FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 5, May, 2025, pp 114 - 117
DOI: https://doi.org/10.33003/fjs-2025-0905-3456

HISTOPATHOLOGY OF WATERMELON ROOT INFECTED WITH ROOT-KNOT NEMATODE (*Meloidogyne* spp.) IN KWARA STATE, NIGERIA

***[1,2]Apalowo, Oluropo Ayotunde, [2]Izuogu, Nkechi Betsy, [2]Baba, Halimat Shola, Olajide, Comfort Motunrayo, [2]Balogun, Olusegun Samuel, [3]Nnabuihe, Emmanuel Chinweike and [3]Okafor, Maduabuchi Johnbosco**

[1]Department of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka
[2]Department of Crop Protection, University of Ilorin, Ilorin
[3]Department of Soil Science and Land Resources Management, Nnamdi Azikiwe University, Awka

*Corresponding authors' email: oa.apalowo@unizik.edu.ng Phone: +2348062142261

ABSTRACT

This study was carried out to determine the feeding pattern of root-knot nematode (RKN) infecting the root of watermelon in Ilorin, Nigeria. A commonly cultivated variety, kaolak was obtained and planted in a screen house. The screenhouse experiment was set out in a Completely Randomized Design (CRD). Two treatments were used; inoculated and uninoculated seedlings. Each treatment was replicated thirty times, making a total of sixty treatment combinations. Roots from each treatment were removed and cut into 4 to 5 cm pieces. The root materials were immediately fixed in formalin-aceto-alcohol (FAA). Results of this experiment showed the disorganization of the vascular tissue in the infected root. Visible galls were observed in the roots after 29 days which revealed similarity in feeding and reproduction of root-knot nematode in parasitized plant. The study advanced our knowledge on RKN feeding pattern in watermelon and the cosmopolitan nature of the pathogen.

Keywords: Histopathology, Kaolak, Nematode, Root, Watermelon

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai belong to the family Cucurbitaceae (Greco and Di vito, 2009; Adams et al., 2024). It is usally cultivated for its nutritional and economic purposes. However, root-knot nematode (*Meloidogyne* spp) has been identified as one of the most devastating biotic agent, limiting watermelon production in the tropical and temperate regions of the world. Generally, the root-knot nematode (RKN) feed by invasion of plant root tips during their second stage juvenile (j2). Thereafter, they locate a feeding site and establish themselves inside the parenchyma root cells, where they continue to develop at the detriment of the host plant (Davis *et al*., 2000). The feeding in the vascular bundle of host plants usually cause histological changes in form of hypertrophy and hyperplasia (Akhtar and Hisamuddin, 2015). As the infection continues, a visible gall could be observed in the root (Escobar et. al., 2015). The presence of galls drains nutrients that should be transported to different plant parts (Wesemael et. al., 2011). This feeding pattern could also make the plant susceptible to other pathogens. Severe infection of RKN might lead to stunting and wilting of the aerial part of plants (Ibrahim et al., 2019). Therefore, the aim of this experiment was to carry out the histological and physical changes of watermelon root infected with RKN.

MATERIALS AND METHODS

Planting conditions and experimental design

Sandy-loam topsoil was collected inside a drum and steam-heated for 24 hours to sterilize soil (Gautam and Goswami, 2002). It was later allowed to cool and transferred to a 5-litre plastic pots (a total of 60 pots) and kept inside screen house until they are ready for use. A watermelon variety, Kaolak, was selected for this experiment because of its wide cultivation in Kwara state.

The experiment was laid out using a Completely Randomized Design (CRD), with two treatment levels: inoculated and uninoculated. Each treatment was replicated thirty times, resulting in a total of sixty experimental units.

Sowing of seed and inoculation of watermelon seedling

A total of 4 seeds were planted per pot but later thinned to one after germination. A pure culture of *Meloidogyne* spp, was obtained from the Nematology unit of International Institute of Tropical Agriculture (IITA), Ibadan. About 4,000 nematode juveniles was determined by Doncaster counting slide and identified under a compound microscope for inoculation. Five days after germination, small holes were created around the root zone of each watermelon seedling, and a suspension containing 4,000 nematode eggs and juveniles was applied to each plant. The inoculation sites were immediately covered with soil to ensure proper establishment.

Preparation of root samples

Forty-eight hours post-inoculation, and at subsequent 48-hour intervals, two watermelon seedlings were carefully uprooted from both inoculated and uninoculated treatments, ensuring minimal disturbance to the root systems. The roots were gently washed with water to remove adhering soil particles and then sectioned into segments measuring approximately 4 to 5 cm in length.

