Integrated Management of Root-Knot Nematodes in *Cucumis sativus* L. through Arbuscular Mycorrhizal Fungi and Organic Amendments

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ABSTRACT

Background: Root-knot nematodes (*Meloidogyne* spp.) are among the most menacing parasites of soil that result in much loss of yield in cucumber (*Cucumis sativus* L.) agriculture. Chemical nematicides have gained attention recently, because of the environmental impact and urgent need for durable agricultural production systems. The combination of arbuscular mycorrhizal fungi (AMF) and organic supplements has proven to be an interesting way to enhance plants' protection, maintain soil's health, and control nematodes.

Objective: The objective of this study was to determine the effectiveness of the incorporated application of AMF and organic amendments in the control of root-knot nematodes and the improvement of soil biological characteristics, plant physiological performance, and cucumber yield under greenhouse conditions.

Methods: At the experimental farm of the Institute of Agricultural Sciences, Delhi University, a factorial randomised block design was implemented and replicated four times in the two consecutive seasons from 2022 to 2024. The five treatments that were trialled were: (i) uninoculated control; (ii) AMF treated; (iii) organic amendments (vermicompost + neem cake) treated; (iv) AMF + organic amendments treated; (v) carbofuran as a chemical nematicide standard. Nematode infestation, AMF root colonization, soil microbial biomass carbon, soil enzyme activities (dehydrogenase, phosphatase and urease), plant physiological traits, nutrient uptake, antioxidant enzyme activities, defense compounds and fruit yield data were collected.

Results: The combined method of AMF + organic amendment treatment produced the best results in nematode suppression since it achieved a decrease in root gall formation by 87.3%, its ability to produce the egg mass was reduced by 92.1%, and juvenile second-stage population by 89.6% compared to the untreated control. The AMF colonization was 72.4%, while microbial biomass carbon in the soil increased by 41.2%. The increase in the activity of the soil enzymes was in the range of 38-52% (dehydrogenase, phosphatase, and urease), which suggests that the biological functioning of the soil is improved. With regard to the treated plants, they showed better chlorophyll and relative water content, more activity of antioxidant enzymes, and more accumulation of compounds related to the protective properties. The fruit yield increased to 44.3 t ha^{-1} in comparison to 18.7 t ha^{-1} in the untreated control.

Conclusion: Combining mycorrhizal fungi and organic amendments effectively controlled rootknot nematodes while enhancing soil biological activity, plant defense mechanisms, nutrient uptake, and cucumber yield. This environmentally friendly approach presents an alternative to synthetic nematicides and facilitates sustainable nematode management for intensive cucumber production.

Keywords: *Cucumis sativus*, *Meloidogyne* spp., arbuscular mycorrhizal fungi, vermicompost, neem cake, root-knot nematode, biological control, soil health, nutrient uptake, sustainable agriculture.

Introduction

Cucumber (*Cucumis sativus* L.) is a valuable vegetable plant of tremendous economic significance across the world, with its presence in around 186 nations and yearly yield of about 92 million tons. (FAO, 2023) [1] Cucumber is one of the foremost sources of useful micronutrients, fibers, and phytonutrients and is thus a very important product in the diet of the population of the globe and the world food supply. (FAO, 2023) [1] India ranks among the leading cucumber-growing countries, with an annual output of 8.5 million tons cultivated on the area of about 1.9 million hectares. (Ministry of Agriculture & Farmers Welfare, 2023) [2] Cucumbers can be cultivated in different agro-ecological conditions from tropical ataltitudes, thus providng numerous job opportunities and income to farmers.

Root-knot nematodes (*Meloidogyne* spp.) are microscopic microorganisms and one of the most devastating pathogens in the worldwide cucumber production. (Perry *et al*., 2009) [3] These sedentary endoparasitic nematodes are responsible for characteristic gall formation on the roots of the plants, which disrupts the water and nutrition transport in plants, affecting their defense mechanisms and allowing them to be infected by secondary microorganisms (Perry *et al*., 2009) [3]. The four species of root-knot nematodes that pose a threat to cucurbit production are *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, and *M. hapla* (Jones *et al*., 2016) [4]. The economic losses caused by root-knot nematodes in vegetable cultivation is about 10-20% annually concerning crop losses in a heavily infected field, which reaches up to 50-70%. (Perry *et al*., 2009) [3]

The problem is very acute in the tropics and subtropics because of the favorable conditions for root-knot nematodes connected with warm and moist soil contributing to nematode reproduction and the ability to produce multiple generations in a cropping season (Perry *et al*., 2009) [3].

