GROWTH AND NUTRITIONAL STATUS OF SWEET PEPPER (*CAPSICUM ANUUM* L.) ARE CO-INFLUENCED BY NITROGEN APPLICATION AND *MELOIDOGYNE* SPP. INFECTION

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Abstract

Sole ammonium is known to negatively affect plant growth and also reduce rhizophere pH. Interestingly low pH negatively affects nematode growth. On the other hand, nitrates enhance plant growth but increase plant exudates that attract nematodes. A greenhouse experiment was set up at Kenyatta University to evaluate the effect of nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations with and without nitrification inhibitor applied at 50,100 and 200 parts per million (ppm) nitrogen (N) on sweet pepper (*Capsicum annum* L.) infected with root-knot nematode *Meloidogyne* spp on growth and nutrient composition. The plant height, leave area, plant weight, and the concentration of N, phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) in the leaves were determined at 60 days after nematode inoculation. NH_4^+-N increased the tissue concentration of total N, P, K, and Mn. *Meloidogyne* spp. caused reduced dry weight of shoots and roots, with decrease in P, Mn and Fe and increase in leaf tissue Mg and Ca in highly infected plant compared to the less infected. Sweet pepper plants fertilized with lower rates of nitrogen treatments (50 ppm N and 100 ppm N) had higher Fe and Zn but with much low Mn than the treatment with more nitrogen (200 ppm N). There was high P uptake in treatments comprising of ammonium-N with the nitrification inhibitor. Although ammonium with nitrification inhibitor (NH_4^+-N+NI) at 200 ppm N exhibited the lowest nematode reproduction and root galling, the same treatment also had reduced shoot growth and dry weights. NH_4^+-N+NI at 100 ppm N had higher tissue nutrient contents with reduced nematode population and higher total dry weight. The study therefore demonstrates that NH_4^+-N fertilization with an inhibitor can reduce nematode population when plants are grown in a soil infested with the root-knot nematodes. The study also demonstrated that NH_4^+-N+NI a reduction *Meloidogyne* spp, rational nitrogen utilization with increased plant growth and plant nutrient concentration under 100 ppm N.

Keywords*:* *Capsicum annum* L., *nitrogen, nitrification inhibitor, cations, mineral antagonism, rhizosphere pH*

Introduction

Sweet Pepper (*Capsicum annuum*) has increased in popularity, value and importance over a long period, thus making it an indispensable part of the daily diet of millions of people (Abu-Zahra, 2012). It has high contents of bioactive compounds and strong antioxidant capacity and it is among the most common fresh vegetables worldwide due to its combination of color, flavor, and nutritional value (Blanco-Ríos, 2013). Pepper (*capsicum* sp) comprising sweet pepper has been recognized as second most significant vegetable after tomatoes in the nightshade family and is now commonly cultivated in all parts of the world (Benson *et al.,* 2014).

Root-knot nematodes (*Meloidogyne* spp.) cause considerable economic losses in sweet pepper production. An average of 10% of loss in the yield is frequently cited for sweet pepper (Smith *et al*, 2001). Knowledge on the interaction between nematodes and fertilizer (NPK) as an agricultural input and soil amendment is still limited (Olowe, 2012), which has led to low yields among farmers who have inadequate information and knowledge on appropriate mineral fertilizer use. Nematode infestation on plants depresses root growth and activity, nutrient uptake and the overall host physiology (Wilhelm *et al*., 1985; Melakeberhan *et al*., 1990, Farahat *et al.,* 2007). While penetrating the plant and feeding on the tissues, the root-knot nematodes draw off plants nutrients and cause mechanical damage and physiological changes. Nutrients can reduce or increase disease severity, affect the environment to attract or deter pathogens and also induce resistance or tolerance in the host plant ((Santana-Gomes *et al* 2013; Agrios, 2005). Ferraz *et al.,* (2010) argued that fertilizer application can reduce nematode-induced damage by stimulating plant development. James *et al.,* (1996) showed that patterns of dry matter distribution and nutritional status depend on how nutrient application has been managed. Diby *et al*., (2011) deduced that proper plant growth assessment cannot be made unless nutritional control of growth is practiced. However, balancing nutrients in plants is difficult to accomplish when plant-parasitic nematodes cause nutrient imbalances within the plant (Hurchanik *et al* 2004).

