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SCRUTINY OF ROOT-KNOT NEMATODE INFESTATION ON MARKETED CARROTS (*Daucus carota* L.) IN ABEOKUTA AND EFFECTS OF THE NEMATODE ON NUTRITIONAL VALUE OF THEIR TUBERS

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ABSTRACT

Examination of some carrot tubers was made in two different markets in Abeokuta, to determine the occurrence and severity of the root-knot nematodes, *Meloidogyne* spp. damage in their tubers as well as testing the inherent phytochemicals in them. The tubers were randomly sampled once a month for three consecutive months. Twenty-five (25) tubers were selected from five sellers in each of the two markets and conveyed to the Crop Protection Laboratory, Federal University of Agriculture, Abeokuta (7.2437º N, 3.3433º E). Each carrot sample was thoroughly washed with distilled water, mopped dry and scored for number of galls and fitted into an index scale for the determination of the severity of the root-knot disease. The nematodes were thereafter extracted from infested tubers to determine its population. Data obtained were subjected to Analysis of Variance and significant means were separated using the New Duncan Multiple Range Test at 5% probability level. Phytochemical test was carried out on the tubers using standard procedure for the identification of the constituents. Results showed that marketed tubers were variously infested with the *Meloidogyne* spp. with average galls ranging from 2 to 11 in Lafenwa and Kuto, respectively. October was the most vulnerable (80%) period for the manifestation nematodes (27 - 55) per tuber damaged by the nematode for both markets. Furthermore, there were reductions in the presence of alkaloids, tannins, and flavonoids contents due to the presence of the nematodes in the tubers with the likelihood of reduced nutritional value of *Meloidogyne*-infected carrot tubers.

Keywords: *Nutraucetics, Food safety, Organic farming, Nematodes, Phtyochemicals*

INTRODUCTION

Carrot (*Daucus carota* L.) is the most important crop of Apiaceae family which was originally used for medical purposes before now becoming food item (Carlos and Dias, 2014). The tuber is very rich in bioactive compounds like carotenoids and dietary fibres and contained appreciable levels of several other functional components like carbohydrates and minerals like Ca, Fe, Na, K, Mg, Cu, Zn, carotenes, thiamine, riboflavin, niacin, vitamin C (Acharya *et al*., 2008; Arscot and Tanumihardio 2010; Sharma *et al*., 2012). Research documentations (Acharya *et al*., 2008; Leja *et al*., 2013) ranked carrot 10th among 39 fruits and vegetables, due to its multiple nutritional and medicinal benefits. The authors asserted that due to its nutritional antioxidants (vitamins A, C, and E) constituent and valuable amount of non-nutritional antioxidants like β-carotene, flavonoids, alkaloids, and phenolics, they remain highly valued. Furthermore, there are several research reports that documented that carrot has a unique combination of three flavonoids: kaempferol, quercetin and luteolin (Lila, 2004; Dias, 2014, Ahmad *et al*., 2019) in addition to being rich in other phenols, including chlorogenic, caffeic and p-hydroxybenzoic acids along with numerous cinnamic acid derivates. Among hydroxycinnamic acid and its derivatives, chlorogenic acid represents 42.2% to 61.8% of total phenolic compounds detected in different carrot tissues (Zhang and Hamauzu, 2004). Phenolics or polyphenols in plants, including carrots, have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumor activities. They have been reported to be a potential scavenger of free radicals, which are harmful to our body and food systems (Nagai *et al*., 2003). Consequently, the crop has been regarded to be of significantly beneficial influence on human health, including immune boosting against pathogens and diseases they may cause. Consequently, the consumption of carrots and carrot products grows steadily which has also stimulated its large-scale production particularly in Northern Nigeria and other value chains including storage, marketing, processing, and consumption all over the country.

The other aspect of the economic importance of carrot is that the presence of alkaloids and other secondary metabolites in plants enhances plant reproductive rates, either by improving defenses against biotic and abiotic stresses or by affecting pollinators and seed/fruit dispersal visitation. Defensive strategies include predator repellent by toxicity or bitterness taste or damage repair by antioxidant system (Vilariño and Ravetta, 2008; Matsuura and Fett-Neto, 2013).

