NUTRITIONAL WATER PRODUCTIVITY OF HOT CHILLI (*CAPSICUM ANNUUM*) UNDER INFECTION WITH *MELOIDOGYNE JAVANICA* AND *MELOIDOGYNE INCOGNITA* RACE 2

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MINI-DISSERTATION SUBMITTED FOR THE DEGREE MASTER OF SCIENCE IN SOIL SCIENCE, DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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2019

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DECLARATION

I, Mogwale Janet Ramputla, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Agriculture (Soil Science) has not been submitted previously by me or anybody for a degree at this or any other university. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

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DEDICATION

To my parents (Maite and Jappie Ramputla), my brother (Isaiah Ramputla) and grandmother (Annah Bopape) with gracious love.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God the Almighty for granting me the opportunity to undertake and complete this milestone. He had given me strength, wisdom, knowledge and understanding. It has not been easy, but through His mercy, I completed. To my supervisory team Professor P.W. Mashela and Dr P.E. Dlamini, words cannot express how thankful I am for having dedicated supervisors like you. Professor P.W. Mashela, you have been a good trainer, I would like to thank you for believing in me and introducing me to the various aspects of research through scientific writing skill and oral presentations. Dr P.E. Dlamini, special thanks also for technical assistance at the Soil Science laboratory. Again, I would like to express my gratitude to Dr Z.P. Dube for technical assistance throughout the research study. Special thanks also go to the Green Biotechnologies Research Centre of Excellence (GBRCE) team for helping in establishment, termination and data collection for this research project. You have been great colleagues throughout. I also send my gratitude to the Department of Plant Production, Soil Science and Agricultural engineering, Green Biotechnologies Research Centre of Excellency and Limpopo-Agrofood Technology Station for technical assistance and analysis of the samples. Most importantly, I send my sincere gratitude to the loving, caring and supportive family I have, Miss Anna Bopape (my grandmother), you have been encouraging me day and night, telling me not to give up, but to work hard. To Mr Jappie and Mrs Maite Ramputla (my parents) and Isaiah Ramputla (my brother), words cannot express how grateful I am for the enduring love, support, courage you have been giving me in achieving this milestone.

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ABSTRACT

Nutritional water productivity (NWP) is an assessment tool, which describes the amount of water that has been used to produce selected mineral malnutrition (MMN) elements and micronutrient malnutrition (MNMN) substances. Therefore, it links agricultural production to human nutrition. Deficiencies in MMN elements and/or MNMN substances in human nutrition referred to as malnutrition, had been linked with fatal diseases. Agricultural soils could be affected by soil-borne pathogens such as plant-parasitic nematodes, which could limit the availability of MMN elements and MNMN substances. In some communities, vegetable crops, including chilli are regarded as a major source of MMN elements and MNMN substances. Effects of root-knot (*Meloidogyne* species) nematodes on NWP of chilli (*Capsicum annuum* L.) have not been documented. The objective of the study was to determine the effects of increasing population densities of *M. incognita race 2* and *M. javanica* on the NWP of hot chilli plants. A microplot trial was conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa. Pots were filled with 10-L steam-pasteurised (300^{o}C) sandy clay loam soil sourced from GBRCE and Hygromix-T (Hygrotech, Pretoria North) growth medium in the ratio 3:1 (v/v). Thereafter, three-week-old hot chilli cv. 'Serrano' seedlings were transplanted into each pot, with inoculum prepared by extracting eggs and second-stage juveniles (J2) of *M. incognita race 2* and *M. javanica* from roots of grown nematode susceptible tomato cv. 'Floradade' (*Solanum lycopersicum* L.) in a 1% NaOCl solution. Fourteen days after transplanting, treatments 0, 50, 125, 250, 625, 1250 and 2000 eggs and second-stage juveniles (J2) of *M. incognita race 2* and *M. javanica* were separately inoculated using a 20 ml plastic syringe into 5-cm-deep holes in pots. At 56 days after the initiation of the treatments, *Meloidogyne* species decreased soil pH and increased organic carbon, contributing 29 and 43% in total treatment variation (TTV) of the respective variables. Treatment effects caused the pH to decrease. NWP variables against increasing nematode numbers exhibited quadratic relations, with coefficients of determination ranging from 59 to 86% for *M. incognita race 2* trial and 80 to 98% for *M. javanica* trial. *Meloidogyne* species population densities against plant variables did not show any significant relationship, except for root galling and chlorophyll content where treatments contributed 76, 98 and 47% TTV of the respective variables. Generally, root galling increased with increase in *Meloidogyne* species population densities, whereas chlorophyll content decreased with increasing inoculum levels. Nematode variables against their increasing population exhibited quadratic relationship with the model explained by 44 to 95% for *M. incognita race 2* and 28 to 82%, association, respectively for *M. javanica*. In conclusion, *Meloidogyne* species interfered with NWP of mineral elements in chilli plant and therefore, nematode management practices should be done to reduce the nematode population densities that would confer quality to agricultural produce for human health benefits.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

The nutritional water productivity (NWP) concept provides empirical-based information on the amount of water that would be used in the assimilation of mineral malnutrition (MMN) elements and micronutrient malnutrition substances (MNMN) (Renault and Wallender, 2000). Globally, more than 2 billion people suffer from deficiencies of MMN elements and MNMN substances (Delfine *et al*., 2000), with fatalities of over 3.1 million children (The Guardian, 2013). Deficiencies of MMN elements cause diseases like hypokalaemia, diabetes, osteoporosis, anaemia and hypertension (Primm, 2013), whereas deficiencies of MNMN substances had been linked with rickets, scurvy, night blindness, beri beri and pellagra (Primm, 2013). Technically, NWP refers to producing more nutrients per mm of water used (Masango *et al*., 2014), with specific reference to MMN elements and MNMN substances.

The deficiencies of MMN elements and MNMN substances had been fatal to people across all socio-economic groups (WHO, 1996). In crop husbandry, crops are being produced for increased yield, which is proportional to biomass, whereas MMN elements and MNMN substances are inversely proportional to biomass (Mabotja and Mashela, 2018). Generally, with regards to NWP, production of several crops which are rich in MMN elements and MNMN substances need to be considered. Chilli (*Capsicum annuum* L.) berries are rich in MMN elements and MNMN substances including high Fe, K and Mg content, high vitamin A and C content, with the ability to boost the immune system and lower cholesterol levels (Grubben and Mohamed, 2004). Furthermore, certain abiotic factors such as frequent irrigation interval have been associated with reduced bioaccumulation of certain MMN elements (Mabotja and Mashela, 2018). Mashela *et al.* (2016) reported that nematode infection could interfer with some of the MMN elements, specifically K in African ginger (*Siphonochilus aethiopicus*) and as a result, limiting their availability in human nutrition. Additionally, the citrus nematode (*Tylenchulus semipenetrans*), induce imbalances in the partitioning of MMN elements (Mashela and Nthangeni, 2002). Currently, there is limited information on the association of biotic factors such as plant-parasitic nematodes with NWP of crops.

In Limpopo Province, climate change projections predict an increase in temperatures by 2°C per annum from 2030, coupled with increased incidents of drought spells (Steyn *et al*., 2014). Such conditions increase the need for climate-smart agriculture, which comprises matching various crops to the predicted conditions, including increases in soil-borne pathogens such as plant-parasitic nematodes. Thies *et al.* (2008) reported that *Meloidogyne* species could reduce yield from as high as 50% to complete crop failure in crops without nematode resistance. Nematode infection affects various MMN elements in crops and as such, infected plants could display a NWP that is different to that of nematode-free plants (Mashela *et al*., 2016).

1.1.2 Impact of the research problem

The NWP concept introduced water-food-nutrition-health linkages, therefore, it creates approaches to improve human nutrition and health (Chibarabada *et al*., 2017). However, malnutrition continues to be the major challenge with millions of people, including women and children dying daily, due to failure to access nutritious food. Nematodes could affect NWP (Mashela *et al*., 2016) and therefore, exacerbate the conditions under which malnutrition occur.

1.1.3 Possible causes of the research problem

Nutritional water productivity quantifies water-food-nutrition linkages (Chibarabada *et al*., 2017). Most conducted studies focused on crop production in terms of food availability or nutrition separately (Chibarabada *et al*., 2017). Nematodes could affect NWP since they have been reported to influence MMN elements in other crops (Mashela *et al*., 2016). However, there had been limited information on the effects of nematodes on NWP in agricultural production systems.

1.1.4 Proposed solutions

The NWP challenges in crops infected by nematodes are critical in understanding the extent of the effects of *Meloidogyne* species population densities on human nutrition. Empirically-based studies using the concept of NWP could provide some information on how crops respond to stress-induced by nematode infection.

1.1.5 General focus of the study

The study focused on generating information on the effects of *Meloidogyne* species on NWP on chilli plants under microplot conditions.

1.2 Problem statement

Food had been viewed as the source of MNMN substances and MMN elements (Newell-McGloughlin, 2008). Human-health is been linked with various food-related diseases, most of which have been shown to be fatal if not attended to (Newell-McGloughlin, 2008). Generally, in food production, improved biomass is inversely proportional to improved nutritional quality. Plant-parasitic nematodes have been shown to affect the nutritional content of crops by directly affecting certain nutrient elements (Mashela *et al*., 2016).

