niğde province

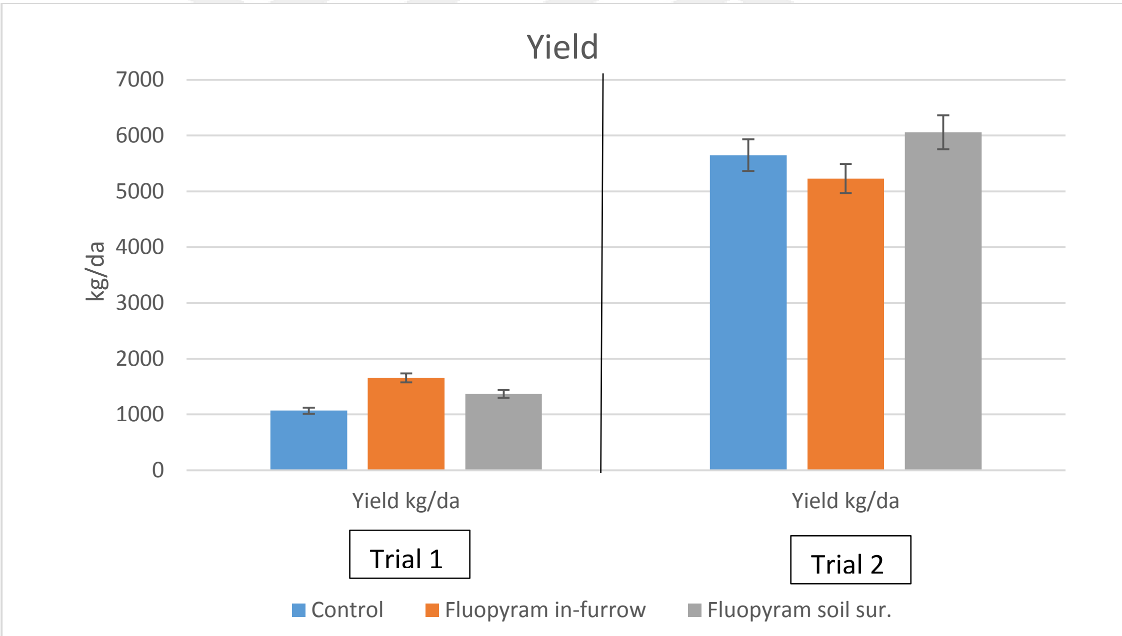

Figure 4.6. Effect of different nematicide application techniques effects against to *Heterodera schachtii* on yield performance of sugar beet in the Bor district of Niğde province

Fluopyram treated plots decrease between initial and final population (Figure 4.5). Yield and nematode cyst populations were recorded as the most direct indicators of treatment success (Figure 4.5). A significant yield difference has been observed between the two trials, despite similar initial populations of *H. schachtii* at both locations. The yield discrepancy is primarily attributed to differences in soil quality and organic matter content between the two sites. Notably, Trial 1 has experienced severe soil compaction issues. Fluopyram-treated plots, particularly those with in-furrow application, showed significantly higher yields and lower cyst populations compared to both the surface application and control groups. For instance, in trial 1, the in-furrow application resulted in a yield of 1655.64 kg, compared to 1369.72 kg in the surface application and 1068.98 kg in the control. In trial 2, yields were significantly higher, with 5229.90 kg in the in-furrow treatment, 6059.85 kg in the surface treatment, and 5647.59 kg in the control (Figure 4.6).

A one-way ANOVA conducted on yield data statistically not significant difference between treatments ($p > 0.05$). The yield improvements observed under the field conditions and cyst reductions in the fluopyram application can suggest that these methods is the most effective for managing *H. schachtii* populations in sugar beet fields. The placement of fluopyram directly into the furrow likely creates a protective zone around the root system, inhibiting nematode feeding and reproduction.

The field trials show that fluopyram has positive effect on sugar beet growth and yield, while also reducing *Heterodera schachtii* cyst populations. While some early-stage growth parameters such as plant count and canopy closure did not reach statistical significance, the final yield results were conclusive. The in-furrow and soil surface applications of fluopyram consistently outperformed untreated controls, demonstrating its effectiveness in managing nematode infestations and promoting healthy plant growth. Further trials over multiple growing seasons would be beneficial to confirm these findings and refine application techniques for optimized results.

This detailed approach highlights the importance of precise nematode control strategies and their potential economic benefits for sugar beet farmers.

