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ASSESSMENT OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) ASSOCIATED WITH GRAPEVINES IN THE POTHOHAR REGION OF PUNJAB, PAKISTAN

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ABSTRACT

Root-knot nematodes (RKN), particularly *Meloidogyne* spp., are major soil-borne pests that cause substantial damage to grapevines worldwide. A survey was conducted to determine the incidence, prevalence, species composition, and severity of RKN infestation in grape orchards across the Pothohar region of Pakistan. The study revealed an overall prevalence of 60.35% and an incidence of 19.9% in the surveyed orchards. Among the four districts, Rawalpindi exhibited the highest prevalence (74.28%) and incidence (21.90%), whereas Jhelum showed the lowest prevalence (31.42%) and incidence (9.52%). Seven grapevine varieties were recorded during the survey. Among these, NARC Black showed the highest prevalence (87.5%) and incidence (30.83%) of RKN infestation, whereas Sughra One and Flame Seedless exhibited the lowest prevalence (35%) and incidence ($\leq 10\%$), respectively. Two species, *Meloidogyne incognita* and *M. javanica*, were identified, with *M. javanica* being the dominant species in most districts, except in Jhelum where *M. incognita* predominated. Sole infestations of *M. incognita* and *M. javanica* were recorded in 36.5% and 43.25% of orchards, respectively, while mixed populations occurred in 20.25% of infested orchards. The highest galling index among varieties (2.5) was recorded for NARC Black in Rawalpindi, whereas the lowest (0.5) occurred in Flame Seedless in Jhelum. District-wise, Attock exhibited the highest mean galling index (2.0), while Jhelum had the lowest (0.7). These findings demonstrate the widespread distribution and variability of RKN infestations in grape orchards of the Pothohar region and emphasize the need for integrated nematode management strategies tailored to regional conditions and varietal susceptibility. The differences in prevalence and incidence among districts were statistically significant ($p \leq 0.05$).

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INTRODUCTION

Grapes (*Vitis vinifera* L.) are among the oldest and most widely cultivated fruit crops globally. They are highly versatile and are consumed fresh as table grapes or processed into wine, raisins, juice, and other value-added products (Gurak *et al.*, 2012; Williams, 2017). Grapevines are climbing perennial plants that exhibit remarkable adaptability to diverse soils and climatic conditions,

making them suitable for cultivation across many regions of the world (Soneji and Nageswara-Rao, 2011). Although the genus *Vitis* comprises several species, *V. vinifera* is the most commercially important, representing approximately 94% of the global vineyard surface area (Bavaresco, 2019). Globally, grape production is substantial, with major producers including Italy, France, the United States, Spain, and China, together accounting for nearly half of the

world's grape output (McKenzie and Pathirana, 2007). China alone produced 14.8 million tons in 2020, highlighting its significant contribution to global grape production (Liu *et al.*, 2022). In Pakistan, despite suitable agro-climatic conditions, grapes remain a minor fruit crop, particularly in the Pothohar region, which remains underutilized for commercial grape production (Khaliq *et al.*, 2025). Although Pakistan's diverse climate supports the cultivation of numerous fruits, the horticulture sector has traditionally emphasized citrus, mango, and date palm (Khana *et al.*, 2013). However, information on the status of grapevine-associated nematodes in Pakistan, particularly in the Pothohar region, remains scarce. Most previous studies have focused on vegetable crops, with limited attention to perennial fruit systems such as grapevine.

Grape production in Pakistan faces several constraints, including the scarcity of registered and well-characterized cultivars, which limits the development of a strong grape industry (Khaliq *et al.*, 2025). In addition, plant-parasitic nematodes, particularly root-knot nematodes, pose a serious threat to grapevine health and productivity (Gondal *et al.*, 2012; Gondal *et al.*, 2015; Kayani *et al.*, 2017; Mukhtar *et al.*, 2017; Smith *et al.*, 2018; Li *et al.*, 2020). Sustainable management strategies and the development of resistant rootstocks are essential to mitigate these challenges and enhance grape productivity in the region (Kayani and Mukhtar, 2018; Nour El-Deen *et al.*, 2018; Smith *et al.*, 2018).

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive pests of grapevines worldwide, causing substantial yield reductions (Smith *et al.*, 2017; Knoetze, 2020; Esmenjaud *et al.*, 2023). These endoparasitic nematodes induce root galling, leading to stunted growth, nutrient deficiencies, and reduced plant vigor (Wang *et al.*, 2014; Wann *et al.*, 2016; Mukhtar and Kayani, 2019, 2020; Haq *et al.*, 2022). The most prevalent species damaging grapevines include *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* (Somavilla *et al.*, 2012; Smith *et al.*, 2017; Esmenjaud *et al.*, 2023).

Root-knot nematodes frequently interact synergistically with soil-borne fungi and bacteria, leading to disease complexes that exacerbate plant damage beyond that caused by individual pathogens. These interactions can result in severe yield losses across various cropping systems (Asghar *et al.*, 2020; Ahmed *et al.*, 2021; Yaseen and Mukhtar, 2024; Yaseen *et al.*, 2023, 2024, 2025).

In grapevines, typical symptoms of root-knot nematode infestation include root galling, chlorosis, stunted growth,

and reduced yield potential (Wang *et al.*, 2014; Wann *et al.*, 2016; Daware *et al.*, 2022). Infested plants experience impaired water and nutrient uptake, resulting in poor establishment and accelerated decline under environmental stress (Mukhtar and Hussain, 2019; Gagnier *et al.*, 2024). The economic impact of root-knot nematodes on grape production is considerable, reinforcing the need for effective management strategies (Somavilla *et al.*, 2012; Wann *et al.*, 2016). Despite extensive global research on root-knot nematodes, there is a lack of comprehensive data on their distribution, species composition, and impact on different grapevine varieties under the agro-ecological conditions of the Pothohar region.

The present study aims to investigate the prevalence and incidence of root-knot nematodes in grape orchards of the Pothohar region of Pakistan. Given the substantial challenges posed by root-knot nematodes to grape production, understanding their distribution and associated damage is crucial for developing effective and sustainable management strategies. By addressing these objectives, this study seeks to support the sustainable development of grape cultivation in Pakistan and contribute to improved yield and fruit quality in the region.

MATERIALS AND METHODS

Study Area

The assessment of root-knot nematodes was carried out in the Pothohar region of Pakistan during the years 2019-2022 to determine the prevalence, incidence, and infestation of root-knot nematodes (*Meloidogyne* spp.) in grape orchards.

The Pothohar Plateau of Pakistan spans approximately 28,489 square kilometers and is located between 32°10' to 34°09' N latitude and 71°10' to 73°55' E longitude. It is bordered by the Jhelum River in the east, the Indus River in the west, the Margalla Hills in the north, and the Salt Range in the south. It has an average elevation of about 300-600 m above sea level and is characterized by hills, valleys, and plains.

This region includes four districts of the Punjab province of Pakistan: Attock, Chakwal, Rawalpindi, and Jhelum. The soil pH ranges from 7.5 to 8.5, and the annual rainfall in the Pothohar region is between 500-1,000 mm. Maximum rainfall occurs in the northwest areas, while the minimum is observed in the southwest areas. About 70% of the rainfall is received during the monsoon season (July and August).