1.3 Rationale of the study

Nutritional water productivity (NWP) had been viewed as being an assessment tool, which could describe the amount of water that was used to produce selected MMN elements and MNMN substances (Renault and Wallender, 2000). The concept is important because it links agricultural production to human nutrition (Wenhold *et al*., 2007). Lack of these elements and/or substances in human nutrition could be associated with malnutrition. Agricultural soil is replete with plant-parasitic nematodes (Mashela *et al*., 2015), which were reported to limit the availability of MMN elements, and MNMN substances. In some communities, vegetable crops, including chilli, had been viewed as the major source of MMN elements and MNMN substances (Dias, 2012). Therefore, chilli could be used to alleviate some of the challenges related to malnutrition deficiencies. The relation between NWP and increasing nematode numbers would provide empirically based evidence on the need to manage nematode population densities in cropping systems.

1.4 Purpose of the study

1.4.1 Aim

Establishment of the influence of increasing nematode population densities on NWP in vegetable production.

1.4.2 Objective

To determine the effects of increasing populations of *M. incognita race 2* and *M. javanica* on NWP of hot chilli plants under microplot conditions.

1.4.3 Hypothesis

Increasing populations of *M. incognita race 2* and *M. javanica* would have effects on NWP of hot chilli plants under microplot conditions.

1.5 Reliability, validity and objectivity

Reliability of data would be based on statistical analysis of data at the probability level of 5%, validity would be achieved through repeating the experiments in time, whereas objectivity would be achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias would be minimised by ensuring that the experimental error in each experiment was reduced through replications. Also, randomly assigning treatments within an appropriate research design would reduce bias (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The findings would provide insight into the influence of biotic factors (*Meloidogyne* species) on nutritional water productivity by revealing their impact on soil quality, nutritional content and water use of chilli plants. Interference of nematodes on MMN elements would be documented, whereby several nematodes management practices would then be recommended not only for economic purposes but also for human health benefits to alleviate the issue of malnutrition deficiency.

1.8 Structure of the mini-dissertation

Chapter 1 focused on the research problem, with Chapter 2 being the literature review that comprised work done and work not yet done on the research problem followed by Chapter 3 that constituted the study objective. In the final chapter, the significance of findings were summarised followed by the significance and the recommendations with respect to the future research. The latter was followed by conclusions, which tied the findings of the study. In the citations and references, the Harvard style was used, with author-alphabet as approved by the Senate of the University of Limpopo.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The potential of crops to meet human nutrition had been assessed on the basis of nutrient composition in edible plant organs, with the main focus being on high yield (Chibarabada *et al*., 2017). However, due to the vagaries of malnutrition in human health systems (Muller and Krawinkel, 2005), recent advances have since focused on nutrient-linked deficiencies that induce diseases in food, with the coining of the concept of NWP (Nyathi *et al*., 2016). The NWP concept offers the possibility of linking water-use and nutrition in food systems (Mabhaudhi *et al*., 2016). Renault and Wallender (2000) linked the concept of more crop per drop with nutrition in the context of NWP, which had technically been defined as nutritional content per volume of water used ($NWP= (Ya/Eta) \times NP$), where Ya is yield, Eta is the actual evapotranspiration and NP is the nutritional content. Most of the NWP studies (Mabhaudhi *et al*., 2016; Nyathi *et al*., 2016) were conducted outside the auspices of nematode infection, with nematode work focusing on components of NWP exclusively.

2.2 Work done on the problem statement

2.2.1 Impact of nematode infection on MMN elements

Root-knot nematodes are among the most destructive nematodes in crop production (Fourie *et al*., 2001). Their impact on plant nutrient composition could devalue the quality of food produced for human nutrition. Previous study showed that root damage by nematodes especially root-knot nematode (*Meloidogyne* species) negatively affects MMN elements by influencing nutrient uptake and translocation (Jagadeesh, 2011). Mashela *et al*. (2016) observed that infection by *M*. *incognita* race 2 had no effects on other MMN elements, except for K, which was reduced by 23−45% in African ginger pseudostems. Generally, K was found to be sensitive to nematode infection, whereas this mineral element is of significance value in the human diet. Jagadeesh (2011) reported the reduction in Fe and Zn content of rice leaves infected with *M. graminicola* as the inoculum level was increasing. The same study further explained that nematode reduced the uptake of nutrients from soil to shoot part of the plant. Failure of crops to access nutrients could lead to deficiency in nutrients, which could in turn result in lower nutrient concentrations than is required for human nutrition. According to Mashela *et al*. (2016), nematode infection to root system interfered with MMN elements in various plant species. Similar observations were made on banana plants, whereby nematode infection and damage to roots disrupted nutrient elements distribution in plants (Talwana *et al*., 2003).

2.2.2 Impact of nematode infection on MNMN substances

An adequate supply of micronutrient substances to human nutrition depends on food produced from agricultural crops. However, nematode infection causes a change in the distribution of nutrient level and substances in different plant parts. Previous study conducted on the lima bean (*Phaseolus lunatus* L.) reported the decline in the vitamins and macro-nutrient (McClure and Viglierchio, 1991). Several studies conducted focused mostly on the effects of root-knot nematodes on mineral malnutrition elements, where their findings also reported decline in nutrient content to infected crops (Jagadeesh, 2011; Mashela *et al*., 2016).

2.2.3 Impact of nematode infection on water uptake

According to Bird (1970), infection of roots by *Meloidogyne* species induces the formation of galls, which disrupt the normal flow of water and nutrients to leaves. Water is essential for two processes, namely mass flow and diffusion, which are responsible for nutrients transport and contact towards roots. Therefore, changes in plant water use could significantly alter nutrient uptake. A previous study by Alam and Saxena (1975) on nematode infection suggested that different inoculum levels of *M. incognita* reduced water absorption capacity of tomatoes as compared to uninfected plants. A study conducted by Fatemy and Evans (1986) reported a reduction in water uptake on potato cultivars due to nematode infection. Reduction of water uptake particularly leads to the decrease in nutrients uptake by mass flow.

Supply of Ca to plant roots occurs through a mass flow in most soils. Therefore, amount of Ca taken up by plants probably relate to the amount of water transpired. As such, crops, which transpire large amount of water, accumulate greater concentration of Ca in their leaves (Evans, 1982). Nematode attack causes water stress, which result in plant using less water than the required, thereby resulting in less Ca accumulation in their leaves. Most studies reported decline in water uptake, which resulted from nematode damage (Kirkpatrick *et al*., 1991).

2.3 Work not done on the problem statement

Most of the studies as reviewed above, were conducted on the components that constitute NWP, without directly using the concept. The information on NWP in relation to nematode is not yet documented.

CHAPTER 3

INFLUENCE OF *MELOIDOGYNE INCOGNITA RACE 2* AND *MELOIDOGYNE JAVANICA* ON NUTRITIONAL WATER PRODUCTIVITY IN HOT CHILLI

3.1 Introduction

Malnutrition continues to be the greatest killer of humans in both undeveloped and developing countries and is increasingly affecting both the rich and the poor (Muller and Krawinkel, 2005). Malnutrition is caused by deficiencies of mineral malnutrition (MMN) elements and/or micronutrients malnutrition (MNMN) substances. The MMN elements include Zn, Fe, Mg, Ca and K (Welch, 2002), whereas the MNMN substances include vitamins A, B and C. The availability of nutrients that would not result in malnutrition is dependent on food choices by consumers, with the available staple food containing mainly carbohydrates, which are not part of malnutrition compounds. Unfortunately, the availability of nutrients is determined by the output of the agricultural systems (Welch, 2002), which are mainly a function of profits. The latter resulted in most producers focusing on biomass (Mabhaudhi *et al*., 2016), which could be inversely proportional to malnutritional compounds as shown recently in irrigation interval studies (Mabotja and Mashela, 2018).

The linkage of agricultural production malnutrition compounds had been introduced using the concept of nutritional water productivity (Wenhold *et al*., 2007). Nutritional water productivity (NWP) is the measure of yield and nutritional outcome per unit of water used (Renault and Wallender, 2000). It is further defined as the amount of water used to produce certain mineral elements such as Ca, Mg, Fe and vitamins (Nyathi *et al*., 2016).

The NWP of most crops reported to be a factor of abiotic factors such as irrigation interval, seasonal effects and crop choices (Nyathi *et al*., 2016). Mabotja and Mashela (2018) demonstrated that the nutritional content of MMN elements such as Ca, Mg, P, K, Na, Fe and Cu in nightshade (*Solanum retroflexum* Dun.) and irrigation interval exhibited positive quadratic relations, with the models explained by 98, 92, 97, 86, 88, 83 and 87%, respectively. The latter implied that at the irrigation interval that crops are generally subjected to, the malnutrition compounds could be compromised. Limited information on NWP-biotic factor relations exists, whereas it is documented that certain soil-borne pathogens like root-knot (*Meloidogyne* species) nematodes affect the availability of water in plants (Kirkpatrick *et al.,* 1991). In general nematode injury to root system decreases water absorption and translocation to the shoots (Jagadeesh, 2011).

