Influence of Inoculum Levels of Root Knot Nematodes (*Meloidogyne* spp.) on Tomato (*Solanum lycopersicum* L.)

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ABSTRACT--- ***The root knot nematodes (Meloidogyne spp.) are important group of plant parasitic nematodes which poses threat to tomato (Solanum lycopersicum L.) production. The study was conducted in shade net house located at the Agronomy Department, Faculty of Agriculture, University for Development Studies, Nyankpala from September to December, 2011 to investigate the effect of inoculum densities of root knot nematodes on the growth of tomato cv. Pectomec and the inoculum level that will cause the highest nematode infestations. The experiment was laid out in a completely randomized design with four treatments and replicated four times. The inoculum levels were: 0, 500, 1000, and 2000 freshly hatched second stage juveniles (J_2) of root knot nematodes /1kg soil/pot. All pots were inoculated with root knot nematode J_2 a week after transplanting of the tomato seedlings. Data were taken on plant girth, plant height, number of leaves, root galls, nematode eggs population density and root weights (fresh and dry weights). From the results obtained, it was observed that all the inoculum levels reduced the stem girth, plant height, number of leaves, and fresh and dry root weights. Increasing the nematode inoculum level resulted in corresponding increased in number of galls and nematode population build up. The reduction in growth parameters and nematode infestations were found to be proportional to the inoculum level.***

Keywords— *Meloidogyne* spp., Inoculum level, *Solanum lycopersicum*, Root galls

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the important vegetable crops grown throughout the world and ranks next to potato in terms of production area but ranks first as a processing crop (Mohammed, 2013). It is a major horticultural crop with an estimated global production of over 120 million metric tons (FAO. 2007). It is one of the most widely used food crops in world vegetable economy (Chapagain and Wiesman, 2004). Total land area for production in Ghana increased from 28,400ha in 1996 to 37,000ha in 2000 (GIPC, 2001). Vegetables account for 9.6% of total food expenditure and 4.9% of total expenditure in Ghana, and tomato alone makes up to 38% of the vegetable expenditure (Wolff, 1999). Tomatoes are major sources of lycopene, a dietary carotenoid found in high concentrations in processed tomato products according to Di Mascio *et al.* (1998). Tomato production has been an important source of income for smallholder farmers for many years. In recent years, domestic tomato production has seen a rise across Ghana but local production is not able to meet the domestic high demand and this has resulted in tomatoes been often imported, mainly from Burkina Faso (Horna *et al.*, 2006).This situation is as a result of a number of constraints in tomato production, among them are root knot nematodes which play prominent role. Root knot nematodes *(Meloidogyne* spp.) are economically important pests of a wide range of vegetables throughout the world (Castagnone-sereno, 2006). Hemeng (1981) reported an average yield loss of 73-100% in the Guinea Savannah zone of Northern Ghana due to root knot nematodes. Root knot nematodes have a wide host range and are considered the greatest threat to global agriculture (Ameer-Zareen *et al*., 2003). The short life cycle of six to eight weeks enables root knot nematode populations to survive well in the presence of a suitable host and their populations build up to a maximum usually as crops reach maturity (Shurtleff and Averre, 2000). The potential host range of *Meloidogyne* species encompasses more than 3000 plant species (Abad *et al*., 2003).The most economically important species are *M. arenaria, M. incognita, M. javanica* and *M. hapla*. The nematode infection acts as energy sink absorbing photosynthate required by the plant for growth and fruit production, hence crop yields are reduced and harvested produce is of poor quality and reduced storage life. Root knot nematodes damage leads to symptoms similar to those caused by nutrient deficiency, water stress or soil-borne diseases. Heavily infested plants do not respond to water and fertilizer application because they severely damage the conducting tissues of the roots. The damage predisposes the crop to other pathogens by leaching of nutrients into the soil which