Pulses, wheat, maize, and oilseed crops are mostly

cultivated in this region. It is also suitable for fruit cultivation. Overall grape production in this region is comparatively low due to disease constraints, especially nematode infestation.

Infestation Assessment of Root-Knot Nematodes on Grapes

Collection of root and soil samples

For the assessment of infestation of root-knot nematodes on grapes in the Pothohar region, 10 orchards were selected based on the ecological zones of Pothohar. Each orchard was 15-20 km apart, and the orchard age ranged from 3-5 years. Orchards were selected using a random sampling approach to ensure representation of different agro-ecological conditions.

Twenty grape plants were selected from each orchard to evaluate the prevalence and incidence of root-knot nematodes. This sample size was considered sufficient to represent within-orchard variability. Sampling was done in an X-plus manner to ensure uniform coverage of the orchard, with five plants selected from each row. Samples were collected from the rhizosphere, 45-60 cm away from the main trunk.

The upper 5 cm of soil was removed, and soil and root samples were collected in polythene zip bags, labeled, and transferred to the laboratory. A total of 250 g of soil was taken from each of the four sides of the plant. These subsamples were mixed to form a composite sample of one kilogram. Roots were examined for galls to determine the presence or absence of root-knot nematodes. Permission was obtained from orchard owners prior to sample collection.

Determination of incidence and prevalence of root-knot nematodes

To determine root-knot nematodes infestation, root samples from each plant in each orchard were examined for the presence of galls. The incidence of each orchard was calculated using the formula described below:

$$\text{Incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of observed plants}} \times 100$$

Similarly, the incidence was calculated for each orchard, district, and grape variety. Each plant was considered an experimental unit for incidence assessment, while each orchard served as a unit for prevalence estimation.

The prevalence of root-knot nematodes in each district was determined by dividing the number of infected orchards by the total number of orchards:

$$\text{Prevalence (\%)} = \frac{\text{Orchards infected with RKN}}{\text{Total orchards visited}} \times 100$$

Determination of galling index

The galling index of root-knot nematodes was determined from the root systems of infected plants in each orchard using the scale suggested by Bridge and Page (1980). Galling indices were also calculated for each district and grape variety. The galling index was rated on a 0-5 scale, where 0 = no galls, 1 = 1-10% roots galled, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, and 5 = more than 75% roots galled.

Identification of Root-Knot Nematodes Species

Morphological identification of nematodes was carried out at the species level based on the distinctive morphological features of second-stage juveniles, adult females, and perineal pattern characteristics (Li *et al.*, 2020).

For preparation of perineal pattern mature females were extracted from the infected roots using a fine needle and placed in Petri dishes containing 45% lactic acid. The posterior end of each female was excised using a fine needle, and the cuticle portion containing the vulva with the perineal pattern was transferred to a drop of glycerin on a slide, covered with a cover slip, and observed under a microscope. Perineal pattern were used for species identification following Taylor and Netscher (1974). Perineal pattern from 20 females per orchard were examined to determine the percentage composition of root-knot nematodes species associated with grapes.

Molecular Identification

The females morphologically identified based on perineal patterns were further confirmed through molecular analysis. Species identification was confirmed using species-specific SCAR primer sets: Fjav (5'-GGT GCG CGA TTG AAC TGA GC-3') and Rjav (5'-CAG GCC CTT CAG TGG AAC TAT AC-3') (Zijlstra *et al.*, 2000), and MI-F (5'-GTG AGG ATT CAG CTC CCC AG-3') and MI-R (5'-ACG AGG AAC ATA CTT CTC CGT CC-3') (Meng *et al.*, 2004).

DNA extraction

DNA was extracted from single females collected from infected grape roots using the NaOH digestion method (Hübschen *et al.*, 2004). Five replicates were prepared for both *M. javanica* and *M. incognita*. Each replicate consisted of an individual female nematode.

Polymerase chain reaction (PCR)

PCR was performed using a Bio-Rad T100 Thermal Cycler in a 25 µl reaction volume containing 12 µl of 2× OneTaq Hot Start Master Mix (New England Biolabs), 1 µl DNA, 0.25 µl of each primer from a 10 µM stock, and 11.5 µl sterile water. Amplification conditions included initial denaturation at 95°C for 15 min, followed by 35 cycles of 95°C for 30 sec, annealing at 64°C for *M. javanica* and 62°C

for *M. incognita* for 30 sec, and extension at 72°C for 60 sec. A final extension was carried out at 72°C for 10 min. Negative controls (without DNA template) were included in each PCR run to ensure the absence of contamination.

Gel electrophoresis

DNA samples were run on 1.25% agarose gel (1.00 g agarose + 4.5 µl GelRed in 100 ml) and visualized under UV light using a Gel Doc™ EZ Imager (Vegetable and Fruit Nematology Lab, GCREC). The expected fragment sizes ranged from 600-1000 bp, consistent with reported values for *M. javanica* and *M. incognita*.

Statistical Analysis

The collected data on incidence, prevalence, and galling index were subjected to analysis of variance (ANOVA) using Statistix 8. Mean comparisons were performed using the Least Significant Difference (LSD) test at a significance level of $p \leq 0.05$.

RESULTS

Incidence and Prevalence of Root-Knot Nematodes in Grape Orchards in the Pothohar Region

An overall prevalence of 60.35% and an incidence of 19.9% of root-knot nematodes in grape orchards were recorded in the Pothohar region (Figure 1). A total of 10 orchards and 200 plants were assessed during the survey. Among the surveyed districts, the highest prevalence (74.28%) and incidence (21.90%) were observed in Rawalpindi, while the lowest prevalence (31.42%) and incidence (9.52%) were recorded in Jhelum (Figure 2). A clear increase in infestation was observed from Jhelum to Rawalpindi.

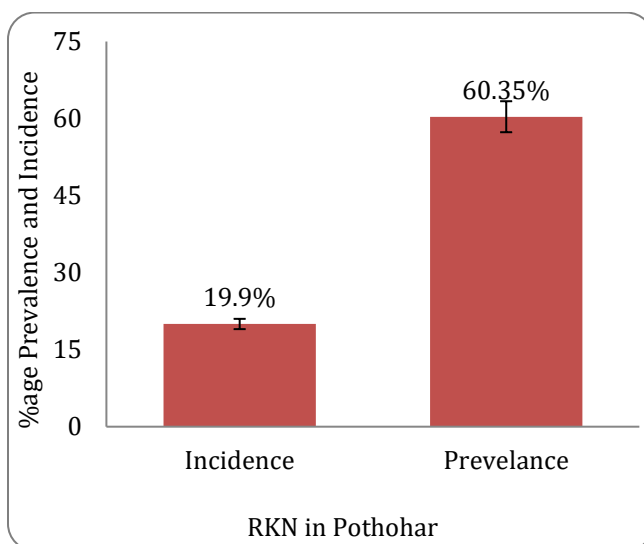


Figure 1. Overall prevalence and incidence of root-knot nematode infestation in grapes in the Pothohar region.