Vegetable crops, including chilli, are known to be a major source of MMN elements and MNMN substances (Dias, 2012). Chilli is regarded as an important commercial crop grown internationally for its edible fruit that is rich in protein and certain essential mineral nutrients (Thiyagarajan and Kuppusamy, 2014). Most chilli cultivars are moderately susceptible to *Meloidogyne* species (Aluvilu *et al*., 2010). Generally, chilli plants wilt when subjected to high population densities of *Meloidogyne* species, (Noling, 2009), and when the soil is at field capacity. As such these factors could influence the NWP of the chilli fruit. Therefore, the objective of this study was to determine the effects of increasing populations of *M. incognita race 2* and *M. javanica* on the NWP of hot chilli plants.

3.2 Materials and methods

3.2.1 Description of the study site

The microplot study was conducted at Green Biotechnologies Research Centre of Excellence (GBRCE) of the University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). The site situated on Westleigh soil form (MacVicar *et al*., 1991), with 65% sand, 30% clay, 5% silt. The location has hot dry summers (November−January) with daily maximum temperatures from 28 to 38^{o}C and average summer rainfall being less than 500 mm. The study was initiated during autumn (February-April) in 2017 (Experiment 1) and validated in 2018 (Experiment 2).

3.2.2 Treatments and research design

The experiment was laid out in a randomized complete block design with seven treatments replicated ten times (Figure 3.1; Figure 3.2). The seven levels of nematode treatments comprised 0, 50, 125, 250, 625, 1250 and 2000 eggs and second-stage juveniles (J2) of *M. incognita race 2* and *M. javanica* using a 20 ml plastic syringe into 5 cm deep holes in the pots.

Figure 3.1 Experimental layout showing arrangement of treatments.

3.2.3 Procedures

The microplot experiment was conducted whereby; 30 cm diameter plastic pots were filled with 10-L steam-pasteurised (300^{o}C) sandy clay loam soil sourced from GBRC and Hygromix (Hygrotech, Pretoria North) growth medium in the ratio 3:1 (v/v). The microplot constituted 70 pots for each experiment, which were inserted 25 cm deep into soil, leaving 5 cm above the soil to avoid overflow of running water. The pots were arranged at 0.6 m inter and intra spacing. Thereafter, three-week-old seedlings of hot chilli cultivar Serrano, purchased from WD Saailinge/Seedlings in Tzaneen were transplanted into each pot. The inoculum was prepared by extracting eggs and second stage juveniles (J2) *M. incognita race 2* and *M. javanica* from roots of grown nematode susceptible tomato ‘Floradade’ (*Solanum lycopersicum* L) in a 1% NaOCl solution (Hussey and Barker, 1973). Fourteen days after transplanting, treatments were initiated. The pots were irrigated back to field capacity with 500 ml tap water every second day based on the amount of depleted water. Plants were fertilised with

5 g NPK 2:3:2 (26), 5% Ca and 0.5% Zn at transplanting and after inoculation with 5 g NPK 2:3:2 (43) Multifeed to provide a total of 0.175 mg N, 0.16 mg K, 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml water. Pots were then mulched with maize straw to minimize soil evaporation.

Figure 3.2: Chilli plants during fruit development.

3.2.4 Data collection

<u>Soil variables</u>: Prior to planting soil samples were collected from microplot for physical and chemical analysis. Physical soil properties such as bulk density were determined using intact core method (Blake and Hartge, 1986), particle size distribution following Bouyoucos (1962) and soil water content using gravimetric method. Phosphorus were determined using Bray-1 method (Bray and Kurtz, 1945), pH with pH meter (HI2002-01, Johannesburg), and soil EC using EC meter (HI2003-01, Johannesburg) and organic carbon (OC) using the Walkley-Black method

(Walkley and Black, 1993). After harvesting, core soil samples were collected for soil physical and chemical analysis.

Plant variables: At harvest (56 days after initiation of the treatments), chilli fruits were hand-picked from each plant. Then plant height was measured with meter stick ruler, stem diameter using digital calliper (DC-515) and chlorophyll content using chlorophyll meter (SPAD-502 Plus, Japan). The above ground biomass of chilli was harvested by cutting each plant at the base.

Nematode variables: The roots from each pot were uprooted, immersed in water to remove soil particles, dry-blotted and then weighed to calculate nematode density per total root plant. Root galls were assessed using the North Carolina Differential Rating Scale (Taylor and Sasser, 1978). Nematodes were first extracted from 10 g roots per plant by maceration and blending for 30 seconds in 1% NaOCl solution (Hussey and Barker, 1973). The nematodes were also extracted from a thoroughly mixed soil, in which a 250 cm^3 soil sample was collected, using the sugar-floatation and centrifugation (Jenkins, 1964). Eggs and J2 were counted from a 10 ml aliquot extract using the stereomicroscope (Z45V, China). The number of nematode from the 10 ml aliquot extract per plant was recalculated for the total root system.

Nutrient elements: In the laboratory, the aboveground biomass excluding the chilli fruits were weighed, oven-dried at 70ºC for 72 hours, and then reweighed to determine total dry biomass per pot. The chilli fruits were weighed, cut into small pieces, oven-dried at 60ºC until constant mass, ground to pass through 0.75 mm sieve for analysis of MMN elements. Prior to nutrient analysis, the ground samples were digested in 5% nitric acid then thoroughly mixed with vortex meter. Thereafter, the samples were incubated in a water bath for an hour at 95ºC, left to cool at room temperature, and then filtered and covered with foil (SW-846 EPA Method 3050B). Samples were quantified for MMN elements, namely, Zn, Mg, Ca, Fe and K, using an Inductively Coupled Plasma Emission (ICPE-9000).

Nutritional water productivity, the amount of water used to produce MMN elements was then calculated using the following equations:

Water use (WU) was calculated from irrigation amount plus rainfall amount (rain that fell during the growing season) minus water available at harvest. Thus: WU = I + R – water at harvest (equation 1). Then water use efficiency or water productivity was calculated from

$$WUE = \frac{yield}{water\ use} \qquad (2)$$

where, WUE is the plant water use efficiency (kg/m^3), yield (kg/ha) and amount of water used in mm. Water use was then converted to m^3/ha (1mm = 10 m^3/ha). The yield was converted to (kg/ha) using proportions of population densities.

Nutrient content (NC) calculation: the sample was extracted from 0.1g dry chilli powder to prepare the aliquot of 40 ml, which was placed on the Inductively Coupled Plasma Emission (ICPE-9000) to give the concentration of nutrient in the sample. Then proportions were done to convert nutrient concentration to nutrition unit per kg (mg/kg).

Then nutritional water productivity was calculated following (Renault and Wallender, 2000):

$$NWP = WP \times NC \tag{3}$$

where NWP is the nutritional water productivity (mg/m^3), WP is the water productivity (kg/m^3) and NC is the nutrient content (mg/kg).

3.2.5 Data analysis

Nutritional water productivity (NWP) values for Ca, K, Fe, Mg and Zn were calculated and subjected to statistical tests that include analysis of variance and regression analysis using Statistix software. Unless otherwise stated, treatment effects were discussed at the probability level of 5%.

3.3 Results

3.3.1 Soil variables

In Experiment 1, infection of plants by *M. incognita race 2* did not have any significant effect on soil electrical conductivity (EC), bulk density (Table 3.1) and particle size (Appendix 3.13; Appendix 3.14; Appendix 3.15). However, the variable had significant effects on soil pH, contributing 29% in TTV of the variable (Table 3.1). Treatment effects were not different from 0 to 625 inoculum levels but were different at 1 250 inoculum level (Table 3.2). Treatment effects caused the pH to decrease (Figure 3.3). Nematode infection had no effects on soil P, but significantly affected organic carbon (OC), contributing 43% in TTV of the variable (Table 3.1). Treatment effects were not different from 0 to 625 inoculum levels but were different at 1250 inoculum level (Table 3.2). In contrast, *M. javanica* did not have effects on all soil variables in Experiment 2 (Table 3.1).

Table 3.1 Partitioning of mean sum of squares for soil variables: electrical conductivity (EC), phosphorus (P), organic carbon (OC), soil pH (pH) and bulk density (BD).

Meloidogyne incognita race 2											
		EC		P		OC		pH		BD	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	1637.54	29	72.95	31	0.84	49	0.4	57	4.775×10^{-3}	28
Treatment	6	2136.62	38^{ns}	107.01	45^{ns}	0.74	43***	0.02	29***	7.419×10^{-3}	44^{ns}
Error	54	1800.97	33	58.29	24	0.13	8.	0.01	14	4.757×10^{-3}	28
Total	69	5575.13	100	238.25	100	1.71	100	0.07	100	0.016951	100
Meloidogyne javanica											
Replication	9	1921.76	39	73.18	29	0.72	62	0.10	43	8.37×10^{-3}	46
Treatment	6	1986.71	40^{ns}	118.58	47^{ns}	0.28	24^{ns}	0.08	35^{ns}	5.66×10^{-3}	31^{ns}
Error	54	1080.81	21	60.45	24	0.16	14	0.05	22	4.27×10^{-3}	23
Total	69	4989.28	100	252.21	100	1.16	100	0.23	100	0.0183	100

TTV% = (MSS/TOTAL) × 100, ^{ns} Non-significant at $P>0.05$ *** Significant at $P \leq 0.05$.

Table 3.2 Influence of *Meloidogyne incognita* race 2 on organic carbon and soil pH.

