

Biocontrol Agents and Organic Matters for Meloidogyne incognita Infection of Beta vulgaris L.

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Abstract - Currently, the utilization of *M. incognita*-resistant cultivars is regarded as a nematode management strategy that is both economically feasible and ecologically benign, with no discernible risks to human health. The primary aim of the investigation was to assess the effects of screening for root-knot nematodes, specifically *Meloidogyne incognita*, on the development, biochemistry, and pathology of beetroot and spinach cultivars. The research investigations detailed in the dissertation were carried out both *in vitro* and *in vivo* within the Department of Botany at Aligarh Muslim University, located in Aligarh (U.P.). The present study selected *Beta vulgaris* L. Cv. Ruby Queen and Spinach (*Spinacea oleracea* L. cv. All Green) as experimental plants, belonging to the Amaranthaceae family. The statistical analysis of the trials was performed utilizing the one-way analysis of variance (ANOVA) method and the R statistical software. The results indicate that the effects of *M. incognita* on various plant growth parameters (e.g. shoot and root length, shoot and root fresh weight, and number of leaves per plant), biochemical parameters (e.g. total chlorophyll and carotenoid contents), and pathological parameters (e.g. number of eggmasses per root, number of eggs per eggmass, nematode population per 250 gm soil, and root-knot index) were not consistent across all cultivars that were analysed.

Keywords - *Meloidogyne incognita*, Cultivars, Nematode, *Beta vulgaris*, *Spinacea oleracea*

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INTRODUCTION

Vegetables represent a cost-effective and highly nutritious dietary option that is accessible to individuals facing financial hardship. It offers a range of macronutrients and micronutrients, including carbohydrates, protein, fat, minerals, vitamins, fibers, and phytochemicals, that play a crucial role in bolstering the body's immune system, eliminating carcinogens, mitigating muscular degeneration, and safeguarding against infectious diseases. Legumes are of significant importance in the human diet due to their provision of essential nutrients. It is widely recognized that the consumption of vegetables is essential for promoting and sustaining a healthy lifestyle, a fact that has been acknowledged globally (Khan et al., 2021). Vegetables possess medicinal properties and are significant contributors to India's nutritional security and national economy. The reason for this phenomenon is attributed to the rapid growth rate, short duration, and higher yield per unit of vegetables in comparison to cereal crops. Over the past few decades, vegetable production has gained

significant importance in the global market. Additionally, the availability of flexible high-income has led to a rise in the demand for vegetables.

The prolonged exposure of vegetables to pathogenic agents leads to an increased vulnerability to diseases, ultimately resulting in a decrease in crop yield. It is plausible that the aforementioned phenomenon is associated with the advantageous climatic conditions that facilitate the expansion of the illnesses. Pests and parasites are considered the foremost obstacles in terms of impeding vegetable yield, posing significant challenges to agricultural production. Plant-parasitic nematodes tend to infest and cause significant damage to the majority of cultivated vegetable crops (Sharma et al., 2006; Anwar and Mc Kenry, 2007; Tileubayeva et al., 2021). Plant-parasitic nematodes are a highly detrimental and economically significant pest that inflicts damage upon a diverse range of cultivated crops worldwide, with a particular emphasis on tropical and subtropical regions (Mesa-Valle et al., 2020). According to Gamalero and Glick's (2020)

findings, there was a significant disparity in production losses caused by nematodes between underdeveloped nations and industrialized countries in tropical and subtropical regions. Specifically, the former experienced losses that were 14.6% higher than the latter, which only experienced losses of 8.8%. According to Jain et al. (2007), significant phytonematodes have caused an estimated annual crop loss of Rs. 242.1 billion in India. *Meloidogyne*, a genus of phytonematodes, are prevalent and highly detrimental root-gall nematodes in agricultural ecosystems.

Beta vulgaris L., commonly referred to as table beetroot, is an herbaceous biennial plant belonging to the Amaranthaceae family. *Beta vulgaris* subsp. *vulgaris* is a cultivated variation that encompasses various edible taproots grown in different regions of the world (Wruss et al., 2015).

