Root Knot Nematode Presence and Its Integrated Management in Pomegranate Orchards Located in Indian Arid Areas

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Abstract: Nematodes are a serious problem across pomegranate-growing areas worldwide, but the severity is higher in light sandy soils of arid regions. The present study was carried out to explore the integrated approaches for the control of nematodes in pomegranate. Three different experiments were carried out during 2017–2020 to (a) delineate nematode abundance in major pomegranate areas, (b) screen pomegranate genotypes against nematode, and (c) assess the efficacy of integrated management for the control of root knot nematode in pomegranate. The survey results revealed that none of the pomegranate orchards were found to be free from nematode infestation. Moreover, the majority of the orchards (78%) showed moderate incidence (10.1 to 40%) of infestation. A significant yield reduction (40.2%) and a decrease in fruit size was observed in nematode-affected trees. Pattern of cuticular markings in the perineal area of the mature female confirmed the occurrence of Meloidogyne incognita only in all the surveyed orchard of pomegranate. All the evaluated genotypes and varieties were found susceptible to root knot nematodes, but the severity of the attack varied among them. Hence, more detailed screening is needed on a larger population. Nematode population (number of galls g⁻¹ root) can be minimized significantly with the combined applications of Carbofuran at 20 g + Fluensulfone at 20 g per plant or Neemcake 500 g + Paecilomyces lilacinus at 25 mL + Carbofuran at 20 g + Fluensulfone at 20 g per plant in April and August.

Keywords: root knot nematode; Punica granatum; bioagents; nematicides; neemcake

1. Introduction

Pomegranate (Punica granatum L.) is an underutilized crop that can be cultivated in several climatic conditions (Mediterranean, subtropical, and tropical), and this indicates its wide adaptability. Low water requirement, good response to modern horticultural practices, high economic returns, and great global demand have made this species one of the most popular commercial cash crops at a global scale [1]. Global production of pomegranates is estimated around 6.3 million metric tons (MT) from an area of 556 thousand hectares [2]. At global level, India is a leading producer (3186 thousand MT) followed by China (1600 thousand MT), Iran (1100 thousand MT), Turkey (220 thousand MT), the USA (210 thousand MT), Afghanistan (150 thousand MT), and Spain (53.18 thousand MT) [2]. The international export market is estimated to be around 362.6 thousand MT, and India has recorded a constant increase in foreign earning by exporting 80,547.74 MT worth USD 92.46 million in 2019–2020 [3]. Recent export trends depict a high amenability in supply–demand of Indian pomegranate in international market with increased prices. This ambiance provides opportunities to stretch the growing area of pomegranate from its traditional belt, viz., Central and Southern India to arid and semi-arid northwestern Indian
Pomegranate is attacked by several non-insect pests. Among these, root knot nematode has emerged as a major threat to sustainable production of pomegranate in these regions [5]. Although the infestation of nematodes is a serious problem throughout its growing areas worldwide, the severity of their attack is higher in arid climates and light sandy soils [6]. Root knot nematodes can be spread by water or by soil or farm equipment or through infested planting materials [7,8]. Substrate mixture used during seedling preparation may also harbor these nematodes [9]. Their feeding leads to impaired root functions such as nutrient and water uptake due to gall formation, and consequently plants become progressively sick (yellow aspect, tip drying, and stunted growth) and may be exposed to secondary infections of fungi, bacteria, and nutritional deficiencies that can even cause plant death [8]. These nematodes are responsible for 30 to 40% yield losses with poor quality fruits in the current season [6,10]. The association of different species and population density of root knot nematode has not been well documented in affected pomegranate orchards thusfar; however, Holland et al. [9] reported that *Meloidogyne incognita* and *M. javanica* are the main species attacking this tree crop species. Darekar et al. [11] reported 10 different species of plant parasitic nematodes associated with pomegranate orchards in the Maharashtra state. The root knot nematode *M. incognita* is one of the main species causing considerable yield losses in pomegranate [10,11]. Application of nematicides has remained the most common short-term management strategy against root knot nematode [12]. Several horticultural practices such as intercropping of marigold, inoculation of various bio-formulations with neem or castor cakes, etc., have been recommended, but their effectiveness in controlling nematodes varies depending on the growing conditions [13,14]. Presently, there is barely any information on the nematode tolerant/resistant genotypes in pomegranate, which can be used as rootstocks or as a source of resistance genes to be used in future breeding programs [15]. In other fruit crops such as grape, citrus, and stone fruits, there are several successful examples of steady solutions to different abiotic and biotic problems including nematodes. Nematode abundance, knowledge of nematode species, use of resistant/tolerant genotypes, organic amendments, and use of biocontrol agents can be important components of an integrated nematode management approach. In light of the above, the present investigation included three experiments that were carried out during 2017–2020 at the ICAR-Central Arid Zone Research Institute, Jodhpur, to delineate nematode abundance in pomegranate orchards, screen tolerant genotypes, and study integrated management strategies of nematodes in field conditions.