	OC		pH	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)
0	1.59^{c}	-	6.20^{ab}	-
50	2.15^{ab}	35	6.24^{a}	1
125	1.93^{b}	21	6.21^{ab}	0
250	2.32^{a}	46	6.15^{bc}	-1
625	2.30^{a}	45	6.15^{bc}	-1
1250	2.35^{a}	48	6.11^{c}	-1
2000	2.18^{ab}	37	6.12^{c}	-1

R.I. (%) = ([treatment mean/control] – 1) × 100.

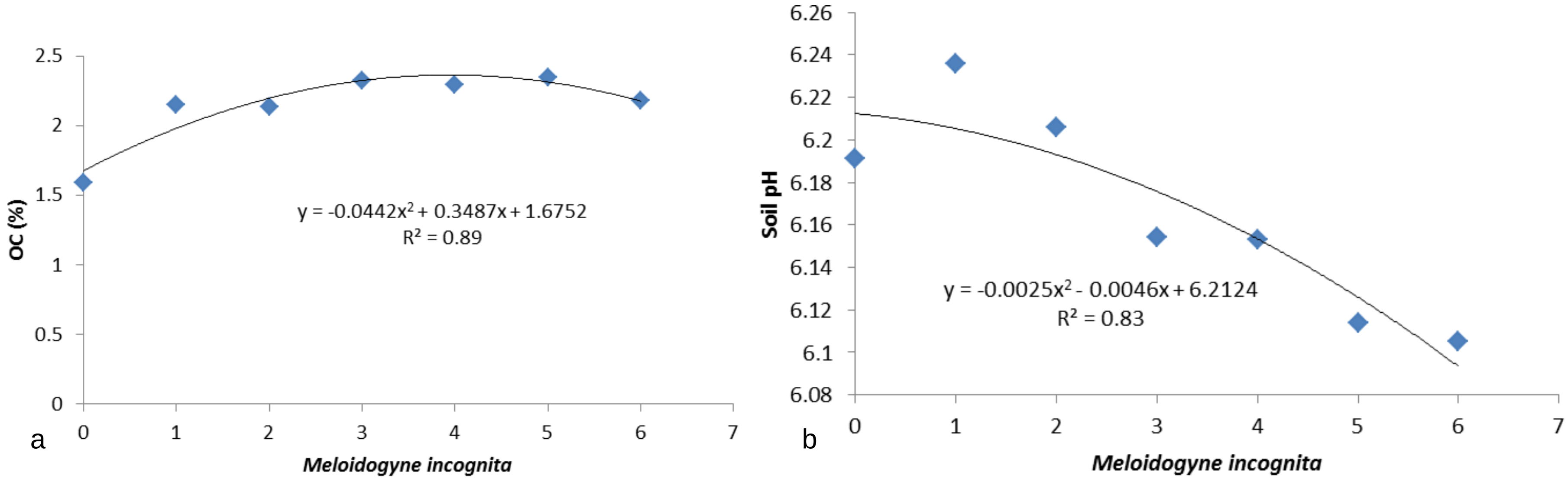

Figure 3.3 Effects of *Meloidogyne incognita* race 2 on soil variables organic carbon and soil pH.

3.3.2 Nutritional water productivity

Means of NWP of Ca, K, Mg Fe and Zn under *M. incognita* race 2 and *M*. *javanica* were summarised (Table 3.3). Subjecting NWP variables and nematode levels to lines of the best fit, exhibited quadratic relations under both *M. incognita* race 2 and *M*. *javanica* trials (Figure 3.4; Figure 3.5; Figure 3.6). Under *M incognita* trial, the models for Ca, K, Mg, Fe and Zn were explained by 78%, 86%, 70%, 70%, and 72% respectively (Figure 3.4; Figure 3.5; Figure 3.6). Whereas under *M. javanica* trial, the models explained by 98%, 92%, 93%, 87%, and 80% relations (Figure 3.4; Figure 3.5; Figure 3.6).

Table 3.3 Mean nutritional water productivity of selected mineral nutrient elements in hot chilli under different inoculation levels of *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

	Meloidogyne incognita race 2					*Meloidogyne javanica*				
Nematode	NWP_{Ca} mg/m^3	NWP_{K} mg/m^3	NWP_{Mg} mg/m^3	NWP_{Fe} mg/m^3	NWP_{Zn} mg/m^3	NWP_{Ca} mg/m^3	NWP_{K} mg/m^3	NWP_{Mg} mg/m^3	NWP_{Fe} mg/m^3	NWP_{Zn} mg/m^3
0	28.89	222.55	54.22	2.74	2.84	21.73	380.78	44.72	1.20	0.77
50	33.65	265.69	59.98	3.72	2.79	32.25	480.07	61.74	1.75	1.12
125	33.54	278.80	57.92	3.95	2.90	36.23	527.03	67.17	2.00	1.11
250	37.66	316.53	69.14	3.65	3.24	38.95	550.50	68.41	2.31	1.20
625	39.14	307.39	70.34	3.07	2.67	41.11	621.01	73.97	2.15	1.30
1250	34.29	309.10	63.68	3.09	2.23	40.70	558.22	60.50	1.99	1.09
2000	35.91	227.63	55.62	7.84	4.49	38.46	581.87	60.33	2.05	1.13
CV	67.95	64.40	60.67	124.7	84.57	74.68	69.51	64.50	70.49	69.42

NWP = Nutritional water productivity for selected mineral elements.

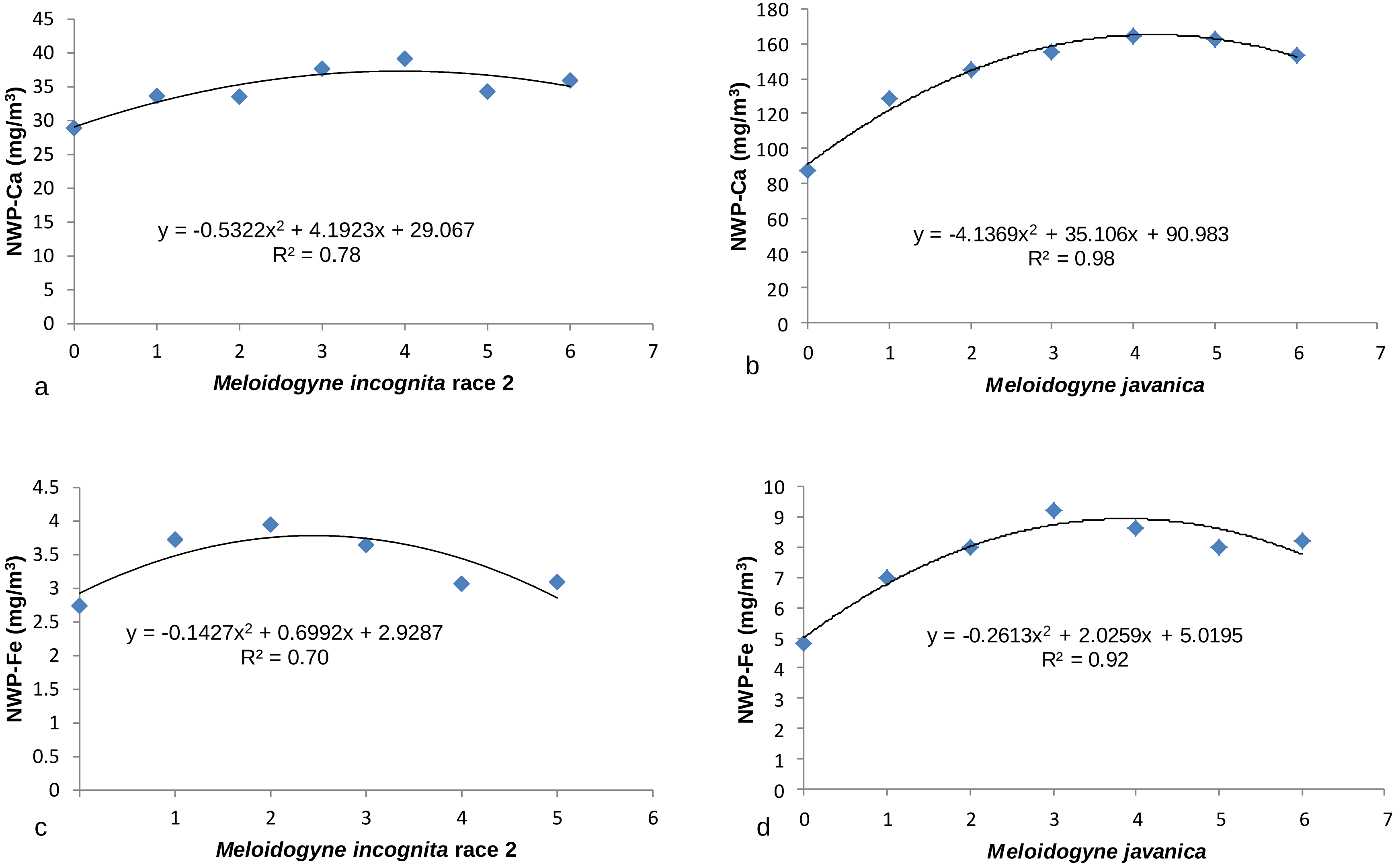

Figure 3.4 Effects of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* on nutritional water productivity of Ca and Fe at 56 days after inoculation.

