UNIVERSITY FOR DEVELOPMENT STUDIES

FACULTY OF AGRICULTURE, FOOD AND CONSUMER SCIENCES

DEPARTMENT OF HORTICULTURE

EFFECT OF BIOCHAR AMENDMENT RATES AND BIO-NEMATICIDE ON GROWTH, YIELD AND ROOT-KNOT NEMATODES (*Meloidogyne* spp.) INFESTATION OF TOMATO (*Solanum lycopersicum* L.)

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(UDS/MHT/0006/19)

MASTER OF PHILOSOPHY DEGREE IN HORTICULTURE

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BY

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THESIS SUBMITTED TO THE DEPARTMENT OF HORTICULTURE, FACULTY OF AGRICULTURE, FOOD AND CONSUMER SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN HORTICULTURE

OCTOBER, 2022

DECLARATION

I, Agyemang Christopher, hereby declare that, this thesis is the result of my own effort and that it has neither in whole nor in parts been submitted anywhere for the award of degree. All works that serve as source of references have been duly cited and acknowledged in the list of references.

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ABSTRACT

Pot and field experiments were conducted at the Nyankpala Campus of University for Development Studies from January 2020 to March 2022. The objective was to test the efficacy of peptide-based nematicide (Nemanol) and its concentration on root-knot nematodes and growth and yield of tomato in biochar amended soil. In experiment 1, the treatments which were applied in a completely randomised design (CRD) with three replications were; Soil with no biochar and no nematicide (T1), Soil with 5 ml of peptide with no biochar (T2), Soil with 1% biochar and 5 ml peptide (T3), Soil with 3% biochar and 5 ml peptide (T4) and Soil with 5 % biochar and 5 ml peptide (T5). In experiment 2, the treatments which were applied in a randomized complete block design (RCBD) with three replications were; Soil with no biochar and no nematicide (T1), Soil with 20 ml of peptide but no biochar (T2), Soil with 20 ml of peptide with biochar 2 t/ha (T3), Soil with 20 ml peptide with biochar at 4 t/ha (T4) and Soil with 20 ml peptide with biochar 6 t/ha (T5). In experiment 3, the effect of different rates of Nemanol (0 ml, 15 ml, 20 ml and 25 ml) on RKN was assessed with tomato as the test crop. The results of experiment 1 and 2 showed that, biochar applied at low rates (1%, 3%, 2t/ha) significantly enhanced the effectiveness of the Nemanol against RKN and promoted the growth, development and yield of tomato. Results of the experiment 3 showed that the application of 20 ml of Nemanol was the best since it was able to control the root-knot nematodes and promoted the growth of the tomato plants. These experiments have demonstrated that lower rates of biochar enhanced the effectiveness of Nemanol against RKN and promoted the performance of tomato. The application of 20 ml of the Nemanol per plant was also more effective than the other rates. Therefore, it is recommended that the Nemanol be tested on farm for validation of the results presented in this thesis.

DEDICATION

I dedicate this work to my mother, Mrs. Boamah Grace.

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I am most grateful to Lord God Almighty for His loving kindness and in making this work a success. I am also very grateful to my supervisors, Dr. Mohammed Mujitaba Dawuda and Prof. Abdul-Halim Abubakari, for the guidance given me throughout the period of the project work. I wish to thank Telluris Biotech India and their staff, particularly, Mr. Venu Polineni, Glenda W. Polack, and Alejandro Calderón-Urrea, for providing the nematicide and funds for the research work. I also wish to express my sincere gratitude to Rev. Dr. Benjamin Ahiabor, Dr. Kwabena Darkwa, Dr. M.O. Opoku Agyemang, Elder Daniel and Elder Charles Banie, for the love and support given me. Many thanks to the technicians at the Spanish laboratory, UDS; Nematology laboratory, KNUST; and those at CSIR-SARI, Nyankpala, for assisting me with the laboratory works. Finally, to my beloved family members including Obeng Nelson, Ohene Solomon, Appiah Desmon, Obeng Rose and Abigail Mensah I am most grateful for the love, support and encouragement they gave me.

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LIST OF ACRONYMS

BAK1…… Brassinosteroid Receptor-Associated Kinase1

BC…… Black carbon

BIK1……. Botrytis-Induced Kinase1

CEC……. Cation Exchange Capacity

CEC……. Cation-Exchange Capacity

CRD……. Completely Randomised Design

ET……. Ethylene

ETI…… Effector-Triggered Immunity

EU……... European Union

FAO…… Food and Agriculture organization

GNTPF…… Ghana National Tomato Producers' Federation

IUPAC…….. International Union of Pure and Applied Chemistry

NADPH……. Nicotinamide Adenine Dinucleotide Phosphate

NDPC…… National Development Planning Commission

NGO……. Non-Governmental Organization

OM……... Organic Matter

PEPR1…….. Perception of the Arabidopsis Danger Signal Peptide

PTI…… Pattern-Triggered Immunity

PPNs……. Plant Parasitic Nematodes

RBOHD…… Respiratory Burst Oxidase Protein D

RKN……. Root-Knot Nematode

SOM……. Soil Organic Matter

TSWV…….. Tomato Spotted Wilt Virus

USA……..... United States of America

WPTC…….. World Processing Tomato Council

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Root-knot nematodes (*Meloidogyne* spp.) are widespread over the world, inhabiting damp conditions and infecting a variety of crops. Huang et al. (2016) reported that, an estimation of about $80 billion in agricultural losses annually is caused by Plant Parasitic Nematodes (PPNs). Among the sedentary endoparasites, root-knot nematodes are found to be one of the most devastating agricultural pests, affecting relatively an extensive array of crops, including agricultural, silvicultural, and horticultural production, thereby resulting in substantial crop losses (Gisbert et al., 2013). Horna et al. (2006) also reported that, the root-knot nematode is a major challenge which poses significant threat to tomato growers in the Upper West, Northern, Bono, Upper East and Ashanti Regions of Ghana.

The use of synthetic nematicides like cadusafos has been particularly effective in nematode management (Hashem and Abo-Elyousr 2011). Traditional nematode management methods include crop rotation and the use of the ubiquitous fumigants such as methyl bromide (CH_3Br) and peptides (Molinaris, 2011). In Ghana, studies have been conducted to find alternative root-knot nematode control techniques. The use of chicken manure (Dawuda et al., 2011), sheep dung (Asiedu et al., 2007), and mucuna (Atta-Poku et al., 2014) in root-knot nematode management have been tested. Plant peptides that have exhibited inhibitory effects on both fungal and bacterial plant diseases and pests have also made significant advances in the field of plant protection (Candela et al., 2021). Peptides have been shown to be effective against root-knot nematodes on cucumbers, and they are relatively cheaper to apply (Calderón-Urrea and Polineni

2019). Peptides were discovered to be quite effective against pathogenic fungus in a previous study, and they were also found to be ecologically acceptable and relatively inexpensive to employ (Calderón-Urrea and Polineni 2019).

Biochar can be used as a fuel directly in place of pulverised coal. However, one of the primary differences between biochar and charcoal (or char) is that the former is made with the intention of being applied to a soil for carbon sequestration and soil quality improvement (Ahmad et al., 2014). By raising soil pH, increasing moisture holding capacity, attracting more beneficial fungi and microorganisms, boosting cation exchange capacity (CEC), and keeping nutrients in soil, biochar has been shown to increase soil fertility and quality (Ahmad et al., 2020).

1.2 Problem Statement

Many common nematicides used to control plant-parasitic nematodes have been linked to groundwater contamination and ozone layer depletion. They are also potentially damaging to humans and animals' health, as well as other beneficial organisms in the rhizosphere (Molinari.S, 2011). Non-fumigant nematicides such as cadusafos is being restricted due to its high toxicity and mixed outcomes in nematode control (Laquale et al., 2015). Several superior nematicides, such as methyl bromide (CH3Br), have recently been subjected to increased global regulatory pressure and are no longer available to growers (Ntalli et al., 2011). Dawuda et al. (2011) and Asiedu et al. (2012) reported on the successful usage of organic amendments such as chicken manure and sheep dung in root-knot nematode control. The requirement for substantially larger volumes of these organic amendments for use in commercial fields is a significant barrier (Dawuda et al., 2011). Interestingly, peptides play important roles in plant growth regulation as they influence cell-to-cell signaling, pest and disease resistance via toxins and elicitors, and

heavy metal detoxification via sequestration (Pahar et al., 2020). A multidisciplinary group of EU researchers produced and tested completely new biopesticide classes based on plant pests and insects (University of Glasgow, 2015-2019). The use of peptides in the management of plant pests and pathogens has been found to lower the danger of pesticide use in human health while being non-toxic to the environment and beneficial insects and also providing long-term crop protection (University of Glasgow, 2015-2019). The use of biochar in soil has the potential to improve the environment by limiting the loss of nutrients and so saving water supplies (Bhuvaneshwari et al., 2019). Raising soil pH, boosting moisture holding capacity, attracting more beneficial fungi and microorganisms, and improving cation exchange capacity (CEC) in soil are all benefits of using it as a soil amendment (Bian et al., 2018). To the best of our knowledge, the efficiency of peptides in controlling root-knot nematodes in biochar amended soil has not been tested in Ghana, thus, the need for this research.

1.3 Hypotheses

This research was based on two hypotheses, as follows:

i. Biochar amendment to the soil can enhance the effectiveness of Telluris peptide nematicide (Nemanol) in controlling root-knot nematodes infestation of tomato.
ii. Higher concentration of nemanol is more effective against root-knot nematodes for improved tomato growth.

1.4 Main Objective

i. The main objective of this research was to test the efficacy of nemanol and its concentration on root-knot nematodes and growth and yield of tomato in biochar amended soil.

1.5 Specific Objectives

The specific objectives of this research were;

i. To determine the effect of biochar rates and nemanol on root-knot nematodes and growth of tomato seedlings.

ii. To determine the effect of biochar rates and nemanol on root-knot nematodes, flowering and yield of tomato.

iii. To determine the effect of different concentrations of nemanol on root-knot nematodes population and growth of tomato seedlings in biochar amended soil.

CHAPTER TWO

LITERATURE REVIEW

2.0. Origin of Tomato

Tomato (*Lycopersicon esculentum* L.) belongs to the Solanaceae family. This family also includes other well-known crop species, such as potato, tobacco, peppers and egg- plant (FAOSTAT, 2014). The World Processing Tomato Council (WPTC) has indicated that tomato is one of the most important vegetables crops utilised worldwide (WPTC, 2020). The WPTC estimates for tomato production for 2020 was raised slightly to 39.2 million metric tonnes, mainly due to the increase of the Chinese forecast to produce 5.6 million tonnes (Ahmed et al., 2012). Tomato originated from the South American Andes. The cultivated tomato was brought to Europe by the Spanish conquistadors in the sixteenth century and later introduced from Europe to southern and eastern Asia, Africa and the Middle East (Ayandiji et al., 2011). More recently, wild tomato has been distributed into other parts of South America and Mexico (Shankara et al., 2019).

Tomatoes contribute to healthy, well-balanced diet due to its high levels of minerals, vitamins, essential amino acids, sugars and dietary fibres (Babolala et al., 2010). The fruits contain vitamin B and C, iron and phosphorus (Ayandiji et al., 2011). Tomato fruits are consumed fresh in salads or cooked in sauces, soup and meat or fish dishes. They can be processed into purées, juices and ketchup. Canned and dried tomatoes are economically important processed products (Shankara et al., 2019).

2.1 Economic Importance of Tomato

Tomatoes are one of the most prevalent and important vegetables in the world (Danjain, 2012; FAO, 2011). It is one of the most widely grown vegetables in most parts of the world, and it is only second in significance to potatoes (Yeboah, 2011). In terms of production, Asia and Africa account for roughly 79 % of total tomato land and 65 % of global productivity (Workneh et al., 2012). Tomato is the third most important vegetable in the United States, according to the USDA (with a total farm value of $2.062 billion), after potato ($2.564 billion) and lettuce ($2.064 billion). In 2013, the total harvested area for tomato in the United States was over 430,000 hectares (130,000 hectares for fresh market tomatoes and 300,000 hectares for processing tomatoes), with a farm value of around $3.00 billion ($1.6 billion for fresh market and $1.4 billion for processing). Furthermore, Pennsylvania is the leading grower of process and fresh market tomatoes in the United States (Adenuga et al., 2013).

The Lycopene pigment is responsible for the distinctive reddish coloring of ripe tomato fruits and related products (Hurst, 2010). According to Beckles, (2012), the crop has received worldwide attention as a natural antioxidant due to its genetic and physical properties. The fruits are commonly consumed raw in salads or cooked in sauces, soups, and meat and fish dishes (Adenuga et al., 2013).

Tomatoes can be used to make purées, liquids, and ketchup. When preserved and dried, they are a valuable commodity (Tan el al., 2010). It is an excellent source of vitamins A and C, as well as minerals such as calcium, potassium, phosphorus, magnesium, and iron, as well as carotenoids, flavonoids, and phenolic acids for human consumption (Horneburg and Myers, 2012). Tomato

has medicinal properties, according to Kaushik et al., (2011), and is utilised for blood purification and the treatment of gastrointestinal illnesses.

Apart from its nutritional and medicinal properties, tomatoes are a popular product for both domestic and international markets, and they provide a way out of poverty for smallholder growers in developing countries (Tewodros and Asfaw, 2013). Following their debut in Italy, tomatoes were mostly grown as ornamentals. Nigeria is Africa's second greatest tomato grower and the world's 13th largest, producing 1.701 million tons of tomatoes each year, an average of 25-30 tons per hectare (Arah, 2015). Tomatoes are very significant and popular vegetable crop in Ghana, and their cultivation is a key source of revenue for low-income farmers (Anang et al., 2013). Almost every Ghanaian home consumes tomatoes on a daily basis (Osei et al., 2010). Tomato is also used in big quantities as a flavoring in stews and soups, as well as in raw form in pepper sauces and salads. They can also be processed into secondary products such as tomato paste, tomato puree, and ketchup by manufacturers (Osei et al., 2010). Tomatoes have a high market value on both domestic and international markets because they are a staple in most people's diets around the world. Tomato consumption per capita in Ghana is slightly more than 100,000 metric tonnes per year (Asare-Badiako et al., 2010).

Tomatoes are often considered among the commercially important crops on the planet. Tomatoes are extremely valuable since they produce higher yields (Arah, 2015). Tomatoes are also a key element in a wide range of cuisines and goods available in shops across the world. Tomatoes are also a popular choice among gardeners who want to raise their own fruits and veggies (Ayandiji et al., 2011). The high demand for tomatoes has made it a very profitable business enterprise for individuals around the world (WPTC, 2020). Tomato farming provides producers with higher revenues and more job chances in rural areas because it is a labor-intensive crop all over the

world (Innes, 2014). Ghana produces 510,000 metric tonnes of tomato per year, according to the Ghana National Tomato Producers' Federation, while importing up to 7,000 tonnes per month from its neighbors and 27,000 tonnes of processed tomato from Europe (Anang et al., 2013). Tomatoes are a cornerstone in Ghanaians' daily diets, accounting for 38.0 % of overall vegetable spending in the country. Roma VF, Laurano, Raki, Chocó TP, Power Reno, Rasta, Italy Heinz, and Petomech are some of the most popular kinds planted in Ghana, and they are primarily appropriate for processing (Anang et al., 2013).

Tomato cultivation is a significant agricultural endeavor in the Upper East area, Ashanti region, Bono region, Volta region, and Eastern region, with a lot of room for expansion and job creation (Yeboah, 2011). The tomato sector continues to be one of the top agricultural investment opportunities in the country, owing to the fact that tomato production is a profitable business with high returns on investment. According to a survey conducted by Trade Aid Integrated, an NGO, tomato cultivation employed 11,728 farm households in the Upper East region, and with an average family size of five people, tomato production benefits 58,640 people (Yeboah, 2011).

2.2 Constraints in Tomato Production

Samuel et al. (2010) outlined the following as the major constraint involved in tomato production worldwide;

1. Physiological issues (blossom end rot, cracking, sunburn, or scald), as well as agronomic restrictions (incidence of illnesses and pests): Crop yields and quality are greatly influenced by insect pests and illnesses. Losses of 20% to 30% of overall production have been estimated in crops with substantial pesticide use and cultivars with insect and disease tolerance (Samuel et al., 2010). There is a scarcity of data on the extent of

damage caused by insect and disease pressure in small-scale vegetable cultivation. Lack of availability to insecticides with restricted use and cultivars with inadequate insect and disease resistance may result in significant losses. Crop protection measures that are improved may also result in considerable increases in production efficiency (Samuel et al., 2010).

2. Institutional obstacles, such as a lack of improved varieties, storage facilities, fertilizer (manure and inorganic fertilizers) shortages, and transportation issues, have all hampered tomato output (Samuel et al., 2010).
3. Market restraints, such as price fluctuations, have also harmed tomato production (Samuel et al., 2010). Limited cash for investment, an insufficient market for output, the use of improved varieties and fertilizer, an abandoned tomato processing factory, high production costs, poor prices for products, and other factors all contribute to the country's low tomato production.
4. In comparison to developed countries, tomato production per farmer per acre in Ghana is quite low. This can be due to a number of factors, including high labor expenses (land preparation, transplanting, and harvesting), which account for more than half of overall production costs (Robinson and Kolavalli, 2010). Droughts and heavy rains bring periods of excess and shortage, which affect and cause market price volatility (Robinson and Kolavalli, 2010). Input costs are high, transportation from farm gates is difficult, and storage and processing facilities are poor (Robinson and Kolavalli, 2010).
5. The most serious of these is the tomato crop's susceptibility to a variety of illnesses, including fungal, viral, bacterial, and root-knot nematode diseases (Horna et al., 2006). Foliar (leaf), fruit, stem, and root infections are caused by fungi and bacteria. Farmers

may experience large yield losses as a result of disease-related damage (Robinson and Kolavalli, 2010). Fungi, like other infections, generate greater problems due to the fact that some species may thrive in soil and seeds. They are only observed when the severity of the disease is great, and yield decline is equally high (Robinson and Kolavalli, 2010). Synthetic pesticides and fungicides are often prohibitively expensive for the average farmer, accounting for around 2% of total production costs.

2.3 Diseases and Pests of Tomato

Diseases and insect pests are two of the most major factors that reduce tomato production and productivity (Silme et al., 2010). Insect pests are thought to be responsible for roughly 15% of crop losses on average (Doumbouya et al., 2010). However, output losses of up to 95 % have been documented in some areas and under certain local farming conditions (Nguessan et al., 2012). The severity of disease and pest infestations determines the magnitude of yield losses. As a result of the existence of distinct pathogen races, biotypes, or strains, crop and fruit losses due to a single disease vary from one location to the next (Okhuoya et al., 2012).

2.3.1. Damping Off Disease in Tomatoes

For tomato seedlings, damping off, a generic term for a series of devastating seedling diseases, is fatal. Several fungi (Pythium, Rhizoctonia, or Phytophthorathat) attack tomato seeds, sensitive stems, and roots, causing the disease (Nirmaladevi and Sirnivas, 2012). Tomatoes are very vulnerable to humidity, especially if the soil is chilly and moist. The most vulnerable are young seedlings or plants. A damping off disease epidemic might be a dreadful way to start a fresh tomato season (Nguessan et al., 2012). Despite the fact that fungus grow in soil and water, spores

migrate in the air and can quickly spread from one seed tray (or garden row) to the next. Plants that have been affected appear to have had their roots chopped off. It's difficult to salvage even a few of your plants after the process has started (Nguessan et al., 2012).

The stems of healthy young tomato plants affected with damping-off disease seem pinched or cut off at the base. They wilt, droop, wither, and eventually die. On the soil surface and on dead plants, a white mold-like growth may form (Doumbouya et al., 2010).

According to (Doumbouya et al., 2010) there are two types of damping off: pre-emergent and post-emergent.

i. Pre-emergent damping-off: seeds rot in the soil or seedlings decay before they push through the soil

ii. Post-emergent damping-off: seedlings sprout, but then pale, curl, wilt, or collapse at the soil line. The stem is water-soaked and turns gray, brown or black before disintegrating.

2.3.1.0 How to Prevent Damping-Off

i. Use sterile containers. If you are re-using last year's flats, be sure to wash them thoroughly and rinse them in a bleach solution before planting (Nguessan et al., 2012).

ii. Start tomato seeds in a sterile potting medium. Damping-off fungi flourish unhygienic conditions. Soil-less potting mix provides a healthier environment and eliminates potential pathogens from the start. (Read more about sterile

growing mediums and find inexpensive seed starting mix to buy) (Nguessan et al., 2012).

iii. Avoid overly damp conditions. Many new gardeners start tomato seeds in damp basements – a perfect breeding ground for damping-off fungi. Choose a seed-starting area with good circulation. You can even run an electric fan in the growing area to keep the air moving (Okhuoya et al., 2012).

iv. Maintain a steady temperature. Drafty, cool conditions encourage damping-off.

v. Sprinkle soil surfaces. Spread a thin layer of sand, perlite, or sphagnum peat moss on the surface of the potting mix or garden soil to discourage fungi and bacteria (Okhuoya et al., 2012).

vi. Work for a low pH. As the potting mix or garden soil pH rises, so does a tomato plant's susceptibility to damping off. Commercially-prepared germination mixes have an average pH around 5.5, while tap water tends to be alkaline. As you water the seed pots and your seedlings with tap water, the pH in your pots gradually increases. Simultaneously, so will the plants' susceptibility to damping-off diseases (Nguessan et al., 2012).

vii. Use a preventative fungicide. Water potting mix, soil, and seeds with soluble copper spray (Nguessan et al., 2012).

viii. Separate infected plants. Damping-off disease spreads quickly from one plant or seed tray to another. Monitor new seedlings carefully. At the first sign of damping-off, move affected plants away from healthy ones (Okhuoya et al., 2012).