Fixing and dehydration of root samples

The root samples were immediately fixed in Formalin-Acetic Acid-Alcohol (FAA), prepared using 90 mL of 50% ethanol, 5 mL of glacial acetic acid, and 5 mL of 37% formaldehyde. The fixative was dispensed into separate, securely corked and properly labeled Kilner jars. Both inoculated and uninoculated root tissues were immersed in the fixative and left for four weeks to ensure thorough preservation prior to histopathological processing. After the fixation period, the root segments were removed and transferred into a conical flask for dehydration through a graded ethanol series of 70%, 95%, and absolute ethanol, each for 30 minutes.

Infiltration of root and mounting on microscope slide

The dehydrated roots were infiltrated by passing them through three different containers of xylene and then through different changes of paraffin wax, the last stage was made in an oven

set at a temperature of 60^0c. On completion of the infiltration process, the root tissues were embedded in motten paraffin wax to section them after hardening. Once the wax was cools and become solid, tissue blocked was produced and pruned. Rotary microtome was used to cut it into transverse and longitudinal sections of 14 µm in thickness

Cut out parts were floated in a water bath and picked via microscope slide, and transferred for drying in the oven. It was later rehydrated by passing them through ethanol series (absolute, absolute, 95%, 75%, 70%) and then stained in Safranin and Counterstained in fast green (Daykin and Hussey, 1985). It was mounted on DPX (Distyrene, Plasticizer and Xylene) in preparation for viewing under microscope. Finally, the micrograph pictures of each slide was taken starting from 24 hours after inoculation to day 30 (Osunlola and Fawole, 2015) at a magnification of 100.

RESULTS AND DISCUSSION

The micrograph from the inoculated root after 48 hours revealed that j2 had invaded the root. The histology of both healthy and infected roots showed that infected root had cellular disorganization in the vascular tissues, which also brought about disruption of cells (Plate 1). This showed that j2 have migrated through the root tips inter-cellular to the area of cell elongation and vascular cylinder of the plant. At this stage, no physical damage to the root and no gall formation were observed. The examination of the root at ten days after inoculation (DAI) revealed a very dense cytoplasm which was induced by the presence of nematode secretions which caused dramatic cytological changes in parasitized cells that was already disorganized. The fourth juvenile stage of root-knot nematode was observed inside the root at 6 day after inoculation (DAI). The micrograph of the root showed that the nematode already molted to the fourth stage juvenile. At 28 DAI, the nematode had already completed their life cycle by becoming an obese matured female adult (Plate 2). The root cells surrounding the adult female were also compressed. The nematode was also surrounded by many disintegrated cells in locations where they emerged. At this time, visible galls were observed in the roots and the root tips were severely distorted in areas where galls appeared (Plate 3).

