Efficacy of conventional and nano-formulated seed treatments for the control of Hessian fly (*Mayetiola destructor*) in wheat.

by

Kokou Selom Amego

B.S., University of Lome, 2014

MPhil., University of Ghana, 2019

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Entomology

College of Agriculture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2025

Approved by:

Major Professor

Dr. Jeff Whitworth

Copyright

Abstract

The Hessian fly is one of the most destructive pests of wheat, causing significant yield losses worldwide. The most effective management methods include using resistant varieties and applying cultural practices. However, resistant varieties often produce lower yields than susceptible ones, and wheat resistance can be significantly affected by temperature. Therefore, seed treatments, especially neonicotinoids, may serve as an effective strategy against Hessian flies. Still, their efficacy is only observed up to four weeks after planting, and there has been no recent evaluation of their effectiveness. This study aims to reassess the current effectiveness of conventional neonicotinoid insecticides and to develop and test carbon nano-insecticides both in the laboratory and in the field. Initially, two conventional neonicotinoid-treated seeds at the label-recommended rate were evaluated: commercially treated thiamethoxam and laboratory-treated imidacloprid. Results showed that thiamethoxam-treated seeds were highly effective, maintaining infestation levels near zero for up to 30 days. Untreated seeds showed infestation levels over 90%. Imidacloprid-treated seeds were moderately effective, with infestation levels ranging from 30% to 60%. Additionally, carbon nano-imidacloprid was tested at both the commercially recommended rate and half that rate in the laboratory and the field. Results indicated that nano-imidacloprid at a high commercial rate was the most effective in controlling Hessian fly infestation in both settings. The nano-imidacloprid at half the rate showed somewhat lower control levels compared to the high rate but was similar to commercial imidacloprid seed treatment.

Keywords: Carbon nano-insecticide, Hessian fly, Neonicotinoid, Seed treatment, Wheat.

Table of Contents

List of Figures

List of Tables

List of graphs

Acknowledgements

I am truly grateful to everyone who supported me during this journey. I want to thank my family for their encouragement and support. Special thanks to my wife Ruth; your love, patience, and belief in me have continually been a source of strength.

I would also like to thank my committee for their time, guidance, and support. A very special thanks to my main advisor, Dr. Whitworth, for your exceptional leadership, mentorship, and dedication. Your advice, constant encouragement, and the sacrifice of your weekends to review my work have been invaluable. Dr. Chen, I appreciate your willingness to address my questions about Hessian fly biology and your provision of the flies that facilitated my experiments. Dr. Zolnerowich, thank you for your guidance throughout my research and for always being available to provide scientific and administrative support and advice whenever I needed it. I would like to express my sincere gratitude to Dr. Amie Norton for her invaluable assistance with the nanotechnology aspects of my research and for her kindness and generosity throughout my journey. Her guidance was important in shaping this work. Special thanks to Dr. Xiu Ming, who ensured I always had enough insects for my experiments and guided me on how to work with the insects. Your dedication and support were truly appreciated. I would also like to thank JR Erwing and Dr. Cohnstaedt for their meaningful suggestions that helped make my work more efficient and manageable. I would also like to thank the Department of Entomology at Kansas State University for providing a supportive academic environment and my classmates for their camaraderie and encouragement.

Finally, I extend my sincere appreciation to the Fulbright Program for making this journey possible. Your support has been instrumental in helping me pursue and complete this work.

Chapter I: Introduction

Wheat (*Triticum aestivum* L.) serves as a primary food source for a substantial proportion of the global population, as a staple food (Alomari et al., 2023). It is a crop of economic importance in the United States, as the third-largest crop in terms of area covered, yield, and gross agricultural income (USDA-ERS, 2025). The United States accounts for approximately 8% of the wheat produced worldwide (Helman & Bonfil, 2022) and consistently ranks among the top five global wheat exporters (USDA-ERS, 2025). Wheat is thus an essential crop for local consumption and international trade in the United States.

According to the U.S. Department of Agriculture, Economic Research Service (USDA, ERS) (2025), winter wheat constitutes a significant portion of the wheat produced in the United States. In 2024, winter wheat production (1,348,930,000 BU) accounted for approximately 68.42% of the total wheat produced in the USA. Kansas typically leads the United States in winter wheat production, with most wheat grown in Kansas being of a winter wheat variety. Kansas contributes about 26% to total US annual wheat production, although this figure varies from year to year (Obembe et al., 2021). Wheat is the primary crop in Kansas, which has historically been recognized as the "Breadbasket of the World" and nicknamed the “Wheat State” (Paulsen, 1998; Zhang et al., 2024). Overall, wheat is crucial for the Kansas economy, significantly contributing to the national wheat supply and global markets.

Wheat plants are subject to effects from a wide range of biotic and abiotic threats, with insect pests representing the predominant biotic threat (Güllü et al., 2025). Over one hundred arthropod species have been identified as wheat pests (Hatchett et al., 1987). These arthropods can cause important economic damage to wheat plants by feeding on various parts. Arthropod damage can cause an average potential global crop loss of 7.9% (Güllü et al., 2025). More than 30 insect and mite species in the United States infest wheat, although most of these pests are occasional or only found in specific areas (Hatchett et al., 1987). A limited number of these pests are of significant economic importance, capable of inflicting substantial damage across extensive regions (Hatchett et al., 1987). The Hessian fly (HF), *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is one of these pests that can cause significant plant damage, resulting in yield reduction (Buntin, 2025; Chen et al., 2023).

The Hessian fly (HF) may infest winter wheat and inflict substantial damage in the fall and spring. This damage can be categorized into four types: 1) plant stress or death in the fall, 2) head reduction or prevention during spring, 3) lodging before harvest, and 4) grain weight reduction due to larval feeding (Hill & Smith, 1925). Nine years of study conducted by Butin (1999) on how HF affects winter wheat yield has shown a direct correlation between yield loss and an increase in the percentage of infestation of tillers during autumn and stems in the spring.

Their study showed an economic reduction in yield with the infestation of ≥5-8% tillers in autumn and ≥13-20% infestation of stems in the spring. These results suggest that to control the HF infestation in the field effectively, it is important to limit its growth. However, HF is a wheat stem-feeding insect with a cryptic larval stage, making it challenging to detect and manage (Hatchett et al., 1987). It is traditionally managed by using resistant cultivars, destroying volunteer wheat, selecting a later planting date to avoid early infestation in fall, and applying an insecticide treatment to seed (Chen et al., 2023; Whitworth et al., 2009). Implementing these methods of control faces challenges. Wheat plant-resistant genes lose effectiveness with increasing temperature (Ratanatham et al., 1986; Chen et al., 2014; Brahmi et al., 2021;

Subramanyam et al., 2025). Zhu et al. (2020) posited that exposure to extreme heat may impede the processes within wheat plants that facilitate the synthesis and mobilization of essential chemical resources necessary for the synthesis of defensive compounds. They suggested that this may result in decreased strength of cell walls and cuticles, reduced 12-oxo-phytodienoic (OPDA) signaling, and enhanced auxin signaling, consequently leading to the suppression of resistance and the induction of susceptibility. Recent research indicated that planting date selection to avoid HF infestation is ineffective (Chen et al., 2023). Adult activity of the HF was observed in Kansas later in the fall season than had been earlier documented (Schwarting, 2014). The efficacy of delayed planting of wheat in managing the Hessian fly population in the southeastern United States is limited (Butin and Chapin 1990). This is due to the recurrent periods of warmer weather that extend from fall into the early winter months (Butin and Chapin 1990). Seed treatment has been demonstrated to offer short-term control of fall infestations of Hessian flies in winter wheat, with an efficacy duration of approximately 30 days. However, they offer minimal to nonexistent protection against spring infestations of HF. (Wilde et al., 2001; Whitworth et al., 2009; Howell et al., 2017). Extensive laboratory and field studies on the efficacy of wheat seed insecticide treatment for the control of HF has been reported during the years 1980 and 1990. A few recent studies conducted mainly in the field using neonicotinoid insecticides have also been reported. Considering the potential building of resistance in the HF population, it is important to test the current efficacy of seed treatment against HF. It is also crucial to conduct more research to improve the current management of Hessian flies.

According to Vivekanandhan et al. (2024), nanotechnology impacts pest management by providing efficient, targeted, and sustainable options for insecticide formulation using nanomaterials. These nano-formulations enhance the solubility, stability, and controlled release of pesticide active ingredients, allowing for more precise pest management. It has been suggested that overcoming pesticide resistance might be possible with nanotechnology (An et al., 2022). The application of nanotechnology could affect wheat seed treatment. Since conventional seed treatment involves the use of neonicotinoid insecticides, which have limited effectiveness (Buntin, 2025; Wilde et al., 2001; Howell et al., 2017), nano-formulated pesticides may offer some advantages over traditional pesticide formulations. They could improve the effectiveness of seed treatment by extending its duration and requiring a smaller quantity of insecticide. Nano insecticides may provide tailored pest control while remaining environmentally friendly, making them ideal for sustainable agriculture.

Thus, the objective of this study is:

1. To evaluate the effectiveness of conventional seed treatments against the Hessian fly and their residual effectiveness in the laboratory.

2. To develop and test nanotechnology-enhanced seed treatments in the laboratory.

3. Test nanotechnology-enhanced seed treatments in the field.

Chapter II: Literature Review

2.1. The biology of the Hessian fly (HF)

2.1.1 Taxonomy and origin of the Hessian fly

The Hessian fly (*Mayetiola destructor* Say) is an insect classified in the Diptera order, the Cecidomyiidae family, and the Mayetiola Genus (Edde, 2022). Stuart et al. (2012) suggested that HF originated in the Middle East, especially from the Fertile Crescent. It can be found in most regions where wheat is produced (Edde, 2022). Hessian fly was first recorded in the United States in New York City in the 1770s (Pauly et al., 2002). It is widespread across an expansive region, including the United States (Hatchett et al., 1987). The Hessian fly's preferred host is wheat (Chen et al., 2009), and it is one of wheat's major insect pests in the United States (Hatchett et al., 1987).

2.1.2 Hessian Fly Life Cycle

Hessian fly is holometabolous; thus, its life cycle comprises four stages: adult, egg, larva, and pupa. HF has an approximate life cycle of 28 days, which can be extended due to aestivation and diapause (Stuart et al., 2012).

- Adult

The adult HF (Figure 2.1) is a small gall midge, measuring approximately three millimeters. It has long legs and two wings, and its color ranges from dark gray to black (Hatchett et al., 1987). As adults, HF live for only one or three days, rarely exceeding four days under room temperatures without supplementing food such as honey water (Harris et al., 2003). It appears to be more active after autumn rain with relatively warm temperatures (Davis et al., 2010). Adult females have a reddish-brown color due to the maturation of orange eggs in their abdomen

(Hatchett et al., 1987). Female Hessian flies are semelparous; their reproductive effort is all at once laying their eggs in several discrete clutches and dying shortly after ovipositing (Fritz et al., 1982). On average, the adult female's lifespan is between two and three days, during which they produce 100–400 eggs and do not consume any food (Harris & Rose, 1989; Flanders et al., 2013). Females deposit their eggs on the surface of recently developed leaves (Hatchett et al., 1987).

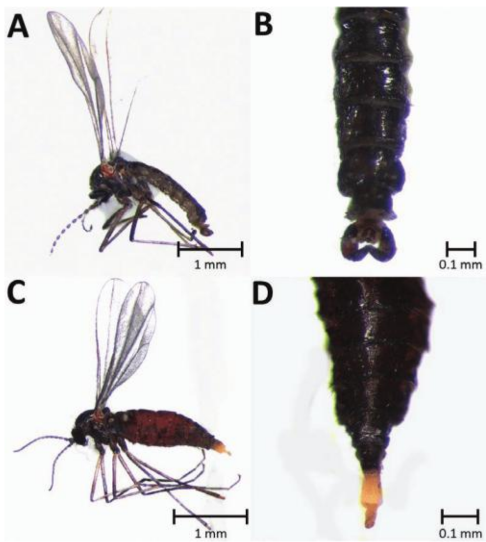


Figure 2. 1: Adult Hessian fly.

(A) Male. (B) Male genitalia. (C) Female. (D) Female genitalia. (Photo credits: Alan Burke)

- Eggs

The HF egg (Figure 2.2) is characterized by a glossy red coloration, a cylindrical form, and a length of approximately 0.5 millimeters (Hatchett et al., 1987). Hessian flies employ visual and chemical signals to discriminate between grasses and non-grasses; they perceive surface leaf details through their tactile and/or kinesthetic senses to make oviposition decisions (Kanno & Harris, 2000; Harris et al., 1993). The oviposition process is significant for the survival of HF offspring, given that larvae cannot migrate from the plant on which the female HF has deposited eggs to another plant (Harris et al., 2001). The mature female HF, possessing the capacity to oviposit, goes from plant to plant, depositing two to five eggs on young grass leaves (Harris & Rose, 1989). Eggs are usually deposited in one or several clutches on the adaxial surface, in a parallel orientation to the long axis of the leaf (Figure 2.2), within the grooves formed by the veins of the leaf (Fritz et al., 1982; Harris & Rose, 1989). Kanno & Harris (2000) have demonstrated that the number of eggs deposited increases with the number of grooves.

According to Foster et al. (1975), a mean of 27 (48 for °F) thermal units (TU) was required to develop HF eggs, with 12.2 °C (53.96 °F) as the base. They also showed that 21.1 °C (69.98 °F) was the optimal temperature for HF development. The "clutch size" refers to the number of eggs deposited by a female in a single reproductive bout. Withers et al. (1997) posited that when the likelihood of survival is high and host plants are available, female Hessian flies produce smaller-sized clutches per plant. Conversely, when there is little expectation of survival and host plants are scarce, females produce larger clutches in response. Hessian fly eggs initially display an orange hue for four to five days, after which they undergo a color change and become white (Flanders et al., 2013).

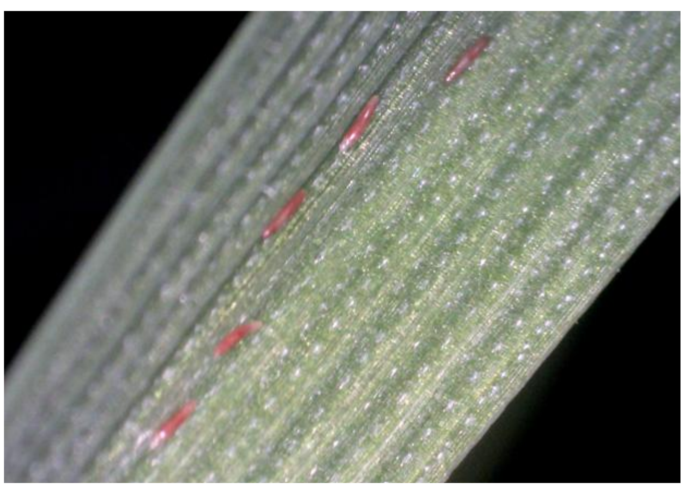


Figure 2. 2: Hessian Fly Eggs (Photo credits: Kokou Amego)

- Larvae

Larvae (Figure 2.3) emerge from eggs three to ten days after being deposited (Flanders et al., 2013). After hatching, larvae crawl down between the leaf sheaths near the plant's crown and initiate feeding. (Hatchett et al., 1987). Those larvae that fail to attach to a feeding site on the plant move along the surface of the stem and die after a few days. The growing second leaf carries the dead larvae up and out of the leaf sheath (Grover, 1995).