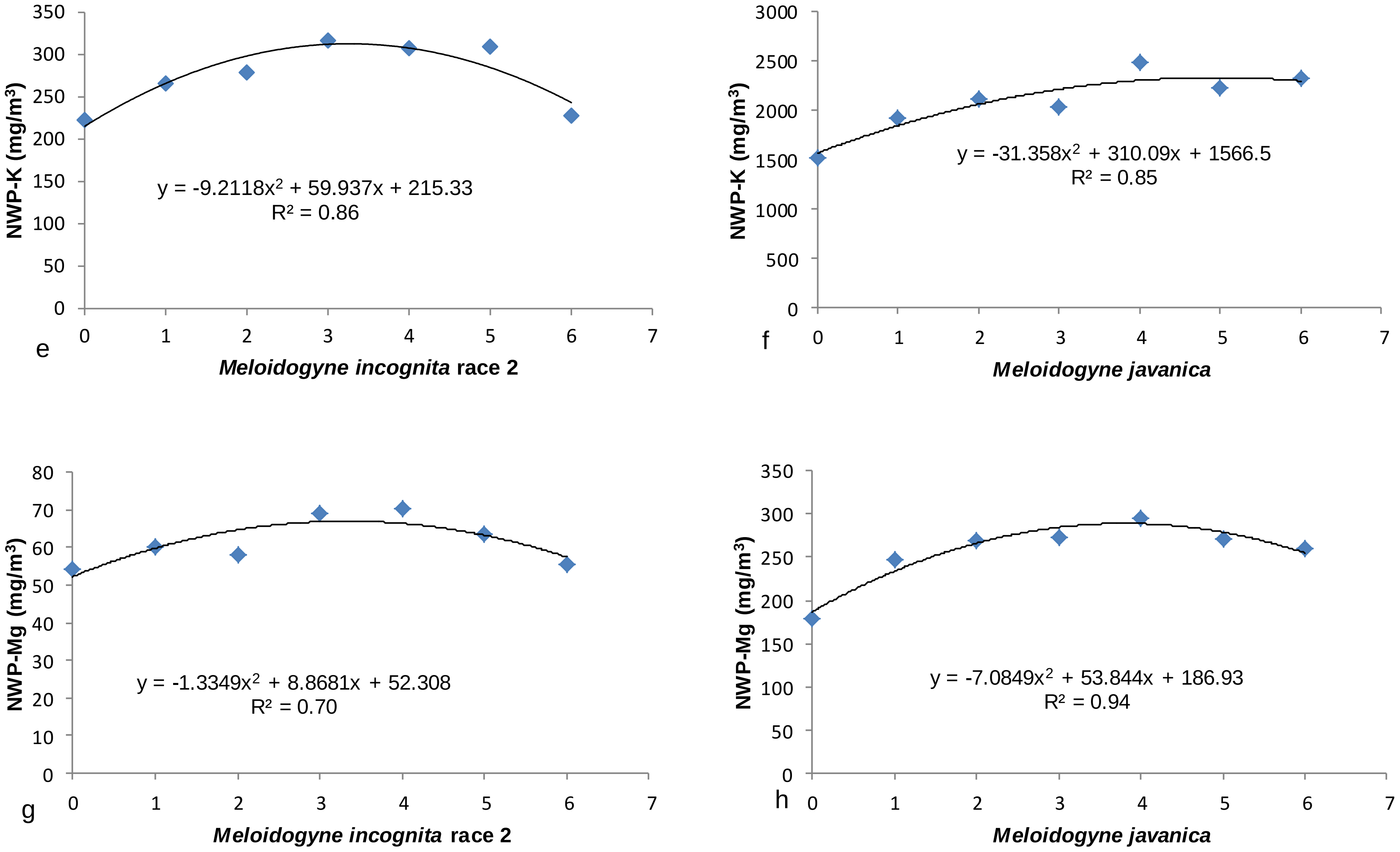

Figure 3.5 Effects of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* on nutritional water productivity of K and Mg at 56 days after inoculation.

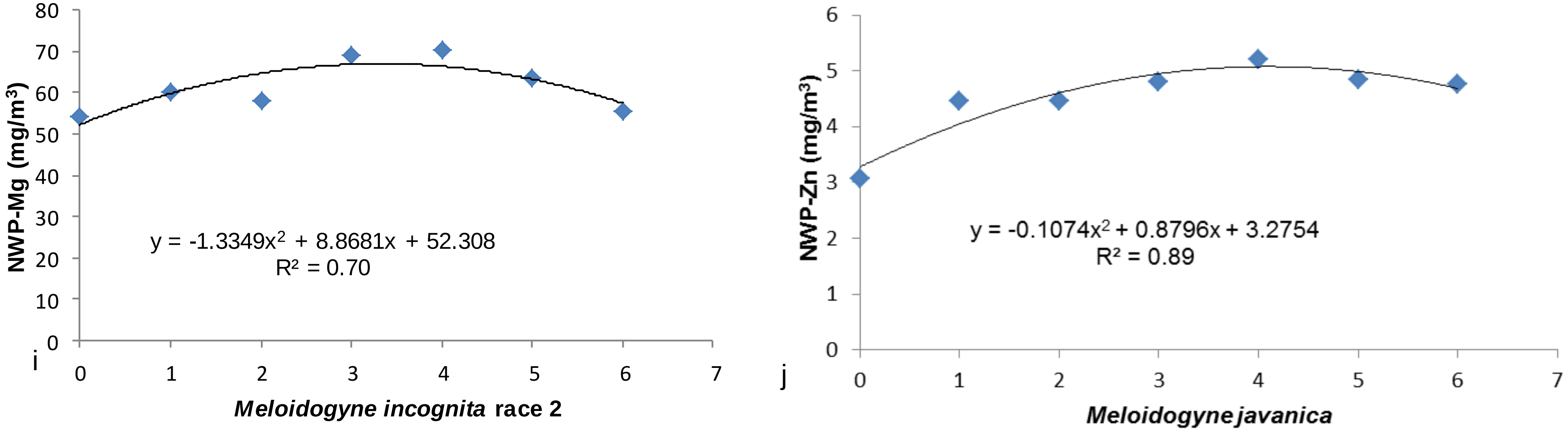

Figure 3.6 Effects of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* on nutritional water productivity of Zn at 56 days after inoculation.

Optimum values: The optimum values for *M. incognita race 2* were attained at treatment 4 (625 eggs and J2), treatment 2 (125 eggs and J2) and treatment 3 (250 eggs and J2) respectively (Table 3.4). The optimum values for *M. javanica* were attained at treatment 3, treatment 4 and treatment 5 respectively (Table 3.4). Therefore, for both experiments, NWP for Ca and Mg declined at same treatment of 625 initial population densities of *Meloidogyne* species*.*

Table 3.4 Quadratic relations for nutritional water productivity of mineral malnutrition element over *Meloidogyne incognita race 2* and *Meloidogyne javanica.*

	Quadratic relation	R^2	X^x	Y^z
	Meloidogyne incognita race 2			
NWP_{Ca}	$y = -0.5322x^2 + 3.9606x + 30.225$	78	4	37
NWP_{Fe}	$y = -0.1427x^2 + 0.6992x + 2.9287$	70	2	4
NWP_K	$y = -9.2118x^2 + 59.937x + 215.33$	86	3	312
NWP_{Mg}	$y = -1.3349x^2 + 8.8681x + 52.308$	70	3	67
NWP_{Zn}	$y = -0.0824x^2 + 0.3238x + 2.7244$	72	2	3
	Meloidogyne javanica			
NWP_{Ca}	$y = -1.0343x^2 + 8.777x + 22.746$	98	4	41
NWP_{Fe}	$y = -0.0652x^2 + 0.5056x + 1.256$	92	4	2
NWP_K	$y = -9.9152x^2 + 89.975x + 388.47$	93	5	589
NWP_{Mg}	$y = -2.0445x^2 + 14.094x + 46.7$	87	3	71
NWP_{Zn}	$y = -0.0305x^2 + 0.2259x + 0.8213$	80	4	1

[x] Calculated optimum treatment level (*Meloidogyne* species) $x = -b_1/2b_2$, where b_1 = coefficient of x and b_2 = coefficient of x^2 on the quadratic equation, then x was the optimum inoculum level.

[z] Nutritional water productivity at optimum level.

3.3.3 Plant growth variables

The treatment, *M. incognita race 2* population densities, had no significant effects on all measured plant variables, except for root galling, where the treatments contributed 76% in total treatment variation (TTV) of the variable (Table 3.5).

Generally, root-galling effects increased with increase in *M. incognita race 2* population densities (Figure 3.7).

Meloidogyne javanica population densities had no significant effects on other measured growth variables. However, the treatment had significant difference on chlorophyll content and root gall rating, contributing 47 and 98% in total treatment variation (TTV) of the respective variables (Table 3.5). Chlorophyll content decreased with increase in *M. javanica* population densities (Table 3.6), whereas root gall rating increased with the latter.

Table 3.5 Partitioning of mean sum of square of plant height (PHT), stem diameter (STD), chlorophyll content (CHR), dry shoot mass (DSM), dry fruit mass (DFM), and gall rating (GLR).

