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An overview of root-knot nematodes and their management

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Abstract
Root-knot nematode is a serious pest of various plants worldwide. It infests more than three thousand plant species. In Pakistan, root-knot is a major pest of vegetable, fruit trees, and ornamentals. Among the vegetables, tomatoes, eggplants and potatoes are severely infested by this pest. Moreover, this pest is responsible for causing up to 60-90% yield losses in potatoes, tomatoes and brinjal. The nematode is a sedentary and semi-endo parasite and causes galls in the roots. As a result, the transportation of water and nutrients to the shoots of plants is severely affected. The nematode is controlled by various control measures, including growing of resistant crops, by applying pesticides and biocontrol agents (BCAs) and by cultural practices. However, none of this control measure is purely effective against root-knot nematode. But nowadays novel approaches have been used to manage this pest. Keeping in view the importance of this pest, the present review is written to update information about the biology, epidemiology, symptomology and recent management strategies of this pest.

Keywords: Root-knot nematode, galls, biology, epidemiology, symptomology, control

Introduction
The root-knot nematode is a major pest of vegetables in the world and Pakistan [1]. This nematode was first discovered in England in a greenhouse on cucumber and in Holland [2, 3]. By the turn of the century, this pest was known in several areas of Western Europe. It attacks many plants of economic importance and known hosts include 3,000 plant species [4, 5]. Singh [6] reported that RKN imposes an important problem in cultivated fields as its infection leads to roots' malfunctioning. Brown [7] was the first scientist who conducted a survey of some plant-parasitic nematode problems in Pakistan and reported a complex disease of tomato of papaya caused by RKN in association with soil fungi. Several research workers carried out a survey and assessed the disease infestation and association of different species of RKN. Saeed *et al*. [8] carried out a survey and recorded several plant-parasitic nematodes, including *Meloidogyne* spp. Maqbool [9] reported the occurrence of RKN from Pakistan, and bottle gourd, coleus, oxalis, cucumber, jute, mint, tuberose, water melon was infected with *Meloidogyne* spp [10]. These nematodes' associations with fungi and bacteria cause disease complex to rank them high on the list of disease-causing agents affecting agricultural productivity [11]. Based on geographical distribution and infestation of plant-parasitic nematodes on vegetables and fruit, crop losses were estimated by the number of research workers in Pakistan and the world as well. Duncan and Noling [12] developed the methodology for determining damage functions and crop losses. Sasser [13] estimated 29% annual crop losses in the tropics. Lamberti [14] reported 50-60% losses in tomato and eggplant due to RKN. Bhatti and Jain [15] estimated a loss of 46.2% in tomato due to *M. incognita*. In areas where RKN are not controlled, average crop yield losses are estimated to be in the neighbourhood of 25% with damage in individual fields ranging as high as 60% [16]. Koenning *et al*. [17] estimated 4-8% average yield losses for any solanaceous crop. Alam and Jairajpuri [18] noted that nematodes are responsible for causing up to 70-90% yield losses in tomato and brinjal. In the warmer, drier areas with sandier soils, losses of up to 95% were reported in tomato crops [19]. On account of alarming losses caused by diseases, it requires immediate and due attention to minimize its predation. In this study, lettuce was assessed for its potential as an accurate and efficient indicator of *Meloidogyne* root galling. The number of days following germination required for lettuce to exhibit 70-80% galling acted as a measurement of its efficiency as an indicator.