Nitrogen forms are largely used in vegetable production in Kenya among other countries and it is the largest agricultural chemical utilized for plant growth and development (Wang *et al*., 2008). Nitrogen is a fundamental structural unit of protein. The N is required for formation of amides, amino acid and eventually protein; which is also important in improvement of growth features through efficient metabolic activity and increased rate of photosynthesis (Marschner, 1995). It stimulates root growth and crop development as well as uptake of the other nutrients (Stevenson and Cole, 1999; FAO, 2002). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) fertilizers are the main forms of inorganic nitrogen fertilizers that are widely used in agro-ecosystem for increased production (Gweyi-Onyango *et al*, 2009). The form of nitrogen that is supplied to plants affects the uptake of other cations and anions, cellular pH regulation in the rhizosphere (Marschner, 1995). In addition, depending on soil pH, the uptake of ammonium may lead to production of fumes (ammonia gas). Both ammonia gas emission and pH changes (associated with ammonium nutrition) are known to adversely affect the nematodes (Munyasi 2017). Besides, the different forms have differential growth promoting effect, hence conferring plant resistance to nematode attack. Application of fertilizers can influence nutrient availability and changes in soil environment leading to the control of plant diseases in an integrated pest management system (Huber and Graham, 1999; Graham and Webb, 1991). Use of nitrification inhibitors has been identified as a way to overcome the challenges associated with N fertilizer application, such as high loss and low recovery of applied nitrogen due to leaching and de-nitrification, nitrate accumulation in vegetables and grasses resulting in nitrate toxicity to human and animals, and nitrate accumulation in groundwater due to continuous N fertilizer application (Gnanavelrajah *et al*, 1998). The correlation of plant growth and tissue nutrients in different nitrogen fertilization of infected crop is envisaged to provide an insight into nutrient-disease interactions in this study.

Material and Methods

Site Description

The study was carried out at Kenyatta University which is located within co-ordinates 1°81'10.0" South and 36°28'41.0" East in Kiambu County. The experiment was carried out under a partially controlled greenhouse conditions with temperatures ranging from 25±3 ºC day-time to 15±3 ºC night-time, a 12:12 hour light:dark photoperiod and a relative humidity of 80-90%.

Table 1 Physiochemical soil properties for the greenhouse experiment

Physical properties	
Sand %	56.60
Silt %	20.40
Clay	25.00
Chemical properties	
Soil pH	6.80
Total N %	0.04
Organic carbon %	0.26
Extractable P ppm	5.00
Potassium ppm	117.00
Calcium ppm	400.00
Magnesium ppm	236.40
Sodium ppm	62.10
Iron ppm	21.60
Manganese ppm	43.95
Copper ppm	1.24
Zinc ppm	3.05

Experimental Design, Treatments and Data Collection

Preparation of nutrient solution

Three nutrient solutions were used from modified full-strength nutrient solutions, one set containing only NO_3^- and the other set containing only NH_4^+ in stabilized and un-stabilized form of nitrogen nutrition. They were stabilized using nitrification inhibitor (dicyandiamide and 3, 4 methylpyrazole phosphate). The 'NO_3^- solution' consisted of: 1M calcium hydrogen phosphate [3Ca $(H_2PO_4)2.H_2O$]; 1M magnesium sulphate [$MgSO_4.7H_2O$]; 1M potassium nitrate [KNO_3] and the 'NH4$^+$ solution': 1M calcium chloride [$CaCl_2.2H_2O$]; 1M potassium phosphate (monobasic) [KH_2PO_4]; 1M magnesium sulphate [$MgSO_4.7H_2O$]; 1M ammonium sulphate [NH_4^{2+}-N]. The micronutrients were supplied to both treatments as: Fe-EDTA; $MnSO_4.10H_2O$;H_3BO_3; $ZnSO_4$. $7H_20$; $CuSO_4.5H_2O$; and $(NH_4)_6Mo_70_{24}$. Ammonium sulfate fertilizer was intended to provided ammonium-N, potassium nitrate to provided nitrates-N, the rates for nitrogen were adjusted to provide 50, 100, and 200 ppm N.

Greenhouse experiment

Five weeks old seedlings of sweet pepper cv. California wonder (about 15cm tall) were transplanted singly in the plastic 1500 cm^3 pots filled with sterilized sandy loam soil set in greenhouse and arranged in a completely randomized design and replicated three times. One week after transplanting, the following nitrogen treatments were applied NO_3^--N, NH_4^+-N, NH_4^+-N + NI at the following rates 50, 100, and 200 ppm N. Each combination was represented by eight experimental units and every unit contained one seedling. Nematodes were inoculated by pipetting nematode suspension into 4 holes in the soil around the root system of each plant. Each pot was inoculated with 2500 infective stage of root-knot nematodes. The experiment was terminated at 60 days after inoculations,

Data collection

Data on growth parameters criteria (plant height, leaf area, shoot fresh and dry weight as well as root fresh weight) and nutrient analysis were recorded.

Data analysis

All data obtained was subjected to analysis of variance (ANOVA) using SAS 9.1 computer software and where significant means separated using Least Significant Difference (LSD) at (5%) probability level.

Results

Plant growth and morphology as affected by N forms and rates

The results revealed that shoot length and root length was significantly affected by the form and rate of nitrogen nutrition (Table 1).

