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Nano formulated chitosan for management of root knot nematode, *Meloidogyne enterolobii* in guava

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ABSTRACT: A study was conducted to assess the efficacy of chitosan nano formulation against root knot nematode, *Meloidogyne. enterolobii* infecting guava. Under *in vitro* conditions, the nano formulation exhibited strong nematicidal activity. In glasshouse experiment, application of chitosan nano formulation @5ml/plant reduced the nematode population in soil by 64.52 per cent and the number of egg masses and adult female nematodes by 67.61 and 46.80 per cent respectively. Under field conditions, infective juvenile population in soil was reduced by 52.97 per cent compared to untreated control. In addition, it also resulted in 31.42 and 31.03 percent reduction in the number of egg masses and adult female nematodes in roots, respectively.

Keywords: Chitosan, nano formulation, guava, root knot nematode, *Meloidogyne enterolobi*

INTRODUCTION

Guava is a high value fruit that holds economic importance in tropical and subtropical regions (Jameison *et al.,* 2021). Guava root-knot nematode (*Meloidogyne enterolobii*) is a highly pathogenic and invasive nematode species. Farmers are even unaware about this nematode until galled roots observed during harvest (Schwarz *et al.,* 2019). It has a broad host range including many vegetable crops, ornamental plants and weeds. It has been identified as a high priority pest in the biosecurity plans for the ginger, papaya, potato, sweetpotato and vegetable industries (Schwarz *et al.,* 2020). Like other root-knot nematode species, guava root-knot nematode induces galls on the roots of infected plants. In severe cases, extremely large and numerous galls can be found. Above-ground symptoms include stunted growth, wilting and leaf yellowing (Collet, 2020). Crop yield can be drastically reduced, and the quality of fruits severely affected. It can reduce the yield of about 65% which is severe root galling than the other root knot nematode species (Castagnone-Sereno and Castillo, 2014). In addition, guava root-knot nematode infection may favour further attacks on roots by secondary plant pathogens, such as root-rotting fungi (Khan *et al.,* 2022). Guava root-knot nematode is very damaging due to its ability to develop and reproduce on crops that are resistant to other species of *Meloidogyne* (Selvam *et al.,* 2025).

Nematicides are a widely used means of managing root knot nematodes. However, chemical application is discouraged due to potential risks to the environment and human health (Elgawad *et al.,* 2024). Chitosan is a naturally occurring polysaccharide obtained from chitin, which is a major structural component in the exoskeletons of crustaceans and insects, fungal cell walls, and fish scales. Structurally, it is a cationic polymer made up of (1–4)-2-amino-2-deoxy-β-ᴅ-glucan units. Its favorable characteristics—such as responsiveness to pH, compatibility with biological systems, and inherent bioactivity—make it more appealing for various applications than chitin itself (Shivasankari *et al.,* 2017). Chitosan is used in plant protection against numerous pests and diseases, as well as in preened post-harvest, as microbial biocide (bio-agent) by increasing the antagonist microorganism action, supports beneficial plant microorganism symbiotic relationships and plant growth regulation and development. Chitosan nanoparticles are used as nanopesticides and carriers of fungicides, insecticides, herbicides, plant hormones, elicitors, and nucleic acids (Hijazi *et al.,* 2019; Mohan *et al.,* 2024). This study was conducted to Application of chitosan nanoformulation, which significantly reduces gall formation, nematode population density, and improves plant growth parameters in guava infected by *M. enterolobii* compared to untreated controls.

MATERIALS AND METHODS

Chitosan nano formulation was prepared by ionic gelation method as described by Mouniga *et al.*(2023). The size and stability of the nano formulation were determined by using a Particle size analyzer.Root knot nematode *M.enterolobii* culture was obtained from the collection maintained at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore

Invitro study on the effect of chitosan nano formulation on *Meloidogyne enterolobii*

Effect of chitosan 1% nano formulation on hatching of eggs

Egg masses of *M. enterolobii* were sterilized with 0.5% sodium hypochlorite solution for 1 minute. Different concentrations *viz.,* 25%, 50 %, 75% and 100% were prepared from the 1% chitosan nano formulation. From each concentration, 2ml was taken in a 5cm diameter Petri plate. One egg mass of root knot nematode was placed in the Petri dish. The experiment was conducted in a Completely Randomized Block Design with five treatments and four replications were maintained for each treatment. Numbers of hatched juveniles were observed at different time intervals *viz.,* 24 h, 48 h and 72 h. Egg hatching study was evaluated by the Abbott formula

