Pakistan Journal of Nematology, 2015, Vol. 33(1): 31-37

Histopathology of sweetpotato roots infected with *Meloidogyne incognita*

O.S. Osunlola† and B. Fawole[1]

Department of Crop Production, Kwara State University, Malete, Kwara State, Nigeria
[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria

†Corresponding author email: remiosunlola@gmail.com

Abstract

Meloidogyne incognita infected roots of sweetpotato (*Ipomoea batatas* (L.) Lam.) were observed for histopathology changes included cellular disorganization in the root cortex, endodermis and vascular cells. Female nematode displaced root cells and occupied large cavities and the surrounding cells were compressed. The nematode induced formation of an average of four giant cells each with 7 ± 0.5 nuclei around the head of nematode close to vascular cells. Nematode eggs were seen in the cortical cells but more eggs were recorded near the root surface. Multiple infections were also observed.

Keywords: *Meloidogyne incognita*, sweetpotato, root, histopathology

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous plant which belongs to the family Convolvulaceae. Out of about 50 genera and more than 1000 species, *I. batatas* is the only important economic plant in the family (Woolfe, 1992). Sweetpotato originated from tropical America (Mexico, Central America and Caribbean) and the North Western part of South America (FAO, 1990). It is widely cultivated in the tropical and warmer parts of the temperate regions of the world for its starchy tubers which serve as staple food in some countries (Gosh *et al*., 1988). The crop is the fourth most important after rice, wheat and corn in China (Lu *et al.,* 1989) and the world's second most important root and tuber crop after Irish potato (Lenne, 1991).

Sweetpotato is a fat-free and cholesterol-free tuber crop. It is a rich source of vitamins A, B and C (Ndolo *et al*., 2001; Odebode, 2008) produced more energy, protein and dry matter than any other crops (Horton *et al*., 1989). It is also a source of calcium, phosphorus, iron and magnesium (Lu *et al.,* 1989; Woolfe, 1992). It is cultivated for its carbohydrate rich tubers and a primary staple food (Meludu & Ayobami, 2005). The tuber is boiled, baked, roasted or fried. Fresh tubers are also fed directly to livestock (Onwueme & Sinha, 1991; Otoo *et al*., 2001). In some cases, the tubers were sliced, sun-dried and ground into flour which was added to wheat for bread making (Onwueme & Charles, 1994). Sweetpotato starch used in textile manufacturing and in the production of alcohol and leaves were richer than the tuber in protein, minerals and vitamins and used as vegetables and livestock food. Other processed products included syrups, acetone, pectin, acetic acid, dyes, noodles, candy, desserts and jams (Gosh *et al*., 1988; Chivinge *et al*., 2000; Zandratta, 2000).

Sweetpotatoes are frequently damaged by root-knot nematodes (*Meloidogyne* spp.) causing stunting and significant reduction in yield and quality of the storage roots (Clark & Moyer, 1988; Noling, 2002; Olabiyi, 2007). In the Philippines, 50% loss reported in *M. incognita* infested soil (Gapasin, 1984; 1986). Root-knot nematode symptoms on sweetpotato included round to spindle shaped swellings (galls) on fibrous roots and cracks on fleshy storage roots (Lawrence *et al*., 1986; Noling, 2002). The infected plants grow poorly or even died due to vascular dysfunction. In addition, complexes of *M. javanica* with *Fusarium* spp., caused severe wilting and premature death (Jatala, 1988). Being

sedentary endoparasites, *Meloidogyne* spp., have complex feeding relationships with their hosts and they induced the development of elaborate feeding sites called giant cells (Pegard *et al*., 2005). Further, nematode development cannot take placed without this unique response from host roots. Giant cell formation was essential for a successful host/parasite relationship (Hussey, 1985). Giant cells were transfer cells passing nutrients to the nematode. Two mechanisms were involved in giant cell formation, repeated endomitoses without subsequent cytokinesis and dissolution of cell walls and coalescing of their contents. Hussey (1985) reported that the tissues preferred for giant cell formation were the primary phloem and adjacent parenchyma. Fawole (1988) observed that giant cells were always closely associated with xylem tissues in yam. Fademi and Fawole (1992) noticed the development of many oval-shaped giant cells in susceptible rice within 14 days while Pegard *et al*., (2005) reported that *M. incognita*, *M. javanica* and *M. arenaria* induced the development of giant cells in susceptible pepper cultivars 5 days after inoculation. Walters *et al*., (2006) also reported that juveniles of all tested species and races induced giant cell formation in both susceptible and resistant cucumber by five days after inoculation. Vovlas *et al.,* (2005) found that each adult female *Meloidogyne javanica* was surrounded by 3-6 large giant cells in potato tuber.

