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Applying Pseudomonas fluorescens and Arthrobotrys thaumasia together Efficiently Manages On tomatoes, Meloidogyne incognita

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Research Article

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Abstract

One of the most significant horticultural crops in Indonesia is the tomato (Solanum lycopersicum L.). However, root-knot nematode (Meloidogyne spp.) infections contribute to its subpar output. The overuse of chemical nematicides has created ecological and environmental issues, underscoring the need for sustainable substitutes. A viable approach is to combine nematode-trapping fungus (NTFs) with beneficial bacteria like Pseudomonas fluorescens, which may increase biocontrol efficacy through synergistic interactions. In this work, we used in vitro tests and in vivo experiments on tomato plants to assess the biocontrol capacity of Arthrobotrys thaumasia and P. fluorescens against M. incognita. Six treatments were used in the in vivo application: M. incognita as a positive control, a negative control without M. incognita, a chemical control with carbofuran, biocontrol treatments with A. thaumasia and P. fluorescens, and a combination of A. thaumasia and P. fluorescens. Seven, fourteen, and thirty-five days after the immunization, observations were made. The greatest nematode death rate (87%) was caused by A. thaumasia after 48 hours, followed by P. fluorescens (64%), according to the results. There was no antagonistic relationship between the two agents, according to compatibility tests. The combination treatment decreased the quantity of vermiform worms and root-knot formation while increasing tomato plant development, according to in vivo studies. At 35 days following inoculation, the combination therapy of A. thaumasia and P. fluorescens decreased root galling by 90%, followed by A. thaumasia (76%), carbofuran (43%), and P. fluorescens (36%). According to these results, suppressing root-knot nematodes during tomato production may be accomplished more successfully and sustainably by combining A. thaumasia with P. fluorescens.

Keywords: Biocontrol; *Meloidogyne incognita*; Root-knot nematode; *Arthrobotrys thaumasia*; *Pseudomonas fluorescens*

Introduction

Tomatoes (Solanum lycopersicum L.) are one of the most important horticultural crops in the world because of their high economic worth (Padmanabhan et al. 2015). The Food and Agriculture Organization of the United Nations reports that the world's tomato production has surpassed 187 million tons, farmed on more than 5 million hectares (FAO 2023). But producing tomatoes comes with a lot of challenges, including soil-borne diseases that significantly lower crop output and are difficult to control once they get established in the field (Cheng et al. 2021; Pontes et al. 2024). One of these diseases that is known to drastically lower tomato production and cause large financial losses is root-knot nematodes (Barbary et al. 2015; Swe et al. 2024).

Root-knot nematodes, or Meloidogyne spp., have a broad host range; they infect around 5,500 plant species and are estimated to generate 5% of global production losses each year, or USD 78 billion (Ahmad et al. 2021). The primary Meloidogyne species associated with tomato infections worldwide include M. javanica, M. incognita, M. arenaria, M. hapla, and M. enterolobii (Philbrick et al. 2020; Eken et al. 2024). M. incognita is the most prevalent and dangerous of all. Over 2000 plant species globally had their productivity drastically reduced by this polyphagous endoparasite (Boakye et al. 2022). Galls or root knots are common indicators of infestation, which are brought on by infectious second-stage juveniles that penetrate plant roots and promote abnormal cell division (Miyashita et al. 2014). In agricultural contexts, synthetic chemical nematicides continue to be the most widely used means of controlling worms. According to Xiao et al. (2016) and Hahn et al. (2019), prolonged use of these pesticides may result in nematode resistance, harmful residues in the environment, and detrimental impacts on non-target species. Alternative controls that are safer and more sustainable are required because of these detrimental impacts. Nematode-trapping fungi (NTFs), which are natural enemies of nematodes and have the capacity to catch and digest nematodes via the creation of trap structures, are one approach that may be used (Jiang et al. 2016). NTFs are acknowledged to offer promise as biological control agents, but there is still need for improvement, particularly in the creation of commercial goods. According

