Tolerance of tomato varieties to natural infestation by *Meloidogyne* nematodes under controlled conditions in Mocuba District, Mozambique

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Abstract

This study aimed to evaluate the tolerance of five tomato varieties naturally infested with *Meloidogyne* spp. nematodes under controlled conditions in Mocuba District, Mozambique. The experiment was conducted in two phases: the first in an experimental field and the second at the Laboratory of Microbiology, Phytopathology, and Agricultural Entomology of the Faculty of Agronomic and Forestry Engineering (FEAF), Mocuba, where laboratory analyses were performed. The experiment followed a completely randomized design, consisting of five varieties with four replicates per treatment. The following parameters were evaluated: nematode genus identification and the most prevalent developmental stage, galling index, number of symptomatic leaves, stem diameter, plant height, and variety yield. Statistical analyses were performed using STATISTIX 10 software. Residual normality was assessed using the Shapiro–Wilk test, homogeneity of variances was evaluated using Levene's test, and means were compared using Tukey's test at a 5 % significance level. A higher occurrence of *Meloidogyne* at the second-stage juvenile (J2), while the genus *Tylenchus* occurred at a lower frequency. The Cherry variety was considered immune to nematode infestation, whereas the Rio Grande variety exhibited a low infection rate, characterized by few root galls and foliar symptoms, while maintaining high yield under the soil conditions of the study area. Growth characteristics, including plant height and stem diameter, did not differ significantly among varieties as a function of the observed level of infection.

Keywords: *Solanum lycopersicum* L. Root-knot nematode. Monitoring. Infestation.

Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and is one of the most cultivated and economically important vegetables worldwide, being one the most diversified crops, with approximately 10,000 known varieties (FAO, 2022). Global tomato production has shown steady growth, exceeding 188 million tons harvested from approximately 5.1 million hectares, with an average yield of 36,809.80 kg ha^{-1}, making tomato one of the vegetable crops of greatest economic and social value in global agriculture (FAOSTAT, 2024). In addition to its economic value, tomato is recognized for its nutritional benefits, being rich in vitamins, including vitamin C, and bioactive compounds such as the antioxidant lycopene, which has been associated with a reduced risk of chronic diseases, including several types of cancer and neurological disorders (GIOVANNUCCI, 1999).

Tomato is also the second most widely cultivated vegetable crop worldwide, surpassed only by potato, and is fundamental in supplying both the fresh market and the processing industry, including the production of sauces, pastes, and pulp (FAOSTAT, 2023). This crop is highly adaptable to different climatic conditions and production systems, including open-field and protected cultivation, contributing to its expressive geographical distribution and socioeconomic relevance (GIBON *et al.*, 2025). However, tomato production faces significant challenges associated with pests and diseases, which can compromise yield and fruit quality. These challenges reinforce the need for

integrated management and the development of varieties carrying the *Mi-1* resistance gene as key strategies for crop sustainability (SACCO *et al.*, 2015; WILLIAMSON, ROBERTS, 2009 SHANMUGAM *et al.*, 2024).

Among emerging biotic threats, root-knot nematodes (RKN) are among the main challenges facing modern agriculture, due to their widespread geographic distribution and the limited effectiveness of currently available control strategies. These phytonematodes belong to the genus *Meloidogyne*, which comprises more than 90 described species, some of which include multiple physiological races. Among them, *Meloidogyne incognita*, *M. javanica*, *M. hapla*, and *M. arenaria* are the most economically important species because of their high adaptability and the severe damage they cause to various agricultural crops globally (EL-SAPPAH *et al.*, 2019). They are considered the most important among phytonematodes due to their high degree of polyphagy, being capable of infecting thousands of plant species and parasitizing several agricultural crops, especially tomato (MOENS *et al.*, 2009).