4.3 *In-Vitro* Experiments

This study investigated the efficacy of different doses of *Bacillus thuringiensis* (Bt) against *Heterodera schachtii* in sugar beet under controlled in-vitro conditions. The parameters measured included fresh and dry weights of shoots and roots, and the cyst counts per plant after 20 weeks of growth. Each treatment was evaluated using analysis of variance (ANOVA), followed by Tukey's and Duncan's post-hoc tests where significant differences were observed. No phytotoxicity was observed during the development stage of the plants.

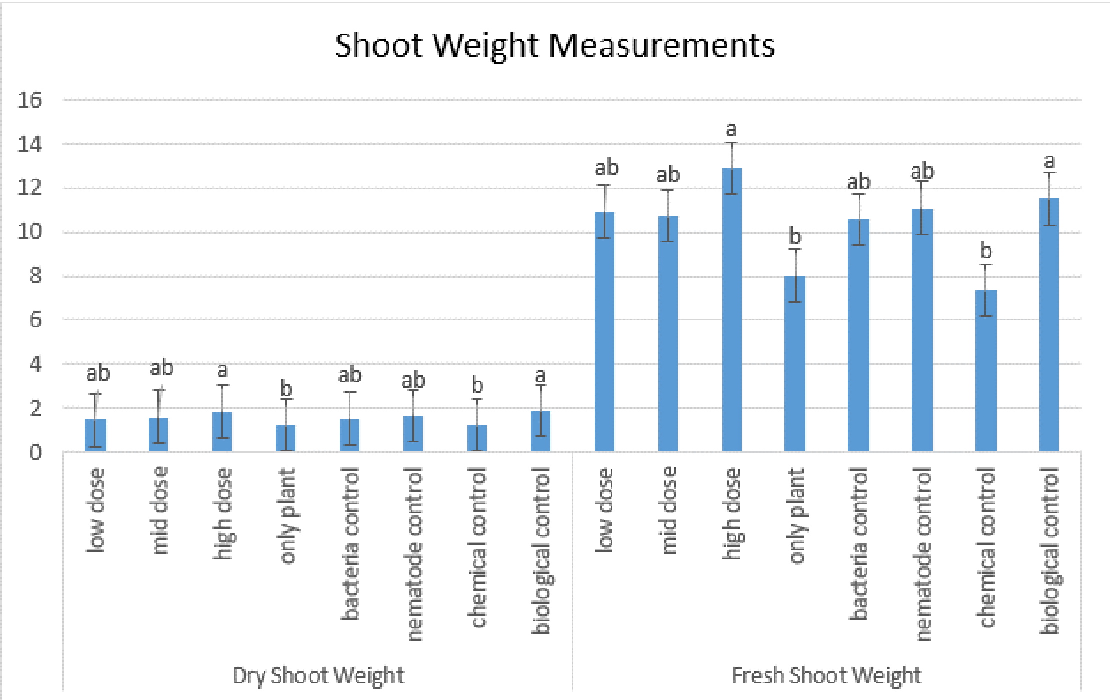

Figure 4.7 Effect of different treatments on shoot weight in sugar beet plants in a pot experiment against sugar beet cyst nematode (*Heterodera schachtii*) after harvest. (Duncan Test for mean comparison if the F-value was significant ($P < 0.05$)

The mean fresh shoot weights for the different treatments varied significantly. The high dose Bt treatment had the highest mean fresh shoot weight at 12.90 g. The mid dose Bt treatment recorded 10.74 g, while the low dose Bt treatment produced 10.94 g. The only plant control (plants without nematodes or bacteria) had a fresh shoot weight of 8.04 g. For the bacteria control (only Bt without nematodes), the fresh shoot weight was 10.56 g, which was similar to the mid dose Bt treatment. In contrast, the chemical control (standard nematicide treatment) had a fresh shoot weight of 7.35 g, which was lower than most Bt treatments. The nematode control (plants infected with nematodes but without any treatment) exhibited the lowest fresh shoot weight at 7.35 g. The mean dry shoot weight followed a similar trend. The high dose Bt treatment yielded the highest dry shoot weight at 1.84 g, followed by the bacteria control at 1.88 g. The mid dose Bt treatment produced a dry shoot weight of 1.64 g, while the low dose Bt treatment recorded 1.22 g. The only plant control had a dry shoot weight of 2.14 g, reflecting healthy plant growth without nematode pressure. The chemical control had a lower dry shoot weight at 1.25 g, while the nematode control produced a mean dry shoot weight of 1.21 g (Figure 4.7).