2. Materials and Methods

2.1. Collection, Isolation, and Identification of Nematode

A field survey was conducted during 2017–2018 in two major pomegranate-growing districts of Rajasthan, India, i.e., Barmer and Jodhpur, in order to ascertain the nematode abundance and to identify their dominant species. In each district, two locations comprising four villages were considered for sample collection. The selection of these fields was based on visual symptoms of nematode infestation, viz., plants with pale green or yellowish leaves, wilting symptoms, reduced growth, and drying twigs (Figure 1).

The number of infected plants in each orchard was recorded to calculate the percentage of nematode incidence. A total of 25 orchards with an area of at least one hectare each (715 trees/ha) with these characteristics and a minimum age of seven years were selected randomly in each location. The percentage of nematode incidence in each orchard was calculated with the following equation:

\[
\text{Nematode Incidence (\%) = No. of plants infected/Total No. of plants} \times 100
\]
were grown in 10 earthen pots and were maintained for three months under protected conditions for mass multiplication of nematode. Simultaneously, around 500 seedlings of 27 genotypes, namely, IC-318790, IC-318723, IC-318728, IC-318754, Wonderful, Joyti, Tabesta, Yercawd-1, Jodhpur collection, Kabuli yellow, Co-white, Ruby, KRS, Kasturi, Amali dana, Bhagwa, CAZRI-Sel., Phule Arakta, P-26, G-137, P-23, Jodhpur Red, Ganesh, Mridula, Dholka, Basein Seedless, and Jalore Seedless, were raised in the nursery. Four 6-month-old saplings of each genotype were shifted in small earthen pots (1 kg capacity) containing soil mixed with well-rotted farmyard manure in a 3:1 proportion to root knot nematode. Sufficient quantity of infected soil and root samples were collected from five randomly selected plants in each orchard and mixed thoroughly to prepare composite samples. One hundred composite samples were brought to the laboratory for assessing the nematode abundance and identification of nematode species for further studies. The egg masses were isolated from soil and root samples and kept in fresh water for hatching. These eggs were obtained from a pure culture established from single egg mass for identification according to the characteristics of the perineal pattern of matured females as previously described [16,17].

2.2. Screening of Genotypes

An experiment was conducted under protected conditions (fan and pad greenhouse with air temperature and relative humidity maintained in the range of 25–32 °C and 40–65%, respectively) during 2018–2019 and 2019–2020 to study the response of several genotypes and varieties of pomegranate to root knot nematode. Sufficient quantity of infected soil from a previously surveyed nematode-affected pomegranate orchard was collected for mass culturing of root knot nematode in earthen pots. The nematode was sub-cultured for mass multiplication by removing egg masses from the mother culture plants. Egg masses were collected and kept in fresh water for hatching. These eggs were obtained from a pure culture established from single egg masses of previously identified species according to the characteristics of the perineal pattern [16,17] and reared under protected conditions. The sterilized soil mixture (sand/farmyard manure in a 3:1 proportion) was used to fill disinfected earthen pots (with a diameter of 30 cm). Three-month-old pomegranate saplings were grown in 10 earthen pots and were maintained for three months under protected conditions for mass multiplication of nematode. Simultaneously, around 500 seedlings of 27 genotypes, namely, IC-318790, IC-318723, IC-318728, IC-318754, Wonderful, Joyti, Tabesta, Yercawd-1, Jodhpur collection, Kabuli yellow, Co-white, Ruby, KRS, Kasturi, Amali dana, Bhagwa, CAZRI-Sel., Phule Arakta, P-26, G-137, P-23, Jodhpur Red, Ganesh, Mridula, Dholka, Basein Seedless, and Jalore Seedless, were raised in the nursery. Four 6-month-old saplings of each genotype were shifted in small earthen pots (1 kg capacity) containing soil mixed with well-rotted farmyard manure (FYM) in a 3:1 proportion. Once seedlings were well established, freshly hatched second-stage nematode juveniles were inoculated to each pot (3000 J2/pots). This experiment was carried out in a completely randomized design under protected conditions. Observations on height and girth of seedlings, as well as the number of root knot galls g−1 root, were recorded after completing three growth cycles of nematode, i.e., nine months after inoculation of seedlings.

Figure 1. Selection of orchards according to visual symptoms of plants: (A) showing symptoms of nematode infestation; (B) healthy plant without nematode infestation.

On the basis of the Nematode Incidence, we categorized the severity of the infestation of surveyed orchards as severe (>40%), moderately severe (10–40%), and mild (0–10%).

Soil and root samples were collected from five randomly selected plants in each orchard and mixed thoroughly to prepare composite samples. One hundred composite samples were brought to the laboratory for assessing the nematode abundance and identification of nematode species for further studies. The egg masses were isolated from soil and root samples and kept in fresh water for hatching. These eggs were obtained from a pure culture established from single egg mass for identification according to the characteristics of the perineal pattern of matured females as previously described [16,17].
2.3. Integrated Management of Nematode

A field experiment was also conducted during 2019–2020 and 2020–2021 in a naturally root knot nematode-infested pomegranate orchard to ascertain the effect of different integrated approaches for the control of nematodes. The experiment was carried out in a 7-year-old pomegranate orchard (3 ha; 715 trees/ha) of own-rooted trees of the Bhagwa variety. This cultivar was selected because it is the most cultivated pomegranate variety in the area where this experiment was carried out (more than 95% of the total area cultivated with this tree crop). Growth, yield, and the number of root knot galls g⁻¹ root were recorded before applying the treatment. The granular nematicide (carbofuran and Fluensulfone), bioagents (Paecilomyces lilacinus and Trichoderma harzianum), and organic amendment (neem cake) were assessed as soil applications for the control of root knot nematode in pomegranate. All the treatments were applied twice in April and August, each year. The experiment was conducted in a randomized block design with three replications and 13 treatments (Table 1) on a total of 78 homogenous trees (6 trees per treatment).