The greatest shoot length was recorded in 100 ppm N in NH_4^+-N+(NI) treatment at 38.83cm, which was 83.16% increase compared to the control. On the other hand, the greatest shoot dry weight (SDW) was observed in 100 ppm N from NH_4^+-N+(NI) (2.73g) and 200 ppm N from NH_4^+-N was 2.58g. There were however no significant difference in their shoot length (Table 1).

The shoot dry weights at higher nitrogen nutrition treatments (200 ppm N) were lower compared to nitrogen nutrition treatments at 100 ppm N. There were significant differences in shoot dry weights at 50 ppm N, 100 ppm N and 200 ppm N for NH_4^+-N +(NI) and NH_4^+-N. In addition, there were no significant differences between 100 ppm N and 200 ppm N in NO_3^--N treatment. From the study, the ratio of shoot dry weight (SDW) to root dry weight (RDW) increased consistently with increase in nitrate N and reduced with increasing ammonium N with the treatment of NH_4^+-N+(NI) eliciting the greatest decrease in ratio compared to NH_4^+-N (Table 1) while the control treatment had the highest ratio of shoot dry weight to root dry weight.

There was significance ($P \leq 0.05$) increase in leaf area amongst all the treatments when compared to the control. The leaf area increased with increase in nitrogen levels from zero (control) to 100 ppm N. However, at higher nitrogen rates of 200 ppm N the leaf area decreased in all the treatments as illustrated in figure 1. It appears that the optimal N concentration was 100 ppm. Beyond this concentration, additional amount led to decrease in leaf area.

The results also revealed significant differences ($P \leq 0.05$) in sweet pepper root dry weights amongst the different forms of nitrogen nutrition (Table 1) There was no significance difference in root dry weights between 100 ppm N and 200 ppm N in terms of NO_3^--N treatments, on the other hand at 100 ppm N and 200 ppm N treatments with NH_4^+-N showed significant difference in relation to root dry weights as shown in table 1. However, the greatest root dry weight (RDW) was recorded in 200 ppm N in NH_4^+-N+(NI) (1.99g) and 100 ppm N in NH_4^+-N+(NI) (1.88.g) (table 1), this might be due to their interaction with root-knot nematodes and the effect of the nitrification inhibitor.

Leaf mineral nutrients concentration as affected by N forms and rates

Table 2 shows the effect of nematode infection and damage on nutrient concentrations in leaves of sweet pepper plants. There was significant difference in tissue nitrogen concentration within the nitrogen sources at different rates. There was were significant differences in tissue potassium levels in 100 ppm N and 200 ppm N (nitrate nutrition), and equally there were no significant difference in tissue potassium at 50 ppm N and 100 ppm N of sole ammonium nutrition but all the rates of ammonium nutrition with nitrification inhibitor were significantly different. The other tissue nutrient concentration behaved in varing ways as a result of root-knot nematode infection and the treatments as shown in table 2.The presence of RKN in the root system definitely affected nutrient uptake by the plant, RKN in the root system resulted in changes in nutrient concentration in leaves and also in total nutrient uptake by the plant. Generally sweet pepper plant grown in soil with ammonium sulfate, the concentrations of Fe, Mn and Zn were higher in plant biomass than those in plants given potassium nitrate.

Table 1 Means of shoot length, root length, leaf area, shoot dry weight, root dry weight, ratio of dry shoot weight to dry root area in sweet pepper weight

Treatments		SL (cm)	Increase %	RL (cm)	SDW (g)	Increase (%)	RDW (g)
Control		21.20g	0	12.95e	1.43d	0	0.70f
NO_3^--N	**50**	27.98f	31.98	13.25de	1.85bcd	29.37	1.08e
	100	33.60cd	58.49	14.20b	2.43abc	69.93	1.38d
	200	35.50bc	67.45	13.50cde	2.38abc	66.43	1.33d
NH_4^+-N	**50**	29.30ef	38.21	13.45cde	1.88bcd	31.47	1.10e
	100	37.75a	78.07	15.98a	2.58ab	80.42	1.68c
	200	38.60a	82.08	14.12b	2.41abc	68.53	1.63c
NH_4^+-N + (NI)	**50**	31.5de	48.58	13.35de	1.79cd	25.17	1.09e
	100	38.83a	83.16	15.46a	2.73a	90.91	1.88b
	200	37.25ab	75.71	13.77bcd	2.6ab	81.82	1.99a

Shoot lengths (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW). Values within a column bearing the same letter are not significantly different (α = 0.05).

