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Synergistic effects of bioagents and botanicals for integrated management of rice root-knot nematode (*Meloidogyne graminicola*)

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ABSTRACT

The study was carried out during rainy (*kharif*) seasons of 2023 and 2024 at ICAR-Indian Agricultural Research Institute, New Delhi to investigate the efficacy of multiple treatment methods in controlling rice root-knot nematode (*Meloidogyne graminicola*), under field conditions. The treatments tested included the nematicide Fluensulfone (trade name Nimitz) @5 g/block, bioagent *Bacillus subtilis* @15 g/plot (CFU 1.5 × 109), and the botanicals garlic oil @2000 ppm and annona oil @20000 ppm, and were applied separately and in combination. The experiment was conducted with and without polythene sheet covering. All the observations were made at 60 days' post-treatment, focusing on plant height and the number of galls/seedling. Results showed that the combined application of garlic oil and *B. subtilis* significantly reduced nematode infestation, with the lowest gall count (9.14/plant) and the tallest seedlings (48.8 cm), especially under polythene sheet covering. Similar patterns were observed with other combinations of garlic oil and *B. subtilis*, showing notable increase in plant height and decrease in gall counts. The influence was more significant with polythene covering compared to without polythene sheet covering.

Keywords: Annona oil, *Bacillus subtilis,* Garlic oil, Management, Polythene cover, Root-knot nematode

Rice (*Oryza sativa* L.) is a primary staple food for a vast population worldwide, yet its productivity is significantly threatened by pests and diseases, including plant-parasitic nematodes (McCouch *et al.* 2016). More than 200 species of nematodes affect rice crops (Prot 1994). Among them, root-knot nematodes (*Meloidogyne* spp.) are particularly problematic in rainfed, upland, and lowland rice systems. Additionally, *Hirschmanniella* spp. cause damage in lowland rice regions across South and South-east Asia (Prot 1994, Gautam *et al.* 2022). *Meloidogyne graminicola* is regarded as the most damaging species in upland rice cultivation (Jones *et al.* 2013, Haque *et al.* 2018), contributing to substantial economic losses in multiple rice-growing environments (Bridge *et al.* 1990, Panwar and Rao 1998).

Meloidogyne graminicola has been identified as a major pest in rice fields worldwide, particularly in well-drained soils. It infests transplanted, deep-water, and upland rice, with severe infestations documented across north India, including Jammu, Punjab, Himachal Pradesh, Haryana, Delhi, and Uttar Pradesh. Reports indicate its increasing presence in rice-wheat cropping systems, raising concerns about its further spread and impact. *M. graminicola* has emerged as a major threat in the Indo-Gangetic plains, particularly in rice-wheat cropping systems. First detected in Bulandshahr, Uttar Pradesh, during the 2009–10 growing season, it caused average yield losses of 20–25%, with severe infestations leading to crop losses of up to 50–60% (Khan and Ahamad 2020). The nematode has the potential to trigger large-scale outbreaks and significant agricultural damage. Surveys have identified infected nursery seedlings as the primary mode of its spread, posing a serious challenge by facilitating its dispersal to new fields.

An effective management strategy at the nursery stage are therefore, important. Conventional chemical-based control poses environmental concerns and risk in developing resistance in nematicide to nematicides. Integrated pest management (IPM), therefore offers an environmentally sustainable approach to nematode suppression. The present study deals with the effectiveness of Fluensulfone, *B. subtilis*, garlic oil, and annona oil alone and in combination against *M. graminicola* in rice.

MATERIALS AND METHODS

Experimental site and design: The study was carried out during rainy (*kharif*) seasons of 2023 and 2024 at ICAR-Indian Agricultural Research Institute (28.08°N latitude and 77.12°E longitude, an elevation of 229.63 m amsl), New Delhi. The soil in this region has been naturally infested with the root-knot nematode (*Meloidogyne graminicola)*,

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and the initial nematode population was estimated to be in the range of 190–210 second-stage juveniles (J2s) per cubic centimeter of soil, as confirmed through pre-experimental sampling.