Hamilton (1966) conducted a greenhouse study on the response of HF larvae to simulated weather. The study showed that newly hatched larvae migrating to the base of the wheat plant exhibit a high degree of susceptibility to unfavorable weather conditions. They noted a high death rate of newly emerged larvae due to low temperature (≤ 1.7 °C (35°F)), low relative humidity, intense rainfall, and wind.

Larvae are exclusively responsible for the damage caused to the wheat plant, by sucking the plant juice. Infested seedlings have a distinctive stunted growth characterized by shorter, erect, and darker leaves, which may die or grow new tillers (Hatchett et al., 1987). Larvae migrate above the node to feed on mature plants, causing damage, which reduces the grain's quantity and quality. Additionally, larval feeding may cause stems to break at the site of feeding, resulting in significant grain loss due to lodging prior to harvesting (Hatchett et al., 1987).

According to Gagné et al. (1989), HF larvae undergo three different molts: the first two instars feed, and a third non-feeding instar. The first instar larvae are mobile and have a six-day feeding period, when most larval development occurs. The second instar larvae are immobile and feed for approximately four days, progressively changing their integument color to dark brown, becoming the cocoon for the third instar. The third instars do not feed and develop into pupae.

Figure 2. 3: Hessian Fly Larva. (Photo credits: Kokou Amego)

- Pupae

The larvae's final development stage is characterized by their maturation within the flaxseed or puparium (Figure 2.3). The puparium is also called flaxseed due to its appearance, like the flax seed. The flaxseed (Figure 2.4) is a protective casing made of cuticles of the second instar larvae, which is three to five mm in size with a shiny, dark brown appearance (Hatchett et al., 1987; Flanders et al., 2013). During summer and winter, Hessian flies overwinter or oversummer on wheat stubble or vernalizing wheat plants. The overwintered flaxseeds metamorphose to adults in the spring, coinciding with or following the wheat breaking dormancy (Davis et al., 2010).

Figure 2. 4: Hessian fly flaxseed (Photo credits: Kokou Amego)

2.2 Management of Hessian Fly

No effective remedial solutions are available post HF infestation (Davis et al., 2010). Applying insecticides to manage HF populations is ineffective (Schmid et al., 2018). The application of foliar insecticide is inefficient not only because of the cryptic life of the HF larvae but also because of the economic aspect and the optimal timing for the application (Schmid et al., 2018). The most effective approaches to minimizing injury and yield loss caused by HF are the integration of preventive measures, such as the use of resistant varieties or insecticide seed treatments (especially for varieties susceptible to HF) (Reay-Jones & Buntin, 2024). Other preventive methods include the destruction of infested stubble and volunteer wheat and using the best pest management planting date (Davis et al., 2010). The most cost-effective and efficient way of controlling HF is planting wheat varieties that are resistant to it (Berzonsky et al., 2002). Despite their efficacy, resistant varieties can also become ineffective. Temperature fluctuation can affect wheat's resistance to the HF (Chen et al., 2014). HF biotypes successfully overcome the resistant gene six to eight years after their release if the resistant cultivar is extensively utilized (Schmid et al., 2018). Burning wheat stubble at a minimum depth of 2 inches and destroying volunteer wheat at a minimum of 2 weeks before germination can help reduce the presence of flaxseed. However, those processes may be ineffective, and some flaxseed may remain for future infestation in the wheat field (Davis et al., 2010).

The timing of wheat planting during late fall is important and can contribute to reducing the proliferation of the pest and its subsequent injury (Davis et al., 2010). Wheat can be planted late between an optimum date in the fall and the onset of cold weather, which can prevent infestation by ovipositing HF. In a study conducted by Buntin and Johnson (1992) utilization of the wheat planting date as a management tool has shown that planting wheat before the advised period resulted in a more significant level of HF infestation, but adherence to the recommended planting date did not guarantee the mitigation of detrimental effects associated with HF infestations during the fall. Recent observations indicate that the timing of flight-free dates in some US regions, such as Kansas, may need reevaluation. This need arises because abiotic factors, including unusual wet or dry periods, can shift the timing of emergence or cause secondary waves of HF emergence. As a result, these conditions alter the emergence timing, requiring an update to the current calendar (Drake and Decker, 1932; Schwarting, 2014).

Insecticide seed treatment can help mitigate HF infestation, but it is only effective for about thirty days after germination (Whitworth et al., 2009).

2.3. Effectiveness of insecticide seed treatments against the Hessian fly.

Wilde et al. (2001) investigated the effectiveness of three insecticides in protecting winter wheat from insect pest infestation. Their study proved that imidacloprid, thiamethoxam, and fipronil effectively controlled early infestations of HF in the fall. They did not effectively protect the plant against infestation during the spring. Similarly, Winn et al. (2023) demonstrated that imidacloprid treatment significantly reduced the proportion of tillers infested by HF larvae and pupae and reduced the mean number of larvae and pupae per tiller. Howell et al. (2017), based on a study on the effect of imidacloprid seed and foliar treatment on HF, concluded that neonicotinoid seed treatments successfully protect susceptible wheat cultivars from HF. However, the foliar insecticide application was not effective.

Butin (2025) assessed the effectiveness of neonicotinoid seed treatments in minimizing HF larval infestations. The results showed that imidacloprid 480FS ("Flowable Concentrate for Seed Treatment") (0.31 g active ingredient (a.i.)/kg of seed) and clothianidin (0.39 g a.i./kg of seed) at higher rates reduced infestation both in autumn and late fall. The lower rate did not provide enough protection against infestation. Similarly, thiamethoxam seed treatment was adequate, but none of the three insecticides effectively controlled spring infestations.

Overall, insecticide seed treatments, especially neonicotinoids, can be effective in negatively affecting HF infestations, but only for a short time (20 to 30 days). They do not protect against later or following infestations, nor for spring and summer infestations (Whitworth et al., 2009; Royer et al., 2015; Butin, 2025)

2.4 Application of Nanotechnology in agriculture, especially in pest management

Nanotechnology and nanoscience are scientific disciplines involving the design, creation, and alteration of particles at a microscopic scale, referred to as the nanometer range (Singh et al., 2018). The nanoscale refers to dimensions measuring 1–100 nanometers (0.000000001 meters) (Singh et al., 2018). The defining feature of nanoparticles is their minute size, but this is not the only distinction that sets them apart. They also exhibit unique physical and chemical characteristics (Zaytseva & Neumann, 2018). Nanoparticles possess properties that differ from those of bulk material, particularly their increased chemical reactivity and solubility. Such properties influence biological activity, mobility, and bioavailability (Margulis-Goshen et al., 2013).

The application of nanotechnologies is extensive across various fields and industries, including agriculture (Malik et al., 2023). Nanotechnologies have many uses in agriculture, particularly in plant cultivation and pest management (Gondal & Tayyiba, 2022). The significant benefits of using nano-agrochemicals may include enhanced application efficiency, improved bio-interaction, diminished losses, a high surface-to-volume ratio, targeted delivery, and the gradual release of nano-agrochemicals, which may increase their efficiency and may render them a cost-effective alternative to conventional agrochemicals (Okey-Onyesolu et al., 2021). The impact of nanotechnology on agricultural production is characterized by enhanced utilization of agricultural inputs with more efficacy in their application. This minimizes losses of chemicals into the surrounding environment and enables precise delivery of fertilizers or other agrochemicals to specific locations of the plant as needed. Furthermore, nanotechnology has been shown to prolong shelf life and reduce recommended dosages of agrochemicals, promoting sustainable agricultural practices through the usage of fewer chemicals to achieve a long-term effect on plants and the release of fewer chemicals into the environment (Reddy & Chhabra, 2022).

Nanoparticles can contribute to achieving a highly effective pest control method (Kannan et al., 2022). For instance, the polymer of polyethylene glycol (PEG) imidacloprid nano-formulation has exhibited enhanced efficacy in controlling wheat green aphid, *Schizaphis graminum* (Rondani)**,** compared with the conventional formulation of imidacloprid at lower concentrations (Ahmadpour al., 2024). The study determined that the lethal concentrations (LC50 and LC30) of pure insecticide and its nano-formulation on adult female aphids were 21.46 and 58.56 mg a.i./L, respectively, and 3.79 and 4.94 mg a.i./L, respectively. This showed that effective control of pests can be achieved with nano-formulations using smaller quantities of insecticide than generally required using standard insecticide formulations. Furthermore, nano-imidacloprid has demonstrated superior durability to conventional treatment, exhibiting prolonged pest control (Ahmadpour al., 2024). Sabry et al. (2021) demonstrated that when tested on second-instar larvae of Egyptian cotton leafworm (*Spodoptera littoralis,* (Boisduval)), the efficacy of indoxacarb and imidacloprid nanoparticles was found to be 12 and 4-fold greater, respectively, compared to conventional formulations of these compounds.

2.5. Carbon nanotubes and their applications

Various formulation types of nano pesticides have been proposed, including nano emulsions, nano capsules, polymer, Layered Double Hydroxides (LDHs), carbon nanotubes, carbon nanofibers, graphene oxide, and products containing immaculate engineered nanoparticles, such as metals, metal oxides, and nano clays (Sarkar et al., 2021; Kannan et al.,2022). Of these nanoparticles, some originate naturally and have persisted in negligible quantities in the environment (Zaytseva & Neumann, 2018). The analysis of geological samples, with a particular focus on fulgurite (a naturally occurring glassy tube or crust formed when lightning strikes sand or rock, resulting in the fusion of the grains) specimens, initially indicated extreme temperature conditions for the detection of naturally occurring fullerenes and nanotubes. However, it has been demonstrated that lower temperatures can be employed to produce carbon nanotubes, thus indicating that a range of carbon phases can be obtained under less extreme conditions (Zaytseva & Neumann, 2018). The presence of carbon-based nanomaterials, including carbon nanotubes and fullerenes, has been ascertained in various natural substances (Velasco-Santos et al., 2003).

Using a laser to vaporize a graphite target in a supersonic cluster beam apparatus, Kroto et al. (1985) created fullerenes in 1985. Fullerene, also referred to as "buckyballs" or "C60" when it was synthesized, is a class of hollow-structured carbon clusters composed of 60 carbon atoms arranged in a spherical structure, resembling a hollow soccer ball. In 1990, significant amounts of fullerenes were synthesized by utilizing an arc process, which involves the application of a high current arc across graphite electrodes. In 1991, Iijima utilized a similar arc process to discover hollow tube analogs of the fullerenes, which consisted of multiple tubes, or shells, one within another. This novel material is designated multiwalled nanotubes (MWNTs) (Figure 2.5), or nanotubules, due to their nanometer-scale diameters.

Carbon nanotubes (CNT) are cylindrical structures made of carbon materials with nanoscale diameters composed of sp2 carbons arranged in a hexagonal network (Figure 2.5) (Popov et al., 2004; Ribeiro et al., 2017). Carbon nanomaterials (CNMs), comprising nanotubes, fullerenes, and graphene, are among the ten foremost fabricated CNMs for industrial applications (Keller et al. 2013). Diverse carbon nanotubes exist based on their length, diameter, chirality, and number of layers (Popov et al., 2004). However, they can be classified into two main groups, including single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) (Figure 2.5) (Ribeiro et al., 2017).

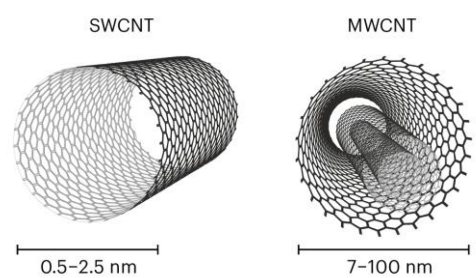


Figure 2. 5: Single-walled carbon nanotube (SWCNT) and multi-walled carbon nanotube (MWCNT) schematic representation (Image by Filchakova, Marina)

The unique cylindrical structure of CNTs allows them to confine nanoscale molecules and atoms within their internal space. Notably, CNT capacity extends to enclosing metal, water, and molecular oxygen. The confined water within the CNT exhibits properties that differ significantly from those of its bulk form. Carbon nanoparticles' properties include their small size, superior mechanical and thermal strength, lightness, rigidity, and high surface area-to-volume ratios (Jakubus et al., 2017; Martins et al., 2019; Safdar et al., 2022). They can be characterized using Raman, electronic, and optical spectroscopies (Popov et al., 2004). CNTs have gained significant attention due to their properties and potential applications (Rudakiya et al., 2019).

Carbon nanotubes (CNTs) possess specific physicochemical characteristics that facilitate their penetration and translocation through plant tissue (Zaytseva & Neumann, 2018). These characteristics include dimensional properties (diameter, length, wall structure, including single or multi-walled CNT), extent of agglomeration (well-dispersed, or clumped CNT), and surface functionalization (attaching chemical group or molecule to the surface of CNT) (Zaytseva & Neumann, 2018). The potential benefits of incorporating carbon nanotubes into agricultural practices include promoting seed germination and plant growth, improving soil fertility, promoting antimicrobial and antifungal activities, and improving insect pest control (Schlogl et al., 2024; Safdar et al., 2022). These benefits could result in a more sustainable, efficient, and productive agricultural production system with the possibility to reduce chemical use and maintain an equal level of effectiveness, benefiting both farmers and the environment.

Various effects of carbon nanomaterials have been observed on plant growth. Cañas et al. (2008) indicated that the effects of carbon nanotubes on plant root systems, mainly the roots' elongation, vary depending on the plant species and the type of nanotubes, with non-functionalized nanotubes generally showing more significant effects, either conducive or detrimental, to the process of root elongation. Research conducted by Lin et al. (2017) on the phytotoxicity of nanoparticles, including carbon nanotubes, on crops has shown that they do not inhibit seed germination or root growth. Rice seeds in the presence of nanotubes had significantly enhanced germination rate. Carbon nanotubes have been demonstrated to significantly impact the amount of water in the germinating seeds. This phenomenon is attributed to the inherent capability of carbon nanomaterials to interact with the rigid external layers of rice seeds, thereby enhancing the process of water absorption (Nair et al., 2012).

CNTs have shown promising properties as pesticides. According to Wang et al. (2007), surface-modified multi-walled carbon nanotubes (MWCNTs) inhibit the germination of the spore of a pathogenic fungus, *Fusarium graminearum* (Schwabe), showing the antifungal activities of CNT. Additionally, Chung et al. (2011) indicated that elevated concentrations of MWCNTs can potentially reduce microbial activity and biomass in soils.

The utilization of nanocomposites in formulating insecticides is a recent development and is still in the preliminary research and experimental stage (Sarkar et al., 2021). The synthesis of carbon nanotube-based insecticides that combine naturally occurring or synthetic insecticide components has only been documented in a few instances. Some recent research includes the direct utilization of carbon nanotubes to control the onion thrips, *Thrips tabaci* Lindeman (Ali et al., 2023), and the Khapra beetle, *Trogoderma granarium* (Everts) (Al-Esawy et al., 2021).

Sarlak et al. (2014) highlighted the potential for improving pest control by combining already available pesticides with carbon nanotubes. They obtained an encapsulated pesticide by trapping the fungicides zineb and mancozeb on functionalized multiwall carbon nanotubes (MWCNT-g-PCA), which exhibited a significantly more potent toxic effect on *Alternaria alternata* fungi than the standard pesticide.