Table 1. Description of treatments applied for integrated management of root knot nematode.

| Treatment ID | Treatment Description |
|--------------|-----------------------|
| T1           | Carbofuran at 40 g/plant |
| T2           | Fluensulfone at 40 g/plant |
| T3           | Carbofuran at 40 g + Fluensulfone at 40 g |
| T4           | Carbofuran at 20 g + Fluensulfone at 20 g |
| T5           | Neemcake at 500 g + P. lilacinus at 25 mL + Carbofuran at 40 g |
| T6           | Neemcake at 500 g + T. harzianum at 50 g + Carbofuran at 40 g |
| T7           | Neemcake at 500 g + P. lilacinus at 25 mL + Fluensulfone at 40 g |
| T8           | Neemcake at 500 g + T. harzianum at 50 g + Fluensulfone at 40 g |
| T9           | Neemcake at 500 g + P. lilacinus at 25 mL + Carbofuran at 40 g + Fluensulfone at 40 g |
| T10          | Neemcake at 500 g + T. harzianum at 50 g + Carbofuran at 40 g + Fluensulfone at 40 g |
| T11          | Neemcake at 500 g + P. lilacinus at 25 mL + Carbofuran at 20 g + Fluensulfone at 20 g |
| T12          | Neemcake at 500 g + T. harzianum at 50 g + Carbofuran at 20 g + Fluensulfone at 20 g |
| T13          | Control |

2.4. Observations Recorded

2.4.1. Growth, Yield, and Quality Parameters

Plant growth in terms of plant height and canopy spread (measured in east–west and north–south directions) were recorded in November (middle of fruiting season). On each picking, fruit yield was recorded, and the average cumulative yield per tree was calculated. On each picking, 20 randomly selected fruits from all the tree canopy sides were sampled and weighed with a top pan digital balance, and their average weight was calculated. Fruit size (fruit length and width) was measured on the same fruits with a digital vernier calliper. Fruit juice was extracted using a mechanical juicer and grinder followed by squeezing and filtering with a muslin cloth. The quantity of extracted juice was measured with a graduated measuring cylinder, and fruit juice content was expressed as percentage on a fresh fruit weight basis. Total soluble solids (TSS) of the juice were determined in the laboratory using a digital hand held refractometer (Model: Brix 54, Bellingham + Stanley Ltd., Tunbridge Wells, Kent, United Kingdom), which was calibrated using distilled water before measurements. The acidity of fruit juice was determined with the titration method described by Ranganna [18].

2.4.2. Nematode Population

In each treatment, 500 g composite soil-root samples were collected at 30–60 cm depth from four sides of a tree, each 45–60 cm apart from the tree trunk, at the beginning and at
the end of the experiment. Soil from 15 different locations within each site was collected and mixed thoroughly, and composite samples were brought to the laboratory to count the root knot nematode population, root galls, and egg masses. The soil samples were processed by Cobb’s decanting and sieving method [19], as previously described [20,21]. Randomly, 5–10 mature females were separated from infested roots using needles and forceps, teased with the stereoscopic binocular microscope to create perineal patterns for identification and confirmation of the root knot species in collected samples [16,17].

2.5. Statistical Analysis

Data of the different measured parameters were subjected to analysis of variance (ANOVA), followed by the Tukey HSD test \( (p < 0.05) \) for mean comparison. Before running the ANOVAs, the assumptions for normality of data distribution and homogeneity of variance were tested using the Shapiro–Wilk test and Levene’s test, respectively. Counts of the number of galls per gram of root, number of eggs per egg mass, and number of nematodes per 200 cc soil were analyzed with the general linear model procedure using the negative binomial model, which best fitted the data, followed by the least-squares means (with \( p \)-value adjustment using Tukey method) for multiple comparison. All analyses were performed using SAS 9.2 (SAS Ins., Cary, NC, USA). A cluster analysis of different genotypes was conducted using the statistical package JMP Pro 10 (SAS Institute Inc., Cary, NC, USA). To study the similarity among the genotypes in terms of tolerance to the root knot nematode, we conducted a hierarchical cluster analysis (Ward linkage method) using the data of root knot galls g\(^{-1}\) root.

3. Results and Discussion

3.1. Growth, Yield, and Fruit Quality of Nematode-Infested Pomegranate

The survey results revealed that none of the pomegranate orchard was found free from nematode infestation in both the investigated districts. Thirteen percent of the surveyed orchards had severe incidence of nematodes (>40.0% incidence), 78% had moderate incidence (10–40%), and 9% had a mild nematode incidence (<10%) (Table 2). In this part of the Indian arid region, commercial cultivation of pomegranate is relatively recent, and nematode infestation is a new emerging problem. This may be a reason for the moderate severity level of nematode we found in this study. However, the severity of nematode incidence was higher in both the locations in Barmer district compared to Jodhpur (Figure 2). Nematodes multiply faster in light soil when available soil moisture is constant and temperature is high [9]. In the region where this survey was carried out, pomegranate is planted under high density with regular supply of water through drip system in light-sandy soils. These factors may explain the high incidence of root knot nematode in this region, as also suggested by the findings of Dasgupta and Gaur [7].