to Kumar et al. (2015) and Jiang et al. (2016), infections may sometimes persist even after biocontrol agents are used, suggesting that the pathogen inoculum is not entirely eradicated and control efficacy is still not at its best. Using two or more helpful microorganisms at once is one method to increase the effectiveness of biological control (Meyer and Roberts 2002). David et al. (2018) claim that antagonistic agent combinations, as opposed to large populations of a single antagonistic agent, are the main source of natural biological control. Combining biological control agents may increase the range of control activity and more nearly mimic natural settings, improving control efficacy and dependability. There is still little usage of NTF in conjunction with bacteria, particularly when it comes to combating root-knot nematodes. Even in the absence of nematodes, a number of bacteria belonging to the genera Bacillus, Delftia, Enterobacter, Pseudomonas, and Serratia are known to be able to cause trap formation in NTFs (Wernet and Fischer 2023). According to research by Lafta and Kasim (2019), using P. fluorescens and NTF together in vitro was more successful than applying them separately at preventing egg hatching and getting rid of J2 Meloidogyne spp. One of the bacteria that has been thoroughly investigated in the biological control of nematodes is P. fluorescens (Azam et al. 2018; Sohrabi et al. 2018; Noureldeen et al. 2021). In light of this, the purpose of this research was to assess how well NTF and P. fluorescens work together to suppress Meloidogyne incognita on tomato plants.

Materials and Methods

Culture preparation

Nematode culture: Second-stage *Meloidogyne incognita* juveniles (J2) were obtained from pure cultures maintained on tomato roots. *Meloidogyne* was identified based on the perineal pattern morphology of mature females, according to Taylor and Sasser (1978). After being carefully removed from the ground and cleaned with tap water, infected roots were chopped into pieces that were between one and two centimeters long. Eggs were collected following the procedure described by Hussey and Barker (1973) and immediately incubated in plastic hatching cups at 25 ± 3°C for 72 h to obtain *M. incognita* second-stage juveniles (J2). For *in vitro* and pot tests experiments, J2 were counted using a counting chamber (Hastuti and Faull 2018; Eken *et al.* 2024).

Fungal strain: *Arthrobotrys thaumasia* used in this study was identified in a previous study and isolated from the tomato rhizosphere. After two weeks of growth at 25°C on potato dextrose agar (PDA), sterile water was added to the culture to create a spore suspension. A hemocytometer was used to determine the spore concentration (1×10^7 spores/mL) (Hastuti and Faull 2018).

Bacterial strain: *Pseudomonas fluorescens* (ATCC 17550) was obtained from Agavi Lab, Indonesia. A culture that had been in nutrient broth medium for 48 hours was centrifuged for 10 min at 2500 rpm. Centrifugation was used to rinse the pellets (bacterial cells) three times with sterile distilled water. A concentration of 10^8 CFU mL^{-1} was achieved by adjusting the final solution (Xu *et al.* 2015; Sohrabi *et al.* 2018).

In vitro nematicidal activity

In vitro tests were conducted to determine the nematicidal effects of A. thaumasia and P. fluorescens on M. incognita. 300 M. incognita J2 were individually treated to microbial solutions (107 conidia/ml of A. thaumasia and 108 CFU mL-1 of P. fluorescens) in sterile petri plates. Sterile distilled water served as the control. Each treatment was duplicated five times. During each 48-hour observation period, distinct sets of petri dishes were kept. Nematode mortality was observed by touching the juveniles with a small needle to check for motility (Hashem and Abo-Elyousr 2011; Lafta and Kasim 2019).

In vitro compatibility test

A dual-culture approach was used to evaluate the compatibility of A. thaumasia and P. fluorescens (Dugassa et al. 2021). One half of a Petri plate containing PDA was injected with an isolate of P. fluorescens. A 5 mm-diameter agar plug from a five-day-old A. thaumasia culture was placed on the other side. Additionally, a control plate devoid of bacterial inoculation was used. Every experiment was carried out in triplicate and incubated at 25 ± 1°C. The presence or lack of a clear zone around the bacterial strain was used to determine compatibility.

In vivo evaluation of biocontrol agents

Preparation of growth media and comparative chemical agent:

In this experiment, tomato plants were grown in compost soil with a pH of around 6, 5-6, 8. The soil was air dried and autoclaved for approximately 60 minutes at 121°C to sterilize it. 300 g of sterilized soil mix were placed into each 20-cm-diameter polybag. A week before a 10 mL solution of each organism was added, the tomato seedlings were moved.The concentration of the suspension was the same as that used in the nematicidal activity test. Carbofuran was used as the similar chemical agent in this investigation. A carbofuran solution was made by grinding two milligrams of carbofuran and mixing

