International Journal of Multidisciplinary Education and Research
www.multidisciplinaryjournals.in
ISSN: 2455-4588
Received: 04-04-2023, Accepted: 19-04-2023, Published: 05-05-2023
Volume 8, Issue 2, 2023, Page No. 12-16

A comparative study on the efficacy of Paecilomyces *lilacinus*, Castor cake and grafting for the management of root knot nematode Meloidogyne incognita in tomato - The superiority of grafting as a nematode control strategy

Madhumathi Kondoor[1*], M Madhavi[2]
[1] Research Scholar, Osmania University, Hyderabad, Telangana, India
[2] Professor, Head of Department of Zoology, Osmania University, Hyderabad, Telangana, India

Abstract
A pot culture experiment was conducted under Polyhouse conditions for the management of root knot nematode Meloidogyne incognita infecting Tomato (Solanum Lycopersicum) by using fungal bioagents namely Paecilomyces lilacinus (@ 50g/kg soil), Castor cake (@ 6g/kg soil), and Grafting (cleft grafting by using Solanum torvum as rootstock and tomato plant as scion) and untreated control. The experiment was laid out in a CRBD (completely randomized block design) with three treatments and each replicated five times. Statistical analysis showed that all treatments were found most effective in improving plant growth parameters and reduction in nematode reproduction as compared to untreated check. Among all the treatments Grafting was found to be superior in increasing plant growth parameters and drastic reduction in nematode reproduction over other treatments.

Keywords: meloidogyne incognita, bio fertilizers, paecilomyces *Lilacinus*, castor cake, tomato, grafting

Introduction

Successful raising of vegetable crops is constrained by the attack of plant parasitic nematodes. The damage caused by these nematodes has been estimated to be around $150 billion worldwide. In India an average national loss of rupees 21,068.73 millions has been estimated due to plant parasitic nematodes (NIPHM Rajendra Nagar). Among the plant parasitic species, Root-knot nematodes, are one of the major economically damaging genera of plant-parasitic nematodes on horticulture and field crops (Almohithef *et al*., 2018) [1]. Root-knot disease is caused by various species of Meloidogyne. There are several species of Root-knot nematodes found in India, including M.incognita, M.javanica, M.arenaria and M.hapla.

On an average 5% of global yield losses are guessed to be due to root knot nematodes only, which may be higher in tropical and subtropical areas. (Anes & Gupta, 2014; Ngele & Kalu, 2015) [21]. RKN's are obligate parasites of the roots of many plant species, and more than 3000 plant species are parasitized due the Root-knot nematodes (Hussey and Janssen 2002) [5]. Among the varied crops vegetables are worst affected, barely any vegetable crop escapes the infection by these nematodes. It was reported that RKN causes yield loss among several economically important vegetable crops and it was estimated around Rs. 6,035.2 million for tomato, Rs. 3,499.12 million for brinjal and Rs.2480.86 million for okra (Kumar *et al*., 2020) [14].

Tomato (Solanum lycopersicum) is grown worldwide and is an important vegetable crop for its edible fruits which is accountable for correcting malnutrition in countries like India. Lycopene - a chemical present in tomatoes, plays an important role in preventing various types of cancer. Body can use lycopene which comes from tomato preparations like tomato paste or tomato juice, rather than from fresh tomatoes. Tomatoes also help in reducing risk of heart disease as well as sun damage.

There are several limitations to the successful cultivation of tomatoes. Nematodes alone can cause about 20.6% loss in yield worldwide (Sasser, 1989) [7]. Subramaniyan, Vedivelu and Rajendran (1990) reported yield loss 42.05–54.42% due to *Meloidogyne incognita* from India. Jain, Dabur, and Gupta (1994) also reported 47.3 and 71.9% yield loss in vegetable crops due to *M. javanica* and *M. incognita*, respectively. The vegetable crops are greatly affected with root knot nematodes in all parts of India and elsewhere (Siddiqui & Shaukat, 2003; Sikora & Fernández, 2005) [33] and strenuous crop pests to control (Chitwood, 2002) as they have high reproduction rates (Ananhirunsalee, Barker, & Beute, 1995). yield losses in tomato due to *Meloidogyne* spp. ranges from 35 to 50% in India (Jain, 1991; Jonathan, Kumar, Devarajan, & Rajendran, 2001) and globally as high as 85% (Sasser, 1979; Taylor & Sasser, 1978).