The experimental field measured 25 m in length and 15 m in width and was ploughed thoroughly, leveled, and pulverized to achieve a fine tilth suitable for uniform seed germination and application of treatments. A basal dose of recommended fertilizers, namely 3.75 kg N, 1.125 kg P_2O_5, and 1.50 kg K_2O, was incorporated into the soil prior to seed sowing following standard agronomic practices for rice nursery bed preparation. Following this, the field was divided into discrete experimental plots, each measuring 1 m × 1 m, with sufficient spacing between plots to avoid treatment interference. The field was divided into two main sections based on solarization treatment: one section was covered with transparent polyethylene sheets for solarization, while the other remained uncovered and served as the non-solarized control.

The experiment was conducted in randomized block design (RBD) with two replications for each treatment. Plot randomization was carried out to minimize variability due to micro-environmental differences across the field. The rice variety used in this study was Pusa Basmati 1121, a popular aromatic cultivar known for its susceptibility to root-knot nematodes, thereby serving as an ideal host for assessing the efficacy of various nematode management treatments. The seeds were sourced from the ICAR-Indian Agricultural Research Institute, New Delhi.

Treatment application and solarization protocol: Various treatments, comprising chemical, biological, and botanical agents, were evaluated alone and in combination under both solarized and non-solarized conditions [T_1, Control (solarization: with and without); T_2, Bioagent- *Bacillus subtilis* @15 g/block; T_3, Garlic oil @2000 ppm; T_4, Annona oil @20000 ppm; T_5, Fluensulfone @5.0 g/block (PC); T_6, Fluensulfone + Garlic oil; T_7, Fluensulfone + Annona oil; T_8, Bioagent- *Bacillus subtilis* + Garlic oil; T_9, Bioagent- *Bacillus subtilis* + Annona oil; T_{10}, Annona oil + Garlic oil] . Treatments were administered using the soil drenching method, wherein a freshly prepared test solution of each agent was applied directly to the soil. Specifically, a volume of 200 ml/seed line of a 1% stock concentration was uniformly applied across each plot. Immediately after treatment application, the treated soil surface was lightly covered with dry soil, and seeds were broadcasted over the treated area.

To achieve effective soil solarization, plots assigned to the solarization treatment were first well-tilled and leveled to break clods and ensure uniform heating. Moisture was incorporated into the soil by irrigating up to 50% of the water-holding capacity (WHC) one day prior to covering with a polyethylene sheet. A transparent polyethylene sheet (100 µm thickness; dimensions 2.5 m × 1.5 m) was then laid over each plot, with the edges buried firmly into the soil to prevent displacement by wind or water. This solarization treatment was maintained for a duration of 15 days during peak summer (average ambient temperature ~41.3ºC), allowing adequate heating of the upper soil layers to target nematode mortality and soil pathogen suppression.

Chemicals and bio-pesticides inputs: The chemical nematicide Fluensulfone (Nimitz) was incorporated at the rate of 5 g/plot by mixing it thoroughly into the soil prior to seed sowing. This active ingredient is known for its efficacy against migratory and sedentary nematodes, particularly *Meloidogyne* spp., and was chosen as a positive control for chemical nematicidal activity.

The microbial bioagent *Bacillus subtilis* (colony forming unit count of 1.5 × 10^9 CFU/ml) was procured from ICAR-Indian Agricultural Research Institute, New Delhi. It was incorporated into the soil in combination with well-decomposed farmyard manure (FYM) to facilitate its establishment and proliferation in the rhizosphere. The use of FYM also helped improve soil structure, water retention, and microbial diversity.

Botanical treatments included Annona oil and garlic oil, each evaluated alone or in combination with either Fluensulfone or *B. subtilis*. These oils were selected based on prior literature supporting their bioactive properties and compatibility with integrated pest management (IPM) strategies.

Seed preparation and sowing: Prior to sowing, rice seeds (Pusa Basmati 1121) were subjected to surface sterilization to prevent contamination from seed-borne pathogens. Seeds were immersed in 0.1% mercuric chloride solution for 2 min, followed by repeated washing with sterile distilled water to eliminate any residual chemical. The sterilized seeds were then shade-dried at room temperature for 24 h to attain suitable moisture content for sowing.

