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ASSESSING THE EFFICACY OF MANNITOL FOR MANAGING ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) INFESTATION IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

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ABSTRACT

Mannitol, a noncyclic sugar alcohol with six carbon atoms, is naturally synthesized in significant quantities across various plant species. Recent discoveries have highlighted its pivotal role in plant reactions to pathogen incursions, suggesting its potential significance in both biotic and abiotic stress responses. This research aimed to assess the efficacy of mannitol in managing infestations of the root-knot nematode Meloidogyne incognita in tomato plants under controlled in-vitro pot conditions. Seedlings of tomato plants, aged two weeks, had their roots treated with varying concentrations of mannitol solution (0.5 mM, 1.0 mM, 1.5 mM, and 2 mM). Subsequently, these treated seedlings were transplanted into 2 kg pots containing sterilized potting mixture. Following one week from the mannitol treatment, the pots were introduced to infective second-stage juvenile nematodes at a rate of 1 juvenile per gram of soil. After 35 days of nematode inoculation, growth parameters, root-knot index, egg mass index, and the count of female nematodes per 5g of root sample were measured. The results of this study revealed significant distinctions among growth parameters of the control group, untreated infected plants (0 mM), and mannitol-treated infected plants (0.5 mM, 1.0 mM, 1.5 mM, and 2 mM). Conclusively, the most favourable outcome was observed in tomato plants treated with 2 mM mannitol concentration during glasshouse pot experiments. This treatment is recommended for further investigation. Furthermore, this alternative shows promise as a potential substitute for synthetic, hazardous, and costly chemical nematicides like carbofuran, among others, in the future. However, thorough efficacy evaluations under field conditions are imperative before widespread adoption within the agricultural community.
Keywords: Mannitol, root-knot nematode, *Meloidogyne incognita*, tomato, growth parameters, root-knot index, egg mass index, female nematodes.

Introduction

Tomato (*Solanum lycopersicum* L.), an herbaceous plant belonging to the Solanaceae family and originating from western South America, stands as one of the most widely cultivated vegetable species globally. Recent data indicates that over 5 million hectares are dedicated to tomato production, with China leading the way with over a million hectares under cultivation, followed by India, Turkey, the United States, and Egypt. Collectively, these countries contribute to more than 60% of the world's tomato production (FAO, 2019).

Available tomato cultivars exhibit limited genetic diversity due to intense selection and genetic bottlenecks resulting from historical evolution and domestication processes (Bai & Lindhout, 2007; Blanca *et al.*, 2015). Consequently, the susceptibility of tomato plants to various plant pathogens has notably increased. Tomatoes are vulnerable to more than 200 diseases caused by diverse pathogens, both in the field and during postharvest processing (Singh, Singh, & Kumar, 2017). A range of pathogens, including fungi, oomycetes, bacteria, phytoplasmas, viruses, and nematodes, contribute to these diseases. Notably, nematode diseases, particularly those caused by *Meloidogyne* spp., play a pivotal role and result in substantial losses of over 30 billion USD annually (Sastry & Zitter, 2014).

Among the nematode threats, root-knot nematodes (RKN) pose a significant challenge to tomato cultivation. *Meloidogyne* spp., sessile endoparasitic nematodes, are widely distributed and cause extensive damage globally (Sasser, 1989). In India, the most prevalent species is M. *incognita*, inducing visible root gall symptoms. Root-knot nematodes, such as *Meloidogyne incognita*, can predispose the host plants to soil-borne fungal pathogens, leading to a synergistic effect. *M. incognita* creates wounds in the plant roots through its stylet, providing entry points for other fungal pathogens and resulting in complex disease incidences (Batten and Powell, 1971; Chahal and Chabra, 1984).

With an extensive host range, RKN causes substantial crop losses. Various methods, including crop rotation, flooding, hot water treatment, steam treatment, predaceous nematodes, chemical treatments, and breeding for resistant varieties, have been employed to control RKN. However, achieving a sustainable control method remains a challenge.