Information is scanty on root-knot nematode histopathology on sweetpotato. In this investigation, a histological study of the relationship of root-knot nematode *M. incognita* with the cells of sweetpotato roots was carried out.

Materials and Methods

Meloidogyne incognita susceptible sweetpotato cultivar, cv TIS 4400 (Osunlola & Fawole, 2004) was used for this study. Galled roots and infected tubers were carefully harvested sixteen weeks after planting in root-knot nematode infested soil. Healthy roots and tubers from nematode-free soils were used as control. They were washed free of adhering soil debris under running tap water. The materials were immediately fixed in formalin-aceto-alcohol (FAA) (comprising 90 ml of 50% ethanol + 5 ml of glacial acetic acid + 5 ml of 37% formaldehyde) in separate Kliner jars securely corked and appropriately labeled. The root tissues remained in the fixative for six weeks prior to further processing. The fixed materials were then dehydrated in a graded ethanol series viz., 70%, 95% and absolute ethanol for thirty minutes each. The dehydrated tissues were infiltrated by passing them through three different containers of xylene and then through three different changes of paraffin wax, the last change made in an oven set at a temperature of 60 °C. On completion of the infiltration process, the tissues were embedded in molten paraffin wax. When wax was cooled and solidified, tissue blocks made and trimmed. Transverse and longitudinal sections 12 µm thick were cut with a rotary microtome (Minot- Microtome Type 1212, Ernest Leitz GMBH, WETZLAR, Germany).

The sections were floated out in a water bath and the sections picked up with pre-coated microscope slides, labeled and taken to the oven for drying. The sections were rehydrated by passing them through ethanol series (absolute, 95%, 75%, 70%) stained in safranin and counterstained in fast green (Dayking and Hussey, 1985). All sections were mounted in DPX (a colourless synthetic resin mounting medium containing Distyrene, Plasticizer and Xylene) prior to examination under a compound microscope (X 250).

Results

Adult females of *M. incognita* were observed from sections made from infected roots. They displaced the root cells and created large cavities (Fig. 1 A-C; Fig. 2 A and D). The root cells surrounding the nematode were compressed (Fig. 1 A-D). The histopathology study also showed that nematode eggs were deposited inside the cortical cells of infected sweetpotato roots (Fig. 2 A and D) but more eggs were observed near the

root surface (Fig. 2 B). Comparative histological observation of healthy (Fig. 2 C) and infected sweetpotato roots showed cellular disorganization in the cortex, endodermis and vascular cells (Fig. 1 A, B and D; Fig. 2 A and D).

Meloidogyne incognita induced the formation of giant cells which were large and multinucleate and were formed close to the vascular cells (Fig. 1 and 2). Their formation brought about the disruption and disorganization of vascular cells. An average of four giant cells comprising 7-8 nuclei each were found around the head of an adult female nematode. One adult female usually occupied a cavity but multiple infections were also encountered in this investigation as more than one nematode was observed in some galls (Fig. 1 D; Fig. 2 D).