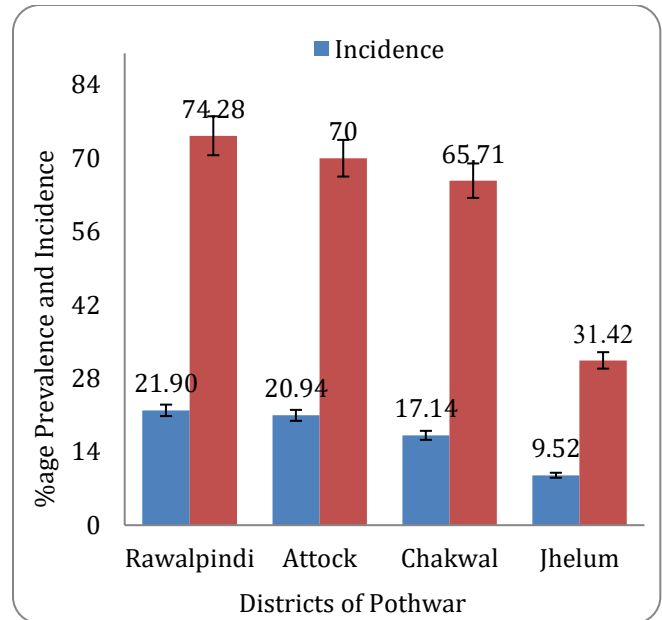


Figure 2. District wise prevalence and incidence of root-knot nematodes in the Pothohar region.

During the survey, seven grape varieties were encountered in the region. Among these, the highest prevalence of root-knot nematodes (87.5%) was recorded in the NARC Black variety, whereas the lowest (35%) was observed in Sughra One. Similarly, the highest incidence (30.83%) was noted in NARC Black, while the lowest was recorded in the Flame Seedless variety (Figure 3). Overall, NARC Black exhibited the highest susceptibility, whereas Flame Seedless and Sughra One showed relatively lower susceptibility to root-knot nematodes. Significant variation in incidence and prevalence was observed among grape varieties ($p \leq 0.05$). The variety-wise incidence and prevalence of root-knot nematodes across the four districts of the Pothohar region are presented in Table 1.

Occurrence of Root-Knot Nematode Species

Two species of root-knot nematodes, *Meloidogyne incognita* and *M. javanica*, were found infecting grapevines in the region. The overall prevalence of *M. javanica* was higher than that of *M. incognita* (Figure 4). *M. javanica* was dominant in all districts except Jhelum, where *M. incognita* was found to be more prevalent (Figure 5). This dominance of *M. javanica* was consistent across most surveyed locations.

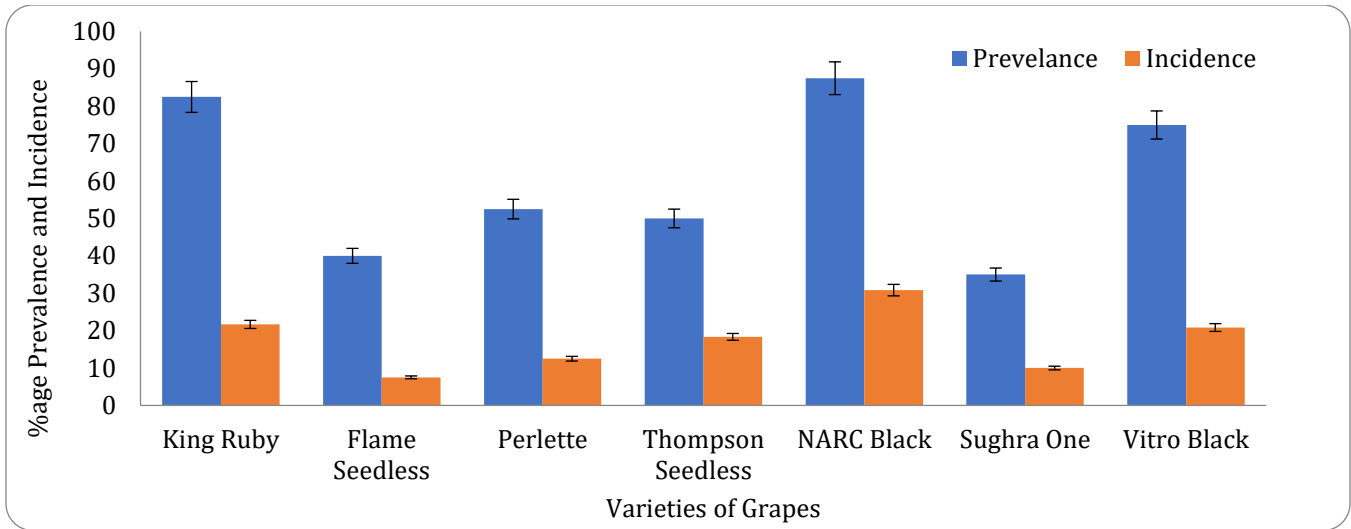


Figure 3. Variety wise prevalence and Incidence of root-knot nematodes in the Pothohar region.

Table 1. Variety wise prevalence and incidence of root-knot nematodes in the four districts of Pothohar region.

| Variety | % Prevalence and incidence of root-knot nematodes in | | | | | | | |
|----------------|--|-----------|------------|-----------|------------|-----------|------------|-----------|
| | Rawalpindi | | Attock | | Chakwal | | Jhelum | |
| | Prevalence | Incidence | Prevalence | Incidence | Prevalence | Incidence | Prevalence | Incidence |
| King Ruby | 100.0 | 23.33 | 100.0 | 26.66 | 70.0 | 23.33 | 60.0 | 13.33 |
| Flame Seedless | 50.0 | 10.00 | 50.0 | 13.33 | 60.0 | 6.66 | 0.00 | 0.00 |
| Perlette | 70.0 | 16.66 | 60.0 | 13.33 | 80.0 | 20.0 | 0.00 | 0.00 |
| Thompson | 60.0 | 23.33 | 50.0 | 26.66 | 50.0 | 10.00 | 40.0 | 13.33 |
| NARC Black | 100.0 | 40.00 | 100.0 | 33.33 | 80.0 | 26.66 | 70.0 | 23.33 |
| Sughra One | 40.0 | 13.33 | 50.0 | 16.66 | 50.0 | 10.0 | 0.00 | 0.00 |
| Vitro Black | 100.0 | 26.66 | 80.0 | 16.66 | 70.0 | 23.33 | 50.0 | 16.66 |

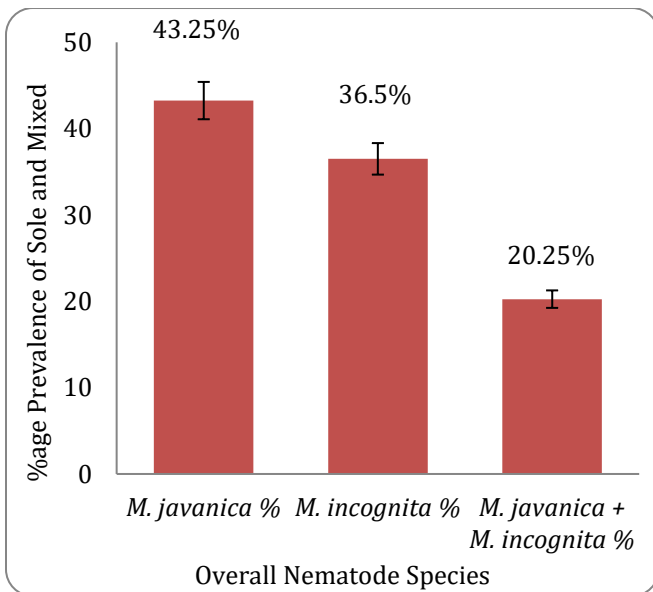


Figure 4. Overall prevalence of *M. incognita* and *M. javanica* as sole and mixed population in the Pothohar region.