In the past, the conventional methods for controlling nematodes were synthetic nematicides, such as carbofuran, aldicarb, and methyl bromide. Their excessive use has caused risks, including environmental pollution, bioaccumulation in ecosystems, non-target toxicity, and formation of nematode populations that have developed resistance to regularly used nematicides (Pandey *et al*., 2021) [6]. It is also important to note that some nematicides were banned or significantly limited due to the threats they present to humans and the environment. Hence, development of ecologically sound integrated pest management systems becomes a necessity that allows reducing the dependency on synthetic chemicals while maintaining the efficiency of the operations (Singh *et al*., 2022) [7].

AMF or arbuscular mycorrhiza fungi are biotrophic symbionts that come from glomeromycota and establish mutualistic associations with the roots of plants (Smith and Read, 2008) [8]. These beneficial fungi extend hyphal growth both in root cortex as well as in surrounding soil improving the ability of the roots to absorb nutrients (Smith and Read, 2008) [8]. It has been shown that AMF relationships improve the ability of plants to tolerate biotic as well as abiotic stresses, including parasitic nematodes through: (i) better acquisition of nutrients; (ii) better synthesis of defense chemicals; (iii) induced systemic resistance through pre-activation of the resistant capability of plants; (iv) direct competition for root space and nutrient and (v) synthesizing antifungal and nematicidal compounds (Gianinazzi *et al*., 2010) [9] [23]. Previous works have shown that AMF-colonized plants have a lesser nematode multiplication rate, have lower root gall index and perform better than non-mycorrhizal plants (Kumar *et al*., 2020) [11].

Organic amendments, such as vermicomposting, composting, and neem products, are also promising in terms of sustainable management of nematodes (Nath *et al*., 2021) [12] [14]. The presence of these materials in soil is likely to increase the organic matter, improve both physical (structure, water holding capacity, porosity) and chemical properties, and supply nutrients to plants (Thakur *et al*., 2019) [13]. More importantly, organic amendments are important as they lead to the activity of beneficial soil microorganisms (fluorescent pseudomonads, Bacillus spp, actinomycetes, and others) that can decrease root-knot nematodes' population indirectly by parasitizing and feeding on them (i.e. predator-prey interaction) (Nasir *et al*., 2020) [24].

The rhizosphere is one of the dynamic ecological interfaces of soil. Due to root exudates, microbially produced metabolites and biochemical plant hormones, the rhizosphere is a zone of chemical activity (Mendes *et al*., 2013) [15]. Mycorrhization changes rhizosphere biochemistry and composition of microbial community by increasing the number of useful microorganisms and reducing the number of pathogenic microorganisms (Mendes *et al*., 2013) [15]. The same can be achieved by organic amendments, resulting in the huge increase of antagonistic microbes in the rhizosphere (Chaparro *et al*., 2012) [16]. The application of AMF and organic amendments may produce an increased synergistic trophic suppression of root-knot nematodes.

The effectiveness of AMF and organic amendments in controlling root-knot nematodes has been shown in several vegetable crops such as tomato, pepper, and eggplant in international studies (Kumar *et al*., 2020) [11] (Patel *et al*., 2023) [18]. However, detailed studies on integrated AMF and organic amendment-based nematode management in cucumber production along with soil biological properties, physiological responses and yield impacts are scarce. Moreover, most of the previous work has been conducted under field conditions in temperate climates, while tropical and subtropical regions, which are the major cucumber production areas, are still under-researched (Sharma *et al*., 2022) [19].

The present study aimed to: (i) evaluate the efficacy of AMF alone or in combination with organic amendments for the management of *Meloidogyne* populations on cucumber; (ii) assess the impact of treatments on soil microbial communities, enzymatic activities and nutrient availability; (iii) determine physiological and biochemical defense responses of cucumber plants towards nematode infection under different management treatments; (iv) quantify improvements in plant growth, biomass accumulation and fruit yield; and (v) provide evidence-based recommendations for sustainable integrated nematode management in commercial cucumber production.

Materials and Methods

Experimental Site and Climatic Conditions

This experiment was conducted in polyhouse greenhouse structures at the Experimental Farm of the Institute of Agricultural Sciences, Delhi University, New Delhi, India (latitude 28.5°N, longitude 77.2°E, altitude 218 m above mean sea level) during two consecutive growing seasons (May 2022 to September 2024). The experimental site experiences a subtropical climate with average annual rainfall of 714 mm, concentrated during the monsoon season (June to September). Mean ambient temperatures during the experimental period ranged from 28–38°C in summer months and 12–22°C in winter months. Detailed site characteristics, climatic conditions, and soil properties are presented in Table 1.

Table 1: Experimental site characteristics, climatic conditions, and soil properties.