Seeds were broadcasted manually in the prepared nursery plots following the farmer's traditional broadcasting practice, ensuring even distribution across the plot area. A thin layer of fine soil was applied over the seeds to protect them from desiccation and bird predation. Regular irrigation was carried out post-sowing to maintain adequate moisture in the soil for germination and seedling establishment.

Observation and data collection: To evaluate the effectiveness of the treatments, three key nematological and plant growth parameters were recorded at defined time intervals.

Seedling height (30 and 60 days after sowing, DAS): Five rice seedlings were randomly sampled from each plot at 30 and 60 DAS. Plants were carefully uprooted to avoid root breakage, and adhering soil was gently washed off with water. Shoot length was measured using a scale from the base of the stem (cut end) to the tip of the tallest leaf. Measurements were taken in cm and averaged to calculate the mean seedling height for each treatment.

Root gall index: At both 30 and 60 DAS, the same seedlings were evaluated for root galling caused by *M. graminicola*. Washed roots were visually inspected using a 10× hand lens, and the total number of galls per plant was counted. Roots were then stained with acid fuchsin following the method of Byrd *et al.* (1983) to enhance the visibility of nematode-induced root structures. The degree of galling served as an indicator of nematode infestation intensity.

Eggs/egg mass (at 60 DAS): To determine the reproductive potential of the nematodes, egg masses from stained root samples were dissected using fine forceps under a stereoscopic binocular microscope. Egg counts were performed on three 1 ml sub-samples of egg suspensions collected from crushed egg masses. The average number of eggs/egg mass was then calculated for each treatment plot.

All treatments were terminated at 60 DAS, and final observations were compiled for statistical analysis.

Weather monitoring: Meteorological data during the crop growth period (Temperature, humidity, and rainfall) were recorded using standard weather instruments installed at the experimental site. These data were used to interpret treatment efficacy in the context of environmental conditions, especially the solarization period, where elevated soil temperature played a critical role in nematode suppression.

Experimental replication and statistical analysis: The entire field experiment was replicated twice under randomized block design (RBD) conditions, and each treatment was applied to four separate plots (replications). Data from pot and field trials were compiled and analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0. For all measured variables, results were expressed as mean ± standard deviation (SD).

Analysis of variance (ANOVA) was conducted to determine the significance of differences between treatment means. Post hoc comparison of treatment effects was performed using Duncan's Multiple Range Test (DMRT) at a 5% level of significance ($p<0.05$). This allowed for statistical discrimination among treatments with respect to plant growth enhancement and nematode suppression.

Additional parameters: To further validate treatment impacts, egg hatch rate was monitored under controlled conditions. Samples of nematode eggs extracted from roots were incubated in petri dishes under laboratory conditions, and the percentage of hatched juveniles was recorded after 72 h to assess the influence of each treatment on nematode development. Field uniformity and soil health indicators, including *p*H and electrical conductivity, were monitored before and after the experiment to detect any treatment-induced changes that could influence long-term soil fertility or microbial balance.

RESULTS AND DISCUSSION

The present investigation revealed a significant improvement in rice seedling growth and effective suppression of the root-knot nematode *M. graminicola* under all treatment conditions compared to the untreated control. From the data presented in Fig. 1, it is evident that solarized beds enhanced seedling height notably up to 60 days after sowing (DAS). The most prominent plant height was observed in the treatment involving a mixture of Fluensulfone and Annona oil (45.7 cm), followed closely by Fluensulfone combined with garlic oil (45.5 cm), and Fluensulfone applied alone (44.5 cm). Treatments involving only Annona oil (41.8 cm) and the biocontrol agent *B. subtilis* (41.7 cm) also demonstrated significant improvements in plant growth. These values were considerably higher compared to the control, where unsolarized beds yielded stunted seedling growth averaging 36 cm. This trend supports the conclusion that soil solarization, combined with effective biopesticidal or chemical interventions, promotes healthier and more vigorous seedling development under nematode-infested conditions.