Table 2. Total number of surveyed pomegranate orchards in each district and location and their distribution in three categories of severity of root knot nematode incidence: mild (nematode incidence of 0–10%), moderate (10–40%), and severe (>40%).

| District | Location | No. of Orchards Surveyed | Percent of Orchards (%) |
|----------|----------|--------------------------|-------------------------|
|          |          |                          | Mild | Moderate | Severe |
| Barmer   | Location 1 | 25                       | 12   | 60       | 28     |
|          | Location 2 | 25                       | 0    | 84       | 16     |
| Jodhpur  | Location 3 | 25                       | 12   | 80       | 8      |
|          | Location 4 | 25                       | 12   | 88       | 0      |
| Total    | 100       |                          | 9    | 78       | 13     |
Table 2. Total number of surveyed pomegranate orchards in each district and location and their distribution in three categories of severity of root knot nematode incidence: mild (nematode incidence of 0–10%), moderate (10–40%), and severe (>40%).

| District  Location | No. of Orchards Surveyed | Percent of Orchards (% | Infestation Severity |
|-------------------|--------------------------|------------------------|----------------------|
|                   |                          |                        | Mild  | Moderate | Severe |
| Barmer Location 1 | 25                       | 12                     | 60    | 28       |
| Location 2        | 25                       | 0                      | 84    | 16       |
| Jodhpur Location 3| 25                       | 12                     | 80    | 8        |
| Location 4        | 25                       | 12                     | 88    | 0        |
| Total             | 100                      | 9                      | 78    | 13       |

Figure 2. Root knot nematode population in pomegranate orchards located in two locations (Jodhpur and Barmer). Means of 100 samples across the surveyed orchards in four locations. Separately for each parameter, different letters indicate significant differences between locations assessed with the general linear model procedure followed by the least-squares means.

Plant height, canopy width, fruit yield, fruit weight, fruit size, juice content, TSS, and acidity in nematode-affected vis-à-vis healthy trees were recorded in all the surveyed orchards from all the locations. We found a significant reduction in plant growth, yield, and fruit quality in root knot nematode-affected orchard (Table 3). A significant yield reduction (40.2%) and a decrease in fruit size were measured in nematode-affected trees when the age of most of the orchards was only seven years. Juice content of fruits harvested from affected trees showed significantly lower juice compared to healthy plants, whereas TSS and acidity of juice were not very affected. Khan et al. [10] also reported 30 to 40% yield losses with poor quality fruits in current season in nematode-affected pomegranate orchards.

Table 3. Reduction in growth, yield, and fruit quality of pomegranate affected by root knot nematode compared to healthy plants.

| Parameter            | Healthy Trees | Nematode-Affected Trees | Reduction (%) |
|----------------------|---------------|-------------------------|---------------|
|                      | Mean *        | Range                   | Mean *        | Range       |               |
| Plant height (cm)    | 222.0 b       | 180–280                 | 167.0 a       | 100–230     | 24.6          |
| Tree spread (cm)     | 223.0 b       | 165–250                 | 169.4 a       | 85–222.5    | 24.2          |
| Fruit yield (kg/tree)| 20.9 b        | 17.8–31.3               | 12.5 a        | 2.8–16.6    | 40.2          |
| Fruit weight (g/fruit)| 227.3 b     | 176.0–280.3             | 167.5 a       | 130.2–220.8| 26.3          |
| Fruit length (cm)    | 8.0 a         | 7.15–9.27               | 6.2 a         | 5.8–7.9     | 22.0          |
| Fruit breadth (cm)   | 7.8 b         | 6.9–8.6                 | 5.6 a         | 5.4–7.3     | 28.6          |
| Juice content (%)    | 30.4 a        | 25.0–36.2               | 24.2 a        | 22.5–28.6   | 20.4          |
| TSS (%)              | 16.7 a        | 15.6–18.8               | 15.8 a        | 15.2–17.0   | 5.4           |
| Acidity (%)          | 0.44 a        | 0.38–0.58               | 0.46 a        | 0.36–0.62   | 4.3           |

* Means of 25 healthy and 25 RKN-affected trees across the surveyed orchard. Within each row, different letters indicate significant differences in the measured parameter between healthy and affected trees according to ANOVA followed by the Tukey HSD test (p ≤ 0.05).