2.3.2 Root Knot Nematode Disease of Tomato

Root knot nematodes are microscopic eelworms that dwell in the soil and turn into plant parasites when they use tomato roots as nurseries. Nematodes frequently invade tomato roots through minor wounds. Small feeder roots are killed as their numbers grow, and irregular galls take their place (Huang et al., 2016).

2.3.2.0 Damage Cause to Infested Plant

Root-knot nematodes produce distinctive galls on roots, which can be up to 1 inch in diameter but are usually much less (Gisbert et al., 2013). Infected plants are less vigorous than healthy plants, may be yellowed, prone to wilt in hot weather, and respond poorly to fertilizer (Laquale et al., 2015). Damaged regions typically appear as uneven patches and are often seen in lighter-textured soils. Plants develop slowly and in sporadic spurts, and are often small. Numerous swellings and galls can be seen on the roots of a problematic tomato or pepper plant when dug up (Hashem and Abo-Elyousr 2011).

2.3.2.1 Preventing Problems

Tomatoes are susceptible to root knot nematodes, in part because they thrive in the same hot summer conditions that please nematodes (Hashem and Abo-Elyousr 2011). Good crop rotations prevent nematode buildup in many gardens, but root knot nematodes may be unavoidable in sandy soils in warm climates (Ntalli et al., 2011). Numerous resistant varieties are available in both tomato and pepper. Regularly amend soil with materials that contain chitin, such as seafood meal, eggshells, or shourimp hulls. In the soil, these materials feed microorganisms that chow down on chitin, including nematode eggs (Ntalli et al., 2011).

2.3.2.2 Managing Outbreaks

Pull up badly infected tomato plants; lop off the roots, and dispose of the roots in the trash. Compost the rest (Wieczorek, 2015). Mark the area where the diseased plants grew, and do not grow tomatoes, peppers, okra or carrots there again for some time. If tomato or pepper plants are only slightly infected, they may make a crop if a deep mulch is used to keep the root zone cool and moist (Wieczorek, 2015).

2.3.3 Fusarium Wilt

The fungus *Fusarium oxysporum* causes Fusarium wilt, the most common tomato wilt disease. Fusarium wilt is found all across the world, and it can even impact resistant tomato types (Nguessan et al., 2012). The fungus is spread by the soil and enters the plant through the roots. Fusarium wilt is a soil-borne disease that can live in the soil for many years even if no host plants are present (Kaushik et al., 2011). It does not spread from plant to plant above ground. When the pathogen reaches the root system, it infects each plant independently (Ford et al., 2015). This soil-dwelling fungus causes leaf yellowing and wilt in plants. Plants can be affected at any stage of development; however, symptoms appear most prominently during or just after flowering (Singha et al., 2010).

The older leaves of diseased plants turn yellow first (those nearing the ground). A recognised hallmark of tomato fusarium wilt is vivid yellowing that is limited to one side of the plant or even to leaflets on one side of the petiole (Zhao et al., 2013). The leaves that have been impacted wilt and dry quickly, yet they remain attached to the plant. The wilting progresses from younger to older foliage, eventually killing the plant. On the outside, the stem is firm and green, but the vascular tissue has a small band of brown discoloration (Sundaramoorthy and Balabaskar, 2013).

Slicing vertically through the stem at the soil line and searching for a short column of browning between the central pith region (middle tissue of the stem) and the outside portion of the stem will reveal this discoloration (Solanki et al., 2019). During the fungus' attack, the brown streaking in the vascular tissue of infected plants becomes clogged, causing withering and yellowing of the leaves (Shanmugam et al., 2015). Infected plants frequently die before reaching maturity. The fungus spreads up the plant roots, obstructing water-conducting tissue in the stem and preventing water from reaching the plant's branches and leaves, starving it (Ramarathnam et al., 2011). When the main stem is broken open, brownish streaks from clogged water-conducting tissue can be seen (Patel and Saraf, 2017).

Plants that are affected produce very few tomatoes. Frequently, the entire plant perishes. The disease can strike at any point throughout the growth of a tomato plant. Temperatures between 21^{o} and 32^{o} C, as well as moist conditions, are favorable to the fungus (Pastor et al., 2012). Tomato plants grown in poorly drained soil are more prone to infection than those grown in well-drained soil (Pane and Zaccardelli, 2015). Wet soil enables the fungus to proliferate, making it easier to spread. When root knot nematodes are present in the soil, Fusarium wilt becomes more problematic (Pane and Zaccardelli, 2015).

3.3.4 Bacteria Wilt

One of the most common diseases of tomatoes and other *solanaceous* plants is bacterial wilt. The disease has been reported in the wet tropics, subtropics, and temperate parts of the globe (Pastor et al., 2012). Bacterial wilt is frequently the most destructive disease, resulting in a 60-70 % yield lose (Shanmugam et al., 2015). In the tropical and subtropical parts of the world, bacterial wilt caused by the soil-borne plant pathogen Ralstonia solanacearum is one of the most severe bacterial plant diseases (Solanki et al., 2019). The bacteria enter the plant through root injuries

and natural openings, then travel to the secondary xylem and migrate to the shoots after reaching the primary xylem (Nguessan et al., 2012).

Because it affects a wide range of economically significant crops such as tomato, potato, eggplant, chilli, and *non-solanaceous* crops such as banana and groundnut in India, it has garnered international attention due to its destructive character, large host range, and geographical dispersal (Solanki et al., 2019). The indications of bacterial wilt in tomatoes include withering of the upper leaves, which is followed by full wilting of the plants within a few days. The vascular tissues of the infected stem show brown discoloration and if the stem is sliced crosswise, white or yellowish bacterial slime may be observed (Doumbouya et al., 2010). Extracellular polysaccharides are produced, which clog the xylem, causing wilting and eventual death. They migrate downward to re-enter the soil through the roots after reproducing within plants and provide a source of infection for following crops (Solanki et al., 2019). Most typically where plants have been cut, wounded, or weakened by transplanting, cultivation, insects, or other diseases, through the root or stem of the plant (Pane and Zaccardelli, 2015).. Tomato yields were reduced by 25% as a result of the disease. High temperatures (30–35°C) and high soil moisture, according to (Pane and Zaccardelli, 2015), favor disease development. High soil moisture promotes the pathogen's survival, infection, and development, as well as its transmission through the soil. However, there is no chemical treatment available at this time; the illness can be managed by avoiding physical damage to roots and stems, controlling root-knot nematodes, which are known to weaken tomato roots and give bacteria access to plants, and preventing physical damage to roots and stems (Pane and Zaccardelli, 2015).

2.3.5 Bacterial Soft Rot

Bacterial soft rots are a collection of illnesses that kill more crops than any other bacterial disease on the planet. Bacterial soft rots wreak havoc on succulent plant components like fruits, tubers, stems, and bulbs in plants from practically every plant family (Pastor et al., 2012). Soft rot bacteria destroy pectate molecules, which link plant cells together, causing the structure of the plant to disintegrate over time (Ramarathnam et al., 2011). Woody tissues are immune to the disease. Soft rots are frequent in vegetables like potatoes, carrots, tomatoes, cucumbers, melons, squash, and pumpkins, as well as cruciferous crops like cabbage, cauliflower, and bok choy (Shanmugam et al., 2015). These infections can affect field crops as well as harvested crops in storage. Rot can develop over a wide range of temperatures, with the worst deterioration occurring between 70 and 80°C, especially when oxygen is scarce (Solanki et al., 2019).

2.3.5.0 Source of Bacterial Soft rot

Soft rots are produced by a variety of bacteria, including *Pectobacterium carotovorum* (formerly *Erwinia carotovora*), *Dickeya dadantii* (formerly *Erwinia chourysanthemi*), and *Pseudomonas*, *Bacillus*, and *Clostridium* spp (Doumbouya et al., 2010). Plants can be infected by bacteria through wounds made by tools, insects, extreme weather such as hail, or natural openings (Singha et al., 2010). Insects, contaminated tools, or movement of infested plant detritus, soil, or contaminated water can all transfer the bacteria from plant to plant. Bacterial soft rots are more common in damp weather and can be more severe when plants don't get enough calcium (Zhao et al., 2013).

2.3.5.1 Damages Caused by Bacterial Soft Rot

Water-soaked patches are caused by bacterial soft rots at first. Over time, these areas expand and become sunken and squishy. Interior tissues beneath the spots become mushy and discolored, ranging from cream to black in hue (Singha et al., 2010). There is a lot of seepage from the impacted locations (Doumbouya et al., 2010). Soft rots have a strong, unpleasant odor that comes with the degradation of plant tissue.

2.3.5.2 How to Prevent Bacterial Soft Rot

Singha et al. (2010) outlined the following as measures to prevent bacterial soft rot;

There are no treatments for soft rot bacteria after they have affected plant tissue. Remove and discard contaminated plants or plant parts as soon as possible. This item should not be buried or composted (Singha et al., 2010).

i. Soft rot can be controlled by avoiding wet environments. Plant vegetables in well-drained soils and regulate watering intervals and amounts to ensure that plants receive appropriate (but not excessive) and consistent watering. Plants should not be crowded; broader spacing promotes faster plant and soil drying. Based on a soil nutrient test, ensure that soil fertility (especially soil calcium) is optimal for the veggies you're cultivating. As needed, add calcium (e.g., bone meal) when planting (Singha et al., 2010).

ii. Rotate soft rot-resistant crops with vulnerable plants in your garden. Corn, snap beans, and beets are examples of vegetables that are resistant to soft rot. Avoid cultivars with flat/concave heads when growing broccoli since they collect moisture and encourage soft rot. Choose cultivars with domed heads, which let water to drain quickly (Singha et al., 2010).

iii. When weeding and harvesting, avoid harming the vegetables. Handle soft-rotted plants as little as possible, but if you must (e.g., to remove them from the garden), wash your hands with soap and water afterward. Decontaminate garden tools before and after use by soaking them in 10 % bleach or, preferably (because to its lower corrosive qualities), 70 % alcohol for at least 30 seconds. The alcohol content of rubbing alcohol and many spray disinfectants is normally around 70%. Insects that can wound vegetables, such as cabbage maggot, should also be controlled (for further information, see University of Wisconsin Garden Facts XHT1030 "Cabbage Maggot") (Singha et al., 2010).

iv. Harvesting should only be done when the weather is dry. Inspect vegetables from polluted gardens that will be stored for an extended period of time to ensure they are not diseased. If required, cure vegetables before keeping them. Store vegetables in a cool, dry, well-aerated environment to avoid bacterial growth (Singha et al., 2010).

v. Remove any infested plant debris from your garden at the end of the growing season and dispose of it by burning or landfilling it. If soft rot is a persistent problem in one region of your garden, don't plant sensitive crops there for at least three years (Singha et al., 2010).

2.3.6 Leaf Spot Disease

A fungus causes leaf spot, often known as Septoria blight (*Septoria lycopersici*). It is one of the most frequent tomato foliar diseases (Ford et al., 2015). Warm temperatures (20-25°C) and high relative humidity, as well as extended periods of leaf wetness induced by overhead irrigation, rain, or heavy dews, encourage the illness (Ford et al., 2015). The majority of infection is most likely caused by infested plant debris left in the soil from a previous tomato crop. Septoria leaf spot spreads swiftly, defoliating and weakening plants to the point where they are unable to yield

fruit to maturity (Kaushik et al., 2011). The Septoria fungus thrives on and in the earth, on fallen tomato plant detritus and weeds. Water and wind, which splash up on the plants from the earth, transfer it to the plants (Kaushik et al., 2011).

Lower leaves are the first to become affected, and the illness spreads upward, attacking stems and blooms, but rarely fruit. Infection can strike at any stage of plant development, but it is most common once the plant has started to bear fruit (Nirmaladevi and Sirnivas, 2012). It begins as little, water-soaked patches on the undersides of older leaves and the plant's bottom, then progresses to circular spots measuring 1/16-1/8 inch in diameter (Nirmaladevi and Sirnivas, 2012). Gradually, the lesions form grayish white cores with dark margins. The most distinguishing feature of Septoria leaf spot is the light-colored core of the dots. Fungal fruiting bodies emerge as small black specks in the middle of the spots when conditions are suitable (Soro et al., 2008).

These are spore-producing fruiting structures. Splashing rain spreads spores to new leaves. This will weaken the plant, causing it to wilt, and result in solar blistering of the exposed, unprotected tomatoes (Okhuoya et al., 2012). Leaves that have been heavily affected turn yellow, white, and eventually fall off. Defoliation occurs from the plant's base upwards, and it can be severe following extended periods of warm and wet weather (Okhuoya et al., 2012). Fruits that have lost their leaves may become sun scalded. Septoria defoliation looks a lot like early blight disease. The larger dark leaf spots with concentric rings of early blight, on the other hand, are clearly distinguishable from the tiny Septoria leaf spots (Okhuoya et al., 2012).

2.3.7 Tomato Pith Necrosis

Pseudomonas corrugata and other soil-borne Pseudomonas species cause tomato pith necrosis. While high tunnels and standard greenhouses provide optimal circumstances for the growth of early season tomatoes, they also give ideal settings for a newly emergent disease of greenhouse tomatoes (Nguessan et al., 2012). This disease is most common in early-planted tomatoes when night temperatures are chilly, humidity is high, and the plants are growing rapidly due to high nitrogen levels. Long periods of overcast and chilly weather are also linked to the condition (Nguessan et al., 2012).

2.3.7.0 Identification of Tomato Pith Necrosis

Yellowing and wilting of young leaves are common early symptoms that develop just as the first fruit clusters reach the mature green stage (Silme et al., 2010). Chlorosis and wilting of upper portions of plants, as well as brown to black lesions on infected stems and petioles, are symptoms of serious infections (Silme et al., 2010). The center of the stem (pith) may be significantly discolored, hollow, and/or deteriorated when stems are sliced longitudinally. Infected stems may swell, develop multiple adventitious roots, and shourink, break, or collapse (Silme et al., 2010).

2.3.7.1 Life Cycle of Tomato Pith Necrosis

The disease's epidemiology is unknown; it's probable that the bacteria are spread via seeds and that they persist in the soil in connection with diseased tomato debris (Nguessan et al., 2012).

2.3.7.2 Cultural Controls and Prevention

Appropriate ventilation to avoid high humidity levels (especially during cloudy weather), avoiding excessive nitrogen levels to prevent vigorous plant growth, incorporation of crop debris to speed decomposition of residue and associated bacteria, and crop rotation are all preventive measures to minimize the occurrence of this disease in high tunnels (Okhuoya et al., 2012).

2.3.7.3 Chemical Controls and Pesticides

There is no effective treatment for this disease; however, affected plants may recover if environmental conditions improve (warm, sunny weather) (Okhuoya et al., 2012).

2.3.8 Early Blight

Early blight, commonly known as *Alternaria* leaf blight, is a widespread disease in which infection usually begins on the older, more vulnerable lower leaves. Lesions start out as small, black, irregularly shaped spots that grow into larger, concentric rings over time (Doumbouya et al., 2010). A golden halo forms around the surrounding tissues, resembling a bulls-eye target.

As the illness progresses, it might result in leaf death and lower yields. Although infection usually starts in the bottom part of the plant, it can spread to the top leaves, stems, and fruits at any time (Doumbouya et al., 2010). Early blight resistance is unusual, and even in these kinds, resistance does not provide complete disease immunity; rather, resistant plants can postpone and decrease disease signs long enough to maintain production (Okhuoya et al., 2012). The fungus that causes early blight can come from a variety of places. It can be found in the soil, on purchased seeds or seedlings, and it can overwinter in damaged tomato plant detritus, where it can last for at least a year (Nguessan et al., 2012).

When foliage comes into contact with contaminated dirt or dead plants, the fungus can survive the freezing temperatures of winter and infect new plantings. The fungus lives in the soil by generating resistant spores in the presence of damaged tomato waste that can last a year or more. Infection spreads quickly in warm, humid environments (Okhuoya et al., 2012). Thousands of spores are produced in diseased leaf patches, with the potential to spread infection (Nguessan et al., 2012). Wind and rain, irrigation, insects, employees, and tools and equipment can all disseminate fungal spores. They become the most important source of fresh spore generation and are responsible for rapid disease transmission once the original infections have occurred (Okhuoya et al., 2012).

When plants are challenged by nitrogen deficiency, dryness, or a heavy fruit load, early blight can develop swiftly in the middle to late season (Kaushik et al., 2011). Early blight can harm the leaves, stems, and fruits of tomato plants, among other things. Although the plants are unlikely to die, they will be weakened and produce fewer tomatoes than usual. The disease's most visible sign is the premature loss of lower leaves (Kaushik et al., 2011). Brown to black spots (lesions) up to 12 inches in diameter with dark edges and a pattern of concentric rings occur on infected leaves, giving the spot the "target" appearance suggested by the common name. Spots frequently merge later, resulting in uneven blotches (Ford et al., 2015). Defoliation spreads upward from the lower plant, with infected leaves finally becoming brown and falling off, exposing the fruits to sun scald. The dark sores on the stems begin tiny and sunken. They expand as they grow larger, and concentric marks, like the spots on the leaves, begin to appear (Nguessan et al., 2012).

Spots that occur close to the earth might produce stem girdling or collar rot. Although the plants will live, they will not thrive or yield many tomatoes. Early blight is more common in older

plants, although it can also affect seedlings. If seedlings are infected with early blight, the affected seedlings will have dark spots on their leaves and stems (Nguessan et al., 2012).

2.3.9 Late Blight

Late blight, caused by *Oomycete phytophthora* infestations, is one of the most devastating tomato diseases, incurring major economic losses each year (Ford et al., 2015). The pathogen is well recognised for its part in the Irish potato famine, which resulted in the deaths of over a million people. It can kill a tomato crop in a matter of days if left unchecked (Singha et al., 2010). The pathogen's success is due to its efficient asexual and sexual life cycles, as well as its extraordinary ability to overcome plant resistance genes quickly (Ford et al., 2015). Its ability to reproduce both asexually and through sexual mating leads to rapid reproduction, epidemics that spread quickly, and greater genetic variety and survival (Singha et al., 2010). Sporangia have mycelia growth at lower temperatures by directly producing and releasing zoospores (asexual spores), which germinate and generate new infections at an even faster rate (Nguessan et al., 2012). Reduced yield, lower fruit quality (such as low specific gravity), decreased storability, and greater costs linked with fungicide applications are all examples of economic losses (Singha et al., 2010). Fruit decay can be severe, and all sections of the plant are affected. Late blight can affect leaves that are young (upper) or elderly (below) (Nguessan et al., 2012).

When the leaves are moist or the humidity is high, it starts as pale green water-soaked dots at the leaf tips that quickly develop, becoming uneven, greenish black blotches. The plant frequently develops white mold around the edges of afflicted areas, giving it a frost-damaged appearance (Singha et al., 2010). When conditions are favorable for the disease, entire plants can be swiftly defoliated. Brown streaks will appear along the stems if the stems and petioles are affected, and

the sections above these infections will wilt and die (Nguessan et al., 2012). Large, irregularly formed brown spots appear on infected green or ripe fruit, usually starting at the stem. Fruits that have been infected quickly decompose into foul-smelling lumps (Singha et al., 2010). Additionally, light brown lesions appear, extend, and wrap the stem and petioles, shattering them and instantly killing the plant. Infection of the stem is more severe in high-temperature and high-relative-humidity environments (Nguessan et al., 2012).

2.3.10 Tomato Leaf Mold

The fungus *Passalora fulva* (previously called *Cladosporium fulvum* or *Fulvia fulva*) causes leaf mold.

2.3.10.0 Identification Tomato Leaf Mold

Symptoms of leaf mold on the upper leaf surface of tomato plant (Zhao et al., 2013)

i. The first infection occurs in oldest leaves.
ii. Pale greenish-yellow spots occur on upper sides of leaves, usually with no definite margins and less than $^1/_4$ inch.
iii. Olive-green to brown velvety mold occurs below leaf spots in the lower leaf surface.
iv. Velvety sporulation in shades of olive green to brown on the lower leaf surface.
v. Leaf splotches coalesce and darken as a group. Although they wither and die, leaves frequently stay affixed to the plant.
vi. Severe leaf mold infection kills leaves.

vii. Blackened and dead blooms from infected plants.

viii. Fruit infections first appear as a smooth, dark, erratic patch on the fruit's stem end. The affected area becomes depressed, dry, and leathery as the condition worsens.

2.3.10.1 Environmental Conditions of Tomato Leaf Mold

i. Relative humidity levels above 85% are ideal for growth.

ii. While disease can develop at temperatures as low as 50 °C and as high as 90 °C, the ideal temperature range is between 71 °C and 75 °C.

2.3.10.2 Biology and Disease Cycle of Tomato Leaf Mold

Spores of P. fulva can survive for 6 months to a year above ground at room temperature (Zhao et al., 2013).

i. In Minnesota's climate, it is unknown if spores will endure on the surface of stakes, tools, and high tunnel walls from one season to the next.

ii. Within infected plant waste, the virus creates black, rigid resting structures.

iii. When exposed to air, these structures will generate a large number of fresh spores (Zhao et al., 2013).

iv. They are *P. fulva* best chance of surviving from one season to the next. They are the most likely means for P. fulva to survive from one season to the next.

v. The leaf mold pathogen can survive on and in tomato seed and may be introduced to a new area by this route (Zhao et al., 2013).

vi. Spores of P. fulva can start an infection at a wide range of temperatures.

vii. Relative humidity at or above 85 % will favor severe leaf mold epidemics.

viii. Some disease can occur at humidity less than 85 %.

ix. New spores form on the lower surface of infected leaves within 10 to 12 days.

x. If humidity remains over 85%, these spores will infect new leaves (Zhao et al., 20013

xi. Within the growing season, multiple generations of the pathogen can be completed.

xii. It can spread from leaf to leaf and plant to plant by wind, rain/overhead irrigation, tools, workers and perhaps insects (Zhao et al., 2013).