The potential for future research on carbon nanotubes in agriculture is significant. Such research could improve efficiency and sustainability in agriculture and plant science (Schlogl et al.,2024). However, the effects of carbon nanomaterials (CNMs) on cells and living organisms are not yet fully established. Some studies have reported no toxicity, while others have highlighted potential risks of carbon nanomaterials, such as lung damage (granulomas formation and lung fibrosis) (Bussy et al., 2013; Freixa et al., 2018; Martins et al., 2019). Further studies are required to comprehensively understand the environmental and health implications of CNTs.

Chapter III: Materials and Methods

3.1. Insect materials

Hessian flies were obtained from Dr. Ming Chen's USDA laboratory at Kansas State University, Manhattan, Kansas. The flies were always provided in a wooden box (Figure 3.1) covered with mesh and plastic sheeting to create a moisture barrier. The box was placed in a plastic bag to slow drying and kept in the freezer to maintain a dormant state, ensuring the viability of the pupae for subsequent experimentation.

Figure 3. 1: Wooden box with Hessian fly colony

3.2. Plant materials used for this research

The wheat variety KS Big Bow was used as the host plant for this research. Wheat seed was obtained from Dr. Ming Chen's USDA laboratory and used as the medium throughout the study. KS Big Bow is a hard winter wheat cultivar developed by the Kansas State University wheat breeding program at the Hays Agricultural Research Center and released in 2022 by the Kansas Agricultural Experiment Station. It is a potentially high-yield wheat cultivar adaptable to dryland production in western Kansas. It has excellent disease resistance against wheat streak mosaic virus, stripe and stem rust, and intermediate resistance to Barley Yellow Dwarf Virus. KS Big Bow is susceptible to Hessian flies with a mean germination index of 84.5%. It is a mediumheight cultivar with good straw strength, below-average winter hardiness, and a freeze damage rating (visual assessment scale used in agronomic and plant breeding studies, to quantify the extent of cold or freezing injury to crops such as winter wheat) of 4.1 on a scale of 1 to 9 (Zhang et al., 2024).

During the research, all plants were grown in containers filled with soil (Figure 3.2). They were maintained in environmental chambers at 20°C, with a relative humidity of 65–75% and a photoperiod of 14:10 (light/dark).

3.3. Evaluation of commercially treated seed

3.3.1. Planting

Three treatments, using KS Big Bow, treated at the commercially recommended rate with imidacloprid and thiamethoxam, plus untreated (control) seed, were used to evaluate the effectiveness of the commercial rate of treated seeds. Commercially treated KS Big Bow seeds with thiamethoxam were obtained from Kansas State University Foundation Seed. Seeds were treated with thiamethoxam insecticide and fungicides at the manufacturer's recommended rate of 0.013 mg ai/seed for thiamethoxam.

To obtain imidacloprid-treated seed at a commercial recommended rate of 2.9 ml ai/5 lbs. wheat seed (2.9 ml ai/2268 g wheat seed), 0.3 ml of imidacloprid was measured in a 250 ml beaker and added to 200 ml of distilled water. The mixture was placed on a plate with a magnetic stirrer and stirred continuously for 30 minutes. The solution obtained was used to treat 220 g of KS Big Bow wheat seeds. The seeds were then placed in closed plastic bags and thoroughly mixed for about 10 minutes. The mixture of seeds and solution was spread out on a tray to dry overnight.

Twenty commercially treated KS Big Bow seeds from each treatment were planted in a soil-filled container (Figure 3.2), with 30 containers allocated to each treatment. Containers were maintained in an environmental chamber at 20°C, with a relative humidity between 60–75% and a photoperiod of 14:10 (light/dark).

Figure 3. 2: Growing container

3.3.2. Plant infestation with Hessian fly

Plants germinated two days after planting, then 10 days after planting (DAP), or eight days after emergence of the seedling, and at the two-leaf stage, six containers (six replicates) were thinned to 10 plants per treatment group to be oviposited on by female HF. The container of flaxseeds was removed from the freezer so adults could develop. Each container was covered with a cylindrical plastic cage with an infestation portal (Figure 3.3). Ten adult Hessian flies, male and female (with a minimum of seven females), were collected and released in each covered container through the infestation portal, which was then closed. The seedlings, plus adult Hessian flies, were placed in an environmental chamber at 20°C, relative humidity between 60–75%, and a photoperiod of 14:10 (light/dark) in a completely randomized pattern (Figure 3.4). Flies were allowed to oviposit on the plants for 48 hours, after which the cages and adults were removed. This process was repeated using 24 seedlings four times after the first infestation, every five days, to obtain a consistent infestation on the 15^{th}, 20^{th}, 25^{th}, and 30^{th} days after planting.

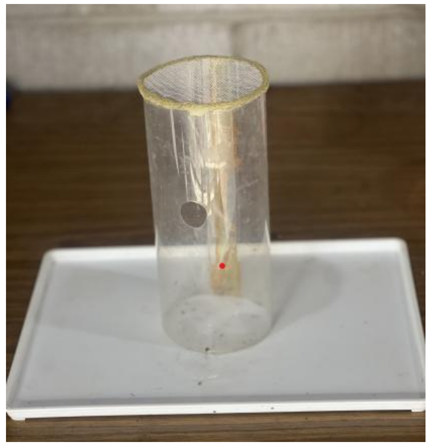


Figure 3. 3: Cylindrical cage

Figure 3. 4: Wheat seedling infestation

3.4. Evaluation of Nano-insecticide seed treatment

3.4.1 Reagents

The imidacloprid insecticide solution was obtained from Mid Kansas Cooperative (MKC), and the carbon nanotubes, multi-walled 10-20 nm (diameter), 5-15 µm (length), were purchased from TCI America.

3.4.2 Synthesis of Nano-insecticide

Carbon nanotubes (Figure 3.5) were selected as the ideal materials for the nano-insecticide formulation due to their nanoscale structural characteristics, including their surface area and stability, which can enhance the efficacy of the formulations and concomitantly reduce environmental runoff. Furthermore, it is hypothesized that nanotubes may potentially result in overcoming resistance mechanisms through enhanced and controlled delivery of insecticide, improved bioavailability, and lower dosage requirements.

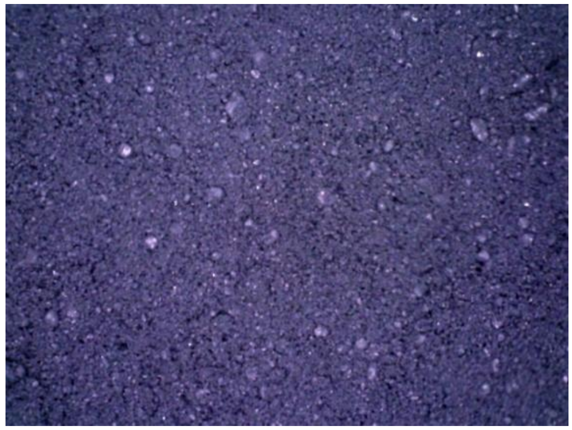


Figure 3. 5: Carbon nanotube powder

Two nano-insecticide formulations were prepared by mixing carbon nanotube powder, water, and a solution of imidacloprid to obtain the commercially recommended rate of 2.9 ml ai/5 lbs. wheat seed (2.9 ml ai/2268 g wheat seed) and a half rate of 1.45 ml ai/5 lbs. (1.45 ml ai/2268 g wheat seed).

To obtain a nano-insecticide at a rate of 2.9 ml ai/5 lbs. wheat seed, 0.3 ml of imidacloprid was measured in a 250 ml beaker. 0.25 g of carbon nanotubes (CNT) were added to the insecticide, and 200 ml of distilled water was added to the mixture of insecticide and CNT. The mixture was placed on a plate with a magnetic stirrer and stirred continuously for 30 minutes to obtain a suspension.

To obtain a nano-insecticide at a rate of 1.45 ml ai/5 lbs. (1.45 ml ai/ 2268g wheat seed), 0.15 ml of imidacloprid was measured in a 250 ml beaker. 0.25 g of CNT added to the insecticide, and 200 ml of distilled water added to the mixture of insecticide and CNT. The mixture was placed on a plate with a magnetic stirrer and stirred continuously for 30 minutes to obtain a suspension.

The CNT treatment was used as experimental or treatment control. To obtain the CNT treatment, 0.25 g of CNT was mixed with 200 mL of distilled water. The mixture was placed on a plate with a magnetic stirrer and stirred continuously for 30 minutes to obtain a suspension.

3.4.3 Characterization of Nano-insecticide

The solutions were characterized by using Dynamic Light Scattering (DLS) spectroscopy and scanning electron microscopy (SEM) to analyze and describe their different properties and understand their effectiveness as a nano-insecticide formulation.

Zetasizer Pro was used to perform the DLS spectroscopy analysis, which provided the particle size distribution (DLS) needed to validate the stability and homogeneity of the nano-formulation.

The SNE-ALPHA Nano Eye SEM was used to characterize the two nano-formulations by dropping the solutions onto a conductive substrate and drying them under controlled conditions. The equipment produced high-resolution photographs of the particles, which revealed their size distribution and surface appearance, which can reveal the behavior of the nano-insecticides, which are important to understand their stability and effectiveness.

3.4.4 Application of Seed Treatment

Three solutions, including 1) a commercially utilized nano-insecticide formulated solution, 2) a half-rate nano-insecticide formulated solution, and 3) a carbon nanotube only solution, were used for the treatment of the KS Big Bow wheat seed.

For each of the three solutions, 220 g of seeds were placed in three closed plastic bags and thoroughly mixed for about 10 minutes. The mixtures of seeds and solution were poured into a tray to dry overnight (Figure 3.6).

Figure 3. 6: Dried wheat seeds treated with nano-insecticide in the tray

3.4.5 Germination test

A germination test was conducted to determine if the seed treatment affected the germination potential of the treated seeds. The germination test was performed in Petri dishes. The bottom of each Petri dish was lined with filter paper. The filter paper was moistened with distilled water (Al-Yahya, 2001). Five treatments were included: 1.) seeds treated with the nano-insecticide commercially used rate formulation of 2.9 ml ai/5 lbs. wheat seed, 2.) seeds treated with a half nano-insecticide rate formulation of 1.45 ml ai/5 lbs. wheat seed, 3.) seeds treated with commercially utilized rate of 2.9 ml ai/5 lbs. wheat seed, 4.) seeds treated with carbon nanotubes and 5.) untreated seeds as a control. Four replicates were utilized for each treatment. A replicate consisted of 20 seeds evenly spaced on filter paper in a Petri dish. Different colors of tape were used to label each treatment set.

To test seed germination, the Petri dishes were covered and placed in an environmental chamber at 20°C, a relative humidity between 60–75%, and a photoperiod of 14:10 (light/dark) using a completely randomized pattern. The humidity in each Petri dish was monitored daily, and distilled water added as needed to keep each dish moist. The number of germinated seeds was recorded each day. After five days, the number of germinated seeds in each Petri dish was recorded to calculate the germination percentage.

The difference between the germination rates of the different treatments was analyzed using a one-way ANOVA. Tukey's post-hoc multiple tests were conducted to determine the significant difference between the mean percentage of germination of the four different treatments. The analysis was conducted using GraphPad Prism version 8.0.1."

3.4.6. Laboratory experiment of nano-insecticide

3.4.6.1 Seed planting

Four treatment groups were considered for the laboratory experiment including: 1.) seeds treated with the nano-insecticide commercially used formulation of 2.9 ml ai/5 lbs. wheat seed, 2.) seeds treated with a half nano-insecticide formulation of 1.45 ml ai/5 lbs. wheat seed, 3.) seeds treated with carbon nanotubes and 4.) untreated seeds, as a control. Twenty seeds were planted in a container filled with soil, with 30 containers per treatment. The containers were maintained in an environmental chamber at 20°C, relative humidity between 60–75%, and a photoperiod of 14:10 (light/dark) utilizing a completely randomized design.

3.4.6.2 Plant infestation with hessian fly

Seedlings started emerging two days after planting. The same infestation procedure used to evaluate the commercially treated seed was used to infest seedlings of the six treatments, starting from ten days after planting (DAP) or eight days after emergence of the seedlings, at the two-leaf stage. Additional infestations were completed on the 15^{th}, 20^{th}, 25^{th}, and 30^{th} days after planting for each of the six treatments, with six replicates made of six containers. Seedlings were reduced to 10 per container before providing them to adult Hessian flies for oviposition. Treatments were placed in an environmental chamber under the conditions of 20°C, 60–75% relative humidity, and a 14:10 (light/dark) photoperiod utilizing a completely randomized design. Flies were allowed to oviposit on the plants for 48 hours, after which cages and adults were removed.

3.4.7 Field experimentation of nano-insecticide

3.4.7.1 Seed planting

Four treatment groups all using KS Big Bow seeds were utilized for the field experiment, including 1.) seeds treated with imidacloprid at the rate of 2.9 ml ai/5 lbs. wheat seed, 2.) seeds treated with a half nano-insecticide rate of 1.45 ml ai/5 lbs. wheat seed, 3.) seeds treated with nano-insecticide commercially utilized rate of 2.9 ml ai/5 lbs. wheat seed as a positive control, and 4.) untreated seeds as a control.

Treatments were planted in Dickinson County, Kansas. The plot's surface was prepared by filling in the soil and leveling the ground. Four plots were prepared for each treatment. The plot surface was irrigated before planting to ensure proper moisture for seed germination. Each plot was marked before the seeds were planted. Most seeds germinated eight days after they were planted.

3.4.7.2 Plant infestation with Hessian fly

Twenty-one days after planting, sufficient seedlings were carefully removed from each plot from each treatment and brought to the laboratory. Seedlings were transplanted into containers for each treatment at the rate of 10 seedlings per container. Six containers representing six replicates were transplanted for each treatment. Containers were placed in an environmental chamber in a completely randomized design. Seedlings were watered and left overnight to acclimate. Conditions in the growing chamber were the same as in the previous experiments. The following day (22 days after planting), seedlings were subjected to adult Hessian flies. Infestations were completed following the same procedure described previously for the laboratory trial.

Twenty-nine days after planting, the same procedure was followed to infest plants on the 30th day.

3.5. Data collection

The same parameters were measured for all experiments to determine the efficacy of seed treatments in controlling HF. The infested seedlings were assessed through destructive sampling 21 days after infestation to check for the presence of HF larvae. The number of wheat seedlings with HF larvae and the number of HF larvae on each seedling were recorded per container. The percentage of seedlings infested per container was determined with the formula:

% infestation = $\left(\frac{Sum\ of\ seedlings\ with\ \inf estation\ in\ a\ container}{Total\ number\ of\ seedlings\ assessed\ in\ the\ container}\right) \times 100$

The mean percentage infestation per treatment was determined by adding the percentage of infestation of the six replicates and dividing by six.

The mean number of larvae collected per seedling, and container was determined considering only the seedlings on which larvae were found, using the formula:

Mean number of larvae/seedlings/containers =

$$\left(\frac{Sum\ of\ the\ number\ of\ in\sec ts\ collected\ on\ \inf ested\ seedlings\ in\ a\ container}{Sum\ of\ seedlings\ assessed\ with\ \inf estation\ in\ the\ container}\right)$$

The mean number of larvae per treatment per day of infestation was determined by adding the mean number of infestations of the six replicates and dividing by six. The data on the percentage of infestation and the number of larvae collected were analyzed using a two-way ANOVA to estimate the main effects of the different treatments. Following the ANOVA test, a post-hoc Tukey’s multiple comparison test was performed to identify significant differences between the different treatment group means."