These nematodes are biotrophic parasites that can infect more than 2,000 plant species. *Meloidogyne* spp. was first reported on cassava (*Manihot esculenta*) by Neal (1889). In most crops, nematode infection compromises plant health and growth, as these parasites invade the root system and induce the formation of giant feeding cells, thereby reducing water and nutrient uptake. As a result, infected plants may manifest symptoms such as wilting and stunting, as well as increased susceptibility to other pathogens, culminating in significant yield losses, with symptoms generally being more severe in tropical species than in species adapted to temperate or cold regions (MOENS *et al.*, 2009; EL-SAPPAH *et al.*, 2019).

Among the available management strategies, the use of genetically resistant varieties is an efficient, sustainable, and environmentally friendly alternative for controlling RKN in susceptible crops such as tomato (PINHEIRO *et al.*, 2014).

Natural resistance genes, particularly *Mi-1*, have been instrumental in controlling RKN (*Meloidogyne* spp.) in tomato and remain one of the main tools for integrated management strategies for this phytopathogen. Resistance to RKN in tomato was first observed by Bailey (1941) in the wild species *Lycopersicon peruvianum* L. Mill. P.I. 128657. Subsequently, using the embryo rescue technique, Smith (1944) transferred this characteristic to the cultivated species *Lycopersicon esculentum* L. Later studies demonstrated that this resistance is controlled by a single dominant gene, designated *Meloidogyne incognita*-1 (*Mi-1*), located on chromosome 6 (Ho *et al.*, 1992). The *Mi-1* gene confers resistance to *M. incognita*, *M. arenaria*, and *M. javanica*, but not to *M. hapla* (CAP *et al.*, 1993), and remains the only commercially available source of resistance. *Mi*-mediated resistance is expressed early, as soon as two weeks after germination, and can be detected in both leaves and roots (MARTINEZ *et al.*, 2001).

Given the significant yield losses caused by RKN, especially in Mocuba District, Mozambique, this study aimed to evaluate the tolerance of five tomato varieties naturally infested with *Meloidogyne* spp., under controlled conditions in this region. Additionally, the study sought to identify viable management alternatives with the use of tolerant cultivars, possibly carrying the *Mi* resistance gene, as a strategy to mitigate the damage caused by this pathogen. As tomato is the country's main vegetable crop and an important source of household income, the varieties evaluated were selected based on their widespread use by local producers, their high cultivation frequency in Mozambique, and the lack of studies analyzing their response to RKN, thereby justifying this study.