Galling increased dramatically after 42 days, with 70-80% galling being reached on the 45th day. These results confirm that lettuce can function as a rapid indicator of nematode galling and shorten the screening time from 45-90 days [20]. The occurrence of RKN with the various field, vegetable crops, fruit trees, and ornamentals in Pakistan and other countries has been reported [21, 22]. *Meloidogyne* species are highly specialized plant-parasitic nematodes, which root galls of variable size. This group is one of the most intensively investigated among plant-parasitic nematodes. Haider *et al.* [23] surveyed in 2004 from eggplant fields in Damascus's rural area and collected 65 samples showing that 28 samples (43.07%) had *Meloidogyne* species. Other plant-parasitic nematodes identified were: *Tylenchorhynchus, Pratylenchus, Paratylenchus, Helicotylenchus, Ditylenchus, Rotylenchus, Longidorus, Xiphenema, Aphelenchus, Aphelenchoides,* and *Tylenchus.* Also, free-living nematodes such as *Cephalobus, Eucephalobus, Panagrolaimus, Chiloplacus, Rhabdetis, Rhabdtophora, Dorylaimus, Eudorylaimus, Acrobolus, Pelodera, Monhystera, Mononchus, Aporcelaimus* were identified. There are useful fungi at the eggplant's rhizosphere, the endomycorrhizal fungi, improving production and increasing plant resistance to some diseases. Testing 84 samples collected from eggplant fields in Rural areas around Damascus in 2004 showed six genera of endomycorrhizal fungi, namely *Glomus, Gigaspora*, and *Acaulospora, Endogone, Entraphospora*, and *Modicella. Glomus* detected in 82 samples (97.61%), followed by the genus Gigaspora detected in 71 samples (84.52%). The present study suggests that growing resistant varieties or moderately resistant brinjal varieties can substantially reduce nematodes populations in infested soils. Furthermore, resistant varieties may release antimicrobial or nematicidal compounds, which may directly contribute to the nematode suppression mechanism or otherwise may induce systemic acquired resistance (SAR) in plants.

Epidemiology

The root-knot nematode was first discovered by Berkley [2] in 1885 on cucumber in greenhouses of the UK. It is parasitic on many plant species. The world's food production is greatly affected by plant-parasitic root-knot nematodes [24]. They are most prevalent in warmer and moderate warmer regions of the world, where summer is more prolonged than winter. According to Sasser, crop losses, root-knot nematodes in prominent geographical areas of the tropics ranged from 5-43%. Plant-parasitic root-knot nematodes have extensive host range and distribution [25]. Abad *et al*. [4] elucidated that the host range of *Meloidogyne* spp. is about 3000 plant species. The damages due to root-knot nematodes that varied from 22-80%; however, global losses have been reported up to five hundred million dollars [26]. Kayani *et al*. [27] investigated root-knot nematodes, *Meloidogyne* spp. in cucumber infested fields in district Rawalpindi and reported a maximum disease incidence of 31.11% in tehsil Taxila followed by 26.11% in Murree. In overall distribution, *M. incognita* (74.17%) was the most prevalent species, followed by *M. javanica* (21.69%), *M. hapla* (1.81), and *M. arenaria* (2.33%). Hussain *et al.* [28] surveyed 17 districts of Punjab's vegetable growing area to find prevalence. The intensity of root-knot nematodes in okra cultivations. Their results revealed that root-knot nematodes prevailed in 85.2% of okra fields with an average incidence of 38.89%. Prevalence was recorded 100% in Multan, Okara, Dera Ghazi Khan, Bahawalnagar, Vehari, Rahim Yar Khan, and Rawalpindi districts, and the lowest incidence of 22.4% was recorded in district Lodhran. The incidence was above 60% in Bahawalnagar, Rahim Yar Khan, Dera Ghazi Khan, and Vehari. *M. incognita* occurred with the highest frequency (74.74%), followed by *M. javanica* (24.02%), *M. arenaria* (24.02%), and *M. hapla* (0.78%). Root-knot nematodes reside in soils and cannot move more than one meter in the soils within their lifetime. Their movement from one field to another or within the same area is aided by any agencies that disperse plant parts and soil particles, including; farm equipment, muddy shoes contaminated with nematode infested soil, water during floods, and irrigation [29]. If plant quarantine is not well observed, then the seeds, infected plants, and bulbs disperse internationally [30]. The nematodes can survive in adverse conditions and can make their dissemination even in the absence of water moisture blown by winds or survive in plant debris and through birds [31].