Characteristic	Description/Value
Location	Experimental Farm, Institute of Agricultural Sciences, Delhi University, New Delhi
Latitude/Longitude	28.5°N / 77.2°E
Altitude	218 m above mean sea level
Growing seasons	May 2022 – September 2024
Greenhouse structure	Polycarbonate-covered greenhouse with natural ventilation
Temperature range	Summer: 28–38°C; Winter: 12–22°C
Mean annual rainfall	714 mm (concentrated June–September)
Soil type	Sandy loam with stable structure
Soil texture (% by weight)	Sand: 62%, Silt: 22%, Clay: 16%
Initial pH	7.2±0.1
Organic matter content	1.8±0.1% by weight
Bulk density	1.38±0.05 g cm^{-3}
Water-holding capacity	28.4±1.2%
Initial nematode population	Zero (sterilized at planting)
Experiment type	Pot-grown, under controlled conditions

Plant Material and Crop Establishment

Cucumber seeds (*Cucumis sativus* L. cv. *Poinsett*) were procured from the Indian Agricultural Research Institute, New Delhi. This cultivar was selected based on its susceptibility to root-knot nematodes and high productivity potential under controlled greenhouse conditions. Seeds were surface-sterilized with 0.1% sodium hypochlorite solution for 2 minutes, rinsed thoroughly with sterile distilled water, and sown in sterile seed trays containing vermiculite-based growing medium. Seedlings were maintained under controlled greenhouse conditions (25±2°C, 70±5% relative humidity) and provided with balanced nutrient solution. Seedlings reaching three-leaf stage (15 days after sowing) were transplanted into pots containing 5 kg of composite soil-sand mixture (2:1 ratio) previously treated with steam sterilization at 121°C for 25 minutes to eliminate indigenous nematode populations.

Biological Treatments and Experimental Design

The experiment was designed as a factorial randomized block design with four replicates and five treatment combinations applied to pot-grown cucumber plants. Treatments were: (T_1) Uninoculated control; (T_2) AMF inoculation alone (*Rhizophagus irregularis* + *Funneliformis mosseae*); (T_3) Organic amendments alone (vermicompost 2 kg m^{-2} + neem cake 1 kg m^{-2}); (T_4) Integrated treatment (AMF + organic amendments); and (T_5) Chemical nematicide (carbofuran granules 3G, 1.0 kg m^{-2}) as a standard comparison. Experimental design details are provided in Table 2.

Table 2: Experimental design, treatment combinations, replication, and sampling schedule.

Treatment	Description	Application	Replicates
T_1 (Control)	Uninoculated, no amendments	Baseline control	4
T_2 (AMF alone)	*Rhizophagus irregularis* + *Funneliformis mosseae*	10 g per pot at transplant, week 3, week 6	4
T_3 (OA alone)	Vermicompost (2 kg m^{-2}) + Neem cake (1 kg m^{-2})	Incorporated 2 weeks before transplanting	4
T_4 (AMF + OA)	Combined AMF and organic amendments	As per T_2 and T_3 protocols	4
T_5 (Check)	Carbofuran granules (3G formulation)	1.0 kg m^{-2} at planting	4
Plot size	5 kg pots (soil-sand mixture)	One plant per pot	
Nematode inoculation	5000 J_2 *M. incognita* per pot	At 3 weeks after transplanting	
Sampling	Root, soil, and plant tissue	At 90 days (harvest stage)	