Chapter IV: Results

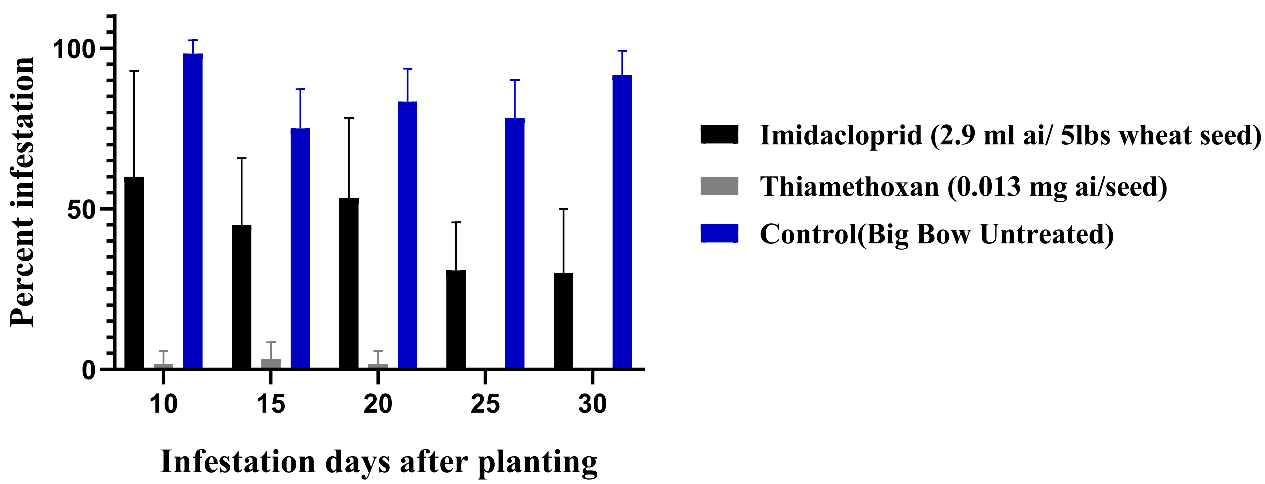
4.1 Laboratory evaluation of conventional seed treatment

Graph 4.1 shows the percentage of infestation 21 days following exposure to HF oviposition. Seedlings were exposed to adult HF 10, 15, 20, 25, and 30 days after planting. They were obtained from untreated and thiamethoxam-treated seeds at 0.013 mg ai/seeds and nano-imidacloprid at 2.9 ml ai/5 lbs. wheat seed. Seeds germinated two days after planting. Ten days after planting, or eight days after germination, the seedlings were at the two-leaf stage. They reached the three-leaf stage after 15 and 20 days. On the 25^{th} and 30^{th} days after planting, most seedlings reached the four-leaf stage. Data were recorded from 60 seedlings per treatment. Seedlings were still at the four-leaf stage when the first two counts were recorded, and the five-leaf stage for the rest of the data collection.

Overall, the thiamethoxam treatment was highly effective in controlling HF larvae. Ten days after planting, the untreated plants exposed to HF adults had 90% of seedlings infested. Few larvae were found on plants with the thiamethoxam seed treatment, with a mean infestation rate of 1.667%. Seedlings treated with imidacloprid showed a relatively higher infestation, with 60% of the seedlings infested. Although there is a numerical difference between the imidacloprid treatment and the control at 10 days after planting, this difference was not statistically significant. There is a clear difference in effectiveness between the treatment of the thiamethoxam and the other two treatments.

Throughout the observation period, and for seedlings infested 15, 20, 25, and 30 days after planting, this trend continued, as the thiamethoxam treatment significantly reduced infestation rates compared to the imidacloprid and the untreated control.

The ANOVA, followed by Tukey's multiple comparisons post hoc tests presented in Table 4.1 below, showed a significant difference ($P < 0.05$) between the thiamethoxam treatments and the untreated for all days of infestation with adult HF after planting. Seeds treated with thiamethoxam and resulting plants infested 25 and 30 days after planting had no HF larvae. The results suggest that thiamethoxam, a neonicotinoid insecticide, is still effective for the control of HF larvae for up to 30 days after planting. The imidacloprid treatment was not as effective as the thiamethoxam in controlling the HF infestation but showed effectiveness compared to the control, with a statistically significant difference on the 15th, 25th, and 30th days after planting. The control showed a high percentage of infestation from 10 DAP to 30 DAP, which confirmed the existence of sustained pest pressure in the absence of seed treatment.



Graph 4. 1: Percent seedling infestation per day after infestation

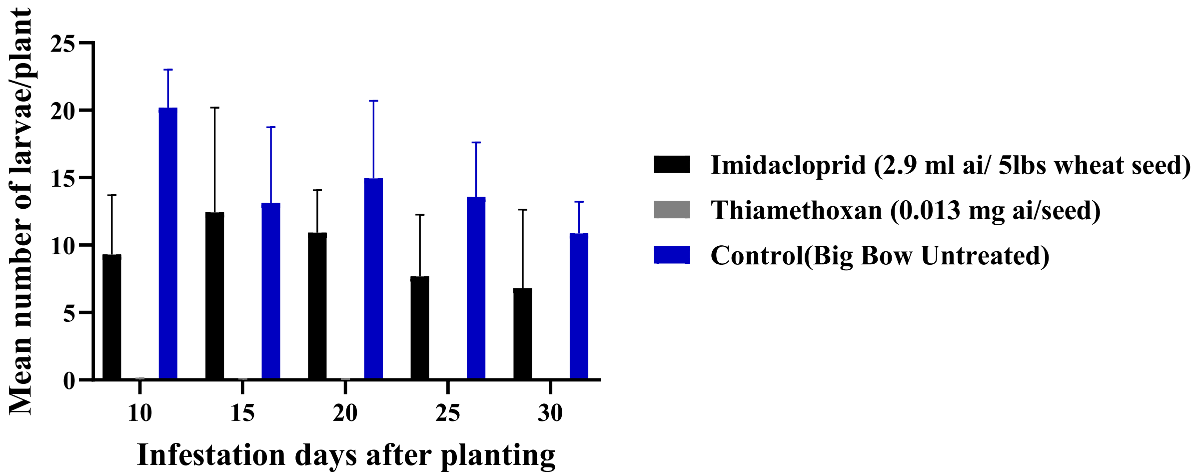
Table 4.1: Mean percent of larvae infestation per treatment

	Treatments		
DAP	Imidacloprid (2.9 ml ai/5 lbs. wheat seed)	Thiamethoxan (0.013 mg ai/seed)	Control (Big Bow Untreated)
10 days	60.00 a	1.667 b	98.33 a
15 days	45.00 a	3.333 b	75.00 c
20 days	53.33 a	1.667 b	83.33 a
25 days	30.83 a	0.000 b	78.33 c
30 days	30.00 a	0.000 b	91.67 c

Means followed by a different letter in the same row are significantly different, while means with the same letter are not significantly different. ($P < 0.05$).

Graph 4.2 presents the mean number of HF larvae recorded per treatment on the indicated days after planting. Few larvae were found on the thiamethoxam at 0.013 mg ai/seed treatment. Thiamethoxam consistently maintained larval counts near zero across all periods of infestation, indicating its ability to provide effective and prolonged protection against HF infestation.

There is a significant difference between larval counts on the thiamethoxan treatment vs. the other two treatments ($P < 0.05$) (Table 4.2). Significantly more larvae survived on the untreated seedlings. Although the number of larvae in the seedlings treated with imidacloprid was lower than the number of larvae in the control, their presence was significant, particularly in the early stages, and declined with time. The thiamethoxam seed treatment offered consistent protection against Hessian fly larvae. The imidacloprid seed treatment was moderately effective, especially during the initial infestation stage. The untreated was consistently highly infested, highlighting the significance of preventative chemical seed treatment in areas susceptible to HF pressure.



Graph 4. 2 : Mean number of larvae per seedling per treatment and days of infestation

Table 4. 2: Mean number of larvae per seedling for each treatment

	Treatments		
DAP	Imidacloprid (2.9 ml ai/5 lbs. wheat seed)	Thiamethoxan (0.013 mg ai/seed)	Control (Big Bow Untreated)
10 days	9.292 a	0.03333 b	20.20 c
15 days	12.42 a	0.03333 b	13.13 a
20 days	10.92 a	0.01667 b	14.94 a
25 days	7.667 a	0.000 b	13.56 c
30 days	6.792 a	0.000 b	10.87 a

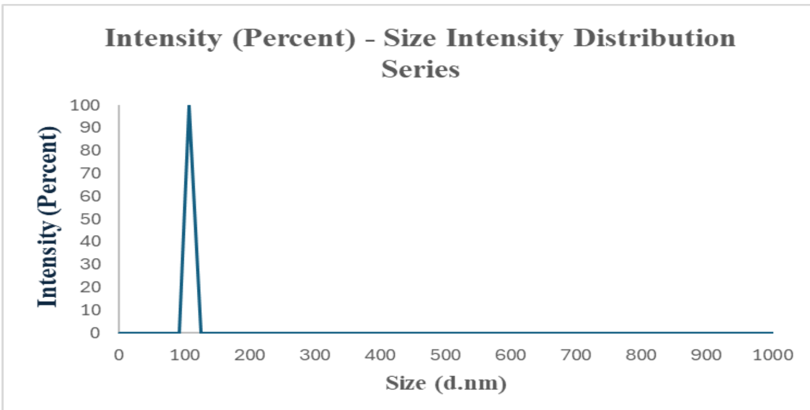
Means followed by a different letter in the same row are significantly different, while means with the same letter are not significantly different. ($P < 0.05$).

4.2 Evaluation of nano-insecticide seed treatment

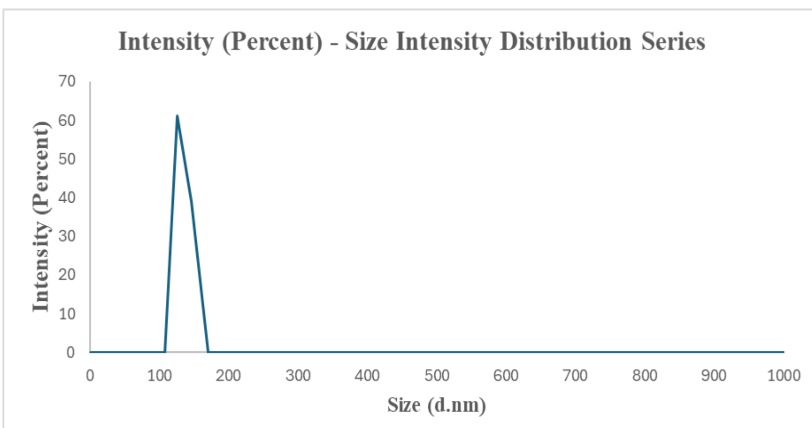
4.2.1 Characterization of nano-insecticide

4.2.1.1 DLS spectroscopy result

Graph 4.3 represents the result of the Dynamic Light Scattering (DLS) analysis of the nano imidacloprid (1.45 ml ai/5 lbs. wheat seed). The result shows the size intensity distribution of particles present in the solution. It indicates the intensity of one peak around 100 nm. This reveals that most particles within the nano-imidacloprid formulation have a hydrodynamic size of 100 nm. The one peak observed suggests that the nano-imidacloprid solution analyzed has a narrow size distribution and uniform particle size. It confirms the formation of nanoparticles with a size close to 100 nanometers. This result both validates the successful formation of nanoparticles and indicates a uniform formulation, which is important for reliable effectiveness in the intended pesticidal application.



Graph 4. 3: Size intensity distribution series of the nano-imidacloprid (1.45 ml ai/5 lbs. wheat seed) formulation

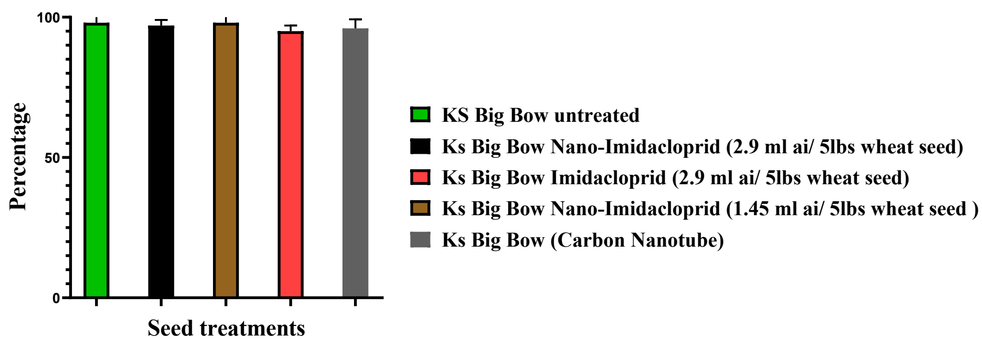


Graph 4. 4: Size intensity distribution series of the nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed formulation

Graph 4.4 represents the result of the Dynamic Light Scattering (DLS) analysis of the nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed). The result shows the size intensity distribution of particles in the solution (Goldburg,1999). The size intensity distribution peaks at approximately 150 nm, with a peak intensity of less than 70%. This reveals that most particles within the nano-imidacloprid formulation have a hydrodynamic size of 150 nm.

4.2.2 Seed germination test

Graph 4.5 presents the mean percent germination of the four treatments. It shows high germination and no statistical difference between treatments.



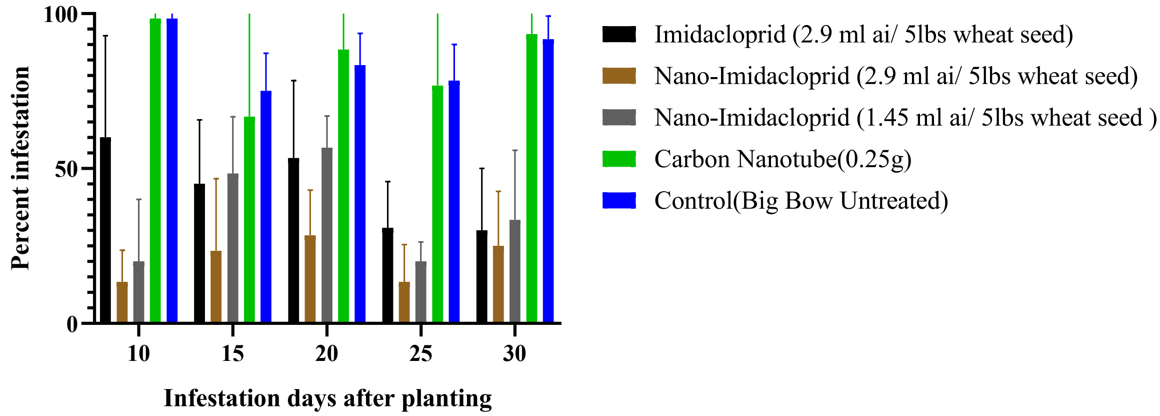
Graph 4. 5: Germination test results

Table 4. 3: Mean germination percent of seed germination per treatment

	Treatments				
	Nano-Imidacloprid (2.9 ml ai/5 lbs wheat seed)	Nano-Imidacloprid (1.45 ml ai/5 lbs wheat seed)	Imidacloprid (2.9 ml ai/5 lbs wheat seed)	Untreated (Control)	Carbon Nanotube
% of germination	97 a	98 a	95 a	98 a	96 a

Means followed by a different letter in the same row are significantly different, and means with the same letter are not significantly different. ($P < 0.05$).