Meloidogyne incognita race 2

		PHT		STD		CHL		DSM		DFM		GLR	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	55.52	38	4.52	25	97.85	45	36.44	28	14.42	44	0.90	13
Treatment	6	53.67	36^{ns}	9.02	50^{ns}	71.84	33^{ns}	45.57	35^{ns}	4.99	15^{ns}	5.45	76^{***}
Error	54	39.08	26	4.61	25	47.57	22	48.95	37	13.26	41	0.80	11
Total	69	148.27	100	18.15	100	217.64	100	130.96	100	32.67	100	7.15	100
Meloidogyne javanica													
Replication	9	106.36	38	14.69	49	681.79	36	162.50	50	60.49	53	0.006	1
Treatment	6	118.67	42^{ns}	7.49	25^{ns}	894.69	47^{***}	110.28	34^{ns}	12.93	11^{ns}	0.49	98^{***}
Error	54	57.17	20	7.94	26	336.73	17	51.35	16	40.70	36	0.003	1
Total	69	282.2	100	30.12	100	1913.21	100	324.13	100	114.12	100	0.50	100

TTV (%) = (MSS/TOTAL) × 100. ^{ns}Not significant at P ≤0.05, ***Significant at P ≤ 0.05.

Table 3.6 Response of chlorophyll content to increasing population of *Meloidogyne javanica.*

Nematode	Variable	R.I. (%)
0	59.27^{ab}	–
50	71.64^{a}	20
125	62.07^{ab}	5
250	246.67^{b}	−21
625	51.15^{b}	−13
1250	46.39^{b}	−22
2000	49.20^{b}	−16

R.I (%) = ([treatment mean/control] – 1) × 100.

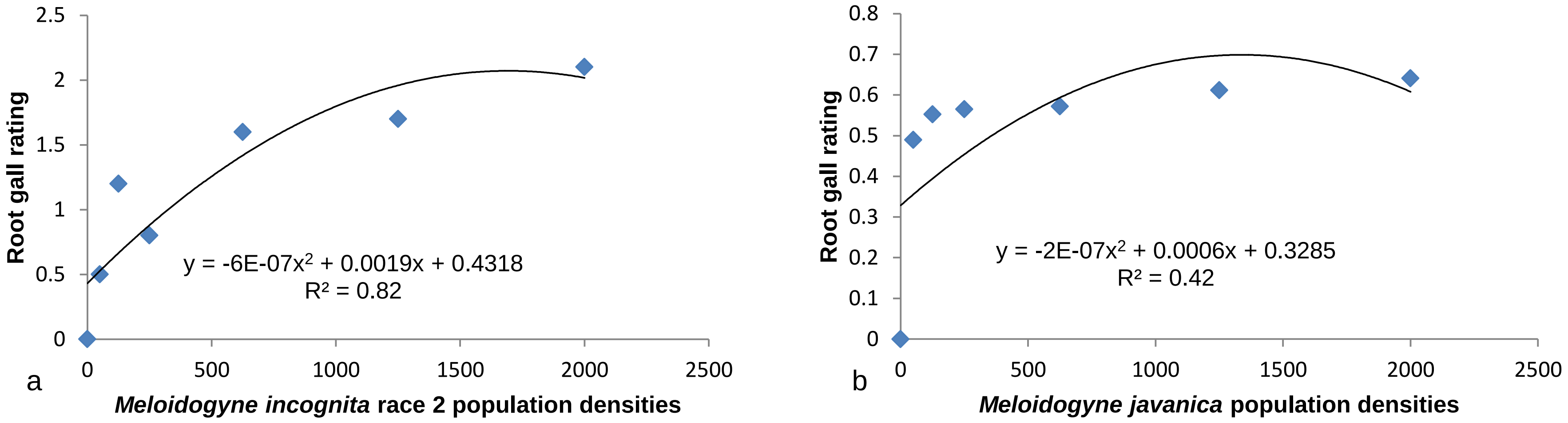

Figure 3.7 Effects of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* on root galling in chilli plant.

3.3.4 Nematode variables

Increasing initial population densities for *M. incognita race 2* (Table 3.7) and *M. javanica* (Table 3.8) had significant effects on final nematode population densities. Furthermore, when subjected to lines of the best fit, nematode variables against their increasing population exhibited quadratic relationship with the model explained by 95, 44 and 93% for *M. incognita race 2* (Figure 3.8). The variables also exhibited positive quadratic relations with the coefficient of determination suggesting 82, 28 and 82%, association, respectively for *M. javanica* (Figure 3.8; Figure 3.9).

Table 3.7 Initial nematodes (Pi), eggs and juveniles in roots, Juveniles in soil ($J2_{soil}$) and final population (Pf) of *Meloidogyne incognita race 2* on nutritional water productivity of chilli.

	Meloidogyne incognita race 2		
Pi	Eggs and $J2_{roots}$	$J2_{soil}$	Pf
50	2.66^{bc} (620)	2.54^{a} (376)	2.95^{b} (996)
125	1.81^{d} (144)	2.45^{a} (299)	2.61^{c} (443)
250	2.24^{cd} (199)	2.55^{a} (365)	2.73^{c} (564)
625	2.71^{b} (662)	2.49^{a} (317)	2.97^{b} (979)
1250	3.71^{a} (6506)	2.50^{a} (326)	3.79^{a} (6832)
2000	3.47^{a} (4687)	2.56^{a} (387)	3.61^{a} (5074)
CV	20.12	6.82	14.37

^{a}Column means with the same letter were not statistically different ($P \leq 0.05$).

$^{()}$Untransformed means.

Table 3.8 Initial nematodes (Pi), eggs and juveniles in roots, juveniles in soil ($J2_{soil}$) and final population (Pf) of *Meloidogyne javanica* on nutritional water productivity of chilli.

Meloidogyne javanica			
Pi	Eggs and $J2_{roots}$	$J2_{soil}$	Pf
50	3.06^{ab} (1501)	2.20^{a} (190)	3.14^{ab} (1691)
125	3.02^{ab} (1520)	2.15^{a} (338)	3.16^{ab} (1858)
250	2.96^{ab} (1301)	2.15^{a} (262)	3.06^{ab} (1563)
625	3.14^{a} (2236)	2.08^{a} (200)	3.21^{ab} (2436)
1250	3.21^{a} (2807)	2.27^{a} (262)	3.31^{a} (3069)
2000	2.73^{b} (978)	2.09^{a} (162)	2.91^{b} (1140)
CV	17.62	20.64	13.44

[a] Column means with the same letter were not statistically different ($P \leq 0.05$).

() Untransformed means.

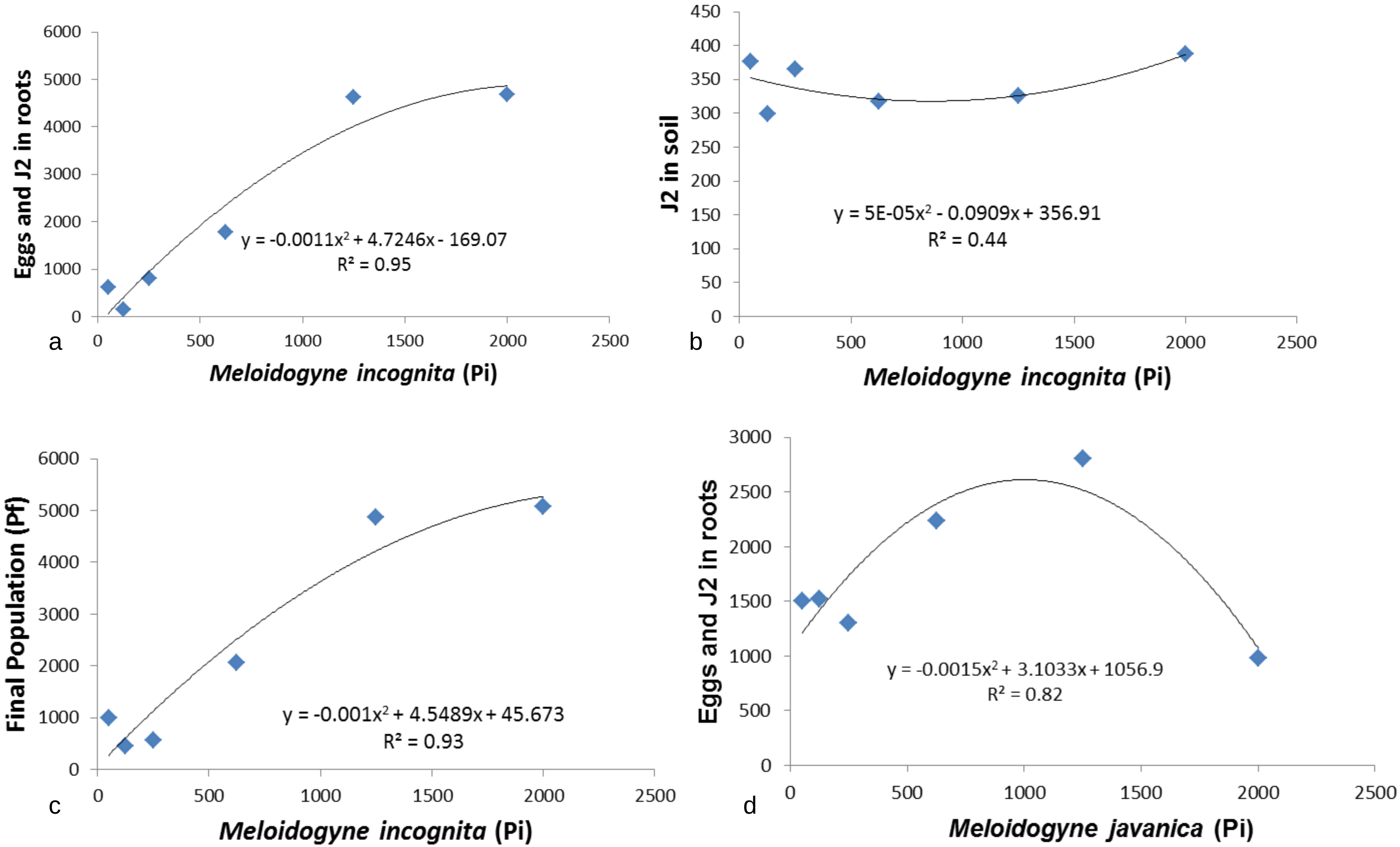

Figure 3.8 Response of nematode variables against increasing population for *Meloidogyne incognita* race 2 and *Meloidogyne javanica.*

