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### Biocontrol Potential of *Aspergillus oryzae* against the Root-knot Nematode *Meloidogyne javanica* in Carrot (*Daucus carota* L.)

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#### Abstract

**Background:** Carrot (*Daucus carota* L.) is an economically and nutritionally important vegetable crop severely affected by root-knot nematodes, particularly *Meloidogyne javanica*. This study evaluated the biocontrol potential and plant growth-promoting effects of *Aspergillus oryzae* against *M. javanica* in carrot.

**Methods:** *M. javanica* was isolated from infected carrot roots and identified based on perineal-pattern morphology and molecular characterization. *A. oryzae*, isolated from the rhizosphere of healthy carrot plants, was identified using cultural, microscopic, and molecular characteristics. Its *in vitro* nematicidal activity was evaluated based on egg-hatching inhibition and J<sub>2</sub> mortality. Greenhouse experiments assessed the effects of different *M. javanica* inoculum levels and amounts of *A. oryzae* fresh mycelial biomass on plant growth, biochemical responses, and nematode-related parameters.

**Results:** Inoculation with 2000 J<sub>2</sub> caused the greatest reduction in plant growth and photosynthetic pigments, whereas phenolic and proline contents and antioxidant enzyme activities increased. *A. oryzae* significantly inhibited egg hatching and increased J<sub>2</sub> mortality in a concentration- and time-dependent manner. Among the combined treatments, T11 (2000 J<sub>2</sub> + 2.0 g *A. oryzae* fresh mycelial biomass suspended in 10 mL DW) produced the greatest improvement in plant growth, photosynthetic pigments, and biochemical responses, whereas T12 (2000 J<sub>2</sub> + 3.0 g *A. oryzae* fresh mycelial biomass suspended in 10 mL DW) showed the greatest suppression of gall formation, egg masses, soil nematode population, and reproduction factor.

**Conclusion:** *A. oryzae* showed plant-growth-promoting effects and nematode-suppressive activity and may serve as a promising component of sustainable *M. javanica* management in carrot. Further field validation and mechanistic studies are required to establish its efficacy, consistency, and mode of action.

**Keywords:** *Aspergillus oryzae*; biocontrol; carrot; *Meloidogyne javanica*; root-knot nematode; plant growth promotion

## 1. Introduction

Carrot (*Daucus carota* L.) is a biennial herbaceous crop belonging to the family Apiaceae and is widely cultivated across Asia, Europe, North Africa, and North and South America (Ellison *et al.*, 2018). It is an important root vegetable valued for its nutritional composition, including vitamins, minerals, dietary fiber, and antioxidant compounds, which contribute to its health-promoting properties (Que *et al.*, 2019). Carrot also contains bioactive compounds such as falcarinol, a polyacetylene associated with potential health benefits (Soares *et al.*, 2018). Despite its nutritional and economic importance, carrot production is affected by several abiotic and biotic stresses. Among the major biotic constraints are soil-borne pathogens and pests, including viruses, bacteria, fungi, and plant-parasitic nematodes. In particular, *Meloidogyne* species (root-knot nematodes) and *Heterodera carotae* (carrot cyst nematode) are important nematode pests associated with carrot production (Gowda *et al.*, 2017).

Root-knot nematode infection can cause substantial yield losses depending on nematode population density, host susceptibility, soil properties, and environmental conditions, with losses ranging from moderate reductions to severe yield loss under favorable conditions (Collange *et al.*, 2011). Infection by *Meloidogyne* spp. is characterized by the formation of galls on roots, which can cause root deformation, forking, and stunted taproot development, thereby reducing yield and market quality. Above-ground symptoms may include chlorosis, wilting, reduced plant vigor, and uneven growth, while below-ground symptoms include gall formation, root distortion, and impaired root development (Joshi and Walia, 2024). These effects are particularly important in carrot because the taproot constitutes the primary marketable product.

Management of root-knot nematodes has traditionally relied heavily on chemical nematicides. However, the extensive use of nematicides has risen environmental and health concerns because some compounds may persist in the environment, contaminate soil and water, adversely affect non-target organisms, and leave undesirable residues (Dutta *et al.*, 2019; Muñoz-Bautista *et al.*, 2025). These concerns have increased the need for sustainable and environmentally compatible alternatives for nematode management. Biological control using naturally occurring microorganisms with antagonistic activity against plant-parasitic nematodes represents a promising approach. Among fungal biocontrol agents, several genera, including *Trichoderma*, *Pochonia*, *Paecilomyces*, and *Purpureocillium*, have demonstrated potential for suppressing plant-parasitic nematodes (Siddiqui and Mahmood, 1996). In addition to direct nematode suppression, beneficial microorganisms may promote plant growth by alleviating biotic stress and improving nutrient availability, thereby contributing to plant health and productivity (Roca-Couso *et al.*, 2021).

The genus *Aspergillus* has also attracted attention as a potential source of biologically active metabolites with nematicidal properties. For example, *A. oryzae* has been reported to exhibit nematicidal activity against *M. incognita*, with fungal culture products and associated metabolites showing inhibitory effects on juvenile survival and egg-hatching (Kim *et al.*, 2016). Other *Aspergillus* species have likewise been investigated for their nematicidal potential against root-knot nematodes (Yang *et al.*, 2024). However, the biocontrol efficacy of individual *Aspergillus* species and isolates may vary depending on their biological characteristics, metabolite profiles, concentration, and interaction with the host plant. Therefore, evaluation of a specific *Aspergillus* isolate under both *in vitro* and greenhouse conditions is important for determining its potential as a biocontrol agent. In the present study, the activity of the isolated *A. oryzae* was evaluated experimentally, without attributing its effects to any specific metabolite that was not characterized in the isolate. In this context, the present study aimed to isolate and identify the root-knot nematode associated with infected carrot roots and to isolate and characterize a fungal biocontrol agent with potential nematode-suppressive and plant growth-promoting properties. The nematicidal activity of *A. oryzae* against *M. javanica* was evaluated *in vitro* based on egg-hatching inhibition and second-stage juvenile (J<sub>2</sub>) mortality. Furthermore, greenhouse experiments were conducted to determine the effects of different *M. javanica*

inoculum levels on carrot growth, photosynthetic pigments, biochemical responses, and antioxidant enzyme activities, and to evaluate the effects of different amounts of fresh *A. oryzae* mycelial biomass on plant growth and nematode development and reproduction.

## 2. Materials and Methods

### 2.1 Isolation and Identification of Root-knot Nematode

Adult females were carefully isolated from infected carrot roots using dissecting needles under a stereoscopic microscope. The nematode species was initially identified based on perineal-pattern characteristics following (Eisenback and Hirschmann, 1981). For molecular identification, genomic DNA was extracted from adult females of *M. javanica* and used as the template for PCR amplification of the 18S rRNA gene region. The primers PP21 (Nem\_18s\_Forward: 5'-CGCGAATRGCTCATTACAACAGC-3') and PP22 (Nem\_18s\_Reverse: 5'-GGGCGGTATCTGATCGCC-3') were used for amplification. PCR amplification was performed in a total reaction volume of 20 µL under the following conditions: initial denaturation at 94°C for 30 s (1 cycle), followed by 32 cycles of denaturation at 98°C for 10 s, annealing at 52°C for 15 s, and extension at 68°C for 30 s, followed by a final extension at 68°C for 3 min (1 cycle). The amplified products were separated by electrophoresis on a 1.5% agarose gel and visualized using a gel documentation system to confirm successful amplification. The PCR products were purified and subjected to Sanger sequencing. The resulting nucleotide sequences were compared with reference sequences available in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). For phylogenetic analysis, the obtained sequence was aligned with representative *Meloidogyne* sequences retrieved from GenBank, and a phylogenetic tree was constructed using the Maximum Likelihood method implemented in MEGA 11. The identity of the nematode isolate was confirmed based on the combined evidence of morphological characteristics, sequence similarity, and phylogenetic placement. The nucleotide sequence generated in this study was deposited in the NCBI GenBank database under accession number PZ886039.