4.2.3 Laboratory evaluation of nano-insecticide



Graph 4. 6: Percent infestation of seedlings per treatment

Table 4. 4: Mean percent infestation per treatment

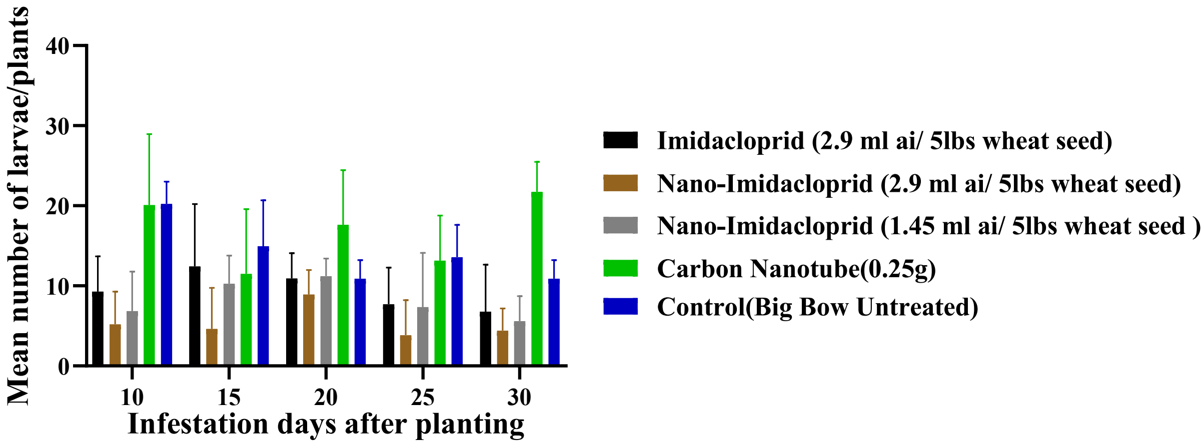
	Treatments				
DAP	Imidacloprid (2.9 ml ai/5 lbs. wheat seed)	Nano-Imidacloprid (2.9 ml ai/5 lbs. wheat seed)	Nano-Imidacloprid (1.45 ml ai/5 lbs. wheat seed)	Carbon Nanotube (0.25g)	Control (Big Bow Untreated)
10 days	60.00 ab	13.33 b	20.00 b	98.33 a	98.33 a
15 days	45.00 ab	23.33 b	48.33 ab	66.67 ab	75.00 a
20 days	53.33 abc	28.33 c	56.67 b	88.33 a	83.33 a
25 days	30.83 bc	13.33 b	20.00 b	76.67 ac	78.33 a
30 days	30.00 a	25.00 a	33.33 a	93.33 b	91.67 b

Means followed by a different letter in the same row are significantly different, and means with the same letter are not significantly different. ($P < 0.05$)

Graph 4.6 presents the results comparing the effect of conventional imidacloprid seed treatment vs two different rates of nano-imidacloprid seed treatments in controlling HF infestations on wheat seedlings vs. two control treatments, including seeds treated with carbon nanotubes and untreated. Results showed the percentage of infestation of seedlings (60 for each treatment) by counting larvae in wheat seedlings five times after planting. As described for the commercial seed treatment experiment, the seedlings were at the same leaf stage described previously when exposed to HF adults and when the data were collected.

The graph shows different larval infestation levels of the seedlings over time, with the nano-imidacloprid at the full dosage (2.9 ml ai/5 lbs. wheat seed) demonstrating an enhanced level of effectiveness among all the treatments. For the seedlings infested at 10 DAP, the nano-imidacloprid at 2.9 ml ai/5 lbs. wheat seed treatments had a comparatively low infestation rate of 13.33%. In comparison, the conventional imidacloprid treatment at 2.9 ml ai/5 lbs. wheat seed resulted in an infestation rate of 60%, while the carbon nanotube and control treatments led to significantly higher infestations at 98.33%.

There is an increase in larval infestation, with a peak in seedling infestation exposed to flies on the 20th day after planting for the nano-imidacloprid treatment, and a decrease in larval infestation for plants provided with flies on the 25^{th} and 30^{th} days after planting. The nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed) with the highest concentration of imidacloprid showed the lowest larval infestations throughout the experiment. This suggests that the nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed) effectively controls HF larvae. It is confirmed to have a significant difference ($P<0.05$) in the level of infestation compared to the carbon nanotube and the control treatment. The conventional imidacloprid treatment with the same level of active ingredient as the nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed), a higher level of active ingredient than the nano-imidacloprid (1.45 ml ai/5 lbs. wheat seed), exhibited a moderately higher percentage of larval infestation throughout the experiment. The nano-imidacloprid (1.45 ml ai/5 lbs. wheat seed) caused reduced infestation compared to conventional imidacloprid. This suggests that the nano-insecticide formulation with a lower concentration of active ingredients may provide better control of HF larvae than the conventional insecticide with a higher concentration of active ingredients, however further research is needed.



Graph 4. 7: Mean number of larvae per seedling, per treatment, and days of infestation

Table 4. 5: Mean number of larvae per plant per treatment

	Treatments				
DAP	Imidacloprid (2.9 ml ai/5 lbs. wheat seed)	Nano-Imidacloprid (2.9 ml ai/5 lbs. wheat seed)	Nano-Imidacloprid (1.45 ml ai/5 lbs. wheat seed)	Carbon Nanotube (0.25g)	Control (Big Bow Untreated)
10 days	9.292 bc	5.167 c	6.850 cb	20.07 ab	20.20 a
15 days	12.42 a	4.625 a	10.24 a	11.47 a	14.94 a
20 days	10.92 a	8.925 a	11.17 a	17.62 a	10.87 a
25 days	7.667 ab	3.805 b	7.360 ab	13.15 ab	13.56 a
30 days	6.792 ac	4.372 c	5.570 ac	21.70 b	10.87 a

Means followed by a different letter in the same row are significantly different, and means with the same letter are not significantly different. ($P < 0.05$).

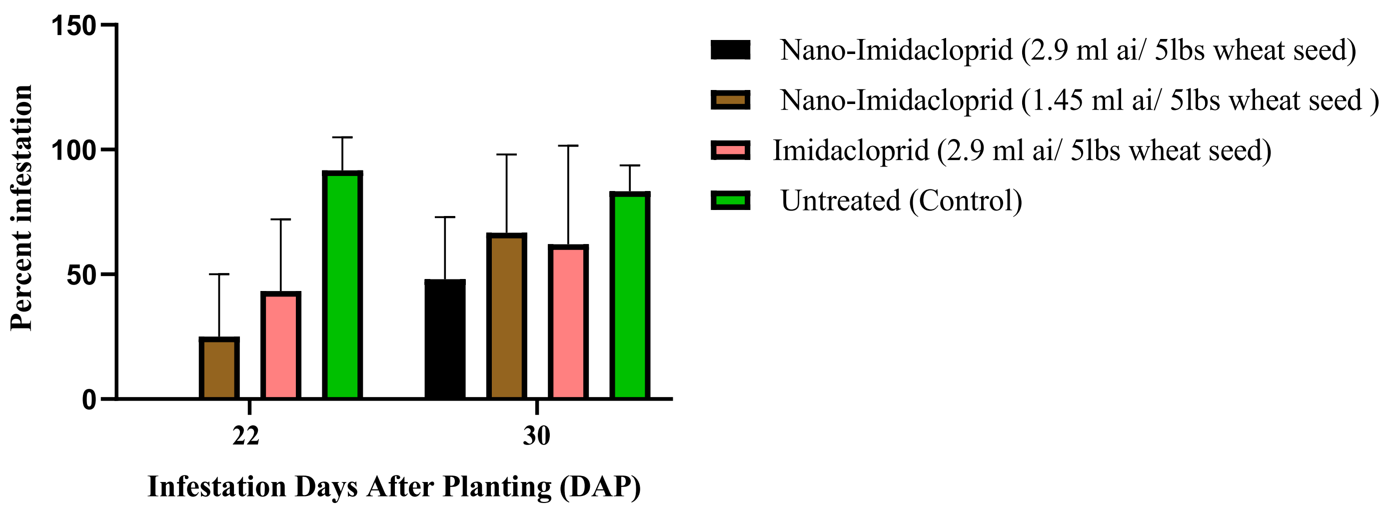
Graph 4.7 presents the mean number of HF larvae on seedlings for the different treatments over time. It reveals some differences in the mean number of HF larvae on the seedlings of the different treatments. As the case for the percentage of infestation, the number of larvae peaked for seedlings infested by adult HF 20 days after planting for the two nano-imidacloprid formulations, followed by a decrease in the number of larvae on seedlings infested 25 and 30 days after planting. The full-dose nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed) treatment had 5.17 larvae per plant at 10 DAP, while the standard imidacloprid (2.9 ml ai/5 lbs. wheat seed) treatment averaged 9.29 larvae per plant, and the carbon nanotube and control treatments had levels exceeding 20 larvae. The nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed) continuously caused the lowest number of HF larvae per seedling for all infestations, indicating that it might provide the most effective control.

The nano-imidacloprid (1.45 ml ai/5 lbs. wheat seed) exhibited intermediate efficacy, with the mean number of larvae consistently higher than the mean number on the nano-imidacloprid (2.9 ml ai/5 lbs. wheat seed) treatment but less than the mean number on conventional imidacloprid treatments. This implies that the nano-imidacloprid (1.45 ml ai/5 lbs. wheat seed) treatment offers some protection, but its efficacy is lower than the nano-insecticide treatment with a higher concentration of imidacloprid. Conventional imidacloprid consistently had the most larvae per seedling among the treatments, except for the seedlings infested twenty days after planting, thus seeming to be the least effective.

The ANOVA, followed by the post-hoc Tukey multiple comparison tests, indicated no significant difference among the different insecticide treatments at 15 and 20 DAP (Table 4.5). This indicated that all treatments achieved some control of HF larvae. Even though there were not always statistically significant differences in the mean number of larvae among all treatments, the trend observed implies that the nano-imidacloprid formulation, especially at a higher rate, consistently decreased the level of larval infestation more than the conventional imidacloprid at the same rate.

4.2.4 Evaluation of nano-insecticide from field grown plants.

Graph 4.8 presents the mean percent of HF larvae on seedlings grown in the field, and Graph 4.9 presents the mean number of larvae recorded from the seedlings. Data were collected on all 60 seedlings representing the six replicates for each treatment per day of infestation. The nano-imidacloprid formulation with a higher insecticide concentration completely suppressed the development of HF larvae 22 days after planting. There was a significant difference ($P<0.05$) between the percent infestation (0%) of the nano-imidacloprid formulation with a higher concentration of insecticide and the percent infestation of other treatments (Table 4.6). The nano-imidacloprid formulation with a lower concentration showed a mean larval infestation of 25%, which is lower than the mean percent infestation of seedlings obtained from high-rate treated seeds with conventional imidacloprid. Seedlings from untreated seeds exhibited the highest larval infestation (91.67%) among all treatments at 22 days after planting.



Graph 4. 8: Percent infestation of seedlings per day of infestation and per treatment

Table 4. 6: Mean percent of plants infested per treatment

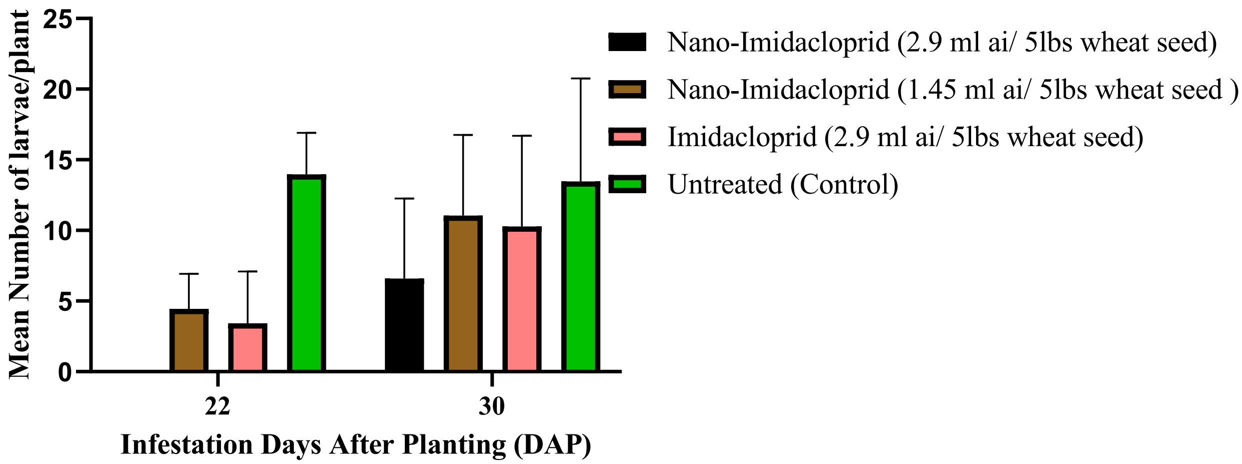
	Treatments			
DAP	Nano-Imidacloprid (2.9 ml ai/ 5lbs wheat seed)	Nano-Imidacloprid (1.45 ml ai/ 5lbs wheat seed)	Imidacloprid (2.9 ml ai/ 5lbs wheat seed)	Untreated (Control)
22 days	0.000 a	25.00 ab	43.33 b	91.67 c
30 days	48.00 a	66.67 a	62.00 a	83.33 a

Means followed by a different letter in the same row are significantly different and means with the same letter are not significantly different. ($P < 0.05$).

Larval infestations increased for all treatments for seedlings infested by HF eggs 30 days after planting. However, the nano-imidacloprid treatment at 2.9 ml ai/5 lbs. wheat seeds had fewer larvae compared to the other treatments, with 48% infested tiller. The conventional imidacloprid and the nano-imidacloprid at the lower concentration had similar levels of infestation. Untreated seedlings were the most infested at 83.33 %. Thus, it seems that the nano-imidacloprid formulations provided significant control of HF larvae initially, but their effectiveness declined over time.

The nano-imidacloprid formulation with a higher insecticide concentration caused fewer larvae per seedling for seedlings provided with flies 22 days after planting. The nano-imidacloprid with a lower concentration and the conventional imidacloprid treatments showed similar larval counts (4.453 and 3.417, respectively). At the same time, seedlings from untreated seeds had the highest larva count (13.98 insects per seedling). There were no statistically significant differences in the mean number of larvae among the different treatments (Table 4.7). Thirty days after planting, the number of larvae per seedling provided with flies increased for all treatments; however, the nano-imidacloprid with the highest concentration still had the lowest number of larvae.

Overall, the nano-imidacloprid formulations seemed more effective than the conventional imidacloprid treatments in controlling HF larvae. The increase in percent infestation and the mean number of larvae implies that the treatment's effectiveness, however, decreases over time.



Graph 4. 9: Mean number of larvae per seedling per treatment and days of infestation

Table 4. 7: Mean number of larvae per plant for each treatment

	Treatments			
DAP	Nano-Imidacloprid (2.9 ml ai/ 5lbs wheat seed)	Nano-Imidacloprid (1.45 ml ai/ 5lbs wheat seed)	Imidacloprid (2.9 ml ai/ 5lbs wheat seed)	Untreated (Control)
22 days	0.000 a	4.453 a	3.417 a	13.98 c
30 days	6.600 a	11.05 a	10.23 a	13.48 a

Means followed by a different letter in the same row are significantly different and means with the same letter are not significantly different. ($P < 0.05$).