3.2. Nematode Abundance and Identification of Species

Pattern of cuticular markings and the presence of high, squarish dorsal arch with smooth to wavy striation in the perineal area of the mature females confirmed the occur-
rence of only *M. incognita* in collected samples from all the locations in both the districts (Figure 3). In contrast to the present study, the association of more than one species of root knot nematode in pomegranate has been reported by different workers worldwide. Khan et al. [10] observed *Helicotylenchus digitus* and *M. incognita* to be the most frequently observed nematodes in pomegranate orchards. Similarly, Holland et al. [9] reported that *M. incognita* and *M. javanica* are the main species, whereas Darekar et al. [11] reported 10 different species of plant parasitic nematodes in pomegranate orchards with *M. incognita* being the most abundant. Singh et al. [6] summarized that *Meloidogyne* spp. are the most active at higher temperatures and optimum field capacity that favor their rapid multiplication, and this might be the reason of the dominance of *M. incognita* in arid regions where temperature remains high, and the orchards are planted under high density with regular water supply through drip irrigation systems.

![Figure 3. Morphology of female root knot nematode: (A) anterior extremity of the body; (B) posterior extremity of the body; (C) pattern of cuticular markings in the perineal area of mature female. Scale was approximately 10 μm in fluorescence microscopy with LEICA DM 3000; 1000× magnification.](image)

### 3.3. Screening of Genotypes

One of the most important sustainable ways to manage root knot nematodes may be the adoption of resistant or tolerant genotypes against nematodes, either as rootstocks or breeding source to develop in the long term as improved resistant varieties. In order to have resistant or tolerant genotypes/cultivars against nematodes, we evaluated 27 different genotypes and cultivars of pomegranate collections. The results showed that the number of root knot galls g⁻¹ root ranged from 28.5 to 56.5 at the third cycle of nematode growth (Table 4).

Therefore, the screening was unable to detect genotypes/varieties resistant or tolerant to *M. incognita*. Ahmadi et al. [22] also could not find resistant or tolerant cultivars to nematodes among 27 evaluated cultivars, whereas Shelke and Darekar [23] reported that, out of various genotypes, seven were moderately resistant to *M. incognita* race-2. Similarly, Ahire [15] reported that, out of 11 evaluated genotypes, 4 were moderately resistant, whereas the remainders were susceptible to root knot nematode. However, the present study clearly indicated that although all the genotypes were found susceptible to *M. incognita*, the relative abundance of infestation differed among the evaluated genotypes. It was also noted that variations in growth of nematode-inoculated vis-à-vis healthy seedlings of the genotypes IC318728, IC-318790, and IC-318723 were meager along with relatively lower number of root knot galls g⁻¹ root.

In this part of the Indian arid region, commercial cultivation of pomegranate is relatively recent, but nematode infestation is becoming a major limiting factor for sustainable pomegranate production. Therefore, identifying tolerant/resistant genotypes or cultivars can be further used for the development of resistant rootstock or cultivars.
| Genotypes         | Plant Height (cm) * | Stem Girth (mm) * | Root Knot Galls g⁻¹ Root in Inoculated Plants ** | Assigned Cluster |
|-------------------|---------------------|-------------------|-------------------------------------------------|------------------|
|                   | Inoculated Healthy  | Variation (%)     | Inoculated Healthy Variation (%)               |                  |
| Amidiana          | 43.2 ± 1.2 de       | 15.8 ± 3.9 bc     | 6.7 ± 0.3 a                                    | 40 ± 1 abc       |
| Bassein seedless  | 43.7 ± 1.6 def      | 43.4 ± 2.4 ijk    | 6.4 ± 0.5 a                                    | 51.7 ± 2.0 lmn   |
| Bhagwani          | 40.5 ± 2.1 cd       | 36.7 ± 2.7 ghi    | 6.5 ± 0.7 a                                    | 35.8 ± 2.6 eghi  |
| CAZRI Sel.        | 50.4 ± 2.1 efgi     | 39.8 ± 2.6 hiij   | 5.7 ± 0.9 a                                    | 45.0 ± 2.3 jkln  |
| Co-white          | 51.7 ± 2.0 fghi     | 16.4 ± 4.1 bc     | 9.4 ± 0.9 a                                    | 28.8 ± 2.9 def   |
| Dholka            | 29.8 ± 2.7 a        | 34.5 ± 2.9 fghi   | 6.5 ± 0.8 a                                    | 31.6 ± 2.8 defgh |
| G-137             | 50.2 ± 2.1 efgi     | 20.3 ± 3.8 cd     | 9.5 ± 0.9 a                                    | 41.9 ± 2.5 jk    |
| Ganesh            | 46.7 ± 2.2 defg     | 39.4 ± 2.8 hiij   | 6.7 ± 0.9 a                                    | 44.7 ± 2.5 kjl   |
| IC-318723         | 58.5 ± 2.0 ijh      | 10.5 ± 5.4 ab     | 7.2 ± 0.8 a                                    | 20.0 ± 3.7 bc    |
| IC-318728         | 50.6 ± 2.1 efgi     | 36.9 ± 3.3 a      | 7.8 ± 1.1 a                                    | 11.4 ± 5.0 a     |
| IC-318754         | 42.9 ± 2.3 defg     | 15.0 ± 4.5 bc     | 8.1 ± 0.8 a                                    | 23.6 ± 3.5 cd    |
| IC-318790         | 45.2 ± 2.3 defg     | 6.8 ± 6.7 a       | 8.2 ± 1.1 a                                    | 9.7 ± 5.5 a      |
| Jalore seedless   | 41.6 ± 2.4 cde      | 50.2 ± 2.4 kgh    | 5.8 ± 1.0 a                                    | 53.4 ± 2.3 mn    |
| Jodhpur Red       | 25.7 ± 3.1 a        | 48.6 ± 2.4 k      | 8.8 ± 1.0 a                                    | 51.7 ± 2.3 lmn   |
| Jodhpur Sel.      | 45.7 ± 2.4 defg     | 58.5 ± 2.8 defg   | 8.3 ± 1.2 a                                    | 43.1 ± 2.4 jkdl  |
| Jyoti             | 33.5 ± 2.9 abc      | 52.5 ± 3.1 bcde   | 6.4 ± 1.0 a                                    | 29.8 ± 3.0 defg  |
| Kabuli yellow     | 30.2 ± 3.1 ab       | 23.5 ± 3.2 cde    | 7.5 ± 1.3 a                                    | 29.3 ± 3.1 defg  |
| Kasturi           | 33.3 ± 3.1 abc      | 44.6 ± 3.6 ab     | 8.8 ± 1.3 a                                    | 37.5 ± 2.8 efghi |
| KR5               | 45.6 ± 2.8 defg     | 56.6 ± 3.3 bc     | 9.0 ± 1.3 a                                    | 60.7 ± 2.3 n     |
| Mridula           | 44.8 ± 2.9 defg     | 23.5 ± 3.6 cde    | 10.2 ± 1.3 a                                   | 40.0 ± 2.4 gj    |
| P-23              | 42.8 ± 3.0 defg     | 46.2 ± 2.7 jk     | 9.8 ± 1.3 a                                    | 42.0 ± 2.3 jk    |
| P-26              | 45.5 ± 3.1 defg     | 37.1 ± 3.0 ghi    | 11.2 ± 1.5 a                                   | 27.3 ± 2.8 cde   |
| PhuleArakta       | 38.5 ± 3.5 bc       | 31.3 ± 3.3 efghi  | 7.0 ± 1.2 a                                    | 10.5 ± 1.5 cd    |
| Ruby              | 42.6 ± 3.5 defg     | 29.6 ± 3.6 efg    | 8.8 ± 1.4 a                                    | 49.1 ± 2.2 klm   |
| Tabesta           | 46.3 ± 3.6 defg     | 20.3 ± 4.7 cd     | 8.9 ± 1.4 a                                    | 14.1 ± 2.2 ab    |
| Wonderful         | 42.7 ± 3.8 defg     | 59.7 ± 3.0 m      | 8.3 ± 1.4 a                                    | 27.6 ± 1.8 cd    |
| Yercaud-1         | 55.2 ± 3.4 gh       | 58.4 ± 3.5 defg   | 7.0 ± 1.2 a                                    | 12.9 ± 1.5 ab    |