### 2.2 Isolation and Identification of Fungal Biocontrol Agent

Rhizospheric soil samples were collected from healthy carrot plants belonging to the family Apiaceae during November–December. Fungal isolation was carried out using the serial dilution technique to obtain individual fungal colonies. The purified fungal isolate was cultured on Potato Dextrose Agar (PDA) medium and incubated at 25±2°C for 5–7 days. Preliminary identification of the isolate was performed based on macromorphological and microscopic characteristics.

For molecular identification, genomic DNA was extracted from the purified fungal culture, and the internal transcribed spacer (ITS) region of the fungal ribosomal DNA was amplified by PCR and sequenced through a commercial molecular diagnostic facility. PCR amplification was performed to obtain an approximately 600-bp fragment of the ITS region. The reaction conditions consisted of an initial denaturation at 95°C for 3 min (1 cycle), followed by 32 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min (1 cycle). The PCR products were analyzed by electrophoresis on a 1.5% agarose gel prepared in Tris-Cl-sodium acetate-EDTA buffer containing ethidium bromide at a concentration of 0.5 µg mL<sup>-1</sup> and visualized under UV illumination. Positive amplicons were purified using the FavorPrep™ GEL/PCR Purification Kit (Cat. No. FAGCK 001) and subjected to bidirectional Sanger sequencing. The resulting nucleotide sequences were analyzed and compared with reference sequences available in the NCBI GenBank database. The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei, 1993), and a phylogenetic tree was constructed using MEGA 11 to confirm the molecular identity of the fungal isolate.

### 2.3 Preparation of Nematode and Fungal Biocontrol Agent Inocula

The *M. javanica* population used throughout the experiments isolated from the carrot root population as described in Section 2.1. Healthy brinjal (*Solanum melongena* L.) plants were used only as a suitable host for multiplication and maintenance of the same nematode population. Thus, no new nematode population was introduced during the experimental period. The second-stage juveniles (J<sub>2</sub>) used in the *in vitro* and greenhouse experiments were obtained from this continuously maintained population. Egg masses were collected from heavily infested brinjal roots, washed thoroughly with sterile distilled water, and transferred onto mesh sieves lined with double-layered tissue paper. The egg masses were incubated at 25°C to obtain freshly hatched second-stage juveniles (J<sub>2</sub>). The resulting J<sub>2</sub> suspension was collected and quantified using a counting dish under a stereoscopic microscope. The nematode suspension was subsequently adjusted to approximately 200 ± 5 J<sub>2</sub> mL<sup>-1</sup> and used for subsequent experiments.

Richard's medium was used for mass multiplication of *A. oryzae* because its nutrient composition provides a suitable growth substrate for fungal biomass production. The fungus was cultured in a 500-mL Erlenmeyer flask containing 280 mL of Richard's medium and inoculated with 3–5 mycelial plugs. The culture was incubated at 25±2°C for 15–20 days. Following incubation, the mycelial mat was harvested and separated from the culture medium by filtration. The fresh fungal biomass was then weighed, and 1.0, 1.5, 2.0, and 3.0 g quantities of *A. oryzae* mycelial biomass were separately suspended in 10 mL of distilled water (DW). Each suspension was homogenized thoroughly to obtain a uniform biomass-based inoculum before application to the root zone according to the respective treatments.

### 2.4 *In vitro* Nematicidal Activity of *A. oryzae*

#### 2.4.1 Egg-Hatching Inhibition (%)

Egg masses obtained from heavily infested brinjal roots bearing the continuously maintained *M. javanica* population were handpicked using sterilized forceps and transferred to Petri plates containing 10 mL of cell-free culture filtrate of *A. oryzae* at 5, 25, 50, and 75% (v/v), prepared by dilution with distilled water. Since cell-free culture filtrate rather than a spore or viable-cell suspension was used, CFU counts were not applicable. Five egg masses were placed in each Petri plate. Distilled water was used as the untreated control. The plates were incubated at 28°C, and observations were recorded after 24, 48, and 72 hrs using a stereobinocular microscope. Each Petri plate was considered an independent experimental unit, with three independent plates used per treatment. Egg-hatching inhibition was calculated according to the formula described by (Ravichandra, 2010);

$$\text{Egg - hatching inhibition (\%)} = \frac{\text{No. of eggs in control} - \text{No. of eggs hatched in treatment}}{\text{No. of eggs in control}} \times 100$$

#### 2.4.2 Juvenile Mortality

Ten milliliters of each *A. oryzae* culture-filtrate concentration (5, 25, 50, and 75% (v/v)) were transferred to separate Petri plates containing 100 freshly hatched J<sub>2</sub> of *M. javanica*. Distilled water was used as the untreated control. Each Petri plate was considered an independent experimental unit, with three independent plates used per treatment. The plates were incubated at 28°C, and J<sub>2</sub> mortality was recorded after 24, 48, and 72 hrs using a stereobinocular microscope. Juveniles that remained immobile and exhibited a straight body posture were considered dead, whereas mobile and curved juveniles were considered alive (Rokiek & Nagdi, 2011). J<sub>2</sub> mortality was calculated as:

$$\text{J}_2 \text{ mortality (\%)} = (\text{Number of dead J}_2 / \text{Total number of J}_2) \times 100$$

### 2.5 Greenhouse Experiment

A greenhouse experiment was conducted from October 2025 to January 2026 to evaluate the efficacy of *A. oryzae* against *M. javanica* in carrot. Sandy loam soil was collected from the field of the Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. The soil was air-dried and passed through a 20-mesh sieve to remove debris, stones, and plant residues. A potting mixture consisting of soil, river sand, and well-decomposed organic manure was prepared in a ratio of 3:1:1 (v/v/v), and 1 kg of the prepared mixture was transferred to earthen pots measuring 15 cm in diameter. Before sterilization, 250 mL of distilled water was added to each pot to maintain appropriate moisture content. The soil mixture was sterilized by autoclaving at 121°C and 15 psi pressure for approximately 20–30 min. The physico-chemical characteristics of the soil used in the experiment are presented in Table 1.

Carrot seeds were surface-disinfected with 2% sodium hypochlorite solution and subsequently rinsed three times with sterile distilled water before sowing. The experiment was arranged in a completely randomized design (CRD) with three replications per treatment. The experiment was conducted under greenhouse conditions with natural light, an average temperature of 25–30 °C, and relative humidity of 60–65%. The treatments comprised an uninoculated untreated control, *M. javanica*-inoculated plants, *A. oryzae*-treated plants, and plants inoculated with *M. javanica* and treated with different amounts of fresh *A. oryzae* mycelial biomass (1.0, 1.5, 2.0, and 3.0 g), each suspended in 10 mL of distilled water (DW), as described in section 2.3.

**Table 1.** Physico-chemical properties of experimental soil.

| Soil property                      | Value      |
|------------------------------------|------------|
| Soil type                          | Sandy loam |
| Sand (%)                           | 74.70      |
| Silt (%)                           | 16.20      |
| Clay (%)                           | 9.10       |
| Water holding capacity (%)         | 52.06      |
| Soil porosity (%)                  | 62.28      |
| Bulk density (g cm <sup>-3</sup> ) | 1.38       |

### 2.5.1 Plant Growth Parameters

Plant growth parameters were recorded 60 days after nematode inoculation. Plants were carefully uprooted, and shoot and root lengths (cm), fresh weight (g), and dry weight (g) were measured separately.

### 2.5.2 Biochemical Parameters

For the estimation of biochemical parameters, fresh leaf tissue was collected from the experimental plants. Photosynthetic pigments, including total chlorophyll and total carotenoid contents, were estimated according to (Arnon, 1949; MacLachlan & Zalik, 1963), respectively, and expressed as mg g<sup>-1</sup> fresh weight. Total phenolic content was estimated according to (Swain & Hillis, 1959) and expressed as mg gallic acid equivalents (GAE) g<sup>-1</sup> fresh weight (FW). Proline content was estimated following (Bates *et al.*, 1973) and expressed as µmol g<sup>-1</sup> fresh weight (FW).