Spinach, which is botanically referred to as *Spinacia oleracea* L., is a type of verdant vegetable that is grown annually for its consumable leaves. Its remarkable nutritional content makes it a popular choice among individuals of all age groups. According to Robert and Moreau (2016), the essential nutrients play a crucial role in maintaining the overall health and proper functioning of the human body. Furthermore, it is noteworthy that spinach exhibits a substantial abundance of bioactive constituents, including glucuronic acid derivatives of flavonoids and p-coumaric acid derivatives, that possess potent antioxidant attributes (Xu and Leskovaar, 2015). The substance in question is a rich source of biological tannins and phenolic active phytochemicals, including alkaloids, flavonoids, steroids, glycosides, and terpenoids. These compounds exhibit diverse bioactivities, such as antimicrobial, anti-carcinogenic, and antioxidant properties, as reported by Vázquez et al. (2013) and Singh (2016). Apart from its nutritional constituents, it serves as a valuable reservoir of tannins and phenolic bioactive phytochemicals.

Root-Knot Nematodes: *Meloidogyne*, a genus of phytonematodes, is considered to be a highly detrimental and noteworthy group in the field of agriculture due to its destructive nature. Specifically, the root-knot nematodes fall under this classification. The aforementioned nematodes are classified as sedentary obligate endoparasites, and have the ability to infect a vast majority of higher plant species. Whilst their distribution is global, their prevalence is highest in regions characterised by tropical or subtropical climates. The genus *Meloidogyne* comprises approximately one hundred distinct species worldwide, as reported by Elling in 2013. The predominant species responsible for over 95% of the instances are *M. incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax*, and *M. hapla*. These species exhibit the most extensive distribution and are prevalent in numerous locations. According to Khan et al. (2014), a total of fourteen discrete species of root-knot nematodes have been identified in India. *M. incognita* and *M. javanica* are the most commonly encountered

species and exhibit the most extensive geographical range across the country. The distribution of *M. hapla* is limited to regions characterised by temperate mountainous terrain.

Management of Root-Knot Nematode:

The term "**cultural practises**" pertains to human endeavours that are directed towards the management of diseases through the cultural alteration of plants. Comprehending the biology of the pathogen and the host's response to infection is ultimately imperative for assessing the efficacy of cultural control measures. This knowledge facilitates managerial decision-making aimed at targeting parasites during their most vulnerable life cycle stages for effective control. Exemplary instances of agricultural management practises of this nature encompass crop rotation, employment of cover crops, utilization of trap crops, implementation of fallowing, application of soil solarization, and utilization of floods.

Crop rotation is a well-established agricultural practise that involves the sequential cultivation of diverse crops in a given area. This technique has been widely adopted and is considered to be one of the most traditional and prevalent agricultural practises. Crop rotation is a farming practise that aids in the mitigation of diseases and weeds that require management by improving soil fertility, moisture, and structure. For a considerable period, the traditional cultural practise has been efficacious in the management of root-knot disease.

Its purpose is to preserve a healthy equilibrium between the nematode population and the frequency of cropping by allowing sufficient time to elapse between each host crop and the next one so that the nematode population might fall below the economic threshold level of the sensitive host crops. The discovered nematode, its host range, the susceptibility degree of various hosts, population dynamics, and the link between population density of nematode and productivity loss must all be known for rotation to be effective. Crops that generate substances that actively kill nematodes, such as marigold (*Tagetes spp.*) and castor bean (*Ricinus communis*), are commonly advocated as rotation crops.

Soil solarization is a pre-planting technique that does not require the use of chemical agents and is considered a safe method. According to Gill et al. (2017), this particular method has proven to be efficacious in the inhibition of soil-borne microorganisms such as fungi, bacteria, and nematodes. The pre-planting technique entails covering moist soil with transparent polyethylene sheets for a duration of four to six weeks during the summer season, when the soil can effectively absorb maximum solar radiation. The absorption of radiant heat energy from the sun by the soil leads to an increase in soil temperature, typically ranging from

44°C to 45°C. This elevated temperature has been observed to have a toxic effect on soil-borne bacteria.