As a sole population, *M. incognita* was detected in 36.5% and *M. javanica* in 43.25% of the root-knot nematode-infested grape orchards. Furthermore, mixed populations of both species were observed in 20.25% of the infected orchards (Figure 4). This indicates the co-existence of multiple species within the same orchard environments. The district-wise and variety-wise occurrence of root-knot nematode species, both as sole and mixed populations, is detailed in Table 2.

Molecular Characterization of Root-Knot Nematodes

Molecular characterization using species-specific SCAR primers confirmed the identity of *Meloidogyne incognita* and *M. javanica* detected through morphological analysis. The primer set specific for *M. javanica* amplified a fragment of approximately 670 bp, while the primer set for *M. incognita* produced a fragment of approximately 1000 bp. The molecular results were consistent with morphological identification based on perineal pattern

analysis, confirming the presence of both species in the surveyed orchards. No amplification was observed in

negative controls, indicating the specificity and reliability of the PCR assays.

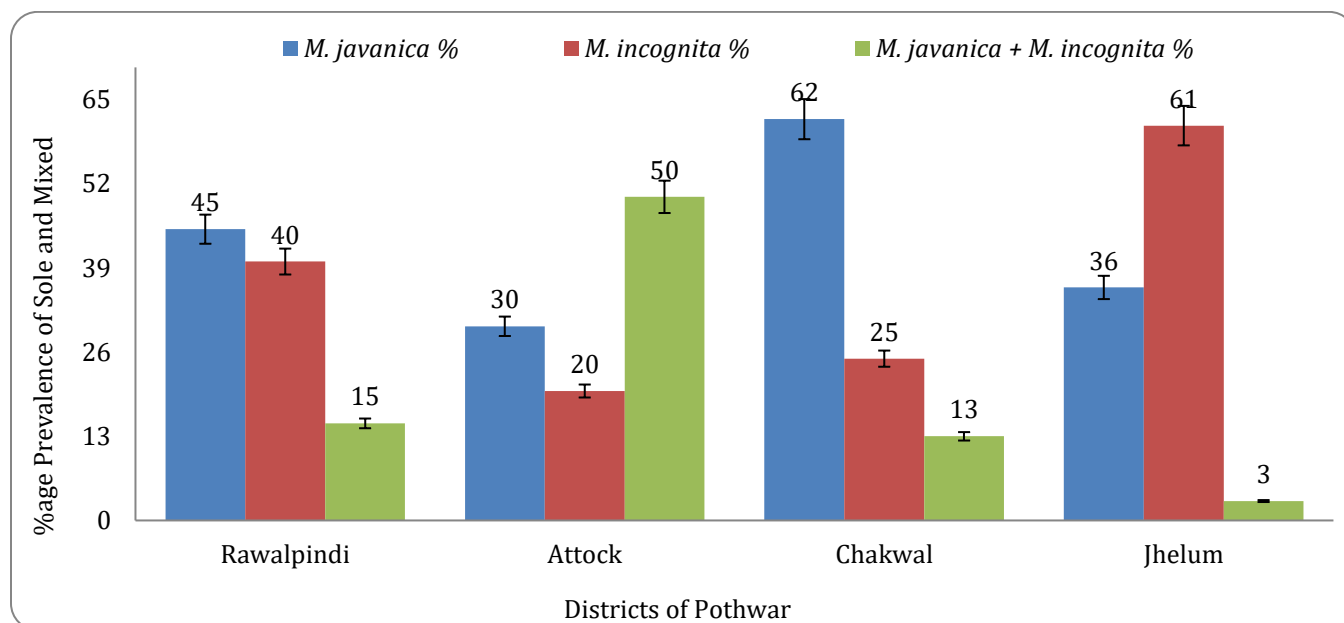
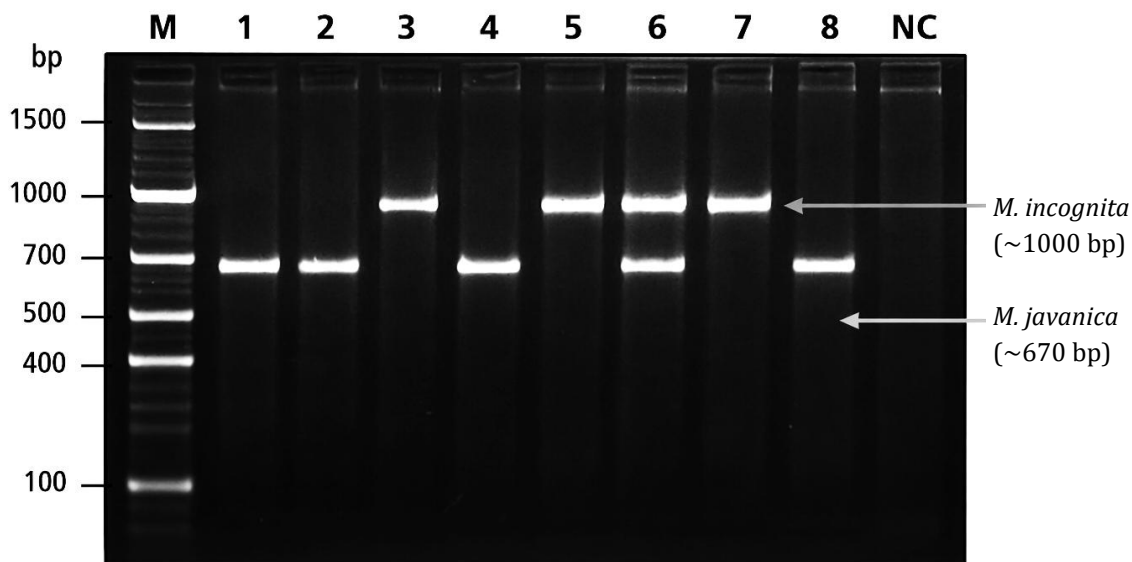


Figure 5. District wise prevalence of root-knot species in the Pothohar region.

Table 2. Variety wise occurrence of root-knot nematodes as sole and mixed populations in the four districts of Pothohar region.

| Varieties | Root-knot species | % occurrence of Root-Knot Nematodes species | | | |
|-------------------|--|---|--------|---------|--------|
| | | Rawalpindi | Attock | Chakwal | Jhelum |
| King Ruby | <i>M. incognita</i> | 8.00 | 6.00 | 7.00 | 14.00 |
| | <i>M. javanica</i> | 10.00 | 5.00 | 16.00 | 10.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 3.00 | 10.00 | 3.00 | 1.00 |
| Flame Seedless | <i>M. incognita</i> | 3.00 | 0.00 | 2.00 | 6.00 |
| | <i>M. javanica</i> | 4.00 | 3.00 | 5.00 | 4.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 0.00 | 0.00 | 1.00 | 0.00 |
| Perlette | <i>M. incognita</i> | 4.00 | 3.00 | 3.00 | 8.00 |
| | <i>M. javanica</i> | 5.00 | 4.00 | 7.00 | 5.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 2.00 | 4.00 | 1.00 | 1.00 |
| Thompson Seedless | <i>M. incognita</i> | 4.00 | 2.00 | 2.00 | 3.00 |
| | <i>M. javanica</i> | 4.00 | 3.00 | 4.00 | 4.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 2.00 | 8.00 | 0.00 | 0.00 |
| NARC Black | <i>M. incognita</i> | 9.00 | 4.00 | 8.00 | 19.00 |
| | <i>M. javanica</i> | 13.00 | 8.00 | 19.00 | 7.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 5.00 | 16.00 | 4.00 | 0.00 |
| Sughra One | <i>M. incognita</i> | 5.00 | 0.00 | 0.00 | 1.00 |
| | <i>M. javanica</i> | 3.00 | 3.00 | 0.00 | 2.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 0.00 | 4.00 | 0.00 | 1.00 |
| Vitro Black | <i>M. incognita</i> | 6.00 | 5.00 | 3 | 10.00 |
| | <i>M. javanica</i> | 7.00 | 4.00 | 11.00 | 4.00 |
| | <i>M. javanica</i> + <i>M. incognita</i> | 3.00 | 8.00 | 3.00 | 0.00 |