Mannitol, a six-carbon noncyclic sugar alcohol with the chemical formula $C_6H_{14}O_6$, stands as the most prevalent polyol in nature. Also known as Mannite or manna sugar, it appears as a white crystalline solid. Mannitol serves as a crucial osmolyte and is synthesized in significant quantities in numerous plant species (Su *et al.*, 1999; Mitoi *et al.*, 2009). Apart from its osmotic adjustment role, mannitol acts as an antioxidant, scavenging hydroxyl radicals (OH.) (Shen *et al.*, 1997; Srivastava *et al.*, 2010). While limited data is available on its stress tolerance mechanisms, particularly concerning biotic stress, this study aims to investigate the impact of different concentrations of mannitol on various parameters of the "Prabhav" tomato plants when infested with the root-knot nematode *Meloidogyne incognita*.

Materials and Methods

Experimental Location

The research was carried out at the Department of Botany, Bharathi College Postgraduate and Research Centre, located in Bharathinagara, Mandya District, Karnataka State. The objective was to assess the impact of mannitol in managing the infestation of the root-knot nematode Meloidogyne incognita on tomato plants under controlled in-vitro pot conditions.

Preparation and Application of Mannitol

To prepare the mannitol solution, 18.217 gm of Mannitol was dissolved in a minimum quantity of distilled water until no granules were left over and the final volume was made up to 100 ml using a standard flask to get 0.1M Mannitol stock solution. From this stock solution, different concentrations of 0.5 mM, 1.0 mM, 1.5 mM, and 2 mM mannitol were prepared for further studies, while distilled water served as the control (Sharada *et al*., 2016). The roots of two-week-old "Prabhav" tomato seedlings were immersed in the respective concentrations of mannitol solution for approximately 10 minutes. Subsequently, these seedlings were transplanted into 2kg pots containing sterilized potting mixture.

The impact of different mannitol concentrations was investigated within a controlled glasshouse environment against *M. incognita*, utilizing the "Prabhav" tomato cultivar. A week following the mannitol treatment, the treated plants were exposed to infective juveniles at a rate of 1 juvenile (J2) per gram of soil. After 35 days from the nematode inoculation, various parameters were assessed, including growth parameters (shoot length, root length, shoot and root fresh weight, shoot, and root dry weight), root-knot index, egg mass index, and the count of female nematodes per 5g of root tissue.

Five treatments, viz. 0.5 mM, 1.0 mM, 1.5 mM, and 2 mM of mannitol were used in this study. In each treatment, 10 plants were maintained and replicated three times for studies of the M. *incognita*-infested Tomato “Prabhav” after 35 days of nematode inoculation.

Shoot and Root Length

After a 35-day interval post-inoculation, the shoot length of tomato plants, randomly chosen from each treatment group, was assessed. The calculated mean values were presented in centimetres (cm). For each individual plant, the uprooting procedure encompassed both the shoot and its associated roots. The total plant length was then measured and subsequently expressed in centimetres (cm).

Fresh Shoot and Root Biomass

After a period of 35 days post-inoculation, the shoots and roots of tomato plants were carefully uprooted. These uprooted portions were thoroughly washed and cleaned to eliminate any attached water and soil particles. The fresh weights of both the roots and shoots were then measured and reported in grams per plant (g/plant).

Dry Shoot and Root Biomass

After 35 days from inoculation, the tomato plant shoots and roots were carefully uprooted for analysis. The fresh biomass of both the shoots and roots was determined. Subsequently, the uprooted plant material was subjected to an initial air-drying process, followed by oven drying at a temperature of 65°C until a consistent weight was achieved. The weights of the shoots and roots were then measured and reported as grams per plant (g/plant).

Root-Knot Index and Egg Mass Index

The galled root system and Egg mass index were scored by using a 0 to 5 disease rating scale given by Taylor and Sasser (1978).

