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***Research Article** (Open Access)*

# EFFECT OF SALINITY STRESS ON ROOT-KNOT NEMATODES INFECTIVITY IN TOMATO CULTIVARS

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## ABSTRACT
*Meloidogyne incognita* is a prevalent species of *Meloidogyne*, which is a type of root-knot nematode, characterized by its small size and worm-like appearance, and is commonly found in soil. RKN, or root-knot nematode, invades the roots of tomato plants and can lead to stunted growth, decreased leaf size, leaf discoloration, and eventual wilting. RKN infection is widespread in saline soils. Salinity and root-knot nematodes are two factors that harm tomato production, which in turn has a significant impact on agricultural yield. The present study investigates the combined stress of salt and RKN on tomato cultivars (Pusa Ruby and Pusa Early Dwarf). Experiment set up in triplicate with a completely randomized design. To assess the effects of saline soil on root-knot nematodes in tomato cultivars, applied the following treatments: After inoculating the tomato cultivars (Pusa Ruby and Pusa Early Dwarf) with 1000 juvenile (J2) of *M. incognita*, irrigated them with a 100 mM salt concentration. The untreated control consisted of J2 inoculation without salt, while the treated control involved inoculation with 1000 J2 and 100 mM salt. In the treated and untreated checks, Pusa Ruby appeared to be a more tolerant cultivar compared to Pusa Early Dwarf. In the treated check experiment, giant cell formation initiated 15 days after inoculation (DAI), which delayed RKN growth compared to the untreated check. The rate of penetration increased from 41.33% to 54% for Pusa Ruby and from 170.67% to 177% for Pusa Early Dwarf. The incidence of galls and egg mass formation increased from 97% to 115.67% for Pusa Ruby and from 43.67% to 62% for Pusa Early Dwarf, respectively. Salinity stress also had an impact on the percentage of giant cell formation: in the treated control, the initiation of nematode growth coincided with giant cell formation, while in the untreated control, nematode growth was delayed.

***Keywords:*** *Salinity, Root Knot Nematodes, M. incognita, Root Gall, Egg Mass, Tomato*

## INTRODUCTION
The three most economically significant groups of plant-parasitic nematodes (PPNs) are root-knot nematodes, cyst nematodes, and lesion nematodes. Root-knot nematodes (*Meloidogyne* spp.) are the primary plant-parasitic nematodes that cause significant damage among PPNs. RKN are members of the *Meloidogyne* genus, which consists of over 98 species. *M. javanica, M. incognita,* and *M. arenaria* are recognized as the prevailing species. The above three species are causing significant damage to economically vital crops such as tomato, maize, cowpea, banana, potato, and sweet potato. *Meloidogyne incognita* are sedentary endoparasites that infect a wide range of hosts and have a negative impact on numerous crops, leading to a substantial decrease in both the quantity and quality of food produced (Shakeel *et al*., 2022). The term "root-knot nematodes" has been assigned to these nematodes due to the distinct symptom of swollen areas (galls or knots) that appear on the infected roots (Subedi *et al*., 2020). As obligatory plant parasites, RKN also induces atypical symptoms that resemble those caused by other biotic and abiotic stresses, such as root swelling and diminished root systems. In addition, the harm caused by these pests includes stunted plant growth, discoloration of leaves, and yellowing, drooping, and eventual loss of foliage. As a result, plants become more vulnerable to other pathogens and stresses (Caillaud *et al*. 2008; Favery *et al*., 2016; Kyndt *et al*., 2017; Liu and Park 2018). Identification of