The data indicate that higher Bt doses, particularly the high dose Bt treatment, resulted in greater shoot development in both fresh and dry weight measurements. Notably, plants under the bacteria control and only plant control also demonstrated significant growth, suggesting the effectiveness of *Bacillus thuringiensis* in promoting plant health under nematode-free conditions.

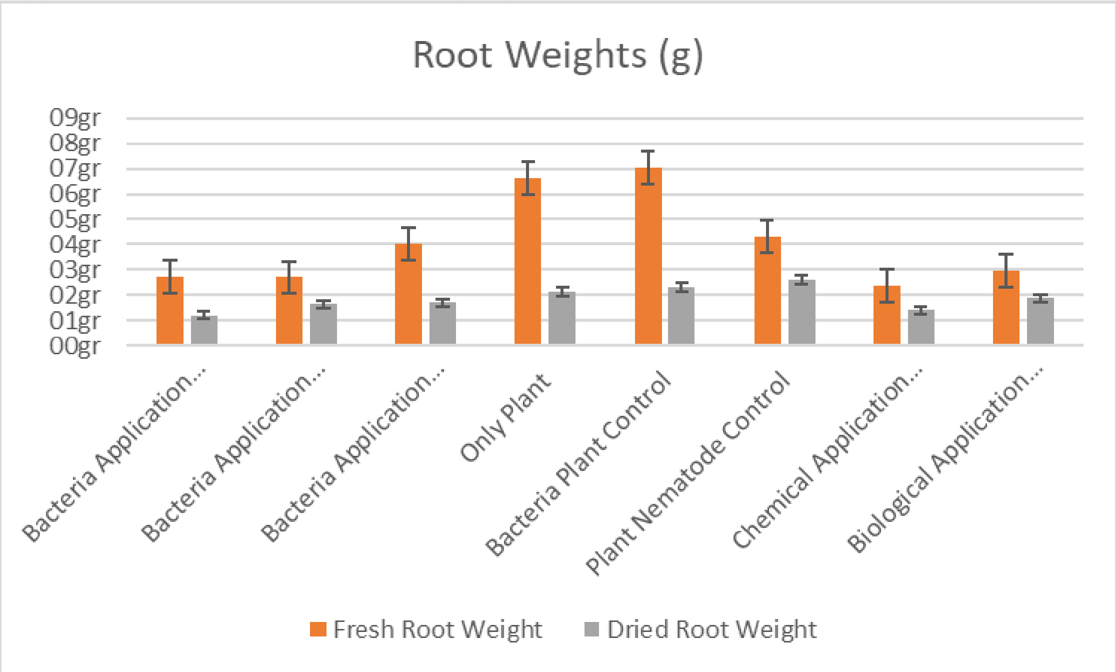

Figure 4.8. Effect of different treatments on root weight in sugar beet plants in a pot experiment against sugar beet cyst nematode (*Heterodera schachtii*) after harvest

The fresh root weights also varied across treatments. The high dose Bt treatment recorded the highest mean fresh root weight at 4.02 g, followed by the mid dose Bt treatment with 2.7 g, and the low dose Bt treatment with 2.71 g. The only plant control (nematode-free, untreated) exhibited a fresh root weight of 6.6 g, reflecting normal root development in the absence of nematodes. The bacteria control (plants treated with Bt but without nematodes) showed a fresh root weight of 7.02 g, which was higher than the treated plants infected with nematodes. The nematode control had a fresh root weight of

4.3 g, while no root growth was recorded in the chemical control treatment. For dry root weights, the high dose Bt treatment again produced the highest value at 2.31 g, followed by the mid dose Bt treatment with 2.7 g and the low dose Bt treatment at 2.71 g. The only plant control exhibited a dry root weight of 6.6 g, while the bacteria control recorded 7.02 g. Similar to fresh weights, no root growth was observed in the chemical control group, reflecting the adverse effect of nematode infestation without effective control. The nematode control had a dry root weight of 1.64 g, which was significantly lower than the other treatments. The data for root weights indicate that higher doses of Bt and the presence of Bt in the bacteria control promote more robust root growth, even under nematode pressure. However, untreated plants infected with nematodes (nematode control) exhibited stunted root development (Figure 4.8).