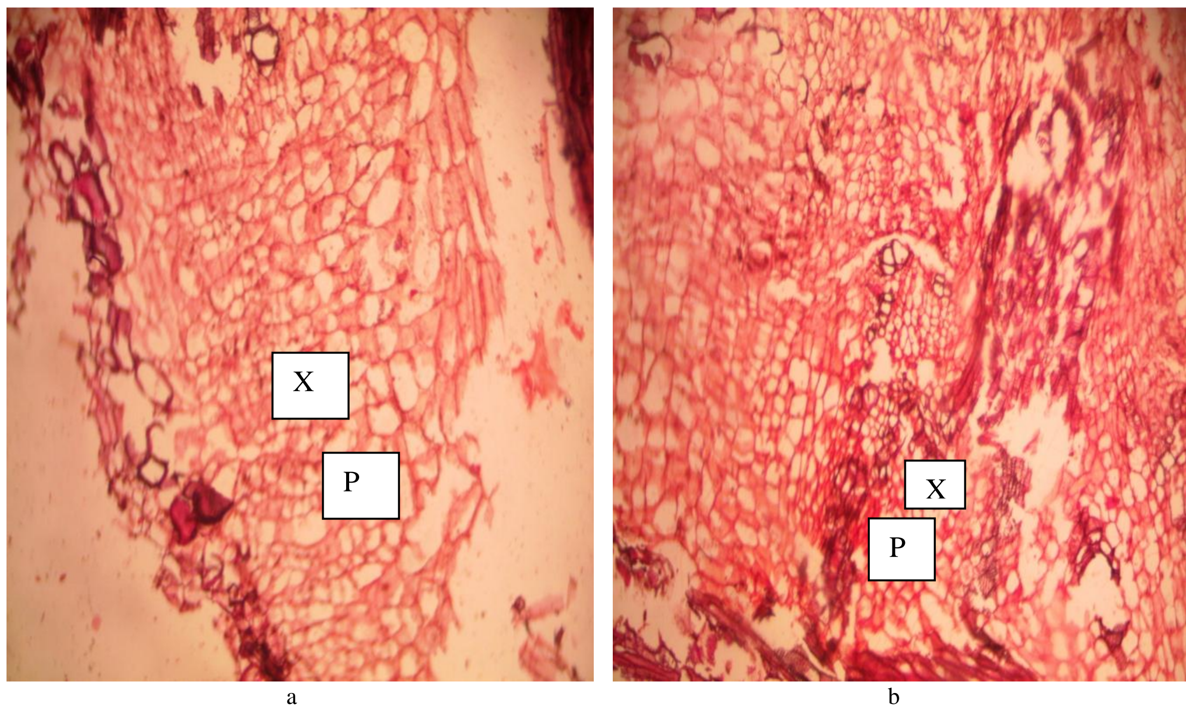


Plate 1: Transverse section of a *Citullus lanatus* root at 10 days after inoculation
a = uninoculated root with the phloem (P) and xylem cell (X)
b = inoculated root with a dense and disorganized cell in the phloem (P) and xylem (X)

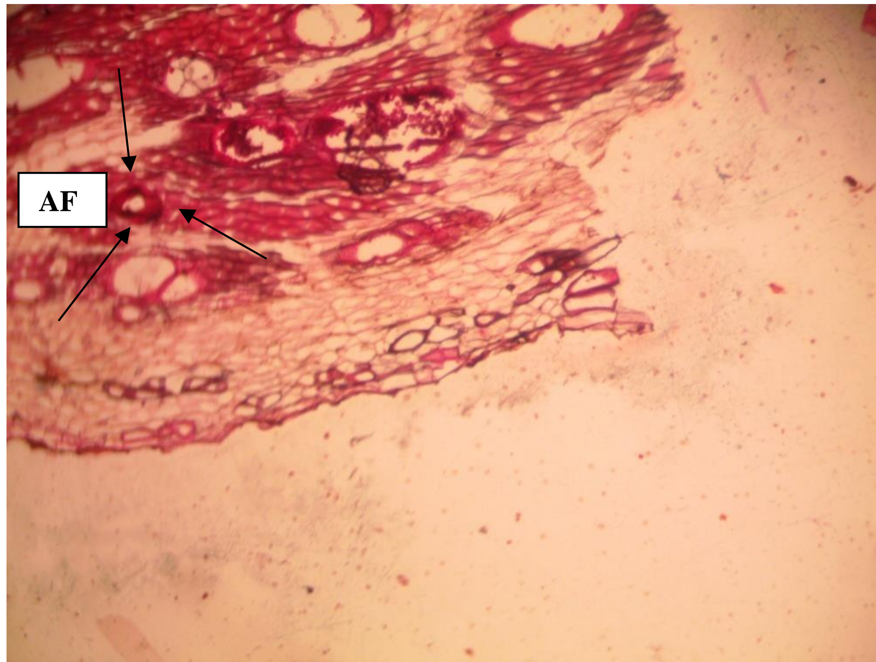


Plate 2: Adult female (AF) of a root-knot nematode in the root of *Citullus lanatus* at 28 days after inoculation with a degenerated cells in the phloem and xylem

Plate 3: The root of *Citullus lanatus* at 28 days after inoculation
a = inoculated root of *Citrullus lanatus* showing root galls
b = uninoculated root of *Citrullus lanatus* with a clean root and no galls

Discussion

The histopathology of watermelon revealed the typical life cycle and feeding habit of root-knot nematode infection in plant. In this study, the second-stage juvenile invaded the root tips within forty-eight hours of inoculation and then moved inter-cellular to the vascular cylinder in search of a feeding site. The invasion of the root within 48 hours was in line with the observations of Derek et al. (2013), that invasion of the root by root-knot nematodes happens between twenty-four to forty eight hours. Generally, the infective stage of rootknot nematode was the second stage juvenile which was also the case in this study. However, no gall or physical damage was observed during the movement of the juveniles in the root. This was also similar to the report of Akhtar and Hisamuddin (2015), where they observe the cells along in the pathway of second-stage juveniles did not show any sign of damage. However, a dense cytoplasm showed that the nematode had already become sedentary, thereby establishing a feeding site to withdraw nutrients. Root-knot nematodes rely totally on their host by injecting esophageal gland secretions into the plant to produce a giant cell which they use their stylet to withdraw. This swelling of giant cell also makes surrounding