Chapter V: Discussion

5.1. Evaluation of conventional seed treatment

The results of Graphs 4.1 and 4.2 suggest that the commercially treated thiamethoxam seed treatment effectively reduced the level of HF infestation in wheat seedlings. It consistently and effectively maintained a low infestation rate, with percent infestation of seedlings remaining close to 0% and larval survival remaining at insignificant levels up to 30 days after planting (DAP). The mean percent infestation of wheat seedlings from seeds treated with thiamethoxam had a maximum average percent infestation of 3.333% obtained for seedlings exposed to HF adults fifteen days after planting. The conventional imidacloprid applied to seed in the laboratory at the rate of 2.9 ml ai/5 lbs. showed an intermediate or moderate level of control of HF. The HF infestation rate was between 30% to 60% throughout the evaluation, and larval densities were comparably higher than those of thiamethoxam treatment. There was a statistically significant difference in the infestation level between the thiamethoxam and the imidacloprid treatments. Both neonicotinoid insecticides perform better than the untreated seedlings. Results from untreated seedlings, in contrast to those from treated seedlings, showed a maximum infestation of 91.67% when infested 25 days after planting. Throughout the study, treated seedlings maintained consistently lower infestation levels, even up to an advanced stage, with zero infestations for plants exposed to adults 30 days after planting, especially for the thiamethoxam. Thiamethoxam-treated seeds may offer wheat producers an effective and timely option for controlling HF infestations, given their potential to protect young wheat seedlings during their early stages and reduce the subsequent occurrence of future infestations, particularly in areas with a known history of significant HF pressure. As only a negligible number of treated plants were infested, the infested plants had a correspondingly low average number of larvae (0.03333 larvae per plant). In contrast, untreated plants had an average of up to 11.65 larvae. The statistical significance of these differences was confirmed by the results of the ANOVA test, which revealed a highly significant difference ($P < 0.05$) for all dates the plants were infested. These results demonstrate a significant mortality effect of these neonicotinoid insecticides on developing HF larvae in treated wheat seedlings.

Neonicotinoids, including thiamethoxam and imidacloprid, are a class of systemic insecticides absorbed by plants, which protect all parts of the plant (Kundoo et al., 2018). They are extensively utilized as seed treatments and are among the most widely applied insecticides globally (Kundoo et al., 2018) and are considered the most efficient class of agrochemicals for the management of sucking insect plant pests (Elbert et al., 2008). The consistently lower infestation of the treated seedlings can be explained by the bioavailability of the insecticide within the plant. This systemic activity provides a mechanism for the absorption of insecticide by the growing seedling, which then results in its translocation throughout the plant. Hessian fly larvae feed on wheat by inserting their mandibles into the plant cell wall and injecting salivary secretions into the plant cells, creating nutritive tissues that allow them to feed by sucking from the plants (Harris et al., 2006; Hatchett et al., 1990). The presence of insecticides could impede the HF larvae's ability to establish a feeding site, as neonicotinoids are known to be active against a wide variety of early season sucking insect pests that feed on the aerial parts of several crops (Elbert et al. 1998), which may result in their mortality. The neonicotinoid compounds, such as imidacloprid, demonstrate notable xylem mobility, which renders them highly effective for seed treatment applications (Elbert et al. 1998).

These results suggest that neonicotinoids are still effective in affecting HF infestation, but with different levels of control depending on the insecticides and are best used for the control of HF’s during the first 30 days after planting to reduce HF damage, especially for winter wheat prior to dormancy. Hessian fly has not yet developed resistance against thiamethoxam, which is a second-generation neonicotinoid (Seifert, 2005). Imidacloprid, which was less effective against the HF than thiamethoxam, which represents the first generation and the first commercially successful of the neonicotinoids, was produced a decade before thiamethoxam (Seifert, 2005). They have been used for several years longer than thiamethoxam; there could be some development of resistance, which should be tested with further studies. The difference in the effectiveness of the different neonicotinoid insecticides can be due to different water solubilities of the insecticides, their degradation rates, or insecticide–soil interactions (Ding et al. 2018). The consistent efficacy of thiamethoxam can also be explained by its optimal uptake and systemic distribution within plants, facilitated by root uptake, and its capacity to be further transformed into clothianidin, another neonicotinoid chemical entity that may facilitate its sustained or protracted activity within both plants and insects as previously reported by Nauen et al., (2003).

It was observed that the level of HF control achieved using thiamethoxam was consistently higher than that achieved using imidacloprid. However, compared with the untreated, imidacloprid demonstrated control.

The present observations are consistent with earlier research indicating that neonicotinoid seed treatments offer effective protection against the initial stages of infestation of wheat plants by HF (Wilde et al., 2001; Whitworth et al., 2009; Howell et al., 2017; Winn et al., 2023; Buntin, 2025). These studies have demonstrated that neonicotinoid insecticides can significantly reduce HF infestations. As posited by Butin (2025), neonicotinoid insecticide seed treatments at high doses constitute a practical approach for controlling HF in susceptible winter wheat varieties. He also demonstrated that the efficacy of thiamethoxam was comparable to that of imidacloprid and clothianidin used at equivalent rates. This result demonstrates the potential efficacy of neonicotinoid insecticides in controlling the proliferation of HF populations in the field during the initial stages of planting. Seed treated with this insecticide can be a valuable component of an integrated pest management strategy for enhancing plant stands, which ultimately impacts yield.

However, it is important to note that neonicotinoids demonstrate limited effectiveness when applied to control infestations that occur up to 30 days after the initial emergence of wheat (Wilde et al., 2001; Whitworth et al., 2009; Howell et al., 2017; Winn et al., 2023). Furthermore, there are potential environmental concerns associated with the application of a higher rate of neonicotinoid insecticide, including contamination of soil and water, harmful effects on pollinators, and aquatic organisms (Hladik et al., 2018). It is therefore essential to implement targeted efforts to reduce neonicotinoid use in field crops. A comprehensive approach that incorporates reduced use of neonicotinoid insecticides, along with additional measures to reduce potential risks, is important to ensure economic sustainability for producers and environmental safety. This strategy should minimize the likelihood of pest resistance, outbreaks of non-target pests, and environmental contamination (Douglas & Tooker, 2015).

In general, the results provide substantial evidence in support of the utilization of commercial thiamethoxam-treated seeds as a reliable management tool against HF during earlier stages of plant growth. Additional research is necessary to investigate whether alternative formulations, including nanoformulations, might offer similar effectiveness as thiamethoxam or improved efficacy with the imidacloprid while reducing potential environmental risks.

5.2 Germination test

The results of the germination test indicate that the application of insecticide and carbon nanotube treatment to wheat seeds had no significant impact on germination. The absence of a statistically significant difference in the data suggests that the treatments, while potentially influential in other areas such as growth rate, do not impede the initial germination process. Consistent with these findings, Talib et al. (2021) reported that carbon nanotube treatments at concentrations ranging from 25 to 100 parts per million (ppm) did not affect the germination percentage of wheat. Consequently, the application of these treatments can be conducted with a reasonable degree of confidence that they will not compromise the viability of the seeds.

Additionally, given the potential implications regarding the validation of nanoformulations as agronomically safe alternatives to conventional formulations, the safety of nanoformulations must be thoroughly validated.

5.3 Laboratory evaluation of nano-insecticide

Neonicotinoids are grouped according to their application in resistance management programs, due to the prevailing scientific consensus that they all act at the same target site, namely the nicotinic acetylcholine receptor (nAChR) (Nauen et al. 2003). They exhibit a high degree of binding affinity to [^{3}H] imidacloprid binding sites on insect acetylcholine receptors (nAChRs), except for thiamethoxam, which has been demonstrated to exhibit a relatively low affinity for the [^{3}H] imidacloprid binding site (Wiesner& Kayser, 2000; Nauen et al., 2003). Nonetheless, the relative biological efficacy of thiamethoxam is analogous to that of other neonicotinoid insecticides in most cases (Nauen et al., 2003). As previously demonstrated, the conventional thiamethoxam outperformed imidacloprid to control most HF infestations. Since thiamethoxam was highly effective, it seemed more prudent to test a nanoformulation of imidacloprid, which is considered the most prominent member of the class of neonicotinoid insecticides (Nauen et al., 2003), to evaluate if it would improve its efficacy and show some promising effect in reducing the quantity of active ingredient needed to maintain efficacy and have extended efficacy.

As presented in Graphs 4.6 and 4.7, the higher rate nano-imidacloprid treatment proved to be the most effective among all treatments in controlling the level of infestation of HF larvae on wheat seedlings. The carbon nano-imidacloprid treatment applied at a rate of 2.9 ml ai/5 lbs. wheat seed resulted in consistent effectiveness compared to the other treatment at all points of infestation after planting. This was evident through the observation of the lowest percent of seedlings infested and the lowest mean number of larvae recorded per seedling throughout the experiment. The effectiveness of nano-formulated insecticides at higher rates may be attributed to their multiple intrinsic properties, which may include efficient ''adsorption, reduced volatilization, improved tissue permeation, controlled release" (Kannan et al. 2023).

Similarly, the nano-imidacloprid treatment with a lower insecticide rate of 1.45 ml ai/ 5lbs wheat seed also caused a reduced infestation level compared to the conventional imidacloprid with a higher rate of insecticide. This effectiveness might also be attributed to the intrinsic properties of nano-formulated insecticides. However, it was less effective compared to the nano-imidacloprid formulation with a higher rate. This can be explained by the finding that neonicotinoids exhibit increasing efficacy in controlling insect pests with increasing application rates, as demonstrated by Yu et al. (2023).

However, as previously observed, the conventional insecticide treatment showed enhanced efficacy compared to the control and the carbon nanotube treatment, which exhibited a significantly higher infestation, resulting in a higher number of larvae present on infested seedlings. These results indicate the potential for enhanced efficacy of carbon nano-insecticide formulations compared to conventional insecticides (Kah et al., 2018) and the improvement of the insecticidal effectiveness of nano-insecticide formulations even when the active ingredient levels are reduced, facilitated by the controlled release, targeted delivery, enhanced bioavailability, and improved stability of the active ingredients (Kah et al., 2013; Kah et al., 2018; Gupta et al., 2021). These results align with previous research indicating that carbon nano pesticides can significantly enhance the biological availability of chemicals, and their precision in their delivery system, thereby improving pest management outcomes (Sarlak et al., 2014; Wang et al., 2017; Al-Esawy et al., 2021; Ali et al., 2023).

Neonicotinoids are capable of translocating across all plant tissues, irrespective of the method of treatment and the pathway of entry into the plant, thereby resulting in toxicity to any insects that feed on the plant (Simon-Delso et al., 2015). It provides a protective barrier against direct damage from insects, particularly those that feed on sap. (Simon-Delso et al., 2015). According to Lahiani et al. (2013), carbon nanotubes can penetrate the seed coat of plants like corn, barley, and soybean. A study conducted on the impact of CNT on corn, barley, and soybean by the application of multi-walled carbon nanotubes (MWCNTs) has revealed the presence of the MWCNTs inside the seeds and later in the root of the plant, based on Raman spectroscopy and TEM imaging (Lahiani et al., 2013). Carbon nanotubes (CNTs) have both a high adsorption capacity and reactivity (Ogunsola et al., 2024).

According to these findings, both the carbon nanotube and the neonicotinoid insecticide can translocate into plant tissue. Thus, carbon nanotube-based insecticides, with the insecticide compound adsorbed on the surface of the CNTs, may improve efficacy by translocating through the roots, enabling better systemic delivery of the active compound throughout plant tissues. This might enhance the protective barrier that systemic insecticides can create, ensuring the availability of a relatively higher concentration of the insecticide at the feeding site of the insect and increasing toxicity to insects like the Hessian fly compared to conventional insecticide formulations. It is clear from the results that CNTs alone cannot create a protective barrier against the feeding of HF larvae, as CNT-treated seedlings were heavily infested. The improved effectiveness of the carbon with nanotubes imidacloprid might be due to the combined action of the insecticide and CNTs.

Further analysis of the infestation patterns indicated a notable increase in the percent infestation at approximately 20 days after planting (DAP), followed by a subsequent decrease at 25 and 30 DAP. This finding was observed consistently among the various treatments, suggesting a possible alteration in plant susceptibility or a decrease in insecticide activity, over time.

The analysis of variance, followed by Tukey's multiple comparison tests, showed significant differences among the imidacloprid treatment (including both nano-formulated and conventional treatments) and the non-imidacloprid treatment (CNT and untreated). The present experiment corroborates the earlier finding that seeds treated with neonicotinoids can reduce the level of infestation of HF in wheat (Wilde et al., 2001; Butin et al., 2025). However, it should be noted that the level of control of infestation obtained with this test was comparatively lower than the level obtained with commercially treated seed. This could also result from the difference in the methods of application between the commercially treated seed and the laboratory-treated treated.

From a practical standpoint, integrating commercially treated seeds at a high rate into an IPM program may not adhere to the objective of minimizing insecticide usage. In general, the number of larvae recorded from the half rate of 1.45 ml ai/5 lbs. nano-imidacloprid treatment was less than the number recorded from the conventional higher rate imidacloprid treatment, but there is no statistical difference between the two treatments. This indicates that the reduced nano-imidacloprid formulation showed the same level of control as the high-rate conventional imidacloprid treatment. Consequently, this observation may suggest significant implications for environmental safety and cost-effectiveness. Additionally, these findings suggest that the utilization of nano-imidacloprid in integrated pest management (IPM) could be a promising approach, as it has the potential to enhance the efficacy of insecticide treatment while potentially reducing the amount of active ingredient required. nano-formulated insecticides can contribute to mitigating environmental contamination and potentially reducing non-target effects, a critical concern in neonicotinoid applications (He et al., 2023).

Although the present study has shown the potential effect of nano-imidacloprid in reducing HF larval infestation, additional research is critical to assess the prolonged residual activity and controlled release in plant tissues, as it has been confirmed that conventional seed treatment is only effective during the 30 days following planting. It is also important to assess the possible benefit of functionalized carbon nanotubes in improving the insecticide encapsulation and stability for an improved result. Additionally, further study should investigate the commercial application of the nano-imidacloprid formulation, as we observed less effectiveness for laboratory-treated seeds. Moreover, additional studies should investigate the molecular mechanisms of nano-insecticide uptake and translocation in wheat seedlings to optimize their formulation and application strategies.

5.4 Evaluation of nano-insecticide from plants grown in the field

A parallel can be drawn between the laboratory experiment using commercial and nano-neonicotinoid insecticides and the field evaluation of nano-imidacloprid seed treatment. The field evaluation revealed similar effectiveness in reducing HF larval infestation, with results aligned with those observed in the laboratory. Results obtained from the experiment conducted on seedlings primarily grown in the field and later transplanted and exposed to adult HF in the laboratory have confirmed that the nano-imidacloprid at a higher concentration effectively controls the level of HF larvae infestation, showing a statistically significant reduction in percent infestation compared to both conventional imidacloprid treatments and untreated control. These results provide additional evidence to support the hypothesis that the nano-imidacloprid formulation may exhibit superior pest management effectiveness. The improved efficiency of the nano-imidacloprid formulations was demonstrated by the total suppression of HF larvae 22 days after planting, or 14 days after seedling emergence, with 0 % plants infested. The enhanced effectiveness of the nano-imidacloprid treatment may be explained by some advantages of nanoformulations over conventional pesticides, which include increased adsorption, reduced volatilization, enhanced tissue permeation, and controlled release (Kannan et al. 2023). In a natural environment, conventional imidacloprid, after its application, can undergo degradation through the action of microorganisms or photolysis, a process that involves the breakdown of the insecticide's chemicals by the action of sunlight (Pang et al. 2020). Thus, nanoformulations might protect insecticides from rapid environmental degradation.

