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Inoculum Level and Inoculation Method Influences on the Pathogenic Activities of *Meloidogyne incognita* in Studied Model Plant Okra (*Abelmoschus esculentus* L. Moench)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Agricultural activities such as watering crops with nematode-infested water from wells and boreholes, and using infected plant debris as manure or mulch increase root-knot nematode infection. So, this study aims at assessing the influence of the inoculation method and inoculum level of *Meloidogyne incognita* on the development of root galls on okra plants. Two *M. incognita* inoculation methods (suspension of individuals and galled root explants) and six inoculum levels (0, 10, 100, 500, 1000 and 2000 second-stage larvae/plant) were studied. The gall index, total numbers and reproductive factor of *M. incognita* were used to assess the effect of treatments on root gall development. Unlike the reproductive factor, gall index and the total numbers of *M. incognita* increased with their inoculum level. The pathogenic activities of *M. incognita* were most significant when crop soils were infested with galled root explants. However, an inverse relationship was found between the inoculum levels of *M. incognita* and the okra plant's development. It is reflected by negative correlation coefficients ranging from -0.90 to -0.62. It is therefore important to burn roots infected with root-knot nematodes left in fields so that they do not act as an inoculum for crops.

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1. INTRODUCTION

Originally from Ethiopia [1], okra (*Abelmoschus esculentus* L. Moench) spread to North Africa, the Mediterranean, Arabia and India in the 12th century [2]. It is cultivated nowadays in the tropics, particularly in tropical Asia, in eastern, central and western Africa, as well as in the Caribbean [3]. It is one of the most popular vegetables in the world [4]. Okra is a multipurpose plant owing to its immature pods, fresh leaves and seeds [5]. Okra is an important constituent of most local dishes in West Africa. Okra immature pods, eaten as a vegetable, can be used in salads and soups [6].

Okra is mainly cultivated for its immature and edible pods [7]. The composition of 100 g of immature okra pods is: 88.6 g of water, 2.1 g of protein, 8.2 g of carbohydrates, 0.2 g of fat, 1.7 g of fiber, 84 mg of calcium, 90 ml of phosphorus and 120 mg of iron [8]. Consumption of immature okra pods provides 4550 kcal/kg for humans [9]. Young okra leaves, rich in vitamins A and C, protein, calcium, and iron, are also eaten in many African countries [7]. Mature seeds, in contrast, are used to produce oil [8] and biofuel [10].

Moreover, with 120 thousand tons of immature pods produced in 2019, Côte d'Ivoire is ranked as the 2nd African okra producer after Nigeria [11]. Okra is one of the most consumed vegetables in Côte dIvoire [12]. Its cultivation and marketing provide significant income for farmers and traders. Okra cultivation generates, on 1 ha, a net profit of 156,884.81 Naïra, that is, about 410 US dollars [3]. Nowadays, the price per kilogram of immature okra pods in Côte d'Ivoire varies between 700 and 1500 CFA francs depending on the market [13]. Okra is cultivated in Côte d'Ivoire throughout the country [14] in rural, urban and peri-urban areas.

Like most vegetables in the tropics, okra cultivation is prone to infection by root-knot nematodes. Okra is host to several root-knot nematode species, such as *Meloidogyne arenaria*, *M. incognita* and *M. javanica* [15]. Root-knot nematodes are responsible for yield losses ranging from 70 to 90% [16]. They cause wilt, chlorosis, stunted growth, gall formation, root reduction, and poor yield if their populations exceed the economic threshold [15]. Gall development is the main symptom of root-knot nematode infection [17]. It is influenced by host cultivar, root-knot nematode species or race, soil infestation level and the environment [18].

Root-knot nematodes, like other plant parasitic nematodes, are transported to their hosts by several factors. These include nematodes themselves through their movement, wind, humans through work tools, runoff, and infected plant material [19]. Disseminated root-knot nematodes, thanks to these factors, supplement the pre-existing ones in production plots. They thus increase parasite pressure on their hosts. Runoff, flooding, gravity irrigation, and watering crops with water contaminated by root-knot nematodes play an important role in root-knot nematode dissemination and crop infection. In fact, nematodes move more easily in moist soils [20], offering them the best conditions for achieving their life cycle [21]. Soil moisture, according to Djian-Caporalino et al. [20], improves nematode egg hatching. Furthermore, infected plant debris (infected roots), brought back to the soil as manure or used as mulch against sunstroke on ridges and beds, plays a role in crop infection. Such practices help to reach the economic threshold of root-knot nematodes for crops. In view of the above, this study was designed so as to assess the influence of the inoculation method and inoculum level of *M. incognita* on its pathogenic activities on the okra model plant.