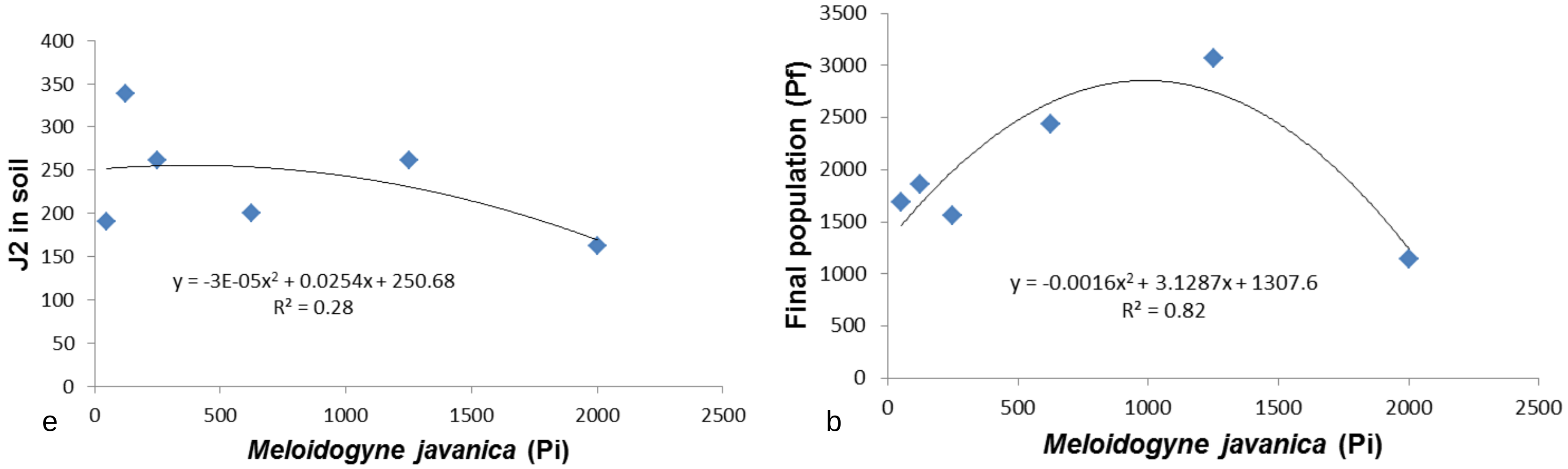

Figure 3.9 Response of nematode variables against increasing population for *Meloidogyne javanica.*

3.4 Discussion

3.4.1 Soil variables

Meloidogyne incognita race 2 infections had no effects on soil electrical conductivity (EC) particle size and bulk density. Interestingly, *M. incognita race 2* infections significantly affected soil pH by contributing 29% in total treatment variation. An increase of *Meloidogyne* infection from 50 to 125 eggs and J2 reduced soil pH. Similarly, a study by Mashela (2002) found that infection by nematodes alone decreased soil pH by 3 to 4% on tomato cultivar. Wallace (1973) postulated that infection of roots by *Meloidogyne* species results in the release of various amino acids from the root galls into the soil solution through root exudation, which reduces soil pH. A study conducted by Aluvilu *et al*. (2010) suggested that some chilli cultivars are tolerant to *M. incognita* race 2 while other cultivars are susceptible. However, tolerant species develop different chemical behaviour with respect to the accumulation of amino acids in stressed root cells (Aluvilu *et al*., 2010). Therefore, results in soil pH change. Soil pH is regarded as a major factor driving the availability of soil nutrients to plant. As the soil pH change the uptake rate of nutrients is affected. Reduction in pH leads to pH moving from alkaline to acidic whereby exchangeable bases including K, Na and Mg will be less available for plant uptake. As such, it affects the soil fertility status. Therefore, limited nutrients in the soil lead to a deficiency in the plant, whereby produced crop also lacks some mineral nutrients.

Nematode infection had no effects on soil phosphorus but significantly affected organic carbon (OC) by contributing 43% in total treatment variation. Organic carbon is contained within organic matter, which favours the growth of plants and microorganism. The latter considered to be important component of soil as it improves soil quality and productivity (Widmer *et al*., 2002) through improving soil structure, water holding capacity and availability of nutrients. In this study, OC was increasing with increase in *M. incognita race 2* population but at the highest population, it dropped. Since, OC releases nutrients to soil, its reduction indicate decline in nutrients for plant growth. This might be the results of nematodes utilizing the nutrients for their own growth through their feeding mechanism on the root system.

3.4.2 Nutritional water productivity

The findings of this study indicated that at lower nematode levels there was gradual stimulation of NWP for Ca, Mg, K, Fe and Zn. Nutritional water productivity was increasing with increasing *M. incognita race 2* population densities, with gradual levelling off that leads to the inhibition phase, in context of density-dependent growth (DDG) patterns (Mashela *et al*., 2016). At higher levels of nematode population, NWP decreased. Currently, there is no information on the effects of increasing nematode population on NWP. However, previous study focused mostly on the effects of nematode infection on the shoot water relation and root hydraulic conductivity of seedling cotton (Kirkpatrick *et al*., 1991). Previous work also reported on response of mineral malnutrition elements in African ginger pseudostems to nematode infection (Mashela *et al*., 2016). Jagadeesh (2011) investigated nutrient translocation and accumulation. Other studies focused on nutritional water productivity under certain environmental conditions (Renault and Wallender, 2000; Chibarabada *et al*., 2017). Therefore, this study is the first to investigate the response of NWP to biotic factors such as *Meloidogyne* species.

Kirkpatrick *et al.* (1991) noted that nematode infection decreases the water uptake by the plant. Jagadeesh (2011) reported a reduction in Fe content from 162.63-172-79 ppm to 46.99-52.69 ppm at high infestation level. Similar observations were made with regard to Zn content, whereby it was reduced from 37.23-40.59 ppm to 3.25-3.96 ppm. In general, studies on the uptake of water under infection with nematodes indicated that nematode injury to root system decreases both water and solute absorption (Kirkpatrick *et al*., 1991). Nutritional water productivity definition implies that any factor influencing yield, nutrient content and water uptake would affect NWP directly or indirectly. Therefore, increase in the NWP in this study might be the fact that nematode was still establishing feeding site on the crop, which resulted in the gall formation on the root system. Then the reduction in NWP in the context of this study was the result of low nutrient absorption rate and low water uptake due to highly infested root system whereby nematodes reproduced. This reduction in NWP leads to failure of accessing nutritious food by the human population, due to the availability of nutritious food being dependent on the agricultural output. Lack of the latter will induce the serious health concern such as malnutrition, a condition where human body does not get enough nutrients to function well.

The findings of this study from the prediction models suggested that at *M. incognita race 2* population densities of above 125 eggs and juveniles, nematode management would be required to sustain the NWP_{Fe} and NWP_{Zn} for human health purposes. However, under infection with *M. javanica*, management practices would be required at population density of above 625 eggs and J2 for NWP_{Zn}. These results suggest that nematode infection could induce malnutrition at higher infestation levels only. Previous study reported that root-knot nematode damage on crops caused yield losses (Thies *et al.*, 2008), which negatively affected the economy. Current study then, suggested that high infestation of *Meloidogyne* species reduced NWP for MMN elements, which could negatively pose threat to human health.

3.4.3 Nematode variables

Nematodes variables against their increasing population exhibited quadratic relationship. The variables increased with increasing initial population for both *M. incognita race 2* and *M. javanica* indicating that the nematodes where able to feed and reproduce on the roots.

3.5 Conclusion

In conclusion, findings of this study suggested that moderate nematode infection improves NWP for selected elements, whereas high nematode infection lowers NWP of the crop for selected MMN elements. At moderate infection levels, nematode infection almost always stimulates regeneration of new rootlets, which increase the surface area for absorption. Therefore, at low to moderate infection levels, nematodes would improve the availability of selected MMN elements, thereby preventing malnutrition in humans, whereas the opposite would be true at high inoculation levels. Also, results suggested that *Meloidogyne* species had effects on soil quality and productivity as they affected other soil properties such as pH, which also affects the availability of selected MMN elements. In order to improve NWP of the current test crop, nematode management would be necessary at higher infestation levels.

CHAPTER 4

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of findings

Due to malnutrition being a health concern, vegetable crops such as chilli can be used as supplement since they regarded as a major source of the mineral malnutrition elements such as Ca, K, Zn, Fe and Mg. The main aim of this study was to determine the effects of increasing population of *Meloidogyne incognita race 2* and *Meloidogyne javanica* on nutritional water productivity (NWP) of chilli plant. Main findings of the study suggested that infection by root-knot nematodes affect the NWP, whereby at a low population the former stimulated NWP but as the population of nematodes increases it caused a slow decline in NWP of the elements. The NWP for Ca increased and dropped at a population density of 1250, whereas for other elements NWP decline occurred at 2000 eggs and J2 population densities.