Within each column, different letters indicate significant differences between genotypes evaluated with (*) ANOVA followed by the Tukey HSD test (p = 0.05) or (**) generalized linear model followed by the least-squares means.

The hierarchical clustering suggests that the tested genotypes could be grouped in terms of susceptibility to the nematodes. Using the Ward linkage procedure, we built a dendrogram and classified these genotypes into five possible groups (Figure 4). The groups no. 1, 4, and 3 showed the highest number of root galls per gram of root, ranging from 38.5 to 56.5. In group 1, this parameter ranged from 42.3 to 48.3 and included 11 genotypes. In group 4, it ranged from 38.5 to 56.5 and included seven genotypes, whereas in group 3, it ranged from 51.5 to 56.5 and included three genotypes. The group no. 2 had a lower number of root galls per gram of root, ranging from 31.0 to 35.2, and included five genotypes. The group no. 5 showed a tolerant reaction against the nematode infestation, having the lowest number of galls per gram of root (18.5). Genotypes of group No. 2 and especially those of the group no. 5 showed resistance/tolerance reaction against root knot nematode; hence, they should be additionally studied to explore their suitability as a resistance source for the development of new resistant cultivar or as rootstocks for the effective long-term sustainable management of nematodes.
without neem cakes was also reported to be effective by various researchers in different practices in pomegranate cultivation, no significant variation in vegetative growth amongst the tested genotypes. In group 4, it ranged from 38.5 to 56.5 and included seven genotypes, whereas groups no. 1, 4, and 3 showed the highest number of root galls per gram of root, ranging from 38.5 to 40.0 and included 11 genotypes. In group 1, this parameter ranged from 42.3 to 48.3 and included 11 genotypes, and especially those of the group no. 5 showed resistance/tolerance reaction against the nematode infestation, having the lowest number of galls per gram of root (18.5). Genotypes of group no. five genotypes. The group no. 5 showed a tolerant reaction against the nematode infestation of root knot nematode; hence, they should be additionally studied to explore their suitability as a resistance source for the development of new resistant cultivar or as rootstocks for fruit crops. Secondary metabolites produced by organic amendments were shown to cause a significant reduction of the reproduction of *M. incognita* on tomato in a field experiment [28]. Mechanisms involved in nematode control by endophytes such as *Paecilomyces* and *Trichoderma* with higher rate of organic materials may also trap and kill root knot nematode in the soil or root systems [25,26,29,30]. The other widely recognized mechanisms of bio-agents along with soil amendments include the production of toxins, enzymes, and other metabolic products, as well as the promotion of plant growth and induction of systemic resistance of host plants to pathogens [31].