A distinct decline in the number of root galls formed on infected rice seedlings was recorded in all treated plots, as indicated in Fig. 2. This reduction was apparent as early as one-week post-sowing, with 1–2 galls/seedling

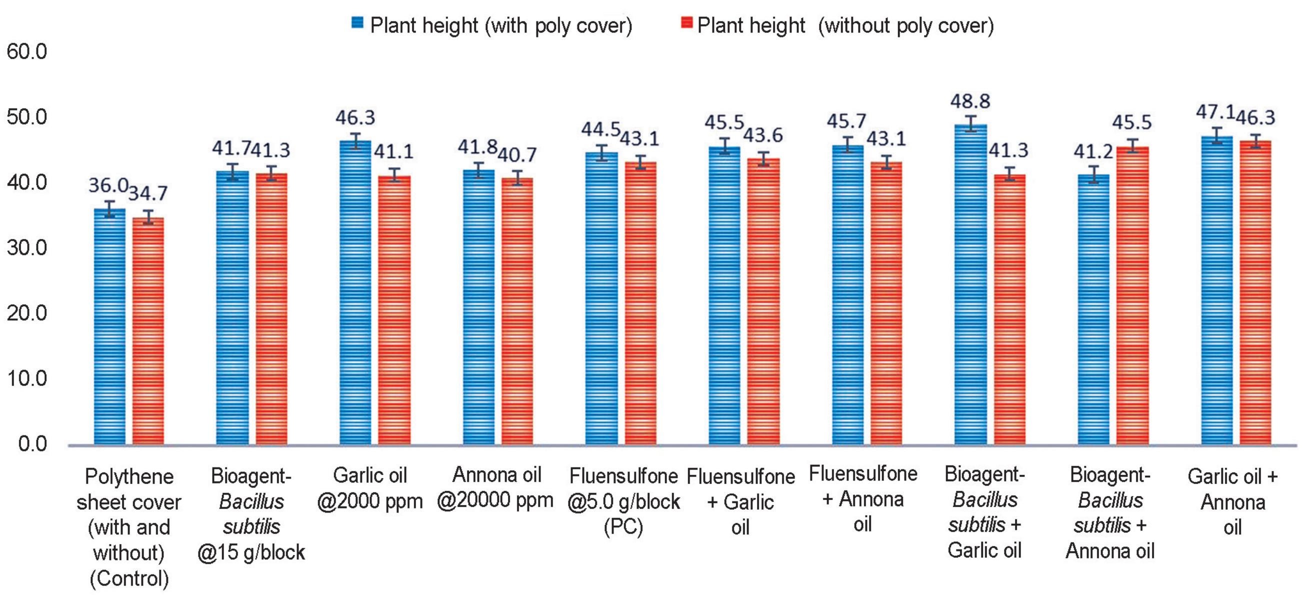

Fig. 1 Effects of nematicide, bioagent, and botanical management techniques on the rice plant/seedling height grown under polythene and open fields (60 DAS).

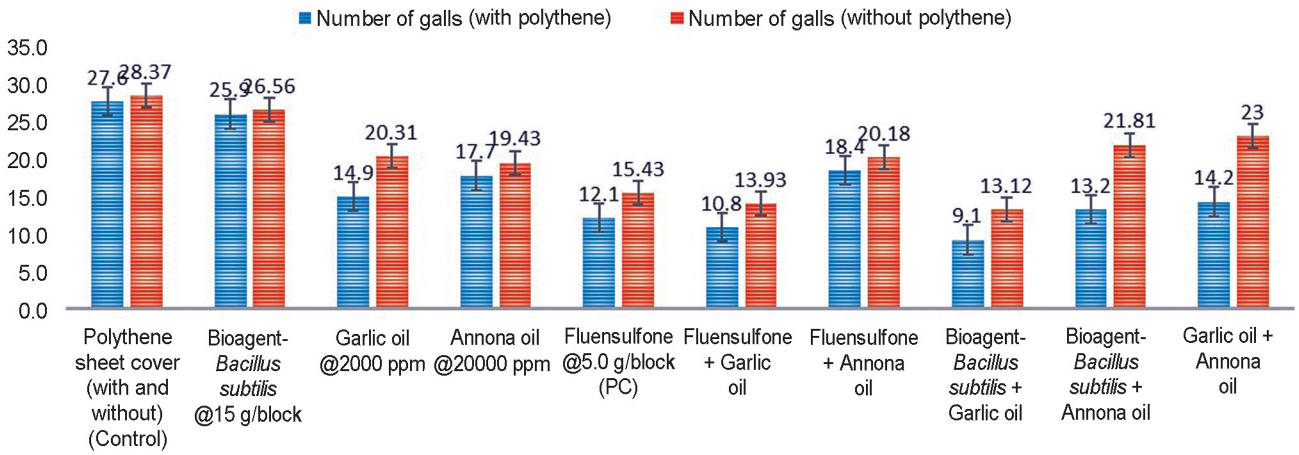

Fig. 2 Effects of nematicide, bioagent, and botanical management techniques on the number of galls in rice grown under polythene and open fields (60 DAS).