In the Figures 4.7 and 4.8 clearly seen bacterial treatments increase the dried and fresh root weights. That means that in it carries potential to increase the yield of tuber and sugar under the field conditions. And Bt carries some antagonistic effect of plant health.

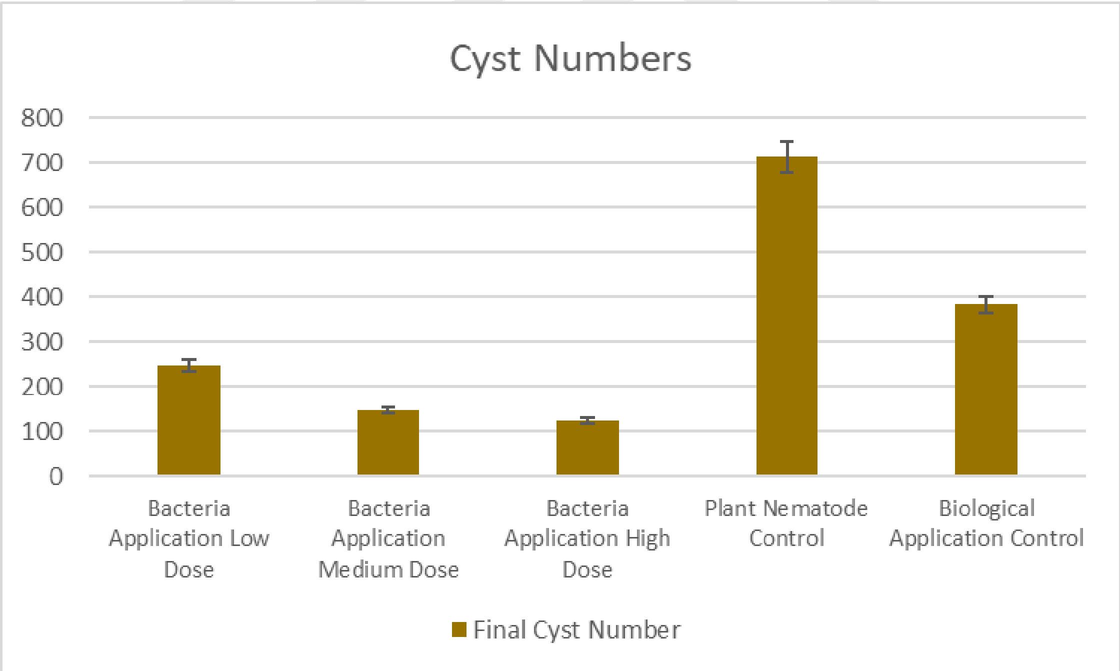

Figure 4.9. Effect of different treatments on cyst counts in sugar beet plants in a pot experiment against sugar beet cyst nematode (*Heterodera schachtii*) after harvest. (Depending on the Duncan test a is the better than c which is worst application)

The number of cysts present in each treatment was a critical measure of the efficacy of the Bt treatments in reducing *Heterodera schachtii* populations seen on Figure 4.9. The high dose Bt treatment resulted in an average cyst count of 123.8, which was significantly lower than the low dose (248.4 cysts) and mid dose treatments (147.8 cysts). The bacteria control and the only plant control both recorded 0 cysts, as expected, since no nematodes were present in these treatments. In contrast, the nematode control exhibited a high cyst count of 713.4, indicating the severity of the nematode infestation in untreated plants. The chemical control resulted in 383.6 cysts, demonstrating a moderate reduction in cyst population compared to the untreated nematode control.

The *in-vitro* experiments demonstrate that *Bacillus thuringiensis* is effective in managing *Heterodera schachtii* infestations in sugar beet, particularly at higher doses. The high dose Bt treatment consistently resulted in improved shoot and root growth and significantly reduced cyst counts. Both fresh and dry weight measurements support the conclusion that *Bt* positively impacts plant health, even in the presence of nematodes. Notably, the bacteria control (without nematodes) performed well, indicating the potential of *Bt* to promote plant growth under optimal conditions. As shown in Figure 4.9, bacterial treatments significantly decrease the number cyst. Even effective than the biological control plots (*Purpureocillium lilacinus*). These results suggest that the highest dose of *Bacillus thuringiensis* was effective in significantly reducing *H.schachtii* populations.