2. MATERIALS AND METHODS

2.1 Soil Preparation

Okra plants were cultivated on a sterilized topsoil. A sample (80 kg) of topsoil from the forest was sterilized twice with an autoclave (Adolf Wolf Sanoclav 01028) for 45 minutes at 121 °C. The sterilized topsoil was distributed in perforated plastic bags (720 g per bag).

2.2 Okra Plant Acquisition

Okra *Hiré* seeds, used in this trial, were obtained from a company specializing in seed marketing. Okra seeds were soaked in distilled water in order to choose viable seeds. The floating seeds were discarded because they were not viable. In contrast, the ones at the bottom of the jar were selected because they were viable.

The viable seeds were sown in a previously sterilized topsoil distributed in vermiculite trays.

Three viable seeds were sown per cell to get sufficient vigorous seedlings for the pathogenicity test. Seedlings were watered every 48h for 7 days. The vigorous 7-day-old okra seedlings were transplanted into each perforated plastic bag filled with 720g of sterilized soil. There was one okra seedling transplanted per bag. Okra seedlings were maintained in the greenhouse of Nangui Abrogoua University, Abidjan, Côte d'Ivoire, for the next inoculation.

2.3 Inoculum Source and Preparation

Second-stage larvae (J2) of *M. incognita* were used for this trial. They were previously maintained on potted okra *Hiré* plants in the greenhouse. They served as a source of inoculum. Okra plants were uprooted and washed with tap water. The roots were cut into explants of about 5 mm. Root explants were homogenized to constitute a composite sample. The eggs of *M. incognita* were extracted from 10 × 5 g of root explants according to the Hussey and Barker [22] method. Egg suspension of *M. incognita* was incubated at 26 °C for 72h in an incubator (UF55-memmert). Second-stage larvae from *M. incognita* hatched eggs were used as inoculum.

2.4 Pathogenicity Test

2.4.1 Inoculation methods and inoculum levels of *M. incognita*

Two inoculation methods of *M. incognita* were studied on okra plants. These included inoculation by suspension of individuals from galled roots and inoculation by galled root explants. Six levels of *M. incognita* inoculum which include 0, 10, 100, 500, 1000 and 2000 individuals per plant were tested for each inoculation method.

To determine the inoculum levels per weight of root explants, second-stage larvae of *M. incognita* from 5g of root explants were counted from 50 ml of nematode suspension. According to the obtained results from 50 ml of J2 suspension, root explants weighing 0.1 g, 1 g, 4.81 g, 9.62 g or 19.23 g equated to inoculum levels of 10, 100, 500, 1000 or 2000 individuals, respectively.

2.4.2 Soil infestation by *M. incognita*

Four holes of 1 cm in diameter and approximately 5 cm deep were made 2 cm around each okra plant. Results obtained from 50 ml of J2 suspension allowed to correspond to 0.2 ml, 0.5 ml, 2.5 ml, 5 ml and 10 ml of suspension to the inoculum levels of 10, 100, 500, 1000 and 2000 individuals, respectively. The aliquot, for each inoculum level, was distributed in the holes around the okra plant.

Moreover, aliquots of 0.1 g (10 individuals) to 19.23 g (2000 individuals) of galled root explants, depending on inoculum level, were incorporated into okra crop soils. The aliquot, for each level of inoculum, was distributed in the holes around the okra plant. Holes were closed after soil infestation. The soils of control plants were not infested with *M. incognita*.

In the greenhouse, the inoculated plants were arranged in a randomized complete block design with eleven treatments, each with ten replicates. For this trial, 110 okra plants were used. The inoculated plants were watered every 72h with 100 ml of water per plant.

2.4.3 Evaluation of the effects of *M. incognita* on root gall development

The effects of *M. incognita* inoculation method and inoculum level were assessed by determining the development parameters of okra root galls. These included the plant's phytosanitary state (gall description), gall index, number of *M. incognita* in soils and okra roots and its reproductive factor. Okra plants were watered the day before they were uprooted after two months of cultivation. The purpose of this watering was to loosen the soil and uproot the okra plants without damage. Uprooted okra plants were grouped per inoculum level and *M. incognita* inoculation method.