M: DNA ladder; 1-8: samples; NC: Negative control (no amplification)

Figure 6. Agarose gel electrophoresis showing PCR amplification of *Meloidogyne javanica* (~670 bp) and *M. incognita* (~1000 bp) using species-specific SCAR primers. M: DNA ladder; lanes 1-8: samples.

Galling Index

The highest variety-wise galling index of root-knot nematodes was recorded for NARC Black (2.5) in district Rawalpindi, while the lowest (0.5) was observed for Flame Seedless in district Jhelum (Figure 6). Among districts, the highest mean galling index (2.0) was

recorded in Attock, and the lowest (0.7) in Jhelum (Figure 7). The differences in galling index among districts and varieties were statistically significant ($p \leq 0.05$). The variety-wise and district-wise mean galling indices of root-knot nematodes are provided in Table 3.

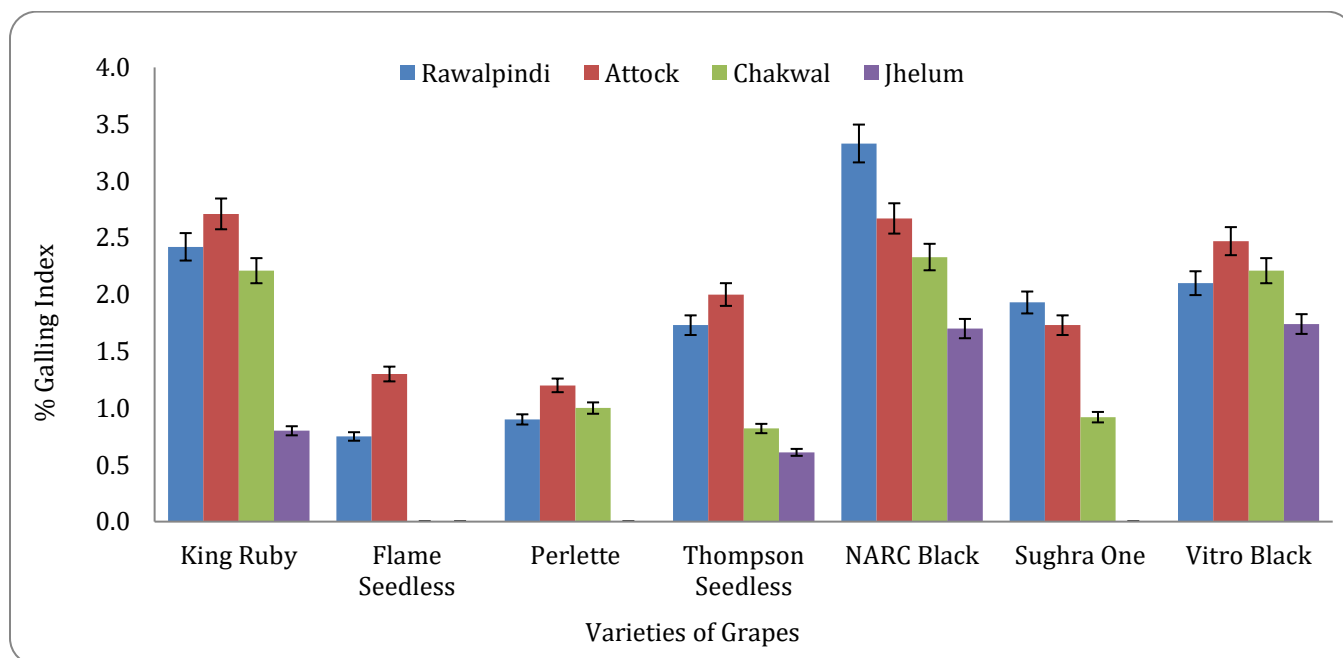


Figure 6. Variety wise galling index of root-knot nematodes in the Pothohar region.

Table 3. Variety wise galling index of root-knot nematodes in the four districts of Pothohar region.

| Variety | Variety wise galling index of root-knot nematodes in district | | | |
|-------------------|---|--------|---------|--------|
| | Rawalpindi | Attock | Chakwal | Jhelum |
| King Ruby | 2.4 | 2.7 | 2.2 | 0.8 |
| Flame Seedless | 0.8 | 1.3 | 0.0 | 0.0 |
| Perlette | 0.9 | 1.2 | 1.0 | 0.0 |
| Thompson Seedless | 1.7 | 2.0 | 0.8 | 0.6 |
| NARC Black | 3.3 | 2.7 | 2.3 | 1.7 |
| Sughra One | 1.9 | 1.7 | 0.9 | 0.0 |
| Vitro Black | 2.1 | 2.5 | 2.2 | 1.7 |

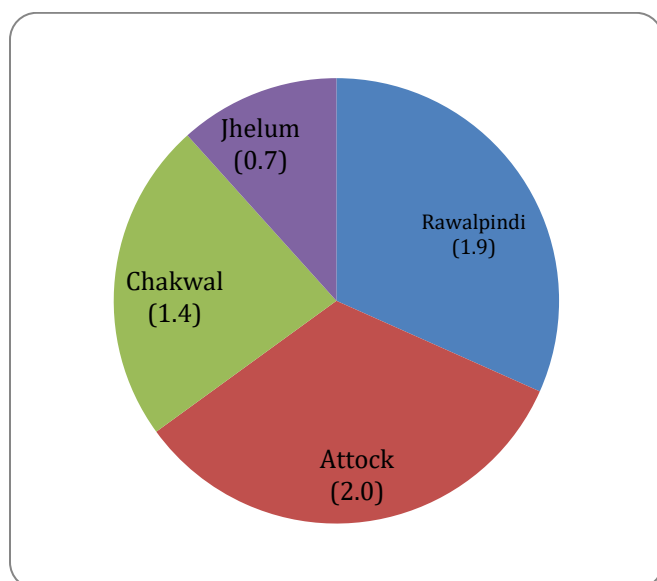


Figure 7. District wise mean galling index of root-knot nematode in the Pothohar region.