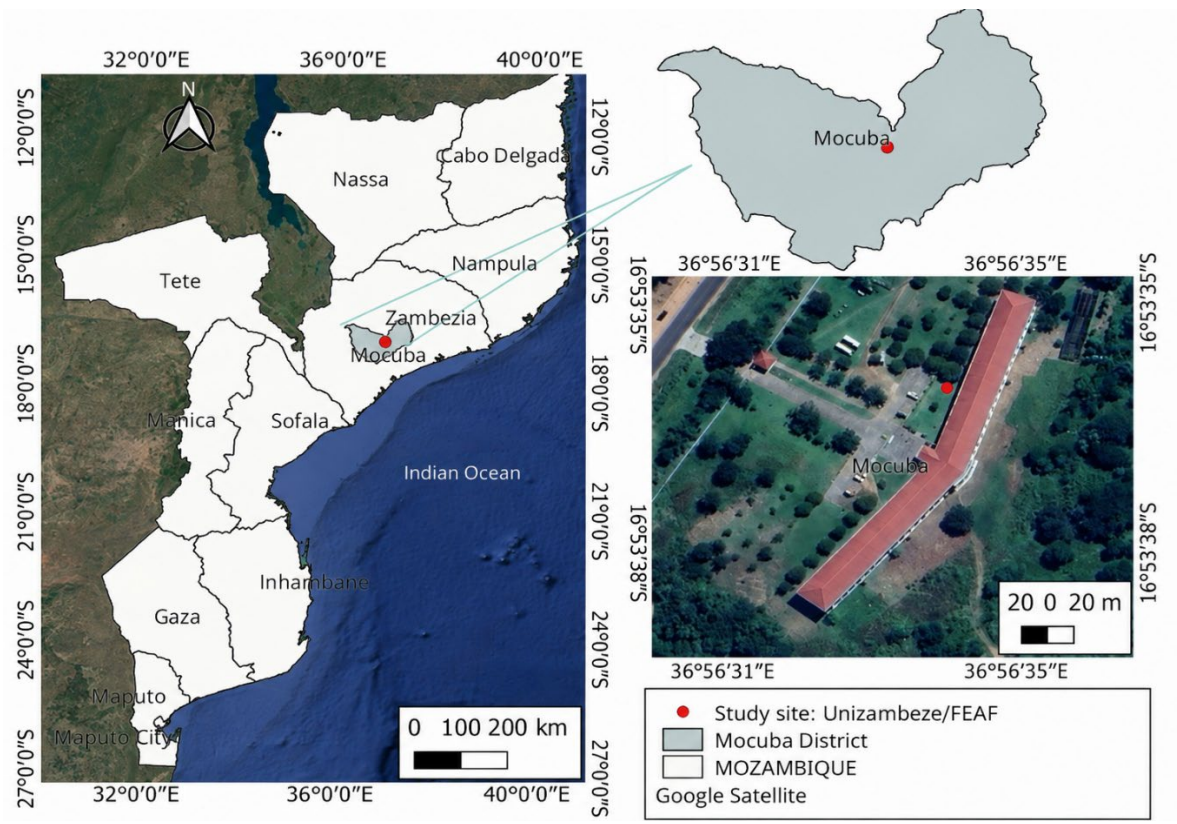
Material and methods

The study was conducted at the Laboratory of Microbiology, Phytopathology, and Agricultural Entomology of the Faculty of Agronomic and Forestry Engineering, Universidade Zambeze, located in Mocuba District, Zambézia State, central Mozambique, with geographic coordinates 16°53'36" S, 36°56'34" E (Figure 1). Mocuba District is located in the central part of Zambézia Province and covers an area of 8,802 km^2, representing approximately 8 % of the provincial territory. It borders Lugela and Errego districts to the north, Maganja da Costa, Namacurra, and Morrumbala districts to the east, Morrumbala District to the south, and Milange District to the west (MAE, 2014). According to the Thornthwaite climate classification, the climate of Mocuba District is subtropical and influenced by the Intertropical Convergence Zone. The average annual rainfall is 1,175 mm, and the average monthly temperature varies from 20 to 27 °C, with maximum temperatures between 27 and 35 °C and minimum temperatures between 15 and 22 °C. Monthly thermal amplitude ranges from 10 to 16 °C (PEDDM, 2014).

The topography of the district has a stepped relief, rising from the plains to the plateaus and from the plateaus to the mountains. Predominant soils include moderately deep red clay soils and, in the plains, black clay soils occurring in broad valleys, often under hydromorphic conditions (MAE, 2014). The identification of nematodes in the soil and the determination of the level of infestation were performed using the sieving method.

Initially, soil samples were collected from an experimental field with a history of occurrence of nematodes of the genera *Meloidogyne*, *Heterodera*, *Pratylenchus*, and others, as well

Figure 1. Geographic location map of the study area.



Source: authors (2025).

as free-living, non-phytopathogenic nematodes. Sampling was conducted at different locations in the area, adopting a systematic X-shaped sampling scheme with ten sampling points. At each point, soil samples were collected using a hoe to a depth of 30 cm. The individual samples were placed in plastic bags and thoroughly homogenized, forming a composite sample. Subsequently, the material was sent to the laboratory to analyze the presence of nematodes.

The extraction and observation of soil nematodes followed the sieving technique described by Jenkins (1964). For this purpose, the composite sample was carefully homogenized, and 200 g of soil were weighed and transferred to a 1,000 mL beaker, using a proportion of 200 g of soil to 1 L of water. After the soil aggregates were broken up with the aid of water, the suspension was kept at rest for 15 seconds and then poured successively through a 200-mesh sieve placed over a 500-mesh sieve. The washing and sieving procedure was repeated five times, until a clear supernatant was obtained, using running water.