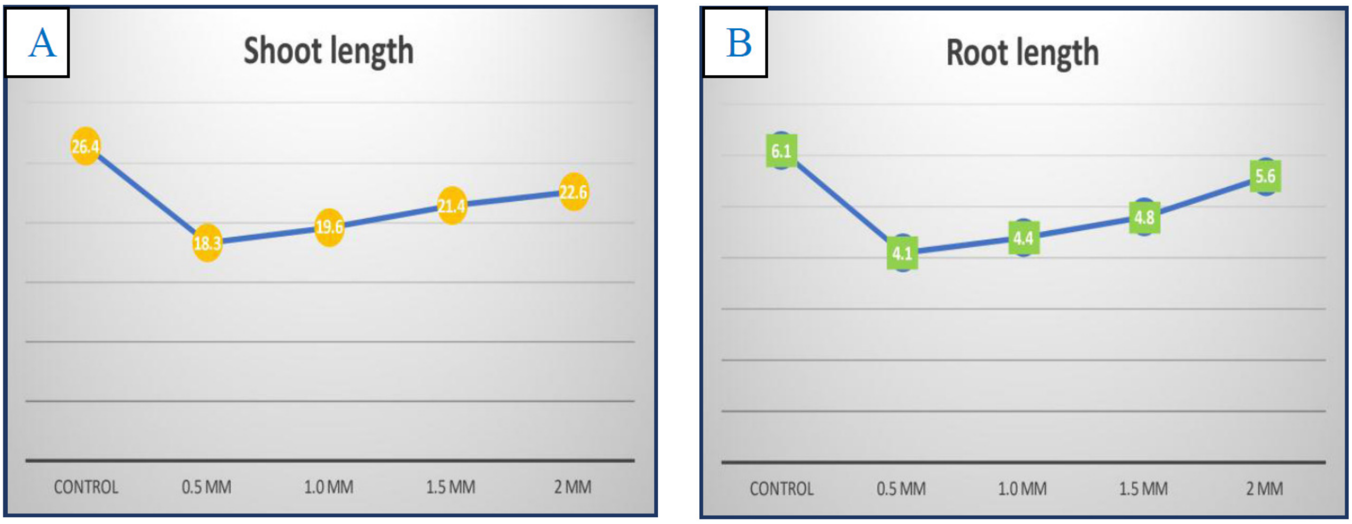
Grade	Description
0	No galls / No Egg mass
1	1-2 galls per root system / 1-2 Egg mass per root system
2	3-10 galls per root system / 3-10 Egg mass per root system
3	11-30 galls per root system / 11-30 Egg mass per root system
4	31-100 galls per root system / 31-100 Egg mass per root system
5	> 100 galls per root system / >100 Egg mass per root system

Female Nematode Population in Roots

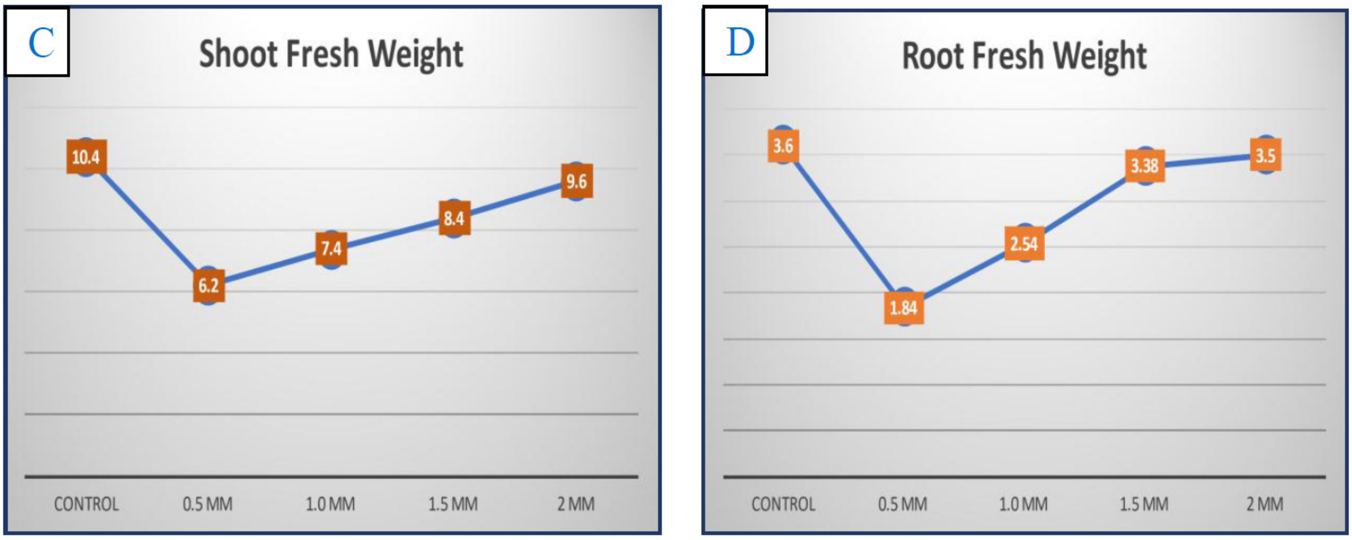
After a period of 35 days following inoculation, the population of *M. incognita* samples was evaluated from the root systems of the plants. This was accomplished by delicately extracting the roots and immersing them in an enamel basin filled with water. Subsequently, 5 grams of root material were carefully dissected into smaller fragments, and a microscopic examination was carried out to identify the existence of egg masses. To enhance visibility, the samples were stained using acid fuchsin lactophenol, followed by a de-staining process in plain lactophenol. The microscope was then employed to observe and count the number of female nematodes present.