Materials and Methods

Experimental site for the experiment

Experiment was carried out in a Poly House, Public Gardens, Nampally, Hyderabad, Telangana, India, Latitude 17.399994°, Longitude 78.468158° during the period from 01 September, 2022 to 30 November, 2022.

Pathogen and Test plant

Meloidogyne *incognita* (Kofid and White, 1919; Chitwood 1946) selected as test pathogen, and Tomato (Solanum lycopersicum) was selected as test plant to evaluate the effect of different soil amendments i.e Paecilomyces lilacinus, Castor cake and grafting on the management of root knot nematode Meloidogyne *incognita.*

Castor Cake, and a bioformulation containing Paecilomyces lilacinus as the active ingredient were obtained from B. N. Bio Science Pvt Lmt, Bachupally, Miyapur, Hyderabad, Telangana 502325.

Collection of infected roots and Identification of Meloidogyne species

Root knot nematode infected roots were collected from Maheshwar mandal, Ranga Reddy district, Telangana State, India by observing above ground symptoms like yellowing of leaves, wilting, and stunted growth (Birat, 1963; Good, 1968). Infected plants were gently removed from the soil without damaging the roots and kept in polythene bags and brought to the laboratory for the examination.

Species of Meloidogyne was identified based on the perineal pattern observed under Stereo binocular microscope and was identified as Meloidogyne *incognita.*

Inoculum preparation

In order to obtain a pure culture of the field populations, a single mature egg mass was inoculated in pots along with the roots of the susceptible host plant tomato cv. Pusa Ruby. Sub-culturing was done by inoculating new plants with at least 15 egg masses each, obtained from the pure culture to maintain sufficient inoculum for further studies. Large numbers of egg masses were hand picked with the help of sterilized forceps from heavily infected roots of tomato, washed thoroughly in distilled water and placed in mesh-sieves carrying tissue paper and placed in the petri dishes containing water, in such a way that was deep enough to touch egg masses. After every 24 hours, the hatched J_2 (second-stage juveniles) were collected and fresh water was added to the petri dishes. The inoculum density was adjusted to 1000 J_2 in order that each 10 ml of this suspension contained about 1,000 freshly hatched second-stage juveniles.

Inoculation technique

Inoculation was done by making three holes nearby to the roots at the same distance without damaging the roots in the pots.

Raising of test plants

Meanwhile seedlings of tomato and Solanum torvum (wild brinjal for grafting resistant rootstock) were produced in a nursery at Poly house, Horticulture department, Public grounds, Hyderabad District. Wild brinjal plants around four weeks old were used for grafting as rootstock on the scion of four weeks old tomato plants, by cleft grafting technique. The grafted area (junction) was covered with paraffin. The plants were then transferred for hardening to the shade nursery house in an open environment for 7 to 8 days at 25° ± 2°C. After a week, the healthy grafted plants were selected for transplanting into plastic pots. Before transplanting, all the leaves grown below the "grafted junction" were removed.

Application of treatments/experimental design

The experiment was carried out in a completely randomized block design in plastic pots of 12" size filled with Compost and sterilized sandy loam soil mixed in the ratio of 3:1. Pots were washed and cleaned with water, and also disinfected before use by rinsing through a four percent (4%) formalin solution. Before filling soil into the pots soil is first mixed with soil amendments (i.e castor cake @ 50 grams/ kg and talk formulation of fungus Paecilomyces lilacinus @ 6 grams/ kg soil thoroughly) to ensure equal distribution of both fungus and oil cake with soil. The pots were watered everyday to ensure proper decomposition of the organic matter. After one week, four weeks old test plants i.e seedlings of tomato raised in the nursery (plastic seed tray), of equal size were transplanted along with grafted plants.