The nano-imidacloprid formulation at a higher rate outperformed the nano-imidacloprid formulation with half the commercially recommended rate. This confirms the finding of Yu et al. (2023), who found that the efficacy of neonicotinoid insecticides, including imidacloprid and thiamethoxam, increases with increasing application rate. More insecticide may be absorbed and translocated into the plant with an increasing application rate (Yu et al. 2023). This results in a higher residue level present in the plant with the higher application rate (Yu et al. 2023), providing enhanced protection against pests like HF.

However, field results revealed a more evident time-dependent decline in the effective protection conferred by the nano-imidacloprid treatment and conventional imidacloprid, resulting in increased larval infestation at 30 days post-planting. Despite this, the nano-imidacloprid at the higher concentration maintained greater effectiveness among all treatments, remaining more effective than conventional imidacloprid. The results also revealed a consistent performance of the nano-imidacloprid treatment for both laboratory and field experiments, indicating its potential to improve the bioavailability and systemic delivery of the CNTs and imidacloprid within plant tissues. The nano-imidacloprid at the low-rate infestation was comparable to conventional imidacloprid at the high rate. This finding is consistent with the hypothesis that environmental factors, including temperature fluctuations, UV radiation, and microbial degradation present in the field, may indeed reduce treatment persistence; nevertheless, nano-formulations continued to demonstrate a relative advantage. Results of this study are consistent with the findings of previous studies, which suggest that carbon nanotube-based insecticides can promote insecticidal delivery and longevity through the mechanisms of increased adsorption and penetration into plant tissues, amplifying the efficacy of insecticide application(Sarlak et al., 2014; Wang et al., 2017; Kah et al., 2018; Al-Esawy et al., 2021; Gupta et al., 2021; Ali et al., 2023).

Neonicotinoid seed treatments are known to be effective on wheat seedlings for about 30 days after the plants' emergence (Wilde et al., 2001; Whitworth et al., 2009; Howell et al., 2017; Winn et al., 2023). The enhanced efficacy of the nano-imidacloprid-based insecticide in controlling HF larvae, compared to conventional and non-imidacloprid treatments, up to 30 days post-planting, suggests the potential for nano-imidacloprid formulation to extend the period of its efficacy compared to conventional insecticides. Additional research is necessary to enhance the stability and controlled release characteristics of nano-imidacloprid within plant tissues.

All carbon nanotubes have a notable drawback: their insolubility in any solvent. It is only possible to synthesize suspensions of the nanotubes (Kuzmany et al., 2004). One solution to this challenge is the functionalization and solubilization of CNTs (Dubey et al., 2021). Functionalization can contribute to a significant expansion in the range of applications of CNTs (Dubey et al., 2021). The CNTs' surface functionalization process involves the modification of the material's surface chemistry to achieve specific properties, which can enable the molecular attachment process (Speranza, 2019). The oxidation treatments result in the introduction of carboxyl and carbonyl functional groups onto the surface of MWCNTs, a process that substantially increases their chemical reactivity. These functional groups added onto the surface of carbon nanotubes enhance bonding interactions with other molecules, including active ingredients contained within pesticides, by facilitating the formation of covalent bonds or robust electrostatic interactions. Consequently, this enhanced reactivity should contribute to an improvement in the stability, dispersibility, and delivery efficiency of MWCNTs in nanoformulations by avoiding aggregation (Mohl et al. 2008).

Future studies should focus on evaluating the extended activity of nano-imidacloprid under field conditions. These studies should focus on methods to extend the effectiveness of nano-imidacloprid formulation beyond 30 days post-germination, through winter, and up to spring. Furthermore, additional molecular-level research is necessary to understand the uptake and translocation of nano-imidacloprid in wheat seedlings. Conducting this research could provide valuable knowledge that could be used to improve the systemic action and formulation strategies of other insecticides, herbicides, fungicides, and all pesticides in general.

In summary, the findings of this study support the promising potential of nano-insecticide formulations for sustainable pest management. Notably, laboratory and field results indicate that carbon nano-imidacloprid has consistently demonstrated enhanced efficacy compared to conventional formulations. To determine the viability of this technology for commercial applications and its incorporation into IPM programs, further refinement and validation under a variety of field conditions are required.

Conclusion

Results of this study demonstrated that the neonicotinoid insecticide remains effective when utilized as a wheat seed treatment, particularly during the initial 30 days following planting. Specifically, the second-generation neonicotinoid thiamethoxam demonstrated significant efficacy in managing the HF. Although the first-generation neonicotinoid, imidacloprid, exhibited reduced efficacy compared to thiamethoxam, it nevertheless demonstrated a substantial improvement in performance over the control group. The carbon nano-imidacloprid formulation, particularly at a high rate, exhibited enhanced efficacy in managing HF infestation when compared to the conventional commercial seed treatment at a high rate. The findings of the study demonstrated that the application of carbon nano-imidacloprid at a reduced dosage exhibited a comparable level of efficacy to the conventional treatment. However, the carbon nanotube alone treatment did not demonstrate any control over the HF infestation. Furthermore, the level of infestation observed in the carbon nanotube-treated seeds was comparable to that observed in the untreated seeds. The germination test revealed that none of the treatments harmed the reproductive potential or the viability of the treated seeds. In consideration of the results obtained, it can be inferred that thiamethoxam can be applied as a seed treatment to control the early infestation of HF in winter wheat production before the plant undergoes dormancy. The carbon nanotube formulation presented notable advantages over the conventional formulation. In addition, it can be used to reduce the active ingredient required to maintain the same efficacy of a conventional insecticide. While the potential benefits of carbon nanotubes for the environment are promising, further research is necessary to fully assess their environmental impact. Additional experiments must be carried out, particularly through the application of functionalization techniques, to achieve a homogeneous formulation that would facilitate commercial utilization.

A notable limitation of the study was the duration of the treatment, which was only tested for up to 30 days. It was observed that there was a decline in the effectiveness of the treatment over time. Further research is necessary to utilize the nanoformulation to extend the effectiveness of both thiamethoxan and imidacloprid beyond 30 days and up to the spring season, using the extended release of the nanoformulation. The laboratory-treated seeds exhibited a lower level of effectiveness in comparison to the commercially treated seeds. To ensure that the observed difference was not attributable to the methods of treatment, it would be necessary to test the commercially treated formulation of the nanoformulation. It is essential to conduct additional field tests, both during the winter season and to assess the economic viability of employing nanoformulations. This assessment would involve a cost-effective analysis, which is a necessary step for determining the commercial scalability of the nanoformulations.

References

Ahmadpour, R., Dastjerdi, H. R., Golizadeh, A., Yangjeh, A. H., Abedi, Z., & Mahdavi, V. (2024). Nanoformulation of imidacloprid insecticide with biocompatible materials and its ecological and physiological effects on wheat green aphid, Schizaphis graminum Rondani. *Journal of Asia-Pacific Entomology*, *27*(4), 102332.

Al-Esawy, M. T., Alshukri, B. M., & Kahdum, B. J. (2021). Carbon nanotubes as control agents against the Khapra beetle, Trogoderma granarium Everts (Coleoptera: Dermestidae). *Revista Brasileira de Ciências Agrárias*, *16*(4), 1-9.

Al-Yahya, S. A. (2001). Effect of storage conditions on germination in wheat. *Journal of Agronomy and Crop Science*, *186*(4), 273-279.

Ali, A., Jawad, S., & Mohammed, A. (2023). Carbon Nanotubes (CNTS) and Frankincense Nanoparticles as Promising Insecticides to Control Onion Thrips. *Tekirdağ Ziraat Fakültesi Dergisi*, *20*(4), 773-783.

Alomari, D. Z., Schierenbeck, M., Alqudah, A. M., Alqahtani, M. D., Wagner, S., Rolletschek, H., & Röder, M. S. (2023). Wheat grains as a sustainable source of protein for health. *Nutrients*, *15*(20), 4398.

An, C., Sun, C., Li, N., Huang, B., Jiang, J., Shen, Y., & Wang, Y. (2022). Nanomaterials and nanotechnology for the delivery of agrochemicals: strategies towards sustainable agriculture. *Journal of Nanobiotechnology*, *20*(1), 11.

Berzonsky, W. A., Ding, H., Haley, S. D., Harris, M. O., Lamb, R. J., McKenzie, R. I. H., & Shanower, T. G. (2002). Breeding wheat for resistance to insects. *Plant Breed Rev*, *22*, 221-296.

Brahmi, H., Lazraq, A., Boulamtat, R., El Fakhouri, K., Bassi, F. M., & El Bouhssini, M. (2021). Effect of temperature on the expression of resistance to Hessian fly (Diptera: Cecidomyiidae) in durum wheat cultivars. *Phytoparasitica*, *49*(3), 357-362.

Buntin, G. D., and J. W. Chapin. 1990. Biology of Hessian fly (Diptera: Cecidomyiidae) in the Southeastern United-States: geographic-variation and temperature-dependent phenology. *J. Econ. Entomol. 83: 1015–1024.*

Buntin, G. D., Ott, S. L., & Johnson, J. W. (1992). Integration of plant resistance, insecticides, and planting date for management of the Hessian fly (Diptera: Cecidomyiidae) in winter wheat. *Journal of Economic Entomology*, *85*(2), 530-538.

Buntin, G. D. (1999). Hessian fly (Diptera: Cecidomyiidae) injury and loss of winter wheat grain yield and quality. *Journal of Economic Entomology*, *92*(5), 1190-1197.

Buntin, G. D. (2025). Hessian Fly (Diptera: Cecidomyiidae) Management Using Seed Treatments in Winter Wheat1. *Journal of Entomological Science*, *60*(1), 129-140.

Bussy, C., Ali-Boucetta, H., & Kostarelos, K. (2013). Safety considerations for graphene: lessons learnt from carbon nanotubes. *Accounts of chemical research*, *46*(3), 692-701.

Cañas, J. E., Long, M., Nations, S., Vadan, R., Dai, L., Luo, M., ... & Olszyk, D. (2008). Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. *Environmental Toxicology and chemistry*, *27*(9), 1922-1931.

Chen, M. S., Liu, X., Wang, H., & El-Bouhssini, M. (2009). Hessian fly (Diptera: Cecidomyiidae) interactions with barley, rice, and wheat seedlings. *Journal of economic entomology*, *102*(4), 1663-1672.

Chen, M. S., Wheeler, S., Wang, H., & Whitworth, R.J. (2014). Impact of temperatures on Hessian fly (Diptera: Cecidomyiidae) resistance in selected wheat cultivars (Poales: Poaceae) in the Great Plains region. *Journal of Economic Entomology*, *107*(3), 1266-1273.

Chen, M. S., Ghori, N., Bai, G. H., & Liu, X. M. (2023). The Hessian fly: a destructive pest of wheat and barley.

Chung, H., Son, Y., Yoon, T. K., Kim, S., & Kim, W. (2011). The effect of multi-walled carbon nanotubes on soil microbial activity. *Ecotoxicology and Environmental Safety*, *74*(4), 569-575.

Davis, H. N., Whitworth, R. J., & Sloderbeck, P. E. (2010). Crop Insects in Kansas. Kansas State University. (Revised January 2023).

Ding, J., Li, H., Zhang, Z., Lin, J., Liu, F., & Mu, W. (2018). Thiamethoxam, clothianidin, and imidacloprid seed treatments effectively control thrips on corn under field conditions. *Journal of Insect Science*, *18*(6), 19.

Douglas, M. R., & Tooker, J. F. (2015). Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environmental science & technology*, *49*(8), 5088-5097.

Drake, C. J., & Decker, G. C. (1932). Late fall activity and spring emergence of the Hessian fly in Iowa. *Annals of the Entomological Society of America*, *25*(2), 345-349.

Dubey, R., Dutta, D., Sarkar, A., & Chattopadhyay, P. (2021). Functionalized carbon nanotubes: Synthesis, properties and applications in water purification, drug delivery, and material and biomedical sciences. *Nanoscale Advances*, *3*(20), 5722-5744.

Edde, P. A. (2022). Arthropod Pests of Small Grains: Wheat (Triticum Aestivum L.) and Barley (Hordeum Vulgare L.). *Field Crop Arthropod Pests of Economic Importance; Elsevier: London, UK*, 536-611.

Elbert, A., Haas, M., Springer, B., Thielert, W., & Nauen, R. (2008). Applied aspects of neonicotinoid uses in crop protection. Pest Management Science: formerly Pesticide Science, 64(11), 1099-1105.

Elbert, A., Nauen, R., & Leicht, W. (1998). Imidacloprid, a novel chloronicotinyl insecticide: biological activity and agricultural importance. In *Insecticides with novel modes of action: mechanisms and application* (pp. 50-73). Berlin, Heidelberg: Springer Berlin Heidelberg.

Flanders, K. L., Reisig, D. D., Buntin, G. D., Winslow, M., Herbert Jr, D. A., & Johnson, D. W. (2013). Biology and management of Hessian fly in the Southeast.

Foster, J. E., & Taylor, P. L. (1975). Thermal-unit requirements for development of the Hessian fly under controlled environments. *Environmental entomology*, *4*(2), 195-202.

Freixa, A., Acuña, V., Sanchís, J., Farré, M., Barceló, D., & Sabater, S. (2018). Ecotoxicological effects of carbon based nanomaterials in aquatic organisms. *Science of the total environment*, *619*, 328-337.

Fritz, R. S., Stamp, N. E., & Halverson, T. G. (1982). Iteroparity and semelparity in insects. *The American Naturalist*, *120*(2), 264-268.

Gagné, R. J., & Hatchett, J. H. (1989). Instars of the Hessian fly (Diptera: Cecidomyiidae). *Annals of the Entomological Society of America*, *82*(1), 73-79.

Gondal, A. H., & Tayyiba, L. (2022). Prospects of using nanotechnology in agricultural growth, environment and industrial food products. *Reviews in Agricultural Science*, *10*, 68-81.

Goldburg, W. I. (1999). Dynamic light scattering. *American Journal of Physics*, *67*(12), 1152-1160.

Grover, P. B. (1995). Hypersensitive response of wheat to the Hessian fly. *Entomologia experimentalis et applicata*, *74*(3), 283-294.

Güllü, M., Gözüaçık, C., & Konuksal, A. (2024). Wheat Insect Pests. In *Advances in Wheat Breeding: Towards Climate Resilience and Nutrient Security* (pp. 391-451). Singapore: Springer Nature Singapore.

Gupta, A., Sahu, P. K., & Tiwari, R. K. (2021). Nanotechnology in Insect Pest Management. *Molecular Approaches for Sustainable Insect Pest Management*, 377-394.

Hamilton, E. W. (1966). Hessian fly larval strain responses to simulated weather conditions in the greenhouse and laboratory. *Journal of Economic Entomology*, *59*(3), 535-538.

Harris, M. O., & Rose, S. (1989). Temporal changes in the egglaying behaviour of the Hessian fly. *Entomologia experimentalis et applicata*, *53*(1), 17-29.