Despite, root-knot nematodes (*Meloidogyne* species) remain one of the major damaging groups of plant-parasitic nematodes affecting almost all major crops, including carrot. The plant and its tubers are susceptible to infection by the nematode. Leading to induced expansion of root cells, swellings, or galls develop on the roots of infected plants. The galls vary in size from slight thickenings to lumps 5 to 10 cm across. Galls increase the susceptibility of the root system to be invaded by disease-causing fungi and bacteria (Rahman, 2003) and diseased plants may show symptoms of nitrogen, potassium, or phosphorus deficiencies even though adequate levels of these minerals are in the soil. During hot daytime temperature, infected plants wilt and then recover at night. The roots also showed characteristic of shorter and bushier than on healthy plants (Nurul, 2016). Often more than not the infected carrot tubers are sold in the markets across the country without sorting adequately the galled from the gall-free ones.

Therefore, nematode infected carrot tubers are usually consumed ignorantly with attendant implication of health issues arising from less defence mechanism as immunity would have been negatively affected. Therefore, there is need to investigate the effect of *Meloidogyne incognita* (Mi) on the phytochemical values of marketed carrot tubers, and to create awareness for the need for adequate management of the nematodes in the field where they are being cultivated. The objectives of this study were to: 1) assess the incidence and severity of root-knot nematode, *M. incognita* damage in marketed carrots tubers in Abeokuta, and 2) determine the effect of Mi on the phytochemical values on marketed carrots in Abeokuta**.**

Infection of root-knot nematodes starts with injection secretions into the host plants. In the zone of root elongation, the worms hatch as second-stage juveniles and actively invading the host. They migrate intracellularly to the root apex and then to the vascular cylinder. This is where permanent feeding sites are established, and second-stage juveniles undergo three moults to develop into adults. While the females remain sedentary, producing large egg masses, males migrate out from the plant (Abad *et al*., 2003). Then, the formation of galls will take place, later on this gall formation will prohibit or only have limited ability to absorb and transport water and nutrients to the plant. This situation will severely affect plants and they will easily wilt. The worst situation is that the infection usually takes place at the early stage of plant's development and at this stage the invading root-knot nematode can directly kill the host plant.

MATERIALS AND METHODS

Location, Carrot Tuber Sampling and Damage Determination

The survey was carried out in Kuto and Lafenwa Markets in Abeokuta, Ogun State, Southwestern Nigeria. The laboratory analysis was conducted at the Crop Protection Department, Federal University of Agriculture Abeokuta and SMO laboratory, Office 5, Joyce B Complex, Off Mobil Road, Ring-Road, Ibadan, Oyo State, Nigeria. Carrot tubers freshly brought to Kuto and Lafenwa Markets for sale to unsuspecting consumers were randomly sampled once a month for three consecutive months. Twenty-five (25) carrot tubers were randomly selected from five sellers in each of the two markets by physically picking the tubers using hand gloves, placed in black nylon bags and conveyed to the Crop Protection Laboratory, Federal University of Agriculture, Abeokuta (7.2437º N, 3.3433º E). Each carrot sample was thoroughly washed with distilled water, mopped dry and scored for number of galls to ascertain the occurrence of root-knot nematode damage symptoms and fitted to Taylor and Sasser (1978) Gall Index Scale for the determination of the severity of the root-knot disease.

Incidence of *Meloidogyne* species and Severity on Carrot Tubers and Identification

Root-knot nematode incidence was accessed via the presence or absence of galls on the carrot tubers and percentage incidence were calculated by using formula below while the severity was determined using the Gall Index rating scale of Taylor and Sasser (1978) as shown in the Table 1. The nematodes were extracted from the carrot tubers samples using Whitehead and Hemming (1965) modified tray method. The galled parts of the carrot tuber samples were severed, chopped and placed in plastic sieves sandwiched with a double ply tissue paper, then placed in a bowl containing 250 ml of distilled water for 24 hours. The suspension was concentrated to 25 ml by removing excess water *supernatant* through the settling-siphon method. The water-nematode suspension was poured into 500 ml Nalgene bottles and left for 5 hours for the nematodes to settle at the bottom of the bottles. The concentrated nematode suspension was placed on a Doncaster (1962) counting Dish for nematode identification and census. The identification of *Meloidogyne* species in the aqueous suspension was determined using a compound microscope. Nematode identification was done with the aid of Pictorial Key of Mai and Lyon (1975).