favours the growth of bacteria and fungi (Sasser, 1989). Root knot nematode increases the incidence and severity of *Fusarium* wilt and bacteria wilt by providing an infection court for the pathogens (Agrios, 1997). According to Kinloch (1982) the growth of a plant is inversely proportional to the initial population density of *Meloidogyne* species; hence as nematode population rises above the economic threshold, control becomes more difficult. It is, therefore, important to control the potential rise of root knot nematodes so that they remain below levels at which they reduce the yield of crop plants (Bridge, 1996). Various control strategies have, therefore been employed to manage the root knot nematode. Some of these have been the use of nematicides, biological control and resistant varieties. Infections of non efficient or efficient hosts by low densities of *Meloidogyne* spp. may enhance growth and yield of host and or have no effect, or cause severe damage to the crop. The objective of this study was therefore to investigate the effect of different inoculum densities of root knot nematodes on the growth and root galling of tomato cv. Pectomec.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in shade net house located at the Department of Agronomy, University for Development Studies, Nyankpala from September to December, 2011. The area is located 16km West of Tamale with latitude of 9°25'N and longitude 0°58'W (SARI, 2008). Nyankpala has unimodal rainfall pattern with mean annual rainfall of 1000 mm distributed from April to September. Mean minimum temperature of 23.4^{o}C and maximum of 34.5^{o}C with minimum relative humility of 46% and maximum of 76.8% (SARI, 2008).

2.2 Soil Sterilization

Soil for pot experiments was sterilized using the steam sterilization method. Top soil was sifted to remove foreign materials such as plant debris, plastic materials, broken pots and glasses. It was then mixed with river sand at 3:1 ratio (v/v) and sterilized using a metal tray for 30 minutes at 100ºC with fire wood as the source of heat. The sterilized soil was spread on a large metal sheet after heating and left over night to cool off.

2.3 Raising of Tomato Seedlings

The farmer's preferred cultivar of tomato seeds cv. 'Pectomec' was nursed in steam sterilized top soil in a seed box. The seedlings were transplanted into one litre size pots filled with 1 kg sterilized topsoil three weeks after germination.

2.4 Filling of Pots and Transplanting of Tomato Seedlings

The sterilized soil (1 kg) was put into a plastic pot of one litre size. After two weeks of seed nursing, hand trowel was used to transplant the seedlings from the seed box to the pots. Two seedlings were planted in each pot. Each pot was labelled and watered with equal amount volume of water**.**

2.5 Extraction and Counting of Root Knot Nematode J2

Cabbage plants infested with root knot nematodes were collected from a farm near Tamale. Extraction of nematode J_2 was done using modified method of Hussey and Barker (1973). Root knot nematodes infested roots were washed, dabbed dry and then cut into pieces with a pair of scissors. 30 g of the chopped roots was placed in a big jam bottle and 0.5% sodium hypochlorite (NaOCl) solution was added to cover the roots and then covered. The content of the bottle was agitated vigorously for four minutes. The chopped roots and NaOCl mixture was collected and rinsed with tap water on 200 µm-pore mesh sieve over 500 µm-pore mesh sieve and rinsed with tap water. Water was added to obtain the egg-water suspension. Root knot nematode J_2 were counted using a counting tray with the aid of a stereo microscope. Counting was done three times per entry.

2.6 Inoculation of Tomato Seedlings

The seedlings were inoculated with 0, 500, 1000, and 2000 *Meloidogyne* spp. J_2 per pot a week after transplanting. Three holes were made in a triangular form 2cm from the plant stem and the egg suspension was poured into them using 10 ml micropipette. Four replicates were kept for each of the inoculum levels, including a control without any inoculation (0 J_2). The pots were arranged in a complete randomized design and kept in the plant house and watered once every two days.

2.7 Experimental Design

The design for the experiment was completely randomized design with four treatments. Each treatment was replicated four times and they were as follows: Control (water), 500 *Meloidogyne* spp. J_2, 1000 *Meloidogyne* spp. J_2 and 2000 Meloidogyne spp. J_2.

2.8 Harvesting of tomato plants

The test plants were harvested twelve weeks after inoculation. To ensure easy removal of the plants from the soil, the soil was watered and kept overnight to soften it. The sides of the plastic pots were pressed to loosen the soil. The soil was then removed from the roots by gently shaking the plants.

2.9 Assessment of root galls

The roots of the harvested tomato plants were each washed separately and dabbed dry with tissue paper. Galling was scored on the scale of 0-10 rating chart by Bridge and Page (1980). Fresh weight of roots of each entry in the screen was measured, using an electronic balance.