Results

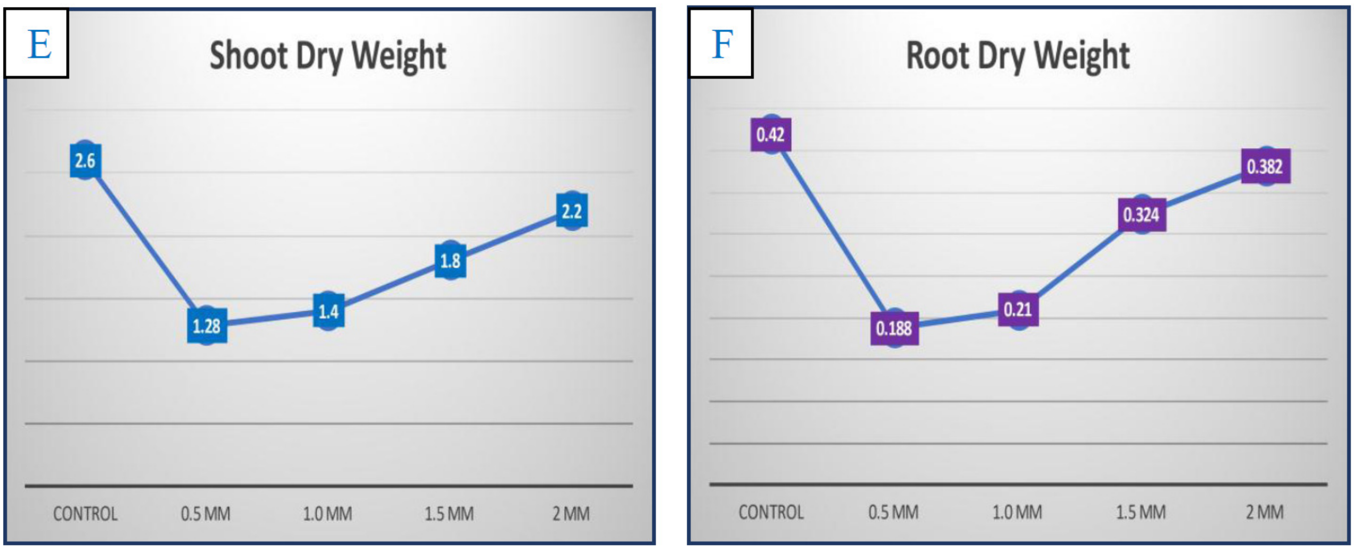
This study examined the impacts of varying Mannitol concentrations, specifically 0.5 mM, 1.0 mM, 1.5 mM, and 2 mM, on a range of parameters including growth, physiology, root-knot index, egg mass index, and the count of females per 5g root sample in tomato plants of the "Prabhav" variety. These analyses were conducted 35 days subsequent to the inoculation of the plants with the root-knot nematode, *M. incognita*.