Experiment was designed as follow:

T1 - Castor cake @ 50 g/kg soil + 1000 J2

T2 - Paecilomyces *lilacinus* @ 6 g/ kg soil + 1000 J2

T3 - Grafted plants on solanum torvum + 1000 J2

T4 - control (untreated inoculated with 1000 J2)

Each treatment was replicated five times.

The experiment was terminated after 60 days and plants were uprooted and observations on growth parameters like shoot length (cm), root length (cm), fresh root and shoot weight (g) and number of galls were taken. The root systems were rinsed under clean running tap water to remove soil. (Bybd DW *et al* 1983).

The plants were cut with a sharp knife just above the zone of root emergence. Length of the plants were measured with the help of measuring tape from the top i.e from the first leaf to the end of stem and recorded in centimeters (cm). Fresh weight of the plants were recorded in grams by using analytical balance. The number of galls per root system was also counted with the help of a magnifying lens. At the time of harvesting, pots were slightly watered in order to loosen the soil, and were removed from the pot. Soil from the pots were combined together to make a bulk sample. 100cc of soil was taken and Nematodes were extracted by using Cobb's decanting and sieving method.

Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) and mean comparison was conducted using LSD (least significant difference) test at 5% level of probability by using SPSS 25.0 version.

Results

The results of the pot experiment revealed that all the three treatments (i.e Castor cake, Paecilomyces *lilacinus* and Grafting) tested were found to be effective against Meloidogyne incognita. The data presented in the table indicates that among all the treatments Grafting was found to be superior to the other treatments not only in improving plant growth characters but also in recorded lowest root knot index, when compared with control (RKI 5.0), and other two treatments Castor cake, and Paecilomyces *lilacinus.* All the treatments showed statistically significant variation in reducing the population of nematodes both in soil as well as in roots.

Table 1.0: Effect of Castor cake, P.lilacinus and Grafting on Root-Knot Nematode Meloidogyne incognita On Tomato under pot experiment:

Treatments	Plant growth characters				Nematode reproduction		
	Shoot height (cm)	Shoot weight (g)	Root height (cm)	Root Weight (g)	No of galls	RKI	Larval Population in 100 cc soil
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Castor cake (T1)	65.14	214.8	26.1	32.60	25.4	3	221.2
P. lilacinus (T2)	67.3	230.8	29.52	29.60	21.4	2.8	208.8
Grafting (T3)	80.58	286	51.1	44.00	3.8	1.2	31.4

Control (T4)	41.6	168.8	20.7	20.80	109	5	639.8
p-Value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S Ed	3.351	6.907	1.777	3.237	4.531	0.400	22.564
C.D	7.103	14.643	3.768	7.154	9.606	0.848	47.836

The data presented in the table indicates that there was a great increase in plant growth parameters i.e fresh shoot and root weight (gms), shoot and root length (cm) in all the treatments over untreated check. But among all the treatments, T3 grafting registered the highest percentage of increase in shoot height and root height, fresh shoot weight and root weight over the check.

Tomato plants inoculated by J2 of Meloidogyne T4 (control) highly reduced the plant shoot height (61.5 cm) than other treatments as shown in the table. Highest plant height was recorded by grafted plants (80.58 cm) followed by P.*lilacinus* (67.3 cm) and Castor cake (65.14 cm). Significance differences of plant height were not noticed in the treatments T1 (Castor cake) and T2 (P.*lilacinus*) compared to control. In case of root height, highest root root height (51.1) was recorded in T3 followed by T2 (29.52cm) and T1(26.1cm).

Comparative efficacy of grafting and other treatments on weight of root in tomato was also recorded. Highest amount of fresh root weight (44.0 g) was found in treatment T3 followed by T2 (29.60g) and T1 (32.60g). Highest shoot weight was also observed in T3 (286.0g) followed by T2 (230.8g) and T1(214.8 g).