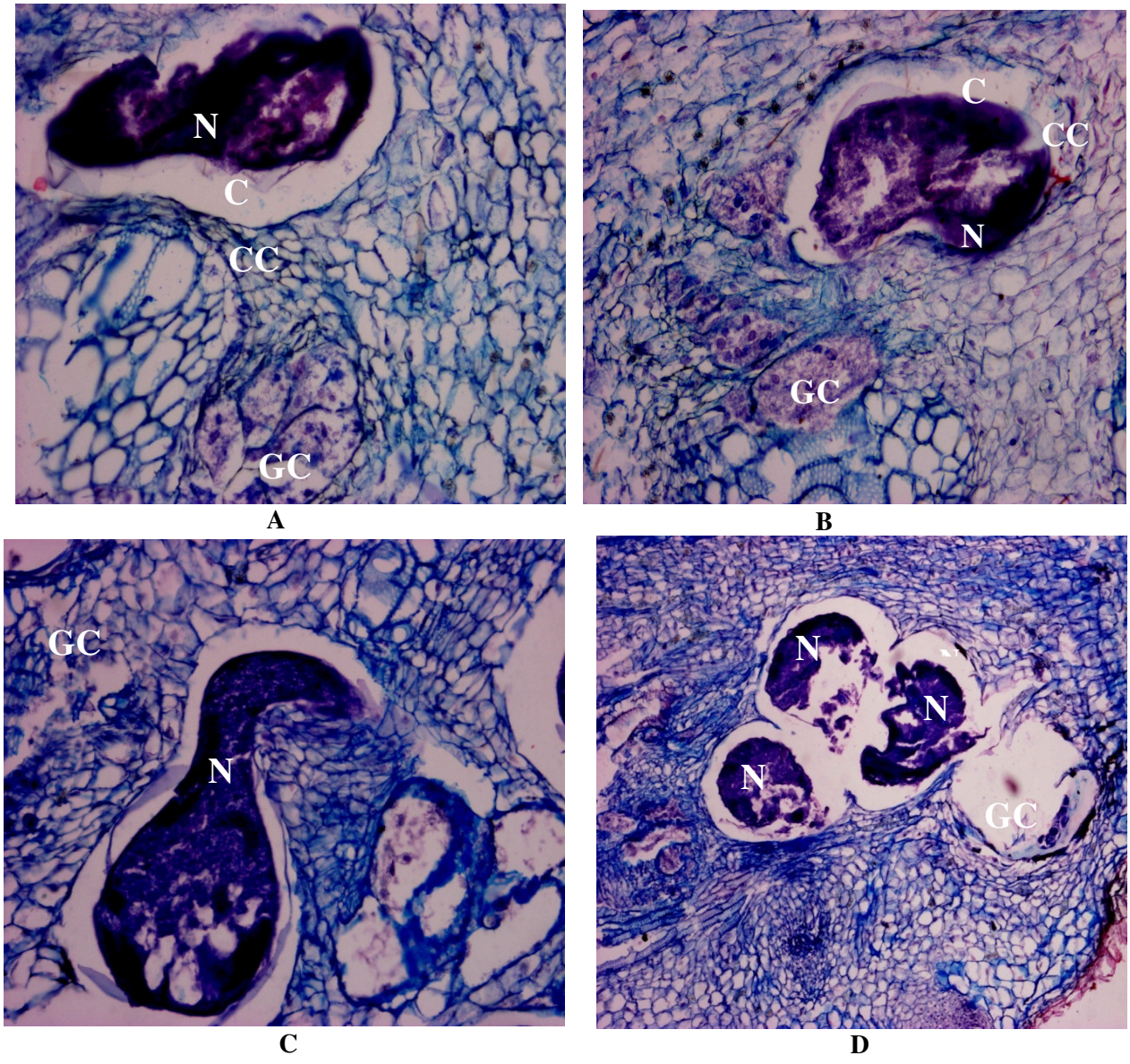

Fig. 1 (A-D). Transverse section (TS) of infected sweetpotato root showing adult female nematode (N), giant cells (GC), cavity (C) and compressed cells (CC).