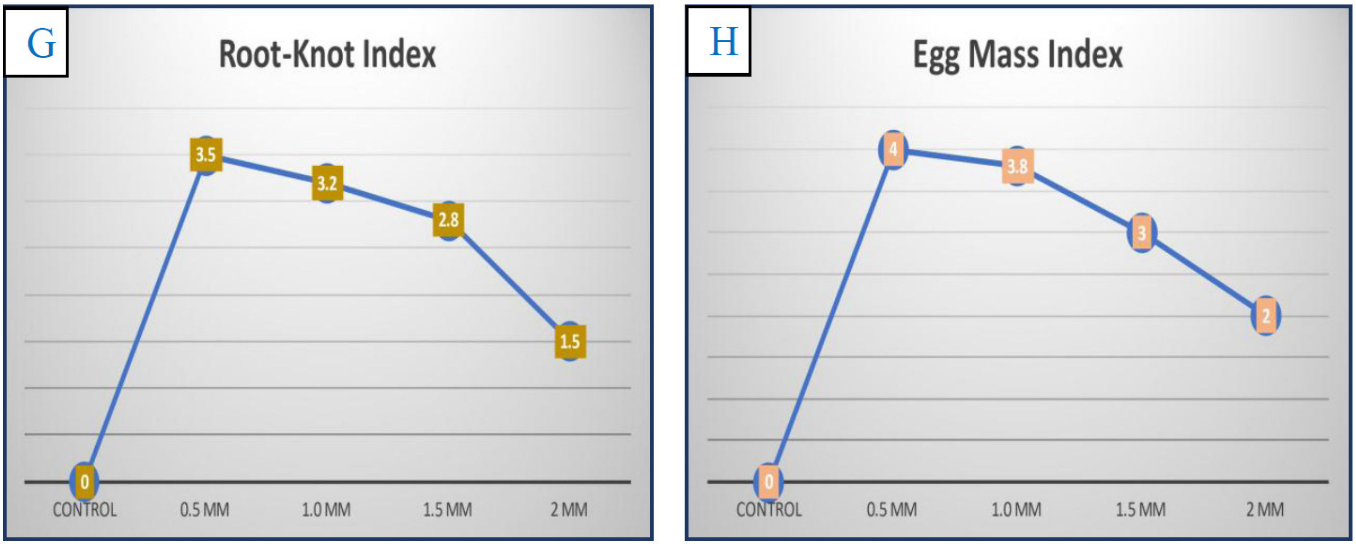
Figs. A and B: Effect of mannitol on shoot and root length of tomato plants.



Figs. C and D: Effect of mannitol on shoot and root fresh weight of tomato plants.



Figs. E and F: Effect of mannitol on shoot and root dry weight of tomato plants.



Figs. G and H: Effect of mannitol on root-knot index and egg mass index.

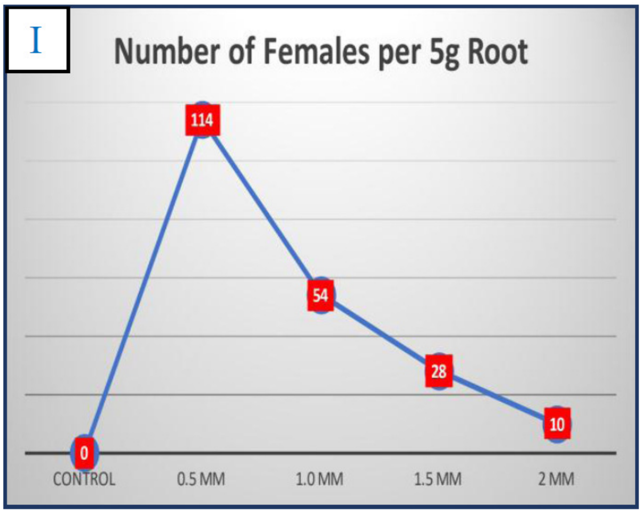


Fig. I: Effect of mannitol on the number of female nematodes per 5g root sample.

Effects of Mannitol on the Growth Parameters of *M. incognita*-Infected "Prabhav" Tomato Plants

Shoot and Root Length

After 35 days from nematode inoculation, measurements of shoot and root lengths were taken, revealing an increase in both parameters across all plants treated with different concentrations compared to the untreated control (0mM) utilized in this study. Among the various treatments, the application of 2 mM mannitol yielded the most significant enhancement, leading to a maximum shoot length of 22.6 cm and root length of 5.6 cm (Figures: A & B).