### 2.5.3 Antioxidant Enzyme Activity

Fresh leaf tissue was collected from the experimental plants for the analysis of antioxidant enzyme activities. Peroxidase (POD) activity was estimated according to Chance and Maehly (1955), catalase (CAT) activity according to Aebi (1984), superoxide dismutase (SOD) activity according to Beauchamp and Fridovich (1971), and polyphenol oxidase (PPO) activity according to Kumar *et al.* (2008). Enzyme activities were expressed as U min<sup>-1</sup> g<sup>-1</sup> fresh weight (FW).

### 2.5.4 Nematode-Related Parameters

At the termination of the experiment, carrot roots were carefully uprooted, washed with water, and examined for the number of galls and egg masses per root system. Soil samples from each pot were collected to determine the nematode population per 250 g of soil using Cobb's sieving and decanting method followed by the Baermann funnel technique, and the reproduction factor ( $R_f$ ) of *M. javanica* was calculated from the initial and final nematode populations (Southey, 1986). The root-knot index was used to assess the severity of nematode infection (Taylor & Sasser, 1978).

## 2.6 Statistical Analysis

The experimental data obtained from the different treatments were subjected to one-way analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 5% level of significance ( $p \leq 0.05$ ), following the procedure described by (Dospikhov, 1984). Since the combined *M. javanica*–*A. oryzae* treatments were evaluated only at the selected nematode inoculum level, the experimental design did not constitute a complete factorial arrangement; therefore, factorial ANOVA was not applied.

## 3. Results

### 3.1 Identification and Molecular Characterization of *Meloidogyne javanica*

The root-knot nematode isolated from infected carrot roots was identified as *M. javanica* based on characteristic perineal-pattern morphology (Fig. 1). Molecular characterization further supported the morphological identification. The amplified nematode sequence was identified as *M. javanica* isolate MJ-9 and was deposited in the NCBI GenBank database under accession number PZ886039. BLAST analysis showed 100% sequence identity with corresponding *M. javanica* sequences available in the NCBI GenBank database. Phylogenetic analysis using the obtained sequence placed isolate MJ-9 within the *M. javanica* clade, supporting its species-level identification (Fig. 2).



Fig. 1. Perineal pattern of an adult female of *M. javanica*.

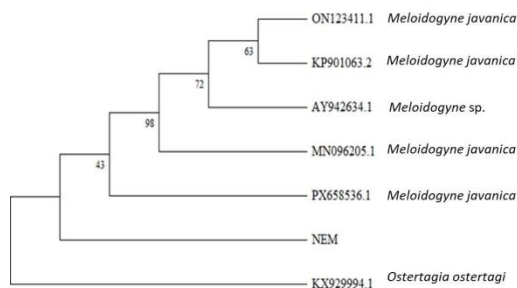


Fig. 2. Phylogenetic analysis of *M. javanica* (NEM) based on 18S rRNA gene sequences using the maximum likelihood method in MEGA 11. Bootstrap values (%) are shown at the respective internal nodes.

### 3.2 Identification and Molecular Characterization of *Aspergillus oryzae*

The fungal isolate obtained from the rhizosphere of healthy carrot plants was identified as *A. oryzae* based on cultural and microscopic characteristics. On Potato Dextrose Agar (PDA), the isolate produced characteristic yellowish-green colonies. Microscopic examination revealed septate hyphae, conidiophores, vesicles, phialides, and globose conidial heads, consistent with the morphological characteristics of *A. oryzae*. Molecular characterization of the isolate based on the ITS sequence further supported its morphological identification. The isolate was designated as *A. oryzae* ASP and deposited in the NCBI GenBank database under accession number PZ856299. BLAST analysis showed 100% sequence identity with *A. oryzae* sequences available in the NCBI GenBank database. Phylogenetic analysis further placed isolate ASP within the *A. oryzae* clade, confirming its molecular identity (Figs. 3 & 4).

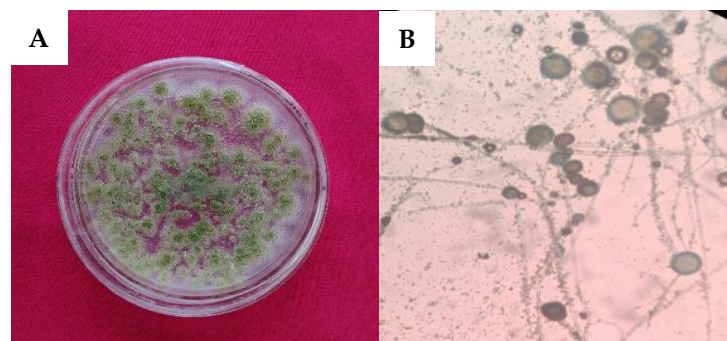


**Fig. 3.** A) Colony morphology of *A. oryzae* grown on PDA medium, B) Microscopic characteristics of the fungus.

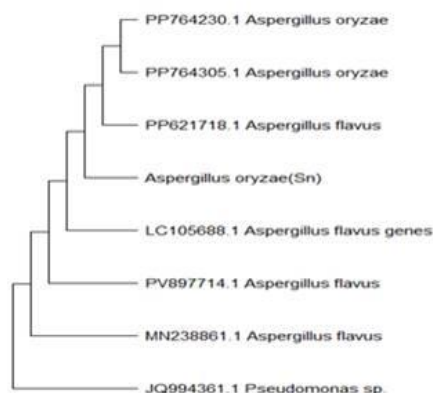


**Fig. 4.** Phylogenetic analysis of *A. oryzae* based on ITS sequences using the maximum likelihood method in MEGA 11. Bootstrap values (%) are shown at the respective internal nodes.

### 3.3 *In vitro* Nematicidal Activity of *A. oryzae*

*A. oryzae* culture filtrate significantly inhibited egg-hatching of *M. javanica*, and the magnitude of inhibition increased with both culture-filtrate concentration and exposure period. The maximum inhibition of egg hatching, 86.23%, was recorded at 75% (v/v) culture filtrate after 72 hrs. At each tested concentration, egg-hatching inhibition was greater at 48 and 72 hrs than at 24 hrs, indicating a progressive inhibitory effect with increasing exposure period (Fig. 5). A similar concentration and time-dependent response was observed for J<sub>2</sub> mortality. *A. oryzae* culture filtrate significantly increased J<sub>2</sub> mortality compared with the distilled-water control. The highest mortality was recorded at 75% (v/v) culture filtrate after 72 hrs, when 92 of the 100 exposed J<sub>2</sub> were immobile and classified as dead, corresponding to 92.0% mortality (Fig. 6). Thus, under the conditions tested, the highest culture-filtrate concentration and longest exposure period produced the greatest inhibition of egg-hatching and J<sub>2</sub> mortality.

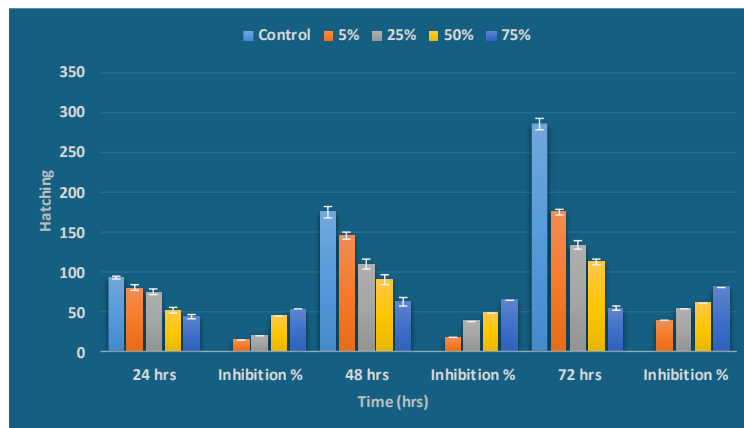


**Fig. 5.** Effect of different concentrations of *A. oryzae* culture filtrate on *M. javanica* egg-hatching at 24, 48 and 72 hrs.