The root system of the okra plants was rinsed with tap water so as to remove soil clods. The okra plants were placed on blotting paper to absorb excess water. The root system of okra plants was examined in order to note the root gall severity level depending on inoculum level and inoculation method according to the following Bridge and Page [23] scale.

0: No galls on roots
1: Few small galls, difficult to find
2: Small galls only but clearly visible. Main roots clean
3: Some larger galls visible. Main roots clean
4: Larger galls predominate but main roots clean
5: 50% of roots affected. Galling on some main roots. Reduced root system

6: Galling on main roots
7: Majority of main roots galled
8: All main root, including tap root, galled. Few clean roots visible
9: All roots severely galled. Plant usually dying
10: All roots severely galled. No root system. Plant usually dead.

The gall index (GI) was calculated according to the Zewain [24] formula.

$$GI = \frac{\sum (ssi \times ni)}{TN}$$

GI: Gall index
ssi: Severity score assigned to galls i found on the plant root system,
ni: Number of plants to which the score ssi was assigned,
TN: Total number of plants with root galls.

The root system was detached from the okra plants at their crown. The root system was weighed per okra plant. The roots of each okra plant were cut into approximately 5 mm explants using a pair of scissors. Individuals of *M. incognita* were extracted from okra plant roots by the Baermann maceration method [25]. Individuals of *M. incognita* were extracted from 100 ml of soil according to the Whitehead tray method [25]. Five repetitions were performed per *M. incognita* inoculum level. After extraction, *M. incognita* individuals were counted and, its reproductive factor (Rf) was calculated per treatment according to the following formula.

$$Rf = \frac{Pf}{Pi}$$

Rf: Reproductive factor
Pf: Final number of *M. incognita* (total number in the soil + total number in the roots)
Pi: Initial number of *M. incognita*

2.4.4 Evaluation of the effects of *M. incognita* on okra plant development

The effects of *M. incognita* inoculation method and inoculum level on okra plant development were assessed by determining agronomic parameters. These included plant size, leaf number per plant, and root and shoot weights. Thus, plant size was measured with a tape measure (Stanley PowerLock) from the crown to the apical end. The leaves were counted per plant. The fresh root and shoot weights were measured with a digital electronic scale.

2.5 Statistical Analysis

Before statistical analysis, the total numbers of *M. incognita* and the leaf number per plant were transformed by the log10 (x + 1) function where x is the total number of nematodes or the leaf number. Root-gall and okra-plant development parameters were subjected to one-way analysis of variance (inoculum level or inoculation method). Statistica 7.1 software was used for statistical analysis. In the event of a significant difference at the 5% level, Fisher's LSD test or Dunnett's test was used, depending on the analysis, to obtain homogeneous groups. Two-way analysis of variance (inoculum level and inoculation method) was used to demonstrate the interaction between the effects of inoculum level and the inoculation method. Pearson's correlation coefficient was used to identify the relationship between inoculum level and development parameters of root galls and okra plants.

3. RESULTS

3.1 Root Gall Development on Okra Plants

3.1.1 Okra plant phytosanitary states

Okra plants, after two months of cultivation on *M. incognita*-infested soils, exhibited root galls, except for control plants. Root galls were in the form of spherical outgrowths of varying diameters, ranging from 0.3 mm to 4 mm (Fig. 1a). The root gall number varied according to the inoculation method and inoculum level. Okra plants varied in size depending on inoculum level (Fig. 1b). Cases of dead okra plants were noted.

3.1.2 Effect of *M. incognita* inoculum level on root gall development

The gall index, final number and reproductive factor of *M. incognita* varied depending on its inoculum level (Table 1). The inoculum level had a significant effect, regardless of *M. incognita* inoculation method, on okra root gall development ($P < 0.05$). The gall index and final number of *M. incognita* increased with the level of inoculum, unlike reproductive factor. Statistical analysis showed that root gall development was greater in okra plants infected with 2000 *M. incognita* individuals compared to other plants.