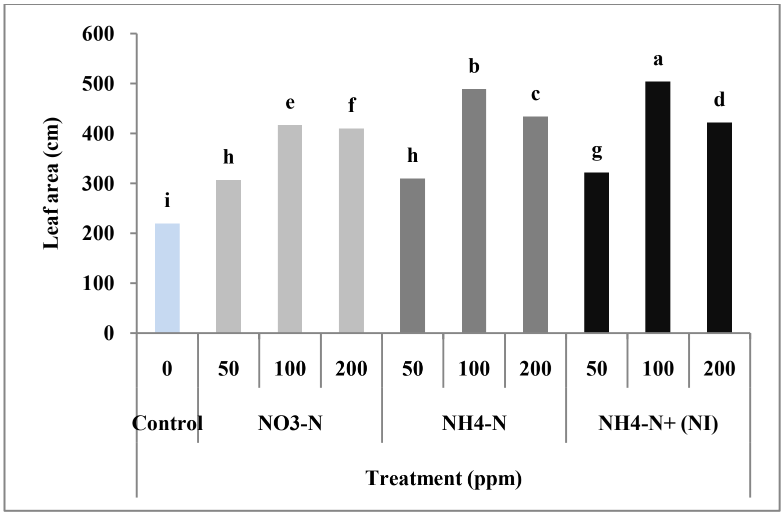

Figure 1**:** Effect of different nitrogen form, rate and nitrification inhibitor on leaf area. Values wth the same letter are not significantly different (α = 0.05)

In all treatments, total N% content of leaves increased considerably compared to the control. The N concentration increased in the tissue as the treatment rate increase except for 200 ppm N in NH_4^+-N+(NI) treatment, where were some observed. Generally, there were significant differences in nitrogen concentration in all forms of nitrogen treatment at 100 ppm N irrespective of the forms. There was no significant difference in nitrogen concentration between treatment NH_4^+-N +(NI) and NO_3^--N at 200 ppm (Table 2). There was no significant differences between 100 ppm N of NH_4^+-N +(NI) and 200 ppm N of NH_4^+-N.

Effects of nitrogen forms and rates on concentrations of potassium, calcium, phosphorus and reproduction factors of nematodes in sweet pepper

Increase in calcium and potassium levels resulted in reduction in reproduction factor as shown in figure 2. The levels of calcium were higher with ammonium and potassium were lower in ammonium nutrition with or without nitrification inhibitor as compared to nitrate nutrition.

Total net uptake of K in NH_4-fed plants was markedly smaller than that of NO_3 plants at both nutrient levels. The sole NH_4^+-N+(NI) N-source also caused a decrease in potassium uptake. Ammonium decreased shoot Ca concentration, increased shoot K and had no significant effect on shoot Mg concentration in NH_4^+-N and NH_4^+-N+(NI) (Table 2) treatments rates, however as the N rates increased, the shoot Mg content also increased and this may be associated with increased growth rate with increased nitrogen fertilizer rates and reduction in nematode infection severity in NH_4^+-N and NH_4^+-N +(NI) treatments.

Potassium uptake was enhanced in NH_4^+-N + (NI) compared to control and sole NH_4^+-N treatment which may be attributed to increased growth rate. Potassium (K) was significantly higher in NO_3^--N treatments compared to NH_4^+-N+(NI) and NH_4^+-N treatments. However, the potassium levels was also significantly higher NH_4^+-N+(NI) when compared NH_4^+-N fed plants.

The phosphorous percent was relatively higher in ammonium treatments compared to nitrate treatments (Figure 3). Phosphorous levels were significant higher in NH_4^+-N +(NI) when compared to NH_4^+-N, however there was no significant differences in phosphorous level at 100 ppm N and 200 ppm N for NH_4^+-N+(NI) (Table 2). There were no significant differences among 50 ppm N of NH_4^+-N+(NI), 100 ppm N of NH_4^+-N and 50 ppm N of NH_4^+-N Table 4.4). The root-knot nematode population and reproduction factor reduced with increasing levels of tissue phosphorus.

Nitrogen forms on manganese, zinc, and iron and reproduction factor of nematodes in sweet pepper

Unexpectedly, manganese concentration was high in ammonium treatments compared to nitrate, however they were much higher in NH_4^+-N + (NI) than in NH_4^+-N (Figure 4). Naturally, inhibition transformation of ammonium to NO_3 resulting in the competition between NH_4^+ and Mn^{2+} on clay surface is expected and may have been the cause less Mn. Like in case of other microelement shown above, the trend of nematode reproduction factor as well as zinc concentrations (Fig 5) was affected by N forms and rates and showed similar trend with manganese (Fig, 4 and 5). Nitrate fertilizer plants had lower zinc concentration compared to ammonium fertilized plants.

The Fe was high in ammonium treatments at high rates of nitrogen nutrition compared to nitrate treatments; however they were much higher in ammonium with inhibitor than ammonium alone. NH_4^+-N +(NI) may have encouraged Fe uptake due to increasing of assimilation process of enzymes and hormones formation (Fig 6). Similar trends were observed with the other two microelement (manganese and zinc) (Figs 4 and 5).