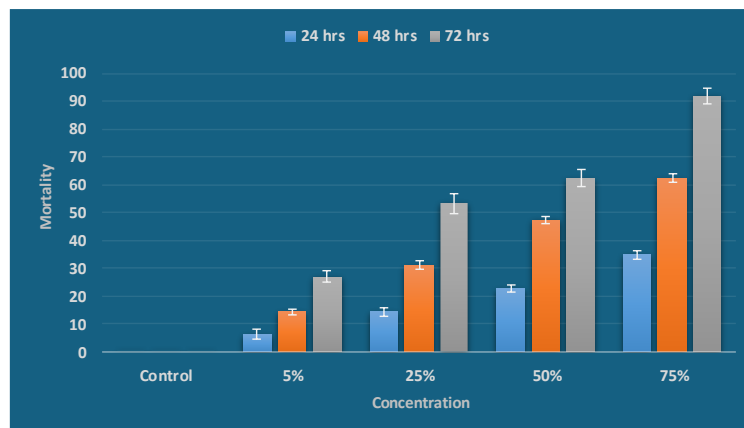


**Fig. 6.** Effect of different concentrations of *A. oryzae* culture filtrate on mortality of J<sub>2</sub> of *M. javanica* at 24, 48 and 72 hrs.

### 3.4 Effect of Different Treatments on Plant Growth Parameters

Increasing *M. javanica* inoculum levels progressively reduced carrot plant growth. Among the nematode-only treatments, T4 (2000 J<sub>2</sub>) produced the lowest growth values, with shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight of 33.70 cm, 23.40 cm, 13.46 g, 0.89 g, 25.80 g, and 3.79 g, respectively. These values were lower than those recorded for the untreated control, indicating a marked adverse effect of the highest nematode inoculum level on plant growth. Among the *A. oryzae*-only treatments, T7 (2.0 g fresh mycelial biomass suspended in 10 mL DW) recorded the highest values for all measured growth parameters. Shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight in T7 were 52.76 cm, 49.10 cm, 26.62 g, 5.46 g, 45.50 g, and 16.33 g, respectively (Table 2).

In plants inoculated with *M. javanica*, application of *A. oryzae* improved plant growth relative to the corresponding nematode-only treatment. Among the combined treatments, T11 (2000 J<sub>2</sub> + 2.0 g fresh *A. oryzae* mycelial biomass suspended in 10 mL DW) recorded the highest values for all measured growth parameters. Shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight in T11 were 39.80 cm, 30.76 cm, 18.76 g, 3.28 g, 32.77 g, and 11.77 g, respectively (Table 2). Thus, T7 showed the highest growth promotion among the *A. oryzae*-only treatments, whereas T11 showed the strongest growth response among the combined *M. javanica*–*A. oryzae* treatments.

**Table 2.** Effect of different inoculum levels of *M. javanica* and amounts of fresh *A. oryzae* mycelial biomass on growth parameters of carrot plants.

| Treatments | Plant length               |                            | Shoot weight               |                           | Root weight                |                             |
|------------|----------------------------|----------------------------|----------------------------|---------------------------|----------------------------|-----------------------------|
|            | Shoot length (cm)          | Root length (cm)           | Shoot fresh weight (g)     | Shoot dry weight (g)      | Root fresh weight (g)      | Root dry weight (g)         |
| C          | 44.36 ± 0.28 <sup>d</sup>  | 32.26 ± 0.29 <sup>e</sup>  | 20.88 ± 0.38 <sup>ef</sup> | 3.32 ± 0.24 <sup>cd</sup> | 35.32 ± 0.24 <sup>e</sup>  | 12.03 ± 0.39 <sup>def</sup> |
| T1         | 43.60 ± 0.45 <sup>d</sup>  | 32.02 ± 0.37 <sup>ef</sup> | 20.34 ± 0.18 <sup>ef</sup> | 3.10 ± 0.17 <sup>cd</sup> | 35.01 ± 0.25 <sup>ef</sup> | 11.48 ± 0.24 <sup>ef</sup>  |
| T2         | 42.28 ± 0.27 <sup>e</sup>  | 29.20 ± 0.34 <sup>g</sup>  | 20.11 ± 0.20 <sup>f</sup>  | 2.90 ± 0.05 <sup>de</sup> | 32.80 ± 0.57 <sup>g</sup>  | 10.16 ± 0.23 <sup>f</sup>   |
| T3         | 37.13 ± 0.17 <sup>h</sup>  | 27.06 ± 0.13 <sup>h</sup>  | 16.26 ± 0.29 <sup>h</sup>  | 1.95 ± 0.08 <sup>gh</sup> | 30.73 ± 0.29 <sup>h</sup>  | 8.46 ± 0.17 <sup>g</sup>    |
| T4         | 33.70 ± 0.32 <sup>j</sup>  | 23.40 ± 0.40 <sup>k</sup>  | 13.46 ± 0.17 <sup>i</sup>  | 0.89 ± 0.01 <sup>i</sup>  | 25.80 ± 0.23 <sup>j</sup>  | 3.79 ± 0.21 <sup>i</sup>    |
| T5         | 44.69 ± 0.32 <sup>cd</sup> | 37.61 ± 0.40 <sup>d</sup>  | 21.87 ± 0.15 <sup>d</sup>  | 3.49 ± 0.26 <sup>ef</sup> | 38.25 ± 0.28 <sup>d</sup>  | 13.20 ± 0.23 <sup>d</sup>   |
| T6         | 47.60 ± 0.40 <sup>b</sup>  | 44.95 ± 0.07 <sup>b</sup>  | 25.20 ± 0.34 <sup>b</sup>  | 3.59 ± 0.18 <sup>bc</sup> | 40.70 ± 0.39 <sup>b</sup>  | 14.60 ± 0.34 <sup>c</sup>   |
| T7         | 52.76 ± 0.49 <sup>a</sup>  | 49.10 ± 0.26 <sup>a</sup>  | 26.62 ± 0.26 <sup>a</sup>  | 5.46 ± 0.23 <sup>a</sup>  | 45.50 ± 0.23 <sup>a</sup>  | 16.33 ± 0.17 <sup>a</sup>   |
| T8         | 45.73 ± 0.46 <sup>c</sup>  | 39.08 ± 0.23 <sup>c</sup>  | 23.62 ± 0.32 <sup>c</sup>  | 3.97 ± 0.17 <sup>b</sup>  | 39.61 ± 0.29 <sup>c</sup>  | 15.42 ± 0.23 <sup>b</sup>   |
| T9         | 34.79 ± 0.40 <sup>i</sup>  | 24.36 ± 0.29 <sup>j</sup>  | 14.29 ± 0.31 <sup>i</sup>  | 1.75 ± 0.13 <sup>h</sup>  | 27.91 ± 0.44 <sup>i</sup>  | 5.63 ± 0.23 <sup>h</sup>    |
| T10        | 36.70 ± 0.36 <sup>h</sup>  | 25.78 ± 0.16 <sup>i</sup>  | 15.73 ± 0.29 <sup>h</sup>  | 2.33 ± 0.15 <sup>fg</sup> | 28.33 ± 0.22 <sup>i</sup>  | 5.72 ± 0.26 <sup>h</sup>    |
| T11        | 39.80 ± 0.41 <sup>f</sup>  | 30.76 ± 0.34 <sup>f</sup>  | 18.76 ± 0.31 <sup>f</sup>  | 3.28 ± 0.16 <sup>cd</sup> | 32.77 ± 0.32 <sup>g</sup>  | 11.77 ± 0.37 <sup>e</sup>   |
| T12        | 38.38 ± 0.18 <sup>g</sup>  | 27.76 ± 0.42 <sup>h</sup>  | 17.20 ± 0.24 <sup>g</sup>  | 2.53 ± 0.16 <sup>ef</sup> | 30.20 ± 0.41 <sup>h</sup>  | 8.16 ± 0.26 <sup>g</sup>    |
| LSD        | 1.06                       | 0.86                       | 0.80                       | 0.50                      | 0.98                       | 0.75                        |

Values represent mean ± standard error (SE) of three replicates. Means followed by the same letter within a column do not differ significantly according to DMRT at  $p \leq 0.05$ . C = untreated control; T1–T4 = carrot plants inoculated with *M. javanica* at 500, 1000, 1500, and 2000 J<sub>2</sub>, respectively; T5–T8 = carrot plants treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW; T9–T12 = carrot plants inoculated with *M. javanica* (2000 J<sub>2</sub>) and treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW.