2.10 Extraction of root knot nematode juveniles

Extraction of root knot nematode juveniles was from infested roots of tomato, using modified Baermann tray method (Whitehead and Hemming, 1965). The roots were chopped with a pair of scissors and 10g of each entry in the study were placed separately in a plastic sieve lined with a two- ply tissue paper placed in a plastic plate. Tap water was poured carefully into the plastic plate in which the sieve was resting until the tissue became moist. The set up was left for 48h and were then poured separately into beakers and left for about 24h for the juveniles to settle at the bottom. The volume of each suspension was standardized to 50ml. Each suspension was taken with a pipette into a counting tray and counting done with the aid of a stereo microscope. Each suspension was homogenized by blowing air through with a pipette. Counting was done three times to obtain the mean number of juveniles.

2.11 Data Collection

Data collection on growth parameters was done at two weeks interval until flowering. Data was collected on the following parameters; plant height, number of leaves per plant, plant girth, fresh and dry root weights, root gall indices and number of root knot nematode J_2 /5g chopped tomato roots at harvest.

2.12 Statistical Analysis

Data collected were analyzed, using the Genstat statistical package (Discovery edition 8.1). Least significant difference (LSD) at 5% was used for comparing mean differences. All counting data were transformed using square root transformation of $\sqrt{(x+0.5)}$, where x is the mean count.

3. RESULTS AND DISCUSSION

3.1 Plant Height

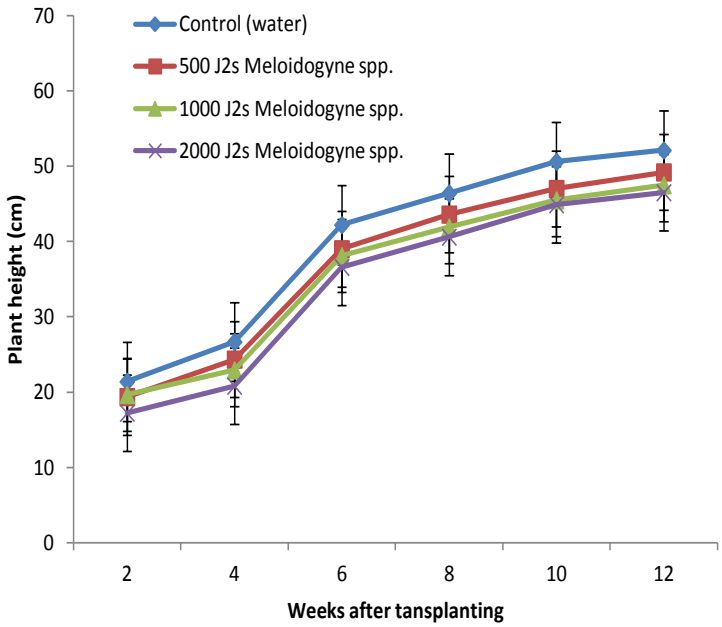

Figure 2: Effect of Inoculum levels of *Meloidogyne* spp. J2s on plant height of tomato

Plant height was significantly affected by the inoculation of *Meloidogyne* spp. J_2. At 2, 4, 6 and 8 WAT, there were no significant differences ($p>0.05$) between treatments. However at 10 and 12 WAT, significant differences ($p<0.05$) were observed with plants which were inoculated with 2000 J2s producing the shortest plants followed by 1000, 500 and 0 *Meloidogyne* spp. J_2 respectively (Figure 1). The decrease in plant height due to inoculation of *Meloidogyne* spp. J_2 at 10 and 12 week after transplanting compared to the other treatments may be attributed to the damage caused by high population density of the nematode to the plants. The results conform to the findings of Siddique and Alam (1985) who

reported that plants heavily infested with root knot nematodes exhibited stunted growth and poor yield and in some cases the plants die even before reaching maturity (Singh and Khurma, 2007). However, Haider *et al*. (2003) reported that the inoculum level of 100 J_2 *Meloidogyne incognita* /plant caused the significant reduction in growth characters of French bean and pea.

3.2 Plant Girth

There were significant differences ($p<0.05$) between the treatments at 6, 8, 10 and 12 WAT. Plants inoculated with 2000 *Meloidogyne* spp. J_2 produced the smallest girth followed by 1000, 500 and 0 *Meloidogyne* spp. J_2 in that order (Figure 2). The results confirm the findings of Hussey (1985) reported that an increase in stem diameter was due to the uptake and transportation of water and nutrients which are dependent on the health of the roots. Eisenback *et al*. (1991) also observed that heavily diseased plants do not respond to water because of the severity of damaged caused by nematodes to the conducting tissues of the plant at the roots resulting in reduction of stem diameter and top growth of the plant. His view holds true with the present findings where in plant growth was proportionately affected with increase in the number of galls and final nematode population. The progressive decrease in plant girth and nematode multiplication with increasing inoculums of root knot nematode on tomato crop was reported by Di Vito and Ekanayake (1983) with 2000 J_2 being the most pathogenic.