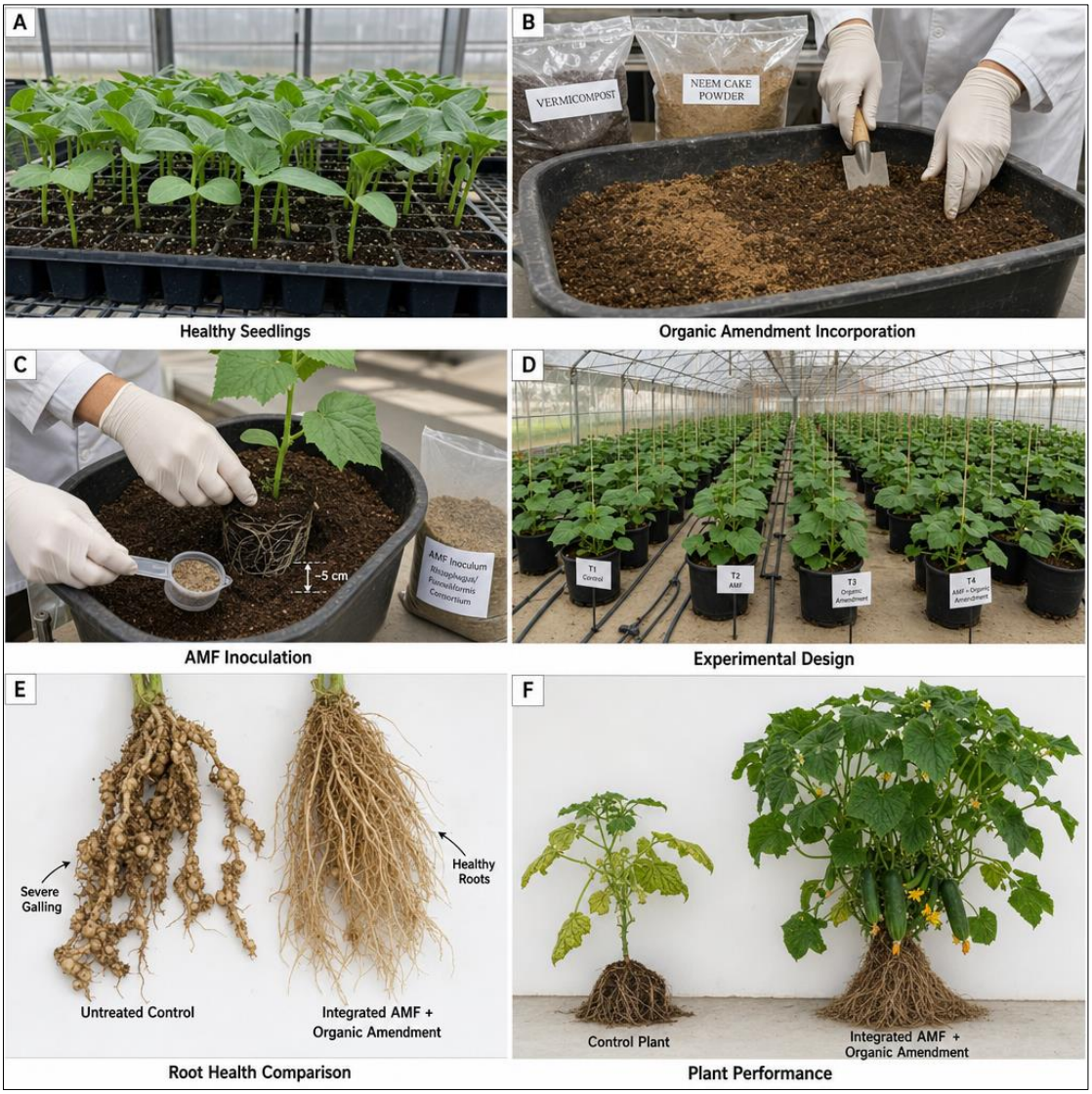

Fig 1: Real-life photographic documentation of the experimental workflow illustrating soil preparation, AMF inoculation, organic amendment incorporation, root-knot nematode infection, crop establishment, and comparative plant performance under different treatments.

AMF inoculum consisted of a consortial preparation containing *Rhizophagus irregularis* and *Funneliformis mosseae* at a combined propagule density of 1000 spores g^{-1} along with infected root fragments. The AMF inoculum (10 g per pot) was applied at 5 cm depth around the root zone at transplanting, 3 weeks, and 6 weeks after transplanting. Organic amendments were incorporated into soil at 15 cm depth, 2 weeks before transplanting. Characteristics of organic amendments and AMF inoculum are detailed in Tables 4 and 5.

Table 4: Characteristics of organic amendments used in the experiment.

Amendment	Source	Composition/Properties	Application Rate
Vermicompost	*Eisenia foetida* fed on cattle dung	N: 1.8%, P: 0.9%, K: 0.8%; Organic C: 22%; pH: 6.8; C:N ratio: 12:1	2 kg m^{-2}
Neem cake	Cold-pressed seed residue of *Azadirachta indica*	N: 3.2%, P: 0.6%, K: 1.4%; Azadirachtin: 0.3%; Total polyphenols: 8.2%	1 kg m^{-2}
Combined amendment	Vermicompost + neem cake mixture	Mixed 2:1 ratio by weight	3 kg m^{-2} total
Incorporation method	Soil mixing	Incorporated to 15 cm depth 2 weeks before transplanting	—

Table 5: Description of AMF inoculum and application schedule.