2.3.10.3 Management of Tomato Leaf Mold

2.3.10.3.0 Resistant Cultivars

Although leaf mold-resistant cultivars are available in many seed catalogs, they may or may not be beneficial in preventing disease in Minnesota (Fondio et al., 2013). As part of an integrated disease management program, resistant varieties should be employed in conjunction with cultural control techniques. Growers with a history of leaf mold should test resistant types on a small scale to see how effective they are in their particular situation (Fondio et al., 2013).

2.3.10.3.1 Cultural Control

i. Use drip irrigation and avoid watering foliage.

ii. Space plants to provide good air movement between rows and individual plants (Fondio et al., 2013).

iii. Stake, string or prune to increase airflow in and around the plant.

iv. Sterilize stakes, ties, trellises etc. with 10% household bleach or commercial sanitize.

v. Circulate air in greenhouses or tunnels with vents and fans and by rolling up high tunnel sides to reduce humidity around plants (Fondio et al., 2013).

vi. Keep night temperatures in greenhouses higher than outside temperatures to avoid dew formation on the foliage.

vii. Remove crop residue at the end of the season. Burn it or bury it away from tomato production areas.

viii. Clean the high tunnel or greenhouse walls and benches at the end of the season with a commercial sanitizer (Fondio et al., 2013).

2.3.10.3.2 Chemical Control

To be most effective, applications should be made before to infection when environmental factors promote disease. The first leaf mold infections of the season were discovered in Minnesota high tunnel tomatoes in the first week of June (Singha et al., 2010). Fungicide applications should be repeated as directed on the label. To avoid the development of disease resistance to certain active components, it's critical to switch between chemical families (Singha et al., 2010).

2.4 Root-Knot Nematode as Pest of Tomato

Many pests and illnesses are wreaking havoc on tomato production, both in terms of quality and quantity. One of them is plant parasitic-nematodes. They are a significant impediment to achieving global food security. Plant-parasitic nematodes are estimated to inflict $80 billion in annual damage (Nicol et al., 2011). Because many growers in developing countries are unaware of the occurrence of plant-parasitic nematodes, this figure is likely to be significantly underestimated (Jones et al., 2013). Nematodes are tiny worms that feed on plant roots and live

in the soil. They are difficult to perceive with the naked eye due to their small size (just a few mm long) (Nicol et al., 2011).

Some nematodes feed on the outside of plants, while others feed inside them. All feed on the sap of the plant, which might diminish the plant's capacity to produce (Jones et al., 2013). Even more harm can be done if viruses or fungi penetrate the plant as a result of the nematode's injuries, making the plant sick and finally killing it (Ntalli et al., 2011). The nematode that causes root knots *Meloidogyne* spp., which includes *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria,* and *Meloidogyne hapla*, as well as a few developing species including M. enterolobii and M. chitwoodi, is responsible for the vast majority of crop damage (Ntalli et al., 2011). *Meloidogyne* spp. cause significant economic losses in a variety of agricultural crops around the world, with catastrophic yield losses in tropical and sub-tropical agriculture (Ntalli et al., 2011). In the tropics, nematodes cause yield losses of up to 30% in tomato. They have an effect on the amount and quality of marketable crops. Tomato roots can be severely harmed by root-knot nematodes. Tropical root-knot nematodes have more symptoms than temperate root-knot nematodes (Wieczorek, 2015). Tomato cultivars are susceptible to distinct *Meloidogyne spp.* to varying degrees. So far, damage and yield loss investigations have revealed a significant variance in susceptibility among tomato varieties (Wieczorek, 2015).

Infested plant material, tools, rains and irrigation water, high winds (which carry infested soil particles), and contaminated soil carried on shoes or animal feet are all possible sources of nematode infestation and transmission. Nematodes may survive in soil if it is kept moist (Nicol et al., 2011).

2.4.1 Root-Knot Nematodes Control Measures

2.4.1.1 Use of Inorganic Compounds in Root-Knot Nematode Control

Applying soil nematicides is justified when severe attacks are observed (high nematode densities in the previous crop) and in cases of replant sickness symptoms following repeated rose crops in the same field or greenhouse (Soheili et al., 2017). Nematicides protect young plantlets during the first stages of their development and allow their satisfactory installation and subsequent productivity (Laquale et al., 2015). None the less, none of the nematicides can eradicate nematodes from the soil because individuals can usually survive in deeper levels where they can escape chemical diffusion; reinfestation of the plants will inevitably occur after a few months or years (Soheili et al., 2017). It is absolutely necessary that preplanting chemical treatments, which act only against nematode stages surviving in the bare soil, are combined with the use of nematode-free nursery plants (Soheili et al., 2017).

In different countries, different ranges of fumigants (e.g., methyl bromide and dichloropropene), precursors of fumigants (e.g., dazomet) and nonvolatile nematicides (in granular form such as aldicarb) are still authorised for use but the list is becoming more and more restricted because of the negative environmental impact of the chemicals (Lopez-Perez et al., 2010). Methyl bromide, for example, which has a remarkably wide-spectrum activity (including weeds, soil fungi, nematodes and insects), was banned in all countries of the European Union in 2005 (Lopez-Perez et al., 2010). Nematicides, such as fosthiazate, have been developed for the control of nematode pests of crops. Fosthiazate is a soil-applied organophosphate, contact-acting nematicide that controls potato cyst nematodes (Laquale et al., 2015).

Certain chemicals in this group are classified as soil sterilants/fumigants, e.g., Dazomet. It is important to remember that a time interval must be observed between the last application of the pesticide and the harvesting of edible crops, as well as the access of animals and poultry to treated areas (Laquale et al., 2015). With some pesticides this interval is longer than others. This is another reason for very careful reading of the manufacturer's instructions (Laquale et al., 2015).

2.4.1.2 Use of Organic Amendments in Root-Knot Nematode Control

Treatments with nematicides (e.g., Temik 5 G) or fumigants (Basamid) result in a clear reduction in the nematode population. Rotate tomato with other crops such as cereals, cabbage, onion, ground nut, cassava, sesame, etc. (Noling, 2019). Do not rotate with Solanaceae. It is not advisable to rotate with crops of the Cucurbitaceae family (e.g., cucumber or pumpkin) or papaya either, as these can also cause the transmission of diseases (Noling, 2019). Remove weeds and plant remains (rotten leaves and fruit). Interplant with plants that emit substances via their roots which nematodes do not like or which kill them, such as sesame or African marigold (Tagetes erecta and other related varieties) (Laquale et al., 2015). Expose the soil to sun and wind. Plough the soil several times. The nematodes will be ploughed up to the surface of the soil and will be exposed to the sun and high temperatures, which kill them (Laquale et al., 2015).

In Ghana, research studies have been conducted to develop alternative strategies such as the use chicken manure (Dawuda et al., 2011), sheep manure (Asiedu et al., 2007) and mucuna (Atta-Poku et al., 2004), for root-knot nematodes control. Soil fumigants are restricted use pesticides that can only be applied by certified applicators. Incorporation of cruciferous green manures such as cabbage, mustard, and rape into soil may also help reduce populations, particularly when

combined with solarization. Many root-knot resistant tomato varieties are available (Laquale et al., 2015).

2.4.1.3 Use of Cultural Methods in Root-Knot Nematode Control

Management of root-knot should focus on sanitation measures for preventing contamination of soils, reducing populations below damaging levels where infestations already exist, and variety selection (Laquale et al., 2015). Sanitation measures include planting nematode-free tomato transplants and avoiding the introduction of nematodes on any other type of transplant stock or with soil (Noling, 2019). This is difficult in reality because soil clinging to plant roots may contain nematodes without obvious plant symptoms. Equipment and boots should be washed free of soil before working clean ground when moving from areas suspected of harboring nematodes. Strategies for reducing nematode populations include starving nematodes by two-year crop rotations with resistant crops like corn, milo, and nematode-resistant soybean varieties; or with clean (weed-free) fallow (Gisbert et al., 2013). Soil solarization may be effective in some situations, but soil fumigation provides more consistent control of nematode populations (Gisbert et al., 2013).

2.4.1.4 Use of Resistant Variety in Root-Knot Nematode Control

Strategies for reducing nematode populations include starving nematodes by two-year crop rotations with resistant crops like corn, milo, and nematode-resistant soybean varieties; or with clean (weed-free) fallow (Lopez-Perez et al., 2010). Several varieties are resistant to nematodes and should be used where nematodes are present. Rotation with resistant varieties and non-host crops is as effective as fumigation (Gisbert et al., 2013). Resistant tomato varieties are not effective against the species *Meloidogyne hapla*, but are effective *against M. incognita, M.*

javanica, and M. arenaria. Cotton is susceptible only to M. incognita and has relatively high tolerance to even that species (Gisbert et al., 2013). Certain varieties of alfalfa and black-eyed peas are resistant to some root-knot species, but *M. hapla* builds to high numbers on alfalfa (Lopez-Perez et al., 2010).

2.5 Chemistry of Peptides

Peptide is an organic compound with components that are structurally similar to proteins but smaller. Many hormones, antibiotics, and other chemicals that engage in the metabolic activities of living organisms are classified as peptide (Machado et al., 2015). Peptide molecules are made up of two or more amino acids that are bonded together by the carboxyl group of one amino acid and the amino group of the next (Candela et al., 2021). A peptide bond is the chemical link that exists between the carbon and nitrogen atoms of each amide group (Tavormina et al., 2015).

Partial or total hydrolysis of the substance can break some or all of the peptide bonds that bind the consecutive triplets of atoms in the chain recognised as the molecule's backbone (Candela et al., 2021). This process, which produces smaller peptides and then individual amino acids, is extensively employed in peptide and protein composition and structure research (Czyzewicz et al., 2013). Perception of the Arabidopsis Danger Signal Peptide (PEPR1) receptor stabilizes a physical interaction between Perception of the Arabidopsis Danger Signal Peptide (PEPR1) and a Brassinosteroid Receptor Associated Kinase1 (BAK1) (Yamada et al., 2016), stimulates the phosphorylation of Botrytis Induced Kinase1 (BIK1) (Liu et al (Kadota et al., 2014; Roux et al., 2011). BIK1 has been shown to phosphorylate Respiratory Burst Oxidase Protein D (RBOHD), a Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that is responsible for the majority of the ROS produced during the oxidative burst associated with race-specific effector

triggered immunity (ETI) and broader spectrum pattern triggered immunity (Macho and Zipfel, 2014).

2.5.1 Use of Peptides in Root-Knot Nematode Control

Plant elicitor peptides (Peps) are broadly dispersed signaling molecules that help plants defend against insects and pathogens on a broad scale (Pahar et al., 2020). Plant peptides that act as signal molecules to activate cell-to-cell communication are critical for plant growth and defense (Czyzewicz et al., 2013). Some plant peptides are involved in plant growth and development, whereas others are involved in plant-microbe interactions and govern defense responses (Van et al., 2019). This preconception is dispelled; however, as more data emerges that growth-related plant peptides also play dual roles in plant defensive responses against various microbial diseases (Van et al., 2019) . The use of peptide to reduce root-knot nematode on cucumber was found to be successful (Calderón-Urrea and Polineni 2019).

Because peptides activate several defensive pathways, including those previously implicated in nematode resistance, they could be a source of broad-spectrum resistance to worms (Van et al., 2019). Peptides coactivate salicylate (SA), jasmonate (JA), and ethylene signaling, according to Arabidopsis transcript profiling (Ross et al., 2014). Nematode infection activates all three pathways, and each one has been linked to plant defenses against nematodes in at least some host plant–nematode combinations and infection phases (Manosalva et al., 2015; Xie et al., 2016; Zhao et al., 2015). Peptides activate signal transduction events and oxidative responses that overlap with plant defense responses against nematodes (Yamada et al., 2016).

2.6 Biochar and its Characteristics

Biochar is a produce of unhurried and imperfect burning of carbon-based materials. Currently biochar is causing attention as a possible soil amendment; this is because soil organic carbon (SOC) accumulation is fundamental to the enhancement in soil properties (Domingues et al., 2017). Addition of C-enriched amendments such a biochar (60-80% C) could enhance soil physical and chemical properties and microbial (Blanco-Canqui, 2017). Biochar addition, particularly to nutrient-deficient and/or drought-prone soils, generally increases the yield of crop plants and trees, as well as enhancing soil carbon sequestration (Zeshan et al., 2014). Biochar varies greatly in their properties; efforts to "design" biochar for specific applications have mainly focused on pyrolysis parameters (Clemente et al., 2018). Biochar pH increases with pyrolysis temperature, but also determined by variation in feedstock chemistry (Chen et al., 2015). Higher pyrolysis temperatures enhance the liming effect of biochar, which generally increases plant available phosphorus (P) and potassium (K) on acid soils and reduces mobility and bioavailability of common toxic metals (Cardoen et al., 2015). Biochar, particularly those generated at relatively high pyrolysis temperatures, also improve soil water retention capacity (WRC) and plant available water content in soils by directly storing water in the pores and indirectly by rearranging soil particles (Zeshan et al., 2014).

2.6.1 Effect of Biochar on Soil Physical Property

Biochar has been shown to improve soil physical structure such as soil aggregate stability and porosity, water-holding capacity and tensile strength and penetration resistance, and soil infiltration and reduce runoff and decrease erosion, soil bulk density, particle sedimentation, specific surface area, thermal properties (Cardoen et al., 2015).

Biochar influence on soil bulk density: Bulk density is an extent of how closely soil particles are compelled together. It is a ratio of mass of oven dry soil to bulk volume (volume of soil particles + volume of pore spaces) (Cao et al., 2018). Bulk density of soil has a substantial effect on soil properties as well as on plant growth e.g., Soils with high bulk density (>1.6 Mg cm-3) has fewer capacity to absorb water and offer great penetration resistance to plant root into the soil, eventually soil characteristic as well as plant growth will be affected (Zeshan et al., 2014). Zeshan et al. (2014) found out that, application of biochar in both years reduced bulk density and increased porosity of the soil significantly compared with the control.

Biochar reduced bulk density and increased porosity as the levels of the biochar increased with 30 t ha− 1 biochar having the least bulk density and highest porosity. (Mukherjee, A, Lal. R., 2013) biochar application decreased the soil bulk density because porosity of biochar is very high and when it used in soil it significantly decreases bulk density by increasing the pore volume whereas (Leonard., 2013) concluded that by increasing the rate of biochar application bulk density was also significantly decreased.

2.6.2 Effect of Biochar on Soil Chemical Property

The soil's chemical properties are inherited from the processes of soil formation, during weathering and transport of the parent material from which the soil has formed (Ameloot, 2013). Thus, the chemical nature of the rocks and minerals and the intensity of the weathering processes are fundamental in determining the chemical properties of the soil (Canal et al., 2020). Application of biochar increased soil chemical properties in the amended plots relative to the control in both years except pH and N in 2017. Also, in both years (except the case of no significant differences between 10 and 20 t ha− 1 biochar levels for N, P, K, and Mg in 2017), biochar increased soil OM, N, P, K, Ca, Mg, and CEC from 0–30 t ha−1 (Aruna et al., 2020).

There were no significant differences in the pH values between 20 and 30 t ha− 1 biochar. The values of SOM, N, P, K, Ca, Mg, and CEC in 2018 were significantly higher than those of 2017 (Aruna et al., 2020). Ameloot, (2013) came into conclusion that, biochar applied at low level do not influence the pH significantly whereas yearly biochar influence on soil chemical property increases. Moreover, some studies showed that biochar addition to soils may influence native soil organic matter (SOM) mineralization (Zimmerman, 2010).

2.6.3 Effect of Biochar on Biological Properties of Soils

The functioning of different biological communities within soils is a complex field of study. The following positive effects have been documented:

1. Improved biological N fixation (rhizobia) (Brewer et al., 2014).

2. Enhanced colonization of mycorrhizal fungi.

3. Earthworms showed preference for biochar amended soils.

4. Raising CH4 uptake.

5. Potential catalyst in lowering N_2O to N_2.

Bian et al. (2018) stated that evidence exists to show that increasing biochar amendments to soil can increase the proportion of N derived from fixation by Phaseolus vulgaris (common green bean) and this increased yields. When preparing acidic soils, the increased alkalinity effect of applied biochar, could help to increase rhizobia numbers, especially when they function optimum in neutral pH (Bhuvaneshwari et al., 2019). According to earthworms show a very distinct preference for biochar amended ferrosol soils, when compared to the control. Ahmad et al. (2020) stated that increased CH_4 uptake was beneficial and available immediately after fresh

biochar application to soil. The reason for the increased CH_4 uptake is unclear. It has been recommended by (Ahmad et al., 2020), that biochar enhances soil aeration, and thus decrease CH_4 production and increase CH_4 oxidation. There have been hypothesised that biochar may have the potential to catalyze the reduction of N_2O to N_2 (Bian et al., 2018), however did not discover supporting proof to these arguments.

This could be due to the fact that we are dealing with case specific scenarios and that each soil type will be affected differently according to the biochar (feedstock and pyrolysis needs to be defined) used and the amount applied under specific climatic conditions (Ahmad et al., 2020). Soils can be observed as complex communities of organisms which are repeatedly shifting in response to soil characteristics and climatic and management factors, especially the addition of organic matter (Bian et al., 2018). Conversely, addition of biochar to soils is probably to have different effects on soil biota (all organisms living within the soil) contrast to addition of fresh organic matter (biomass) (Bian et al., 2018). The differences arise because of the relative stability of biochar and the general lack of energy and biologically useable carbon in comparison with fresh organic matter. Nevertheless, addition of biochar to soils affects the abundance, activity and diversity of soil biotic communities. Biochar addition to soils can stimulate microorganism activity in the soil, potentially affecting the soil microbiological properties (Bian et al., 2018). Relatively supplying microorganisms with a prime source of nutrients, biochar is considered to improve chemical and physical environment in soils to provide microbes with a further favorable habitat (Krull et al., 2010).

Though biochar effects on soil biological processes are not well understood (Lehmann et al., 2011), biochar amendments have been shown to increase microbial biomass due to the presence of labile C fractions and un-pyrolysed feedstocks (Luo et al., 2013; Bruun et al., 2011).

Contrarily, other studies have reported that biochar has no effect on soil microbial biomass as a result of its recalcitrance (Bruun et al., 2011). Dempster, (2012), reported that biochar amendments reduced soil microbial biomass induced by a toxicity effect whiles (Lehmann et al., 2011), concluded that biochar application rates and soil type also affected or response to soil microbial biomass. Explanations for soil microbial biomass change in response to additions of biochar include enhanced available soil nutrients (P, Ca and K), adsorption of toxic compounds and improved soil water and pH status, all of which can influence the activity of soil microorganisms (Lehmann et al., 2011).

2.6.4 Effect of Biochar on Root-Knot Nematode Control

A 1.2 % concentration of biochar added to the potting medium of rice was found to be the most effective at reducing nematode development in rice roots, whereas direct toxic effects of biochar exudates on nematode viability, infectivity or development were not observed (Elad et al., 2011). The increased plant resistance was associated with biochar-primed H_2O_2 accumulation as well as with the transcriptional enhancement of genes involved in the ethylene (ET) signaling pathway Bruun et al., 2011). The increased susceptibility of the Ein2b-RNAi line, which is deficient in ET signaling, further confirmed that biochar-induced priming acts at least partly through ET signaling. Biochar was found to have a high sorption capacity for dichloropropene, a strong anti-nematode fumigant (Graber et al., 2011). As a result, biochar-amendment to the soil can increase the required dose of dichloropropene to efficiently control nematodes (Graber et al., 2011). Biochar does not contain an indigenous consortium of microorganisms that can potentiate disease suppression, and the potential methods by which biochar induces systemic plant defenses against microbes has been documented in a review by (Lehmann et al., 2011). The suppression

of soil pathogens by biochar may stem from several mechanisms, including improved nutrient solubilization and uptake, which helps enhance plant growth and resistance to the stresses of pathogens; microbe stimulation, which promotes direct competition or parasitism against pathogens; or induced plant defense mechanisms (Elad et al., 2011).