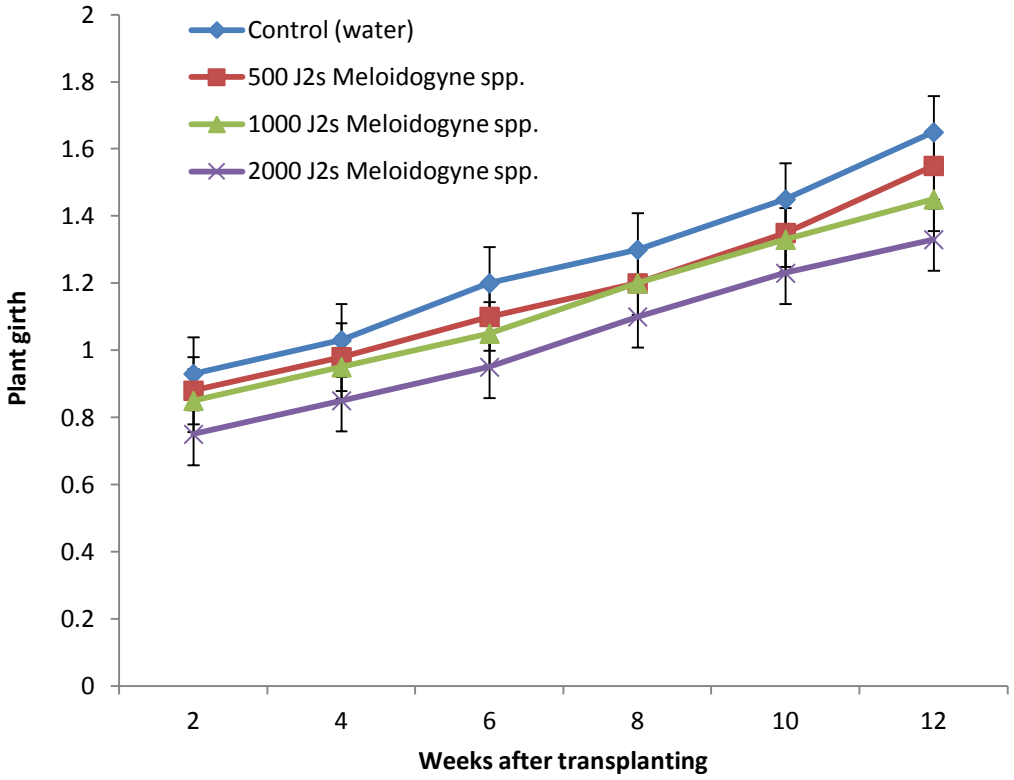

Figure 2: Effect of Inoculum levels of *Meloidogyne* spp. J2s on plant girth of tomato

3.3 Number of Leaves

At 4 WAP, there were no significant differences ($p>0.05$) between treatments. However at 6, 8, 10 and 12 WAT, plants inoculated with 2000 *Meloidogyne* spp. J_2 produced significantly smaller ($p<0.05$) leaves followed by 1000, 500 and 0 *Meloidogyne* spp. J_2 (Figure 3). The reductions in leaves parameters caused at level of 500 J_2 were quite similar to those caused at 1000 J_2 showing that there is no statistical significant difference in these two levels. In a similar study, Akhtar *et al*. (2005) reported that increasing the initial inoculum levels of *Meloidogyne incognita* resulted in a corresponding decreased in number of leaves and that the inoculum level of 500 J_2 /kg soil was pathogenic to mung bean. According to Sikora and Fernandez (2005), the increase in the nematode populations and the subsequent reduction in the growth and yield of crops are directly influenced by the initial density of the nematodes in the soil.