Biology

Females of root-knot nematodes lay eggs on plant material or in soil. Temperature determines the eggs hatching without requiring stimulus from plant roots, whereas hatching sometimes encourages root diffusates [32]. The root-knot nematode has four larval stages, J_2s hatch as a vermiform second stage from the eggs. The first moult occurs inside the egg of nematodes [33]. In the soil, freshly hatched juveniles have a short free-living stage and the host plant's rhizosphere. The juveniles enter the plant through the root tips and feed on the plant cells. The adjacent tissue of root stretches increases to a gall in which the developing juvenile is entrenched. After further feeding, the juvenile undergoes morphological changes and moult three times and finally developed into an adult. The life cycle of root-knot nematodes is 4 to 8 weeks, depending on temperatures, and an adult female may produce up to 2000 eggs [34].

Host range

Meloidogyne spp. have a pervasive host range, and cultivators with a root-knot nematode problem found it challenging to manage the nematode through crop rotation either due to this extensive host range [35]. Over 80 species of *Meloidogyne* have been described worldwide. Three of them are incredibly polyphagous apomictic species namely, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood (Karajeh, 2015). Species like *M. incognita*, *M. arenaria* race 1, and *M. javanica* can infect or survive on alternate hosts of the same fields. There is also a high degree of specialization of different variances in pathogenicity on the precise crop [36].

Symptomology

Root-knot nematodes are sedentary, polyphagous, and semi-endo parasites [37]. It parasitizes all higher plants and an inclusive range of products ranging up to two thousand plant species [38]. They pierce plants roam intercellularly, and developing galls on roots (Fig 1). In solitary cropping seasons, *Meloidogyne* spp. has many generations, and the plants are inhibited from water and nutrient uptake. They pass on a disease to various parts of plants such as taproots and tubers and lowering the economic and qualitative values of vegetable crops [39]. Yellowing of plant leaves and stunting in growth are above-ground symptoms of root-knot nematodes [37]. Wilting occurred due to xylem vessel blockage. The root tissue is exposed by root-knot nematodes to other pathogens, viz., bacteria, fungus, and significant losses increased [40]. Severe yield losses have been caused by nematodes in warmer to moderately warmer parallel to a less warm region of the worlds [37].

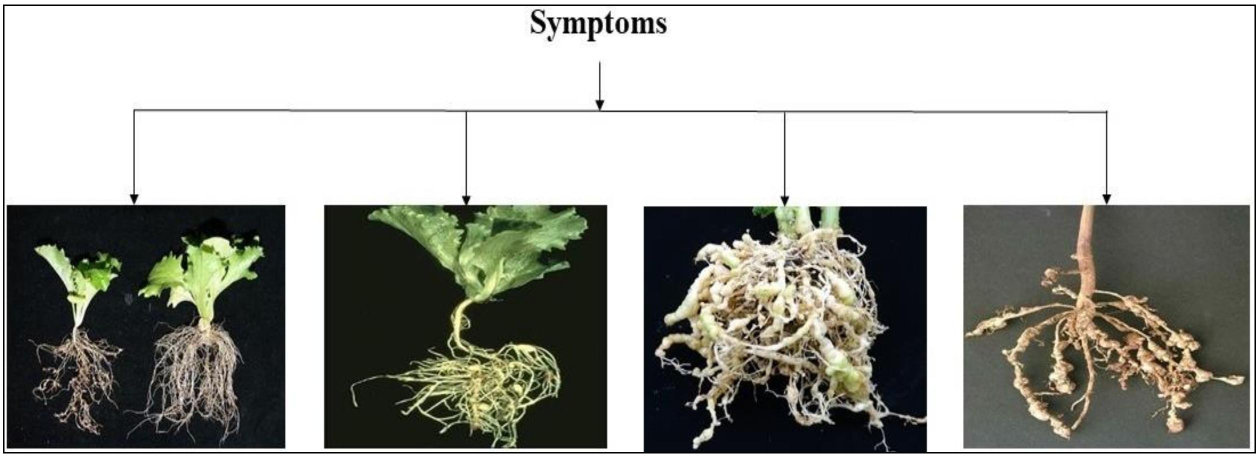

Fig 1: Characteristic disease symptoms of Root Knot Nematode

Management

The following methods help to manage the RKNs (Fig. 2);