### 3.5 Effect of Different Treatments on Photosynthetic Pigments and Biochemical Parameters

Inoculation with *M. javanica* adversely affected photosynthetic pigment accumulation in carrot plants. Among the nematode-only treatments, the lowest total chlorophyll and carotenoid contents were recorded in T4 (2000 J<sub>2</sub>), with values of 1.76 and 4.35 mg g<sup>-1</sup> FW, respectively, compared with 2.66 and 6.56 mg g<sup>-1</sup> FW, respectively, in the untreated control. Among the *A. oryzae*-only treatments, T7 (2.0 g fresh mycelial biomass suspended in 10 mL DW) recorded the highest total chlorophyll and carotenoid contents, reaching 4.84 and 10.53 mg g<sup>-1</sup> FW, respectively. In *M. javanica*-inoculated plants, application of *A. oryzae* improved photosynthetic pigment levels relative to the nematode-only treatment. The maximum values among the combined treatments were recorded in T11, with total chlorophyll and carotenoid contents of 2.35 and 6.17 mg g<sup>-1</sup> FW, respectively (Table 3).

In contrast to the reduction in photosynthetic pigments, *M. javanica* inoculation was associated with increased accumulation of total phenolics and proline. The highest values among the nematode-inoculated treatments were recorded in T4, with total phenolic and proline contents of 39.14 mg GAE g<sup>-1</sup> FW and 15.46 µmol g<sup>-1</sup> FW, respectively. Among the *A. oryzae*-only treatments, T8 (3.0 g fresh mycelial biomass suspended in 10 mL DW) recorded the highest numerical values for total phenolic and proline contents, at 32.76 mg GAE g<sup>-1</sup> FW and 8.89 µmol g<sup>-1</sup> FW, respectively. In *M. javanica*-inoculated plants, combined application of *A. oryzae* further increased these biochemical responses relative to the nematode-only treatment. The highest total phenolic and proline contents among the combined treatments were recorded in T11, reaching 42.79 mg GAE g<sup>-1</sup> FW and 17.50 µmol g<sup>-1</sup> FW, respectively (Table 3). Thus, T11 showed the greatest improvement in photosynthetic pigment accumulation and biochemical responses among the combined treatments.

**Table 3.** Effect of different inoculum levels of *M. javanica* and amounts of fresh *A. oryzae* mycelial biomass on photosynthetic pigments and biochemical parameters of carrot plants.

| Treatments | Total chlorophyll<br>(mg g <sup>-1</sup> FW) | Total carotenoids<br>(mg g <sup>-1</sup> FW) | Total phenolics<br>(mg GAE g <sup>-1</sup> FW) | Proline<br>(μmol g <sup>-1</sup> FW) |
|------------|----------------------------------------------|----------------------------------------------|------------------------------------------------|--------------------------------------|
| C          | 2.66 ± 0.09 <sup>e</sup>                     | 6.56 ± 0.12 <sup>e</sup>                     | 18.30 ± 0.26 <sup>l</sup>                      | 7.46 ± 0.17 <sup>i</sup>             |
| T1         | 2.54 ± 0.07 <sup>e</sup>                     | 6.42 ± 0.02 <sup>ef</sup>                    | 22.76 ± 0.06 <sup>i</sup>                      | 7.96 ± 0.05 <sup>h</sup>             |
| T2         | 2.23 ± 0.02 <sup>f</sup>                     | 6.13 ± 0.05 <sup>g</sup>                     | 35.30 ± 0.05 <sup>f</sup>                      | 12.89 ± 0.09 <sup>f</sup>            |
| T3         | 1.92 ± 0.03 <sup>hi</sup>                    | 5.82 ± 0.03 <sup>h</sup>                     | 38.15 ± 0.04 <sup>e</sup>                      | 14.35 ± 0.22 <sup>e</sup>            |
| T4         | 1.76 ± 0.06 <sup>i</sup>                     | 4.35 ± 0.03 <sup>i</sup>                     | 39.14 ± 0.04 <sup>d</sup>                      | 15.46 ± 0.17 <sup>d</sup>            |
| T5         | 3.41 ± 0.08 <sup>d</sup>                     | 7.43 ± 0.14 <sup>d</sup>                     | 20.80 ± 0.27 <sup>k</sup>                      | 7.12 ± 0.07 <sup>i</sup>             |
| T6         | 4.35 ± 0.04 <sup>b</sup>                     | 9.46 ± 0.08 <sup>b</sup>                     | 26.12 ± 0.03 <sup>i</sup>                      | 7.31 ± 0.05 <sup>i</sup>             |
| T7         | 4.84 ± 0.04 <sup>a</sup>                     | 10.53 ± 0.14 <sup>a</sup>                    | 28.16 ± 0.06 <sup>h</sup>                      | 8.54 ± 0.17 <sup>g</sup>             |
| T8         | 3.94 ± 0.06 <sup>c</sup>                     | 8.56 ± 0.08 <sup>c</sup>                     | 32.76 ± 0.20 <sup>g</sup>                      | 8.89 ± 0.06 <sup>g</sup>             |
| T9         | 1.84 ± 0.05 <sup>i</sup>                     | 4.67 ± 0.04 <sup>i</sup>                     | 39.20 ± 0.23 <sup>d</sup>                      | 15.69 ± 0.10 <sup>cd</sup>           |
| T10        | 2.05 ± 0.04 <sup>gh</sup>                    | 5.76 ± 0.04 <sup>h</sup>                     | 41.39 ± 0.18 <sup>b</sup>                      | 15.95 ± 0.05 <sup>c</sup>            |
| T11        | 2.35 ± 0.04 <sup>f</sup>                     | 6.17 ± 0.03 <sup>fg</sup>                    | 42.79 ± 0.16 <sup>a</sup>                      | 17.50 ± 0.15 <sup>a</sup>            |
| T12        | 2.17 ± 0.05 <sup>g</sup>                     | 5.96 ± 0.13 <sup>gh</sup>                    | 39.74 ± 0.09 <sup>c</sup>                      | 16.84 ± 0.05 <sup>b</sup>            |
| LSD        | 0.17                                         | 0.25                                         | 0.46                                           | 0.36                                 |

Values represent mean ± standard error (SE) of three replicates. Means followed by the same letter within a column do not differ significantly according to DMRT at  $p \leq 0.05$ . C = untreated control; T1–T4 = carrot plants inoculated with *M. javanica* at 500, 1000, 1500, and 2000 J<sub>2</sub>, respectively; T5–T8 = carrot plants treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW; T9–T12 = carrot plants inoculated with *M. javanica* (2000 J<sub>2</sub>) and treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW.

### 3.6 Effect of Different Treatments on Antioxidant Enzyme Activities

Inoculation with *M. javanica* significantly increased the activities of antioxidant enzymes in carrot plants compared with the untreated control, with enzyme activities generally increasing with increasing nematode inoculum level. Among the nematode-only treatments, T4 (2000 J<sub>2</sub>) recorded the highest activities of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and polyphenol oxidase (PPO), with values of 1.64, 2.93, 44.46, and 20.46 U min<sup>-1</sup> g<sup>-1</sup> FW, respectively. Among the *A. oryzae*-only treatments, T8 (3.0 g fresh mycelial biomass suspended in 10 mL DW) recorded the highest numerical activities for all four enzymes, with POD, CAT, SOD, and PPO activities of 0.92, 2.15, 41.25, and 18.75 U min<sup>-1</sup> g<sup>-1</sup> FW, respectively. In *M. javanica*-inoculated plants, combined application of *A. oryzae* increased antioxidant enzyme activities relative to the nematode-only treatment. T11 (2000 J<sub>2</sub> + 2.0 g fresh *A. oryzae* mycelial biomass suspended in 10 mL DW) recorded the highest activities among the combined treatments, with POD, CAT, SOD, and PPO values of 2.43, 3.47, 48.13, and 23.81 U min<sup>-1</sup> g<sup>-1</sup> FW, respectively (Table 4). These results indicate that *A. oryzae* application was associated with enhanced antioxidant enzyme activity under *M. javanica* stress. However, because oxidative damage and reactive oxygen species levels were not directly measured, the present results do not establish whether this enzymatic response reduced oxidative damage.