Figure 4. Dendrogram obtained with the hierarchical cluster analysis (Ward linkage method) using the data of root knot galls g$^{-1}$ root. The numbers on the vertical axis represent a unique ID assigned to the genotypes.

### 3.4. Integrated Management of Root Knot Nematode

Table 5 clearly shows that all the treatments were significantly superior to untreated control in reducing the incidence of nematodes in terms of number of root galls g$^{-1}$ root. However, the soil application of Carbofuran 20 g + Fluensulfone 20 g plant$^{-1}$ was found to be the most effective compared to the other treatments in reducing the number of root galls (49.7%). This was closely followed by the treatment with Neemcake 500 g + *P. lilanici* at 25 mL + Carbofuran at 20 g plant$^{-1}$ + Fluensulfone at 20 g plant$^{-1}$ (48.8%) and Neemcake 500 g + *P. lilanici* at 25 mL + Fluensulfone 40 g plant$^{-1}$ (46.4%). It was interesting to note that the soil application of Carbofuran at 40 g plant$^{-1}$ or Fluensulfone 40 g plant$^{-1}$ alone or in combination was not as much effective as its half dose applied in combination, i.e., Carbofuran at 20 g + Fluensulfone 20 g plant$^{-1}$. On the other hand, nematode population densities continued to increase (38.2%) in the rhizosphere of pomegranate plants in the absence of nematode management combinations (78.5 to 108.5 galls g$^{-1}$ root). Different management treatments did not affect significantly plant growth in terms of plant height and canopy spread, but it was significantly superior to untreated control. Since pruning is a recommended practice in pomegranate cultivation, no significant variation in vegetative growth amongst different treatments was expected as all the plants were pruned uniformly. Selected trees in study were unable to produce any fruit at the time of initiation of experiments, but at the termination of the experiment, treated trees bore few fruits.

Since the fruit yield was negligible, data were not presented and discussed here. Nematicides reduced the populations of various *Meloidogyne* spp. in the soil [10], but once the symptoms have developed, they are incapable of completely eliminating those *Meloidogyne* species already in plant tissues [24]. Integrated approaches involving nematicides, soil amendments with organic sources, and different bioagents were proven to be effective for the control of root knot nematode in pomegranate and other fruit crops [22,23,25,26]. Similar to our study, the use of bioagents, viz., *Paecilomyces lilanici* and *Trichoderma* with or without neem cakes was also reported to be effective by various researchers in different fruit crops [9,14,25,27]. Secondary metabolites produced by organic amendments were shown to cause a significant reduction of the reproduction of *M. incognita* on tomato in a field experiment [28]. Mechanisms involved in nematode control by endophytes such as *Paecilomyces* and *Trichoderma* with higher rate of organic materials may also trap and kill root knot nematode in the soil or root systems [25,26,29,30]. The other widely recognized mechanisms of bio-agents along with soil amendments include the production of toxins, enzymes, and other metabolic products, as well as the promotion of plant growth and induction of systemic resistance of host plants to pathogens [31].
Table 5. Effect of different treatments on number of root knot galls per gram of root, plant height, canopy spread, and fruit yield (means ± standard errors) of pomegranate trees.