Host-Plant Resistance: The concept of "resistance" in nematology pertains to the ability of a host to impede the reproduction of a particular species of nematode, as opposed to the reproduction of the same species on a non-resistant host, as defined by Cook and Evans in 1987. This phenomenon is commonly referred to as host-plant resistance. It is possible to modify the genetic makeup of the host plant in order to reduce its vulnerability to a particular disease, thereby imparting resistance to the plant. The adoption of genetic resistance has been identified as a highly effective approach for preventing diseases, specifically root-knot disease caused by *M. incognita*, as noted by Djian-Caporalino et al., 2011 and Colla et al., 2012.

The implementation of *M. incognita*-resistant cultivars is presently considered as a nematode management approach that is economically viable and environmentally safe, while also posing no threat to human health. The management strategies for nematodes have been documented in a study conducted by Fourie et al. (2013). In 1974, Giebel was credited with being the first individual to discuss the biochemical pathways responsible for conferring resistance to *M. incognita* in plants. Rohde (1965) and the subsequent author identified four common forms of resistance exhibited by host plants towards plant-parasitic nematodes.

Numerous studies have demonstrated that the addition of organic matter to soil can mitigate the detrimental impact of phytonematodes on crops. Numerous organic compounds sourced from flora and fauna have been experimentally evaluated as agents for deterring nematodes. According to Roskopf et al. (2020), the suppression of nematodes by organic matter occurs through various mechanisms. These include the increase in soil organic matter, which creates microhabitats that are less conducive to nematode survival and multiplication. Additionally, organic matter releases volatile organic compounds that are either nematotoxic or nematostatic, and stimulates microorganisms that are antagonistic to nematodes. Organic matter also produces antibiotics and harmful secondary metabolites that directly affect nematodes, and stimulates or induces plant resistance to nematicides.

Biological Control: Biological control has garnered increasing interest as a feasible approach for the management of various phytopathogens, such as plant-parasitic nematodes, due to its environmentally friendly nature. The utilisation of biological control has been proposed as a viable approach for the regulation of diverse phytopathogens, as suggested by Aballay et al. (2011) and Zhang et al. (2016). Bioagents possess antagonistic potential that can efficiently mitigate nematode diseases via diverse mechanisms. The aforementioned mechanisms encompass antibiosis, competition, mycoparasitism, degradation of cell membrane, inductive resistance, promotion of plant

growth, and the ability to colonise the rhizosphere (Khan et al., 2023). The bioagents in question are believed to have been derived from various organisms including nematophagous fungus, predatory nematodes, nematophagous bacteria, mites, plant growth-promoting rhizobacteria (PGPR), as well as arbuscular mycorrhizal fungi (AMF) and arbuscular mycorrhizal fungi (Askary and Martinelli, 2015).

The rhizosphere harbours a significant population of aerobic endospore-forming bacteria, primarily belonging to the *Pseudomonas* genus. According to Tian et al. (2007), these bacteria possess the capacity to counteract plant-parasitic nematodes. Rhizobacteria, such as *Pseudomonas fluorescens*, have been reported to exhibit nematode-suppressive activity. *Pseudomonas fluorescens* exerts its effect against phytoparasitic nematodes by inducing the production of antinematic secondary metabolites, including but not limited to 2,4-diacetylphloroglucinol, pyoluteorin, phenazines, pyrrolnitrin, and hydrogen cyanide. Additionally, it triggers systemic resistance in the plant hosts. The aforementioned capability is executed through the proficiency of *Pseudomonas fluorescens*, as reported by Defago et al. (1990), Siddiqui and Shaukat (2003), and Trivedi and Malhotra (2013).