**Table 4.** Effect of different inoculum levels of *M. javanica* and amounts of fresh *A. oryzae* mycelial biomass on antioxidant enzyme activities in carrot plants.

| Treatments | Peroxidase (POD)<br>(U min <sup>-1</sup> g <sup>-1</sup> FW) | Catalase (CAT)<br>(U min <sup>-1</sup> g <sup>-1</sup> FW) | Superoxide dismutase<br>(SOD) (U min <sup>-1</sup> g <sup>-1</sup> FW) | Polyphenol oxidase<br>(PPO) (U min <sup>-1</sup> g <sup>-1</sup> FW) |
|------------|--------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------------------------|----------------------------------------------------------------------|
| C          | 0.34 ± 0.01 <sup>j</sup>                                     | 0.68 ± 0.07 <sup>j</sup>                                   | 23.90 ± 0.36 <sup>i</sup>                                              | 9.13 ± 0.17 <sup>j</sup>                                             |
| T1         | 0.85 ± 0.05 <sup>h</sup>                                     | 1.05 ± 0.06 <sup>i</sup>                                   | 42.20 ± 0.32 <sup>d</sup>                                              | 19.46 ± 0.10 <sup>e</sup>                                            |

|     |                          |                           |                           |                           |
|-----|--------------------------|---------------------------|---------------------------|---------------------------|
| T2  | 1.25 ± 0.03 <sup>g</sup> | 2.56 ± 0.08 <sup>e</sup>  | 42.33 ± 0.27 <sup>d</sup> | 19.65 ± 0.06 <sup>e</sup> |
| T3  | 1.47 ± 0.04 <sup>f</sup> | 2.74 ± 0.07 <sup>d</sup>  | 44.20 ± 0.23 <sup>c</sup> | 20.44 ± 0.03 <sup>d</sup> |
| T4  | 1.64 ± 0.03 <sup>e</sup> | 2.93 ± 0.06 <sup>c</sup>  | 44.46 ± 0.05 <sup>c</sup> | 20.46 ± 0.06 <sup>d</sup> |
| T5  | 0.43 ± 0.02 <sup>i</sup> | 0.88 ± 0.03 <sup>i</sup>  | 27.80 ± 0.23 <sup>h</sup> | 12.86 ± 0.29 <sup>i</sup> |
| T6  | 0.62 ± 0.04 <sup>i</sup> | 1.43 ± 0.01 <sup>h</sup>  | 32.93 ± 0.43 <sup>g</sup> | 14.26 ± 0.29 <sup>h</sup> |
| T7  | 0.83 ± 0.02 <sup>h</sup> | 1.73 ± 0.05 <sup>g</sup>  | 38.43 ± 0.06 <sup>f</sup> | 15.62 ± 0.04 <sup>g</sup> |
| T8  | 0.92 ± 0.03 <sup>h</sup> | 2.15 ± 0.09 <sup>f</sup>  | 41.25 ± 0.04 <sup>e</sup> | 18.75 ± 0.14 <sup>f</sup> |
| T9  | 1.74 ± 0.04 <sup>d</sup> | 3.23 ± 0.02 <sup>b</sup>  | 46.26 ± 0.12 <sup>b</sup> | 21.39 ± 0.11 <sup>c</sup> |
| T10 | 1.85 ± 0.01 <sup>c</sup> | 3.26 ± 0.04 <sup>b</sup>  | 46.56 ± 0.08 <sup>b</sup> | 21.64 ± 0.06 <sup>c</sup> |
| T11 | 2.43 ± 0.03 <sup>a</sup> | 3.47 ± 0.06 <sup>a</sup>  | 48.13 ± 0.48 <sup>a</sup> | 23.81 ± 0.08 <sup>a</sup> |
| T12 | 2.08 ± 0.01 <sup>b</sup> | 3.35 ± 0.04 <sup>ab</sup> | 47.56 ± 0.18 <sup>a</sup> | 22.34 ± 0.05 <sup>b</sup> |
| LSD | 0.09                     | 0.17                      | 0.76                      | 0.41                      |

Values represent mean ± standard error (SE) of three replicates. Means followed by the same letter within a column do not differ significantly according to DMRT at  $p \leq 0.05$ . C = untreated control; T1–T4 = carrot plants inoculated with *M. javanica* at 500, 1000, 1500, and 2000 J<sub>2</sub>, respectively; T5–T8 = carrot plants treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW; T9–T12 = carrot plants inoculated with *M. javanica* (2000 J<sub>2</sub>) and treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW.

### 3.7 Effect of Different Treatments on Nematode-Related Parameters

Inoculation with *M. javanica* significantly increased root-knot development and nematode reproduction with increasing inoculum level. The highest values were recorded in T4 (2000 J<sub>2</sub>), which showed 37.33 galls and 30.33 egg masses per root system, a soil nematode population of 3219.00 per 250 g soil, and a reproduction factor (R<sub>f</sub>) of 1.60. The corresponding root-knot index was 4. No galls, egg masses, or detectable soil nematode populations were recorded in the *A. oryzae*-only treatments (T5–T8), as these treatments did not receive *M. javanica* inoculum. Application of *A. oryzae* in combination with *M. javanica* reduced nematode-related parameters compared with the nematode-only treatment. The greatest suppression among the combined treatments was recorded in T12 (2000 J<sub>2</sub> + 3.0 g fresh *A. oryzae* mycelial biomass suspended in 10 mL DW), which showed 13.00 galls and 10.00 egg masses per root system, a soil nematode population of 2261.33 per 250 g soil, and an R<sub>f</sub> of 1.13. The root-knot index in T12 was reduced to 3 compared with an index of 4 in T4 (Table 5; Fig. 7). Therefore, T12 produced the greatest suppression of gall formation, egg-mass production, soil nematode population, and reproduction factor among the combined treatments.

**Table 5.** Effect of different inoculum levels of *M. javanica* and amounts of fresh *A. oryzae* mycelial biomass on nematode reproduction and root-knot disease severity in carrot plants.

| Treatments | Number of galls per root system | Number of egg masses per root system | Nematode population per 250 g soil | Reproduction factor (R <sub>f</sub> ) | Root-knot index (0-5) |
|------------|---------------------------------|--------------------------------------|------------------------------------|---------------------------------------|-----------------------|
| C          | 0.00 ± 0.00 <sup>g</sup>        | 0.00 ± 0.00 <sup>h</sup>             | 0.00 ± 0.00 <sup>g</sup>           | 0.00 ± 0.00 <sup>f</sup>              | 0                     |
| T1         | 6.67 ± 0.88 <sup>f</sup>        | 14.00 ± 0.57 <sup>f</sup>            | 640.66 ± 25.46 <sup>f</sup>        | 1.28 ± 0.05 <sup>cd</sup>             | 2                     |
| T2         | 15.00 ± 1.15 <sup>e</sup>       | 17.66 ± 0.88 <sup>de</sup>           | 1343.67 ± 51.38 <sup>e</sup>       | 1.34 ± 0.05 <sup>bcd</sup>            | 3                     |
| T3         | 23.66 ± 1.20 <sup>c</sup>       | 18.33 ± 0.33 <sup>d</sup>            | 2118.00 ± 135.91 <sup>d</sup>      | 1.41 ± 0.09 <sup>bc</sup>             | 3                     |
| T4         | 37.33 ± 2.90 <sup>a</sup>       | 30.33 ± 0.33 <sup>a</sup>            | 3219.00 ± 65.95 <sup>a</sup>       | 1.60 ± 0.03 <sup>a</sup>              | 4                     |
| T5         | 0.00 ± 0.00 <sup>g</sup>        | 0.00 ± 0.00 <sup>h</sup>             | 0.00 ± 0.00 <sup>g</sup>           | 0.00 ± 0.00 <sup>f</sup>              | 0                     |
| T6         | 0.00 ± 0.00 <sup>g</sup>        | 0.00 ± 0.00 <sup>h</sup>             | 0.00 ± 0.00 <sup>g</sup>           | 0.00 ± 0.00 <sup>f</sup>              | 0                     |
| T7         | 0.00 ± 0.00 <sup>g</sup>        | 0.00 ± 0.00 <sup>h</sup>             | 0.00 ± 0.00 <sup>g</sup>           | 0.00 ± 0.00 <sup>f</sup>              | 0                     |
| T8         | 0.00 ± 0.00 <sup>g</sup>        | 0.00 ± 0.00 <sup>h</sup>             | 0.00 ± 0.00 <sup>g</sup>           | 0.00 ± 0.00 <sup>f</sup>              | 0                     |