After sieving, the material retained on the 500-mesh sieve was collected with the aid of a wash bottle and transferred to 9-cm diameter Petri dishes for observation under a stereomicroscope. Subsequently, slides were prepared for observation under a light microscope to identify nematodes based on morphological characteristics. After confirming the presence of nematodes in the soil sample, a representative amount (5 kg) of infested soil was collected and placed in 10-L buckets, and later used to fill bags for transplanting seedlings and establishing the experiment.

Seedlings were transplanted into bags containing naturally infested soil 30 days after sowing, when plants had five to six fully developed leaves. Seedlings were obtained from a nursery and selected based on vigor and health, discarding those with signs of infestation. Transplanting was conducted in the afternoon to promote plant recovery from transplant stress. The procedure was performed using bags with a capacity of 25 kg of soil to enable better root system development. Each bag received three plants, according to the treatment (variety) distribution. Irrigation was managed according to the water requirements of the crop, ensuring adequate conditions for plant growth and development.

Evaluations were conducted beginning at 30 days after transplantation (DAT), when symptoms of leaf wilting and intense gall formation on the roots were observed. Three evaluations were conducted at 30, 60, and 90 DAT. The plants were carefully removed from the bags and evaluated individually for phytopathological variables (galling index and number of symptomatic leaves) and growth variables (plant height and stem diameter). For the quantification of galls and egg masses, the roots were collected monthly, washed under running water, stained with natural dye, and evaluated in the laboratory using a stereomicroscope.

The galling index of the tomato plants was evaluated after careful removal of the plants from the bags, with four plants per variety being analyzed. The number of galls per plant was quantified according to the methodology described by Hussey and Barker (1973), as modified by Bonetti and Ferraz (1981) (Table 1). Root damage (galls) was counted for each treatment using forceps. To facilitate counting, the roots were previously stained with natural dye (Jolly Jus) and analyzed using a bench magnifier. The galling index (GI) was determined according to the rating scale established by Dos Santos (2012).

Yield evaluation considered exclusively fruits classified as marketable, according to the criteria established by the Horticultural Quality Center of CEAGESP, under the Brazilian Program

for the Modernization of Horticulture. Fruits free of serious defects, such as rot, wilting, sunscald, or unhealed damage, were considered marketable. The fruits were harvested per unit area and separated according to the treatments evaluated. Subsequently, they were washed and weighed using a precision scale, and the values were expressed in kilograms (kg). Yield was then calculated and expressed in tons per hectare (t ha^{-1}), according to the methodology proposed by Lima (2013), using the following equation:

$$Rend = \frac{M_{kg}}{Au} x10.000 \quad (1)$$

In which: REND = Yield (kg ha^{-1}); M_{kg} = Total mass of marketable fruits (kg); A_u = floor area.

Results and discussion

Based on the morphological characteristics and identification keys for nematode genera, the genera identified in soil samples collected from the experimental field were *Meloidogyne*, *Tylenchulus*, and *Pratylenchus*. A higher occurrence of *Meloidogyne* was observed, with a predominance of second-stage juveniles (J2), while the genus *Tylenchulus* occurred at a lower frequency.

The highest occurrence of *Meloidogyne* may be explained by soil characteristics of the experimental area, with Moens *et al.* (2009) reporting that soil texture directly influences nematode movement because of its porosity and water-retention capacity. Furthermore, the history of land use, characterized by the continuous cultivation of vegetables, predominantly tomato, as well as other species of the family Solanaceae, may have favored the multiplication and maintenance of high populations of this nematode genus.

Nematodes of the genus *Meloidogyne* occur in different soil types, with a higher incidence in sandy and sandy loam soils due to their greater porosity and aeration, which favor nematode movement and root infection. In clayey and hydromorphic soils, their occurrence tends to be lower, although the presence of the pathogen is not completely excluded (MOENS *et al*., 2009; PERRY, MOENS, 2011). According to Pinheiro *et al.* (2014), while several nematode genera can infect the roots of different vegetables, including tomato, species of the genus *Meloidogyne* are the most frequent and economically important, being responsible for the greatest productivity losses in tomato crops worldwide due to their wide distribution and high adaptability.