in infected roots, which illustrates the early penetration and establishment of *M. graminicola*. In untreated control plots, gall formation continued to increase over time, peaking at 60 DAS. This sustained increase underscores the aggressive and persistent nature of *M. graminicola* when left unmanaged.

Interestingly, even in solarized plots treated with the chemical nematicide carbofuran, gall counts increased, though less aggressively than in untreated plots. This observation suggests that while carbofuran may have initial efficacy, the nematode's population rebound remains a concern. Similar outcomes were reported by Hossain *et al.* (2007), where solarization alone or in combination with nematicides delayed but did not entirely prevent nematode resurgence.

A key metric of nematode reproductive potential, the number of eggs/egg mass was also significantly reduced across treatments, ranging from 42.6–74.4% reduction compared to controls. Among all treatments, the greatest reduction in gall numbers (and therefore nematode reproduction) was achieved in solarized plots treated with garlic oil in combination with *B. subtilis* (9.1 galls/plant) and Fluensulfone combined with garlic oil (10.8 galls/plant). These were closely followed by Fluensulfone alone (12.1 galls/plant). Notably, while the individual application of Fluensulfone (12.1 galls) or garlic oil (14.9 galls) showed some effectiveness, their combined use, especially under solarized conditions, amplified the nematicidal effect significantly. These results aligned with earlier studies by Gaur (1994), Ganguly *et al.* (1996), and Hossain *et al.* (2007), who highlighted that solarization can enhance the efficacy of both chemical and biological nematicides.

Field-based assessments in naturally infested rice soils further substantiated these results. As shown in Fig. 3, solarization using transparent polythene mulch significantly suppressed *M. graminicola* populations, reducing nematode counts to 82 individuals/unit of soil, as opposed to 92 in non-solarized open-field plots. This suggests that increased

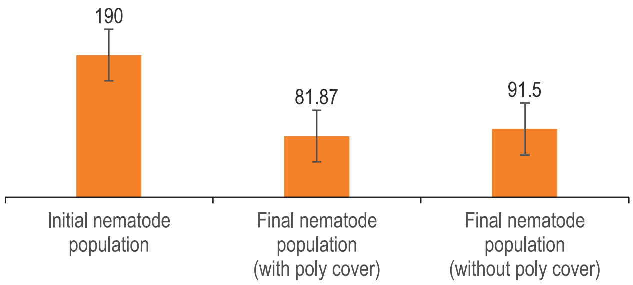

Fig. 3 Effect of solarization and non-solarization treatments on naturally infested population of *M. graminicola* in rice fields/plots.

soil temperature under polythene mulch create unfavourable conditions for nematode survival and reproduction, thereby aiding in effective nematode population management. Based on these outcomes, it is recommended that rice farmers adopt solarization via soil mulching or polythene covers, especially in nursery beds prior to sowing. This simple practice could substantially reduce nematode pressure in the early stages of plant development.

Plant height and gall count analysis: Under polythene-covered (solarized) conditions, the positive control, Fluensulfone, resulted in rice seedlings achieving a height of 48.8 cm with an average gall count of 9.14/plant. Treatments using garlic oil and Annona oil, whether applied individually or in combination, generated comparable results in terms of both plant height and gall suppression. However, the combination of garlic oil and *B. subtilis* under polythene covering emerged as the most effective treatment, resulting in the tallest seedlings and the least number of galls, outperforming even the chemical control.

In open-field conditions (non-solarized), a similar trend was noted, albeit with slightly less pronounced results. Here too, the combination of garlic oil and *B. subtilis* significantly enhanced seedling growth and reduced nematode infestation. Nevertheless, the benefits of treatments were more evident under solarized conditions, emphasizing the synergistic potential of solarization in combination with nematicidal agents.