3.1.3 Effect of *M. incognita* inoculation method on okra root gall development

The gall index, final number and reproductive factor of *M. incognita* varied depending on

inoculation method (Table 2). A significant difference was noted between inoculation methods with respect to gall index, final number and reproductive factor of *M. incognita* ($P < 0.05$). Root gall development was more significant in plants inoculated with galled root explants compared to suspension of individuals. Inoculation with galled root explants induced root galls with an index of 2.87. The total number and reproductive factor of *M. incognita* were 908 individuals and 3.05, respectively.

Fig. 1. Okra plant phytosanitary states after inoculation with galled root explants
a: Galls on okra plant roots; b: Plant states with their root systems
Scale bars: a (10 mm) & b (50 mm)

Table 1. Okra root gall development depending on *M. incognita* inoculum level

Inoculation method	Inoculum level	Gall Index	Final number of nematodes	Reproductive factor
Suspension of individuals	0	0	0	0
	10	1.00 ± 0.06**c**	23 ± 3**e**	2.23 ± 0.5**a**
	100	1.20 ± 0.06**c**	191 ± 52**d**	1.91 ± 0.48**ab**
	500	1.25 ± 0.08**c**	672 ± 254**c**	1.34 ± 0.51**ab**
	1000	1.60 ± 0.19**b**	1052 ± 211**b**	1.05 ± 0.21**b**
	2000	4.40 ± 0.19**a**	1889 ±157**a**	0.94 ± 0.08**b**
	F	*153.58*	*24.17*	*2.26*
	P	*0.000*	*0.000*	*0.042*
Galled root Explants	0	0	0	0
	10	1.00 ± 0.16**c**	78 ± 14d	7.80 ± 1.34**a**
	100	1.20 ± 0.03**c**	385 ± 36cd	3.85 ± 0.36**b**
	500	4.00 ± 0.32**b**	653 ± 166c	1.31 ± 0.33**c**
	1000	5.40 ± 0.19**a**	1277 ± 332b	1.28 ± 0.33**c**
	2000	5.60 ± 0.13**a**	2145 ± 63**a**	1.07 ± 0.03**c**
	F	*199.91*	*28.33*	*19.11*
	P	*0.000*	*0.000*	*0.000*

For each inoculation method, values with the same letter in a column are statistically identical at 5% level

Table 2. Root gall development depending on *M. incognita* inoculation method

Inoculation Method	Gall index	Final number of nematodes	Reproductive factor
Control	0	0	0
Suspension of individuals	1.58 ± 0.26**b**	766 ± 153**b**	1.49 ± 0.18**b**
Galled root explants	2.87 ± 0.42**a**	908 ± 165**a**	3.05 ± 0.59**a**
F	*3.94*	*4.01*	*3.49*
P	*0.023*	*0.037*	*0.036*

Values with the same letter in each column are statistically identical at the 5% level

3.2 Okra Plant Development

3.2.1 Effect of *M. incognita* inoculum level on okra plant development

Plant size, leaf number, root and shoot weights varied with *M. incognita* inoculum level (Table 3). The inoculum level of *M. incognita* significantly influenced okra plant development ($P < 0.05$). The agronomic parameters increased when the *M. incognita* inoculum level decreased. Okra plant development on non-infested or infested soil with a low number (10 to 100 individuals) of *M. incognita* showed the best agronomic characteristics. In contrast, the development of okra plants on infested soil with 2000 *M. incognita* individuals, regardless of the inoculation method, was not improved.

3.2.2 Effect of *M. incognita* inoculation method on okra plant development

Leaf number, plant size, root and shoot weights varied depending on the *M. incognita* inoculation method (Table 4). Statistical analysis revealed a significant difference between *M. incognita* inoculation methods for each aforementioned parameter ($P < 0.05$). Okra plants on non-infested soil presented agronomic parameters higher than those on infested soil with *M. incognita*. Okra plants on non-infested soil presented the largest size (16.2 cm) and the highest leaf number (5.6 leaves per plant). Roots and shoots had the largest weights, 2.38 g for roots and 4.87 g for shoots. Plants inoculated with galled root explants had the least improved development. The plant size was 9.48 cm with 3.08 leaves per plant. Their root and shoot weights were 1.06 g and 2.75 g respectively.

3.3 Interaction between *M. incognita* Inoculation Method and Inoculum Level

The development of okra root galls is influenced by *M. incognita* inoculum level and inoculation method (Table 5). An interaction was noted between the two factors with respect to gall, reproductive factor and final number of *M. incognita*. In contrast, no interaction was found between the effects of *M. incognita* inoculum level and inoculation method with respect to leaf number, plant size, root and shoot weights.