$$\frac{\text{Number of infected carrot tubers}}{\text{Total number of sampled carrots}} \times 100$$

Table 1: Taylor and Sasser (1978) Rating Scale for the Presence of Root-knot Nematode Galls

Number of Galls	Gall Rating	Host Designation Category
0	0	Immune
1-2	1	Resistance
3-10	2	Moderately resistance
11-30	3	Moderately resistance
31-100	4	Susceptible
100^{+}	5	Highly susceptible

Data Collection and Analyses

Data was collected number on galls on each carrot tuber on each market day for three consecutive months for the two central markets selected. The data obtained were subjected to Analysis of Variance using Statistical Application for Sciences and significant means were separated using the New Duncan Multiple Range Test (NDMRT) at 5% probability level. The presence and quantity of phytochemicals such as tannins, saponins, flavonoids, alkaloids, and phenols in the consumable sampled carrot tubers were determined using standard procedure for the identification of the constituents (Trease and Evans, 1989; Sofowora, 1993) and presented as follows:

Test for Tannins

The carrot tuber was prepared into extract of which 1 ml was boiled in 20 ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue–black coloration which confirms the presence of tannins.

Test for Saponins

About 5 ml of the extract was boiled in 20 ml of distilled water in a water bath and filtered. Thereafter, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it observed for the formation of emulsion which confirms a positive presence of saponins.

Test of Flavonoids

A 3ml of 1 % aluminum chloride solution was added to 5 ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. A 5 ml of dilute ammonia solution was added to the above mixture followed by addition of concentrated H_2SO_4. With these, a yellow coloration disappeared on standing.

Test for Alkaloids

Extracted 1 ml of carrot samples (galled and non-galled) was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide- solution) or Wagner's reagent (solution of iodine in Potassium iodide) or Dragendorff's reagent (solution of Potassium bismuth iodide). The formation of a cream colour with Mayer's reagent and reddish-brown precipitate with Wagner's and Dragendorff's reagent give a positive test for alkaloids.

Test for Phenols

The extracted 5 ml carrot samples were pipetted into a 30 ml test tube, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added and left to react for 30 min. Development of bluish green colour was taken as a positive presence of phenol.

RESULTS

Symptoms, Incidence and Severity of *Meloidogyne incognita* on Carrot Tubers

Meloidogyne-infected plants usually show the distinct gall and these were found occurring on the sampled carrot tubers as the symptom of the infection by invading *Meloidogyne incognita*. The highest mean number of carrot tuber galls stood at 11.2 and were obtained from Kuto Market in October. This was statistically ($P<0.05$) different from the mean number of galls in August (3.92) in Kuto and the 3.08 - 6.68 galls recorded in samples obtained from Lafenwa Market from August through October. However, the average number of tuber galls of 11.2 in Kuto by October was not statistically ($P>0.05$) different from the 8.08 in September in the same market. Almost all sampled carrot tuber were asymptomatic with galls, the basic damage and infection that almost all unsuspecting consumers do not consciously or wish to check while purchasing their carrot tubers from the market or roadsides along city traffics. To measure the severity of the carrot tuber gall disease, gall galls indices were evaluated and utilized for host response to the invading Mi over a period of three months. The host status of the sampled tubers were all susceptible (Gall Index = 4.0) across the months and the markets, except in September and October where it was both moderately susceptible at Lafenwa Market with Gall Index of 3.0 (Table 2). Verification of the causal organism for the damage in carrot tuber damage inform of galls showed that the presence of tens of Mi recovered from the carrot samples obtained from Kuto and Lafenwa Markets across the months. *Meloidogyne incognita* population peaked at 80% in October in both markets studied which was the same as August in Kuto Market. However, the percentage population dropped to 60% in August and 28% in September at Lafenwa Market as well as 64% at Kuto Market in September. When partitioned to individual records of Mi population recovered from infected galled carrot tubers in the two markets, from August through September in Figure 1, Kuto scored the highest population of 55.2 in August which was sustained in both September and October. Conversely, Lafenwa Market's Mi population of 36.6 in August dropped to 26.6 in September and remained the same in October in the same market (Figure 1).

