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Pathogenicity Study of Southern Root Knot Nematodes (*Meloidogyne Incognita* Chitwood) on Roma King Tomato Cultivar (CV)

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Abstract

Seedlings of the hybrid tomato 'Roma king' cultivar (cv.) grown in a steam sterilized soil were inoculated with Root knot nematode (Meloidogyne incognita) eggs, under screen house temperature 27±2°C. Serially graded inocula of 2, 000, 4, 000, 6, 000, 8, 000 and 10, 000 eggs that hatched from the second stage juvenile (J2) of the root-knot nematodes, Meloidogyne incognita, extracted from infested roots of a hybrid okra (Abelmoschus esculentus) were applied around the bases of 12 day-old tomato cv. seedlings. At high inoculums levels of 6, 000, 8, 000 and 10, 000 eggs; flowering, number of leaf, plant height and fruit yield were significantly (p > 0.05) reduced. 100% loss occurred on number of fruit and fruit weight of tomato plants inoculated with 10, 000 eggs. Stunted stems with darker linings, poor flowering, small and chlorotic leaves, reduced size and weight of fruits and root galling all increased with the initial nematode population.

Keywords: Roma tomato, Meloidogyne incognita, eggs inoculums and pathogenicity.

Introduction

Tomato Roma cultivar (cv.) is an egg or pear-shaped tomato belonging to the genus *Lycopersicon*; generally available in red and yellow in colder and warmer regions[1]. The plant grows up to 1.2 meters (4 feet) height and its single fruit can weigh about 110 grams[2].

Growing of tomato has crossed from one continent to another and has become one of the world's most produced vegetable crops[3]. In 1987, the world's productivity of tomatoes has increased from 15.34 meters tons/ha to 16 metric tons/ha. But, world register of tomato tonnage has increased from 15.2 million tons in 1976 to 26.1 million tons[4]. In Nigeria, the annual production of tomatoes for 1995/1996 was estimated at 555.630 tons and is expected to rise annually due to high number of population and increased farming activities[5].

Southern root-knot nematodes, (*Meloidogyne incognita*) are microscopic round worms of the Tylenchid family[6]. Most are beneficial members of their ecosystems, but a few are economic parasites of plants and animals[7]. *Meloidogyne* species (*M. hapla* and *M. chitwoodi*) are parasites of many dicotyledonous plants[8]. Some crops that may be severely damaged by *M. incognita* parasites are tomato, pepper, okra, watermelon, cantaloupe, onion, pumpkin, squash, sweet potato, sweet corn, carrot, eggplant, bean and pea[7].

Many vegetables, bedding plants, shrubs and trees are susceptible to plant parasitic nematodes[8,9]. Root-knot nematode (*Meloidogyne incognita*) is an important pest in Nigeria as elsewhere in the tropics [10]. In Nigeria, poor yield of tomato has been attributed to nematode diseases[11,12]. Infection of these nematode species has been reported as one of the limiting factor in tomato cultivation which in some cases results in 90-100% yield loss of the crop[13,14].

This research was undertaken to determine the pathogenicity of root-knot nematodes (*Mloidogyne incognita*) on a hybrid tomato cultivar (cv.) in Sokoto Area

Material and Methods

Pathogenicity Study: The pathogenicity of *M. incognita* on a hybrid tomato cultivar (cv.) "Roma king" obtained from the Local market in Sokoto was carried out in the screen house of the Botanical garden in the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, from May-October, 2009. Eggs of *M. incognita* were extracted from the nematodes culture that was maintained on hybrid okra 'Ladies fingers' (*Abelmoschus esculentus*) by the use of chlorox[15] method.

The nematode inoculum was prepared using infected okra root system with heavy egg masses. The roots of infected portions of the plant were washed very well, removing the debris and the infected portion of the root cut into small pieces of 2-3cm. The segments of the root were placed in a container. A solution of 0.5% sodium hypochlorite (NaOCl) was added to cover the roots and later stirred for 2-3 minutes. The suspension was poured through the sieves of the jar from top to bottom which are of 80 mesh, 200 mesh and 500 mesh. Eggs on 500 mesh sieve were gently washed with slow cold tap water to rinse off residual NaOCl. Eggs were then collected from 500 mesh sieve into a glass beaker. Eggs suspension was brought to known volume and determined in milliliter and eggs of the active juveniles were quantified by agitating the egg suspension. 2ml of the suspension was used and transferred to the counting slide. A chamber made of 24 cells placed under the light microscope and the eggs were counted (three counts are made in 4 of the 24 cells) in the counting slide by random selection and number of eggs were counted in average of the three counts[16].

Serially graded inocula that hatch from the second-stage juveniles (J2s) by number (No.) of nematodes, of approximately 2, 000 4, 000 6,000 8,000 and 10, 0000 eggs per 25cm in diameter plastic pot were applied in the soil around the bases of 12 day old tomato seedlings in a 6-liter steam sterilized soil. Tomato seedlings that were not inoculated with the eggs served as the control. Each level of inoculums was replicated was replicated four (4) times and the pots were laid out in a completely randomized designed pattern in the screen house.