MATERIAL AND METHODS

- **Study area:** The research investigations detailed in the research encompassed both in vitro and in vivo studies, which were carried out within the Department of Botany at Aligarh Muslim University, located in Aligarh (U.P.). Aligarh is a city situated in western Uttar Pradesh, positioned between the rivers Ganga and Yamuna at 27°52' N latitude and 78°51' E longitude. The average elevation of the city is 199 m above sea level.
- **Collection of Seeds, cultivation of seedlings and gathering of Root-Knot Nematode:** The seeds of *Beta vulgaris* L. (beetroot) and *Spinacea oleracea* L. (spinach) were procured from Chola Beej Bhandar located in Aligarh. For each trial, Spinach and Beetroot crops were seeded by placing five surface-sterilized seeds of each crop into autoclaved clay pots containing 1 kg of soil. The planting of each crop was initiated through the sowing of seeds during the month of November, followed by the harvesting process in February. A survey was conducted in the district of Aligarh to gather root-knot nematode-infested samples of brinjal, tomato, and okra from vegetable fields located in Sasni, Agra road, Mathura road, Punjipur, and Mehrawal regions of Uttar Pradesh. The plants that were affected by disease were meticulously extracted from the soil. Subsequently, the roots were placed in plastic bags and dispatched to a laboratory for additional scrutiny. The root samples that were impacted underwent a comprehensive rinsing process using tap water in order to eliminate any dirt that may have been

attached to them. Subsequently, a thorough examination was conducted to identify the presence of galls and eggmasses.

- **Bioagent Inoculum Preparation:** The bacterial bioagents *Bacillus subtilis* (MTCC No. 2274) and *Pseudomonas fluorescens* (MTCC No. 6732) were procured from the Microbial Type Culture Collection and Gene Bank (MTCC) at the Institute of Microbial Technology (IMTECH) in Chandigarh, India. The bacterial strains were subcultured and maintained in a nutrient agar medium. The procedure involved dissolving 14.0 g of nutritional agar in 500 ml of distilled water, followed by sterilisation at 15 pounds (lbs) of pressure for 20 minutes in an autoclave. The culture filtrates were obtained through centrifugation of the nutrient broth at 5000 rpm for 10 minutes subsequent to incubation, as described by Mahesha et al. (2017). Subsequently, the haemocytometer was utilised to ascertain the concentration of colony-forming units (CFU).
- **Screening spinach and beetroot varieties for nematode resistance:** The beetroot and spinach seeds were procured from Chola Beej Bhandar, located in Aligarh, Uttar Pradesh, India. Individually, a set of five seeds that underwent surface sterilisation were sowed into clay pots measuring 15 cm in diameter. The pots were filled with 1 kg of soil that had undergone autoclaving. The study was conducted under controlled greenhouse conditions, utilising a complete randomised block design with ten replications for each cultivar, consisting of five infected and five uninoculated samples. After a period of two weeks from the initiation of seed germination, only a single viable plant per pot was retained subsequent to the thinning process. Following the thinning process, a total of 2000 second-stage juveniles (J2s) of *Meloidogyne incognita* were intravenously administered into the plants after a period of two days. For control purposes, the non-inoculated plants of each cultivar were utilized.
- **Assessing antimicrobial activity of various agents, including plant extracts and chitosan:** The subsequent enumeration comprises a selection of plant species that were scrutinized for their capacity to impede the proliferation of *M. incognita*.

Table 1: Plant species examined for their ability to inhibit the growth of *M. incognita*

Plant	Common name	Family	Part used
<i>Xanthium strumarium</i>	Rough cocklebur	Asteraceae	Fruit
<i>Tridax procumbens</i>	Coatbuttons	Asteraceae	Flower
<i>Malvastrum coromandelianum</i>	False mallow	Malvaceae	Leaves
<i>Hyptis suaveolens</i>	Vilayati-Tulsi	Lamiaceae	Whole plant
<i>Dicliptera paniculata</i>	Panicled foldingwing	Acanthaceae	Inflorescence
<i>Alternanthera pungens</i>	Khaki weed	Amaranthaceae	Whole plant

The antimicrobial activity of different agents was evaluated by conducting experiments on the egg hatching and mortality of second-stage juveniles of *Meloidogyne incognita*. The agents tested

included various plant species, chitosan, emamectin benzoate dilutions, and bioagents, and their efficacy was assessed in response to varying concentrations.