Application of J2 of Meloidogyne spp., and all the three treatments simultaneously increased the amount of weight of fresh root per plant over control. On the other hand T4 inoculated only with J2 of Meloidogyne spp. without any amendments highly decreased fresh weight of root over control.

So far as population growth of Meloidogyne incognita is concerned, results indicate that there was a remarkable reduction in population of nematodes both in soil and roots in all treatments over untreated control. But there was a higher reduction in J2 population (31.4) per 100 cc soil in grafted plants, followed by P. lilacinus (202.8) and Castor cake (221.2).

Significant reduction in RKI (root knot index) was also observed in all treatments over the check. Lowest number of galls (3.8) and RKI (1.2) was recorded in T3 (grafted plants). However Paecilomyces lilacinus (T2) and Castor cake (T1) were at par with each other with gall number (21.4) and (25.4) and root knot index 3.0 in and 2.8. All treatments were effective to suppress the number of females per root system as compared to control. Treatment of Paecilomyces lilacinus (T2) had less number of females per root system followed by Castor cake (T1) but they were statistically at par.

Discussion

The vegetable crops are greatly affected with root knot nematodes in all parts of India and elsewhere (Siddiqui & Shaukat, 2003; Sikora & Fernández, 2005) [33]. The present study showed that all the treatments were beneficial to the tomato plants, in promoting shoot and root growth of the plants (Saravanapriya, B., and M. Sivakumar. 2005) [20]. Abd-Elgawad and Askary (2018) also reported similar results who compiled use of different types of bio-nematicides for integrated management.

Darnetty and Liswani (2018) found that 55-70% suppression of Meloidogyne spp. by P.lilacinus isolates in tomatoes. Hano and Khan (2016) also found that P.lilacinus formulations both in 25% SC and wettable powder 25% were effective in reducing RKN population in soil, and also improving plant growth parameters as well as enhancing tomato yield. The severity of root galling as well as egg mass production was found to be more significantly suppressed by the application of Paecilomyces lilacinus in tomato (Udo *et al*., 2014).

It has been reported that certain microorganisms that contributed to the decomposition of oil cakes produce certain products like fatty acids, formaldehyde, phenols and ammonia. The effect of these combined factors leads to reduced nematode development. The decomposition of organic matter releases nematicidal principle(s) and the residual organic matter increases fungal activity and persistence (Ashraf, M. S., & Khan, T. A. 2010) [4].

While Paecilomyces lilacinus and Castor cake are also effective methods for controlling nematode populations, they have limitations. Paecilomyces lilacinus is a fungal biocontrol agent that can help reduce nematode populations in soil, but its effectiveness depends on several factors such as environmental conditions and the stage of nematode development. Castor cake, which is a byproduct of castor oil production, contains natural compounds that can inhibit nematode activity, but it may also have negative effects on plant growth and can be toxic in high concentrations.

The improvement of plant growth in grafted plants compared to the control indicates the existence of a complex physiological and biochemical beneficial relationship between the scion (tomato) and rootstock (Solanum torvum) have been reported to alter the physiology of the root and reduce root-exudation. Improved nutrient status in the grafted plants resulted in increased growth potential and biomass production (AHA Abdelmageed and Nazim S. Gruda 2009) [2].

Besides combating diseases (bacterial and Virus), grafting has other added benefits, such as increasing yields, crop quality, extended growing seasons, and improved flavor (Charles E. Barr *et al* 2012) [6]. In order for grafting to be successful, the rootstock and the scion must be closely related. Grafting will fail if we try to mix and match plants that belong to different plant families (Mahmoud, A. M. A. 2020) [11].

Conclusions

As the increasing demand for organically produced vegetables and fruits continues to rise, more organic growers will need effective methods of soil-borne disease control. For soil-borne disease management grafting is an effective tool that carries economic considerations with it. In order for grafting to be successful, the rootstock and the scion must be closely related. Grafting will fail if we try to mix and match plants that belong to different plant families. However, commercial rootstocks are limited, thus more study is needed to improve rootstock development under public sector by the use of wild relatives to mitigate biotic and abiotic stresses in changing climatic conditions.