it with ten milliliters of methanol. Ten milliliters of this solution were added to each treatment polybag seven days before the tomato seedlings were moved. To keep the soil wet, plants were irrigated every day (Purba et al.. 2022). **Bioassay test against *M. incognita* on tomato plants:** The bioassay was conducted using a completely randomized design. The procedure followed Hastuti and Faull (2018) with modifications. Thirty-day-old seedlings were transplanted into the prepared polybags according to the treatments: (i) a positive control (K+) inoculated with *M. incognita*; (ii) a negative control (K–), without *M. incognita* or any biocontrol agents; (iii) a chemical control inoculated with *M. incognita* and treated with carbofuran; (iv) plants inoculated with *M. incognita* and treated with *A. thaumasia*; (v) plants inoculated with *M. incognita* and treated with *P. fluorescens* and (vi) plants inoculated with *M.incognita* and treated with a combined application of *A. thaumasia* and *P. fluorescens*. Approximately 1000 *M. incognita* J2 were applied to each treatment, except for the K–. Each treatment was replicated three times and observed at 7, 14, and 35 d after the 7 d application period of *M. incognita*.

Data analysis

Root knots were counted, and various growth parameters of the tomato plants were quantified. Root infection was determined by excavating the plants, thoroughly cleaning the roots, staining them with an acid fuchsin dye and observing the presence of vermiform nematodes and root galls. Tomato plant growth was evaluated by measuring the roots and shoots' fresh and dry weights, and the root and shoot lengths. The collected data were analyzed using analysis of variance with SPSS software version 25.00 (Hastuti and Faull 2018).

Results

***In vitro* assay**

The *in vitro* efficacy of *A. thaumasia* and *P. fluorescens* in suppressing the viability of *M. incognita* J2 is presented in Table 1. Both *A. thaumasia* and *P. fluorescens* significantly reduced the number of viable *M. incognita* J2 after 48 h of exposure. *A. thaumasia* was significantly more effective than *P. fluorescens* in suppressing J2 viability, reducing the number of J2 by 87%, whereas *P. fluorescens* caused a 64% reduction. Based on the observation data and statistical analysis, *A. thaumasia* showed significantly greater efficacy than *P. fluorescens* in suppressing J2 viability.**Table 1:** Effect of biocontrol agents on *M. incognita* mortality

Treatments	*M. incognita* mortality*	
	Viable juveniles† after 48 h	Reduction % of control
Arthrobotrys thaumasia	35 ± 1.70[a]	87
Pseudomonas fluorescens	102 ± 1.22[b]	64
Control	288 ± 0.70[c]	0

*Mortality was evaluated based on motility observations (initial population of J2 = 300)
†Mean ± SE, n = 5
Significant differences ($P \leq 0.05$) between treatments are indicated by different letters

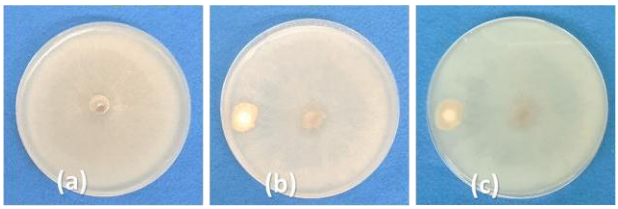


Fig. 1: The mycelial growth *of A. thaumasia* on potato dextrose agar plates with or without inoculated bacterial cells. (**a**) Individual of *A. thaumasia* (control) (**b**) Obverse side view of the *A. thaumasia-P. fluorescens* interaction and (**c**) Reverse side view of the *A. thaumasia* and *P. fluorescens* interaction

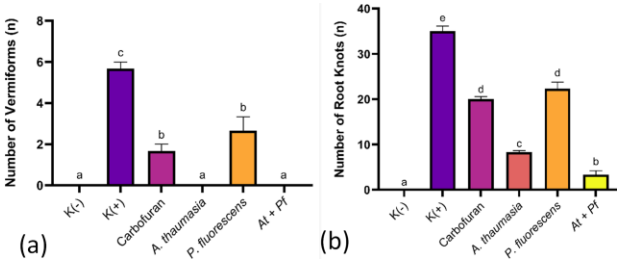


Fig. 2: Effect of *Arthrobotrys thaumasia*, *Pseudomonas fluorescens*, and their combination on (**a**) the number of vermiform *M. incognita* in tomato after 7 d and (**b**) the number of root knots by *M. incognita* in tomato after 35 days. K(–), negative control, *i.e*., no *M. incognita* or isolates; K(+), positive control, *i.e*., only *M. incognita* present. Carbofuran served as the chemical nematicide control

Compatibility test

The compatibility between *A. thaumasia* and *P. fluorescens* was evaluated to determine whether the two biocontrol agents could be co-applied without inhibitory interactions. The dual-culture assay showed no visible inhibition zone between the fungal and bacterial isolates throughout the incubation period (Fig. 1). The absence of growth suppression indicates that the two microorganisms do not directly compete or produce toxic metabolites against one another, confirming that they are compatible and suitable for potential synergistic co-application.