Table 2 Figures showing variations in tissue concentration of some macro and micro elements in leaves as affected by nitrogen forms and rates

Nutrients Treatments		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Control		3.56f	0.35e	4.08e	0.89e	0.34a	90.00h	70.00h	56.70g	13.30a
NO_3^--N	**50**	4.47de	0.44d	5.19a	0.88e	0.32b	273.13b	64.36i	64.59f	4.56g
	100	4.70dc	0.55c	4.35c	0.61fg	0.29bc	236.28e	78.26e	67.81e	5.16f
	200	5.12b	0.54c	4.68b	0.50g	0.27c	164.21g	73.23g	53.77h	5.34e
NH_4^+-N	**50**	4.88c	0.56bc	4.53c	1.06d	0.29bc	200.44f	82.18d	77.60a	8.25b
	100	5.22b	0.57bc	4.08e	2.94b	0.30bc	254.97d	90.79b	78.22a	7.40c
	200	5.73a	0.59b	3.89f	3.10a	0.32b	234.24e	84.28c	75.75b	7.48c
NH_4^+-N + (NI)	**50**	4.44e	0.57bc	5.09a	0.66f	0.29bc	237.00e	76.18f	72.50d	5.73d
	100	5.74a	0.63a	4.23d	1.81c	0.30bc	294.37a	93.02a	73.35c	5.17ef
	200	5.32b	0.65a	3.98ef	3.00ab	0.31b	264.17c	84.65c	78.07a	5.19ef

Data are means of four replications use to compare between nitrogen sources, rate and nitrification inhibitor on root-knot nematode infected plant Values within a row bearing the same letter are not significantly different ($\alpha = 0.05$)

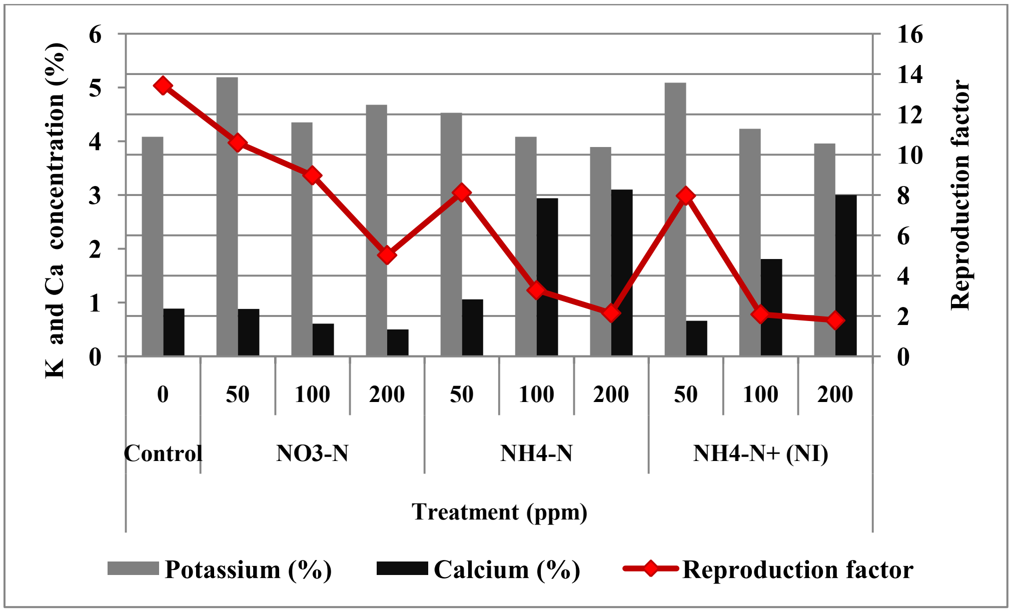

Figure 2 Relationship between treatments and potassium and calcium concentration in root-knot nematode infected sweet pepper. Values within a plot bearing the same letter are not significantly different (α = 0.05)

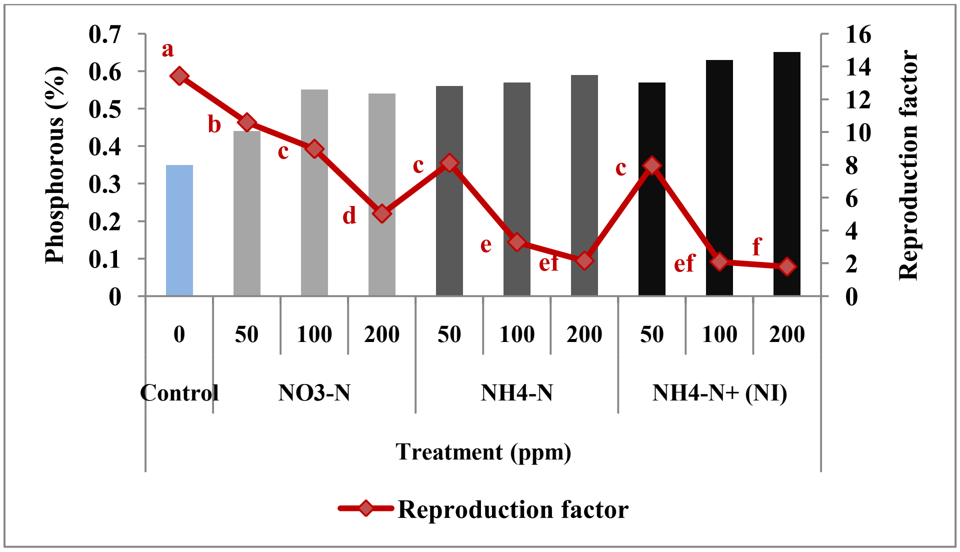

Figure 3: Relationship between treatments and phosphorous concentration in root-knot nematode infected sweet pepper. Values within a plot bearing the same letter are not significantly different (α = 0.05)