References

1. Almohithef *et al*., Prevalence of plant-parasitic nematodes associated with certain greenhouse vegetable crops in Riyadh region, Saudi Arabia. Journal of the Saudi Society of Agricultural Sciences, 2018, 19(1). DOI:10.1016/j.jssas.2018.05.001.

2. Abdelmageed AHA, Gruda N. Influence of grafting on growth, development and some physiological parameters of tomatoes under controlled heat stress conditions. European Journal of Horticultural Science.2009:74(1):16-20.
3. Sasser JN, Freckman DW. A world perspective of Nematology: the role of Society. In: Veech JA, Dickson DW (eds) Vistas on Nematology Society of Nematologists, USA, 1987, 7–14.
4. Ashraf MS, Khan TA. Integrated approach for the management of Meloidogyne javanica on eggplant using oil cakes and biocontrol agents. Archives of Phytopathology and Plant Protection,2010:43:609-614. 10.1080/03235400801972434 [Taylor & Francis Online].
5. Hussey RS, Janssen GJW. 'Root-knot nematodes: Meloidogyne species.', 2002. CABI Books. CABI International. doi: 10.1079/9780851994666.0043.
6. Charles E Barr *et al*. Grafting for Root-knot Nematode Control and Yield Improvement in Organic Heirloom Tomato Production [2012], in HortScience, 2012, 47(5). DOI.
7. Sasser JN. Plant Parasitic Nematodes - The Farmer's Hidden Enemy. North Carolina State University Graphics. Raleigh, USA, 1989.
8. Abd-Elgawad MMM. Biological control agents of plant-parasitic nematodes: a review. Egypt J Biol Pest Cont,2016a:26(2):423–429.
9. FAOSTAT. 2020. Food and Agriculture Organization of the United Nations, Rome. Accessed 17-May-2020.
10. Subramaniyan, Vedivelu and Rajendran (1990) Estimation of loss of tomato due to Meloidogyne incognito and Rotylenchulus reniformis Indian Journal of Nematology,1920:20(2). ISSN 0303-6960.
11. Mahmoud AMA. Tomato Rootstock Breeding: Evaluation of Tomato Interspecific Hybrid Rootstocks Under Greenhouse Conditions. The Horticulture Journal, advpub, 2020. https://doi.org/10.2503/hortj.UTD-199
12. Shilpa Sharma P, Thakur V, Sharma A, Rana RS, Kumar P. A statusquo review on management of root knot nematode in tomato. The Journal of Horticultural Science and Biotechnology,2022:1(14):403-416.
13. Singh S, Singh B, Singh AP. Integrated treatment of root-knot disease of okra caused by Meloidogyne incognita. Indian Journal of Nematology,2014:44(2):172-178.
14. Kumar V, Khan MR, Walia RK. Crop loss estimates due to plant-parasitic nematodes in major crops in India. National Academy Science Letters,2020:43(5):409-412.
15. Cobb NA. Estimating the nema population of soil, with special references to the sugar beet and root-gall nemas, Heterodera schachtii Schmidt and Heterodera radicicola (Greef) Muller, and with a description of Tylencholaimus aequalis n. sp. Agric Tech Circular,1918:1(1918):48.
16. Hussey, Janssen *et al*. Plant Resistance to Parasitic Nematodes, 2002. DOI: 10.1079/9780851994666.004.
17. Forghani F, Hajihassani A. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Front Plant Sci,2020:11:1125. [PMC free article] [PubMed] [Google Scholar].
18. Ali Tauheed et ai., meloidogyne incognita infecting brinjal in Khyber Pakhtunkhwa, Pakistan: its distribution, reproduction and management through resistance, Plant Cell Biotechnology and Molecular Biology,2021:22(27-28):126-138. 2021 ISSN: 0972-2025.