DISCUSSION

The present study revealed variations in the prevalence, incidence, severity, and species composition of root-knot nematodes across surveyed grape orchards in the Pothohar region of Punjab, Pakistan. Several studies have reported the distribution of root-knot nematodes across different regions of Pakistan on various crops, revealing consistent findings (Hussain and Mukhtar, 2019; Saeed and Mukhtar, 2024; Tariq-Khan *et al.*, 2017, 2020, 2025). These nematodes have caused significant reductions in crop productivity in these regions (Mukhtar *et al.*, 2021; Saeed *et al.*, 2023; Afzal and Mukhtar, 2024; Azeem *et al.*, 2021, 2025). The distribution of root-knot nematodes was notably influenced by both biotic and abiotic factors, resulting in observable mutations and population shifts. Environmental and edaphic conditions, such as cropping

history, soil pH, moisture, temperature, and texture, played a critical role in shaping these variations. Several studies have corroborated that fluctuations in agro-climatic and edaphic parameters significantly affect dynamics of root-knot nematodes (Van Gundy, 1985; Kayani *et al.*, 2013). These findings are in agreement with the statistically significant differences observed among districts and varieties in the present study ($p \leq 0.05$).

Among the surveyed districts, Rawalpindi recorded the highest prevalence and incidence of root-knot nematodes, while Jhelum showed the lowest. The higher infestation in Rawalpindi is attributed to several interrelated factors. One major contributor is the cultivation of grapes in areas previously or concurrently used for vegetable production, where nematode inoculum is often already present. Due to a lack of awareness and nematode management knowledge, farmers frequently transplant grapevines into infested soils without applying nematicides or raising nematode-free nursery plants. This practice significantly contributes to the high incidence and severity of root-knot nematodes in the region.

Furthermore, many grape orchards are established adjacent to vegetable plots, which serve as reservoirs for nematode inoculum. The rain-fed topography of the Pothohar region, characterized by seasonal flooding, further facilitates the dispersal of nematodes through surface runoff. The lack of farmer knowledge regarding nematode biology, transmission, and management compounds the problem, as nematodes can survive on alternate hosts such as weeds and vegetables commonly found within orchards.

Smallholder farmers in the region often lack the financial resources to implement effective nematode management strategies. The sandy loam soils prevalent in many parts of the Pothohar region are also conducive to nematode

movement and multiplication. Research has consistently shown that nematode populations are highly influenced by the type of crops grown in a given area (Cuc and Prot, 1992; Kayani *et al.*, 2013).

In contrast, the lower root-knot nematodes incidence in Jhelum may be attributed to its clay-rich soil texture and soil pH ranging from 6.0 to 7.5. The fine and cohesive nature of clay particles restricts nematode mobility, thereby reducing infestation. However, other factors such as crop management practices, irrigation patterns, and the use of infected planting material may also contribute to these variations and require further investigation. Moreover, the lower intensity of vegetable cultivation in Jhelum minimizes the availability of alternative hosts and inoculum sources.

The study also identified varietal differences in root-knot nematodes susceptibility. The NARC Black grape variety showed the highest incidence and prevalence, whereas Sughra One and Flame Seedless exhibited the lowest, likely due to genetic resistance. Resistant cultivars can prevent nematode penetration or, if penetration occurs, restrict nematode development and reproduction within roots.

Two root-knot nematodes species were detected in the surveyed orchards: *Meloidogyne incognita* and *M. javanica*. Overall, *M. javanica* was more prevalent and was the dominant species across all districts, whereas *M. incognita* was more frequently detected in Jhelum. These findings are consistent with global reports identifying *M. incognita* and *M. javanica* as the most widespread *Meloidogyne* species (Trudgill *et al.*, 2000). Similar results have been reported by various researchers worldwide (Bhosle *et al.*, 2004; Rathour *et al.*, 2006). The current study also aligns with earlier reports of *M. incognita* and *M. javanica* distribution in Pakistani soils at 52% and 31%, respectively (Maqbool *et al.*, 1992).

Molecular characterization using species-specific SCAR markers further confirmed the presence of *Meloidogyne incognita* and *M. javanica*, supporting the accuracy of morphological identification. The use of molecular tools enhances the reliability of species identification, particularly in cases where morphological variations may lead to misidentification.

The distribution and establishment of these species across the Pothohar region are influenced by soil characteristics, environmental conditions, crop rotation patterns, and host suitability (Ploeg and Maris, 1999). The relatively lower infestation in Jhelum is likely due to unfavorable environmental conditions for nematode

penetration, reproduction, and development, especially for *M. incognita* (Taylor *et al.*, 1982).

Regarding species distribution, *M. incognita* was found alone in 36.5% of the infested orchards, *M. javanica* alone in 43.25%, and both species co-occurring in 20.25% of the orchards. Similar mixed populations of root-knot nematodes have been reported in Morocco, Iran, and Spain (Nico *et al.*, 2002; Sanei and Okhovvat, 2011; Hamza *et al.*, 2018). The dominance of *M. javanica*, whether alone or in mixed infections, may suggest a competitive advantage over *M. incognita* in this region (Eisenback and Griffin, 1987).

Although the study provides valuable insights into the distribution and species composition of root-knot nematodes in the Pothohar region, it is limited by the relatively small sample size and the absence of seasonal population dynamics. Further studies incorporating long-term monitoring and additional molecular markers are recommended.

CONCLUSION

The survey revealed a prevalence (60.35%) and incidence (19.9%) of root-knot nematodes in grape orchards across the Pothohar region, with Rawalpindi exhibiting the highest and Jhelum the lowest infestation levels. Among the seven grape varieties encountered, NARC Black showed the highest susceptibility, while Flame Seedless and Sughra One exhibited the lowest incidence and prevalence, respectively. Two root-knot nematode species, *Meloidogyne javanica* and *M. incognita*, were identified, with *M. javanica* being the more dominant species in most districts, except Jhelum. Mixed populations were also commonly observed. The galling index mirrored the incidence pattern, with NARC Black and Rawalpindi showing the highest galling severity. These findings provide a baseline for the development of targeted nematode management strategies and support the expansion of grape cultivation in the Pothohar region under sustainable production systems.

RECOMMENDATIONS

To mitigate nematode damage under the agro-ecological conditions of the Pothohar region, it is recommended to adopt resistant or less susceptible varieties such as Flame Seedless and Sughra One, which exhibited lower incidence and prevalence in the present study. Crop rotation with non-host plants, use of certified nematode-free planting material, and application of organic amendments or

biological control agents are also recommended to suppress nematode populations. Regular monitoring and early detection through nematode surveillance should be integrated into vineyard management practices. An integrated nematode management approach combining cultural, biological, and host resistance strategies is essential for effective and sustainable control.

FUTURE DIRECTIONS

Future research should focus on screening and developing resistant grape cultivars and understanding host-nematode interactions at the molecular level, particularly using advanced molecular markers and genomic tools for accurate species identification and resistance breeding.

Additionally, the efficacy of eco-friendly nematode management strategies, including biocontrol agents and organic amendments, should be evaluated under field conditions across different seasons and agro-climatic conditions to ensure sustainable grape production in the region. Such research will support the development of sustainable and region-specific nematode management strategies for grape production in Pakistan.