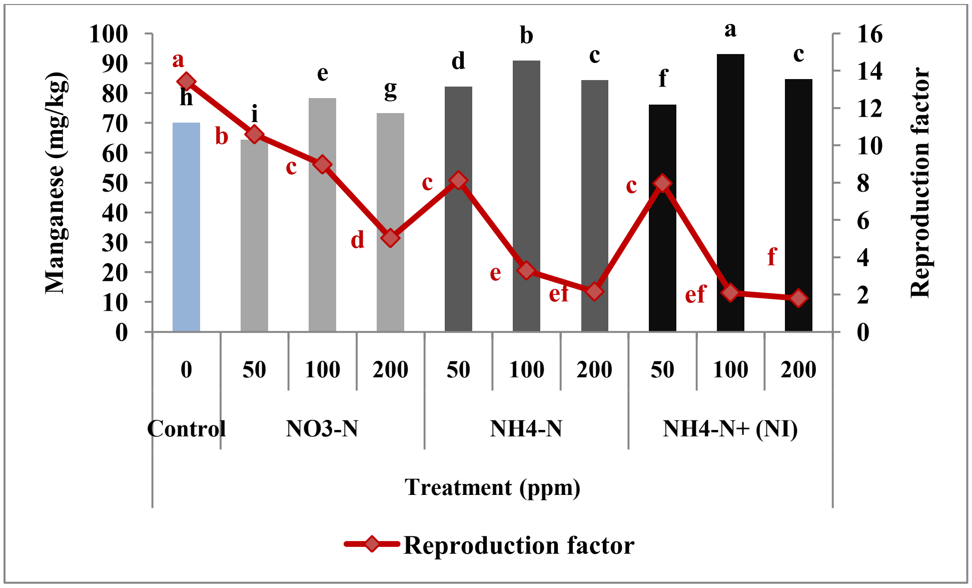

Figure 4 Effects of different N forms on tissue manganese concentration in root-knot nematode infected sweet pepper.

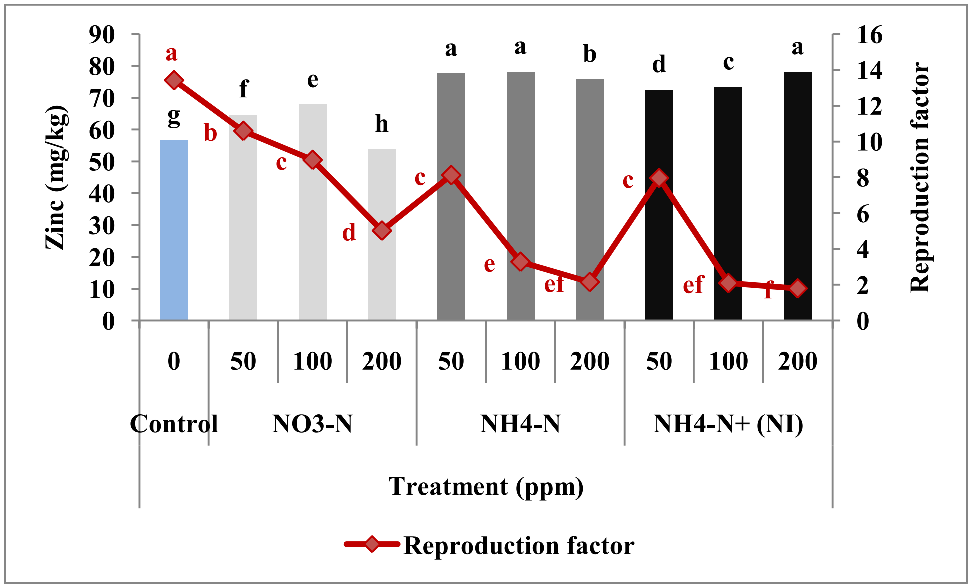

Figure 5: Relationship between treatments and zinc concentration in root-knot nematode infected sweet pepper. Values within a plot bearing the same letter are not significantly different ($\alpha = 0.05$)