|     |                           |                            |                               |                            |   |
|-----|---------------------------|----------------------------|-------------------------------|----------------------------|---|
| T9  | 37.33 ± 1.20 <sup>a</sup> | 28.77 ± 0.57 <sup>ab</sup> | 3187.67 ± 91.66 <sup>ab</sup> | 1.53 ± 0.04 <sup>abc</sup> | 4 |
| T10 | 30.33 ± 0.88 <sup>b</sup> | 22.33 ± 1.20 <sup>c</sup>  | 2763.00 ± 127.01 <sup>b</sup> | 1.38 ± 0.06 <sup>bc</sup>  | 3 |
| T11 | 19.00 ± 1.73 <sup>d</sup> | 16.00 ± 1.15 <sup>f</sup>  | 2453.00 ± 57.86 <sup>c</sup>  | 1.22 ± 0.02 <sup>de</sup>  | 3 |
| T12 | 13.00 ± 0.57 <sup>g</sup> | 10.00 ± 1.15 <sup>g</sup>  | 2261.33 ± 60.05 <sup>cd</sup> | 1.13 ± 0.03 <sup>e</sup>   | 3 |
| LSD | 3.37                      | 2.04                       | 193.50                        | 0.12                       | - |

Values represent mean ± standard error (SE) of three replicates. Means followed by the same letter within a column do not differ significantly according to DMRT at  $p \leq 0.05$ . C = untreated control; T1–T4 = carrot plants inoculated with *M. javanica* at 500, 1000, 1500, and 2000 J<sub>2</sub>, respectively; T5–T8 = carrot plants treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW; T9–T12 = carrot plants inoculated with *M. javanica* (2000 J<sub>2</sub>) and treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW.



**Fig. 7.** Root morphology of carrot plants subjected to different *M. javanica* and *A. oryzae* treatments. C = untreated control; T1–T4 = carrot plants inoculated with *M. javanica* at 500, 1000, 1500, and 2000 J<sub>2</sub>, respectively; T5–T8 = carrot plants treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW; T9–T12 = carrot plants inoculated with *M. javanica* (2000 J<sub>2</sub>) and treated with *A. oryzae* at 1.0, 1.5, 2.0, and 3.0 g fresh mycelial biomass, respectively, each suspended in 10 mL DW.

## 4. Discussion

In the present study, the root-knot nematode associated with carrot was identified as *M. javanica* based on morphological and molecular characteristics, while the fungal isolate obtained from the rhizosphere of healthy carrot plants was identified as *A. oryzae*. The agreement between cultural and microscopic observations, sequence-based identification, and phylogenetic analysis provides support for the identity of both organisms. The study further demonstrated that *A. oryzae* exhibited nematode-suppressive activity against *M. javanica* and was associated with improved growth and altered biochemical responses of carrot plants under nematode stress.

The *in vitro* assays demonstrated that the cell-free culture filtrate of *A. oryzae* inhibited egg-hatching and increased mortality of second-stage juveniles (J<sub>2</sub>) of *M. javanica*, with the magnitude of both responses increasing with culture-filtrate concentration and exposure period. The maximum inhibition of egg-hatching (86.23%) and J<sub>2</sub> mortality (92.0%) was recorded with 75% (v/v) culture filtrate after 72 hrs. This concentration- and time-dependent response is consistent with previous reports describing nematicidal activity among *Aspergillus* species. For example, *A. japonicus* has been shown to produce metabolites with activity against *M. incognita*, while *A. niger* has also been reported to produce nematicidal compounds

capable of inhibiting egg-hatching and causing J<sub>2</sub> mortality (He *et al.*, 2020; Zhang *et al.*, 2023; Yang *et al.*, 2024). These studies support the potential of *Aspergillus* spp. as sources of extracellular nematode-suppressive metabolites. However, the specific compounds responsible for the activity of the present *A. oryzae* isolate were not identified. Therefore, metabolite profiling and bioassay-guided characterization will be necessary to determine the chemical basis of the observed egg-hatching inhibition and J<sub>2</sub> mortality.

Increasing inoculum levels of *M. javanica* progressively reduced carrot growth and biomass, with the greatest reduction observed at 2000 J<sub>2</sub> (T4). Root-knot nematodes establish specialized feeding sites in host roots, and the associated structural and physiological alterations can interfere with normal root function and resource acquisition (Rutter *et al.*, 2022). The reduction in photosynthetic pigments observed with increasing nematode inoculum may therefore be associated with the physiological stress imposed by nematode infection. Similar reductions in plant growth and photosynthetic pigments under root-knot nematode stress have been reported in other crop plants (Collinge *et al.*, 2023). The increase in gall formation, egg-mass production, soil nematode population, and reproduction factor with increasing inoculum level further demonstrated the effect of initial nematode pressure on disease development and nematode multiplication (Pulavarty *et al.*, 2021). Thus, the increasing inoculum series used in the present study provided a clear gradient of *M. javanica* stress against which the effects of fungal application could be evaluated.

Under combined nematode–fungus treatment, T11 (2000 J<sub>2</sub> + 2.0 g fresh *A. oryzae* mycelial biomass suspended in 10 mL DW). Application of *A. oryzae* fresh mycelial biomass in the absence of nematode inoculation was associated with enhanced carrot growth with T7 (2.0 g fresh mycelial biomass suspended in 10 mL DW) recording the highest values for all measured growth parameters among the fungal-only treatments. This response indicates that application of *A. oryzae* can positively influence carrot plant growth under the conditions tested. Previous studies have also reported beneficial effects of *A. oryzae* and other *Aspergillus* species in plant-associated systems (Sun *et al.*, 2018). However, the mechanisms responsible for the growth-promoting response were not investigated in the present study. In particular, nutrient mobilization, phytohormone production, and root colonization were not measured and therefore cannot be inferred from the present data. Under combined nematode–fungus treatment, T11 (2000 J<sub>2</sub> + 2.0 g fresh *A. oryzae* mycelial biomass suspended in 10 mL DW) produced the strongest recovery of the measured growth parameters among the combined treatments. Similar beneficial responses to fungal biocontrol agents under nematode stress have been reported in horticultural crops (Rahman *et al.*, 2024).

Nematode infection also markedly altered the biochemical status of carrot plants. Chlorophyll and carotenoid contents decreased with increasing nematode inoculum, whereas total phenolic and proline contents increased. Such changes are consistent with physiological and metabolic responses associated with *Meloidogyne* infection and feeding-site establishment (Rutter *et al.*, 2022). Phenolic compounds participate in plant defense, while proline may contribute to cellular protection and osmotic adjustment under stress. Similar changes in phenolic and stress-associated metabolites have been reported in plants exposed to biotic stress (Kamle *et al.*, 2020; Mohagheghian *et al.*, 2025). Application of *A. oryzae* modified these responses in nematode-inoculated plants. Among the combined treatments, T11 recorded the highest chlorophyll, carotenoid, phenolic, and proline contents. The enhanced levels of these biochemical parameters indicate an altered physiological and defense-associated response following fungal application, although the molecular signaling mechanisms underlying these changes require further investigation.