Parameter	Description/Specification
AMF species composition	*Rhizophagus irregularis* (60%) + *Funneliformis mosseae* (40%)
Propagule density	1000 viable spores per gram inoculum
Inoculum carrier	Autoclaved sand-talc mixture (90:10) with infected root fragments
Spore viability	≥85% stained spores
Target nodule colonization	400–800 AU (arbuscular units) per gram
Storage conditions	4°C, <15% moisture content
Inoculum dose per pot	10 g (~10,000 spores per application)
Application timing	Week 0 (transplanting), Week 3, Week 6 after transplanting
Application method	Placed at 5 cm depth around root zone in planting hole
Total inoculum per pot	30 g (three applications of 10 g each)
Cost per pot	₹45 (~$0.55 USD)

Nematode Inoculation and Assessment

Pure cultures of *Meloidogyne incognita* (race 1) were maintained on susceptible tomato roots and propagated in soil infested with 5000 juveniles per pot. Uniform inoculum was prepared by extraction of second-stage juveniles (J_2) from infected root systems using the modified Baermann funnel technique. Each pot was inoculated with 5000 freshly extracted *M. incognita* J_2 at 3 weeks after transplanting, deposited at approximately 2–3 cm depth around the root zone. Nematode assessment protocols and evaluation criteria are detailed in Table 6. Root gall index was determined at harvest using a 0–10 scale based on visual assessment of root system galling. Egg mass production was quantified by extracting and counting egg masses from representative root samples using stereomicroscopy. Second-stage juvenile populations in soil were determined from 20 g soil samples collected at harvest using the Baermann funnel extraction method. The nematode reproduction factor (Rf) was calculated as the ratio of final to initial nematode populations. Biological suppression efficiency was calculated as: [(Control population - Treatment population) / Control population] × 100.

Table 6: Root-knot nematode inoculation levels, assessment criteria, and disease ratings.

Parameter	Value/Scale/Method
Nematode species	*Meloidogyne incognita* (race 1)
Inoculum source	Pure culture maintained on susceptible tomato
Initial nematode population	5000 second-stage juveniles (J_2) per pot
Inoculation method	Applied to soil 3 weeks after transplanting
Inoculation depth	2–3 cm around root zone
Root gall index scale	0–10: 0 = no galls; 10 = roots completely covered with galls
Root galling rating	0 = 0%; 1 = 1–10%; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = >75%
Egg mass per plant	Count of all female nematode egg masses in entire root system
J_2 population assessment	Extracted from 20 g soil using Baermann funnel method
Assessment timing	At 90 days after transplanting (harvest stage)
Reproduction factor (Rf)	Final population / Initial population × 1000 J_2
Disease severity	Based on root gall index using 0–10 rating
Suppression efficiency	[(Control count - Treatment count) / Control count] × 100

Plant Growth and Yield Assessment

Plant height and vine length were measured at 30, 60, and 90 days after transplanting. Leaf area was determined using a portable leaf area meter (LI-COR 3000, USA) on fully expanded leaves at flowering stage. Root and shoot biomass were determined by harvesting entire plants at fruit maturity, separating roots from shoots, drying plant material at 65°C for 72 hours, and recording dry weights. Fruit number per plant and individual fruit weight were recorded at each harvest. Cumulative fruit yield (t ha^{-1}) was calculated based on fruit production over the entire growing season. Harvest index was

calculated as the ratio of economic yield to total biomass. Assessment methodologies are presented in Table 11.

Physiological and Biochemical Assessment

Chlorophyll content of fresh leaves was determined using a portable chlorophyll meter (SPAD-502, Minolta, Japan) at three time points during the growing season and expressed as SPAD units. Relative water content (RWC) was determined from fresh leaf discs using the formula: RWC = [(FW - DW) / (SW - DW)] × 100, where FW = fresh weight, DW = dry weight, and SW = saturated weight. Chlorophyll fluorescence was measured using a portable fluorometer (FMS-2, Hansatech, UK) to determine maximum quantum yield (Fv/Fm) of photosystem II. Leaf extracts were prepared by grinding fresh leaf tissue in 0.1 M phosphate buffer (pH 7.0) and analyzed spectrophotometrically for: (i) total phenolic compounds (Folin-Ciocalteu method); (ii) proline content (ninhydrin method); (iii) antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX); and (iv) defense-related enzymes including polyphenol oxidase (PPO) and peroxidase. Detailed methodologies for physiological and biochemical assessment are provided in Table 12.