3.4 Relationship between *M. incognita* Inoculum Level and Root Gall and Okra Plant Development

The study of the relationship between *M. incognita* inoculum level and root gall and okra plant developments revealed a variation in correlation coefficients (Table 6). The increase in *M. incognita* inoculum level, regardless of the inoculation method, strongly favored okra root gall development. It is reflected by positive correlation coefficients ranging from 0.88 to 0.99 between the inoculum level and gall index and the final number of *M. incognita*. In contrast, the inoculum level increased, while the reproductive factor decreased. The increase in inoculum level resulted in reduced development of okra plants with negative correlation coefficients ranging from -0.90 to -0.62. However, the plant size and the leaf number increased with root and shoot weights. This increase was characterized by positive correlation coefficients ranging from 0.67 to 0.97.

4. DISCUSSION

Okra plays an important role in food and local commerce in Côte dIvoire. Its cultivation, despite its food and economic interests, is highly prone to root-knot nematode infections. In this study, all *M. incognita* inoculum levels incorporated into the crop soil, regardless of the inoculation method, induced root galls in okra plants. This result reveals the pathogenicity of *M. incognita* on okra plants. It is also responsible for root galls on the okra plant. Indeed, *M. incognita* second-stage larvae migrate through intercellular spaces by enzymatic digestion of the middle lamella of host cells [26]. Once in the vascular cylinder of host roots, they select host target cells and transform them into giant cells. The latter increase in size and function for the benefit of root-knot nematodes. Meanwhile, the cells around the giant cells divide rapidly, causing localized swelling, hence the typical symptom of root galls [27]. Hussain et al. [28] and Kayani et al. [29] also found that all selected *M. incognita* inoculum levels induced root galls in eggplant and cucumber in Pakistan.

However, root gall development on okra plants is influenced by *M. incognita* inoculum level. It is reflected by the increase in gall index, total numbers of *M. incognita* in soils and okra plant roots with the inoculum level. This increase in root gall development parameters might be due to the increase in *M. incognita* inoculum level brought to crop soils. In fact, the more second-stage larvae, which are the infectious stage of *M. incognita*, are brought to the soil, the more their pathogenic activities are accumulated. In the study by Kankam and Adomako [30], the development parameters of tomato plant root

galls also increased, when *Meloidogyne* spp. inoculum levels switched from 500 to 2000 individuals. The gall index and total numbers of *M. incognita* were higher in okra plants infected with 2000 individuals compared to other inoculum levels. They were, however, low in plants infected by 10 individuals, regardless of the inoculation method. The development of root galls limits the ability of plants to absorb water and mineral salts from the soil, leading to stunted growth [30]. The growth of tomato plants is reduced, according to Azam et al. [31] after infesting tomato crop soils with 500 to 3000 *M. incognita* individuals.

Unlike the gall index and total numbers, *M. incognita* reproduction is reduced as its inoculum level increases in the soil. Indeed, when inoculum levels are low, *M. incognita* individuals might have sufficient nutritional resources for their feeding and development. This situation could justify the high reproductive factors recorded in plants infested with 10 and 100 individuals, regardless of the inoculation method. Ndiaye [32] indicated that the more the host plant develops, the more its root system is significant and the nematodes have more fixation sites and resources favorable to their development. However, when inoculum levels are high, the reproductive factor becomes low. Hussain et al. [28] also noted that the minimum reproductive factor of *M. incognita* was found at the highest inoculum level. So, an inverse relationship was found between inoculum level and reproductive factor. This low reproduction might be due to the destruction of the okra plant root system by the pathogenic activities of *M. incognita* second-stage larvae. The destruction of the root system could lead to competition between individuals for increasingly insufficient resources [30]. Nematodes might be unable to identify new resources necessary for the emergence of new generations of individuals. The scarcity of resources could lead to the death of some individuals, thus reducing reproduction [32]. The destruction of the root system could justify the reduction of development, even the death of plants infested with high inoculum levels of *M. incognita*. Also, the low reproduction might be due to the change in environmental conditions for the nematodes at inoculation as well as the handling [33]. Indeed, after hatching, second-stage larvae of *M. incognita* were exposed to water before okra plant inoculation. This situation may affect the life cycle duration or pathogenic activities of nematodes. Baimey [34] found a decrease in *Scutellonema bradys* population density on yam tubers during the 1st month after inoculation.