2.6.5 Biochar Effect on Plant Growth

Black carbon (BC) being a main component of biochar is produced by incomplete combustion of organic matter (OM) (Bruun et al., 2011), which is relatively resistant to decomposition and degradation due to its condensed aromatic structure. Soil OM of terra preta is composed of up to 35% of BC (Bruun et al., 2011). Terra preta sites exhibit mean biochar contents of 50 Mg ha–1 at 1 m soil depth, which is ≈ 70 times the number of surrounding soils (Elad et al., 2011).. Therefore, biochar addition to soil can be a potential strategy for long-term C sequestration while improving ecosystem services. Biochar has proven positive effects on nutrient retention cation-exchange capacity (CEC) (Elad et al., 2011), water-holding capacity (Major et al., 2010), soil microbial and mycorrhizal activity soil acidity, electric conductivity when applied to soils all of which improves soil fertility and thereby plant growth.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at the University for Development Studies Nyankpala Campus in the Northern Region of Ghana. According to the Ghana Statistical Service (2013), the Northern Region of Ghana is the country's largest administrative territory, covering 70,384 km^2 and possesses the largest (29.5%) portion of the country's land mass. A large number of farmers in the region raise livestock, and the majority relies heavily on rainfall to sustain their agricultural activities (MOFA, 2015). The region is located at Latitude: 9°29' 59.99" N and Longitude: 1° 00' 0.00" W and shares boundary with Bono Region to the south and the Upper East Region to the north. The region also shares an international boundary to the east with Togo and with Cote d'Ivoire to the west. Minimum temperatures in the northern Savannah ecological zones in Ghana has increased by 3.7 % between 1960 and 2010. Annual mean precipitation in the Guinea Savannah ecological zone, which includes the Northern Region, decreased by 120 mm over the same period (Environmental Protection Agency, 2015). The region is usually dry over a longer period, with only one rainy season usually occurring from May to October with an annual rainfall amount of 750 to 1050 mm (Ghana Statistical Service, 2013). Nyankpala, where the experiment was conducted, is a town in the Tolon District, about 16.0934 kilometers south-west of Tamale, the capital of the Northern Region of Ghana. Nyankpala is located between the coordinates 9°24'N and 0°59'W with an elevation of 560 ft (170 m).

3.2 Root-Knot Nematode Soil and Bioassay of Selected Vegetable Growing Sites

3.2.1 Root-Knot Nematode (RKN) Soil and Bioassays

In order to ensure that the soil used for the experiments is naturally infested with RKN, soil and bioassays were conducted. The soil samples were collected from "Water Works", a major vegetable growing area in Tamale. Prior to the collection of soil samples from the farmers' fields, some vegetable plants including cabbage, okra and tomatoes were uprooted for visual assessment. The roots showed the presence of galls which is an indication of the presence of RKN in the soil at that location. In all, twelve soil samples were collected with the aid of soil augur at a depth of 0-15 cm, following a zigzag pattern. The soil samples were then taken to the Nematology Laboratory, Kwame Nkrumah University of Science and Technology, for soil assay. The soils used in all the experiments conducted in this study were collected from the same field at the "Water Works".

Fig 3.1: Collection of soil samples (plate 'a') from okra plots (plate 'b') at 'Water Works' vegetable farms, Tamale.

3.2.2 Soil and bioassays procedures

The samples were spread on separate trays in the laboratory to air dry. After this, the set up was done for the nematode extraction to start using the tray method (Fig. 3.2). The materials used were plates, /trays, two ply tissue paper, plastic basket, water, soil and beakers. After the set up was done the water was allowed to settle for 48 hour. After 48 hours, the water that settled in the trays was poured into the beakers and allowed to settle for 24 hours. Then, the suspended water was poured out gently without shaking the beaker leaving the debris at the base of the beaker. Fifty (50) ml was pipetted onto the counting tray and by the help of the stereo microscope, multiple tally counter, the RKN nematodes in each of the samples were countered.

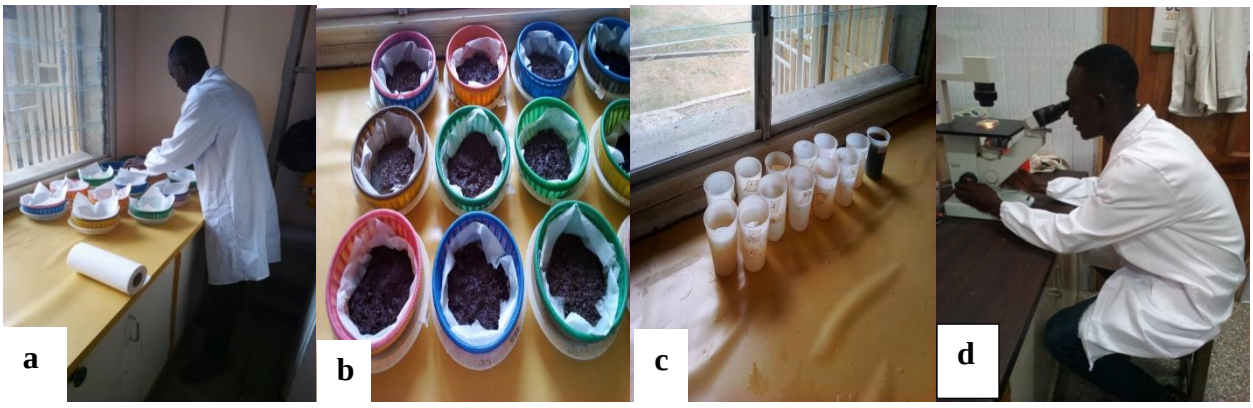

Fig 3.2: Setup for nematode extraction (plate 'a, b and c') and counting (plate 'd') at the nematology laboratory, KNUST, Kumasi, Ghana

The bioassay was conducted in a greenhouse at the Plant Pathology Section of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology in Kumasi. The soil collected from "Water Works" in Tamale was transported to Kumasi for this study. The soil was sieved to remove debris and filled in 1 L capacity plastic pots which were provided with drainage holes. Tomato seeds (Var. Power) were sown in the pots and raised for six weeks. Then, the seedlings were gently uprooted, washed in water and the roots observed under a hand lens for the

presence of galls. This activity confirmed the presence of RKN in the soil used for the experiments.

3.3 Determination of Soil Physical and Chemical Properties

3.3.1 Soil pH (Glass Electrode Method)

The pH of a solution is the negative logarithm of the hydrogen ion activity, which is usually measured potentiometrically. Furthermore, pH value determination is carried out by measurement of the potential difference between electrodes immersed in standard buffers and test solutions (IUPAC, 1993). In the measurement of pH, Glass Electrode finds wide applicability as it shows an immediate response to rapid changes of hydrogen ion concentrations even in poorly buffered solutions. The pH value has long been used to evaluate the acidity/alkalinity of soil and has long been accepted as one of the standard criteria for characterizing soils.

Electrometric method was employed here for this analysis, two forms of soil to solution ratios 1:1, 1:2, or 1:2.5 are involved and either could be used upon request (IUPAC, 1993).

3.3.1.1 Reagents and Apparatus Used

The pH meter, Glass electrode, 50 ml beakers, stirring rods, Spatula and distilled water were used.

3.3.1.2 Procedure

1. Calibration of pH meter was undertaken by immersing electrodes in buffers of pH 4.0 and pH 7.0 respectively.
2. 10g of air-dried soil was weighed into a 50ml beaker.

3. 25mls of distilled water was added and the suspension was vigorously stirred for 20 minutes.
4. Suspension was allowed to settle for about 30 minutes by which time most of the suspended clay have settled.
5. The electrode of the pH meter was inserted into the partly settled suspension.
6. The pH value was recorded.

3.3.2 Cation Exchange Capacity

Methods for measuring Cation Exchange Capacity (CEC) at pH 7 with Ammonium Acetate. Advantages of pH 7 Ammonium Acetate CEC: Many state agencies have traditionally required CEC to be measured by this procedure and a large database exists for soil CEC by this method. This method can readily and cost effectively be implemented by most soil testing laboratories. Disadvantages of pH 7 Ammonium Acetate CEC: The main problem with this method is that, it buffers soil pH at 7.0. Thus, this method will only approximate CEC if a soil's pH is 7.0 and can result in large overestimates of CEC for the many acid soils (Bašić et al., 2011).

3.3.2.1 Equipment (Flame photometer)

1. Funnel filtration apparatus.

2. Balance. 3. 250- and 500-mL Erlenmeyer flasks.

3. Weighing scale.

3.3.2.2 Reagents:

1. 1 M KCl replacing solution: Completely dissolve 74.5 g KCl in distilled water and dilute to a final volume of 1 L.

2. Ethanol, 95%.
3. 1 M ammonium acetate (NH_4OAc) saturating solution: Dilute, in a chemical hood, 57 mLs glacial acetic acid (99.5%) with ~800 mL of distilled H_2O in a 1 L volumetric flask.

Add 68 mL of concentrated NH_4OH, mix and cool. Adjust pH to 7.0 with NH_4OH if needed and dilute to 1 L.

3.3.2.3 Procedure

1. 10.0 g of soil was added to the filter paper on 250 mL Erlenmeyer flask.

2. The soil was leached with 5 separate 25 mL in addition of 1 M KCl,

3 The leaching was repeated slowly 4 times and the leachate was discarded.

4. The soil was washed with 5 separates adding 95% ethanol to remove excess saturating solution. Enough of the ethanol was added to cover the soil surface, and each addition was allowed to filter through before adding more. The leachate was discarded and the receiving flask was cleaned. This process was repeated twice.

5. Leach with 25 mL of the 1 M NH_4OAc,

6. The soil was gently washed four times with 25 mL of the NH_4OAc, allowing each addition to filter through but not allowing the soil to crack or dry.

7. The flame photometer was used to determine Potassium in the leachate as CEC (Cmol+/kg)

3.4 Preparation of Biochar

Rice husk was collected from a rice milling factory in Nyankpala and used to produce the biochar used for the research work. The Kuntan (Japanese stove) pyrolysis method of biochar

production was used in producing the biochar and about 100kg of the biochar was produced and stored for use.

Fig 3.3: Preparation of biochar (plate 'a, b and c') at the Horticulture Department, UDS, Ghana.

3.5 Source of Seeds Used in the Experiment

The seeds of 'Rio Grande' tomato variety, which were obtained from Wumpini Agro Chemical Enterprise, was used as the test crop in this research. The variety has large pear-shaped fruits that mature to deep red in a period of 90 days after sowing.

3.6 Bio-Nematicide Used in the Experiment

The peptide-based nematicide (Nemanol 201) used for the experiment was obtained from Telluris Biotech India, for the purpose of this study. The recommended formulation is mixing 10 mL of the bio-nematicide product to 20 L of water. For field application, 20 mL of the mixture is applied in the planting hole a day or two before transplanting. This formulation is applied at the same 20 ml per plant at 25 days after transplanting. The second application is done by spreading the 20 ml per plant around the root zone of each plant.

3.7 Experiment One: Impact of biochar rates on the effectiveness of Nemanol on root-knot nematodes, yield and yield components of tomato.

A pot experiment was conducted to determine the impact of biochar rates on the effectiveness of Nemanol on root-knot nematodes, growth and development. This experiment was terminated at the seedling stage 70 days after sowing whiles the field experiment was monitored until fruit harvest.

3.7.1 Experimental Designs and Treatments Used in the Pot Experiment

The growing medium was prepared by amending the soil with 0%, 1%, 3% and 5% w/w biochar and the various treatments were used in filling the plastic pots.

3.7.2 Application of Nemanol Treatments

Nemanol was used is in the liquid form. A mixture of 2.5 ml of the peptide plus 997.5 ml was prepared to obtain 1 L of the utilizable solution. The application of the peptide was done by gently spreading 5 ml of the solution in the planting hole before sowing the seeds. The application of 5 ml of nemanol was repeated at 25 days after sowing as recommended by Telluris Biotech India. The various treatments used for the study were as follows;

T1 = Soil with no biochar and no peptide

T2 = Soil with 5 ml peptide but no biochar

T3 = Soil with 1% biochar and 5 ml peptide

T4 = Soil with 3% biochar and 5 ml peptide

T5 = Soil with 5% biochar and 5 ml peptide

3.7.3 Experimental Design

The treatments were replicated three times and arranged in completely randomised design. In all, 150 plastic pots were used in this experiment which was started in a planthouse but the pots were later moved outside and kept under shade net. Each pot was filled with either 800 g soil (no biochar) or 800 g of a mixture of soil plus the required amount of biochar. With the exception of T1, each pot was supplied with 5 ml of nemanol which was spread in the plating hole, about 0.5-1 cm deep. The seeds were sown 24 hours after the peptide application. The application rate was reduced from 20 ml per plant to 5 ml per plant because (1) The volume of soil used per pot was limited compared to planting in field and two (2) Seeds were sown directly and not seedlings as in the case of field planting of tomato. At 25 days after sowing, the second dose of nemanol was applied using the same rate of 5 ml per plant. This was also spread gently around the root zone of each plant.

3.7.4 Sowing and Management of Plants

Two seeds were sown directly in each pot after which watering was done ones a day to promote seedling emergence and plant growth. Weeds were removed manually as and when necessary. After 2 weeks of sowing, the seedlings were thinned to 1 seedling per pot.

3.7.4.1 DATA ANALYSES

The data was analyzed using GenStat (General Statistics) Twelfth Edition. It is a statistical software package with data analysis capabilities, particularly in the field of agriculture science. The analyzed data was then presented in the form of tables and bar-graphs where applicable.

3.7.5 Data Collection

3.7.5.1 Seedling Vigor

% seedling emergence was determined by counting the number of seedlings that emerged by the 5th day after sowing. The % seedling emergence was calculated by dividing the number of emerged seedlings over the number of seeds sown multiplied by 100.

3.7.5.2 Plant Height: Plant height was measured using tape measure and was done at 7 days intervals.

3.7.5.3 Number of Leaves: Number of leaves was counted at 7 days intervals.

3.7.5.4 Stem Diameter: Stem thickness was measured using venire calipers and was done at 7 days intervals.

3.7.5.5. Fresh Shoot and Root Weights of Seedlings: The weight of fresh shoots and roots of the tagged seedlings were determined at 70 days after sowing using an electronic scale. The weights obtained were divided by the number of shoots /roots weighed and the average shoot weight per plant obtained and recorded.

3.7.5.6. Dry Shoot and Root Weights: The fresh shoots and roots harvested at 70 days after sowing were placed in an oven at 80°C for 48 hours after which the dry roots were measured using electronic scale. The weights obtained were divided by the number of sampled shoots/roots for average shoot or root weight per plant.

3.7.5.7. Root-Knot Nematodes Assessment

3.7.5.7.1 Initial and Final Root-Knot Nematode Population in Soil:

Before the experiment, soil samples (500g each) were collected from vegetable growing area and the RKN were extracted and counted.

3.7.5.7.2 Severity of Root Galling: This was done at 70 DAS by using the 1-5 rating scale described by Bridge (1980) as follows:

1. No galls observed, feeder roots intact
2. At least one gall observed
3. Numerous galls observed, about 50% of roots affected
4. Numerous galls, most roots affected
5. Heavy galling on most roots, with necrosis and feeder roots heavily affected or absent

3.7.5.8 Soil Chemical Analysis

a. Soil p^H and Cation exchange capacity: At 70 DAS, soil samples (500g each) of the various treatments were carried to the laboratory for Soil p^H and Cation exchange capacity analyses.

3.8 Experiment Two: Effect of different rates of biochar on the effectiveness of peptides in root-knot nematode control and yield and yield components of tomato grown under field conditions.

3.8.1 Raising of Seedlings

Seedlings were nursed in seedling trays in a Planthouse (Temperature 22-38^{o}C, RH 60-65%), at the University for Development Studies. Pasteurization of nursery soil was done by using an electronic oven set at 65^{o}C for 48hours to kill soil-born micro-organisms. The pasteurised soil was used to fill the seedling trays and the seeds sown. Pricking out was done by moving seedlings from the seedling's trays to a nursery bed where they had enough space to grow well. Prior to pricking out, the prick out bed was also pasteurised by burning a heap of dry grass for about 30 minutes.

3.8.3 Preparation of Field, Transplanting and Management of Crop

The field was prepared by slashing weeds, hoeing, raking and raising of beds. The total land area for the field experiment was 14m x 8m, demarcated into 15 plots (2m x 2m each) in three blocks, separated by 1m walking path. The planting spots were marked and about 10 cm diameter and 15 cm deep of soil was scooped out of the marked spots. The holes created were filled with soil naturally infested by root-knot nematodes which was obtained from a popular vegetable growing site called 'Water Works' in Tamale. Prior to filling the holes, the appropriate biochar rates (equivalent to 0t, 2t, 4t and 6t per ha) were thoroughly mixed with the infested soil. The filled holes were watered and allowed to settle for two days before the first application of nemanol (20 ml per planting hole) was done. The seedlings were then transplanted at 24 hours after the nemanol application. The second application of nemanol was done at 25 days after the

first application as recommended by the peptide manufacturer. The experimental plot was regularly weeded manually to keep weeds under complete control. The plants were staked to prevent them from lodging especially during fruiting. Watering was done as and when necessary to keep the soil moist for good plant growth.

3.8.4 Preparation and Application of Nemanol

The Telluris peptide nematicide (nemanol) used is in the liquid form. A 2.5 ml of the nemanol was mixed with 997.5 ml of distilled to make 1 L of the utilizable solution. The application of the nemanol was done by gently spreading 20 ml of the solution in the planting hole before transplanting. The application of 20 ml of nemanol was repeated at 25 days after transplanting as recommended.

3.8.5 Experimental Design and Treatments

In this experiment, the treatments which were arranged in a randomised complete block design (RCBD) were as follows:

T1 = Only soil with no biochar and peptide

T2 = Soil without biochar plus 20ml of peptide-based nematicide

T3 = 0.8 kg biochar equivalent to 2t/ha soil plus 20ml of peptide-based nematicide

T4 = 1.6 kg biochar equivalent to 4t/ha soil plus 20ml of peptide-based nematicide

T5 = 2.4 kg equivalent to 6t/ha soil plus 20ml peptide-based nematicide

Root-knot nematode infested soil collected from vegetable garden was thoroughly mixed with the various rates of biochar. For T1, the infested soil (5 kg/planting spot) was used without the

addition of biochar and without peptide application. For T2, the infested soil was used without biochar but with the application of 20ml of peptide-based nematicide. For T3, the 5 kg soil was mixed with 88g of biochar (equivalent to 0.8 kg/plot or 2 t/ha) which was then used to fill the planting spot. For T4 177 g of biochar (equivalent to 1.6 kg/plot or 4t/ha) was used to fill the planting spot. For T5, 266 g of biochar (equivalent to 2.4 kg/plot or 6t/ha) was used to fill the planting spot. The application of 20ml of nemanol per plant was repeated at 25 DAT for T3, T4 and T5.

3.8.6 DATA ANALYSES

The data was analyzed using GenStat (General Statistics) Twelfth Edition. It is a statistical software package with data analysis capabilities, particularly in the field of agriculture science. The analyzed data was then presented in the form of tables and bar-graphs where applicable.

3.8.7 Data Collection

3.8.7.1 Plant Growth

3.8.7.1.2 Plant Height: Plant height was measured using tape measure and was done at 7 days intervals.

3.8.7.1.3 Number of Leaves: Number of leaves was done by visual counting and was done at 7 days intervals.

3.8.7.1.4 Canopy Spread: Canopy spread was measured using tape measure calibrated in millimeters and was done at 7 days intervals.

3.8.7.2 Flowering and Fruit Set

3.8.7.2.1 **Days to First Flower Set:** This was done by carefully observing as the plants grow. Number of days to first flower set was counted from the first day after transplanting to the first day the plants started flowering.

3.8.7.2.2 **Days to 50% Flowering:** This was done by carefully observing as the plants grow. Number of days to 50% flowering was counted from the first day after transplanting to the first day at least 50% of the plants per each experimental unit flowered.

3.8.7.2.3 **Days to First Fruit Set:** This was done by carefully observing as the plants grow. Number of days to first fruit set was counted from the first day after transplanting to the first day fruits were observed in each experimental unit.

3.8.7.3 Yield and Biomass Assessment

3.8.6.3.1 Yield per Plot (g): Matured fruits were harvested from the various treatments every three days by gently plucking fruits from the plants.

3.8.6.3.2 **Average Fruit Weight (g):** Fresh fruits harvested were measure using electronic scale.

3.8.6.3.3 **Yield per Hectare (tonnes):** Fresh fruits harvested were measure using electronic scale after which the outputs were being calculated equivalent to tonnes per hectare.

3.8.6.3.4 **Fresh Shoot and Root Weight:** Fresh shoots and roots harvested at 63 days after transplanting were measured for their weight using and electronic scale.

3.8.6.3.5 **Dry Shoot and Root Weight:** Fresh shoots and roots harvested at 63 days after transplanting were placed in an oven at 80°C for 48 hours after which the dry roots were measured using electronic scale.

3.8.6.3.6 **Dry Fruit Weight:** Total number of fruits harvested at 63 days after transplanting per plot were sliced into pieces and placed in an over at the temperature of 65°C for 48 hours after which the dry fruit weight was measured using electronic scale.

3.8.6.4 Root-Knot Nematodes Assessment

3.8.6.4.1 **Severity of Root Galling:** After harvesting, fresh roots were been accessed by carefully observing the level of nematode infection of the roots. The severity of galling was assessed as described in page 51 following J. Bridge chart (1980).

3.9 Experiment Three: Effect of different concentrations of nemanol on root-knot nematode control in pots.

3.23 Study Location: The study was conducted in a field experiment over a period of two months at the University for Development Studies Nyankpala.

3.9.1 Raising of Seedlings

Seedlings were raised in seedling trays in a Planthouse (Temperature 22oC-36^{o}C; RH 65-70%), at the University for Development Studies. Pasteurization of nursery soil was done by exposing the soil to 75^{o}C in an electronic oven to kill soil-born micro-organisms. Seeds were sown in seedling trays and watered as and when necessary.

3.9.2 Pot Experiment

The soil was pasteurised in an oven at a temperature of 75°C for 48 hours, allowed to cool before it was used to fill the pots (800g soil per pot). Six (6) ml of inoculum estimated to contain about 1000 RKN eggs were applied per pot, leaving the control pots without any inoculation. At five days after the inoculation, primed seeds were sown directly into the various pots and monitored for 49 days.