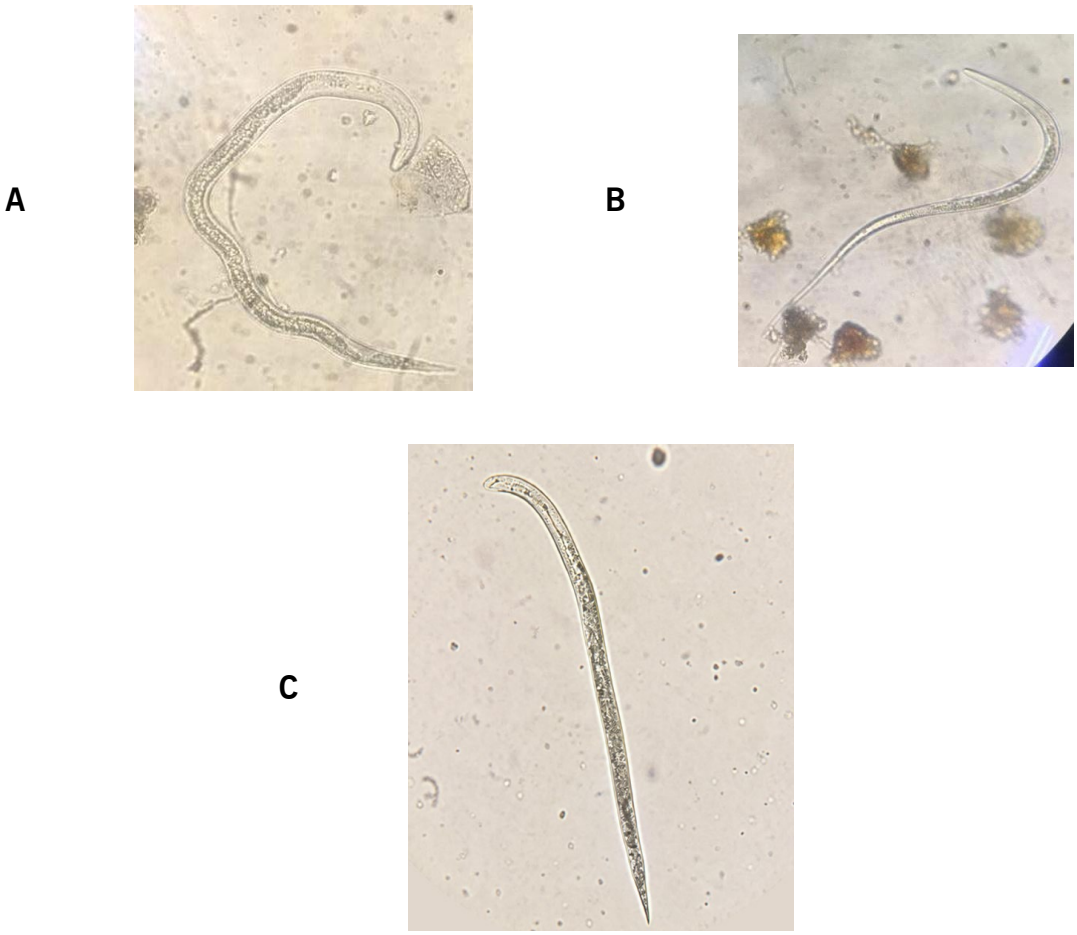
Regarding the galling index, the results presented in Table 2 show that there was no significant difference among varieties at 30 days after transplanting. However, at 60 and 90 days after transplanting, significant differences were observed among varieties when grown in soil

Table 1. Rating scale for assessing plant susceptibility to root-knot nematodes (*Meloidogyne* sp.) based on the number of galls and egg masses.