Juvenile mortality test

Freshly hatched juveniles (J2) were used for juvenile mortality studies. Different concentrations *viz., viz.,* 25%, 50 %, 75% and 100% were prepared from the 1% chitosan nano formulations. From each concentration, 1ml was taken in a 5cm diameter Petri plate to which 100 numbers infective juveniles (J2) were added. Totally five treatments and four replications were maintained in Completely Randomized Block Design.Numbers of dead juveniles were observed during 24 h, 48 h and 72 h time intervals and estimated by Abbott formula

Evaluation under glasshouse condition

A trial was conducted in Nematology Department Glass house, TNAU, Coimbatore. Sterilized pot mixture was taken in earthen pots. Guava seedlings were procured from TNAU orchard. Infective juveniles of root knot nematode *M.enterolobii* were inoculated @ 2 /g of soil after 15 days of planting. The experiment was conducted in a Randomized Block Design with five treatments (Table 1) and four replications were maintained for each treatment. The treatments were applied seven days after nematode inoculation. Observation on plant growth and nematode population in soil and roots were recorded 90 days after application of the treatments.

Field Evaluation

Field trial was conducted in a two-year-old guava plantation with incidence of *M.enterolobii*, at Coimbatore district, Tamil Nadu. The experiment was conducted in a Randomized Block Design with five treatments and four replications were maintained for each treatment. The pretreatment nematode population in soil was uniform throughout the experiment area with 200 guava trees. Ten trees were selected for each replication. Observation on plant growth and nematode population in soil and roots were recorded 90 days after application of the treatments. The treatments (Table 1) were applied as soil drench.

RESULTS AND DISCUSSION

Invitro study on the effect of chitosan nano formulation on *M. enterolobii*

From the observation, the chitosan nanoformulation at 100% concentration exhibited the highest efficacy, completely inhibiting egg hatching of *Meloidogyne enterolobii* when compared to the untreated control. The 75% concentration also proved to be highly effective, resulting in a substantial reduction in egg hatch ranging from 84.39% to 94% within 24 to 72 hours of exposure. In contrast, the lower concentrations (25% and 50%) showed a marked inhibition in egg hatching at 24 hours post-treatment; however, their efficacy declined over time. These results are consistent with findings reported by Ozdemir *et al.* (2022), who noted significant nematicidal activity of 2% chitosan formulations against nematode eggs. Further experiment outcome of Alfy *et al.* 2020 showed egg hatching inhibition 95.3%. after 72 hrs. This may be due to electrostatic interactions, as chitosan is positively charged and the surface of nematode eggs is negatively charged (Rabea and Badawy, 2003). Similarly juvenile mortality evaluated at the same concentrations indicated that at 100% concentration of chitosan nanoformulation caused 97.10% mortality of second-stage juveniles (J2) after 72 hours. The 75% concentration also exhibited strong nematicidal activity,

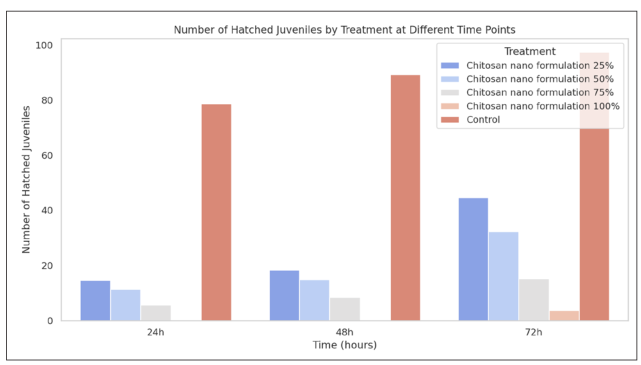

Fig.1. Effect of chitosan nano formulation on egg hatching of *M.enterolobii*

Table 1. Effect of Chitosan nano formulation on plant growth and population of *Meloidogyne enterolobii* in guava under pot culture condition (Mean of four replications)