potentially harmful species of plant parasite nematodes is beneficial success to agriculture which provides regulatory procedure or evaluation of quarantine to minimize their spread and reproduction within host (Abd-Elgawad 2021). Root knot nematodes (*Meloidogyne incognita)* exhibit an intricate parasitism mechanism whereby they are capable of converting particular host cells into specialised feeding cells through the modulation of target gene expression. The life cycle of the root-knot nematode is intricate, and its duration varies depending on environmental conditions such as temperature, moisture, and the presence of a suitable host. It can range from three weeks to several months to complete its life cycle. *Meloidogyne* spp. has the ability to overcome the defense mechanisms of host plants, allowing them to flourish within the roots (Yigezu Wendimu, 2021). On the basis of microbial pathogenesis (damage response framework) some plants species susceptible and competitive with host interaction (Dutta *et al*., 2023). *Solanum lycopersicum L.* (Solanaceae), commonly referred to as tomato is a highly important fruit that is taxonomically classed as a vegetable due to its nutritional characteristics. Tomatoes contain a significant proportion of polyunsaturated and monounsaturated fatty acids, including palmitic and arachidic acid, oleic and linolenic acids, and stearic acids (Kiranmai and Koshta 2023). The presence of elevated salt concentration in the vicinity of the plant's roots resulted in the absorption of sodium ($Na^+$) and chloride ($Cl^-$) ions, leading to an increase in their concentration inside both the roots and shoots of the plants. The accumulation of $Na^+$ ions in living organisms alters the balance of ions and thus impairs the homeostasis of oxidative processes (Kaur *et al.,* 2023). The impact of salinity stress on root knot nematodes infection in plants is contingent upon various parameters, including the specific type of pathogen and the severity of the salt stress applied (El-sheikh and Osman, 2002; Li *et al.,* 2020; Cui *et al.,* 2021; Alsantely *et al.*,2023). The impact of fungal infection under salinity stress varies depending on the severity of salt stress, with either enhancement or suppression observed (Wiese *et al.,* 2004; DiLeo *et al.,* 2010; Dikilitas, and Karakas. 2014; Camacho-luna *et al.,* 2023; Khuna. *et al.,* 2023). The increased vulnerability was ascribed to the buildup of ABA in roots generated by salt, which inhibited the SA-mediated defense mechanism in tomato (Pye *et al.,* 2018; Khuna. *et al.,* 2023).

## MATERIALS AND METHODS

### *Collection of Seed and Root Knot Nematode*

Freshly harvested seeds of tomato varieties (Pusa Ruby and Pusa Early Dwarf) were procured from the Department of Horticulture IARI, New Delhi. *Meloidogyne incognita* (Root-Knot Nematode) infected tomato plants were collected from Dr. Zakaullah Khan, Division of Plant Quarantine, National Bureau of Plant Genetic Resources (NBPGR) New Delhi.

### *Root-knot nematode (Meladogyne incognita) infection in tomato*

### *Soil sterilization and Seed germination*

Sandy loam soil was procured and sterilized in autoclave (121°C at 16 psi) for 60 minutes. After sterilization, soil samples were stored on safe place & used for raising nursery. Seeds were surface sterilized for one minute with 70% ethyl alcohol followed by sterilization with 1% sodium hypochlorite for 2 minutes. Further seeds were subjected to 3 times washing with distilled water.

### *Larval (J2) population in suspension*

The larval suspension was calibrated for the presence of number of juveniles per ml of suspension. One ml suspension sample was drawn and poured in a counting dish. The number of juveniles in each sector were counted and summed to get total number of juveniles per ml suspension and then multiplied with the total quantity of suspension

### *Inoculation of J2 (M. incognita) and salt treatment in tomato cultivars*

1000 juveniles (J2) of *M. incognita* were inoculated into the roots of tomato cultivars (Pusa Ruby and Pusa Early Dwarf) 7 days after seedling transplantation. At 15 days after inoculation (DAI), a 100 mM salt (NaCl) solution was irrigated on alternate days for two weeks. After 60 days of seed sowing, plant samples were harvested and analyzed for larval penetration, number of egg masses, and gall formation.

***J2 inoculation into the root zone of tomato cultivars under saline soil***

When tomato seedlings reached the 3-5 leaf stage, they were inoculated with freshly hatched 1000 J2 nematodes into the root zone of each seedling. A 100 mM salt concentration dissolved in distilled water was used for irrigation in the treated samples, while the untreated samples were inoculated only with J2.

***Acid Fuschin Staining***

Roots were subjected to acid fuchsin staining following the methodology described by Bybd *et al*., (1983) to investigate penetration, gall formation, and the population of egg masses. The root tissues underwent treatment with a clearing solution composed of equal volumes of lactic acid, glycerol, and distilled water. This process lasted 2 to 4 hours at room temperature. Subsequently, roots containing nematodes were rinsed several times with tap water and preserved in acidified glycerol (comprising five drops of 1.0 M HCl in 50 ml of glycerol). Finally, the roots were examined using a Nikon Eclipse 50i light microscope.