3.9.4 Preparation and Application of Nemanol

The Telluris peptide nematicide (nemanol) is in the liquid form. A mixture of 2.5 ml of the peptide was added to 997.5 ml of distilled to make 1 L of the utilizable solution. The solution was then applied at different rates including 15 ml, 20 ml and 25 ml per plant by gently spreading the solution in the planting hole 24 hours before transplanting. The application of 15

ml, 20 ml and 25 ml of nemanol was repeated at 25 days after transplanting as recommended. The various treatments applied were as follows:

T1 = Soil with no peptide and no inoculation

T2 = RKN inoculated soil with no peptide

T3 = RKN inoculated soil with peptide 15 ml

T4 = RKN inoculated soil with peptide 20 ml

T5 = RKN inoculated soil with peptide 25 ml

The treatments were replicated three times and arranged in completely randomised design in a planthouse. In all, 150 plastic pots were used in this experiment and each pot was filled with 800 g soil.

3.9.6 Data Collection

3.9.6.1 Plant Growth

3.9.6.1.1 Plant Height: Plant height was measured from the soil level to the tip of the apex of the plant using tape measure and this was done at 7 days intervals.

3.9.6.1.2 **Number of Leaves per Plant:** Number of leave per plant was counted at 7 days intervals.

3.9.6.2 Biomass Assessment.

3.9.6.2.1 Fresh Shoot and Root Weight: Fresh shoots and roots were harvested at 49 days after transplanting and the weights measured using electronic scale.

3.9.6.2.2 **Dry Shoot and Root Weight:** The harvested shoots and roots were put in envelops and placed in an oven at the 80°C for 48 hours and the dry weights measured using electronic scale.

3.9.6.3 **Root-Knot Nematodes Assessment.**

3.9.6.3.1 **Severity of Root Galling:** After harvesting, fresh roots were accessed by carefully observing the level of nematode infestation on the roots and scoring using a rating of 1-5 as described by Bridge (1980) as described in page 51.

3.9.6.4 DATA ANALYSES

The data was analyzed using GenStat (General Statistics) Twelfth Edition. The analyzed data was then presented in the form of tables and bar-graphs where applicable.

CHAPTER FOUR

RESULTS

4.1 Initial Nematode spp and their Counts in the Soil Used for the Experiment

This study was done to determine the presence of nematodes in the Tamale 'Water Works' soils. The area has been cultivated to vegetables such as tomato, lettuce, cabbage, okra and carrot for many years. The soil assay showed that the *Meloidogyne* spp was the dominant nematode species whiles *tylenchus* spp. was the least (Fig 4.1). It was found that as high as 400 *Meloidogyne* spp were counted from a sample of 100 g, suggesting that the Tamale 'Water Works' soils were highly infested with root-knot nematodes. Other kinds of nematodes found in the soil were Practylenchus, Helicotylenchus, Free-living, Aphelenchoides and Rotylenchus.

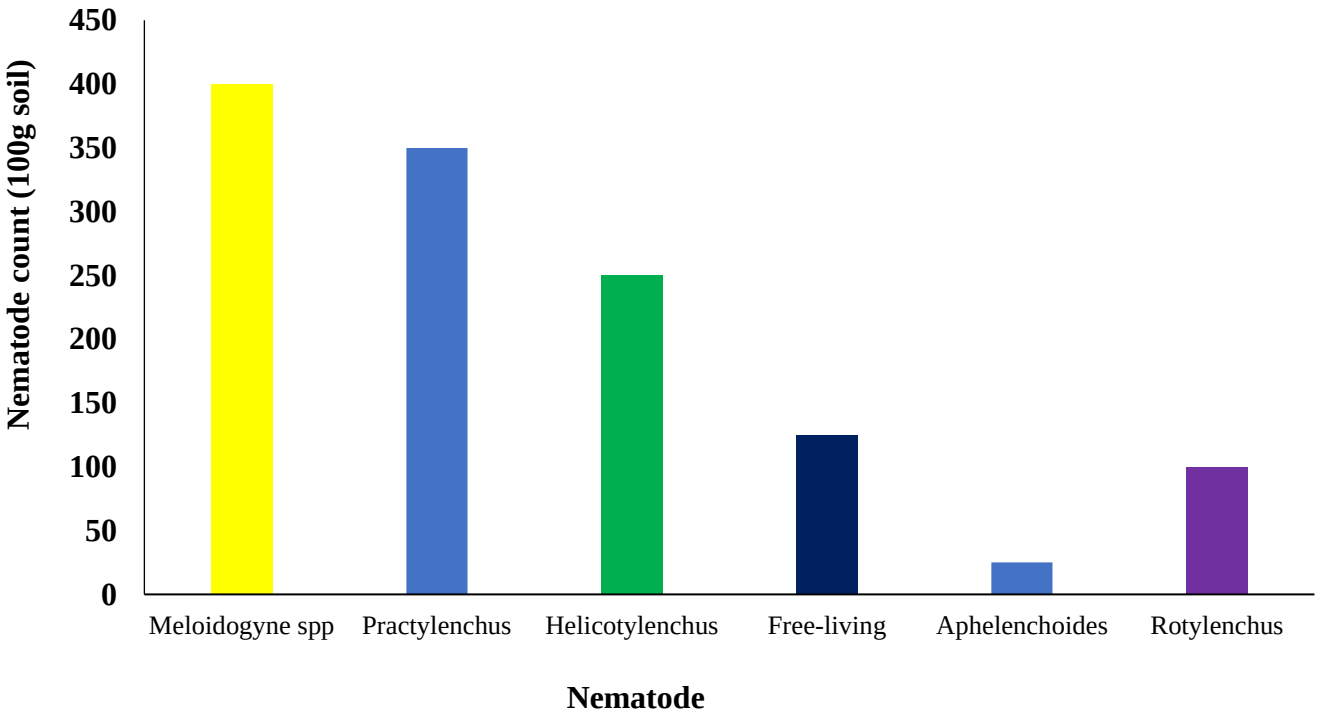

Figure 4.1: Number of nematodes per 100 g samples from 'water works' vegetable farms.

RESULTS OF EXPERIMENT ONE (POT EXPERIMENT)

4.2 Seedling Vigor

This was carried out to determine how the various treatments affected the initial seedling growth at five days after sowing. The results showed that the various treatments did not significantly affect seedling vigor (P> 0.995) in terms of plant height. Even though there were no significant differences recorded among the various treatments, the 3% Biochar + Peptides produced plants which were relatively taller and about 6% taller compared to soil + peptide and 5% Biochar + Peptides treatments (Fig 4.2).

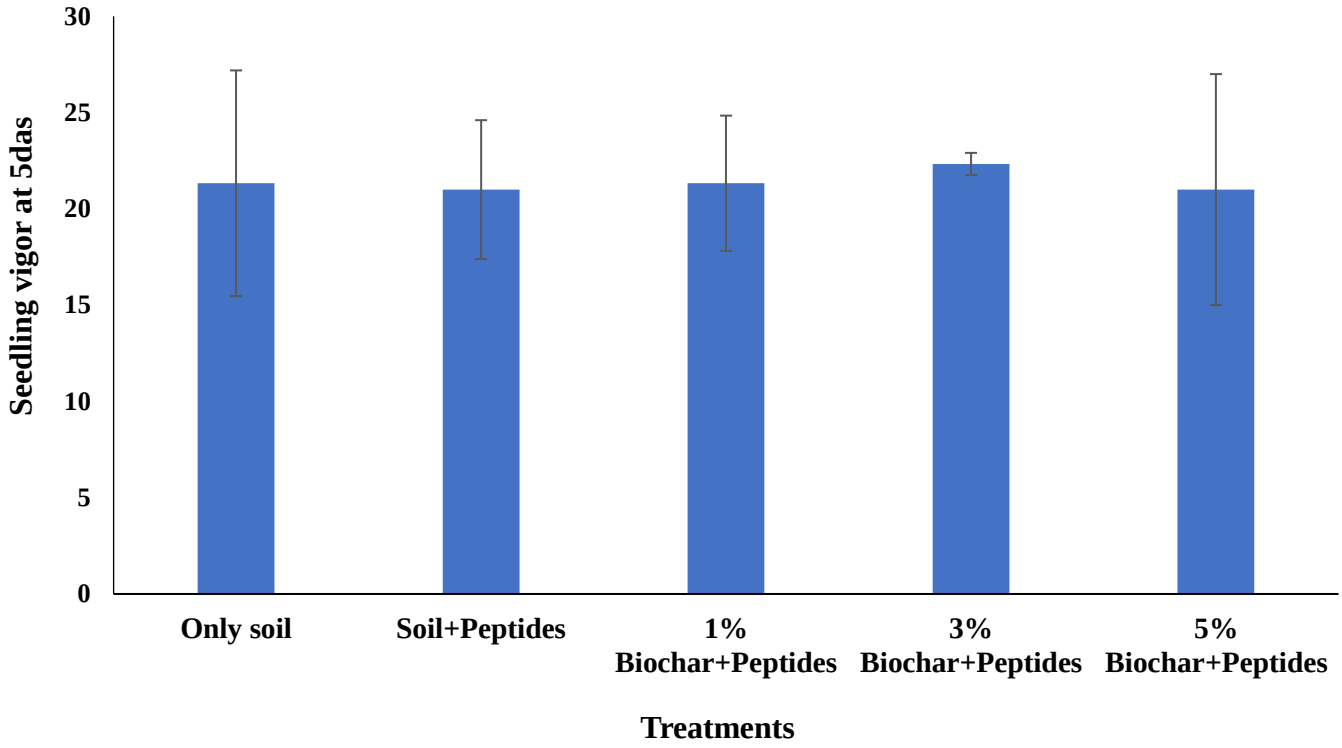

Figure 4.2: Effect of different concentrations of biochar and peptide on germination of tomato seeds. The error bars are represented by standard deviations of the treatment means.

4.3 Plant Height

Figure 4.3 shows that plant height was significantly affected by the different rates of biochar amendments. The 1% Biochar + Peptide treatment gave the highest plant height compared with Soil + Peptide, 3% Biochar + Peptide, Only soil and 5% Biochar + Peptide. The 1% Biochar + Peptides application increased plant height by 14.4% more than the Soil + Peptide treatment which gave the least plant height at 70 days after sowing.

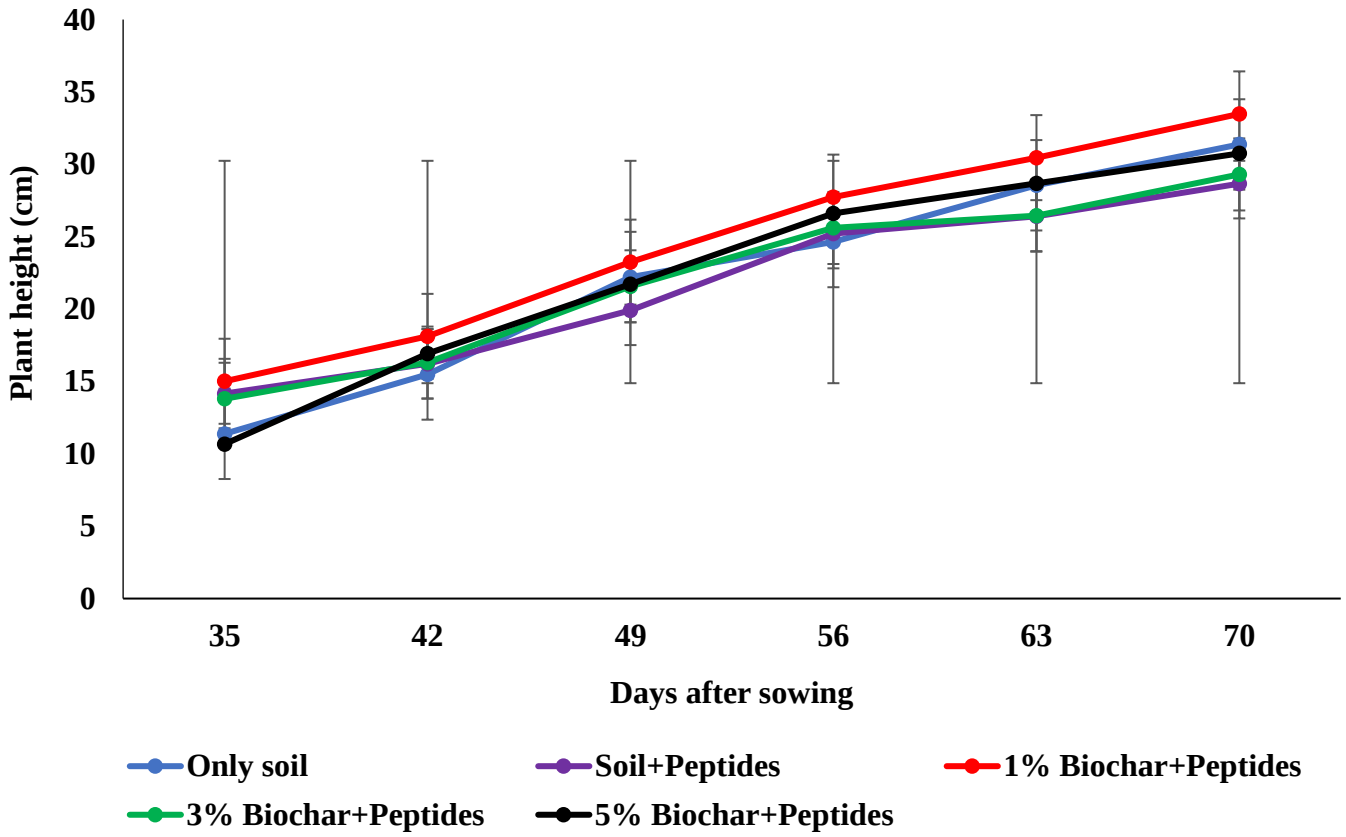

Figure 4.3: Effect of treatments on plant height from 35 to 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.4 Number of Leaves per Plant

The number of leaves per plant was significantly ($P<0.05$) affected by the different treatments (Fig 4.4). The 1% Biochar + Peptide treated plants produced the highest number of leaves compared with the Soil + Peptide, 3% Biochar + Peptide and the Only soil treatments. Remarkably, the 1% Biochar + Peptide treated tomato plants produced 22.6% more leaves than the 3% Biochar + Peptides treated plants which gave the least leaf number per plant.

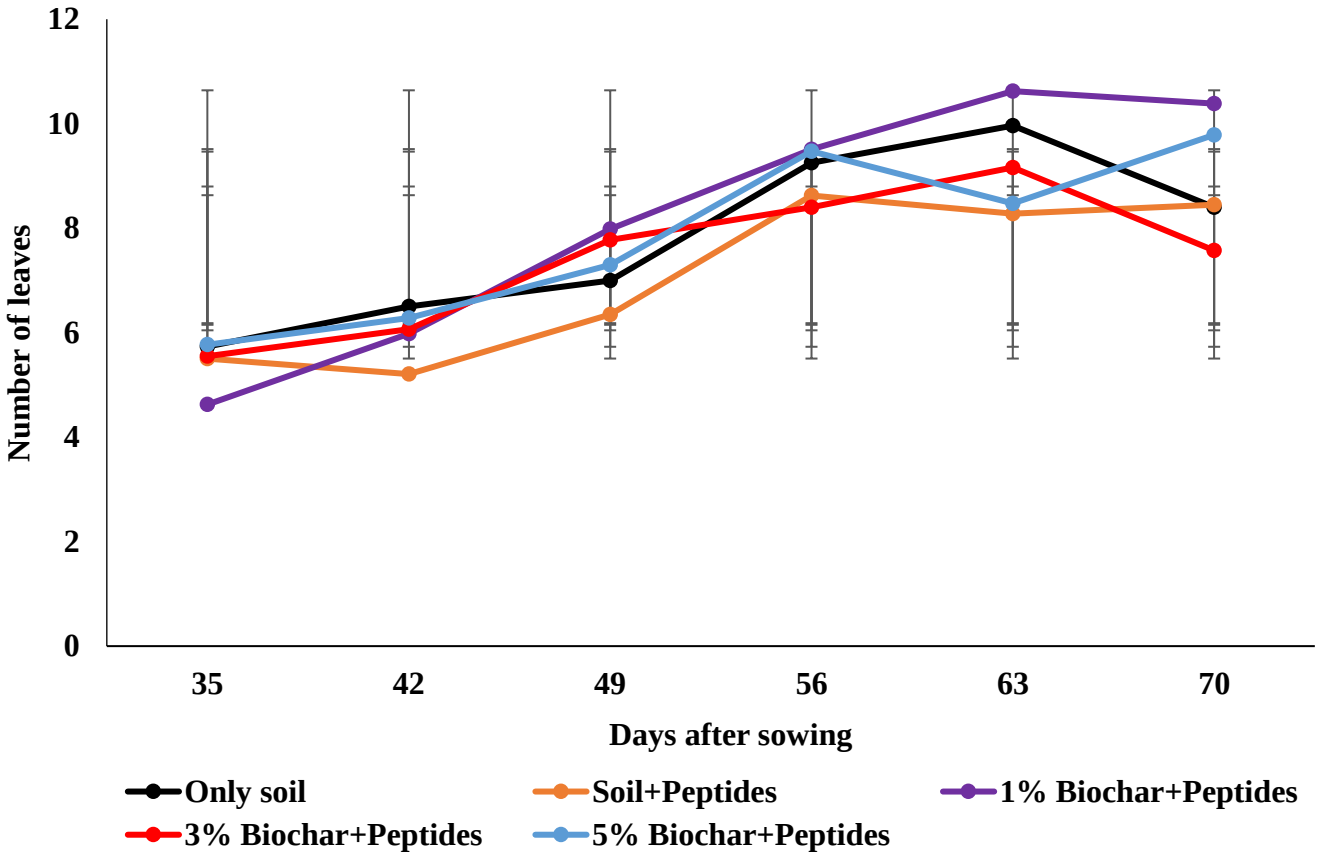

Figure 4.4: Effect of treatments on number of leaves per plant from 35 to 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.5 Stem Diameter

The various treatments significantly (P<.001) affected stem diameter of the plants (Fig. 4.5). The results showed that the 1% Biochar + Peptide, Only soil and the 5% Biochar + Peptide treatments gave the largest stem diameter as compared to the 3% Biochar + Peptide and Soil + Peptide treatments. Moreover, the 1% Biochar + Peptide treatment increased the stem diameter of the plants by 25.3% over the Soil + Peptide treated plants.

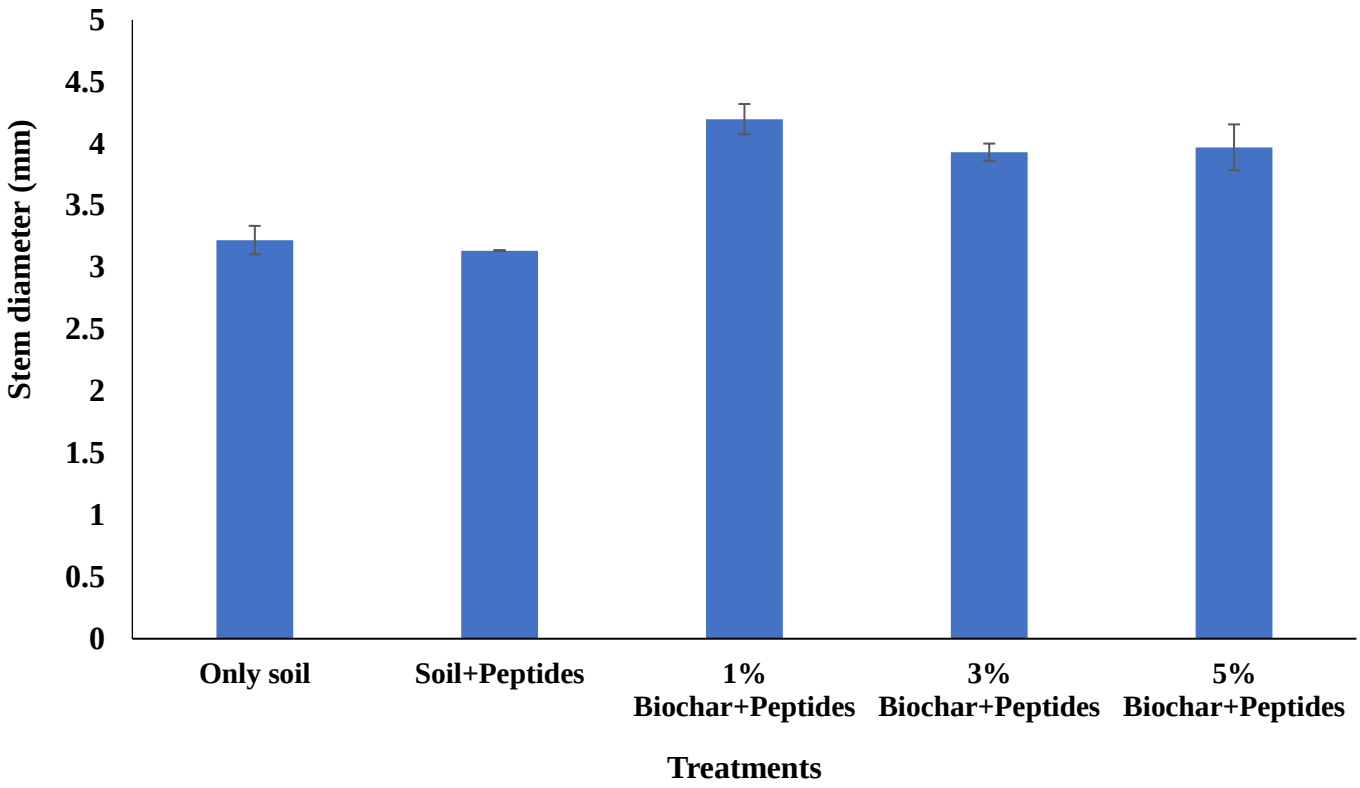

Figure 4.5: Effect of treatments on stem diameter at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.6 Severity of Galling and Root-Knot Nematode Population in Soil

The results of this experiment have shown that there was significant effect of the treatments on severity of galling and root-knot nematode population in soil at 70 days after sowing (Table. 4.1). With respect to the severity of galling, the 1% and 3% Biochar + Peptide treatment were the best in suppressing galling. They decreased galling by 41.8% compared to the untreated (Only soil) plots. Moreover, the 1% Biochar + Peptides treatment greatly decreased RKN population around the root zone of the plants. It also decreased RKN population in soil by 96.5% relative to the control treatment. The results further shows that the 1% Biochar + Peptide treatment decreased egg count by 85.6% compared with the control treatment.