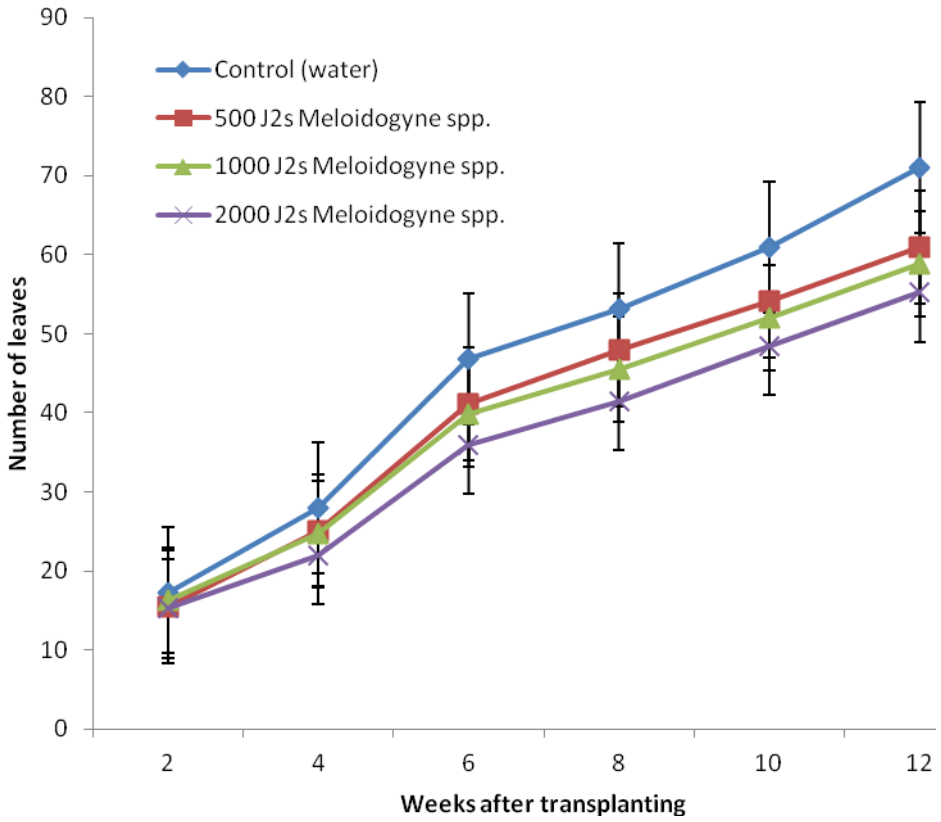

Figure 3: Effect of Inoculum levels of *Meloidogyne* spp. on number of leaves of tomato plant

3.4 Root Weight

The results clearly indicated that there were significant differences ($p<0.05$) between tomato plants inoculated with J_2 *Meloidogyne* spp. and the control in both fresh and dry root weights (Table 1). Plants inoculated with second stage juveniles of *Meloidogyne* spp. produced smaller roots with 2000 J_2 being the least whilst the control plants produced longer and heavier roots. Plants under control treatments recorded a significant increase in both fresh and dry root weights respectively due to less root knot infestation. Plants inoculated with 500, 1000 and the 2000 J_2 inoculum levels produced smaller roots which might be due to higher number of galls formed on their roots. This in turn reduces the uptake of water and transportation of nutrients as reported by Hussey and Boerma (1989). Williamson and Hussey (1996) also reported that root knot nematodes spend maximum time of their active lives within plant roots feeding on host cells. The infective stage second stage juvenile (J_2) penetrates through the root and migrates to a site near the vascular tissue.

Table 1: The effect of inoculum levels *M.* ssp. on fresh root and dry root weight of tomato after harvest

Treatments	Root weight (g)	
	Fresh weight	Dry weight
Control (water)	23.50	1.33
500 J_2 *Meloidogyne* spp.	21.05	1.08
1000 J_2 *Meloidogyne* spp.	17.45	0.90
2000 J_2 *Meloidogyne* spp.	11.85	0.76
LSD (0.05)	1.71	0.13
CV (%)	6.00	8.30