Treatment details	Shoot length (cm)	Root length (cm)	Gall index	No. of egg mass/ g root	No. of female nematodes/ g root	Nematode population/ 200 cc soil
Chitosan nano formulation @ 5ml/plant at planting and 30 DAP	59.62 (40.05)	26.87 (56.95)	2	8.5 (67.61)	18.75 (46.80)	96.5 (64.52)
Pochonia chlamydosporia @ 2.5kg/ha at planting and 30 DAP	56.05 (31.66)	21.62 (26.28)	2	9.5 (63.8)	20 (56.73)	90 (66.91)
Purpureocilium lilacinum @ 2.5kg/ha at planting and 30 DAP	46.25 (8.64)	20.87 (21.90)	3	8.75 (66.66)	21 (40.02)	102.25 (62.4)
Fluropyram 34.48% EC	63 (47.99)	29.87 (74.47)	2	6.75 (74.28)	14.25 (59.57)	62.75 (76.93)
Untreated control	42.57	17.12	5	26.25	35.25	272
SE (d)	1.67	1.34	-	1.34	1.65	3.57
CD (0.05)	3.34	2.68	-	2.68	3.30	7.14

Table 2. Effect of Chitosan nano formulation on plant growth and population *Meloidogyne enterolobii* in Guava under field condition (Mean of four replications)

Treatment details	Gall index	No. of egg mass / g root	No. of female nematodes / g root	Soil nematode population/ 200 cc soil
Chitosan nanoformulation @ 5ml/plant	2	24 (31.42)	30 (31.03)	158 (52.97)
Pochonia chlamydosporia @ 2.5kg/ha	2	25 (28.57)	31.25 (28.16)	159.5 (52.52)
Purpureocilium lilacinum @ 2.5kg/ha	3	26.5 (24.28)	30 (31.03)	159.25 (52.6)
Fluropyram 34.48% EC @500ml/acre	2	19.75 (43.57)	24 (44.82)	134 (60.11)
Untreated control	5	35	43.5	336
SE (d)	-	1.42	1.99	4.02
CD (P=0.05)	-	2.84	3.98	8.04

inducing 63.22% to 84.31% mortality over the 24–72 hour exposure period. Even the lower concentrations (25% and 50%) were effective in causing statistically significant mortality of *M. enterolobii* juveniles. These observations align with the results of Khan *et al.* (2021), who reported highest juvenile mortality at 2500 ppm chitosan concentration. Similarly, Alfy *et al.* (2020) reported that chitosan nanospheres at 2000 ppm inhibited *M. incognita* egg hatching by 95.3% after 72 hours and resulted in 77.5% juvenile mortality, which confirm the current findings. The observed nematicidal activity of chitosan nanospheres leading to disruption of membrane integrity and leakage of intracellular proteinaceous contents of juveniles, as previously described by Rabea and Badawy (2003).

Evaluation under glasshouse conditions

Among the various treatments assessed, the emulsifiable concentrate (EC) formulation of fluopyram demonstrated the most pronounced effect on plant growth, resulting in a 47.99% increase in shoot length and a 74.47% increase in root length compared to the untreated control. Some recent studies have also reported that fluopyram enhances tomato growth and yield (Ji et al., 2019). Additionally, Li *et al.* (2020) reported that fluopyram application contributed

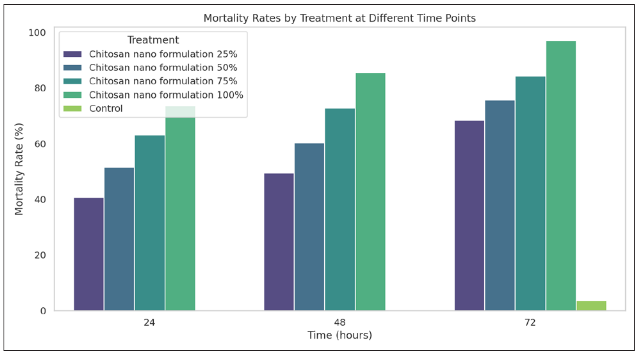

Fig. 2. Effect of chitosan nano formulation on *M.enterolobii* infective juveniles