Table 4.1: Severity of root galling and root-knot nematode population in soil at 70 days after sowing.

Treatments	Severity of galling in roots	RKN population in soil
Only soil	4^{a}	250^{a}
Soil+Peptides	3^{b}	125^{b}
1% Biochar+Peptides	2^{d}	25^{d}
3% Biochar+Peptides	2^{d}	32^{d}
5% Biochar+Peptides	3^{c}	75^{c}
P- value	**<.001**	**<.001**
LSD	**0.203**	**2.369**

Treatment means within the columns with the same letters are not significant different from each other.

4.6 Fresh Shoot Weight

At 70 DAS, the various rates of biochar significantly (P = 0.001) influenced fresh shoot weight (Fig. 4.6). The 1% biochar + peptide treatment produced the highest fresh shoot weight whiles the soil+peptide without biochar treatment gave the least fresh shoot weight. Moreover, the 1% biochar + peptide gave 39.4% more fresh shoot weight compared to the soil+peptide treatment. However, the difference between the control and the 5% biochar + peptide and that of the 3% biochar + peptide and the control was marginal.

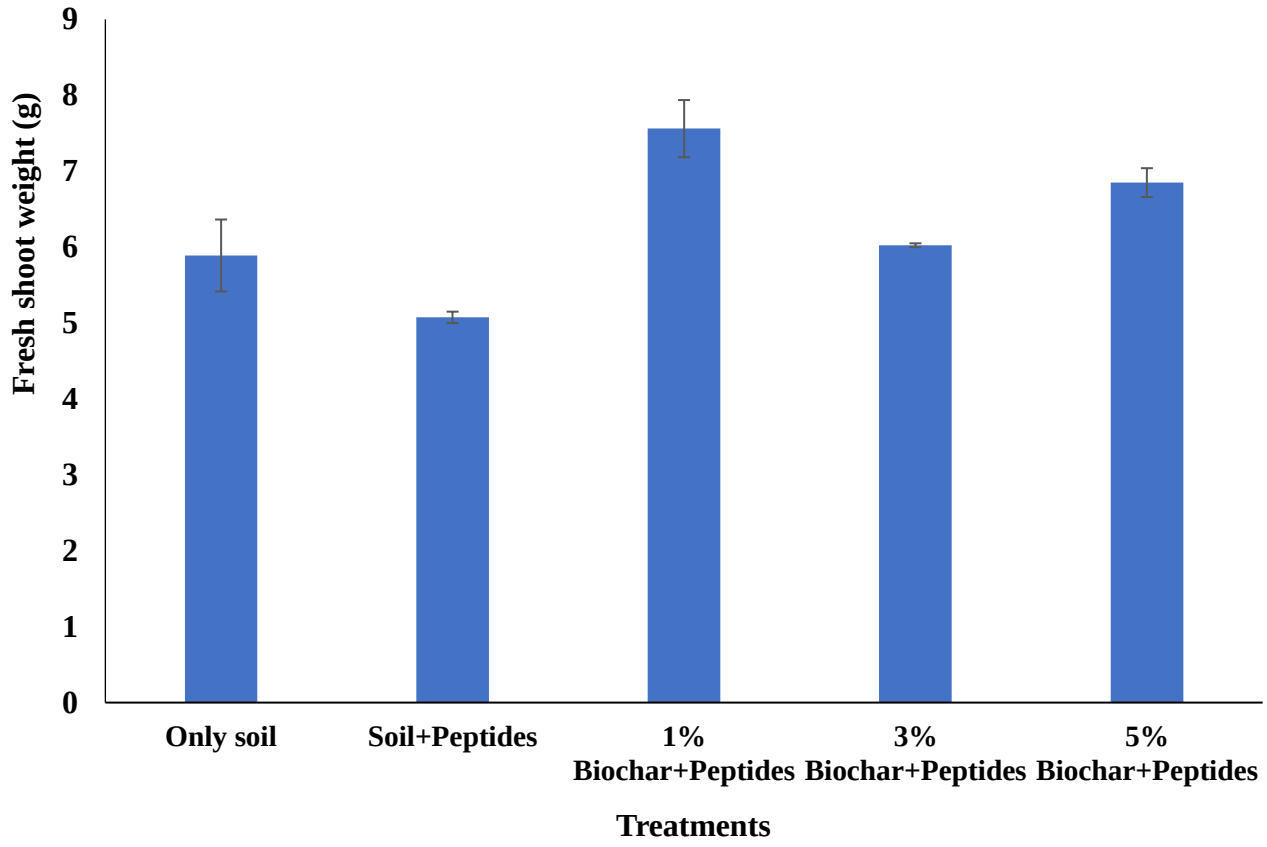

Figure 4.6: Effect of treatments on fresh shoot weight at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.7 Dry Shoot Weight

The results showed that the various rates of soil amendments did not influence dry shoot weight at 70 DAS (P = 0.281) (Fig. 4.7). Although the data showed no significant differences, the 1% Biochar + Peptide treated plants gave the highest dry shoot weight which was 25% more than that of the Soil + Peptide treated plants.

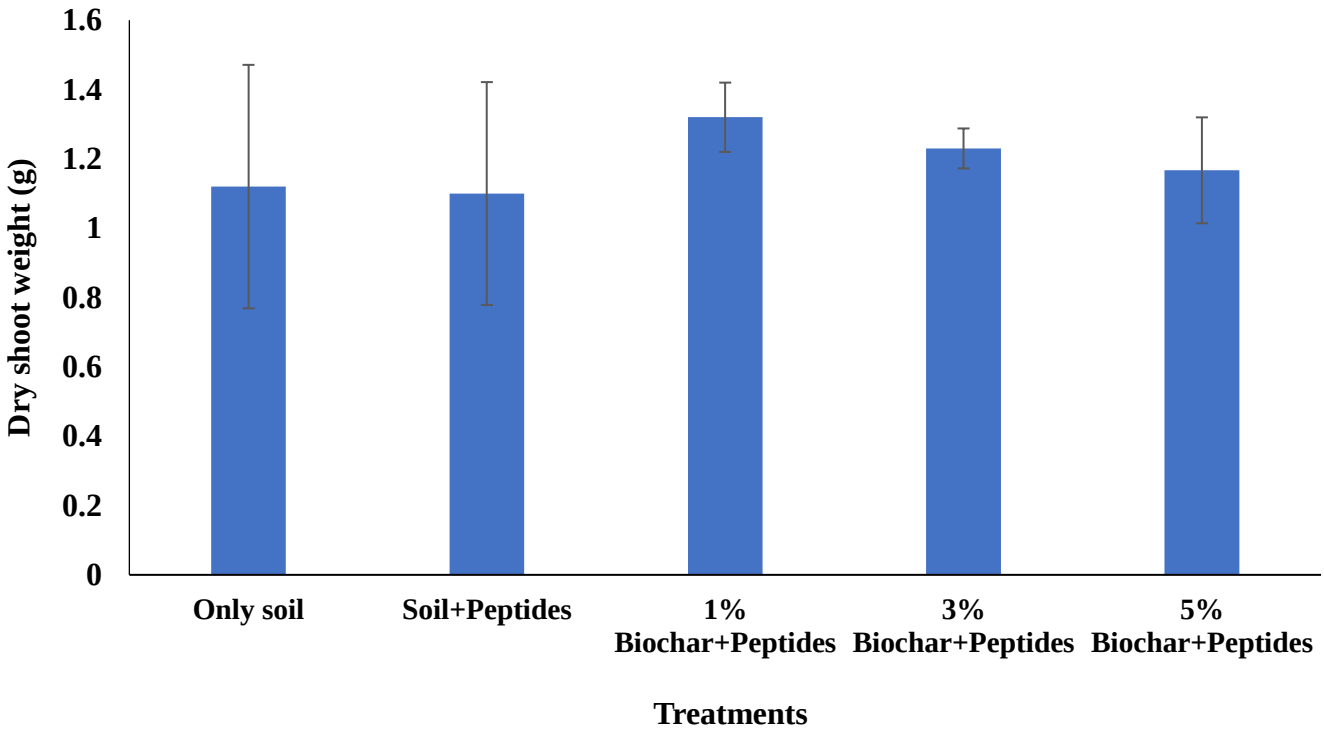

Figure 4.7: Effect of treatments on dry shoot weight at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.8 Fresh Root Weight

The fresh root weight of the plants was significantly (P = 0.001) affected by the various treatments at 70 DAS (Fig. 4.8). The plants from the 1% Biochar + Peptide treated pots gave the highest fresh root weight at 70 DAS compared to the other treatments. The plants from the 1% Biochar + Peptide pots gave 68.9% more fresh root weight than the Soil + Peptide treated plants.

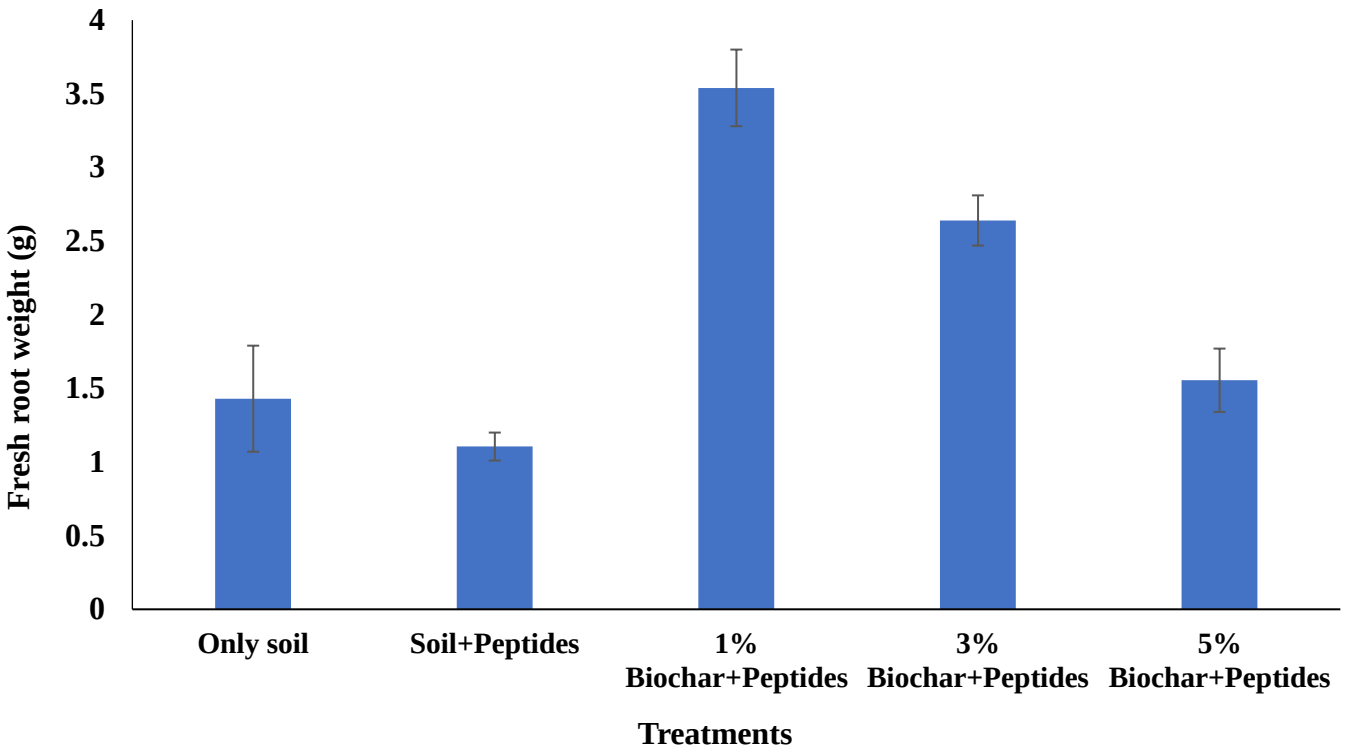

Figure 4.8: Effect of treatments on fresh root weight at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.9 Dry Root Weight

The various rate of soil amendments treatments did not affect (P = 0.390) dry root weight at 70 DAS (Fig. 4.9). Even though the various treatments did not significantly influence dry root weight, the 1% Biochar + Peptide treated plants produced the highest dry root weight which was 41.8% more than that from the 5% Biochar + Peptide treated plants.

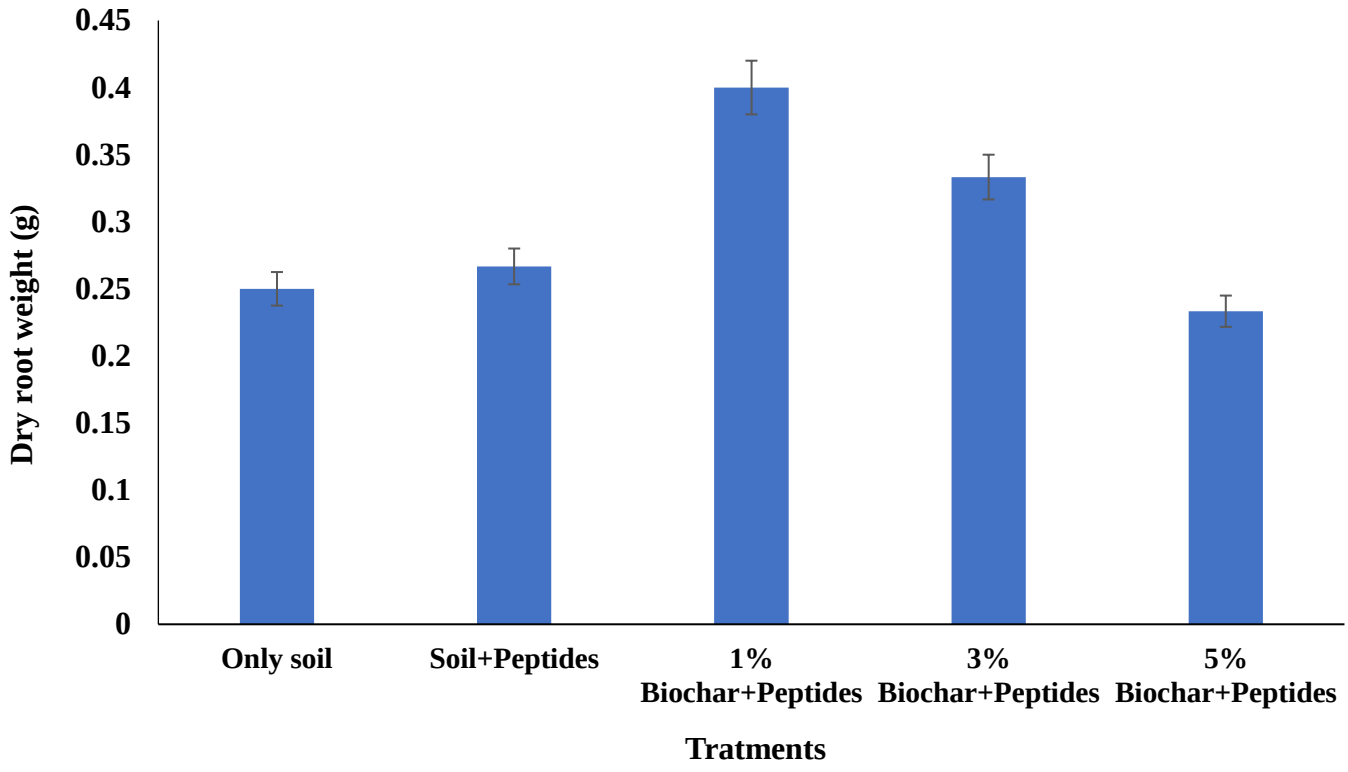

Figure 4.9: Effect of treatments on dry root weight at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.10 Soil pH

The results have shown that soil pH varied significantly (P = 0.002) among the various treatments. The control (only soil) and soil + peptide treatments recorded the lowest soil pH whiles 1%, 3% and 5% biochar + peptide treatments gave the highest soil pH (Fig. 4.10). The treatments without biochar (Only soil and Soil with peptides) had lower soil p^H values than the biochar amended soils. The lower biochar rates (3% and 5%) increased the soil pH from nearly alkaline to slightly acidic which is more suitable for the growth of tomato plants.

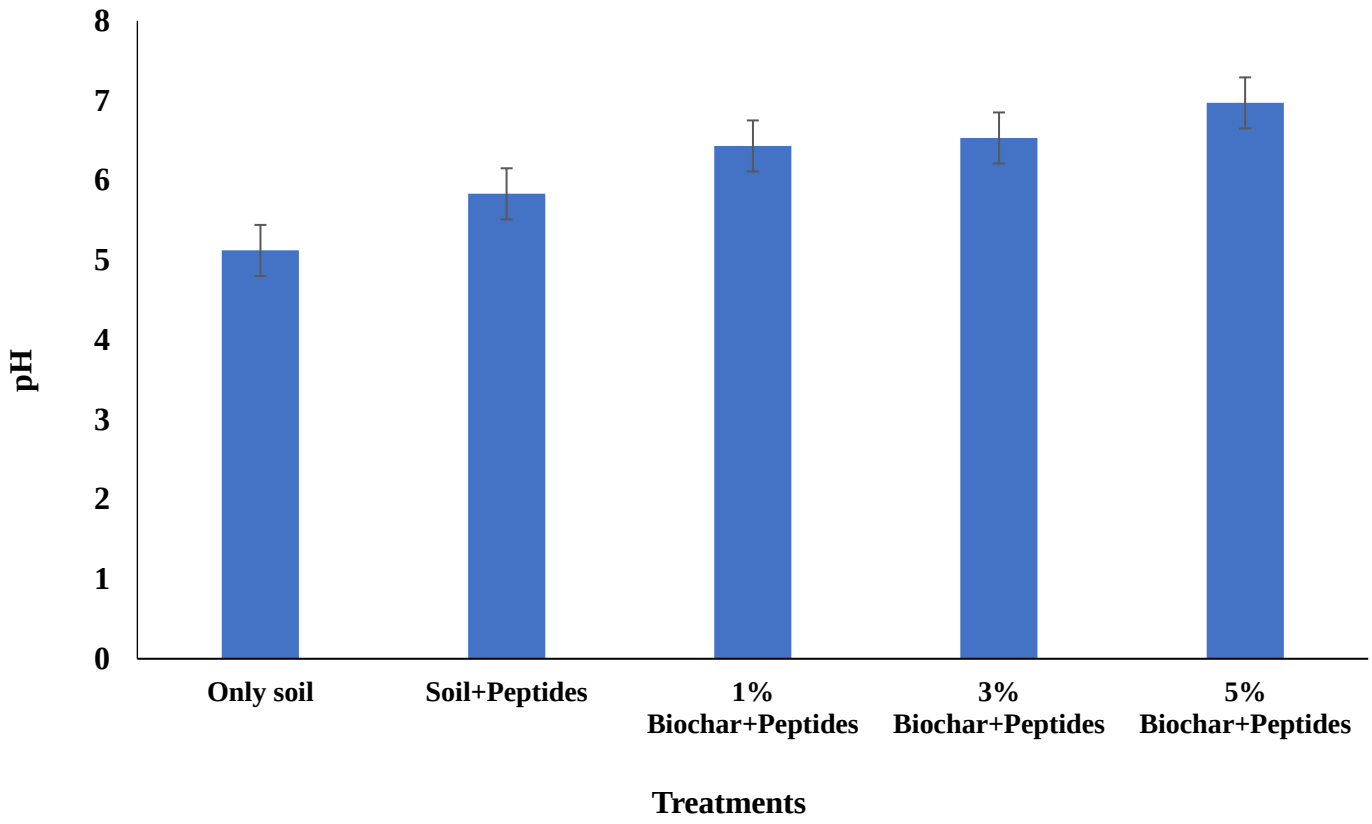

Figure 4.10: Effect of treatments on soil pH at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

4.11 Soil Bulk Density

The results in Figure 4.11 shows that there were no significant differences (P>0.005) among the various treatments with regards to the soil bulk density at 70 days after sowing. This notwithstanding, soil bulk density was highest in the control pots and it was 8.5% higher than the 3% biochar + peptide treated soil.