Further research, including field trials, is recommended to validate these findings and explore the practical application of *Bt* as part of an integrated pest management strategy for controlling cyst nematodes in sugar beet crops. Under the light of that results *Bacillus thuringiensis* has potential to control *H. schachtii* also it can be used as combination with chemical treatments.

CHAPTER V

DISCUSSION

Sugar beet cyst nematode (SBCN) is a significant pest to sugar beet (*Beta vulgaris)* cultivation all over the world. Its ability to cause substantial yield losses that effect the development and need effective and sustainable control strategies to be implemented. Recent advances in chemical, biological, and cultural control methods offer potential pathways to moderate the impact of this pest. This research discusses these strategies, portraying recent studies that highlight new approaches and their challenges.

Chemical nematicides have long been a basis in managing *H. schachtii* populations. Recently, the use of fluopyram, a succinate dehydrogenase inhibitor, has shown promise. Research by Oka et al. (2021) indicates that fluopyram can significantly reduce nematode populations when appropriately applied. However, there is a growing need to balance efficacy with environmental safety. Safer application methods, such as seed treatment or targeted soil applications, may reduce chemical use and environmental impact, as suggested by Ibeawuchi et al. (2022). Oka (2021) and Ibeawuchi (2022) research clearly shows as fluopyram has effect on the suppress the population of *H. schachtii* but for the environmental impacts and additionally production cost keeps in the balance. In that case in-furrow application carrying high importance because when it's suppresses SNB population, it gives less residue to nature with lower costs even not performing as soil surface applications. For future studies we need to be able to investigate the optimum application timing for *H. schachtii* control, optimum time of application means more effective management. The efficacy of fluopyram was confirmed through field results but difference of yield was not found by statistically in field experiments. However, while the treatment effectively suppressed nematode populations, it did not fully eliminate the pest, suggesting that multiple applications over several seasons might be necessary for long-term control. Research by Oka et al. (2021) and Ibeawuchi et al. (2022) supports these findings, emphasizing that fluopyram provides considerable nematode suppression when used correctly. However, concerns regarding the environmental safety of such treatments persist, particularly in regard to chemical residues in soil and potential runoff into water bodies . Therefore, more targeted application methods, like in-furrow treatment, reduce the environmental burden and lower the cost of nematicide application.

Implementing cultural practices such as crop rotation and resistant varieties can provide substantial benefits in managing *H. schachtii*. Crop rotation with non-host plants interrupts the nematode's life cycle, effectively reducing its population over time (Scholte & Vos, 2020). Furthermore, breeding for nematode resistant sugar beet varieties has shown significant promise. Boland et al. (2023) have highlighted recent genetic advancements in developing resistant strains that maintain yield quality under nematode pressure. Using of tolerant sugar beet varieties gives advantages to manage of *H. schachtii* especially if integrated with other types of control methods such as in-furrow fluopyram application or *Bacillus thuringiensis* (Bt) based bio-pesticides. In vivo experiments show Bt has great potential of the use as a bio control agent. Bt agent also should combine with tolerant sugar beet varieties to observe the potential managing of *H. schachtii* for further studies.

Biological control agents offer a sustainable alternative by utilizing natural predators or antagonists to control nematode populations. Studies by Meyer et al. (2020) demonstrated the effectiveness of using fungal parasites such as *Pochonia chlamydosporia*, which attacks nematode eggs, thus reducing the population density. In vitro studies indicate that Bt can significantly suppress nematode populations across various doses (low, medium, and high). Notably, Bt outperformed other biological agents like *Purpureocillium lilacinus* in controlled conditions. When integrated with cultural practices such as the use of tolerant sugar beet varieties, Bt offers a multifaceted approach to nematode management. Moreover, Bt's compatibility with other chemical and biological treatments positions it as a versatile tool in IPM programs. However, the effectiveness of Bt and other biological agents can vary based on environmental conditions, and further research is needed to optimize their application in field settings. However, the variable efficacy of biological agents in different environmental conditions remains a challenge (Greco & Perry, 2019). *Bacillus thuringiensis* is renowned for its efficacy as a biocontrol agent against various pests, including nematodes. It produces crystal proteins (Cry proteins) that are toxic to nematodes, disrupting their midgut cells and leading to larval mortality (Khan et al., 2020). Studies by Zhang et al. (2021) have demonstrated that Bt formulations significantly reduce *H.*

schachtii populations in sugar beet fields. In vitro experiments shows us that Bt has effect in 3 doses of applications (Low-Medium-High) and results shows us Bt suppression of SBN population. Even Bt is more effective than *Purpureocillium lilacinus* under the in vitro conditions, also Bt shows antagonistic effects when we are examining Figure 4.4 and Figure 4.5. Furthermore, Bt's compatibility with other control methods makes it a versatile component of integrated pest management strategies.