Score	Number of galls	Genotype classification
0	0	Immune (IM)
1	1–2	Highly resistant (HR)
2	3–10	Resistant (R)
3	11–30	Moderately resistant (MOR)
4	31–100	Susceptible (S)
5	>100	Highly susceptible (HS)

Source: Dos Santos (2012).

Figure 2. Identification of nematode genera. Male of *Pratylenchus* (A); *Tylenchulus* (B); *Meloidogyne* (C).



Source: authors (2024).

naturally infested with nematodes of the genus *Meloidogyne* (Table 2).

The absence of galls in the varieties evaluated at 30 days after transplanting may be attributed to the initial stage of root system colonization, during which *Meloidogyne* juveniles are still in the process of penetration, intracellular migration, and establishment of feeding sites, with the consequent induction of giant cells. At this initial phase of parasitism, the reproductive activity of the nematode is reduced, which explains the absence or low incidence of visible galls. This finding is supported by Cotter *et al.* (2003), who reported that nematodes of the genus *Meloidogyne*, after penetrating the roots, migrate through plant tissues and establish their feeding sites in giant cells, resulting in a complex nematode–plant interaction. This process induces tissue hypertrophy and hyperplasia, culminating in the formation of root galls, which requires a certain period for their complete development and visible manifestation (PERRY, MOENS, 2011).

The evaluation of the resistance of tomato varieties to RKN (*Meloidogyne* spp.) revealed significant differences among the genotypes analyzed. The Cherry variety did not exhibit gall formation throughout the evaluation period, indicating immunity to the pathogen, while the Rodade and Rio Grande varieties showed lower galling rates in the final evaluations, indicating a resistant behavior under naturally infested field conditions.

Table 2. Average galling index of tomato varieties evaluated at different times after transplanting.

Variety	Gall Index		
	30 DAT	60 DAT	90 DAT
Rodade	0.0	40.7 C	46.0 BC
Roma	0.0	68.0 A	110.7 A
Rio Grande	0.0	72.5 A	41.2 BC
Cal J	0.0	59.5 B	63.0 AB
Cherry	0.0	0.0 D	0.0 C
Average	0.0	48.1	52.2
CV (%)	---	8.9	5.6

Means followed by the same letter in each column do not differ significantly according to Tukey's test at the 5 % probability level. DAT: Days after transplanting.

Source: authors (2025).

These results may be associated with the presence of the *Mi* resistance gene, which limits the reproduction of *Meloidogyne* spp. with the activation of plant defense mechanisms. According to Assunção *et al*. (2010), plants carrying the *Mi* gene express hypersensitive reaction, promoting histological alterations and localized cell death in the roots, preventing the establishment of the second-stage juveniles of the nematode. Thus, the use of resistant varieties constitutes an efficient and sustainable strategy for the management of RKN in tomato cultivation.

Regarding the number of symptomatic leaves, no significant differences were observed during the first two evaluations. However, at 90 days after transplanting, typical symptoms of RKN infection, such as chlorosis and leaf wilting, were observed (Table 3). The Roma variety showed the highest incidence of symptoms, while Rio Grande showed a low incidence, and the Cherry variety did not manifest leaf symptoms. These results are consistent with Perry and Moens (2011), who stated that nematode infection compromises the root system of plants, reducing the absorption and transport of water and nutrients, which results in reduced photosynthetic activity and impaired development of the aerial part.

Table 3. Average number of symptomatic leaves in tomato varieties at different times after transplanting.

Variety	Symptomatic leaves		
	30 DAT	60 DAT	90 DAT
Rodade	0.0	1.0 A	0.5 AB
Roma	0.0	1.2 A	1.5 A
Rio Grande	0.0	0.7 A	0.2 B
Cal J	0.0	0.7 A	0.5 AB
Cherry	0.0	0.0 A	0.0 B
Average	0.0	0.7	0.5
CV (%)	---	10.2	9.9

Means followed by the same letter in each column do not differ significantly according to Tukey's test at the 5 % probability level. DAT: Days after transplanting.