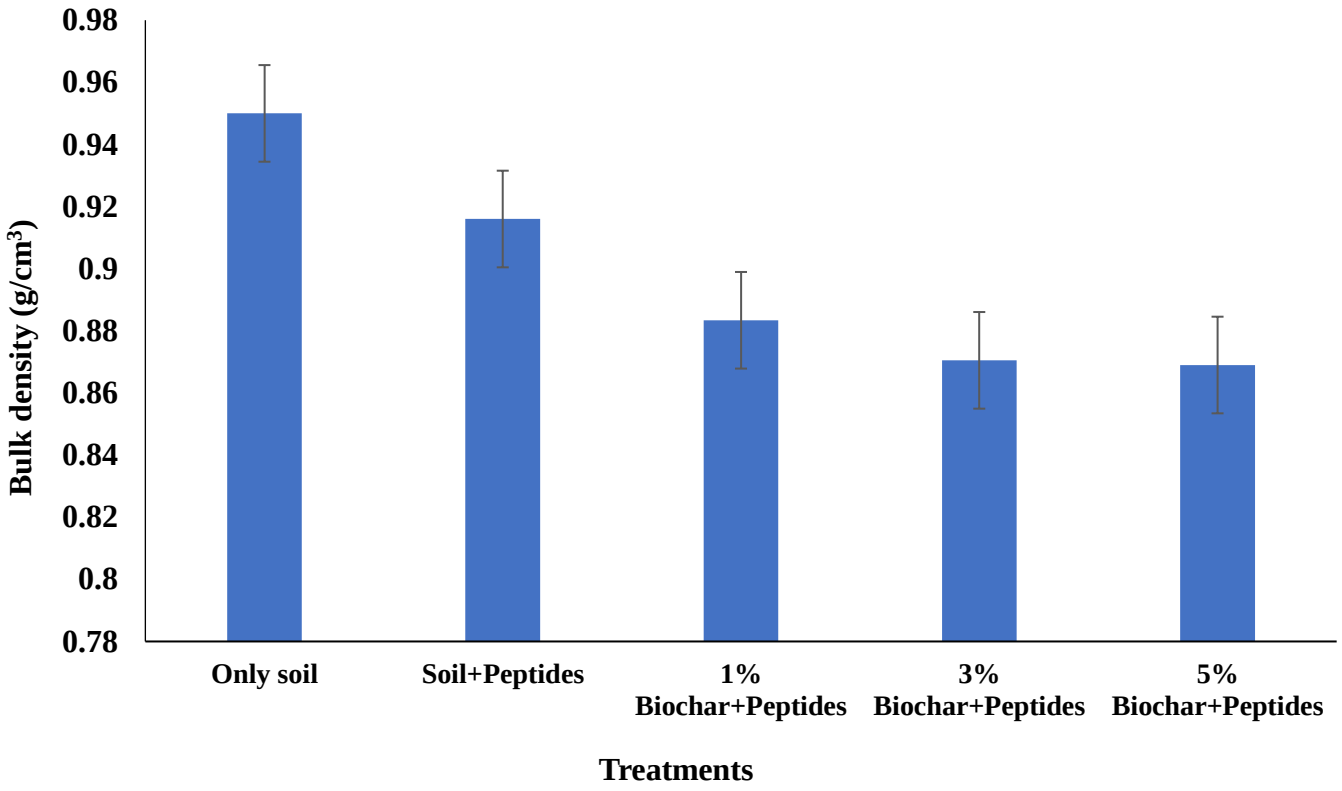

Figure 4.11: Effect of treatments on soil bulk density at 70 days after sowing. The error bars are represented by standard deviations of the treatment means.

RESULTS OF EXPERIMENT TWO (FIELD EXPERIMENT)

4.12 Plant Height

The heights of the plants were significantly ($P<0.001$) affected by the various treatments (Fig. 4 12). The results shows that the lower rate of biochar (2t/ha Biochar + Peptide) treated plants were the tallest compared with the control (only soil), Soil + Peptide, 4t/ha Biochar + Peptide and the 6t/ha Biochar + Peptide treatments. The 2t/ha Biochar + Peptide treatment produced plants which were 42.7% taller than the control plants.

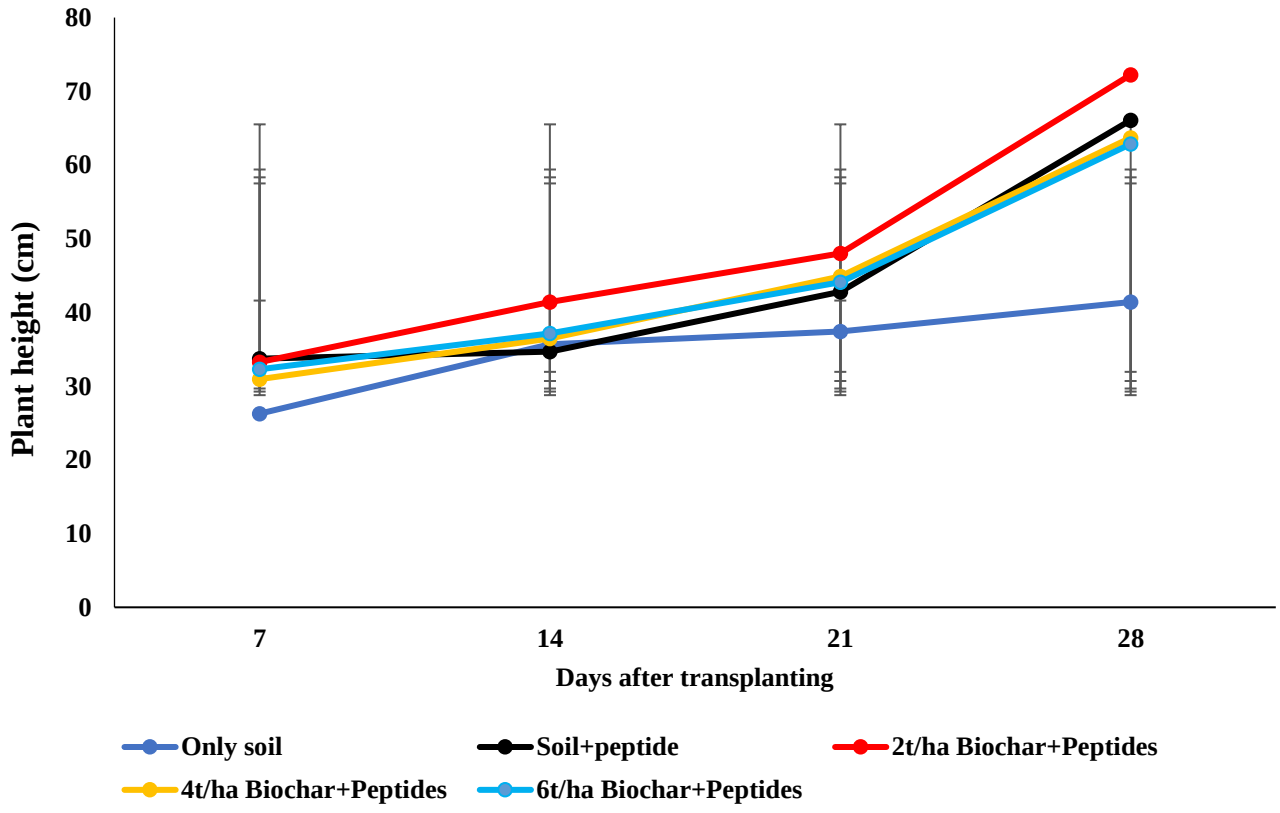

Figure 4.12: Effect of treatments on plant height at 7-28 days after transplanting. The error bars are represented by standard deviations of the treatment means.

4.13 Number of Leaves per Plant

The number of leave per plant was significantly (P<0.001) affected by the treatments at all the points of measurement (7-28 days) after transplanting (Fig. 4.13). The results showed that, the plants produced from 6t/ha Biochar + Peptide and 2t/ha Biochar + Peptide amended plots had the highest number of leaves at 7, 14, 21 and 28 DAT. The 2t/ha Biochar + Peptide treated plants produced 47.4% more leaves than the plants from the control plots (only soil).

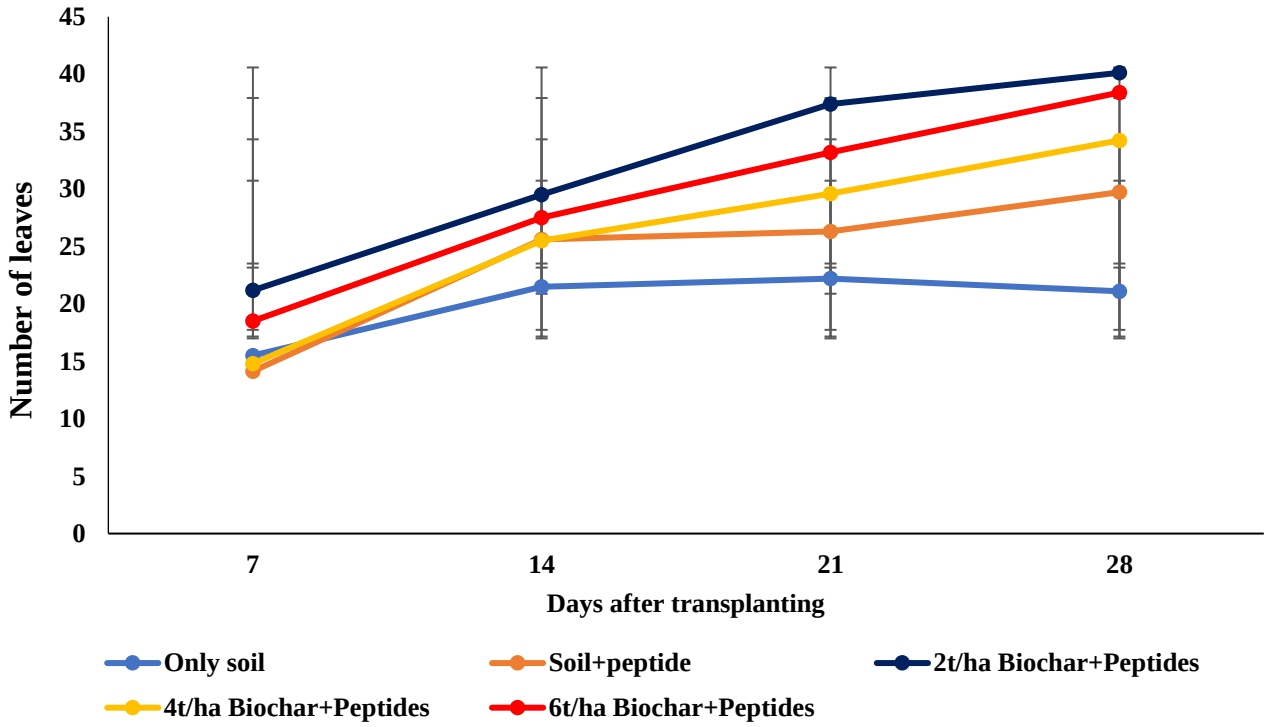

Figure 4.13: Effect of treatments on number of leaves per plant at 7-28 days after transplanting. The error bars are represented by standard deviations of the treatment means.

4.14 Canopy Spread

The canopy spread of the plants was also significantly (P<0.001) influenced by the treatments (Fig. 4.14). The 2t/ha Biochar + Peptide treatment consistently increased canopy spread over the period (14-28 DAT) more than the control, Soil + Peptide, 4t/ha Biochar + Peptide and the 6t/ha Biochar + Peptide treatments. The 2t/ha Biochar + Peptide treatment increased the canopy spread of the plants by 34.9% as compared with the control plants.

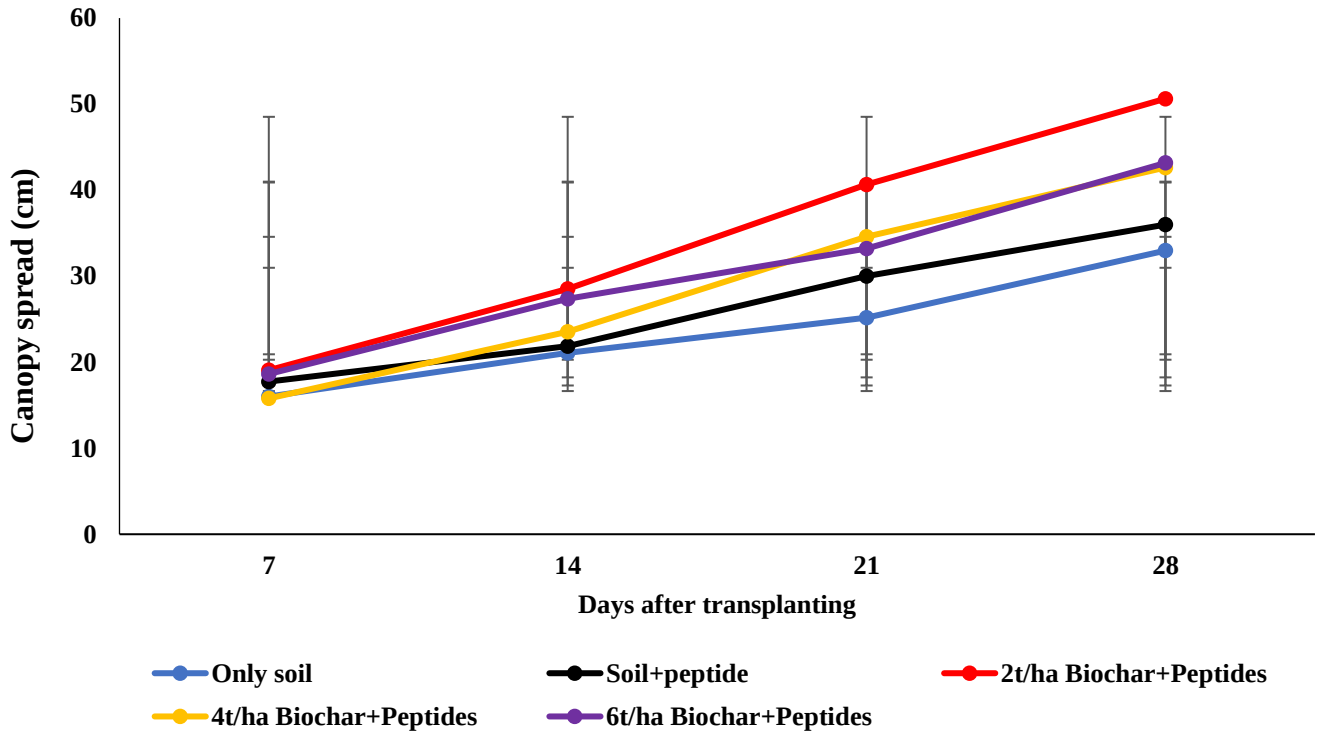

Figure 4.14: Effect of treatments on canopy spread at 7-28 days after transplanting. The error bars are represented by standard deviations of the treatment means.

4.15 Flowering and Fruit Set

The application of the treatments significantly affected days to first flower appearance, days to 50% flowering and days to first fruit set (Table 4.2). The results revealed that the plants produced from the 2t/ha Biochar + Peptide plots were the first to flower, followed by plants from the control plots, the Soil + Peptide treatment, 4t/ha Biochar + Peptide and the 6t/ha Biochar + Peptides, respectively. The 2t/ha Biochar + Peptide treated plants generally produced flowers at seven (7) days before the Soil + Peptide treated plants. Moreover, the 2t/ha Biochar + Peptide treated plants attained 50% flowering seven (7) days before the plants from the control plots and also recorded the first fruit set eight (8) days before the Soil + Peptide treated plants.

Table 4.2: Effect of treatments on flowering and fruit set of tomato plants at 63 days after transplanting

Treatments	**Days to first flower set**	**Days to 50% flowering**	**Days to first fruit set**
Only soil	24^{ab}	32^{a}	36^{a}
Soil+Peptide	26^{a}	28^{ab}	37^{a}
2t/ha Biochar+Peptide	19^{d}	25^{b}	29^{c}
4t/ha Biochar+Peptide	22^{bc}	29^{a}	34^{ab}
6t/ha Biochar+Peptide	21^{cd}	28^{a}	32^{bc}
P- value	**0.002**	**0.018**	**<.001**
LSD	**2.848**	**3.338**	**2.877**

Treatment means within the columns with the same letters are not significant different from each other.

4.16 Yield

Yield (kg/plot) and yield (t/h) at 63 DAT were significantly influenced by the various treatments. The results showed that the plants produced from the 2t/ha Biochar + Peptide treatment gave higher yield per plot (kg/plot) and higher yield per hectare (t/h) compared to the control (Soil only), Soil + Peptide, 4t/ha Biochar + Peptides and 6t/ha Biochar + Peptides (Table 4.3). The results also showed that the 2t/ha Biochar + Peptide treated plants gave 54.3% more yield per plot and 53.76% higher per yield per hectare (tonnes) compared with the control plots.

Table 4.3: Yield (kg/plot) and yield (t/h) as influenced by the various treatments at 63 days after transplanting.

Treatments	**Yield (kg/plot)**	**Yield (t/h)**
Only soil	2.8^{a}	5^{a}
Soil+Peptides	4.1^{b}	7^{b}
2t/ha Biochar+Peptides	5^{d}	9^{c}
4t/ha Biochar+Peptides	4.2^{c}	7.5^{b}
6t/ha Biochar+Peptides	4^{b}	7^{b}
P- value	**<0.001**	**<0.001**
LSD	**48.29**	**0.942**

Treatment means within the columns with the same letters are not significant different from each other.

4.17 Fresh and Dry Biomass of Roots and Shoots

The fresh and dry root weights of plants at 63 DAT were significantly ($P<0.005$) influenced by the various treatments whiles the fresh and dry shoot weight of plants at 63 DAT were not affected ($P>0.005$) by the treatments. The results indicated that the 2t/ha Biochar + Peptide treated plants gave highest fresh and dry root weights relative to the control plants (Table 4.4). The application of 2t/ha Biochar + Peptide increased the fresh and dry root weights by 57.6% and 49% respectively when compared with the control plants.

Table 4.4: Effect of treatments on fresh and dry root and shoot weight of plants 63 days after transplanting.

Treatments	Fresh root weight (g plant^{-1})	Dry root weight (g plant^{-1})	Fresh shoot weight (g plant^{-1})	Dry shoot weight (g plant^{-1})
Only soil	63.1^{b}	12^{c}	481.7^{a}	126.8^{ab}
Soil+Peptide	141.2^{a}	16.45^{bc}	698.3^{a}	155.6^{a}
2t/ha Biochar+Peptide	148.1^{a}	23.53^{a}	705^{a}	165.7^{a}
4t/ha Biochar+Peptide	125.3^{a}	18.28^{ab}	630.1^{a}	155.4^{a}
6t/ha Biochar+Peptide	134.7^{a}	21.06^{ab}	654.9^{a}	159.3^{a}
P- value	**0.037**	**0.01**	**0.22**	**0.684**
LSD	**54.89**	**5.668**	**227.5**	**20.23**

Treatment means within the columns with the same letters are not significant different from each other.

4.18 Severity of Galling

The various treatments significantly (P=0.002) influenced the severity of galling of the roots. The results show that the 2t/ha Biochar + Peptide treated plants had the least galls compared with the control, Soil + Peptide, 4t/ha Biochar + Peptide and 6t/ha Biochar + Peptide treatments (Fig. 4.15). The 2t/ha Biochar + Peptide treatment suppressed galling by 58.3% when compared with the roots of the control plants.

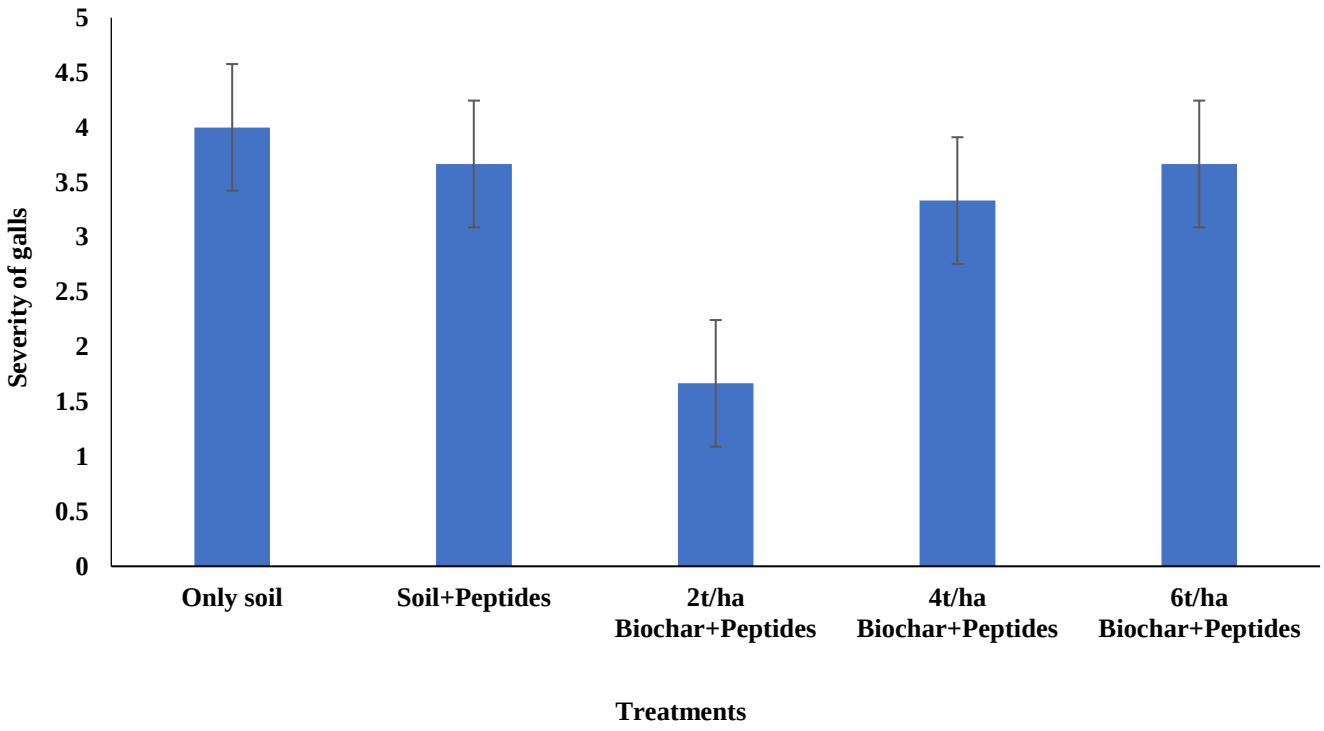

Figure 15: Effect of treatments on severity of root galling at 63 days after transplanting. *The error bars are represented by standard deviations of the treatment means.*

RESULTS OF EXPERIMENT THREE (POT EXPERIMENT)

4.19 Plant Height

The height of the plants were significantly (P<0.001) affected by the different amounts of peptide-based nematicide applied (Fig. 4.16). The results showed that the application of 20ml, 15ml and 25ml of peptide-based nematicide produced plants with the greatest height compared with plants from nematode-Inoculated pots without Peptide application and those from the control plots. The application of 20ml Peptide-based nematicide to the soil inoculated with RKN eggs also produced taller plants which were 18.5% taller the control plants.