3.5 Influence of Inoculum Densities on Root Galling and Nematodes Population

The plants inoculated with second stage juveniles of *Meloidogyne* spp. significantly affected both root galls and nematodes population after harvest. The control treatment recorded the lowest population density of *Meloidogyne* spp. while the 2000 J_2 *Meloidogyne* spp. inoculum recorded the highest. Significant increase in number of galls was also observed at all inoculums levels. Maximum galls were produced at a level of 2000 J_2 followed by 1000, while the galls were the minimum in plants inoculated with 500 J_2. There were however significant differences ($p<0.05$) between the treatment means (Table 2). It is evident from the data presented in Table 1 that the rate of nematode multiplication was reduced with the increase in the inoculum levels of root knot nematodes. This might be due to the destruction of root system by the parasitism of root knot nematode which led to competition for food and nutrition among the developing nematodes within the root rhizosphere. The inability of juveniles to identify new infection courts for subsequent generation might also account for the reduction (Ogunfowora, 1977). The control treatment recorded the lowest count of root galls which might be due to the low presence of root knot nematodes. The presence of galls on roots is a primary symptom associated with root knot nematode infection. Root knot nematodes stimulate formation of root galls which interferes with plant water supply, resulting in stunted and chlorotic growth (Waller *et al.,* 2002). Noling (2009) reported that under heavy nematode infestation, crop transplants may fail to develop, maintaining a stunted condition causing poor stand development. From the results obtained, the presence of root galls and root knot nematode J_2 has a strong negative correlation with the growth parameters. This means that, as root galling of tomato plant and nematode juveniles increases, plant height, number of leaves and girth are negatively affected along with fresh and dry root weights reducing significantly. It was also observed that nematode juveniles have a strong positive correlation with the presence of root galling. This implies that, as nematode population increases in the rhizosphere of the tomato plant, the presence of root galling of tomato plant roots also increases. Ploeg (2001) observed that nematodes have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination.

Table 2: Effect of inoculum levels on root galling index and population densities of *Meloidogyne* spp. recovered from the rhizosphere of tomato plant.

Treatments	Root galling (scale: 0 - 10)	Untransformed mean of *M*. spp.	Transformed mean of *M*. spp.
Control (water)	0.25	20	4.53
500 J_2 *Meloidogyne* spp.	0.50	873	29.56
1000 J_2 *Meloidogyne* spp.	1.50	1322	36.37
2000 J_2 *Meloidogyne* spp.	1.50	2381	48.80
LSD (0.05)	0.86	496.90	22.79
CV (%)	59.60	28.10	5.80

* $\sqrt{x+0.5}$ where x is mean count, 0= no gall, 10= severely galled and plant usually died.

4. CONCLUSION

From the results obtained, it was observed that all the inoculum levels reduced the plant height, stem girth, number of leaves, and fresh and dry root weights. Increasing the nematode inoculum level resulted in corresponding increased in number of galls and nematode population build up. The reductions in growth parameters and nematode infestations were found to be proportional to the inoculum level. It is therefore concluded that *Meloidogyne* species is pathogenic to *Solanum lycopersicum* cv Pectomec at all inoculum levels and the damage is most severe at 2000 J_2/kg soil.

5. REFERENCES

1. Akhtar, H., Anita, S. and Shukla, P.K. Effects of initial inoculums levels of *Meloidogyne incognita* on root knot development and growth of *Vigna radiata* cv. ML-1108. Indian Journal of Nematology, 35; pp.93-94, 2005.

2. Abad, P., Favery, B., Rosso, M. and Castagnone-Sereno, P. Root knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Molecular Plant Pathology, 4: pp.217-224, 2003.

3. Agrios, G.N. Plant Pathology. 4th Edition. Academic Press. USA. pp.635, 1997.

4. Ameer-Zareen, Zaki, J.M. and Javed, N. Nematicidal activity of ginger and its effect on the efficacy *Pasteuria penetrans* for the control of root knot nematodes. Asian Journal of Plant Science, 2(11): pp.858-860, 2003.

5. Bridge, J. Nematode management in sustainable and subsistence agriculture. Plant Parasitic Nematodes in Temperate Agriculture. CAB International, Wallingford, UK, pp.203-209, 1996.

6. Bridge, J. and Page, S.L.J. Tropical Pest Management, vol 26 no. 3, 1980.

7. Castagnone-Sereno, P. Genetic variability and adaptive evolution in parthenogenetic root knot nematodes. Heredity, 96: pp.282-289, 2006.

8. Chapagain B.P, Wiesman, Z. Effect of potassium magnesium chloride in the fertigation solution as partial source of potassium on growth, yield and quality of greenhouse tomato. Scintia Hoticulture 99: pp.279-288, 2004.

9. Di Mascio, P., Kaiser, S. and Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Archives of Biochemistry and Biophysics, 274: pp.532-538, 1998.

10. Di Vito, M. and Ekanayake, H.M.R.K.. Relationship between population density of *Meloidogyne incognita* and growth in resistant and susceptible tomato. Nematologia Mediterranea, 11: pp.151-155, 1983.