Data were taken on plant height, number of leaf, fruit number and fruit weight. Root gall rating was done after termination of the experiment 110 days after planting, using a rating scheme of 0-5 scale, where 0 = no galls, 1 = 1-25% root gall, 2 = 26-50%, 3=51-75%, 4=76-100% and 5 = 81-100% root gall, as described by Hussey R.S.et. al.[17].

Extraction of Root-Knot Nematodes: The nematodes were isolated by Cobb's sieving and decanting process as described by [18]. Active root-knot nematodes were isolated from small sample of soil in the polythene bags and finally pass through sieves, using Cobb's decantation and sieving technique, where soil sample taken to laboratory were transferred from the polythene bags into the buckets and water was added to the sample. The soil was stirred thoroughly and allowed to settled for about three minutes. This allows heavy particles to settle at the bottom of the bucket. The mixture was then poured through a set of scientific sieve of different mesh sizes serially 60, 80 and 120. The siever was then filled up in another bucket. To collect the residue, the siever was stand at the edge of a petri dish and fitting with its undersides upper most and the residue was collected by utilizing gentle stream of water to wash it into the Petri dish. This was then taken to dissecting microscope where it was observed and the nematodes founds were extracted with acid dropper along with curved nylon tooth brush and introduced using needles into preserving bottles.

Data Analysis: The data collected was subjected to analysis of variance ANOVA, using[19] package. Differences between the mean were partitioned using Turkey-Kramer's highest significant difference at 5% (P=0.05) probability level.

Results and Discussion

It was observed from this study that the hybrid tomato cv. "Rama king" is susceptible to active root-knot nematodes (*M. incognita*) attack. The plant height and leaf number was progressively reduced with the increasing nematode eggs inoculums. Stems appeared stunted with darker linings, leaves number per plant are fewer, smaller and chlorotic with patches as seen in figure-1. Plant height was progressively reduced with the increasing nematode population/inoculums levels as shown in table-1. From the 4th week of June, 09 plants inoculated with 2, 000 and 4, 000 eggs per pot were significantly different from the control treatment. Plants with higher inoculums levels of *M. incognita* eggs exhibit swollen root galls.

Similarly, from table-1 the fruit set was very poor and yield of fruit number and fruit weight per plant was progressively reduced. 100% loss was observed on tomato plants inoculated with 8, 000 eggs (that is, on fruit weight per plant) and a 100% loss on number of fruit and fruit weight of plants inoculated with 10, 000 eggs. Hence, at this highest inoculums level of 10, 000 eggs and rarely at 8, 000 eggs i.e from 5th week of July to the 3rd week of August 09; it was observed that the survived plants do not produce fresh fruits, hence, the weight and size of the fruits was reduced due to nematode attack with significant retard growth in height, leaves and fruits of the tomato plants ($p > 0.05$). The control treatment was observed to be superior to the 2, 000 eggs per pot treatment in terms of fruit weight per pot table-1. The higher the level of egg inoculums the lesser the weight and number of fruits per plant as indicated in table-1.

This indicates that fruit weight fruit number was significantly reduced as from 4, 000 eggs and from this level a high root galling was recorded. It could be deduced that, the more or the higher the level of egg inoculums the higher the number of root gall in the tomato plant, which was terminated in the 4th week of September, 09 table-1.

It could be deduced that the retard growth of the tomato plants at 10, 000 inoculums has been found to exhibit large galls or knots figure-2.

It could be seen that, following the extraction of the root-knot population from the soils sampled out, the most frequent diagnosed nematode damage occurs below ground with the plants showing symptoms of nutrient deficiencies along with stunted stems as observed around afternoon hours with hot climate temperature ranging up to 29^{o}C. Moreover, inoculations of the nematode eggs at their second stage juveniles (J2s), i.e the infective stage, were mostly found in the soils and are vermiform or worm-shaped with lips and strongly developed stylets, ranging up to 400 µm in length figure- 3

Discussion: The growth of tomato plants with corresponding decrease in plant height leaves and fruits number has been reported in this research. These poor growths might be as a result of the pathogenic effect of these ground worms and their susceptibility to the tomato cv. plants as influenced by the climatic conditions of the weather. This report is in line with the findings of Southey J.F. et.al.[20] who reported that tomato cv. Roma V was susceptible to root-knot nematode population and *M. incognita* significantly reduced the growth of tomato cv. Roma V.

Table-1
Growth, Yield and Root gall parameters of the hybrid tomato cv. "Roma king" inoculated with *Meloidogyne incognita* eggs

No. of *M. incognita* eggs	Mean plant height (cm) May-June, 2009	Mean No. of leaves/plant May-Jun, 2009	Mean No. of fruits/plant Jul-Aug, 2009	Mean fruit weight (g) Aug-Sep, 2009	Root gall index Oct, 2009
0 (control)	40.2^a	32.5^a	14.5^a	48.5^a	0.0^a
2, 000 eggs and J2	17.2^b	21.3^b	4.0^b	10.7^b	1.6^b
4, 000 eggs and J2	14.0^b	16.5^b	2.1^e	3.3^e	2.5^b
6, 000 eggs and J2	8.5^e	8.3^e	1.0^c	1.6^c	2.5^c
8, 000 eggs and J2	4.4^e	5.2^c	1.0^c	0.2^c	2.8^c
10, 000 eggs and J2	4.2^e	2.4^e	0.0^e	0.0^e	4.0^e

Numbers with same letter in the column show no significant difference. The means were partitioned using Turkey-krammer Highest Significant difference at 5% probability level.