Viable post-treatment vermiform *M. incognita* and root- knot formation

On day 7 after treatment (Fig. 2a), the positive controlealed the greatest quantity of viable vermiform M. incognita, with P. fluorescens and carbofuran treatment coming in second and third. On the other hand, plants treated with A. thaumasia, the combination of A. thaumasia and P. fluorescens, and K– treatments did not exhibit vermiform worms. This implies that A. thaumasia was more successful than P. fluorescens in inhibiting M. incognita's juvenile growth. The K+ treatment had the most root galls on day 35 (Fig. 2b), followed by the P. fluorescens and carbofuran treatments. Gall production was greatly decreased by applying A. thaumasia, and the combined treatment produced the fewest galls, which demonstrated the greatest efficacy in preventing the formation of root knots. The negative control (K−) showed no signs of galls. Consequently, the most effective way to reduce nematode infection and root damage in tomato plants was to apply both treatments at the same time. Microscopic observations provided further evidence for these findings. Fig. 3 shows the various phases of M. incognita infection in tomato roots at 7, 14, and 35 days after treatment.

Tomato growth performance under *M. incognita*

The shoot development characteristics of tomato plants seven, fourteen, and thirty-five days after transplanting were considerably impacted by the treatments (Fig. 4). By day 35, the combined application of A. thaumasia and P. fluorescens treatment substantially outperformed K− and all other treatments, consistently producing the tallest shoots and greatest fresh weights during all observation periods ($P < 0.05$). Additionally, shoot dry weight rose dramatically with the combined treatment, particularly at 35 days, suggesting higher biomass accumulation, perhaps as a result of better plant performance and nematode suppression. When compared to the positive control inoculated with M. incognita and the chemical control treated with carbofuran, treatments with A. thaumasia and P. fluorescens applied separately also produced better shoot growth; however, the combined application demonstrated the strongest synergistic effect on shoot growth under nematode stress. The biological treatments greatly improved root growth (Fig. 5). At every observation period (7, 14, and 35 days), the combination treatment produced the longest roots and the maximum root biomass. Effective nematode suppression was shown by day 35, when the root length and fresh and dry weights in the combination treatment were considerably higher than in all other treatments ($P < 0.05$). Over K+ and carbofuran, separate treatments enhanced root characteristics; however, the combination treatment proved to be the most successful. These findings imply that, despite nematode infection, the synergistic action of A. thaumasia and P. fluorescens improved shoot growth and root development, perhaps as a consequence of decreased nematode

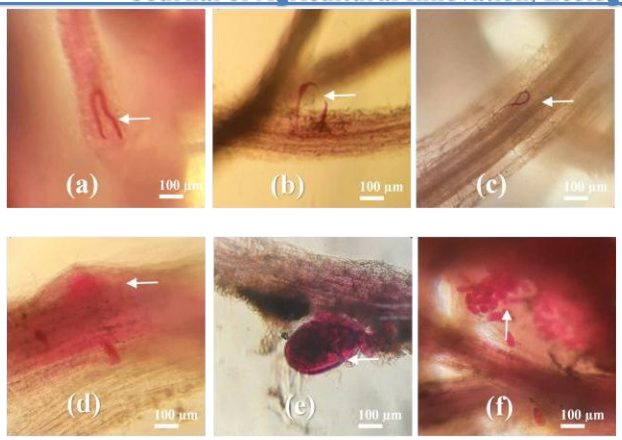
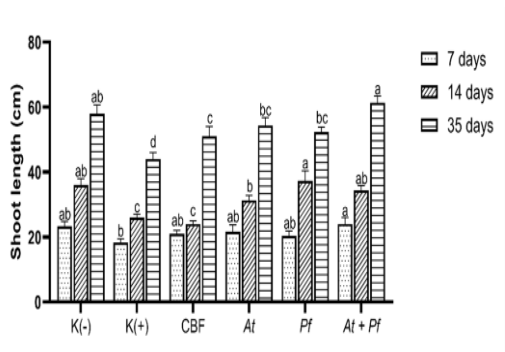