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AUTHORS' CONTRIBUTIONS

MTAK and TM conceptualized the study and designed the methodology. MTAK conducted the survey, collected root-knot nematode-infested samples, and processed them. MTAK and TM identified the nematodes and compiled the data. GI assisted with data collection and nematode identification, while MJA contributed to the molecular studies. MTAK drafted the manuscript, and TM and GI supervised the work. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

- Afzal, A. and T. Mukhtar. 2024. Revolutionizing nematode management to achieve global food security goals - An overview. *Heliyon*, 10: e25325.
- Ahmed, H. M., M. Ashfaq, T. Mukhtar and M. A. Khan. 2021. Categorization of available cucumber genotypes against Zucchini yellow mosaic virus and root-knot nematode (*Meloidogyne incognita*). *International Journal of Agriculture & Biology*, 25(5): 955-961.
- Asghar, A., T. Mukhtar, M. U. Raja and A. Gulzar. 2020. Interaction between *Meloidogyne javanica* and *Ralstonia solanacearum* in chili. *Pakistan Journal of Zoology*, 52(4): 1525-1530.
- Azeem, W., T. Mukhtar and T. Hamid. 2021. Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in tomato. *Pakistan Journal of Zoology*, 53(1): 119-125.
- Azeem, W., T. Mukhtar, M. I. Haq, M. A. Khan, M. S. Ibrahim, A. Hassan, H. Regmi and L. W. Duncan. 2025. The nematicidal potential of *Moringa oleifera* extracts and rhizobacteria against *Meloidogyne incognita* in tomato. *Frontiers in Plant Science*, 16: 1562074.
- Bavaresco, L. 2019. Impact of grapevine breeding for disease resistance on the global wine industry. *Acta Horticulturae*, 7-13.
- Bhosle, B. B., M. Sehgal and S. N. Puri. 2004. Prevalence of phytophagous nematodes in rhizosphere of okra (*Abelmoschus esculentus* L. Moench) in Parbhani District, Maharashtra, India. *Indian Journal of Nematology*, 34(1): 56-59.
- Bridge, J. and S. L. J. Page. 1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. *International Journal of Pest Management*, 26(3): 296-298.
- Cuc, N. T. T. and J. C. Prot. 1992. Effect of changing the agricultural environment on ufra occurrence in the Mekong delta. *International Rice Research Newsletter*, 17(2): 25.
- Daware, S. K., D. B. Kadam and P. R. Palande. 2022. Identification of the race of root knot nematode by differential host test method. *Environment Conservation Journal*, 23: 31-34.
- Eisenback, J. D. and G. D. Griffin. 1987. Interactions with

- other nematodes. In: Veech, J. A. and G. W. Dickson (Eds.), *Vistas on Nematology*. Society of Nematologists, Inc, Hyattsville, MD, USA, pp. 313-320.
- Esmenjaud, D., M. Y. Banora, U. Julien-Portier, M. D. D. Lafargue, J. P. Tandonnet, B. Rubio, A. Walker, C. V. Ghelder and N. Ollat. 2023. A muscadine locus confers resistance to predominant species of grapevine root-knot nematodes (*Meloidogyne* spp.) including virulent populations. *Oeno One*, 57: 219-232.
- Gagnier, B. K., I. A. Zasada and M. M. Moyer. 2024. Impact of vineyard fallow practices on reducing *Meloidogyne hapla* population densities. *Plant Health Progress*, 25: 446-453.
- Gondal, A. S., N. Javed, S. A. Khan and S. Hyder. 2012. Genotypic diversity of potato germplasm against root-knot nematode (*Meloidogyne incognita*) infection in Pakistan. *International Journal of Phytopathology*, 1(1): 27-38.
- Munawar, M., S. A. Khan, N. Javed, I. U. Haq and A. S. Gondal. 2015. Bio-management of tomato wilt complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. *Nematology*, 17(4): 479-485.
- Gurak, P. D., I. Santana, A. P. Gil, S. P. Freitas, M. H. Rocha-Leão and L. Cabral. 2012. Grapes: Cultivation, varieties and nutritional uses. *Grapes: Cultivation, Varieties and Nutritional Uses*, pp. 201-247.
- Hamza, M. A., A. Moukhli, Z. Ferji, O. Fossati-Gaschnard, J. Tavoillot, N. Ali and T. Mateille. 2018. Diversity of plant-parasitic nematode communities associated with olive nurseries in Morocco: Origin and environmental impacts. *Applied Soil Ecology*, 124: 7-16.
- Haq, M. A., T. Mukhtar, M. I. Haq and A. Khalid. 2022. Reproduction of root-knot nematode, *Meloidogyne incognita*, on *Solanum melongena* genotypes determines their host status. *Pakistan Journal of Zoology*, 54(5): 2097-2103.
- Hübschen, J., L. Kling, U. Ipach, V. Zinkernagel, N. Bosselut, D. Esmenjaud and R. Neilson. 2004. Validation of the specificity and sensitivity of species-specific primers that provide a reliable molecular diagnostic for *Xiphinema diversicaudatum*, *X. index* and *X. vuittenezi*. *European Journal of Plant Pathology*, 110: 779-788.
- Hussain, M. A. and T. Mukhtar. 2019. Root-knot nematodes infecting okra in major vegetable growing districts of Punjab, Pakistan. *Pakistan Journal of Zoology*, 51(3): 1137-1143.
- Kayani, M. Z. and T. Mukhtar. 2018. Reproductivity of *Meloidogyne incognita* on fifteen cucumber cultivars. *Pakistan Journal of Zoology*, 50(5): 1717-1722.
- Kayani, M. Z., T. Mukhtar and M. A. Hussain. 2017. Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. *Crop Protection*, 92: 207-212.
- Kayani, M. Z., T. Mukhtar, M. A. Hussain, and M. I. Haque. 2013. Infestation assessment of root-knot nematodes (*Meloidogyne* spp.) associated with cucumber in the Pothohar region of Pakistan. *Crop Protection*, 47: 49-54.
- Khaliq, U. A., I. Ali, A. Ghazy, M. T. Akram, H. Ali, U. Habib, T. Hussain, K. A. Attia, S. Fiaz, A. A. Al-Doss, M. Uzair, A. Anwar and R. M. M. Naz. 2025. Comprehensive ampelographic and biochemical profiling of exotic grapevine (*Vitis vinifera* L.) cultivars grown under semi-arid conditions. *Genetic Resources and Crop Evolution*, 1-20.
- Khana, A. S., S. Ahmad and M. Amjad. 2013. Prospects and challenges of organic fruit production in Pakistan. *Acta Horticulturae*, 57-64.
- Knoetze, R. 2020. The effect of hot water treatment of rooted grapevine nursery stock on the survival of the root-knot nematode, *Meloidogyne javanica* (Nematoda: Heteroderidae). *South African Journal of Enology and Viticulture*, 41: 51-55.
- Li, J., G. Xu, M. Ren, J. Du, P. Gu, J. He. 2020. Symptoms, damage degrees and species of root-knot nematodes in Ningxia grapevine nursery. *Journal of Plant Protection*, 47: 666-678.
- Liu, W., F. Ping, X. Bai, Y. Fang, S. Dong, J. Yang, C. Yuan and J. Lai. 2022. Status and prospect of resource utilization for grape vine stalks. *Nongye Gongcheng Xuebao / Transactions of the Chinese Society of Agricultural Engineering*, 38: 270-283.
- Maqbool, M. A., B. Zarina, K. Nasira and K. Firoza. 1992. Distribution and host association of plant parasitic nematodes in Pakistan.
- McKenzie, M. and R. Pathirana. 2007. Micrografting grapevine for virus indexing. In: *Protocols for Micropropagation of Woody Trees and Fruits*, pp. 259-266.
- Meng, Q., H. Long and J. Xu 2004. PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *Acta Phytopathologica Sinica*, 34: 204-210