Figure-1
Roma tomato indicating poor fruit set-up, stunted stems and chlorotic leaves

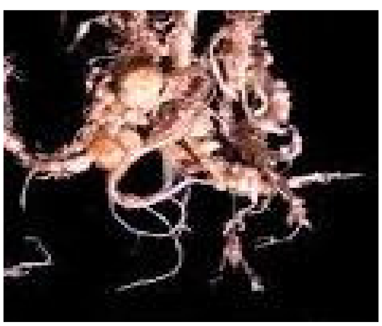

Figure-2
Severe galling with feeding disposition area of *M. incognita* on the tomato root

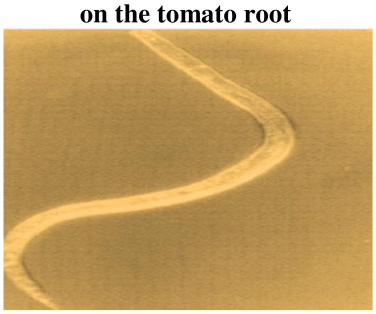

Figure-3
Representative specie of root knot nematode, *M. incognita* extracted from the infested tomato root

It has also been established in this survey that the tomato 'Roma king' cv. was found to inhabit poor flowering, stunted stems with dark linings and yellowed leaves with white patches. 100% loss on leaves per tomato plants inoculated with 8, 000 eggs and a 100% loss on fruit number and weight of fruits with a rapid increase in root galling up to 10, 000 inoculums level of *M. incognita* ($p > 0.05$). This report correlates with the findings of SAS. et. al.[21] who estimated a 69% drop in cowpea yield and [21] reported approximately 75% reduction in tomato yield because of this pathogen Babatola J.O. et.al.[22] also reported that tomato plants infected with root-knot nematodes yield conspicuous root galls.

It was established from this research that, the gradual increase of nematode eggs at their J2s in the tomato plants yield poor flowering set-up and the whole plants loosing nutrients with knots forming on the roots region. This might be because of the hidden nature, their vermiform and morphological structure of needle-like stylets of which the nematode parasites use to protrude the plant root cells. As the density of nematodes increases, galls per tomato plant also increase. This could also be seen that the infected tomato plants usually wilt easily, and are not productive. Sometimes the infection is easy to identify because of the large swellings easily noted on the root portion of the tomato cv. plants. This result agreed with the work of Wilson W.R.et.al.[23] who reported that the plant parasitic nematode (*Heterodera sacchati*) penetrates plant cell with stylet, by injecting the secretory proteins thereby stimulating changes within the parasitized cells; which in turn produce large galls throughout the root system of infected plants Wilson W.R.et.al.[23] added that, root-knot nematode females are globose and sedentary at maturity, ranging from 400 to 1000 µm, but the male ones are vermiform ranging from 1100 to 2000 µm in length Stirling G.R.[24] reported that root knot nematode establish a feeding site on the plant root and permanently remain at that location allowing the giant cell to produce large amounts of proteins which the nematode use to ingest.

It was reported from same study, that the higher the nematode population the greater the rate of infection. This might be due to the fact that some plants like tomato are specific, sensitive and cannot with stand vulnerability against nematode populations in soil because, some or most of the tomato plants in the trials respond to nematode attack. This could also be explained that, regular initial nematode population of *M. incognita* in the field of study had been found to move along shallower temperature gradients. But, at the initial eggs inoculums, the root knot nematodes population was observed to feed and multiply on many tomato plants, although they did not injure the whole plant body but to some extent. Nematodes penetrating roots in close proximity result in larger galls and *M. hapla* produces gradual increase in galling less than half the size of those produced by *M. incognita* on the same plant hosts[25].

It was reported from this study that in most of the second stage infective larvae of the nematode population, it was encountered during hot temperature (27±2^{o}C) of the climatic condition. This will be explained by the fact that, hot dry hot weather in temperature variation may influence or triggers the development and multiplication of root-not nematodes population[26] noticed that vegetable crops grown in warm climates experience severe losses from root knot nematodes. They also reported that root-knot nematode damage results in poor growth, decline in quality and yield of the crop and reduced resistance to other stresses leading to total crop loss.

Conclusion

Hybrid tomato plants are highly sensitive to root knot diseases and root knot nematodes (*Meloidogyne incognita*) at second staged juveniles (2Js) are vulnerable, infecting plant roots leading to the development on the tomato plant roots. The initial nematode populations (eggs inoculums) drain the plants nutrients and multiply the infections causing more decreased in yield. This research should be encouraged in relevant areas of study so that lay men can have knowledge on identifying these parasitic worms and produce/consume such healthy vegetable and other versatile crops that can support nutrient deficiency in human and other domestic animals.

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