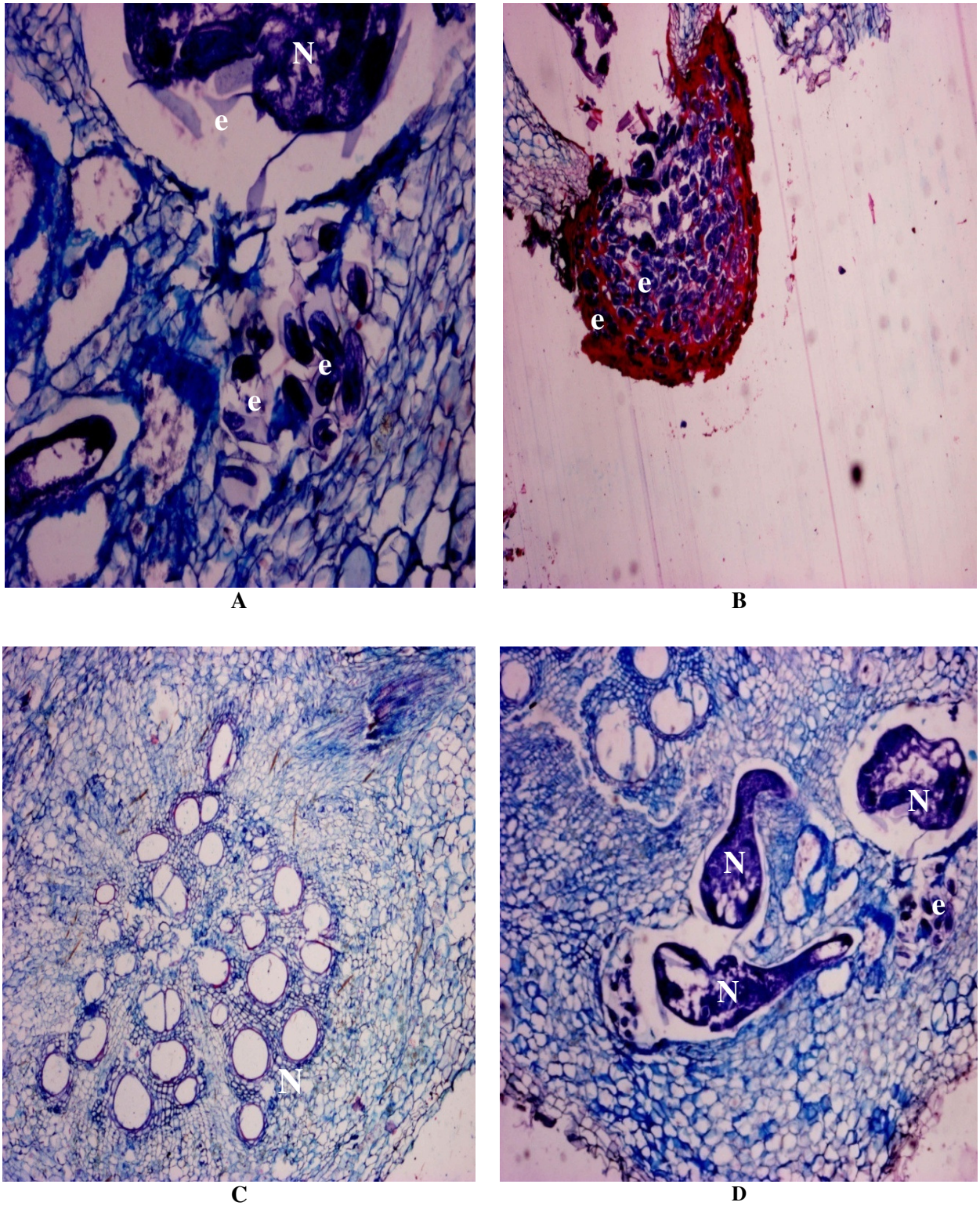

Fig. 2 (A-D). A, B and D: TS of infected sweetpotato root showing nematodes (N) and eggs (e); **C:** TS of healthy sweetpotato root.

Discussion

The result of the anatomical modifications induced by *M. incognita* in the root of sweetpotato (CV TIS 4400-2) revealed a typical susceptible reaction to infection. These changes were consistent with the findings of other researchers on other crops (Fawole, 1988; Di vito *et al*., 2000). In response to feeding activity of the root-knot nematodes, giant cells were formed. Because of their constant association with root-knot nematodes, the causal relationship between root-knot nematodes and giant cells was not in doubt. Bird (1962) established that giant cells were essential for root-knot nematode growth, development and reproduction because they essentially transfer cells passing nutrients to the nematodes. Since nematodes depended on giant cells for feeding, adult nematodes are usually found concentrated in the vascular tissue of the roots. Fawole (1988) found adult females in the 4-6 mm layer of *Dioscorea rotundata* tuber where the first set of vascular bundles are located. Recent cytological evidence suggests that giant cells are formed by repeated endomitosis without subsequent cytokinesis (Hussey, 1985). They however degenerate towards the end of nematode life. The presence of eggs inside the roots of sweetpotato is similar to the findings of Fawole (1988); Di Vito *et al*., (2000); Vovlas *et al*., (2005). Therefore, if infected sweetpotato tubers were used as planting materials in an uninfested field, they served as a primary source of inoculum. The multiple infection sites were observed in this study as reported by Di Vito *et al*., (2000). Roots having galls containing several females are more likely to suffer more damage than ones containing just a single female. Browning of cells around the nematode was not observed as reported by other workers. The formation of giant cells, which pass nutrients to the nematode and disorganization of vascular cells limit water and nutrient translocation from infected roots to above-ground plant tissues (Hussey & Williamson, 1997) with subsequent plant growth and yield reduction.