Although substantial progress has been made in controlling the sugar beet cyst nematode, ongoing research and innovation are crucial. Developing environmentally friendly, cost-effective, and sustainable strategies will require continued collaboration among researchers, practitioners, and policymakers. Future research should focus on optimizing the combination of chemical and biological controls, specifically exploring the timing, dosage, and frequency of applications. Field trials over multiple growing seasons will be essential to understanding the long-term efficacy of integrated treatments. Moreover, exploring Bt's potential synergy with other management methods could further enhance nematode suppression and crop yields.

The integration of *B. thuringiensis* into SBCN management strategies offers innovative and sustainable control options. Continued research focusing on the development of Bt-based formulations and their integration with other control methods will be vital for enhancing their applicability and efficiency. Under the Green Deal, it has been previously stated that countries involved must cut their greenhouse gas emissions by 50% by 2030 and reach carbon neutrality by 2050. Collaborative efforts between researchers and practitioners are essential to refine these strategies for diverse agricultural settings, ensuring they are both effective and environmentally sustainable.

CHAPTER VI

CONCLUSION

The results of this study highlight the potential of different control strategies for managing *Heterodera schachtii*, a major pest in sugar beet cultivation. The application of Fluopyram, both in-furrow and soil surface, showed some reduction in nematode populations and improvements in plant vigor. However, the lack of statistically significant differences among treatments suggests that a single application may not be sufficient for long-term control in heavily infested fields. More comprehensive approaches, including multiple applications over several seasons, could enhance the effectiveness of chemical treatments. the trials showed that fluopyram applications led to reductions in nematode cyst populations. This reduction is critical for long-term crop health, as it prevents the buildup of nematode populations in the soil, which could otherwise persist and damage future crops. By lowering nematode numbers and reducing their impact on the crop, fluopyram provides both immediate and long-term benefits to growers. field trials underscore the potential of fluopyram to significantly improve sugar beet yields and manage *H. schachtii* populations. While early-stage growth parameters did not show statistical significance, the yield results were compelling, particularly in fields with challenging soil conditions. Growers should consider fluopyram, especially when applied in-furrow, as a viable solution for managing nematode infestations and increasing sugar beet production. Further research across multiple seasons would help solidify these findings and optimize application strategies for different field conditions. fluopyram into their integrated pest management (IPM) strategies could be a highly effective approach to controlling nematode infestations and improving sugar beet yields. The in-furrow application method, in particular, shows promise as it targets the root zone directly, where nematodes are most active. This method ensures that the nematicide is concentrated around the roots, offering protection during the early stages of plant development, which is critical for establishing a strong, healthy crop. However, the results also indicate that soil conditions play a significant role in determining the effectiveness of fluopyram. In locations with more compacted soils, the in-furrow application proved to be especially beneficial. This highlights the need for growers to assess their soil conditions carefully before deciding on an application method. In well-structured soils with higher organic matter, surface application might be sufficient and can still provide yield improvements, as seen in the second trial.

In addition to chemical control, the study's in vitro experiments demonstrated the effectiveness of *Bacillus thuringiensis* as a biological control agent. The strains containing the Cry6Aa2, Cry55Aa1, and Cry5B genes were found to significantly suppress nematode populations while promoting plant growth. The bacterial treatments not only reduced the cyst counts more effectively than other biological control agents like *Purpureocillium lilacinus*, but also positively impacted the fresh and dry weights of both shoots and roots. These results indicate that *B. thuringiensis* has strong potential as a sustainable and environmentally friendly option for nematode management in sugar beet cultivation.

In conclusion, combining chemical and biological control methods, particularly the use of *B. thuringiensis* alongside chemical treatments like Fluopyram, may offer a more integrated and effective approach to managing *Heterodera schachtii*. It has previously been stated that under the Green Deal, participating countries are required to reduce their greenhouse gas emissions by 50% by 2030 and achieve neutrality by 2050. Future research should focus on optimizing these strategies, potentially incorporating crop rotation and resistant sugar beet varieties to ensure a more holistic and sustainable management plan for nematode control.

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