The activities of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and polyphenol oxidase (PPO) increased following *M. javanica* inoculation and were generally higher at greater nematode inoculum levels. Such changes are consistent with activation of antioxidant and defense-associated responses during biotic stress. Reactive oxygen species (ROS) participate in plant responses to biotic challenges, while antioxidant enzymes contribute to the regulation of cellular redox homeostasis (Zandi and Schnug, 2022; Sahu *et al.*, 2022). Recent studies have also emphasized the importance of antioxidant responses during

plant–root-knot nematode interactions (Yang *et al.*, 2026). In the present study, application of *A. oryzae* further increased the activities of the four investigated enzymes in nematode-inoculated plants, with T11 showing the highest numerical enzyme activities among the combined treatments. These results indicate an association between fungal application and enhanced antioxidant and defense-related enzyme activities. Importantly, ROS accumulation, lipid peroxidation, and other oxidative-damage markers were not measured in the present study. Therefore, the increased enzyme activities cannot be taken as direct evidence that *A. oryzae* reduced oxidative damage. Instead, they demonstrate an altered enzymatic response associated with fungal application under nematode stress.

The greenhouse experiment further demonstrated that *A. oryzae* suppressed several parameters associated with *M. javanica* reproduction. No galls, egg masses, or detectable soil nematode populations were recorded in the *A. oryzae*-only treatments because these plants were not inoculated with the nematode. In contrast, nematode-related parameters increased with increasing *M. javanica* inoculum in the nematode-only treatments. In the combined treatments, application of *A. oryzae* reduced gall formation, egg-mass production, soil nematode population, and reproduction factor relative to the 2000-J<sub>2</sub> treatment. The magnitude of suppression, however, varied with the amount of fresh fungal biomass. T12 (2000-J<sub>2</sub> + 3.0 g fresh *A. oryzae* mycelial biomass suspended in 10 mL DW) recorded the lowest numerical values for galls, egg masses, soil nematode population, and reproduction factor among the combined treatments. The root-knot index further supported the reduction in root galling following fungal application, with a lower index in T11 and T12 than in the 2000-J<sub>2</sub> treatment. These findings indicate that the treatment producing the strongest plant-growth and biochemical response was not identical to the treatment producing the greatest numerical suppression of nematode multiplication. T11 was the strongest combined treatment for plant growth and biochemical responses, whereas T12 showed the greatest numerical suppression of the measured nematode-reproduction parameters.

The greenhouse suppression of nematode reproduction is also consistent with the nematode-suppressive activity observed in the *in vitro* culture-filtrate assay. The concentration- and time-dependent inhibition of egg-hatching and J<sub>2</sub> survival suggests that extracellular products released by *A. oryzae* may contribute to the observed activity. Comparable nematicidal effects have been reported for other *Aspergillus* species. For example, *A. japonicus* ZW1 fermentation filtrate strongly inhibited *M. incognita* and reduced gall formation and egg production in greenhouse experiments (He *et al.*, 2020), while *A. niger* has been shown to produce nematicidal metabolites active against *M. incognita* (Zhang *et al.*, 2023). More recently, *A. tubingensis* has also been investigated for its nematicidal activity against root-knot nematodes, with oxalic acid implicated in its activity (Yang *et al.*, 2024). Nevertheless, the present study did not characterize the active metabolites of *A. oryzae*, and the relationship between the *in vitro* activity of the culture filtrate and the greenhouse response to fresh mycelial biomass cannot be assigned to a specific compound or mechanism. Metabolite profiling, fungal colonization studies and targeted bioassays will therefore be required to establish the mechanisms underlying the observed nematode suppression.

The differential responses of T11 and T12 are important for interpreting the practical significance of the findings. T11 produced the strongest recovery of plant growth and the highest measured photosynthetic and biochemical responses among the combined treatments, whereas T12 produced the greatest numerical reduction in galls, egg masses, soil nematode population, and reproduction factor. This indicates that increasing fungal biomass did not result in a uniform improvement across all measured variables. Such treatment-dependent responses are consistent with the broader understanding that the efficacy of fungal biocontrol agents can vary according to fungal strain, host plant, nematode species, application conditions, and environmental factors (Rahman *et al.*, 2024). Accordingly, the present results support the potential integration of *A. oryzae* into sustainable nematode-management strategies, but they do not establish a single universally optimal fungal biomass for all management objectives.

Several limitations should be considered when interpreting these findings. First, the active metabolites responsible for the *in vitro* nematode-suppressive activity were not identified. Second, fungal colonization and persistence in the carrot rhizosphere were not quantified, and therefore the establishment of *A. oryzae* in the plant–soil system remains unresolved. Third, traits potentially responsible for plant growth promotion, including nutrient mobilization, phosphate solubilization, and phytohormone production, were not measured. Fourth, the present study assessed antioxidant and defense-related enzyme activities but did not directly measure ROS accumulation or oxidative damage. Finally, the experiments were conducted under controlled greenhouse conditions and therefore do not establish field-level consistency or agricultural applicability. Before practical deployment, further studies should evaluate formulation and shelf stability, appropriate application methods, persistence in soil, compatibility with other management practices, non-target effects, and efficacy under diverse environmental conditions. Field-scale validation will also be necessary to determine the consistency of the nematode-suppressive and plant-growth-promoting effects observed in the present study.

Taken together, the findings demonstrate that *A. oryzae* has measurable nematode-suppressive activity against *M. javanica* and can be associated with improved growth and altered biochemical responses in carrot under nematode stress. The *in vitro* culture filtrate assays demonstrated substantial inhibition of egg-hatching and J<sub>2</sub> survival, while greenhouse application of fresh fungal biomass reduced nematode reproduction and improved plant performance. Importantly, T11 and T12 differed in their strongest responses: T11 was associated with the greatest plant growth and biochemical recovery, whereas T12 showed the greatest numerical suppression of nematode-reproduction parameters. These findings identify *A. oryzae* as a promising candidate for further investigation as a biological component of root-knot nematode management, while highlighting the need for mechanistic studies and field validation before practical recommendations can be made.

## 5. Conclusion

The present study demonstrated the biocontrol potential of *A. oryzae* against *M. javanica* in carrot. *A. oryzae* significantly inhibited egg-hatching and increased J<sub>2</sub> mortality *in vitro* in a concentration and time-dependent manner. Under greenhouse conditions, increasing *M. javanica* inoculum levels progressively reduced plant growth and photosynthetic pigments, whereas phenolic, proline, and antioxidant enzyme activities increased. Application of *A. oryzae* improved plant growth and physiological responses and reduced nematode reproduction and root-knot development. Among the combined treatments, T11 (2000 J<sub>2</sub> + 2.0 g *A. oryzae* fresh mycelial biomass suspended in 10 mL DW) showed the greatest improvement in plant growth, photosynthetic pigments, and biochemical responses, whereas T12 (2000 J<sub>2</sub> + 3.0 g *A. oryzae* fresh mycelial biomass suspended in 10 mL DW) produced the greatest numerical reduction in galls, egg masses, soil nematode population, and reproduction factor. These findings indicate that *A. oryzae* showed both plant-growth-promoting effects and nematode-suppressive activity and may serve as a promising component of sustainable root-knot nematode management in carrot. Further studies under field conditions are required to validate its efficacy and consistency and to determine appropriate formulation, persistence, safety, application methods, and mechanisms of action.

## Statements and Declarations

**Supplementary Materials:** The supplementary materials include [Fig. S1](#) and [S2](#).

## Author Contributions

Conceptualization, R.R.; methodology, S.M., N.F., M.P. and S.A.; investigation, S.M., N.F., M.P. and S.A.; formal analysis, S.M., N.F. and M.P.; data curation, S.M., N.F. and M.P.; writing—original draft

preparation, S.M. and N.F.; writing—review and editing, R.R. and I.M.; supervision, R.R.; project administration, R.R. All authors have read and approved the final version of the manuscript.

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