Table 2: Mean Number of Galls, Occurrence and Severity of Root-Knot Disease on Carrot Tubers in Kuto and Lafenwa Central Markets, Abeokuta in 2018

Month	Kuto				Lafenwa			
	%Occurrence	No. of Galls	Gall Index	Host Designation/ Severity	%Occurrence	No. of Galls	Gall Index	Host Designation/ Severity
August	80	3.92^{bcd}	4	Susceptible	60	3.08^{cd}	4	Susceptible
September	64	8.08^{ab}	4	Susceptible	28	1.92^{d}	3	Moderately Susceptible
October	80	11.2^{a}	4	Susceptible	80	6.68^{bc}	3	Moderately Susceptible

Means followed by the same letter in the columns are not significantly different at 5% level of probability using New Duncan Multiple range test.

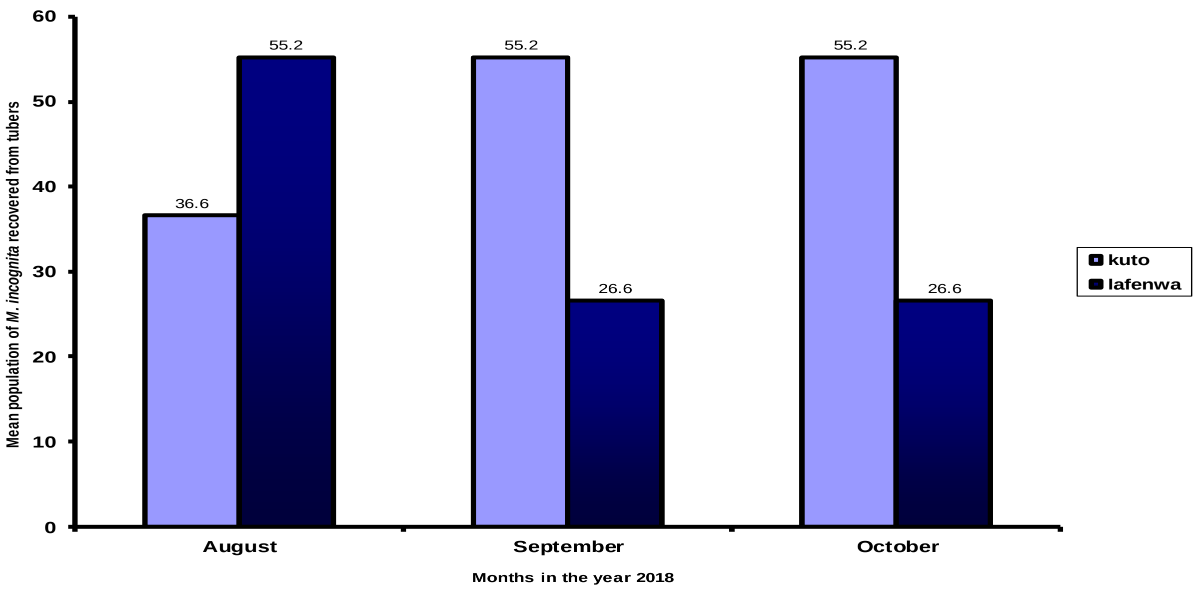

Figure 1: Mean population of *Meloidogyne incognita* Recovered from Infested Galled Carrot Tubers from Kuto and Lafenwa Central Markets in Abeokuta, Ogun State

Occurrence and Level of Phytochemicals Present in Carrot Tubers

Table 3 showed the presence and levels of phytochemicals in both galled and non-galled carrot tubers obtained from Kuto and Lafenwa markets. Alkaloids was found to be present in moderate quantity in non-galled carrot tuber samples obtained from both Kuto and Lafenwa markets. The galled samples from these markets had alkaloids, flavonoids, phenols and saponins consistently in high amounts in both markets. Low quantity of tannins was found in non-galled samples of the carrot tubers samples obtained from the markets (Kuto and Lafenwa) while it was present in medium amount in galled samples from the two markets. Flavonoids was present in medium amount in carrot samples showing no galls in both Kuto and Lafenwa markets.