Several factors found to have effects on the NWP. The findings of this study indicated that infection by *M. incognita race 2* reduced soil pH and increased OC, which directly or indirectly affected the NWP. The latter explained by the reduction in soil pH as the driver of most nutrients needed by the crop for its growth. Most nutrients are pH dependent. Reduction in soil pH cause some of the nutrients to be unavailable in the soil whereby the crop fails to absorb them, hence result in a deficiency of the nutrients in the crop. As such, the NWP is also affected.

4.2 Significance

The findings demonstrated that increasing population densities of *M. incognita race 2* and *M. javanica* positively and negatively affect the NWP of chilli plant, depending on the infestation levels. Quadratic relations showed the relationship between the NWP of the mineral elements and increasing population of nematodes. It was also observed that *M. incognita race 2* affect soil quality, as there was a reduction in soil pH and soil organic carbon.

4.3 Recommendations

In future studies, the interaction of soil water balance, nematodes and NWP should be studied further to validate their relations. Since this study focused on the mineral malnutrition elements, further studies should be conducted on micronutrients malnutrition substances because their deficiency also leads to malnutrition. Due to chilli having high nutritional content it can be recommended as source to supplement malnutritional compounds. However, future research should be conducted on how it should be used since human health respond differently to the capsaicin, which is contained within the chilli fruit.

4.4 Conclusion

In conclusion, *Meloidogyne* species interfered with NWP of the mineral elements in chilli plant. As such, nematode management practices should be done to reduce the population densities while sustaining the quality of agricultural products for human health benefits in areas where the soil is affected by the latter.

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APPENDICES

Appendix 3.1 Analysis of variance for plant height of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P ≤
			Experiment 1		
Replication	9	461.44	51.27		
Treatment	6	307.69	51.28	1.32	0.27
Error	54	2101.46	38.92		
Total	69	2870.59	141.47		
			Experiment 2		
Replication	9	957.28	106.36		
Treatment	6	711.99	118.67	2.08	0.07
Error	54	3087.35	57.17		
Total	69	4756.35	282.2		

Appendix 3.2 Analysis of variance for stem diameter of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P ≤
			Experiment 1		
Replication	9	40.70	4.52		
Treatment	6	54.11	9.02	1.96	0.09
Error	54	248.81	4.61		
Total	69	343.62	18.15		
			Experiment 2		
Replication	9	132.24	14.69		
Treatment	6	44.95	7.49	0.94	0.47
Error	54	428.67	7.94		
Total	69	605.86	30.12		

Appendix 3.3 Analysis of variance for chlorophyll content of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P ≤
			Experiment 1		
Replication	9	880.66	97.85		
Treatment	6	431.05	71.84	1.51	0.19
Error	54	2568.73	47.57		
Total	69	3880.44	300.42		
			Experiment 2		
Replication	9	6136.1	681.79		
Treatment	6	5368.1	894.96	2.66	0.02
Error	54	18183.1	336.73		
Total	69	29687.4	1913.48		

Appendix 3.4 Analysis of variance for dry shoot mass of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P ≤
			Experiment 1		
Replication	9	327.95	36.44		
Treatment	6	273.43	45.57	0.93	0.48
Error	54	2643.09	48.95		
Total	69	3244.47	130.96		
			Experiment 2		
Replication	9	1462.47	162.50		
Treatment	6	661.64	110.27	2.15	0.06
Error	54	2772.89	51.35		
Total	69	4897.00	324.12		

Appendix 3.5 Analysis of variance for number of fruits of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P ≤
			Experiment 1		
Replication	9	1567.7	174.19		
Treatment	6	546.9	91.15	0.61	0.72
Error	54	8005.7	148.25		
Total	69	10120.3	413.59		
			Experiment 2		
Replication	9	2.06	0.23		
Treatment	6	092	0.15	1.60	0.17
Error	54	5.16	0.10		
Total	69	8.13	0.48		

Appendix 3.6 Analysis of variance for dry fruit mass of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P ≤
			Experiment 1		
Replication	9	129.77	14.42		
Treatment	6	29.96	4.99	0.38	0.89
Error	54	716.03	13.26		
Total	69	875.76	32.7		
			Experiment 2		
Replication	9	544.40	60.49		
Treatment	6	77.56	12.93	0.32	0.93
Error	54	2197.79	40.70		
Total	69	2819.75	114.12		

Appendix 3.7 Analysis of variance for root gall rating of chilli (*Capsicum annuum* L.) in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	8.13	0.90		
Treatment	6	32.74	5.46	6.86	0.00
Error	54	42.97	0.80		
Total	69	83.84	7.16		
			Experiment 2		
Replication	9	0.05	0.01		
Treatment	6	2.94	0.49	181.01	0.00
Error	54	0.15	0.02		
Total	69	3.13	0.52		

Appendix 3.8 Analysis of variance for organic carbon in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	7.56	0.84		
Treatment	6	4.45	0.74	5.55	0.00
Error	54	7.22	0.13		
Total	69	19.22	1.71		
			Experiment 2		
Replication	9	6.50	0.72		
Treatment	6	1.68	0.28	1.73	0.13
Error	54	8.70	0.16		
Total	69	16.87	1.16		

Appendix 3.9 Analysis of variance for phosphorus in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	656.57	72.95		
Treatment	6	642.04	107.01	1.84	0.11
Error	54	3147.60	58.29		
Total	69	4446.21	238.25		
			Experiment 2		
Replication	9	658.66	73.18		
Treatment	6	711.48	118.58	1.96	0.09
Error	54	3264.40	60.45		
Total	69	4634.54	252.21		

Appendix 3.10 Analysis of variance for soil electrical conductivity in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	14738	1637.54		
Treatment	6	12820	2136.62	1.19	0.33
Error	54	97252	1800.97		
Total	69	124810	5575.13		
			Experiment 2		
Replication	9	17295.9	1921.76		
Treatment	6	11920.2	1986.71	1.84	0.11
Error	54	58363.8	1080.81		
Total	69	87579.9	4989.28		

Appendix 3.11 Analysis of variance for soil pH in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	0.40	0.04		
Treatment	6	0.14	0.02	3.23	0.01
Error	54	0.39	0.01		
Total	69	0.92	0.07		
			Experiment 2		
Replication	9	0.93	0.10		
Treatment	6	0.49	0.08	1.51	0.19
Error	54	2.90	0.05		
Total	69	4.32	0.23		

Appendix 3.12 Analysis of variance for bulk density in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	0.04	4.78×10^{-3}		
Treatment	6	0.04	7.42×10^{-3}	1.56	0.18
Error	54	0.26	4.76×10^{-3}		
Total	69	0.34	0.016951		
			Experiment 2		
Replication	9	0.07	8.37×10^{-3}		
Treatment	6	0.03	5.66×10^{-3}	1.33	0.26
Error	54	0.23	4.26×10^{-3}		
Total	69	0.33	0.0183		

Appendix 3.13 Analysis of variance for silt in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
Replication	9	238.63	26.51		
Treatment	6	42.97	7.16	0.71	0.64
Error	54	542.17	10.04		
Total	69	823.77	73.71		

Appendix 3.14 Analysis of variance for sand in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P
Replication	9	85.94	9.55		
Treatment	6	26.97	4.50	0.63	0.70
Error	54	384.46	7.12		
Total	69	497.37	21.17		

Appendix 3.15 Analysis of variance for clay in response to *Meloidogyne incognita race 2* and *Meloidogyne javanica*.

Source	DF	SS	MS	F	P≤
Replication	9	78.63	8.74		
Treatment	6	38.40	6.40	1.82	0.11
Error	54	190.17	3.52		
Total	69	307.20	18.66		

Appendix 3.16 Analysis of variance for eggs and juveniles in roots of chilli in response to increasing initial population densities

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	1.06	0.12		
Treatment	6	92.81	15.47	67.55	0.00
Error	54	12.37	0.23		
Total	69	106.23	15.82		
			Experiment 2		
Replication	9	1.37	0.15		
Treatment	6	79.64	13.27	63.80	0.00
Error	54	11.23	0.21		
Total	69	92.24	13.63		

Appendix 3.17 Analysis of variance for Juveniles in soil in response to increasing initial population densities.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	0.15	0.02		
Treatment	6	54.22	9.04	418.43	0.00
Error	54	1.17	0.02		
Total	69	55.54	9.08		
			Experiment 2		
Replication	9	2.74	0.30		
Treatment	6	40.14	6.69	45.95	0.00
Error	54	7.86	0.15		
Total	69	50.73	7.14		

Appendix 3.18 Analysis of variance for final population in response to increasing initial population densities.

Source	DF	SS	MS	F	P≤
			Experiment 1		
Replication	9	0.40	0.04		
Treatment	6	91.36	15.23	122.66	0.00
Error	54	6.70	0.12		
Total	69	98.46	15.39		
			Experiment 2		
Replication	9	1.25	0.16		
Treatment	6	85.01	14.17	108.82	0.00
Error	54	7.03	0.13		
Total	69	93.30	14.46		