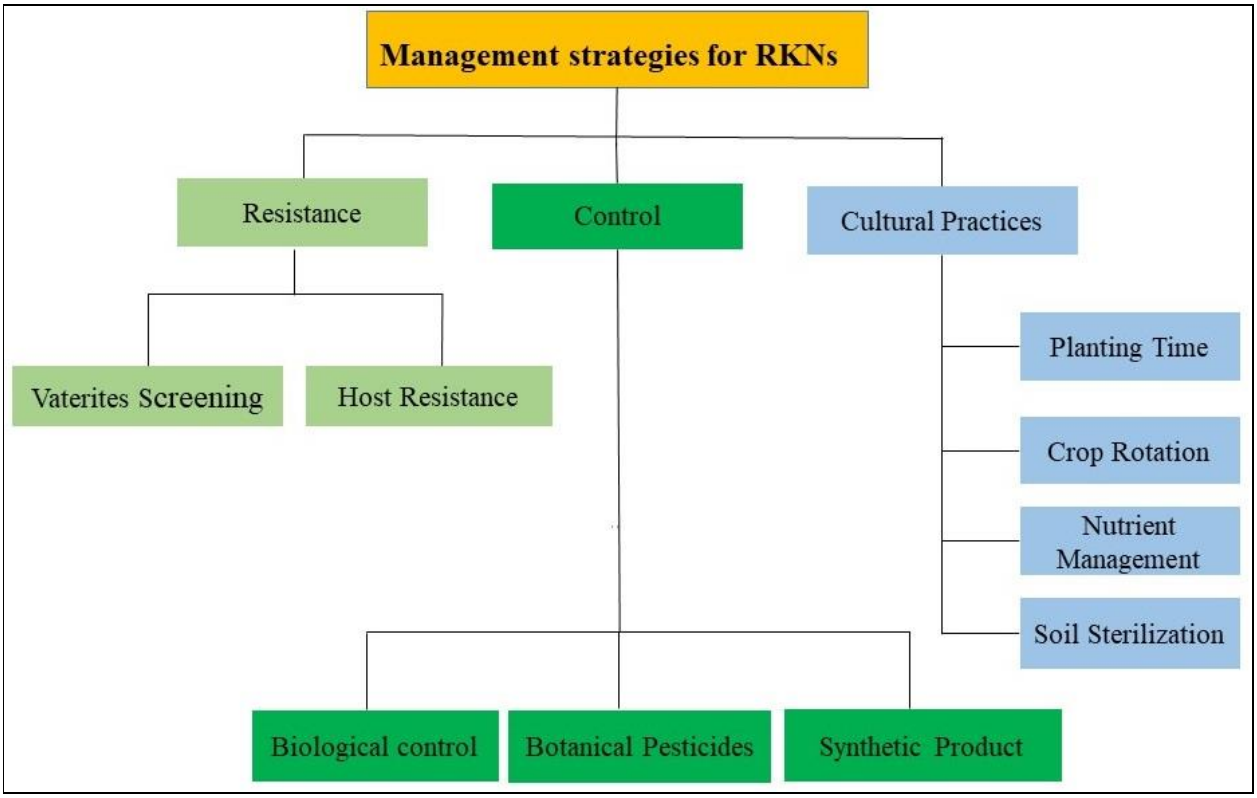

Fig 2: Root-knot nematodes (RKNs) Management

Cultural control

These include various manipulative practices to reduce nematode populations without the use of nematicides. Leaving the soil fallow significantly reduces most nematode populations, especially during hot, dry weather, but is not economical. The planting time can be significant if the crop is such that it can be planted early or late when the nematodes are inactive due to temperature or other conditions [41]. The growing of non-host crops for one or more years, depending on the degree of the infestation, also promises the control of plant-parasitic nematodes (Maqbool, 1988). There are certain oilcake and other organic materials available that reduce nematode infestation, improve plant growth, and increase yield significantly when added into the soil before sowing [42].

Chemical control

The use of nematicides for the control of nematode diseases is a widespread practice. Chemical control has been proved to be a generally useful and reliable means of checking a wide variety of plant-parasitic nematodes [7]. Two nematicides, viz., Furadan and Tenakil, are available in the local market and frequently used for plant-parasitic nematodes control [43].