***Number of galls and egg mass formation***

The collected root-knot infected tomato plants were carefully washed with fresh water and then surface sterilized with 0.1% Mercuric Chloride for 30 seconds. The roots of the root-knot nematode-infected tomato plants were washed again with running water and stored at 15°C for isolation of root-knot nematode egg masses. Single egg masses were extracted and placed in 5 cm diameter Petri dishes for 72 hours at 25°C to obtain freshly hatched juveniles for further inoculation into tomato plant roots.

***Scanning electron microscopy to analyze giant cell of RKN formation***

Giant cells (GCs) formation in root galls was observed using SEM. Adult nematode-containing micro-sliced root-knot specimens were submerged for two hours at 248C in 2.5% glutar-aldehyde in 0.05 M cacodylate buffer, pH 7.2. At 248C, postfixation with 1% osmium tetroxide was conducted for two hours in the same buffer. Following that, the specimens underwent freeze drying after being submerged in t-butyl alcohol (2-methyl-2-propanol) and dehydrated in a series of ethanol grades. The specimens were examined using a field-emission SEM after being coated with 8 nm of platinum using an ion spatter.

## STATISTICAL ANALYSIS

Statistical analyses were performed with statistical software's Graph Pad Prism (version 7.04) and XLSTAT (version 2023.3.1). All measurements were performed in triplicates. Duncan's multiple range test were performed with XLSTAT, to compare in between the cultivars for resistance for root knot nematode under saline soil by evaluated the parameters included rate of penetration, no. of egg mass and no. of gall formation by *M. incognita* under 100mM saline irrigation.

## RESULTS AND DISCUSSION

***Effect of salt stress on J2 penetration into the root of tomato cultivars***

In the treated check experiment, 100 mM salt stress significantly increased the rate of J2 (*M. incognita*) penetration in both cultivars during 100 mM saline irrigation compared to the untreated control (*M. incognita* alone). The percentage increase in penetration ranged from 41.33% to 54% for Pusa Ruby and from 170.67% to 177% for Pusa Early Dwarf, as shown in Fig. 1(A). Pusa Early Dwarf showed higher susceptibility to root-knot nematodes under saline soil conditions compared to Pusa Ruby. These results were statistically significant at p=0.05 using Duncan's multiple range tests.

***Effect of saline soil on number of gall formation***

To study the effect of saline soil on the rate of gall formation by *M. incognita* in the tomato cultivars Pusa Ruby and Pusa Early Dwarf, an experiment was conducted with an untreated check (1000 J2 of *M. incognita* alone) and a treated check (1000 J2 + 100 mM salt). The percentage increase in gall formation ranged from 7.97% to 15.67% for Pusa Ruby and from 43.67% to 62% for Pusa Early Dwarf, as shown in Fig. 1(B). These results were statistically significant at p=0.05 using Duncan's multiple range tests.

***Effect of saline soil on egg mass formation***

The data reveal that saline soil significantly increased the number of *M. incognita* egg masses in tomato cultivars compared to the untreated control (1000 J2 inoculated alone) and the treated check (1000 J2 +

100 mM salt). The percentage increase in egg masses ranged from 3.67% to 11% for Pusa Ruby and from 24.67% to 48.67% for Pusa Early Dwarf, as depicted in Fig. 2. These results were statistically significant at p=0.05 using Duncan's multiple range tests.