significantly to overall plant growth.This growth-promoting effect may be partially attributed to fluopyram's potential to stimulate beneficial soil microbial activity, which in turn could contribute to improved plant health and productivity (Sun *et al.,* 2020). Chitosan nanoformulation was the next most effective, promoting shoot and root growth by 40.05% and 56.95%, respectively. According to Jail *et al.,* 2014 chitosan application increased the plant growth fruit yield by inducing increased the activity phytoalexins. It acts as phytostimulant because it reduces ROS formation and improve defense system in plants (Ullah *et al.,* 2023). The biocontrol agents *Pochonia chlamydosporia* and *Purpureocillium lilacinum* also enhanced plant development, with *P. chlamydosporia* increasing shoot length by 8.64% and root length by 21.9%, and *P. lilacinum* by 31.66% and 26.28%, respectively. These findings are in agreement with the report by Khan *et al.* (2023), who highlighted the growth-promoting effects of *P. lilacinum*, including improved biomass accumulation and enhancement of photosynthetic pigments. In terms of nematode suppression, the chitosan nanoformulation reduced the population of *Meloidogyne enterolobii* juveniles in the soil by 64.52% relative to the untreated control. Furthermore, it reduced the number of egg masses and adult females in the roots by 67.61% and 46.80%, respectively. Despite its growth-promoting potential, fluopyram was superior in reducing nematode infestation in both soil and root systems by affecting ubiquone binding site which blocks electron flow between mitochondrial membrane. Similarly, the biocontrol agents showed notable efficacy in nematode suppression. *P. chlamydosporia* decreased the soil nematode population by 66.91%, the number of egg masses by 66.66%, and the root population by 56.73%. *P. lilacinum* followed closely with reductions of 62.40%, 63.8%, and 40.02% in soil nematodes, egg masses, and root populations, respectively.

These findings are supported by those of López *et al.* (2024), who confirmed fluopyram's superior nematicidal activity among chemical treatments. The current results also align with those of Özdemir *et al.* (2022), who reported that chitosan at a 1% concentration significantly enhanced plant growth and reduced nematode populations without phytotoxic effects. Khalil *et al.* (2022) also observed a 45–66% reduction in nematode populations following chitosan treatment. The efficacy of *P. chlamydosporia* in decreasing nematode density was similarly documented by Arunachalam *et al.* (2025), while *P. lilacinum* was shown by Khan *et al.* (2023) to reduce both nematode populations and gall formation.

Field evaluation

The experiment revealed fluopyram resulted in the highest efficacy, enhancing plant growth parameters by 48% and reducing the nematode population by 60% compared to the untreated control. This was followed by the chitosan-based formulation, which improved plant growth by 40–56% and reduced the nematode population by 53%. *Pochonia chlamydosporia* demonstrated a 31% increase in plant growth and a 52% reduction in nematode population, while *Purpureocillium lilacinum* showed the least impact, with a 21% improvement in plant growth and a 52% reduction in nematode infestation**.**The nematode population also indicates the population reduction of J2 and egg mass count. Hence these reductions, where moderate compared to fluopyram, highlight the potential of these biocontrol agents as sustainable components of integrated nematode management programs. The nematicidal effect of chitosan is closely linked to its biochemical interactions in the soil. Chitosan application enhances the population of chitinolytic microorganisms, which produce chitinase enzymes that degrade the chitin-rich egg shells and juvenile cuticles of root-knot nematodes (Abd El-Aziz and Khalil, 2020). Furthermore, chitosan's ability to induce systemic resistance in plants and generate nematotoxic compounds upon decomposition contributes to its inhibitory effects on nematode development and reproduction (Asif *et al.,* 2017). Previous studies evidence the present findings. Mouniga *et al.* (2023) reported that chitosan nanospheres exhibited strong nematicidal properties against *M. incognita* under laboratory, greenhouse, and field conditions. Alfy *et al.* (2020) demonstrated that chitosan nanoparticles at 2000 ppm treatment led to a 78% reduction in soil nematode populations and a 98% reduction in root infestation in tomato plants, along with enhanced plant growth metrics.

CONCLUSION

Preliminary studies have indicated that chitosan nano formulation to be very effective against root knot nematode *M.enterolobii* in Guava. However, the long-term effect of chitosan nano formulation and its compatibility with nematode biocontrol agents like *Pochonia chlamydosporia* has to be evaluated in order to develop a holistic and sustainable management practice for root knot nematode *M. enterolobii* in guava.

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