Harris, M. O., Rose, S., & Malsch, P. (1993). The role of vision in the host plant-finding behaviour of the Hessian fly. *Physiological Entomology*, *18*(1), 31-42.

Harris, M. O., Sandanayaka, M., & Griffin, W. (2001). Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. *Ecological Entomology*, *26*(5), 473-486.

Harris, M. O., Stuart, J. J., Mohan, M., Nair, S., Lamb, R. J., & Rohfritsch, O. (2003). Grasses and gall midges: plant defense and insect adaptation. *Annual Review of Entomology*, *48*(1), 549-577.

Harris, M. O., Freeman, T. P., Rohfritsch, O., Anderson, K. G., Payne, S. A., & Moore, J. A. (2006). Virulent Hessian fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. *Annals of the Entomological Society of America*, *99*(2), 305-316.

Hatchett, J. H., Starks, K. J., & Webster, J. A. (1987). Insect and mite pests of wheat. *Wheat and wheat improvement*, *13*, 625-675.

Hatchett, J. H., Kreitner, G. L., & Elzinga, R. J. (1990). Larval mouthparts and feeding mechanism of the Hessian fly (Diptera: Cecidomyiidae). *Annals of the Entomological Society of America*, *83*(6), 1137-1147.

He, J., Li, J., Gao, Y., He, X., & Hao, G. (2023). Nano-based smart formulations: A potential solution to the hazardous effects of pesticide on the environment. *Journal of Hazardous Materials*, *456*, 131599.

Helman, D., & Bonfil, D. J. (2022). Six decades of warming and drought in the world's top wheat-producing countries offset the benefits of rising CO2 to yield. *Scientific Reports*, *12*(1), 7921.

Hill, C. C., & Smith, H. D. (1925). The relation of Hessian fly damage to yield. *Journal of Economic Entomology*, *18*(1), 69-73.

Hladik, M. L., Main, A. R., & Goulson, D. (2018). Environmental risks and challenges associated with neonicotinoid insecticides.

Howell, F. C., Reisig, D. D., Burrack, H. J., & Heiniger, R. (2017). Impact of imidacloprid treated seed and foliar insecticide on Hessian fly abundances in wheat (Triticum aestivum L.). *Crop Protection*, *98*, 46-55.

Iijima, S. (1991). Helical microtubules of graphitic carbon. *nature*, *354*(6348), 56-58.

Jakubus, A., Paszkiewicz, M., & Stepnowski, P. (2017). Carbon nanotubes application in the extraction techniques of pesticides: a review. *Critical Reviews in Analytical Chemistry*, *47*(1), 76-91.

Kah, M., Beulke, S., Tiede, K., & Hofmann, T. (2013). Nanopesticides: state of knowledge, environmental fate, and exposure modeling. *Critical Reviews in Environmental Science and Technology*, *43*(16), 1823-1867.

Kah, M., Kookana, R. S., Gogos, A., & Bucheli, T. D. (2018). A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nature nanotechnology*, *13*(8), 677-684.

Kannan, M., Mohan, M., Elango, K., Govindaraju, K., & Mani, M. (2022). Principles and application of nanotechnology in pest management. *Trends in horticultural entomology*, 49-79.

Kannan, M., Bojan, N., Swaminathan, J., Zicarelli, G., Hemalatha, D., Zhang, Y., ... & Faggio, C. (2023). Nanopesticides in agricultural pest management and their environmental risks: a review. *International Journal of Environmental Science and Technology*, *20*(9), 10507-10532.

Kanno, H., & Harris, M. O. (2000). Physical features of grass leaves influence the placement of eggs within the plant by the Hessian fly. *Entomologia experimentalis et applicata*, *96*(1), 69-80.

Keller, A. A., McFerran, S., Lazareva, A., & Suh, S. (2013). Global life cycle releases of engineered nanomaterials. *Journal of nanoparticle research*, *15*, 1-17.

Kroto, H. W., Heath, J. R., O'Brien, S. C., Curl, R. F., & Smalley, R. E. (1985). C60: Buckminsterfullerene. *nature*, *318*(6042), 162-163.

Kundoo, A. A., Dar, S. A., Mushtaq, M., Bashir, Z., Dar, M. S., Gul, S., Ali, M. T., & Gulzar, S. (2018). Role of neonicotinoids in insect pest management: A review. *Journal of Entomology and Zoology Studies, 6*(1), 333–339.

Kuzmany, H., Kukovecz, A., Simon, F., Holzweber, M., Kramberger, C., & Pichler, T. (2004). Functionalization of carbon nanotubes. *Synthetic Metals*, *141*(1-2), 113-122.

Lahiani, M. H., Dervishi, E., Chen, J., Nima, Z., Gaume, A., Biris, A. S., & Khodakovskaya, M. V. (2013). Impact of carbon nanotube exposure to seeds of valuable crops. *ACS applied materials & interfaces*, *5*(16), 7965-7973.

Lin, D., & Xing, B. (2007). Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environmental pollution*, *150*(2), 243-250.

Malik, S., Muhammad, K., & Waheed, Y. (2023). Nanotechnology: a revolution in modern industry. *Molecules*, *28*(2), 661.

Margulis-Goshen, K., & Magdassi, S. (2013). Nanotechnology: an advanced approach to the development of potent insecticides. *Advanced technologies for managing insect pests*, 295-314.

Martins, C. H., de Sousa, M., Fonseca, L. C., Martinez, D. S. T., & Alves, O. L. (2019). Biological effects of oxidized carbon nanomaterials (1D versus 2D) on Spodoptera frugiperda: Material dimensionality influences on the insect development, performance and nutritional physiology. *Chemosphere*, *215*, 766-774.

Mohl, M., Kónya, Z., Kukovecz, Á., & Kiricsi, I. (2008). Functionalization of multi-walled carbon nanotubes (MWCNTS). In *Functionalized nanoscale materials, devices and systems* (pp. 365-368). Springer Netherlands.

Nair, R., Mohamed, M. S., Gao, W., Maekawa, T., Yoshida, Y., Ajayan, P. M., & Kumar, D. S. (2012). Effect of carbon nanomaterials on the germination and growth of rice plants. *Journal of nanoscience and nanotechnology*, *12*(3), 2212-2220.

Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V. L., & Kaussmann, M. (2003). Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pesticide biochemistry and physiology*, *76*(2), 55-69.

Obembe, O. S., Hendricks, N. P., & Tack, J. (2021). Decreased wheat production in the USA from climate change driven by yield losses rather than crop abandonment. *Plos one*, *16*(6), e0252067.

Ogunsola, S. S., Oladipo, M. E., Oladoye, P. O., & Kadhom, M. (2024). Carbon nanotubes for sustainable environmental remediation: A critical and comprehensive review. *Nano-Structures & Nano-Objects*, *37*, 101099.

Okey-Onyesolu, C. F., Hassanisaadi, M., Bilal, M., Barani, M., Rahdar, A., Iqbal, J., & Kyzas, G. Z. (2021). Nanomaterials as nanofertilizers and nanopesticides: an overview. *ChemistrySelect*, *6*(33), 8645-8663.

Pang, S., Lin, Z., Zhang, Y., Zhang, W., Alansary, N., Mishra, S., ... & Chen, S. (2020). Insights into the toxicity and degradation mechanisms of imidacloprid via physicochemical and microbial approaches. *Toxics*, *8*(3), 65.

Paulsen, G. M. (1998). Hard White Winter Wheat for Kansas (1998). *Kansas Agricultural Experiment Station Research Reports*, (12), 57.

Pauly, P. J. (2002). Fighting the Hessian fly: American and British responses to insect invasion, 1776–1789. *Environmental History*, *7*(3), 485-507.

Popov, V. N. (2004). Carbon nanotubes: properties and application. *Materials Science and Engineering: R: Reports*, *43*(3), 61-102.

Ratanatham, S., & Gallun, R. L. (1986). Resistance to Hessian fly (Diptera: Cecidomyiidae) in wheat as affected by temperature and larval density. Environmental entomology, 15(2), 305-310.

Reay-Jones, F. P. F., & Buntin, G. D. (2024). Management strategies for Hessian fly in wheat. Land-Grant Press by Clemson Extension. Clemson University Cooperative Extension. http://lgpress.clemson.edu/publication/management-strategies-for-hessian-fly-in-wheat

Reddy, S. S., & Chhabra, V. (2022, May). Nanotechnology: Its scope in agriculture. In Journal of Physics: Conference Series (Vol. 2267, No. 1, p. 012112). IOP Publishing.

Ribeiro, B., Botelho, E. C., Costa, M. L., & Bandeira, C. F. (2017). Carbon nanotube buckypaper reinforced polymer composites: a review. *Polímeros*, *27*, 247-255.

Royer, T. A., Edwards, J., & Giles, K. L. (2015). Hessian fly management in Oklahoma winter wheat.

Rudakiya, D., Patel, Y., Chhaya, U., & Gupte, A. (2019). Carbon nanotubes in agriculture: production, potential, and prospects. *Nanotechnology for Agriculture: Advances for Sustainable Agriculture*, 121-130.

Sabry, A. K. H., Salem, H. A. N., & Metwally, H. M. (2021). Development of imidacloprid and indoxacarb formulations to nanoformulations and their efficacy against Spodoptera littoralis (Boisd). *Bulletin of the National Research Centre*, *45*, 1-7.

Safdar, M., Kim, W., Park, S., Gwon, Y., Kim, Y. O., & Kim, J. (2022). Engineering plants with carbon nanotubes: a sustainable agriculture approach. *Journal of Nanobiotechnology*, *20*(1), 275.

Sarkar, S., Kundu, A., Chakraborty, R., & Mukhopadhyay, A. (2021). A review on nanocomposites and their role in insecticide delivery. *Journal of Entomology and Zoology Studies*, *9*(1), 1985-1988.

Sarlak, N., Taherifar, A., & Salehi, F. (2014). Synthesis of nanopesticides by encapsulating pesticide nanoparticles using functionalized carbon nanotubes and application of new nanocomposite for plant disease treatment. *Journal of agricultural and food chemistry*, *62*(21), 4833-4838.

Schlogl, A. E., de Sousa, M. S., de Oliveira, A. M., Santos, I. J. B., & de Oliveira Junior, E. N. (2024). Carbon nanotubes fertilizers: properties and applications. In Nanofertilizer Synthesis (pp. 233-246). Elsevier.

Schmid, R. B., Knutson, A., Giles, K. L., & McCornack, B. P. (2018). Hessian fly (Diptera: Cecidomyiidae) biology and management in wheat. *Journal of Integrated Pest Management*, *9*(1), 14.

Schwarting, H. N. (2014). *Examining Hessian fly (Mayetiola destructor) management concepts and quantifying the physiological impact of Hessian fly feeding on post-vernalization selected cultivars of winter wheat in Kansas*. Kansas State University.

Seifert, J. (2005). Neonicotinoids. In P. Wexler (Ed.), *Encyclopedia of Toxicology* (2nd ed., pp. 196–200). Elsevier. https://doi.org/10.1016/B0-12-369400-0/00675-X.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., ... & Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research*, *22*, 5-34.)

Singh, P., Kumari, K., Vishvakarma, V. K., Aggarwal, S., Chandra, R., & Yadav, A. (2018). Nanotechnology and its impact on insects in agriculture. *Trends in insect molecular biology and biotechnology*, 353-378.

Speranza, G. (2019). The role of functionalization in the applications of carbon materials: An overview. *C*, *5*(4), 84.

Stuart, J. J., Chen, M. S., Shukle, R., & Harris, M. O. (2012). Gall midges (Hessian flies) as plant pathogens. *Annual review of phytopathology*, *50*(1), 339-357.

Subramanyam, S., Nemacheck, J. A., Suetsugu, T. E., Flynn, R. D., & Faik, A. (2025). Genetic and phenotypic responses of temperature-independent Hessian fly-resistant durum wheat to larval attack during heat stress. *BMC Plant Biology*, *25*(1), 210.

Talib Al-Esawy, M., Mohsen Alshukri, B., & Jawad Kahdum, B. (2021). Carbon nanotubes as control agents against the Khapra beetle, Trogoderma granarium Everts (Coleoptera: Dermestidae). *Brazilian Journal of Agricultural Sciences/Revista Brasileira de Ciências Agrárias*, *16*(4).

U.S. Department of Agriculture, Economic Research Service. (2025, January 27). *Wheat*. https://www.ers.usda.gov/topics/crops/wheat

Velasco-Santos, C., Martınez-Hernández, A. L., Consultchi, A., Rodrıguez, R., & Castaño, V. M. (2003). Naturally produced carbon nanotubes. *Chemical Physics Letters*, *373*(3-4), 272-276.

Vivekanandhan, P., Kannan, S., Pittarate, S., & Krutmuang, P. (2024). Nanoinsecticide: An Introduction. In *Nano-Insecticide: Today and Future Perspectives* (pp. 1-23). Cham: Springer Nature Switzerland.

Wang, X., Zhou, Z., & Chen, F. (2017). Surface modification of carbon nanotubes with an enhanced antifungal activity for the control of plant fungal pathogen. *Materials*, *10*(12), 1375.

Whitworth, R. J., Sloderbeck, P. E., Davis, H., & Cramer, G. (2009). *Hessian fly*. Kansas: Kansas State University, Agricultural Experiment Station and Cooperative Extension Service.

Wiesner, P., & Kayser, H. (2000). Characterization of nicotinic acetylcholine receptors from the insects Aphis craccivora, Myzus persicae, and Locusta migratoria by radioligand binding assays: relation to thiamethoxam action. *Journal of biochemical and molecular toxicology*, *14*(4), 221-230.

Wilde, G. E., Whitworth, R. J., Claassen, M., & Shufran, R. A. (2001). Seed treatment for control of wheat insects and its effect on yield. *Journal of Agricultural and Urban Entomology*, *18*(1), 1-11.

Winn, Z. J., Reisig, D., & Murphy, J. P. (2023). Yield protection afforded by imidacloprid during Hessian fly infestation in six genotypes. *Agronomy Journal*, *115*(3), 1059-1068.

Withers, T. M., Madie, C., & Harris, M. O. (1997). The influence of clutch size on survival and reproductive potential of Hessian fly. *Entomologia experimentalis et applicata*, *83*(2), 205-212.

Yu, X. R., Tariq, T., Guo, L. H., Wu, S. Y., Tang, L. D., & Zang, L. S. (2023). Assessing the effectiveness of imidacloprid and thiamethoxam via root irrigation against Megalurothrips usitatus (Thysanoptera: Thripidae) and its residual effects on cowpea. *Journal of Economic Entomology*, *116*(5), 1767-1775.

Zaytseva, O., & Neumann, G. (2018). Penetration and accumulation of carbon-based nanoparticles in plants. *Phytotoxicity of Nanoparticles*, 103-118.

Zhang, L., Zhao, H., Wan, N., Bai, G., Kirkham, M. B., Nielsen-Gammon, J. W., ... & Lin, X. (2024). An unprecedented fall drought drives Dust Bowl–like losses associated with La Niña events in US wheat production. *Science Advances*, *10*(31), eado6864.

Zhu, L., Yuan, J., O'Neal, J., Brown, D., & Chen, M. S. (2020). Analyzing molecular basis of heat-induced loss-of-wheat resistance to hessian fly (Diptera: cecidomyiidae) infestation using RNA-sequencing. *Journal of Economic Entomology*, *113*(3), 1504-1512.