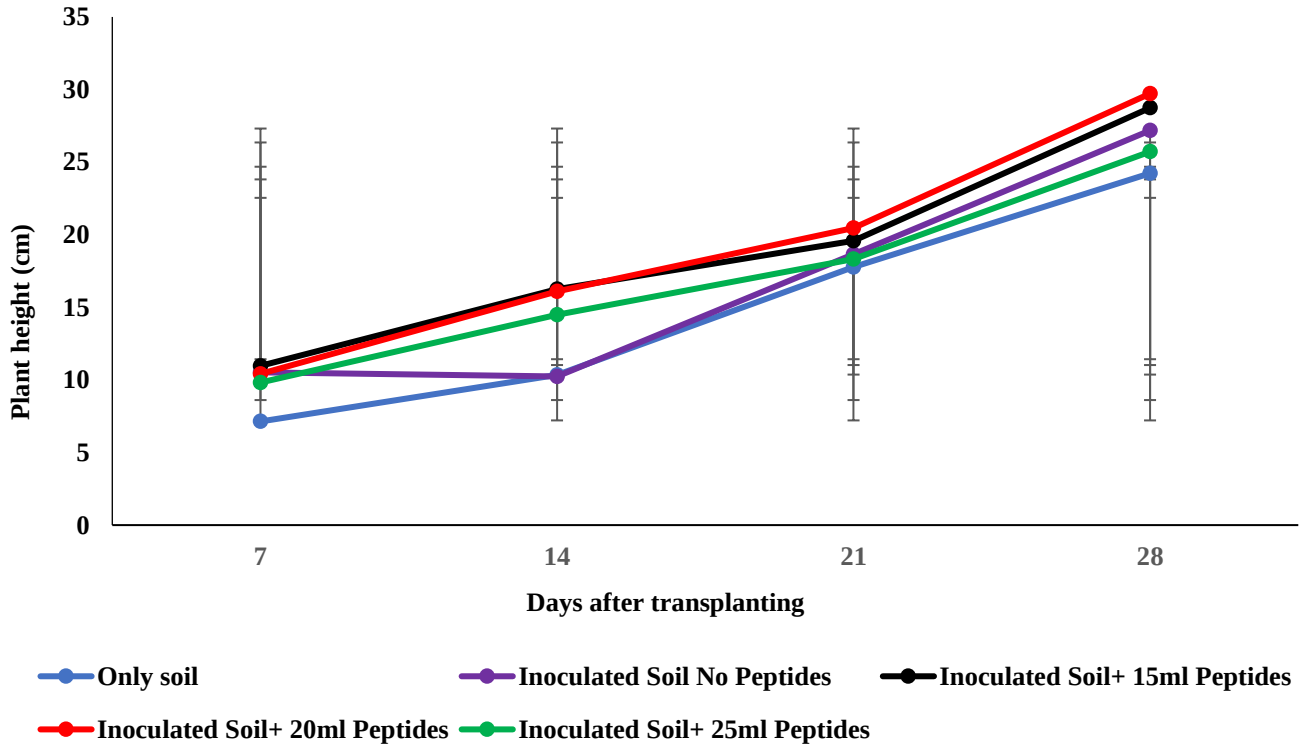

Figure 4.16: Effect of treatments on plant height at 35 days after transplanting, the error bars are represented by standard deviations of the treatment means.

4.20 Number of Leaves per plant

The number of leaves per plant was significantly (P<0.001) affected by the different rates of the peptide-based nematicide. The Inoculated Soil+ 20ml Peptide produced plants taller than those from the Inoculated Soil+15ml Peptides, Inoculated Soil+ 25ml Peptides, Inoculated Soil without Peptide and the control (Fig. 4.17). The Inoculated Soil+20ml Peptide treated plants produced 28% more leaves than the control plants.

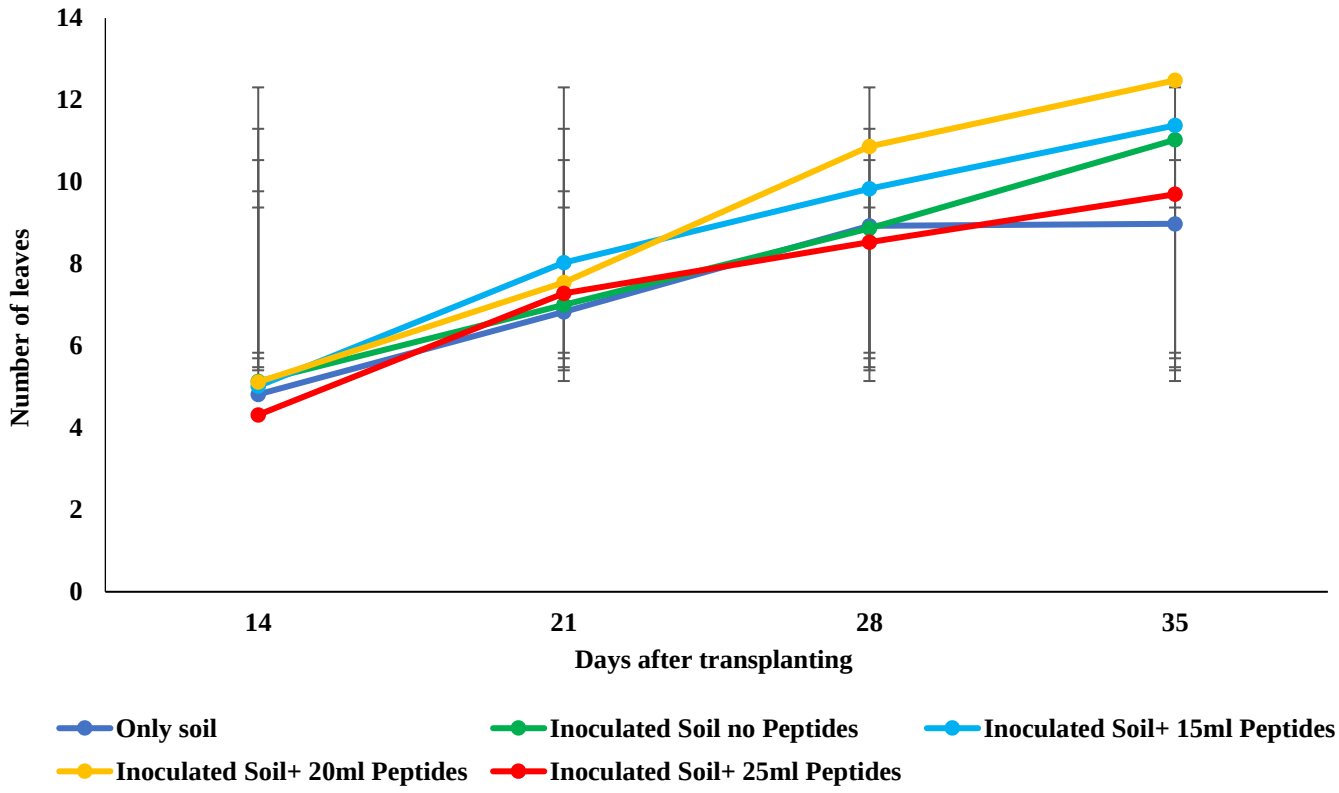

Figure 4.17: Effect of treatments on number of leaves per plant at 35 days after transplanting. The error bars are represented by standard deviations of the treatment means.

4.21 Fresh and Dry Root and Shoot Weights of Plants

The fresh and dry shoot weights of plants measured at 63 DAT were significantly (P<0.001) influenced by the various treatments whiles there were no significant differences recorded for the fresh and dry root weights. The results have shown that the Inoculated Soil+20ml Peptide treatment produced plants with the least weights relative to Inoculated Soil+ 15ml Peptides, Inoculated Soil+ 25ml Peptides, Inoculated Soil No Peptides and control (Table 4.5). The results also indicated that the Inoculated Soil+20ml gave the highest fresh shoot and dry shoot weights which were 44.6% and 39.8% more respectively greater than those from the control plots.

Table 4.5: Fresh and dry shoot and root weight of plants as influenced by the various treatments at 49 days after transplanting (DAT)

Treatments	**Fresh shoot weight (g)**	**Dry shoot weight (g)**	**Fresh root weight (g)**	**Dry root weight (g)**
Only soil	34.9^{d}	7.9^{c}	9.9^{a}	3.7^{a}
Inoculated Soil No Peptides	46.3^{bc}	11.43^{ab}	15.03^{a}	4.233^{a}
Inoculated Soil+ 15ml Peptides	50.93^{b}	11.57^{ab}	12.73^{a}	4.133^{a}
Inoculated Soil+ 20ml Peptides	63.05^{a}	13.13^{a}	14.07^{a}	4.3^{a}
Inoculated Soil+ 25ml Peptides	43.55^{c}	9.63^{bc}	11.9^{a}	3.633^{a}
P- Value	**<.001**	**0.036**	**0.287**	**0.786**
LSD	**6.739**	**3.191**	**5.192**	**1.498**

Treatment means within the columns with the same letters are not significant different from each other.

4.22 Severity of Galling

The various peptide-based nematicide treatments significantly (P<0.001) influenced the severity of galling in roots. The results showed that the plants from the control and Inoculated Soil+ 20ml Peptides treatment had the least galling compared with the Inoculated Soil+ 15ml Peptides, Inoculated Soil+ 25ml Peptides and nematode Inoculated Soil without Peptide application (Fig. 4.18).

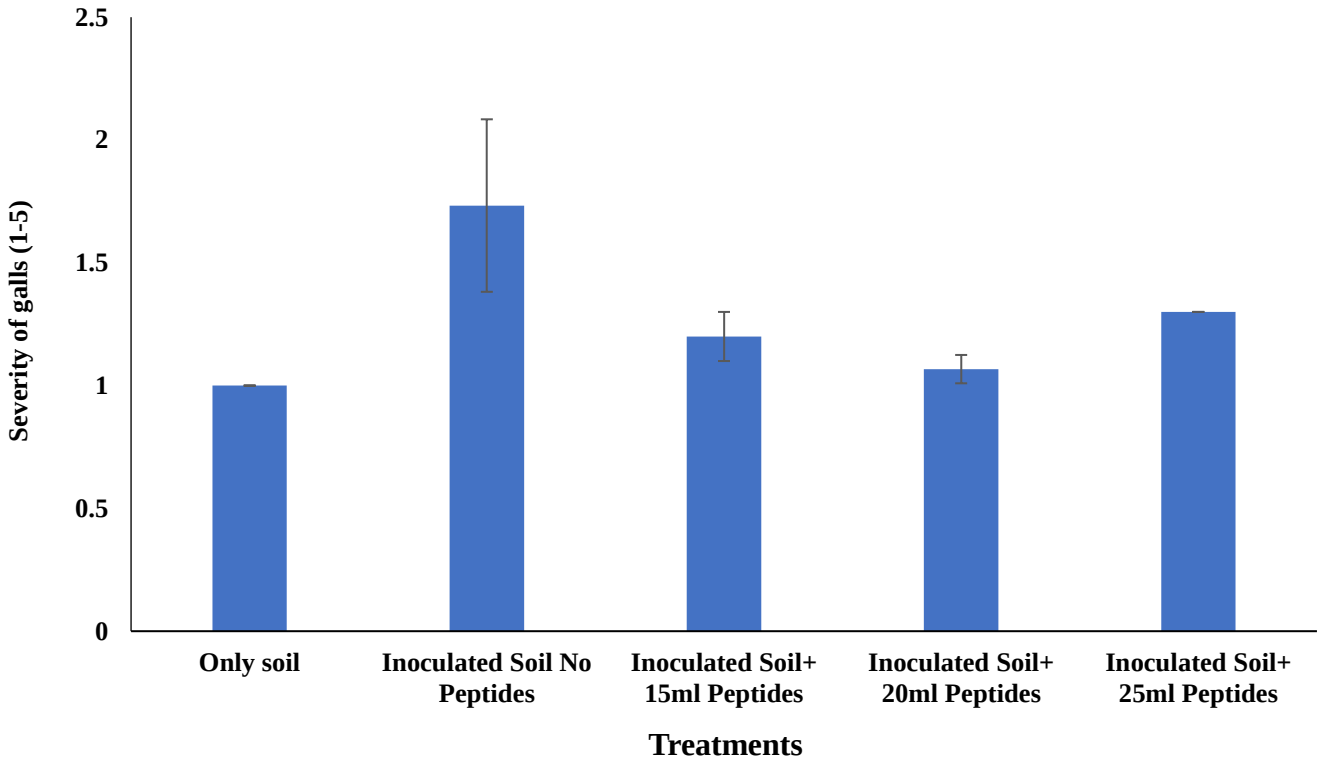

Figure 4.18: Effect of treatments on severity of galling at 70 days after transplanting. *The error bars are represented by standard deviations of the treatment means.*

CHAPTER FIVE

DISCUSSION

5.1. Soil Amendments Effect on Plant Growth and Development (Experiment One and Two)

The application of lower rates of biochar (1% or 2t/ha) to the soil enhanced the effectiveness of nemanol against root-knot nematode (RKN) and also promoted plant growth and development in these experiments. This observation can be attributed to the fact that biochar is known to improve the physical (bulk density, porosity, infiltration etc) and chemical (pH) conditions of soils for improved plant growth and development. In these experiments, the application of lower rates of biochar reduced soil bulk density which probably enhanced root development and plant growth in the RKN infested soil. It was also likely that the improvement in the condition of the soil promoted the growth of the plants and increased their resistance to the RKN whiles the nematicide inhibited the activities of the root-knot nematodes in the soil. This finding is in conformity with an earlier report which state that, biochar application to the soil increased nutrient retention, bulk density, water-holding capacity, cation-exchange capacity, soil microbial and mycorrhizal activity, soil acidity and electric conductivity (Novotny et al., 2009). Moreover, the lower levels of biochar (1% and 3%) applied in experiment one slightly increased soil pH from 6.4 to 6.5, making it more suitable for the growth of the tomato plants. This, probably, also contributed to increasing the resistance of the plants to the RKNs attack. This findings is not in conformity with (Congli et al. 2009) who reported that, in soils with lower pH (>4.5), the activities of RKNs are usually inhibited, promoting root health. In this experiment, the biochar amended soils which were treated with nemanol had slightly higher pH and the root-knot nematode numbers and severity of galling were decreased as compared to the non-biochar

amended soils with lower pH but recorded higher infestation of RKNs. The enhanced protection of the plants treated with biochar plus the peptide-based nematicide can be attributed to the efficacy of the peptide against RKNs and some pathogenic fungi. Huffaker et al. (2007) earlier indicated that peptides embody a potential basis of comprehensive spectrum effect against nematodes because they activate multiple defensive pathways, including defenses previously implicated in nematode resistance. According to Ross et al., (2014), transcript profiling in Arabidopsis showed that peptides coactivate salicylate (SA), jasmonate (JA) and ethylene signaling. All of these three pathways are also activated by nematode infection, and each has been implicated in plant defenses against nematodes in at least certain host plant-nematode combinations and infection stages. The finding is also in conformity with another experiment by Calderón-Urrea and Polineni, (2019) who found that, the peptide-based nematicide was effective in controlling RKNs on cucumber.

5.2. Soil Amendments Effect on Root-Knot Nematode Control (Experiment One and Two)

The application of the lower rates of biochar (1%, 3% per pot or 2t/ha) improved soil structure and enhanced the effectiveness of the Telluris peptide-based nematicide (Nemanol) against RKNs. Moreover, these biochar rates (1%, 3% per pot or 2t/ha) enhanced the effectiveness of the peptide applied in the root zone. Peptides have the potential to trigger calcium signaling and synthesis of defensive proteins at root tips to hinder the activities of RKNs in the soil and in the roots (Huffaker, A. et al., 2007). The lower biochar rates contributed effectively in reducing the soil bulk density which could also contribute to the effectiveness of the nematicide. Eunji et al. (2016) observed severe galling and increased egg-mass formations of *M. incognita* due to increased penetration rates of the nematodes in sandy soil, which provided sufficient aeration due to the coarse nature of the soil. This current finding does not conform to the findings of Eunji et

al. (2016) observation. This is because the biochar amendment slightly decreased soil bulk density and enhanced the effectiveness of the nemanol against RKNs activities as compared to the non-biochar amended soils. In an experiment conducted by Elad et al. (2011), they reported that the application of 1.2 % biochar in a potting medium effectively suppressed nematode development in rice roots. Elad et al. (2011) further explained that the increased plant resistance was associated with biochar-primed H_2O_2 accumulation as well as with the transcriptional enhancement of genes involved in ethylene (ET) signaling pathway. Elad et al. (2011) further found that biochar had a high sorption capacity for dichloropropene, a strong anti-nematode fumigant. As a result, biochar amendment to the soil could increase the required dose of dichloropropene to efficiently control nematodes. Lehmann et al. (2011) also reported that biochar does not contain an indigenous consortium of microorganisms that can potentiate disease suppression, the suppression of soil pathogens by biochar may be due to several mechanisms, including improved nutrient solubilization and uptake, which helps plants develop resistance to the stresses caused by pathogens; microbe stimulation. Huffaker et al. (2007) also reported that the movement of RKNs is through the root tips to a parenchymatous cell in the developing vascular bundle where they then become sessile and secrete effectors to initiate the feeding structure. Plant defensins are located in peripheral cell layers and they have also been found in the xylem, root tips, in stomatal cells and cells that line the substomatal cavity, all of which are sites where first contact and entry of pathogens takes place (Huffaker et al., 2007).

5.3. Peptide-Based Nematicide (Nemanol) Rates Effect on Root-Knot Nematodes (Experiment Three)

In this experiment, the effect of different concentration (0ml, 15ml, 20ml and 25ml) of nemanol on RKNs was determined. The application of 20 ml of the formulated nemanol per plant decreased the nematode population and suppressed galling on the roots better than the other rates. This rate proved to be the most suitable for the soil used in the current experiment, thus the manufactures recommended rate of 20 ml per plant before planting and 20 ml per plant 3 weeks later was the best. This suggests that the dosage below the recommended 20ml per plant or higher dosage above the recommended rate could be less effective against the RKNs in the soil. This finding is in conformity with Bayuh et al. (2013) who reported that, three concentrations of rapeseed cake extracts (5%, 10% and 20%) and two concentrations of BioNem extracts (5% and 10%) significantly reduced ($p<0.05$) the nematode egg hatching capacity as well as juvenile motility over the untreated control. Higher concentration of rapeseed cake (20%) proved most effective in reducing hatching and affecting motility. In another experiment, Bayuh et al. (2013) found that the application of 0.6 and 0.4 g/pot of BioNem at ten days before transplanting and at the time of transplanting resulted in less gall formation, decreased number of eggs per egg mass and final nematode population over the untreated control in a pot experiment. Moreover, the incorporation of 600 kg/ha and 300 kg/ha of rapeseed cake and 150 and 75 kg/ha of BioNem in a nursery bed also reduced the nematode infestation and improved tomato seedling growth as compared to the untreated control.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

A number of experiments were conducted at the University for Development Studies to determine the effect of nemanol on root-knot nematodes and growth and yield of tomato in biochar amended soil. In the first experiment (pot experiment), the 1% Biochar + Peptide treatment significantly promoted the growth (plant height, number of leaves and stem diameter) of tomato seedlings. However, the 3% Biochar + Peptide treatment significantly decreased root-knot nematode egg count, root-knot nematode population in soil and severity of galling. In the second experiment (field experiment), the lower biochar rates (2t/ha Biochar + Peptide) enhanced the effectiveness of nemanol against root-knot nematode. The application of 2t/ha Biochar + Peptide promoted the growth (plant height, number of leaves, canopy spread,) and development (flowering and fruiting) of the tomato plants in the field. Moreover, the 2t/ha Biochar + Peptide's treatment decreased root-knot nematode population in the soil and also suppressed galling. In the third experiment, the effects of different rates (0ml, 15 ml, 20 ml and 25 ml per plant) of the nemanol on RKNs population and severity of galling were studied. The results showed that the 20 ml per plant Telluris peptide (nemanol) was more effective against the root-knot nematodes and it also promoted the growth of the tomato plants. To sum up these studies have demonstrated that lower rate of biochar (1%, 3% per pot or 2t/ha) and 20 ml of nemanol per plant were more effective against the root-knot nematodes and also improved growth and yield performance of the tomato plants.

6.2. Recommendation

Firstly, it is recommended that, for effectiveness of the Telluris peptide-based nematicide (nemanol) in the northern soils, lower rate (2t/ha) of biochar can be applied to the soil.

Secondly, the experiments should be repeated on farmers' fields to validate the results obtained in this research.

Finally, a study to find out whether priming tomato seeds with the Telluris peptide-based nematicide could protecte the emerging seedlings from root-knot nematode attack.

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