19. Khan R *et al*. "Phytochemical Management of Root Knot Nematode (Meloidogyne incognita) Kofoid and White Chitwood by Artemisia Spp. in Tomato (Lycopersicon esculentum L.),2020:80(4):829–38.
20. Saravanapriya B, Sivakumar M. "Management of Root Knot Nematode Meloidogyne incognita on Tomato with Botanicals." Natural Product Radiance,2005:4:158–61.
21. Ngele K, Kalu UN. " Studies on different species of plant parasitic nematodes attacking vegetable crops grown in Afikpo North L.G.A. Nigeria", Direct Research Journal of Agriculture and Food Science DOI: ISSN: 2354-4147 Vol.3 (4), pp. 88-92, April, 2015.Sinno, Martina *et al*., 2020. "Endophytic Fungi of Tomato and Their Potential Applications for Crop Improvement." Agriculture (Switzerland),2015:10(12):1–22.
22. Rawal Sara. "A Review on Root-Knot Nematode Infestation and Its Management Practices Through Different Approaches in Tomato." Tropical Agroecosystems,2020:1(2):92–96.
23. Ahmad, Syed Faheem, Tabreiz Ahmad Khan. "Management of Root-Knot Nematode, Meloidogyne incognita, by Integration of Paecilomyces lilacinus with Organic Materials in Chilli." Archives of Phytopathology and Plant Protection,2004:37(1):35–40.
24. Giri B, Rawat R, Saxena G, Manchanda P, Wu QS, Sharma A. Effect of Rhizoglomus fasciculatum and Paecilomyces lilacinus in the biocontrol of root-knot nematode, Meloidogyne incognita in Capsicum annuum L. Commun Integr Biol,2022:15(1):75-87. doi: 10.1080/19420889.2021.2025195. PMID: 35273677; PMCID: PMC8903792.
25. Monjil MS, Ahmed S. Paecilomyces lilacinus on egg hatching and larval population of Meloidogyne sp.Bangladesh Journal of Plant Pathology,2017:33:71–78.
26. Seid A, Fininsa C, Mekete T, Decraemer W, Wesemael WM. Tomato (Solanum lycopersicum) and root-knot nematodes (Meloidogyne spp.)–a century-old battle. Nematology,2015:17(9):995–1009.
27. Abd-Elgawad MMM. Biological control agents of plant-parasitic nematodes: a review. Egypt J Biol Pest Cont,2016a:26(2):423–429.
28. Calderón-Urrea A, Vanholme B, Vangestel S, Kane SM, Bahaji A, Pha K, *et al*. Early development of the root-knot nematode Meloidogyne incognita. BMC Dev. Biol,2016:16:1–14. doi: 10.1186/s12861-016-0109-x.
29. Ahmed S, Monjil MS. Effect of Paecilomyces lilacinus on tomato plants and the management of root knot nematodes. Journal of Bangladesh Agricultural University,2019:17(1):9–13. https://doi.org/10.3329/jbau.v17i1.40657.
30. Awol Seid, Chemeda Fininsa, Tesfamariam Mekete Mengistu, Toon Janssen. Biodiversity of Meloidogyne spp. from major tomato growing areas of Ethiopia; European Journal of Plant Pathology,2019:153(915):1-16
31. Elling AA. Major emerging problems with minor Meloidogyne species Phytopathology,2013:103(2013):1092-1102.
32. Hallmann J, Meressa BH. 'Nematode parasites of vegetables.', 2018. CABI Books. CABI International. doi: 10.1079/9781786391247.0346.

33. Sikora RA, Fernández E. 'Nematode parasites of vegetables.', CABI Books. CABI International, 2005. doi: 10.1079/9780851997278.0319.
34. Karssen G, Moens M. 'Root-knot nematodes.', CABI Books. CABI International, 2006. doi: 10.1079/9781845930561.0059.
35. Udo *et al*. Management of Root-Knot Disease on Tomato with Bioformulated Paecilomyces lilacinus and Leaf Extract of Lantana camara; Brazilian Archives of Biology and Technology, 2014, 57. (ahead):0-0 DOI:10.1590/S1516-89132014005000022; License CC BY-NC 4.0.