Chuka University
info@chuka.ac.ke | 020 231 0512 / 020 231 0518

PREVALENCE AND DISTRIBUTION OF PARASITIC ROOT KNOT NEMATODES IN SWEET POTATO FARMS OF KIRINYAGA COUNTY

Onchari, N. M., Githae, E. W., Nyabuga. I. and Muraya, M. *Department of Biological Sciences, Chuka University, P. O. Box 109-60400, ChukaEmail: oncharin5@gmail.com, egithae@chuka.ac.ke, moses.muraya@gmail.com. moses.muraya@chuka.ac.ke*

How to cite:
N.M Onchari, Githae, E. W, Nyabuga.I and Muraya, M. (2022). Prevalence and distribution of parasitic root knot nematodes in sweet potatoes farms of Kirinyaga County. In: Isutsa, D.K. (Ed) *Proceedings of the 8th International Research Conference held in Chuka University from 7th to 8th October 2021, Chuka.p142-145*

ABSTRACT
Sweet potato production is constrained by many biotic factors which include parasitic root knot nematodes. Root knot nematodes (RKN) pose a significant threat to a wide range of agricultural crops. The effect of RKN on sweet potatoes include reduced yields and poor quality of the tubers, high costs of production and hence loss of income. Moreover, development of resistance by RKN has partly rendered various pest management strategies ineffective, therefore risking food security. It is likely more losses may be experienced in future due to ongoing withdrawal of nematocides from the market. Information on distribution and management of root knot nematodes is limited. This study aimed in the isolation and characterization of root knot nematodes from soils and root tubers of sweet potato farms in different agro ecological zones of Kirinyaga County. From the undertaken study, prevalence and distribution of root knot nematodes was analyzed based on early cropping of sweet potatoes between one to two months and post harvested farms. Across all sweet potato farms, identification through microscopy revealed parasitic RKNs that were *Meloidogyne* species, *Pratylenchus* species, *Trichodorus* species among other spiral nematodes (*Helicotyllenchus* species and *Scutellonema* species) that are also categorized as parasitic nematodes. Reniformis (*Rotylenchus* species) were also identified as well as predatory nematodes which were singled out too under microscope observations. Root tubers that were stained pink with phloxine B showed large galls with mature female root knot nematodes under microscopy. Based on the questionnaire answers from farmers, they were familiar with nematode symptoms on sweet potatoes however, awareness of nematodes was low.
Keywords: Microscopy, Sweet potatoes, Food security, Agriculture

INTRODUTION
Sweet potato (*Ipomoea batatas* L. Lam) is a short-season tropical crop, which grows on marginal and degraded soils with less input (Kolombia *et al*, 2020). It is ranked as the seventh most important crop in the world with a total production of 138 million tonnes, and a total production of 871,010 tonnes in Kenya (FAOSTAT, 2018). Sweet potatoes form a major staple in diet along with Irish potatoes, bananas and cassava providing food security in rural household (Omotobora *et al*, 2014). Sweet potatoes are highly nutritious, enriched with proteins, carbohydrates and vitamins (Sun *et al*., 2012). Hence, it has a tremendous potential to promote food security, to alleviate poverty and as a supplement to an alternative staple food resource to poor farmers. However, its production has been decreasing due to variety of factors, which include biotic, abiotic and social factors (Karuri *et al*, 2017).

Among the biotic factors that affect sweet potatoes are root knot nematodes (RKN) which are ranked the most economically damaging parasites with a wide host range and environments (Jones *et al*. 2013; Seesayo *et al.*, 2017). They are widely distributed and the host list has become so large that it includes nearly all cultivated plants. They feed on plant tissues resulting to decrease in growth, yield and quality of crops, for example, cucumber (Kayani *et al*., 2017), tomatoes (Centitas *et al*., 2018), soybeans (Lee *et al*., 2018) and potatoes (Mburu *et al.*, 2020). Up to 25% of yield loss for agricultural crops have been recorded by Rocha *et al*., (2017). The presence of RKN in many sweet potato farms has negatively affected its production by lowering yield and quality of sweet potato tubers (Briar *et al*., 2016). An average of 41.8 - 88.4% yield loss of sweet potatoes is experienced by farmers depending with the level of root knot nematodes infection (Akinsanya *et al*., 2019).