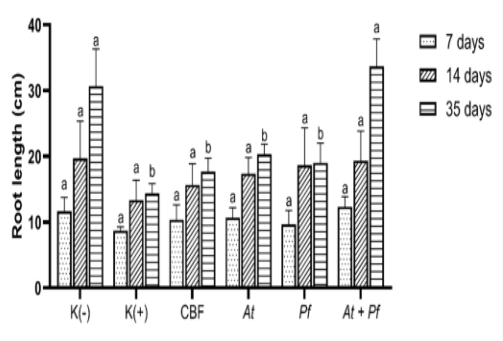
Fig. 3: *M. incognita* in tomato roots at different stages of infection 7, 14, and 35 d after treatment. (**a**–**c**) Vermiform second-stage juveniles penetrating the root tissues at 7 d after inoculation; (**d**) formation of feeding sites (giant cells) at 14 d; (**e**) mature female established within the root and (**f**) egg masses at 35 d post- inoculation. Arrows indicate the location of the nematode or its developmental structures

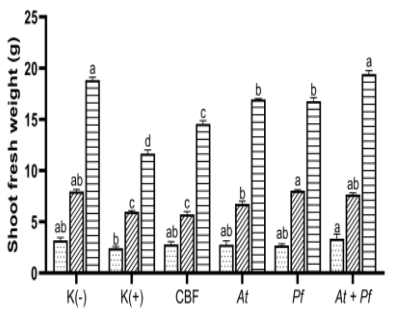
infestation, increased nutrient uptake, and overall healthier root system development.

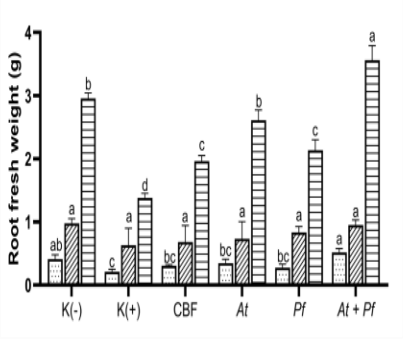
Discussion

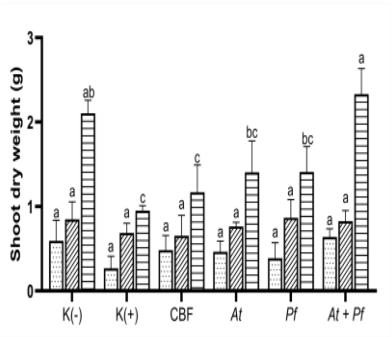
According to in vitro experiments, the biocontrol agents were successful in raising the death rates of M. incognita second-stage juveniles (J2), which were noticeably greater than those of the untreated control group. Among the organisms assessed, A. thaumasia shown a greater capacity to decrease J2 viability, lowering J2 populations by 87% as opposed to P. fluorescens by 64%. These findings are consistent with earlier research that found that the population of infectious M. incognita juveniles was significantly reduced against this fungus. For instance, according to Kassam et al. (2021) and Eken et al. (2024), A. thaumasia was able to lower the number of J2 M. incognita by up to 82% and more than 70%, respectively, in an in vitro test. A. thaumasia's broad-spectrum potential as a biological control agent was further reinforced by Hastuti et al. (2023), who observed an 89% decrease in the population of juvenile M. hapla. The M. incognita population was effectively suppressed by the combined treatment of A. thaumasia and P. fluorescens, which demonstrated a favorable synergistic effect. In comparison to A. thaumasia (76%), carbofuran (43%), and P. fluorescens (36%), this combination therapy reduced the amount of root knots by 90% on day 35. The fact that there were more root knots in the P. fluorescens treatment than in the A. thaumasia treatment suggests that P. fluorescens's capacity to prevent the production of root knots is comparatively reduced when it is not mixed. Similarly, although the carbofuran-treated chemical control decreased the production of root knots when compared to the positive control (K+), it was less successful than the biocontrol treatments, particularly the