- **Statistical Investigation:** The statistical analysis of the trials was conducted using one-way analysis of variance (ANOVA) and the R statistical software (version 3.6.1). The use of the agricolae package library was employed in the study. The statistical analysis involved the utilization of Least Significant Differences (L.S.D.) at a significance level of 0.05 to compare all of the data. The mean of five replicates was reported as the outcome. Duncan's Multiple Range Test was employed to ascertain the presence of a statistically significant difference among the treatments, with a significance level of $P < 0.05$. As per the Duncan's Multiple Range Test, the means that are denoted by identical letters within a column do not exhibit significant differences among them.

RESULTS

- Six cultivars of *Beta vulgaris*, namely Ruby Queen, Sultan, Raktima, Atlas, DDR 303, and Red ACE, were assessed for their susceptibility or resistance to the root-knot nematode, *Meloidogyne incognita*, under controlled greenhouse conditions. The observed impact of *M. incognita* on plant growth characteristics (such as shoot and root length, shoot and root fresh weight, and number of leaves per plant), biochemical characteristics (including total chlorophyll and carotenoid contents), and pathological characteristics (such as the number of eggmasses per root, number of eggs per eggmass, nematode population per 250 g soil, and root-knot index) varied across all cultivars that were examined.

Table 2: Influence of root-knot nematode, *Meloidogyne incognita* on different beetroot cultivars in relation to growth attributes

Cultivar	Inoculation	Length (cm)				Fresh Weight (g)				Leaves/Plant	% Reduction over control
		Shoot	% Reduction over control	Root	% Reduction over control	Shoot	% Reduction over control	Root	% Reduction over control		
Sultan	No	29.4 ^{bc}		16.2 ^{bc}		43.83 ^{cd}		90.39 ^c		12.0 ^{ef}	
	Yes	18.6 ^{ab}	36.73	10.1 ^{ab}	37.65	28.58 ^{ab}	34.80	57.69 ^{ab}	36.18	8.3 ^d	30.83
Ruby Queen	No	31.8 ^a		17.3 ^a		47.76 ^{de}		96.53 ^d		15.3 ^{bc}	
	Yes	16.9 ^a	46.86	8.4 ^a	51.45	26.60 ^a	44.30	48.46 ^a	49.80	8.0 ^d	47.71
Red ACE	No	32.5 ^a		18.6 ^a		50.24 ^d		103.79 ^d		16.7 ^a	
	Yes	27.4 ^{cd}	15.69	15.5 ^{cd}	16.67	42.60 ^{cd}	15.20	87.61 ^{cd}	15.59	14.4 ^{bc}	13.77
Raktima	No	27.3 ^{cd}		14.5 ^{cd}		41.04 ^{de}		80.91 ^c		12.8 ^{bc}	
	Yes	20.4 ^d	25.27	10.5 ^{cd}	27.59	30.91 ^{ab}	24.69	61.71 ^{ab}	23.73	9.8 ^d	23.44
DDR 303	No	25.5 ^{de}		13.8 ^d		38.25 ^{de}		77.00 ^{cd}		14.6 ^{bc}	
	Yes	22.0 ^f	13.73	11.9 ^d	13.77	33.62 ^d	12.10	66.38 ^{ab}	13.79	12.7 ^{bc}	13.01
Atlas	No	30.6 ^{ab}		16.5 ^{abc}		45.28 ^{cd}		92.07 ^{bc}		13.9 ^{bc}	
	Yes	21.9 ^f	28.43	11.6 ^d	29.70	32.52 ^d	28.19	64.91 ^a	29.50	11.1 ^{bc}	20.14

Values represent the average of five replicates. According to Duncan's multiple range test at $P \leq 0.05$, the means in each column following the same letter are not substantially different.