Indiscriminate use of these nematicides causes soil and environmental pollution, and farmers are not careful in using these nematicides and other toxic chemicals. Chemical control is becoming more and more expensive because of increased costs in the synthesis of new compounds. Their use is increasingly undesirable because of their application's environmental hazards [44].

Biological control

Biological control of plant-parasitic nematodes aims at the use of natural enemies as a safe and cheap alternative method to chemical control [45, 46]. Encouraging results were obtained with the use of *Pasteuria penetrans* and *Paecilomyces lilacinus* as biological control agents of nematodes on different crops [47]. Species of *Trichoderma* viz., *T. harzianum,* and *T. viride* have shown nematicidal sound effects on eggs and J_2s of *M. javanica in vitro* conditions [48]. Different inoculum densities of *T. harzianum* and *T. viride* against *M. javanica* on tomato suppressed nematode reproduction and root galling. The efficacy of both fungi increased as their inoculum densities increased [49]. Biocontrol isolates can compete and persist in the environment, rapidly colonize, and efficiently proliferate on newly formed roots [50].

Botanical pesticides

More than 100 plant species have been screened for their nematicidal properties, some of which have shown promising results to kill root-knot nematodes. These *Calotropis procera, Euphorbia caducifolia, Nerium oleander,* and *Azadirachta indica* have exhibited inhibitory effects against root-knot juveniles [51, 52]. Likewise, crude extracts from the stem and roots of different weed species, such as *Fumaria parviflora*, have suppressed eggs and J_2s of *M. incognita* on tomato [53]. Root extracts of *F. parviflora* revealed several nematicidal phytochemicals such as alcohol, sterol, and alkaloids. These compounds have remarkably reduced root galling and nematode reproduction in tomato roots. For example, some of these compounds, Nonacosane-10-ol and 23α- Homostigmast-en-3*β*-ol, showed synergistic effects on J_2s of *M. incognita* in tomato [53].