Shoot length (cm)
7 days
14 days
35 days
K(-)
K(+)
CBF
At
Pf
At + Pf


Root length (cm)
7 days
14 days
35 days
K(-)
K(+)
CBF
At
Pf
At + Pf


Shoot fresh weight (g)
K(-)
K(+)
CBF
At
Pf
At + Pf


Root fresh weight (g)
K(-)
K(+)
CBF
At
Pf
At + Pf


Shoot dry weight (g)
K(-)
K(+)
CBF
At
Pf
At + Pf

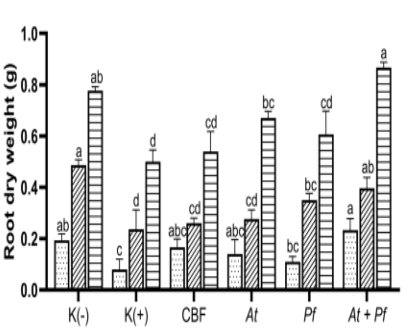

Root dry weight (g)
K(-)
K(+)
CBF
At
Pf
At + Pf

Fig. 4: Effects of various treatments on shoot growth parameters over time. K(–), negative control, *i.e*., no *M. incognita* or isolates; K(+), positive control, *i.e*., only *M. incognita* present; CBF, *M. incognita* present and treated with carbofuran; *At*, *M. incognita* present and treated with *A. thaumasia*; *Pf*, *M. incognita* present and treated with *P. fluorescens*; *At + Pf*, *M. incognita* present and treated with *A. thaumasia* and *P. fluorescens*. Different letters denote statistical significance ($P \leq 0.05$) derived from Tukey's HSD post-hoc test analysis

combined application. The effectiveness of the combined treatment in reducing the number of root knot is likely due to the synergistic interaction between *A. thaumasia* and *P. fluorescens*. *A. thaumasia* is a NTF that infects nematodes through the formation of specialized trap structures, while *P. fluorescens* suppresses nematodes activity through the production of antimicrobial metabolites and lytic enzymes such as proteases and chitinases. Hajjaji *et al*. (2024) demonstrated that enzymatic extracts from *Pseudomonas* spp. caused significant juvenile mortality and that combined enzyme treatments exhibited synergistic nematicidal effects. These findings support the role of *P. fluorescens* in weakening nematode structural defenses, thereby enhancing the trapping efficiency of *A. thaumasia*. The compatibility between *A. thaumasia* and *P. fluorescens*, as indicated by the absence of antagonistic interactions *in vitro*, further supports their effective cooperation in the rhizosphere. Previous studies have shown that several bacterial genera, including *Pseudomonas*, *Bacillus*, *Delftia*, *Enterobacter*, and *Serratia*, can induce trap formation in nematode-trapping fungi such as *A. oligospora* and *A. conoides*, even in the absence of

nematodes. Bacterial signaling molecules including urea and diketopiperazines, which function as triggers for trap creation, facilitate this induction (Wernet and Fischer 2023). The increased nematode suppression seen in the combination therapy may be explained by these bacterial–fungal interactions. The combination of A. thaumasia and P. fluorescens may improve plant defense mechanisms against nematode infection in addition to having direct nematicidal action. According to Arshad et al. (2021), tomato defense-related genes (PR-1b and JERF3) were activated by combination biocontrol treatments, which decreased M. incognita infestation. According to Ayub et al. (2024), Pseudomonas species enhanced tomato vigor and decreased nematode damage by inducing systemic resistance as well as direct antagonistic interactions. Sharma et al. (2021) further shown that by improving nutrient absorption and triggering plant defense mechanisms, the combination application of mycorrhizal fungi and P. fluorescens considerably decreased root-knot nematode infestation. Overall, using A. thaumasia or P. fluorescens alone was less successful than using them together, highlighting the drawbacks of individualbiological control agents in complicated soil environments. The A. thaumasia and P. fluorescens combination therapy outperformed carbofuran, which is consistent with earlier findings that microbial consortia provide more consistent and long-lasting worm suppression than chemical nematicides (Meyer and Roberts 2002; Liu et al. 2012). These findings highlight the possibility of using P. fluorescens and A. thaumasia into sustainable and ecologically friendly integrated nematode control systems for tomato farming.

Conclusion

The greatest efficacy in reducing M. incognita infection was shown by the combined application of A. thaumasia and P. fluorescens, which reduced the number of root knots by 90% as opposed to 76% for A. thaumasia and 36% for P. fluorescens alone. The two agents' suitability for co-application is supported by the lack of adversarial interactions. Additionally, this combination boosted the biomass of tomato plants, suggesting that the combination of A. thaumasia and P. fluorescens provides a more sustainable and successful method of controlling tomato root-knot nematodes than either chemical control or solo biological agents.

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