- The results indicate that all beetroot cultivars that were evaluated and infected with *M. incognita* exhibited a significant reduction in plant growth when compared to their corresponding control groups. Statistically significant variations in total chlorophyll and carotenoid content were observed among all cultivars of beetroot.

Table 3: Effect of various chitosan dilutions on the hatching of *Meloidogyne incognita* eggs after seven days in vitro

Treatment	No. of Emerged Juveniles in different concentrations after 7 days					DW
	3000 ppm	1500 ppm	750 ppm	500 ppm	250 ppm	
Chitosan	58.3 (90.0)	101.7 (82.6)	179.1 (69.3)	289.0 (50.5)	362.2 (37.9)	583.2 (0.0)

Table 4: The effect of various dilutions of emamectin benzoate on the hatching of *Meloidogyne incognita* eggs after seven days in vitro

Treatment	No. of Emerged Juveniles in different concentrations after 7 days					DW
	200 ppm	100 ppm	50 ppm	25 ppm	10 ppm	
Emamectin benzoate	172.4 (70.4)	238.2 (59.2)	352.3 (39.6)	437.8 (24.9)	524.5 (10.1)	583.2 (0.0)

- Significant variation in root-knot index was observed among all assessed spinach cultivars that were infected with *M. incognita*. The correlation between the root-knot index and the reduction in plant growth and biochemical traits was positive.
- This study investigated the impact of varying concentrations (1000, 2000, 3000, 4000, and 5000 ppm) of aqueous extracts derived from different plant parts of *Alternanthera pungens*, *Dicliptera paniculata*, *Hyptis suaveolens*, *Malvastrum coromandelianum*, *Tridax procumbens*, and *Xanthium strumarium* on the hatching of *M. incognita* eggs in comparison to a control group treated with distilled water. The results indicated that all aqueous plant extracts examined inhibited the hatching of *M. incognita* eggs at all doses throughout the experiment. Furthermore, chitosan at 3000 ppm dilution and emamectin benzoate at 200 ppm dilution were found to be highly effective, inducing maximum percent inhibition of egg hatching (90.0% and 70.4%, respectively) after 7 days of exposure. Conversely, 250 ppm dilution of chitosan and 10 ppm dilution of emamectin benzoate induced minimum percent inhibition (37.9 and 10.1).
- Combined application of organic materials and bioagents is particularly efficient for increasing plant development characteristics and preventing root-knot nematode infection. Nevertheless, of all the treatments evaluated, chitosan + *P. fluorescens* was the most effective in inhibiting root-knot formation and promoting the growth of both test crops.

CONCLUSION

The study thus concludes that utilizing *M. incognita*-resistant cultivars is currently regarded as a nematode management strategy that is economically viable, ecologically benign, and poses no discernible hazards to human health. The primary objective of the study was to evaluate the impact of root-knot nematode screening, specifically for *Meloidogyne incognita*, on the development, biochemistry, and pathology of beetroot and spinach cultivars. The research described in the dissertation was conducted both in vitro and in vivo within the Department of Botany at Aligarh Muslim University in Aligarh, Uttar Pradesh. As experimental plants for this research, the Amaranthaceae species *Beta vulgaris* L. Cv. Ruby Queen and Spinach (*Spinacea oleracea* L. cv. All Green) were chosen. The samples were analysed statistically using the one-way analysis of variance (ANOVA) technique and the R statistical software. The results indicate that the effects of *M. incognita* on various plant growth parameters (e.g. shoot and root length, shoot and root fresh weight, and number of leaves per plant), biochemical parameters (e.g. total chlorophyll and carotenoid contents), and pathological parameters (e.g. number of eggmasses per root, number of eggs per eggmass, nematode population per 250 gm soil, and root-knot index) were not consistent across all cultivars that were analysed.

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