Host resistance and varietal screening

Di Vito *et al*. [54] experimented with evaluating the association between *M. incognita* and eggplant yield. Finely chopped nematodes infected pepper roots were added at population densities of 0.062, 0, 0.125, 0.25, 0.50, 2, 4, 8, 16, 32, 50, 64 and 128 eggs and juveniles/cm^3. Nematodes repressed the growth parameters and yield productivity. Results revealed that the maximum degree of reproduction of nematodes was 12,300. However, eggs' viability was greatly significant in water (58%) compared to sodium hypochlorite (12%) for four weeks. Maximum nematodes were collected from brinjal roots when infested soil with egg masses compared to infested soils with sodium hypochlorite dissolved egg masses. Khan *et al*. [55] experimented with studying the effect of different inoculum levels (2000, 4000, and 6000 eggs of *Meloidogyne* spp.) and plant age on tomato roots. Five weeks nursery transplanted showed slightly significant reduction as compared to three to four weeks old nursery. Increasing the inoculum level caused a reduction in plant growth parameters, whereas increased gallons, galling index, and egg masses. Zia *et al*. [56] screened six eggplant germplasms (Nirrala, Purple Queen, Qaiser, VRIB-9901, VRIB-0401, and Bemissal) evaluate their response against root-knot nematode *M. incognita.* The cultivars were inoculated with 2000 newly hatched juveniles. Their results revealed that all cultivars were susceptible to *M. incognita* with maximum galling index, and galls were recorded on VRIB 0401 (50). A minimum galling index (4.0) was found on Narala. In another study, Jaiteh *et al*. [57] conducted an experiment on tomatoes' genome against root-knot nematodes, *Meloidogyne* spp. Tomato seedlings raised in the nursery were inoculated with five different inoculums viz., 100, 500, 1000, 1500, and 2000 eggs on a known amount of soils. Overall, 33 tomato genomes were screened against root-knot nematodes. The inoculum level of 1500 eggs/plants showed the maximum number of eggs, juveniles, and root weight. Out of 33 screened genotypes of tomato, two genomes, viz., T 11 Mongal and Beef Master, were resistant against *Meloidogyne* spp. These two genomes showed minimum number of reproduction (0.71 and 0.53). Nayak and Sharma [58] screened sixteen different varieties of eggplant against *M. incognita.* Seedling of sixteen varieties raised in the nursery was transplanted to a pot containing autoclaved soils (sand and clay 2:1 v/v). Results revealed that two varieties of brinjal Annamalia and Vijay were found resistant (R), whereas four varieties viz., BR 112, Symala, Azad hybrid, and Rajedra were found moderately resistant (MR). Varieties viz., 81 and VNR 125 were moderately susceptible (MS). In contrast, Purple long and Pusa were found highly susceptible (HS), and the rest of the varieties (Green round, Azad Kranti, Aruna, NS 317, Navkiran 23, Sakura 371, and VNR 60) were found susceptible (S). Beghum *et al*. [59] carried out a net house experiment on screening trial using thirteen different brinjal cultivars, namely Bari Begun 7, Bari Begun 9, Bari Begun 10, Bari Begun 4, Bari Begun 5 Tobla, Desi, Irri, Molika Begun-7, BARI Begun-9, BARI Begun 10, BARI Begun-4, Tobla, BARI Begun 5, Irri, Desi, Molika, BARI Begun 1, Khotkhotia, Singnath, and Uttara. The highest number of galls were found on Desi, and the lowest number of galls were found on Uuttora, Bari Begun 7, Bari Begun 9-10, 4, Tobla Bari Begun 5, Irri, Desi, and Molika screened as highly susceptible (HS). Three varieties of brinjal viz., Bari Begun 1, Khotkhotia, and Shingnath were found susceptible (S), whereas Uttora was moderately resistant (MR) and three varieties viz., Bari Begun 1, three varieties Bari Begun 1, khotkhotia and Shingnath were found susceptible. None of the varieties out of sixteen was found resistant (R). Another study was conducted in a net house to evaluate the influence of different inoculum levels (0, 500, 1000, and 2000 J_2s of nematodes/kg soil/pots). The inoculum was added to the soils after one week of transplanting the seedlings. Results revealed that different inoculum levels reduced the plant's growth parameters (height of the plant, number of leaves per branch, fresh and dry root weights, and stem girth). Maximum galls were recorded at 2000 J_2s/kg soil. Results showed that increasing the inoculum levels increased the nematodes population while reducing plant growth parameters [60]. Screening of brinjal germplasms against root-knot nematode (*Meloidogyne incognita*) was evaluated under the net house condition. The results revealed that all fifteen germplasms of brinjal showed varying degrees of susceptibility to *Meloidogyne incognita.* Out of fifteen germplasms, four (JB 09-01, JB 09-12, JB 10-14, and JB 01-16) were found to be susceptible, and eleven were highly susceptible. No germplasm was found to be resistant [61]. In another study, out of one hundred fifty brinjal varieties/cultivars screened against root-knot nematode, only twenty varieties have shown resistant reaction with least gall index (1.1-2.0), fifty-eight varieties/cultivars moderately resistant with gall index of 2.1-3.0, forty-seven varieties were susceptible having gall index of 3.1-4.0 and twenty-five were found highly susceptible with highest gall index of 4.1-5.0 against the test nematode. Plant height of brinjal varieties was decreased by 9.04, 18.48, and 23.50% in varieties Pusa Kranti, Kantabaigan, and Pusa Purple Long, respectively over un-inoculated control, and the formation of giant cells and galls in the roots of both resistant and susceptible brinjal varieties had shown a decrease of shoot weight (34.08%) and root weight (31.67%) of the susceptible brinjal cultivars, Pusa Purple Long which was different from other two resistant varieties showing resistance reaction against the test nematode [62]..

Conclusion and Future aspects

The information presented in this review clearly shows that root-knot nematode is a very severe pest of various plants. The information in this review is merely a fundamental perspective of root-knot nematode. More studies including proteomic, transcriptomic and genomics of this pest will help clarify many unfold stories. The results of such studies can be used to control this pest effectively.

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