Conclusion

The development of a successful host/parasite relationship between the nematode and sweetpotato in this study suggested that infection by *M. incognita* has the potential to severely damaged sweetpotato. Management is essential need to reduce pre-plant nematode population in an infested soil.

References

Bird, A.F. 1962. The inducement of giant cells by *Meloidogyne javanica. Nematropica* 8, 1-10.

Chivinge, O.A., Rukuni, T. & Mutungamiri, A. 2000. Dissemination of sweetpotato production and processing technologies in Zimbabwe. *Proceedings of African Potatoes Association Conference* 5, 157-162.

Clark, C.A. & Moyer, J.W. 1988. *Compendium of sweetpotato diseases*. American Phytopathological Society Press, 74 pp.

Daykin, M.E. & Hussey, R.S. 1985. Staining and histopathological techniques in Nematology. In: Barker, K.R., Carter C.C. & Sasser, J.N. (Eds.). *Advanced Treatise on Meloidogyne Vol. II*: *Methodology*. North Carolina University and USAID, 39-48 pp.

Di Vito, M., Crozzoli, R. & Vovolas, N. 2000. Pathogenicity of *Meloidogyne exigua* on coffee (*Coffea arabica* L.) in pots. *Nematropica* 30, 55-61.

Fademi, O.A. & Fawole, B. 1992. The biology and control of nematode pests of rice in Nigeria. In: Fawole, B., Egunjobi, O.A., Adesiyan, S.O., Babatola, J.O. & Idowu, A.A. (Eds). *Proceedings of the 1st symposium on the biology and control of nematode pest of food crops in Africa.* Hiswill Info Resources Management Limited, Ibadan, 117-126 pp.

FAO. 1990. Roots, tubers, plantains and bananas in human nutrition. Food and Agricultural Organization (FAO). *Food and Nutrition Series* No. 24, 182 pp.

Fawole, B. 1988. Histopathology of root-knot nematode (*Meloidogyne incognita*) infection on white yam (*Dioscorea rotundata*) tubers. *Journal of Nematology* 20, 23-28.

Gapasin, R.M. 1984. Resistance of fifty-two sweetpotato (*Ipomoea batatas* (L.) cultivars to *Meloidogyne incognita* and *M. javanica*. *Annals of Tropical Research* 6, 1-19.

Gapasin, R.M. 1986. Pre-and post-inflectional resistance of sweetpotatoes to *Meloidogyne incognita* and *javanica*. *Annals of Tropical Research* 8, 176-188.

Gosh, S.P., Ramanujam, T., Jos, J.S., Moorthy, S.N. & Nair, R.G. 1988. *Tuber Crops*. Mohan Primlani for Oxford and IBH Publishing Company Pvt. Ltd. New Delhi, India, 305 pp.

Horton, D.E. 1989. Recent trends in world sweetpotato production and use. In: Mackay, K.T., Palomar, M.K. & Sanico, R.T. (Eds.). *Sweetpotato research and development for small farmers*, 17-32 pp.

Hussey, R.S. 1985. Host-parasite relationship and associated physiological changes. In: Sasser, J.N. & Carter, C.C. (Eds.). *Advanced Treatise on Meloidogyne Vol. I. Biology and control*. North Carolina State University and USAID, 143-153 pp.

Hussey, R.S. & Williamson, V.M. 1997. Physiological and molecular aspects of nematode parasitism. In: Barker, K.R., Pederson, G.A. & Windham, G.L. (Eds.). *Plant and nematode interactions*. Madison, WI, USA. American Society of Agronomy, 87-108.