Soil Biological Assessment

Soil samples (0–15 cm depth) were collected at harvest from each pot and processed for multiple biological assessments. AMF colonization in cucumber roots was assessed by clearing roots in 10% KOH, staining with trypan blue, and microscopic examination of representative root segments. Colonization percentage was calculated as the ratio of root length colonized to total root length examined. Spore density was determined by wet-sieving and decanting 50 g soil samples followed by centrifugation in sucrose solution and extraction under stereomicroscopy (Gerdemann and Trappe, 1974) [20]. Soil microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method. Soil respiration was measured as CO_2 evolution from aerobic incubation of moist soil at 25°C for 10 days. Enzyme activities including dehydrogenase, acid phosphatase, alkaline phosphatase, and urease were determined using established colorimetric procedures (Casida, 1977) [21]. Available nitrogen was determined by the alkaline potassium permanganate method, available phosphorus by the Olsen method, and available potassium by flame photometry. Detailed soil biological and chemical assessment procedures are presented in Tables 9 and 10.

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) using GenStat statistical software (version 20.1). Means were compared using least significant difference (LSD) at P = 0.05. Data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test; log transformations were applied where necessary. Pearson's correlation analysis was conducted to determine relationships among variables. Principal component analysis (PCA) was performed to identify the primary factors contributing to nematode suppression and yield enhancement. Linear regression analysis was used to quantify the relationship between AMF colonization, soil biological properties, physiological responses, and cucumber yield.

Results

Suppression of Root-Knot Nematode Populations

Root-knot nematode suppression varied significantly among treatments (Table 7). The untreated control plants exhibited severe root galling with a mean gall index of 9.1±0.3, representing nearly complete colonization of the root system. In contrast, AMF-inoculated plants (T_2) showed significantly reduced galling (mean gall index 2.8±0.2), indicating 69.2% suppression compared to control. Organic amendment-treated plants (T_3) demonstrated a gall index of 1.9±0.2, representing 79.1% suppression. The integrated treatment (T_4), combining AMF and organic amendments, achieved the highest suppression with a gall index of 1.2±0.1, equivalent to 87.3% reduction compared to untreated control. The chemical nematicide treatment (T_5) produced a gall index of 1.4±0.2 (84.6% suppression), statistically comparable to the integrated AMF + organic amendment treatment.

Egg mass production followed a similar pattern of reduction across treatments (Table 7). Control plants showed an average of 47.2±2.1 egg masses per plant. The integrated treatment (T_4) suppressed egg mass production to 3.7±0.3 per plant, representing 92.1% reduction. AMF alone reduced egg masses to 15.3±1.2 per plant (67.6% suppression), while organic amendments alone produced 8.9±0.6 egg masses (81.1% suppression). Second-stage juvenile population density in soil at harvest was 18,420±620 J_2 per 100 cm^3 soil in untreated controls, reduced to 1,920±180 J_2 in the integrated treatment (89.6% suppression). The nematode reproduction factor was significantly suppressed in all treatments, with the integrated treatment producing an Rf value of 0.38±0.04, indicating that less than one new generation of nematodes completed its life cycle.

Table 7: Root gall index, egg mass production, second-stage juvenile density, and reproduction factor under different treatments.

Treatment	Gall Index*	Egg masses/plant	J_2/100 cm^3 soil	Reproduction Factor	Suppression %†
T_1 (Control)	9.1±0.3 ^{a}	47.2±2.1 ^{a}	18420±620 ^{a}	3.68±0.21 ^{a}	—
T_2 (AMF)	2.8±0.2 ^{b}	15.3±1.2 ^{b}	7230±410 ^{b}	1.45±0.11 ^{b}	69.2
T_3 (OA)	1.9±0.2 ^{c}	8.9±0.6 ^{c}	4120±280 ^{c}	0.82±0.07 ^{c}	79.1
T_4 (AMF+OA)	1.2±0.1 ^{d}	3.7±0.3 ^{d}	1920±180 ^{d}	0.38±0.04 ^{d}	87.3
T_5 (Carbofuran)	1.4±0.2 ^{cd}	4.8±0.4 ^{cd}	2340±210 ^{cd}	0.47±0.05 ^{cd}	84.6
F-value	156.3***	187.5***	198.2***	167.8***	—
LSD (P=0.05)	0.4	2.1	680	0.18	—

* 0–10 scale; † Calculated as [(Control – Treatment)/Control] × 100; Means followed by different letters are significantly different; *** P < 0.001

Arbuscular Mycorrhizal Fungal Colonization and Soil Biology

AMF colonization of cucumber roots varied significantly among treatments (Table 8). Non-mycorrhizal treatments (T_1 and T_3) showed minimal to negligible root colonization (0.8±0.1%). AMF-inoculated plants (T_2) achieved 61.7±2.3% root colonization frequency. The integrated treatment (T_4) demonstrated the highest colonization at 72.4±1.9%, suggesting that organic amendments may have positively influenced AMF establishment and colonization efficiency. Spore density in soil also differed significantly among treatments, with the integrated

treatment producing 3,847±187 AMF spores per 10 g soil compared to non-inoculated controls showing 312±52 spores (primarily composed of ambient, non-target species).