cells to divide quickly with several small nuclei and thickened cell wall. Hence, the reason affected root tissue became dense. The development of the nematode inside the root showed it was able to successfully establish a giant cell and form a feeding site. This further affirmed that a feeding site had been established. Bird (1962), reported that there is a need for root-knot nematode to form giant cells in other to feed, grow and reproduce. Similar result was observed by Ayoub and Chnar (2020), in their report of differentiation of root cells into giant cells by root-knot nematode in the root of *Cynodon dactylon* (turf grass). The structure and appearance of the tissue also showed that nutrients and other photosyntates were expeditiously delivered to the nematode by the plant vasculature. The concentration of the nematode in the vascular region also revealed the importance of the location to the growth of the parasite. Distortion of vascular system by root-knot nematodes had also been reported by Bhat and Hisamuddin, (2010). At 28 days after inoculation, the root-knot nematode had already completed their life cycle by becoming an obese adult. At this stage, the formation of galls was visible on the root. The histopathology result also revealed that some of the nematode eggs were deposited inside the root, whereas more eggs were recovered at the root surface. The presence of eggs in the root is also similar to report of Osunlola and Fawole (2015). However, it was not clear whether the eggs were fertilized by males or produced parthenogenetically

The result of this experiment is consistent with the reports from other crops (Ayoub and Chnar, 2020; Akhtar and Hisamuddin; Osunlola and Fawole, 2015; Bhat and Hisamuddin, 2010; Fawole, 1988). Although their investigation was carried out on different crops, however, the similarity in feeding and reproduction of root-knot nematode showed their method of parasitizing plants is the same. The study also revealed the cosmopolitan nature of the RKN.

CONCLUSION

This study reveals the feeding pattern of RKN infecting the root of watermelon. The investigation also showed that RKN causes disintegration of the vascular tissue which provides an insight on how RKN causes infection in plants.

REFERENCES

Adams, S. O., Obonyilo, B. A., & Kolawole, T. B. (2024). Contribution of agricultural output to Nigeria's constant basic price GDP: A panel data regression approach. Fudma Journal of Sciences, 8(6), 118 - 127. Retrieved from https://fjs.fudutsinma.edu.ng/index.php/fjs/article/view/2741

Akhtar, A. and Hisamuddin (2015). Studies on the histopathological responses of *Vigna mungo* towards *Meloidogyne Incognita* at different time intervals. *J Plant Pathol Microbiol* 6(11) 319-329. https://doi.org/10.4172/2157-7471.1000319

Ayoub, A and Fathulla, C. (2020). Histopathological and morphological studies of root knot nematode *Meloidogyne* spp. on turfgrass (*Cynodon dactylon*). *Plant Archives. 19*, 3279-3282.

Bhat, M.Y. and Hisamuddin (2010). The effect of *Meloidogyne incognita* on the histopathology of *Momordica charantia* roots. *Arch Phytopathol Plant Protect. 43*, 1619-1624.

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

Davis E. L., Hussey R. S., Baum T. J., Bakker J., Schots A., et al. (2000) Nematode parasitism genes. *Annu Rev Phytopathol* 38: 365-396.

Daykin, M.E. and 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.

Derek, G., Bartlem, M., Jones, G. K. and Ulrich, Z. (2013).Vascularization and nutrient delivery at root-knot nematode feeding sites in host roots. *Journal of Experimental Botany. 65(*7) 1789–1798, https://doi.org/10.1093/jxb/ert415

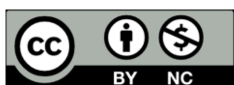
Escobar, C., Barcala, M., Cabrera, J., and Fenoll, C. (2015). Overview of root-knot nematodes and giant cells in *Advances in Botanical Research*, Vol.73, edsC.EscobarandC. Fenoll (Oxford: Elsevier AcademicPress), 1–32

Gautam, C. and Goswani, B.K. (2002). Different Combinations of Neem Cake and Carbofuran against *Meloidogyne incognita* on *Vigna radia*. *International Journal of Nematology. 12*, 106-110

Greco, N. and Di Vito, M. (2009). Population dynamics and damage levels. In: Perry, R.N., Moens, M. & Starr, J.L. (Eds). *Root-knot nematodes*. Wallingford, UK, CAB International, pp. 246-274.

Osunlola, O.S. and Fawole, B. (2015). Histopathology of sweet potato roots infected with *Meloidogyne incognita .Pakistan Journal of Nematology. 33*(1): 31-37

Wesemael, W.M., Viaene, N., and Moens, M. (2011). Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology* 13, 3–16. https://doi.org/10.1163/138855410X526831



©2025 This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International license viewed via https://creativecommons.org/licenses/by/4.0/ which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is cited appropriately.