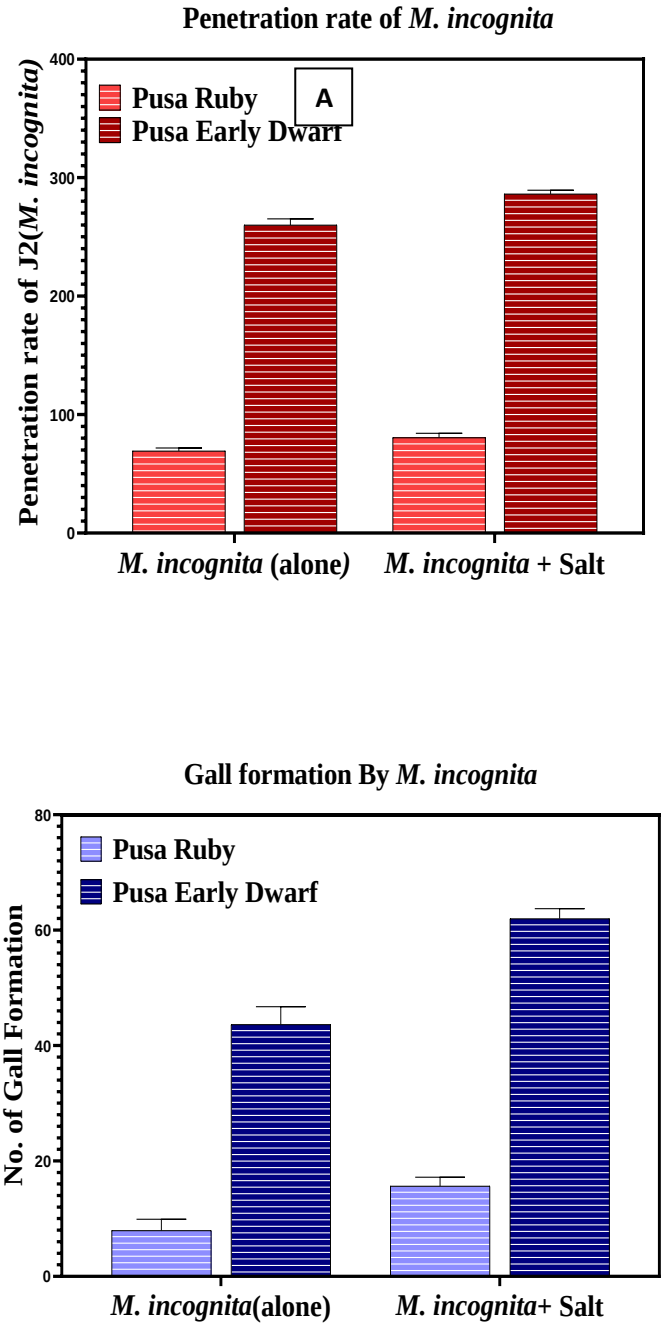


**Figure 1:** Represents untreated check (1000 J2 inoculated alone) and treated check (1000J2 + 100mM) in tomato cultivars (Pusa ruby and Pusa early dwarf) on (A) Impact of saline irrigation (100mM) on the rate of penetration of J2 *(M. incognita)* **(B)** on the number of gall formation by *Meloidogyne incognita* in two tomato cultivars, Pusa Ruby (Resistant) and Pusa Early Dwar (Susceptible).

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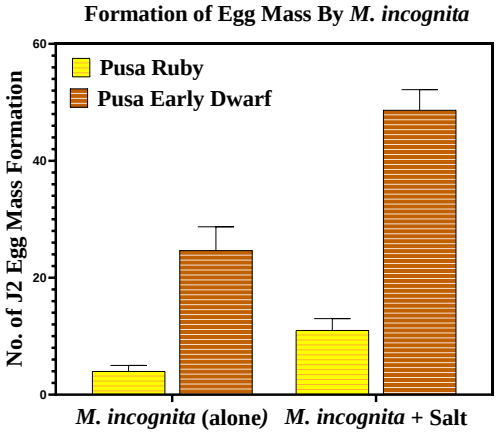


**Figure 2:** Impact of salt irrigation (100mM) on number of egg mass formation by *Meloidogyne incognita* in two tomato cultivars, Pusa Ruby (Resistant) and Pusa Early Dwarf (Susceptible).