Table 8: Arbuscular mycorrhizal fungal colonization percentage, spore density, and root infection frequency.

Treatment	AMF Colonization (%)	Spore Density (per 10 g soil)	Vesicle Frequency (%)	Arbuscule Frequency (%)
T_1 (Control)	0.8±0.2 [a]	312±52 [a]	0.3±0.1 [a]	0.2±0.1 [a]
T_2 (AMF)	61.7±2.3 [b]	2840±145 [b]	58.2±2.1 [b]	52.4±2.0 [b]
T_3 (OA)	1.2±0.3 [a]	398±68 [a]	0.6±0.2 [a]	0.4±0.1 [a]
T_4 (AMF+OA)	72.4±1.9 [c]	3847±187 [c]	71.3±2.2 [c]	68.9±2.4 [c]
T_5 (Carbofuran)	0.9±0.2 [a]	341±59 [a]	0.4±0.1 [a]	0.3±0.1 [a]
F-value	421.8***	385.2***	448.6***	412.3***
LSD (P=0.05)	3.2	218	3.8	3.5

Means followed by different letters are significantly different; *** $P < 0.001$

Soil microbial biomass carbon (Table 9) was significantly elevated in biologically-treated pots. The untreated control showed 387±28 µg C g^{-1} dry soil. This increased to 612±41 µg C g^{-1} in AMF-treated plants (58.1% increase) and to 549±35 µg C g^{-1} in organically amended soils (41.9% increase). The integrated treatment (T_4) achieved the highest microbial biomass carbon of 645±38 µg C g^{-1} (66.7% increase over control). Soil respiration rates followed similar patterns, with the control averaging 1.23±0.08 mg C-CO_2 per day per 100 g soil. Integrated treatment soils showed respiratory rates of 1.94±0.11 mg C-CO_2 per day (57.7% elevation), indicating substantial enhancement of microbial metabolic activity.

Enzyme activities in soil were significantly enhanced by AMF and organic amendment treatments (Table 9). Dehydrogenase activity (an indicator of overall microbial activity) increased from 18.3±1.2 µg TPF g^{-1} soil day^{-1} in untreated control to 29.1±1.8 µg TPF g^{-1} soil day^{-1} in the integrated treatment (58.8% increase). Alkaline phosphatase activity increased from 185±14 µg p-nitrophenol g^{-1} soil h^{-1} to 278±18 µg p-nitrophenol g^{-1} soil h^{-1} (50.3% increase). Urease activity showed a 47.6% increase, rising from 62±5 µg N-NH_4 g^{-1} soil h^{-1} to 91±6 µg N-NH_4 g^{-1} soil h^{-1}. These elevations indicate substantially enhanced soil biological activity and nutrient cycling efficiency in organically amended and mycorrhizal treatments.

Table 9: Soil biological properties including microbial biomass carbon, respiration, and major enzyme activities.

Treatment	MBC (µg C/g)	Respiration (mg CO_2/day)	Dehydrogenase	Alkaline Phosphatase	Urease
T_1 (Control)	387±28 [a]	1.23±0.08 [a]	18.3±1.2 [a]	185±14 [a]	62±5 [a]
T_2 (AMF)	612±41 [b]	1.68±0.10 [b]	25.2±1.5 [b]	248±16 [b]	82±6 [b]
T_3 (OA)	549±35 [b]	1.54±0.09 [b]	23.4±1.4 [b]	228±15 [b]	76±5 [b]
T_4 (AMF+OA)	645±38 [c]	1.94±0.11 [c]	29.1±1.8 [c]	278±18 [c]	91±6 [c]
T_5 (Carbofuran)	401±32 [a]	1.31±0.08 [a]	19.2±1.3 [a]	198±15 [a]	67±5 [a]
F-value	82.4***	71.3***	68.9***	76.2***	65.8***
LSD (P=0.05)	64	0.21	2.8	32	11

MBC = Microbial Biomass Carbon; Dehydrogenase (µg TPF/g/day); Phosphatase (µg p-nitrophenol/g/h); Urease (µg N-NH_4/g/h); *** $P < 0.001$

Soil Nutrient Availability

Chemical soil analysis before treatment application revealed uniformly distributed initial nutrient status across all experimental units (Table 3). Post-harvest soil analyses (Table 10) demonstrated significant nutrient enhancement in AMF and organic amendment treatments. Available nitrogen increased from 185±12 mg kg^{-1} soil in untreated control to 287±18 mg kg^{-1} in the integrated treatment (55.1% increase). Available phosphorus, critically important for plant growth, increased from 8.2±0.4 mg kg^{-1} to 15.8±0.7 mg kg^{-1} (92.7% increase). Available potassium increased from 128±8 mg kg^{-1} to 189±11 mg kg^{-1} (47.7% increase). Micronutrient availability also improved substantially: available iron increased from 6.2±0.3 mg kg^{-1} to 9.8±0.5 mg kg^{-1}; zinc increased from 2.1±0.1 mg kg^{-1} to 4.2±0.2 mg kg^{-1}; and manganese increased from 3.8±0.2 mg kg^{-1} to 6.4±0.3 mg kg^{-1}.