11. Eisenback, J.D., Carmel-Goren, L., Hareven, D. and Triantaphyllou, H.H. Root knot nematodes: *Meloidogyne* spp. and races. In: Manual of Agricultural Nematology. W.R.Nickle and Marsel Dekker,Inc.New York,USA. 1991.

12. FAO (Food and Agricultural Organization) (2007) *FAOStat, core production 2005*. Available online: http://faostat.fao.org/site/340/default.aspx

13. Ghana Investment Promotion Council (GIPC). Available online: www.gipc.org.gh, 2001.

14. Haider, M.G., Dev, L.K. and Nath, R.P. Comparative pathogenicity of root knot nematode, *Meloidogyne incognita* on different pulse crops. Indian Journal of Nematology, 33: pp.152-153, 2003.

15. Hemeng, O.B. Efficacy of selected nematicides for the control of root knot nematode, *Meloidogyne* spp. on tomato in Ghana. Ghana Journal of Agricultural Science, 13: pp.37-40, 1981.

16. Horna, D., Melinda, S. and Falck-Zepeda, J. Assessing the potential economic impact of genetically modified crops in Ghana: Tomato, garden egg, cabbage and cassava. Scientia Horticulturae, 27: pp.189-198, 2006.

17. Hussey, R.S. Host-parasite relationship and associated physiological changes. In: Advance treatise on *Meloidogyne*. Vol. 1. Biology and control. Releigh, North Carolina State University, pp.143-53, 1985.

18. Hussey, R.S. and Barker, K.R. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Report, 57: pp.1025–1028, 1973.

19. Hussey, R.S. and Boerma, H.R. Tolerance in maturity groups of soybean cultivars to heterodera glycines. Supplement to Journal of Nematology, 21: pp.686-692, 1989.

20. Kinloch, R.A. (1982). Galling and yields of soybean cultivars grown in *Meloidogyne arenaria* infected soils. Journal of Nematology, 19: pp.233-239, 1982.

21. Mohammed, M., Ebrahim, I.D., Houshang, N.R. and Ahmad, T. Growth and yield of tomato (*Lycopersicum esculentum* Mill.) as influenced by different organic fertilizers. International journal of Agronomy and plant production. 4(4): pp.734-738, 2013.

22. Noling, J.W. Nematode management in tomatoes, peppers and eggplant. http://www.edis.ifas.ufl.edu/, 2009.

23. Ogunfowora, A.O. Effects of different population levels of *Meloidogyne incognita* on the yield of tomato in south Western Nigeria. Plant Protection, 3: pp.61-67, 1977.

24. Ploeg, A**.** When nematode attack is imp**o**rtant: *California Grower*. pp.12-13, 2001.

25. Sasser, J.N. Plant-parasitic Nematodes: The farmer's hidden enemy. North Carolina State University, Raleigh, North Carolina, pp.11-14, 1989.

26. Savannah Agricultural Research Institute (SARI). *Annual report.* Nyankpala, 2008.

27. Shurtleff, M.C. and Averre, C.W. Diagnosing plant disease caused by plant parasitic nematodes. The American Phytopatological Society, pp.187, 2000.

28. Siddiqui, M.A. and Alam, M.M. Evaluaion of nematicidal properties of different parts of Margosa and Persian Lilac. Neem Newsletter, 2: pp.1-4, 1985.

29. Sikora, R.A., Fernandez, E**.** Nematodes parasites of vegetables. In: Luc, M., Sikora. A., Bridge, J. (Eds). Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, pp.319-392, 2005.

30. Singh, S.K. and Khurma, R.K. Susceptibility of six tomato cultivars to *Meloidogyne inconita*. The South Pacific Journal of Natural Sciences, 13: pp.73-77, 2007.

31. Waller, J.M., Lenne, J.M., and Waller, S.J. Plant Pathologist's Pocketbook. 3rd ed. CABI publishing, Wanllingford. 2002.

32. Whitehead, A.G. and Hemming, J.R. A comparision of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology, 55: pp.25:38, 1965.

33. Williamsom, V.M. and Hussey, R.S. Nematode pathogenesis and resistance in plants. Plant Cell, 8: pp.1735-1745, 1996.

34. Wolff, H. Economics of tomato production with special reference to aspects of plant protection: A case study of two tomato production systems in Brong-Ahafo region, Ghana. Ghanaian-German Project for Integrated Crop Protection. GTZ: Eschborn, pp.131-133, 1999.