- Mukhtar, T. and M. A. Hussain. 2019. Pathogenic potential of Javanese root-knot nematode on susceptible and resistant okra cultivars. *Pakistan Journal of Zoology*, 51(5): 1891-1897.
- Mukhtar, T. and M. Z. Kayani. 2019. Growth and yield responses of fifteen cucumber cultivars to root-knot nematode (*Meloidogyne incognita*). *Acta Scientiarum Polonorum Hortorum Cultus*, 18(3): 45-52.
- Mukhtar, T. and M. Z. Kayani. 2020. Comparison of the damaging effects of *Meloidogyne incognita* on a resistant and susceptible cultivar of cucumber. *Bragantia*, 79(1): 83-93.
- Mukhtar, T., M. Arooj, M. Ashfaq and A. Gulzar. 2017. Resistance evaluation and host status of selected green gram genotypes against *Meloidogyne incognita*. *Crop Protection*, 92: 198-202.
- Mukhtar, T., M. Tariq-Khan and M. N. Aslam. 2021. Bioefficacy of *Trichoderma* species against Javanese root-knot nematode, *Meloidogyne javanica* in green gram. *Gesunde Pflanzen*, 73(3): 265-272.
- Nico, A. I., H. F. Rapoport, R. M. Jiménez-Díaz and P. Castillo. 2002. Incidence and population density of plant-parasitic nematodes associated with olive planting stocks at nurseries in southern Spain. *Plant Disease*, 86(10): 1075-1079.
- Nour El-Deen, A. H., A. S. Alghamdi, A. F. Al-Barty, H. Y. Darwish, B. N. Samra, A. Al-Qurashi, K. Al-Malki and H. Al Osaimi. 2018. Management of root-knot nematode, *Meloidogyne incognita* by novel bio-methods on grapevine. *Bioscience Research*, 15: 3033-3039.
- Ploeg, A. and P. Maris. 1999. Effects of temperature on the duration of the life cycle of a *Meloidogyne incognita* population. *Nematology*, 1(4): 389-393.
- Rathour, K. S., J. Pandey and S. Ganguly. 2006. Community structure of plant parasitic nematodes in Champawat District of Uttaranchal, India. *Indian Journal of Nematology*, 36(1): 89-93.
- Saeed, M. and T. Mukhtar. 2024. Root-knot nematodes (*Meloidogyne* spp.) infecting peach (*Prunus persica* L.) in the Pothwar region of Pakistan. *Journal of Agricultural Science and Technology*, 26(4): 897-908.
- Saeed, M., T. Mukhtar, R. Ahmed, T. Ahmad and M. A. Iqbal. 2023. Suppression of *Meloidogyne javanica* infection in peach (*Prunus persica* (L.) Batsch) using fungal biocontrol agents. *Sustainability*, 15(18): 13833.
- Sanei, S. and S. Okhovvat. 2011. Incidence of plant-parasitic nematodes associated with olive planting stocks at nurseries in northern Iran. *International Journal of Applied*, 1: 79-82.
- Smith, B. P., N. B. Morales, M. R. Thomas and H. M. Smith. 2017. Grapevine rootstocks resistant to the root-knot nematode *Meloidogyne javanica*. *Australian Journal of Grape and Wine Research*, 23: 125-131.
- Smith, H. M., B. P. Smith, N. B. Morales, S. Moskwa, P. R. Clingeleffer and M. R. Thomas. 2018. SNP markers tightly linked to root knot nematode resistance in grapevine (*Vitis cinerea*) identified by a genotyping-by-sequencing approach followed by Sequenom MassARRAY validation. *PLoS ONE*, 13: e0201889.
- Somavilla, L., C. B. Gomes and V. M. Quecini. 2012. *Meloidogyne incognita* report on 'IAC 766-Campinas' rootstock in the State of Pernambuco and reaction of grape rootstocks and cultivars to *Meloidogyne* spp. *Revista Brasileira de Fruticultura*, 34: 750-756.
- Soneji, J. R. and M. Nageswara-Rao. 2011. *Vitis*, Wild Crop Relatives: Genomic and Breeding Resources: Temperate Fruits, pp. 223-239.
- Tariq-Khan, M., A. Munir, T. Mukhtar, J. Hallmann and H. Heuer. 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. *Journal of Plant Diseases and Protection*, 124(3): 201-212.
- Tariq-Khan, M., T. Mukhtar, A. Munir, J. Hallmann and H. Heuer. 2020. Comprehensive report on the prevalence of root-knot nematodes in the Poonch division of Azad Jammu and Kashmir, Pakistan. *Journal of Phytopathology*, 168: 322-336.
- Tariq-Khan, M., T. U. Rehman, T. Mukhtar, M. Mahmood, A. U. Rahman and R. Ahmed. 2025. Population dynamics and virulence patterns of root-knot nematodes (*Meloidogyne* spp.) on tomato in Poonch Highlands, Azad Jammu and Kashmir, Pakistan. *Journal of Phytopathology*, 173: e70060.
- Taylor, A. L., J. N. Sasser and L. A. Nelson. 1982. Relationship of climate and soil characteristics to geographical distribution of *Meloidogyne* species in agricultural soils. *International Meloidogyne Project*, pp. vi-65.

- Taylor, D. P. and C. Netscher. 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. *Nematologica*, 20: 268-269.
- Trudgill, D. L., G. Bala, V. C. Blok, A. Daudi, K. G. Davies, S. R. Gowen, et al. 2000. The importance of tropical root-knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent. *Nematology*, 2(8): 823-845.
- Van Gundy, S. D. 1985. Ecology of *Meloidogyne* spp. Emphasis on environmental factors affecting survival and pathogenicity. In: Sasser, J. N., Carter, C. C. (Eds.), *An Advanced Treatise on Meloidogyne. Biology and Control*, vol. 1. North Carolina State University Graphics, Raleigh, North Carolina, pp. 177-182.
- Wang, F., L. Zhang, Y. Du and H. Zhai. 2014. Research on physiological and biochemical reaction of susceptible grape infected by root-knot nematode. *Acta Horticulturae*, 83-88.
- Wann, S. B., B. Borah, R. Ahmed, B. Gogoi, P. Phukon, J. Baruah, D. K. Sharma and B. S. Bhau. 2016. Isolation, characterization of nematode-controlling bacteria and fungi from nature. In: *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*, pp. 271-295.
- Williams, L. E. 2017. Grape. In: *Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships*, pp. 851-882.
- Yaseen, I. and T. Mukhtar. 2024. Impact of sequential and concurrent inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on the growth performance of diverse okra cultivars. *Plant Protection*, 8(2): 303-313.
- Yaseen, I., T. Mukhtar, A. Mubarik, B. Arshad, N. Sahu and U. Somaddar. 2025. Management of *Ralstonia solanacearum*-*Meloidogyne incognita* complex with *Trichoderma harzianum* in tomato. *Bragantia*, 84: e20240060.
- Yaseen, I., T. Mukhtar, H. T. Kim and B. Arshad. 2023. Quantification of resistance to *Meloidogyne incognita* in okra cultivars using linear and nonlinear analyses of growth parameters and nematode infestations. *Bragantia*, 82: e20230114.
- Yaseen, I., T. Mukhtar, H. T. Kim and B. Arshad. 2024. Interactive effects of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on okra cultivars. *Bragantia*, 83: e20230266.
- Zijlstra, C., D. T. Donkers-Venne and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology*, 2(8): 847-853.

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