Table 3: Effect of *Meloidogyne incognita* on the nutritional value on the marketed carrots in Kuto and Lafenwa Markets

Carrot Tuber Sample	Alkaloids	Tannins	Flavonoids	Phenols	Saponins
Lafenwa non-galled	++	+	++	+++	+++
Kuto non-galled	++	+	++	+++	+++
Lafenwa galled	+++	++	+++	+++	+++
Kuto galled	+++	++	+++	+++	+++

Key: + Present in small amount, ++ Present in medium amount, +++ Present in high amount

Plate 1: A - Non-Galled, and B - Infected (Galled) Carrot Tubers

DISCUSSION

This research has provided information concerning the impact of *Meloidogyne incognita* on marketed carrots tubers in two major central markets in Abeokuta City, Southwestern Nigeria. The basic symptom of *Meloidogyne* species infection observed on the carrot tuber was the appearance of galls (syn: tumor, warts) which is a localized outgrowth on the tuber surface resulting from swellings of internal tissues occasioned by hyperplasia and hypertrophy of the vascular cell (Caillaud *et al*., 2008). Attempts were made to extract and identify the causal organisms associated with this damage (otherwise known as infection) in some of the sampled carrot tubers sampled from Kuto and Lafenwa Markets between August and October 2018. This study, therefore, demonstrated, for the first time in research report, the presence of, damage by and severity of the root-knot nematodes, *Meloidogyne incognita* damage and possible incidence was observed that most carrot tubers sold in both Kuto and Lafenwa Markets in Abeokuta, Ogun State, to unsuspecting consumers were damaged and infected by *M. incognita*. Abad *et al*. (2003) reported that gall formation was the characteristic symptom of *M. incognita* infection on host crops. Galling which is the disease and at the same time below-ground symptom (Atungwu *et al*., 2021) of the infection were reported to affect different parts of the plants, including the roots and tubers of carrot. Our current study indicated that the severity the carrot tuber gall disease was dependent on the reaction of the tubers Mi. The highest percentage incidence of *M. incognita* in the month of October suggested that the high rainfall experienced during that period in the Northern part of Nigeria where carrots are cultivated has a positive impact in the life cycle of the nematodes. This is in line with Olowe (1976) who implicated nematodes to be abundant in irrigated conditions. However, it appear more logical to conduct field studies to authenticate this claim since, in the case of carrots there are suspicions that those grown locally may have been mixed up with those brought in from other parts of the country.

On nutritional/medicinal implications of the galled carrot tubers, the study leveraged on earlier reports that phytochemicals compounds were highly abundant in them elsewhere (Ghasemzadeh *et al*., 2012). According to Allende *et al*. (2006), carrots are rich sources of nutrients that are directly or indirectly associated with homeostasis in human beings it is also said to contain a variety of phytochemicals (also known as bioactive compounds) recognized for their nutraceutical effects and health benefits (Tiwari and Cummins, 2013). The high quantity of alkaloids and flavonoids observed in galled carrot tubers suggested that nematodes infection increased alkaloid and flavonoid contents which was like the report of Westphal and Becker (2011) who stated the that the sedentary endoparasitic lifestyle of root-knot nematodes causes them to form feeding sites where they produce enzyme to digest the nutrients in the site while causing adaptive changes. These enzymes are suggested to alter the content and composition of some phytochemicals. Notwithstanding, the current study contradicted the earlier report, in respect of phenol and saponin contents were not affected by the invading Mi.

The experiment concluded that carrot gall disease caused by root-knot nematodes was visibly evident in marketed tubers in Ogun State and that non-galled samples had higher alkaloids, tannins, and flavonoids than galled ones but phenols and saponins contents did not differ. The implication of the high content of these phytochemicals is their likely effect of forming complexes with nutritionally important nutrients like protein and mineral elements, thereby making them unavailable for absorption and utilization. Therefore, it is recommended that consumers should sort and dispose of galled carrots in food boxes as they are likely to be infested with root-knot nematodes that deprive them of the nutrients expected from the tubers when eaten.

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