Jatala, P. 1988. Important nematodes of sweetpotatoes. In: *Improvement of Sweetpotato in East Africa*. Report of the workshop on sweetpotato improvement in Africa. September-October, 1987, Nairobi, Kenya: ILRAD, 101-102 pp.

Lawrence, G.W., Clark, C.A. & Wright, V.L. 1986. Influence of *Meloidogyne incognita* on resistant and susceptible sweetpotato. *Nematology* 18, 59-65.

Lenne, J.M. 1991. *Diseases and pests of sweet potato*: South East Asia, The Pacific and East Africa. Natural Resources Institute Bulletin No 46, 116 pp.

Lu, S.Y., Xue, Q.H., Zhang, O.P. & Song, B.F. 1989. Sweetpotato production and research in China in Asia. In: *Improvement of sweet potato* (*Ipomoea batatas*) *in Asia*. Report of the Workshop on Sweetpotato Improvement in Asia. ICAR, India, October 24-28, 1988.

Meludu, N.T. & Ayobami, F.T. 2005. Socio-economic contributions of sweetpotato cultivation, processing and utilization: Implications for the development of appropriate technology in Nigeria, Ibadan. *Journal of Agricultural Research* 1, 24-29.

Ndolo, P.J., Mcharo, T., Carey, E.E., Gichuki, S.T., Ndinya, C. & Malinga, J. 2001. Participatory onfarm selection of sweetpotato varieties in western Kenya. *African Crop Science Journal* 9, 41-48.

Noling, J.W. 2002. *Nematode management in sweetpotato* (*including Boniatos*). University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS Extension), 15 pp.

Odebode, S.O. 2008. *Sweetpotato* (*Ipomoea batatas* (*L.*) *Lam.*) *Utilization Manual* (A Recipe Book). Codat Publications, 38 pp.

Olabiyi, T.I. 2007. Susceptibility of sweetpotato (*Ipomoea batatas*) varieties to root-knot nematode, *Meloidogyne incognita. American-Eurasian Journal of Agricultural & Environmental Sciences* 2, 318-320.

Onwueme, I.C. & Charles, W.B. 1994. Tropical root and tuber crops. *Production, perspectives and future prospects*. FAO Plant Production and Protection Paper 126, 230 pp.

Onwueme, I.C. & Sinha, T.D. 1991. *Field Crop Production in Tropical Africa*: Principles and Practice, Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, Netherlands, 480 pp.

Osunlola, O.S. & Fawole, B. 2004. Screening of sweetpotato cultivars for resistance to *Meloidogyne incognita* Race 2 in Ibadan, Nigeria. In: Hanafi A. (Ed.). *6th Triennial Congress of the African Potato Association*, Agadi, Morocco, April 5-10, 258-265 pp.

Otoo, J.A., Misah, A. & Carson, A.G. 2001. Evaluation of sweetpotato for early maturity across different agroecological zones in Ghana. *African Crop Science Journal* 9, 25-31.

Pegard, A., Brizzard, G., Fazari, A., Soucase, O., Abad, P. & Djian-Caporalino, C. 2005. Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum. Phytopathology* 95, 158-165.

Vovlas, N., Mifsud, D., Landa, B.B. & Castillo, P. 2005. Pathogenicity of the root-knot nematode *Meloidogyne javanica* on potato. *Plant Pathology* 54, 657-664.

Walters, S.A., Wehner, T.C., Daykin, M.E. & Baker, K.R. 2006. Penetration rates of root-knot nematodes into *Cucumis sativus* and *C. metuliferus* roots and subsequent histological changes. *Nematropica* 36, 231-242.

Woolfe, J.A. 1992. *Sweetpotato*: *An untapped food resource*. Cambridge University Press, 643 pp.

Zandratta, H.G. 2000. Potatoes and sweetpotatoes for Africa and the world. In: Ekwamu, E., Nampala, P. & Osiru, M. (Eds.). *Proceedings of the 5th Triennial Conference of the African Potato Association, Kampala, Uganda*. 5, 1-6.

(Accepted: April 16, 2014)