Previous studies, including those by Katan (1981), Barbercheck *et al.* (1986) and Stapleton and DeVay (1986), have established the efficacy of soil solarization against a wide range of soil-borne pathogens, including nematodes. Their findings corroborated the current study's results, demonstrating that elevated soil temperature during solarization not only kill nematodes directly but also alter the soil micro-environment in a way that reduces pest establishment. Gaur and Perry (1991) reported similar findings across different crops, further supporting the use of this technique in diverse agro-ecosystems.

The climatic conditions during the *kharif* season characterized by high temperatures and long daylight hours are particularly favourable for solarization. Optimal results are obtained when the process is conducted between June and July, although May, August, and September may also be viable depending on the regional climate (Chandel *et al.* 2002). The duration and intensity of solarization are critical factors influencing its success, and thus, careful timing and implementation are crucial.

In addition to soil solarization, the application of biocontrol agents such as *B. subtilis* has emerged as an effective component of integrated nematode management. Similar to other biocontrol agents like *Pseudomonas fluorescens*, *B. subtilis* operates via multiple mechanisms, including induced systemic resistance, antibiotic and siderophore production, and alterations in root exudate composition that interfere with nematode attraction and penetration (Oostendorp and Sikora 1990, Aalten *et al.* 1998, Muthulakshmi *et al.* 2010, Deepa *et al.* 2011, Ye *et al.* 2022, Pervez *et al.* 2024). In the current study, *B. subtilis* proved to be a robust biological control agent when used alone or in conjunction with botanical oils and solarization.

Faria *et al.* (2024) demonstrated the nematicidal efficacy of volatile and essential oil components against *M. graminicola*, providing mechanistic evidence for the role of botanicals in suppressing nematode activity. In line with these findings, our study showed that combining garlic oil with *B. subtilis* markedly reduced gall formation and enhanced plant growth, particularly under polythene covering, thereby reinforcing the potential of botanical–bioagent synergies for effective nematode management.

Moreover, Kumar *et al.* (2022) also demonstrated that integrated use of organic amendments and bio-agents effectively reduced *M. graminicola* infestation in rice under screen-house conditions, highlighting the potential of combinational approaches for nematode management. In line with these findings, our observations revealed that the combined application of garlic oil and *B. subtilis* under polythene sheet covering resulted in the lowest gall counts and enhanced plant growth, further underscoring the synergistic benefits of bio-agents and botanicals in integrated nematode management.

A separate but relevant study highlighted the effectiveness of garlic oil and *B. subtilis* in reducing *M. graminicola* populations by 46% at 60 DAS. These findings are particularly promising, considering that botanical oils such as garlic oil are biodegradable, environmental friendly, and compatible with organic farming systems.

Overall, the findings of this study provided compelling evidence for the effectiveness of integrated nematode management strategies in suppressing *Meloidogyne graminicola* in rice nurseries. Among all treatments the garlic oil and *Bacillus subtilis* under solarized (polythene-covered) conditions was the most effective, leading to the highest seedling growth and the lowest incidence of root galling. The synergistic interaction between the botanical oil and the biological control agent enhanced nematode suppression beyond what could be achieved through individual applications. Furthermore, the application of Fluensulfone, both alone and in combination with plant-based oils, proved to be an efficient chemical alternative. However, its effectiveness was notably enhanced when applied in solarized nursery beds, indicating that thermal treatment plays a vital role in boosting the performance of nematicidal interventions. This study also reinforces the importance of solarization as a pre-planting strategy for nematode control. The rise in soil temperature under polythene mulch significantly reduces nematode survival rates, making it an ideal technique for nursery preparation during the *kharif* season. This non-chemical, environmentally benign method can be easily adopted by farmers, especially when integrated with low-cost botanical and microbial treatments.

Future research should focus on the long-term impacts of these treatments across different soil types, climatic zones, and cropping patterns. Economic analyses are also needed to assess the cost-effectiveness and scalability of these integrated approaches. Additionally, more studies are warranted to understand the interactions between bioagents and plant physiology under field conditions to optimize dosages, formulations, and timing of applications.

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