Moreover, the *M. incognita* inoculation method influenced root gall and okra plant development. Inoculation with galled root explants favored root gall development more than inoculation with suspension of individuals. This finding might be due to an almost authentic simulation of the natural conditions of infection and development of root-knot nematodes on their host. Indeed, when necrosis invades roots, nematodes move out of the roots by themselves [35]. They search for new resources, including the roots of developing okra plants, and begin another infection cycle. Under these conditions, pathogenic activities could be more significant. In fact, the root-knot nematodes that emerge from infected plant debris supplement those already present in the soil in order to increase the parasite pressure on the crops developing on the plot or those of the next season. This situation could justify a significant reduction in the development of okra plants. Baimey et al. [36] found that dry rot is more developed in yam plants infected with yam peel infected with *Scutellonema bradys* compared to those infected with suspension of individuals. This finding highlights the benefit of burning plant debris infected by nematodes so that it does not act as a source of inoculum for crops.

Table 3. Development of okra plants as a function of *M. incognita* inoculum level

Inoculation method	Inoculum level	Plant size (cm)	Leaf Number	Root weight (g)	Shoot weight (g)
Suspension of individuals	0	16.20 ± 1.98**a**	5.60 ± 0.24**a**	2.38 ± 0.44**a**	4.87 ± 0.21**a**
	10	12.00 ± 1.05**ab**	4.40 ± 0.24**ab**	1.12 ± 0.25**b**	4.39 ± 0.61**ab**
	100	12.00 ± 2.07**ab**	3.80 ± 0.34**bc**	1.11 ± 0.30**b**	4.46 ± 1.49**ab**
	500	13.20 ± 1.16**ab**	4.60 ± 0.24**ab**	1.47 ± 0.10**ab**	5.66 ± 0.54**a**
	1000	10.40 ± 0.98**b**	4.00 ± 0.55**bc**	0.92 ± 0.29**b**	3.03 ± 0.71**c**
	2000	12.20 ± 1.20**ab**	3.00 ± 0.84**c**	1.43 ± 0.41**b**	2.68 ± 0.64**c**
	F	*2.76*	*3.45*	*2.69*	*2.98*
	P	*0.043*	*0.017*	*0.046*	*0.041*

Galled root explants	0	16.20 ± 1.98**a**	5.60 ± 0.24**a**	2.38 ± 0.44**a**	4.87 ± 0.21**ab**
	10	12.20 ± 1.46**ab**	3.60 ± 0.75**ab**	0.70 ± 0.19**b**	3.37 ± 0.75**bc**
	100	11.80 ± 1.62**b**	5.20 ± 0.58**a**	1.67 ± 0.22**ab**	5.36 ± 0.29**a**
	500	9.60 ± 1.81**bc**	3.00 ± 1.26**b**	1.64 ± 0.83**ab**	2.10 ± 0.93**cd**
	1000	7.40 ± 0.51**c**	3.00 ± 0.95**b**	0.69 ± 0.33**b**	2.27 ± 0.53**cd**
	2000	6.40 ± 0.24**c**	0.60 ± 0.40**c**	0.58 ± 0.13**b**	0.66 ± 0.19**d**
	F	*6.27*	*5.38*	*2.96*	*10.26*
	P	*0.001*	*0.002*	*0.032*	*0.000*

For each inoculation method, values with the same letter; in each column, are statistically identical at 5% level

Table 4. Okra root gall development depending on *M. incognita* inoculation method

Inoculation Method	Plant size (cm)	Leaf Number	Root weight (g)	Shoot weight (g)
Control	16.20 ± 1.06**a**	5.60 ± 0.13**a**	2.38 ± 0.24**a**	4.87 ± 0.11**a**
Suspension of individuals	11.96 ± 0.58**b**	3.96 ± 0.23**b**	1.21 ± 0.12**b**	4.04 ± 0.42**b**
Galled root explants	9.48 ± 0.71**c**	3.08 ± 0.46**c**	1.06 ± 0.20**b**	2.75 ± 0.40**c**
F	*14.43*	*9.62*	*12.08*	*5.75*
P	*0.000*	*0.000*	*0.000*	*0.001*

Values with the same letter, in each column, are statistically identical at 5% level

Table 5. Interaction between *M. incognita* inoculum level and inoculation method on root gall and okra plant development