Source: authors (2025).

The greater incidence of foliar symptoms observed in the Roma variety may be attributed to the fact that this cultivar had the highest galling index, indicating a higher level of nematode infestation (Table 2). The high level of root infection compromises the absorption and transport of water and nutrients, resulting in aboveground symptoms such as leaf yellowing and plant wilting, even in properly moistened soils. Conversely, the lower incidence of foliar symptoms recorded in the Rio Grande variety is related to the lower level of root infection, which contributed to a smaller number of symptomatic leaves and better physiological performance of the plants.

These results are supported by Carneiro *et al.* (2003), who reported that plants attacked by RKN exhibit root system disorganization, hindering the absorption and transport of water and nutrients. Consequently, symptoms develop in the aerial part, such as yellowing, wilting during the hottest hours of the day and, ultimately, reduced plant growth.

Regarding plant height, significant differences were observed from 60 days after transplanting onward. At 90 days, greater heights were recorded for the Cherry, Rio Grande, and Rodade varieties in relation to the others (Table 4).

The greater plant height observed in the aforementioned varieties may be explained by the low level of infestation (galling index), which provided adequate root development and greater nutrient uptake, reflecting on the growth of the aerial part. According to Davis *et al*. (2000), RKN directly influence plant development by causing deformations, poor root development, and reduced water and nutrient uptake, resulting in lower growth of the aboveground parts of the plant.

Regarding the yield of the varieties, a significant difference was observed among them. The highest yield was recorded in the Rio Grande variety (5.55 t ha^{-1}), followed by Rodade (4.62 t ha^{-1}), with no statistical difference in relation to Roma and Cal J varieties. The lowest yield was recorded for the Cherry variety (3.775 t ha^{-1}) (Table 5). The low yield of the Cherry variety is related to its genetic characteristics, which result in fruits with lower mass, reflecting lower total yield. According to Thakur *et al*. (2024), this variety produces

Table 4. Average plant height of tomato varieties at different times after transplanting.

Variety	Plant height		
	30 DAT	60 DAT	90 DAT
Rodade	16.2	23.7 B	51.2 BC
Roma	14.5	23.7 B	43.2 C
Rio Grande	15.5	30.0 A	52.0 BC
Cal J	16.2	26.2 B	41.5 C
Cherry	17.5	19.2 C	76.5 A
Average	16.0	24.6	52.9
CV (%)	15.7	27.2	5.4

Means followed by the same letter in each column do not differ significantly according to Tukey's test at the 5 % probability level. DAT: Days after transplanting.

Source: authors (2025).

Table 5. Comparison of mean yield of tomato varieties.

Variety	Yield (t ha-1)
Rodade	4.62 B
Roma	4.5 B
Rio Grande	5.55 A
Cal J	4.58 B
Cherry	3.78 C
CV (%)	5.28

Means followed by the same letter in each column do not differ significantly according to Tukey's test at the 5 % probability level.

Source: authors (2025).

fruits with an average weight ranging from approximately 3.8 g to 64 g, depending on the genotype, which limits yield per hectare.

The higher yield observed in the Rio Grande variety may be explained by the low galling index at 90 days after transplanting, with a value of 41.2, associated with lower incidence of foliar symptoms (0.2), resulting in better productive performance. These results are consistent with Perry *et al.* (2013), who reported that plants attacked by RKN exhibit greater root deformation, which compromises the absorption of water and nutrients, resulting in lower growth of the aerial part and reduced yield.

Conclusion

A predominance of the genus *Meloidogyne* was observed in the experimental field, with second-stage juveniles being the most frequent, while the genus *Tylenchulus* showed lower occurrence. The Cherry variety was immune, with no gall symptoms, while the Rio Grande variety had low infestation and better productive performance. Plant growth was not significantly affected by nematode infection. The importance of using resistant varieties as an effective strategy for the management of RKN in tomato is emphasized, with *Meloidogyne* identified as the most common genus.

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