The effect of root knot nematode in sweet potatoes include stunted vines, discoloured cracked roots and reduced quality and yield of the tubers (Hunja *et al.*, 2017). Males are eel-like and females are pear-shaped. Females remain in the roots or get attached to the plant host tissues. This causes knots or galls to develop in most plants and swellings in others. The galls reduce nutrient and water uptake, this results to formation of root cracks and necrosis

hence reducing market quality of sweet potatoes (Karuri *et al*., 2017; Adomako *et al*., 2020). The tissues of the infested roots often die and decay, especially late in the season. When the root systems of young plants are seriously injured, the plants appear sickly and yields are reduced and plants often die. Root knot nematodes abundance and composition in the soil is influenced significantly by soil property (calcium, potassium and iron), temperature and rainfall (Nielsen *et al*., 2014). Geographical distribution of RKN is largely dependent on environmental factors such as moisture and temperature (Sasser, 1997; Karuri *et al*., 2017). The type of farming system also plays a big role in

the infestation by root knot nematodes (Waiganjo *et al*., 2006). Moreover, RKNs have the ability to rapidly spread and to colonize new localities (Bebber *et al.* 2014). The changes of the environment can modify host immune responses, parasite virulence and the specificity of their interactions. Therefore, there is need for continuous survey and characterization of RKNs to determine the change in host parasite evolutionary trajectories and their potential for speciation under different agroecological zones (Fournet *et al.*, 2016).

Root knot nematodes are characterized using microscopic observation and further by molecular techniques. Microscopic observation is done to identify their morphology including perineal patterns, however it is impractical to differentiate strains (Gine *et al.*, 2016). Molecular techniques have been found to be more reliable and successful in identifying root knot nematode species (Blok *et al*., 2002; Hu *et al*., 2011; Niu *et al.*, 2012). Molecular studies have been based on using root knot nematode rDNA (Ye *et al*., 2019), microRNAs (Medina *et al*., 2017), nuclear ribosomes and mitochondrial gene sequence (Janssen *et al*., 2017). Molecular techniques can underestimate or overestimate species by intraspecific sequence variants and need for further optimization to enhance precision is required (Waeyenberge *et al.,* 2019). Due to new strain formation, molecular characterization is therefore an important and reliable way of identifying the isolated species from both soil and infected sweet potato tubers.

The objectives were to estimate the prevalence and distribution of root knot nematodes in sweet potato farms from different agroecological zones in Kirinyaga County. To isolate and characterize root knot nematodes using morphological techniques from different agroecological zones in Kirinyaga County.

METHODOLOGY

Data Collection

Prevalence and Distribution of Root Knot Nematodes

In each of the selected agroecological zone, sweet potato farms were sampled for prevalence of nematodes. A systematic (zigzag) sampling pattern, was used when collecting soil and sweet potato tubers in the farms. Five transects (plots) laid diagonally in every potato farms were established. In every plot, 500 g of soil were collected (Soil depth of 1-5 cm) using clean sterile soil auger. Fifteen (15) potato tubers were collected randomly from every plot where infection was high and examined for symptoms of nematode infestation. The soil samples and the tubers were packed in separate zip lock bags, labelled appropriately then packed in cool box and transported to Chuka University for nematode extraction. Once in the laboratory, samples were stored at 4^{o}C prior to parasite isolation.

Incidences and Prevalence of Root Knot Nematodes in Soil

- **Laboratory Procedure**

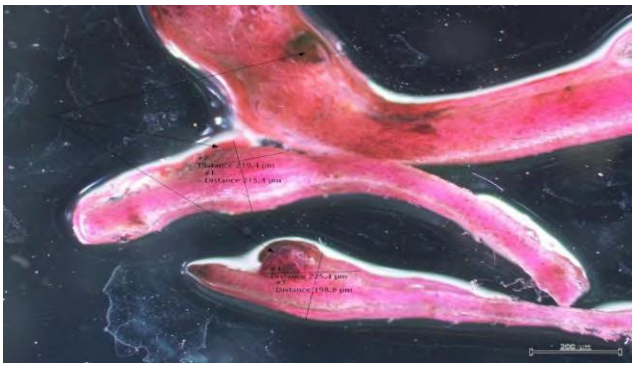
- The following staining process was done to stain the egg masses.