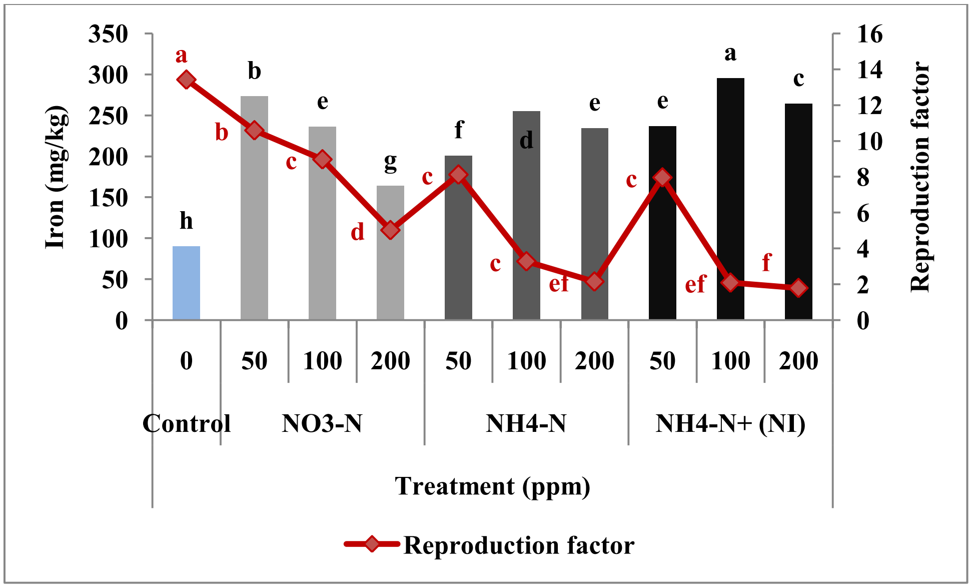

Figure 6: Relationship between treatments and iron concentration in root-knot nematode infected sweet pepper. Values within a plot bearing the same letter are not significantly different (α = 0.05).

Discussion

Abundance of nitrogen may results in the production of new tissues and saps, and can extend the vegetative state and increase the number of feeding sites in the roots, encouraging nematode attack, and when a plant is deficient in nitrogen can become debilitated, suffer slowed growth (Mengel *et al*., 2006) and become more susceptible as illustrated in table 1 for control and 50 ppm N treatments as compared to 100 ppm N and 200 ppm N treatments. Increased growth rate due to the addition of ammonium to nitrate containing nutrient solution also been observe by other researchers in a variety of plant species. Maynard et al., (1969) and Cox et al., (1973) found that the addition of ammonium to nitrate-containing solutions produced increased growth in wheat, but that the maximum growth rate in ammonium-containing nutrient solutions was limited by ammonium toxicity at high levels of nutrient ammonium, this is also observed in the final plant growth height at 200 ppm N of NH4+-N and NH4+-N+(NI) treatments. Badra et al., (1980), illustrated that application of nitrogenous fertilizer significantly affected shoot and total plant weights but root growth was almost unaffected. Regardless of nematode treatment, shoot dry weights from plants treated with NH4+-N or NH4+-N+(NI) were generally better than the other treatments. As expected, shoots from plants supplied with different N sources and rates were larger than the controls (Table 1). There was decrease in shoot dry weight with increase in nitrogen nutrition probably due to reduced plant use of nitrogen efficiency; this is in agreement with the results Tilman et al., (2002). There was a significant increase in sweet pepper growth in terms of shoot and root dry weights in the NH4+-N treatments over the control, whereas the treatments NO_3^--N were lower compared to ammonium treated plants and this is in agreement with a study carried out by Karajeh et al. (2013) reporting on effects of different nitrogen forms and salts on root-knot nematode infected tomato plant. As expected higher proportions of dry weights were observed when the higher rates of N were applied compared to lower rates.

Leaf, area and plant weight increased as both nitrate and ammonium concentrations increased. There was decrease in shoot growth with NH4+-N relative to NO_3^--N nutrition both in presence and absence of NI, which may be associated with increased in plant water stress, root hydrant resistance. The current results are in agreement with previous work reported by Gweyi-Onyango (2009) with tomatoes.

The increase in plant resistance to nematode infection as shown by reduction in reproduction factor (Figure 2) in ammonium nutrition treatment may be due to the utilization of K thus increased thickness of the epidermal cell wall, boosting the structural rigidity of tissues and playing a fundamental role in metabolic reactions in plants, regulating stomata functioning and promoting rapid recovery of injured tissue, due to the accumulation of phytoalexins and phenols around the infection site (Huber *et al*., 1985). The other argument is that higher K^+ concentrations decreased the internal competition of pathogens for nutrient resources as reported Holzmueller *et al.* (2007). Low levels of potassium uptake may result in the impairment of synthesis of high molecular-weight compounds (proteins, starch and cellulose) leading to accumulation of low-molecular-weight organic compounds. Potassium deficient plants have impaired protein synthesis and accumulate simple N compounds such as amides which are used by invading plant pathogens (Dordas, 2008), hence higher observed reproduction factor with nitrates in the current findings.