Notably, treating the plants with 1.5 mM, 1 mM, and 0.5 mM mannitol also resulted in noticeable differences in shoot and root lengths when compared to the untreated control after 35 days from nematode inoculation. Among these observations, the most pronounced impact on shoot and root lengths was observed in plants treated with 2mM mannitol, surpassing the effects of the other concentrations.

Fresh Shoot and Root Biomass

The tomato plants of the "Prabhav" variety, infested by nematodes, exhibited a rise in biomass across the 0.5 mM to 2 mM mannitol treatments. Among these treatments, the 2 mM concentration demonstrated the highest values for fresh shoots and root biomass, reaching 9.6 g and 3.5 g, respectively. Notably, all plants subjected to mannitol treatments at various concentrations displayed elevated biomass values in comparison to the untreated control. This trend was consistent for both shoot and root biomasses (Figures: C and D).

Dry Shoot and Root Biomass

The application of mannitol at concentrations of 0.5 mM, 1 mM, 1.5 mM, and 2 mM resulted in a progressive rise in both shoot and root dry weights of the plants. Notably, when compared to the untreated control, the plants treated with 2 mM mannitol exhibited a more substantial impact on shoot and root dry biomass, registering values of 2.2 g and 0.382 g, respectively (Figure: E and F).

Root-Knot and Egg Mass Index

After 35 days from nematode inoculation, among all the treatments, the plants treated with 2mM mannitol exhibited the lowest root-knot index and egg mass index. Following closely were the plants treated with 1.5mM and 1mM mannitol. In comparison to the untreated control, which displayed a root-knot index and egg mass index of 4.5 and 5 on a 5-scale rating, the tomato plants treated with 2mM mannitol showcased significantly reduced values of 1.5 and 2, respectively. This clearly demonstrates the discernible influence of mannitol on the growth and propagation of root-knot nematodes, as well as its pronounced impact on the root-knot index and egg mass index (Figure: G and H).

Number of Female nematodes per 5g root

The nematode population parameters, specifically the count of female nematodes, were assessed using a 5g root sample collected from tomato plants subjected to various concentrations of mannitol treatment. After a span of 35 days post-nematode inoculation, a reduction percentage became evident in the count of female nematodes per 5g of root in the treated plants as compared to the untreated control. Notably, the most substantial reduction (10) in the count of female nematodes was observed in plants treated with 2mM mannitol, surpassing the effects of other treatments. The outcomes depicted a gradual decline in the percentage of female nematodes per 5g root sample (Figure: I).

Discussion

Nematode control strategies can be categorized into two main groups: cultural and chemical measures. It's important not to rely solely on any single method or measure for nematode management. Instead, a holistic approach should be adopted, where each management procedure is considered in combination with others, creating an integrated nematode management program.

Mannitol ($C_6H_{14}O_6$), found in bacteria, fungi, algae, lichens, and vascular plants (Lewis, 1984), serves various functions such as carbon storage, free radical scavenging, osmoregulation, and acting as a compatible solute (Bieleski, 1982). Some of these roles are especially apparent in stress tolerance mechanisms.

The application of a 2 mM mannitol solution notably enhanced growth parameters, including shoot and root length, as well as fresh and dry shoot and root biomass. Simultaneously, it led to a reduction in both root-knot index and egg mass index, along with the count of female nematodes per 5 g of root sample.

Similar findings concerning growth parameters have been reported by various authors in different crop plants exposed to both abiotic and biotic stresses. Kaya *et al*. (2012) demonstrated that applying mannitol at varying concentrations effectively regulated the growth of maize plants under abiotic stress. Similar observations were also presented by Stoop *et al*. in 1996. Mannitol's prominent role in both abiotic and biotic stress tolerance is well-documented, although limited data is available regarding its role in biotic stress tolerance.

Conclusion

In this study, notable differences were observed in growth parameters between controlled plants, untreated infected plants, and treated infected plants (0.5 mM, 1 mM, 1.5 mM, and 2 mM). In conclusion, the application of 2 mM mannitol concentration to "Prabhav" tomato plants yielded the most favourable results in glasshouse pot experiments, warranting further investigation. This approach could potentially replace synthetic, hazardous, and expensive chemical nematicides, such as carbofuran, in the future. However, before widespread adoption within the farming community, additional efficacy tests conducted under field conditions are imperative.

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