| Treatments | Nematode Galls g⁻¹ Root * | Plant Height (cm) ** | Canopy Spread (cm²) ** | Fruit Yield (kg tree⁻¹) ** |
|------------|---------------------------|----------------------|------------------------|---------------------------|
|            | Initial       | Final         | Reduction (%) | Initial | Final         | Variation (%) | Initial | Final         | Variation (%) |
| T1         | 74 ± 5 ab     | 52 ± 4 bcd    | 29.9 ± 1.5 c  | 200.0 ± 2.9 a | 236.7 ± 3.7 cd | 18.3 ± 3.1 bcd | 172.5 ± 1.5 a | 186.7 ± 4.3 a | 8.23 ± 6.1 ab | 4.8 ± 0.2 ab   | 5.2 ± 0.3 ab   | 8.3 ± 7.0 cd   |
| T2         | 43 ± 4 d      | 30 ± 3 c      | 31 ± 0.9 ed   | 215.0 ± 1.7 bc | 233.3 ± 2.7 bc  | 8.5 ± 5.2 ab   | 180.0 ± 2.6 b  | 197.8 ± 5.2 ab | 9.8 ± 1.9 ab   | 5.3 ± 0.3 bc   | 5.8 ± 0.2 b    | 9.4 ± 5.0 e    |
| T3         | 91 ± 5 a      | 58 ± 4 bcd    | 36.1 ± 1.8 d  | 240.0 ± 4.6 e  | 243.3 ± 3.6 de  | 1.4 ± 3.1 a    | 188.9 ± 2.2 cd | 195.0 ± 6.1 ab | 3.2 ± 2.6 ab   | 5.4 ± 0.2 bd   | 5.9 ± 0.3 b    | 9.2 ± 4.0 de   |
| T4         | 84 ± 5 ab     | 42 ± 4 cde    | 49.7 ± 2.1 e  | 210.0 ± 4.0 b  | 250.0 ± 5.5 ef g| 19.0 ± 2.3 cd  | 210.0 ± 1.9 f  | 221.7 ± 1.9 e  | 5.6 ± 2.0 a    | 4.6 ± 0.2 a    | 5.2 ± 0.3 ab   | 13 ± 0.4 f     |
| T5         | 79 ± 5 ab     | 52 ± 4 bcd    | 33.9 ± 4.9 cd | 220.0 ± 2.7 cd | 246.7 ± 3.4 ef  | 12.1 ± 1.7 bc  | 215.0 ± 2.0 g  | 228.9 ± 2.6 e  | 6.5 ± 1.8 ab   | 6.2 ± 0.2 e    | 7.0 ± 0.6 cde  | 12.9 ± 0.6 f   |
| T6         | 73 ± 4 ab     | 62 ± 4 bc     | 15.2 ± 1.2 b  | 220.0 ± 1.7 cd | 256.7 ± 3.1 g   | 16.7 ± 2.9 bcd | 182.3 ± 1.8 b  | 186.7 ± 2.5 a  | 2.5 ± 1.0 ab   | 6.8 ± 0.1 fg   | 7.3 ± 0.3 e    | 7.4 ± 5.0 c    |
| T7         | 76 ± 5 ab     | 41 ± 3 de     | 46.4 ± 2.3 e  | 210.0 ± 1.2 b  | 260.0 ± 5.2 g   | 23.8 ± 1.7 d   | 193.5 ± 1.0 de | 219.4 ± 2.9 de | 13.8 ± 2.2 b   | 5.5 ± 0.3 d    | 6.2 ± 0.2 bcd  | 12.7 ± 7.0 f   |
| T8         | 66 ± 4 bc     | 47 ± 4 bcd    | 28.9 ± 2.7 c  | 225.0 ± 1.3 d  | 258.3 ± 3.1 g   | 14.8 ± 2.3 bcd | 237.5 ± 0.9 b  | 245.0 ± 3.5 g  | 3.2 ± 1.8 ab   | 5.5 ± 0.2 d    | 5.8 ± 0.2 b    | 5.4 ± 7.0 b    |
| T9         | 94 ± 5 a      | 64 ± b        | 32 ± 2.6 cd   | 210.0 ± 1.7 b  | 236.7 ± 2.3 cd  | 12.7 ± 2.7 bc  | 232.5 ± 1.3 h  | 242.2 ± 2.7 fg | 4.2 ± 1.0 ab   | 5.3 ± 0.2 bcd  | 6.0 ± 0.4 bc   | 13.2 ± 6.0 fg  |
| T10        | 51 ± 4 cd     | 46 ± 4 bce    | 10.5 ± 1.4 b  | 200.0 ± 2.9 a  | 226.7 ± 3.0 b   | 13.4 ± 3.6 bc  | 195.2 ± 1.5 e  | 209.4 ± 5.2 cd | 7.2 ± 0.9 a    | 7.2 ± 0.1 g    | 7.8 ± 0.4 e    | 8.4 ± 6.0 cde  |
| T11        | 79 ± 5 ab     | 40 ± de       | 48.8 ± 2.2 e  | 215.0 ± 1.7 bc | 253.3 ± 1.2 fg  | 17.8 ± 5.5 bcd | 187.5 ± 2.9 c  | 206.7 ± 3.9 bc | 10.3 ± 1.3 ab  | 6.3 ± 0.2 ef   | 7.2 ± 0.4 de   | 14.2 ± 0.4 g   |
| T12        | 71 ± 5 abc    | 47 ± 4 bcd    | 33.7 ± 2.0 cd | 210 ± 2.3 b    | 236.7 ± 1.4 cd  | 12.7 ± 1.7 bc  | 215.0 ± 2.0 g  | 231.1 ± 2.7 ef | 7.5 ± 2.7 ab   | 4.8 ± 0.1 abc  | 5.4 ± 0.2 b    | 12.5 ± 0.4 f   |
| T13        | 78 ± 5 ab     | 108 ± 6 a     | (+)38.2 ± 1.6 a| 215 ± 1.3 bc   | 196.7 ± 2.5 a   | (−)8.5 ± 4.6 a  | 237.5 ± 1.3 h  | 203.9 ± 3.1 bc | (−)6.5 ± 5.2 a | 5.4 ± 0.3 bd   | 4.2 ± 0.6 a    | (−)22.3 ± 0.4 a|

*Within each column, different letters indicate significant differences between genotypes evaluated with (*) generalized linear model followed by the least-squares means or (**) ANOVA followed by the Tukey HSD test (p = 0.05).
4. Conclusions

It is concluded from the present study that the majority of pomegranate orchards in the arid region of western Rajasthan are more or less infected by nematodes. Root knot nematode (*Meloidogyne incognita*) is the single dominant species found in all the pomegranate orchards surveyed during the study. Among the evaluated genotypes and varieties, all of them were found to be susceptible to root knot nematode, but the severity level of its infestation was variable; hence, a more detailed screening is needed on a larger population. Among the tested management approaches, there is no doubt that a combination of nematicides such as Carbofuran and Fluensulfan with their half dose was effective, but integrated approaches involving nematicides, bioagents, and organic amendments are more effective and sustainable, especially when nematode species are already in root tissue.

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