The Ca concentration in nitrate nutrition and control was significantly lower compared to ammonium nutrition alone or with the inhibitor. It is an essential element for the integrity of the plant cell's plasmatic membrane, and more specifically ion-transport selectivity (Epstein et al, 2004). Like other nutrients, calcium must be present in sufficient quantity in the soil, since calcium-deficient plants are more susceptible to nematode attack (Hurchanik *et al*., 2003), this is shown by the higher nematode reproduction factor in nitrate treatment and lower reproduction factor in ammonium treatment. The NH_4^+-N+(NI) decreased shoot Ca concentration and increased shoot K concentration when compared with NH_4^+-N, which may be due to depressed growth of meristematic tissues leading to fewer roots available for infection as a result of reduced translocation calcium in the xylem. Generally, the calcium content of plant tissues affect the incidence of diseases in two ways: a) when calcium levels are low, there is an increase in the efflux of compounds of low molecular weight (sugars) from the cytoplast to the apoplast, and b) calcium polygalacturonates are required for the middle lamella to stabilize the cell wall. Calcium has also been used in the management of fungal diseases infections through its suppressive activity (Von Broembsen and Deacon, 1997; Biggs, 1999).

NH_4^+-N+(NI) indirectly improved the mobilization and uptake of phosphorous in form of phosphate in the rhizosphere compared to NH_4^+-N, this is in agreement withthe findings reported by Hagin *et al*., (1990) and Shaviv, (1993) where NH_4 increased P bio-availability when its nitrification rate was reduced, probably through the rhizosphere acidification mechanism. In this regard, the delay in biological oxidation of ammonia using inhibitors can reduce the loss of nitrogen through leaching process (Zerulla, *et al*., 2001) and this process leads to the reduction of the rhizosphere pH and thus increased uptake of phosphorus increases (Amberger, 1989). Maintaining greater ammonium concentrations through more efficient nitrogen applications benefits plant growth as well as favoring better soil retention of nitrogen and improving phosphate availability and uptake (Fixen, 1983). Manganese (Mn) activates a number of enzymes on the chemical acid and subsequent pathways, leading to the biosynthesis of aromatic amino acids such as tyrosine and a number of other secondary compounds such as lignin and flavenoids (Barker *et al*., 2007). This leads to development of tougher cell walls resulting to reduce nematode penetration and infection. For instance, Mn^{2+} affects the phenylalanine Ammonia-Lyase (PAL) enzyme and stimulates peroxidases necessary for lignin biosynthesis. The lower lignin content in plants deficient in manganese is an indication of the need for this element at a number of stages in lignin biosynthesis and the reduction in the amount of root material contributes to lower plant resistance to pathogen attack (Marschner, 1995).

Zinc deficiency seriously interferes with growth, which is dependent on protein synthesis, which in turn depends on transcription (Barker and Pilbeam, 2007). These considerations suggest that one of the inhibiting effects of zinc deficiency on growth could be due to inadequate protein synthesis (Epstein *et al*., 2004). Plants deficient in zinc contain low levels of superoxide dismutase and therefore high levels of superoxide radicals, promoting membrane lipid peroxidation and a loss of membrane integrity, increasing permeability (Barker and Pilbeam, 2007). Furthermore, the accumulation of free amino acids and amides occurs as a result of protein synthase inhibition due to zinc deficiency, boosting the quantity of these amino acids in root exudates (Cakmak and Marschner, 1988). Since nematodes are attracted by exudates, the higher root exudation in plants deficient in zinc can attract these parasites and, therefore, speed up the infection process (Streeter *et al*., 2001). The reported zinc deficiency with and nematode increase are at variance with the current results (Fig 5). This expected outcome may have been masked by the higher Zn related to nitrate N which also lead to higher exudates production related associated with nematode attack (Munyasi 2017).

Ammonium ion uptake by plant and consequence hydrogen ion efflux occur from root solubilizing enough Fe near the root to overcome chlorosis. NO_3^--N fed plants have reduced Fe (Fig 6) uptake and/or chelation within the plant. Our findings aresimilar to the results obtained by Smith *et al*., (1983). Iron uptake is enhanced at low pH (Mengel and Kirkby, 1978) and it is possible that the external pH rise accompanying with nitrate absorption causes restricted Fe uptake. The reduction of nitrate results in higher levels of free inorganic cations than free inorganic anions. The production of organic acid anions enables of plant to achieve ionic balance (Osmond and Laties, 1969).

Conclusion

Ammonium nutrition led reduced plant growth but decreased plant nematode load as evidenced by the lower reproduction factor. The cations (Ca, P, K, Mg, Zn, Fe) were negatively (partially) affected by concentration of ammonium. The mixture of ammonium and nitrate can be proposed as a trade off since the mixture can enhance plant growth but modifies the pH and hence reduce nematode infestation and also has less effects on cations concentrations.

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