Variation sources	Root gall development				Plant development				
	df	GI	RF	FNN	df	LN	PS	RW	SW
IL	5	*0.000*	*0.000*	*0.000*	5	*0.000*	*0.000*	*0.031*	*0.000*
IM	1	*0.000*	*0.000*	*0.037*	1	*0.085*	*0.006*	*0.676*	*0.012*
IL × IM	5	*0.000*	*0.000*	*0.000*	5	*0.267*	*0.236*	*0.578*	*0.151*

IL: Inoculum level, IM: Inoculation method, GI: Gall index, FNN: Final number of nematodes, Rf: Reproductive factor, PS: Plant size, LN: Leaf number, RW: Root weight, SW: Shoot weight, df: Degree of freedom

Table 6. Correlation coefficients between *M. incognita* inoculum level and its effect on root gall and okra plant development

Variables	IL	GI	FNN	Rf	PS	LN	RW	SW
Suspension of individuals								
IL	1							
GI	0.94**	1						
FNN	0.99***	0.92**	1					
Rf	-0.22ns	0.05ns	-0.20ns	1				
PS	-0.39ns	-0.45ns	-0.42ns	-0.64*	1			
LN	-0.75*	-0.88*	-0.73*	-0.40ns	0.74*	1		
RW	-0.21ns	-0.32ns	-0.25ns	-0.78*	0.97***	0.67*	1	
SW	-0.76*	-0.72*	-0.72*	0.08ns	0.56ns	0.71*	0.39ns	1
Galled root explants								
IL	1							
GI	0.88*	1						
FNN	0.99***	0.90*	1					
Rf	-0.41ns	-0.38ns	-0.40ns	1				
PS	-0.85*	-0.96**	-0.89*	0.14ns	1			
LN	-0.90*	-0.86*	-0.88*	0.11ns	0.87*	1		
RW	-0.62*	-0.64*	-0.63*	-0.38ns	0.78*	0.77*	1	
SW	-0.85*	-0.88*	-0.82*	0.22ns	0.84*	0.97**	0.69*	1

IL: Inoculum level, GI: Gall index, FNN: Final number of nematodes, Rf: Reproductive factor, PS: Plant size, LN: Leaf number, RW: Root weight, SW: Shoot weight

****, **, *: Correlation respectively significant at 1 ‰, 1% and 5% level; ns: Non-significant correlation*

However, an inverse relationship was found between the inoculum levels of *M. incognita* and the okra plant's development. It was observed that all the inoculum levels of *M. incognita* resulted in significant reductions in the leaf number, plant increase, root and shoot weights of the okra plant. The inverse relationship between the inoculum levels of *M. incognita* and the okra plant development might be due to root damage due to penetration and/or feeding of nematodes into the roots. This situation could result in impairment and disruption of water absorption by the infected root systems. Indeed, after penetration into roots, the root-knot nematodes induced gall formation and giant cells in the stellar region and caused disruption of xylem tissues [37]. Due to extensive disruption of xylem vessels, the upward uptake of water and nutrients was greatly reduced. The root-knot infection also greatly affected the permeability of roots to water [37]. Due to induction of nurse cell systems by the females of root-knot nematodes for incessant feeding in infected roots, there was a greater translocation of photosynthates towards these infection sites while the above ground parts experienced acute deficiency of nutrients [38,39]. As the infected plants faced an insufficient supply of nutrients, photosynthates, energy, and water, therefore, the development and growth of leaf tissues and their essential constituents, particularly chlorophyll pigments, were greatly hampered [40]. The stunted and reduced growth of foliar parts results in reduced biomass [41].

5. CONCLUSION

This study was conducted to study the influence of inoculum level and inoculation method on the pathogenic activities of *M. incognita* on the okra plant model. It appears that *M. incognita* is responsible for root galls on okra plants. The pathogenic activities of *M. incognita* increase with its inoculum level. The pathogenic activities of *M. incognita* also increase when soil infestation is made by galled root explants. Therefore, the development of okra plants is severely limited. This study highlights the benefit of burning plant debris infected with root-knot nematodes so that it does not act as a source of inoculum for existing or future crops. Furthermore, infestation of the crop soil with galled root explants, with low inoculum levels, is an excellent method for screening tests in order to identify okra cultivars tolerant and/or resistant to *M. incognita*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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