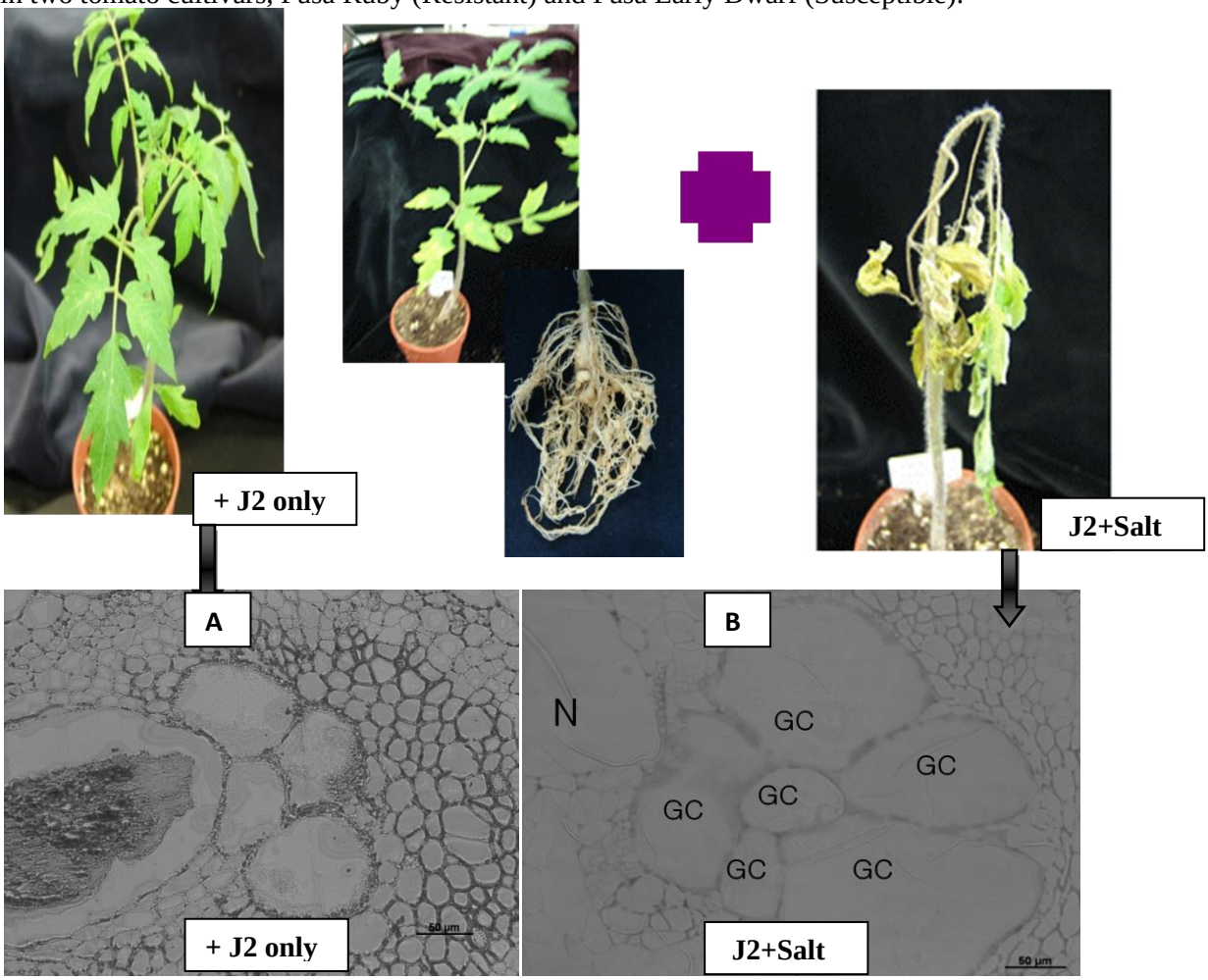


**Figure 3:** Scanning electron microphotographs represents giant cells formations by *M. incognita* into **(A)** GCs formed but not initiated nematode growth at 15DAI in Untreated check (1000J2 inoculated alone) **(B)** Nematode (*M. incognita)* growth started within giant cells i.e., Nematodes feeding site in treated check (1000J2+ 100 mM salt) at 15 DAI of J2.

***Effect of saline soil on giant cell formation***

Upon examination, it was observed that roots irrigated with saline water showed enormous size of giant cell in compared to untreated check (1000J2 inoculated alone) to treated check (1000J2 +100mM salt) . Giant cells (GCs), which are specialised cells that feed on nematodes, are formed when the second-stage juvenile (J2) nematode settles down to begin feeding. In the absence of cell division, they experience synchronous nuclear divisions, resulting in the formation of cells with multiple nuclei. Root-knot nematodes extract nutrients from the giant cells located in the cortex, endodermis, pericycle, and vascular tissues of the roots they infect. They accomplish this by establishing permanent feeding sites. Additionally, the deformities and obstructions in the vascular tissues of the feeding sites impede the movement of water and nutrients, thereby further retarding plant growth and reducing crop yield. Treated check i.e., saline soil increased the damage of plant live by wilting in *M. incognita* infected roots of tomato cultivars Pusa ruby and Pusa early dwarf as shown in **Fig – 3 (A)** and **(B).**

## DISCUSSION

The findings of the current study underscore that saline soil exacerbates the reproductive capabilities of *M. incognita*, accelerating the formation of root galls and egg masses in tomato cultivars (Pusa Ruby and Pusa Early Dwarf). The results above highlight that the Pusa Ruby cultivar shows resistance to RKN in saline irrigated areas compared to Pusa Early Dwarf. According to the findings, the combination of biotic stress (RKN) and abiotic stress (100mM salt) is more detrimental to agricultural yield than either stress alone.

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