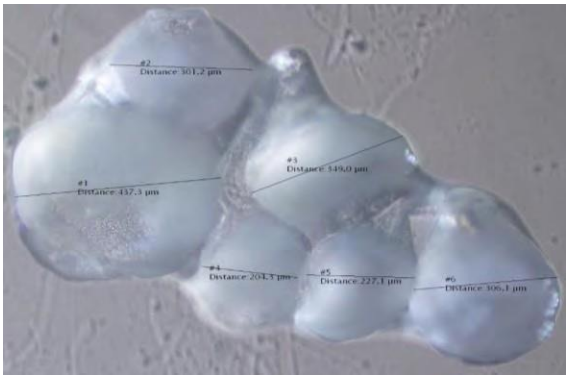
- **Staining root knot nematodes Egg Masses**

- Roots were gently rinsed in tap water free of debris.
- The clean roots were placed in a tray containing Phloxine B 0.15g/L of water for 15-20 minutes.
- The stained Pink-red egg masses were counted first in the original weight capture on the weighing scale and later converted to 10g an extrapolated figure for reporting.
- The isolated root-knots each with an individual female of *Meloidogyne* species were observed.

- **Stained Sweet Potato Root Tubers showing Galls**

Arrows showing stained egg masses, galls where there is only a single female inside each small gall.





Extracted Mature Females

There were differences in shape and size of extracted mature females a clear indication that there were probably different *Meloidogyne*, spp affecting the sweet potatoes.

RESULTS

Tubers with root knot nematode symptoms were presented and analyzed based on galls formed and a significant amount of root knots were identified. Affected root tubers were weighed prior and after staining then observed under microscope for egg mass count. Each gall contained one mature female that laid eggs enough to form galls. There were differences in shape and size of extracted mature females from galls seen under microscope, a clear indication that there were probably different *Meloidogyne* species affecting the sweet potatoes.

Microscopy observation of collected soil samples revealed all three stages of *Meloidogyne* species that were captured; mature females with eggs, J2, and J3. Some of the nematodes observed from soil of different farms were *Meloidogyne* species, *Pratylenchus* species, *Trichodorus* species, spiral nematodes (*Helicotyllenchus* and *Scutellonema* species) as well as reniformis (*Rotylenchus* species). Non parasitic (free living and predatory) nematodes were also observed.

REFERENCES

Adomako, J., Danso, Y., Sakyiamah, B., Kankam, F., & Osei, K. (2020). Plant-parasitic nematodes associated with sweet potato rhizosphere soil in the Semi-Deciduous Forest and Coastal Savannah Zones of Ghana. *Ghana Journal of Agricultural Science*, *55*(2), 1-9.

Akinsanya, A. K., Afolami, S. O., Kulakow, P., & Coyne, D. (2019). The root-knot nematode, *Meloidogyne incognita*, profoundly affects the production of popular bio fortified cassava cultivars. *Nematology*, 1-10.

Blok, V., Fargette, M., Wishart, J., Phillips, M., & Berthier, K. (2002). Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. *Nematology*, *4*(7), 773-781.

Briar, Shabeg S., David Wichman, and Gadi VP Reddy (2016). "Plant-parasitic nematode problems in organic agriculture." *Organic Farming for Sustainable Agriculture*. Springer, Cham, 107-122.

Cetintas, R., Kusek, M., & Fateh, S. A. (2018). Effect of some plant growth-promoting rhizobacteria strains on root-knot nematode, *Meloidogyne incognita*, on tomatoes. *Egyptian Journal of Biological Pest Control*, *28*(1), 7.

Fournet, S., Eoche-Bosy, D., Renault, L., Hamelin, F. M., & Montarry, J. (2016). Adaptation to resistant hosts increases fitness on susceptible hosts in the plant parasitic nematode Globodera pallida. *Ecology and evolution*, *6*(8), 2559-2568.

Giné, A., Carrasquilla, M., Martínez-Alonso, M., Gaju, N., & Sorribas, F. J. (2016). Characterization of soil suppressiveness to root-knot nematodes in organic horticulture in plastic greenhouse. *Frontiers in plant science*, *7*, 164.

Hu, M. X., Zhuo, K., & Liao, J. L. (2011). Multiplex PCR for the simultaneous identification and detection of Meloidogyne incognita, M. enterolobii, and M. javanica using DNA extracted directly from individual galls. *Phytopathology*, *101*(11), 1270-1277.

Hunja, W. E., & Ogechi, B. A. (2017). land use land cover changes and implications for food production: a case study of keumbu region kisii county, Kenya.

Janssen, T., Karssen, G., Orlando, V., Subbotin, S. A., & Bert, W. (2017). Molecular characterization and species delimiting of plant-parasitic nematodes of the genus Pratylenchus from the penetrans group (Nematoda: Pratylenchidae). *Molecular Phylogenetics and Evolution*, *117*, 30-48.

Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Manzanilla-López R, Palomares-Rius JE, Wesemael WIMML, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14:946–961

Karuri, H. W., Olago, D., Neilson, R., Mararo, E., & Villinger, J. (2017). A survey of root knot nematodes and resistance to *Meloidogyne incognita* in sweet potato varieties from Kenyan fields. *Crop protection*, *92*, 114-121.

Kayani, M. Z., Mukhtar, T., & Hussain, M. A. (2017). Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. *Crop Protection*, *92*, 207-212.

Kolombia, Y. A., Kumar, P. L., Adewuyi, O., Korie, S., Viaene, N., Bert, W., & Coyne, D. L. (2020). Distribution, prevalence, and severity of damages caused by nematodes on yam (Dioscorea Rotundata) in Nigeria. *Nematropica*, *50*(1), 1-18.

Lee, M. W., Huffaker, A., Crippen, D., Robbins, R. T., & Goggin, F. L. (2018). Plant elicitor peptides promote plant defences against nematodes in soybean. *Molecular plant pathology*, *19*(4), 858-869.

Mburu, H., Cortada, L., Haukeland, S., Ronno, W., Nyongesa, M., Kinyua, Z. & Coyne, D. (2020). Potato cyst nematodes: a new threat to potato production in East Africa. *Frontiers in Plant Science*, *11*.

Medina, C., da Rocha, M., Magliano, M., Ratpopoulo, A., Revel, B., Marteu, N., ... & Silva, A. C. (2017). Characterization of microRNAs from Arabidopsis galls highlights a role for miR159 in the plant response to the root-knot nematode *Meloidogyne incognita*. *New Phytologist*, *216*(3), 882-896.

Nielsen UN, Ayres E, Wall DH, LiG, Bardgett RD, Wu T, Garey JR (2014) Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties. *Global Ecology Biogeography* 23: 968–978

Niu, J. H., Jian, H., Guo, Q. X., Chen, C. L., Wang, X. Y., Liu, Q., & Guo, Y. D. (2012). Evaluation of loop-mediated isothermal amplification (LAMP) assays based on 5S rDNA-IGS2 regions for detecting *Meloidogyne enterolobii*. *Plant Pathology*, *61*(4), 809-819.

Omotobora, B.O., Adebola, P.O., Modise, D.M., Laurie, S.M. and A.S. Gerrano. 2014. Greenhouse and field evaluation of selected sweet potato (*Ipomoea batatas* (L.) Accessions for drought tolerance in South Africa. *American Journal of Plant Sciences* 5:3328-3339

Rocha, T. L., Soll, C. B., Boughton, B. A., Silva, T. S., Oldach, K., Firmino, A. A., ... & Silva, L. P. (2017). Prospection and identification of nematotoxic compounds from Canavalia ensiformis seeds effective in the control of the root knot nematode *Meloidogyne incognita. Biotechnology Research and Innovation*, *1*(1), 87- 100.

Sasser, J. N. (1977). Worldwide dissemination and importance of the root-knot nematodes, Meloidogyne spp.
Journal of Nematology, *9*(1), 26.

Seesao, Y., Gay, M., Merlin, S., Viscogliosi, E., Aliouat-Denis, C. M., & Audebert, C. (2017). A review of methods for nematode identification. *Journal of Microbiological Methods*, *138*, 37-49.

Sun, M., Mu, T., Zhang, M., & Arogundade, L. A. (2012). Nutritional assessment and effects of heat processing on digestibility of Chinese sweet potato protein. *Journal of Food Composition and Analysis*, *26*(1-2), 104-110.

Waeyenberge, L., de Sutter, N., Viaene, N., & Haegeman, A. (2019). New insights into nematode DNA- metabarcoding as revealed by the characterization of artificial and spiked nematode communities. *Diversity*, *11*(4), 52.

Waiganjo, M. M., Wabule, N. M., Nyongesa, D., Kibaki, J. M., Onyango, I., Wepukhulu, S. B., & Muthoka, N. M. (2006). Tomato production in Kirinyaga district, Kenya, a baseline survey report. *Kenya Agricultural Research Institute, Nairobi, Kenya*, 1-43.

Ye, W., Robbins, R. T., & Kirkpatrick, T. (2019). Molecular characterization of root-knot nematodes (*Meloidogyne* spp.) from Arkansas, USA. *Scientific Reports*, *9*(1), 1-21.