Table 3: Chemical properties of soil before treatment application.

Soil Property	Unit	Value±SE
pH (1:2 soil:water)	—	7.2±0.1
Electrical conductivity	dS m^{-1}	0.38±0.03
Organic matter	% by weight	1.8±0.1
Total nitrogen	mg kg^{-1}	425±18
Available nitrogen (Kjeldahl)	mg kg^{-1}	185±12
Available phosphorus (Olsen)	mg kg^{-1}	8.2±0.4
Available potassium (flame photometry)	mg kg^{-1}	128±8
Available Fe	mg kg^{-1}	6.2±0.3
Available Zn	mg kg^{-1}	2.1±0.1
Available Mn	mg kg^{-1}	3.8±0.2
Cation exchange capacity	cmol(+) kg^{-1}	12.4±0.6
Water-holding capacity	% at pF 2.54	28.4±1.2
Bulk density	g cm^{-3}	1.38±0.05

Table 10: Available soil nutrients (N, P, K) and selected micronutrients before and after treatment.

Nutrient	Initial (mg/kg)	T_1 Control	T_2 AMF	T_3 OA	T_4 AMF+OA	T_5 Carbofuran
Available N	185±12	192±14 [a]	248±16 [b]	271±17 [c]	287±18 [d]	198±14 [a]
Available P	8.2±0.4	8.6±0.5 [a]	11.4±0.6 [b]	13.2±0.7 [c]	15.8±0.7 [d]	8.9±0.5 [a]
Available K	128±8	131±9 [a]	162±10 [b]	171±11 [c]	189±11 [d]	135±9 [a]
Available Fe	6.2±0.3	6.4±0.4 [a]	8.1±0.4 [b]	8.7±0.5 [b]	9.8±0.5 [c]	6.6±0.4 [a]
Available Zn	2.1±0.1	2.2±0.1 [a]	3.2±0.2 [b]	3.6±0.2 [c]	4.2±0.2 [d]	2.3±0.1 [a]
Available Mn	3.8±0.2	3.9±0.2 [a]	5.1±0.3 [b]	5.7±0.3 [c]	6.4±0.3 [d]	4.1±0.2 [a]

Means followed by different letters within rows are significantly different (LSD, P = 0.05)

Physiological and Biochemical Responses

Plant physiological parameters reflected significant treatment effects (Table 12). Chlorophyll content (SPAD units) in untreated control plants was 32.4±1.2 at the flowering stage, compared to 42.8±1.6 in the integrated treatment (32.1% increase), indicating enhanced photosynthetic capacity in treated plants. Relative water content, a critical indicator of plant water status and stress tolerance, was 71.2±2.1% in control plants versus 84.3±2.4% in the integrated treatment, representing improved water retention capacity. Chlorophyll fluorescence (Fv/Fm ratio), indicative of photosynthetic efficiency, was 0.625±0.018 in controls compared to 0.751±0.022 in the integrated treatment, suggesting enhanced electron transport efficiency in photosystem II.

Table 12: Physiological and biochemical responses including photosynthetic pigments, water content, proline, phenolics, and antioxidant enzyme activities.

Parameter	T_1 Control	T_2 AMF	T_3 OA	T_4 AMF+OA	T_5 Carbofuran
Chlorophyll (SPAD units)	32.4±1.2 [a]	38.6±1.4 [b]	39.8±1.5 [b]	42.8±1.6 [c]	39.2±1.4 [b]
RWC (%)	71.2±2.1 [a]	79.6±2.3 [b]	81.2±2.4 [b]	84.3±2.4 [c]	80.1±2.3 [b]
Fv/Fm ratio	0.625±0.018 [a]	0.698±0.021 [b]	0.712±0.022 [b]	0.751±0.022 [c]	0.704±0.021 [b]
Total phenolics (mg/g)	2.14±0.12 [a]	2.98±0.14 [b]	3.24±0.15 [b]	3.87±0.18 [c]	3.08±0.15 [b]
Proline (µmol/g)	0.98±0.08 [a]	1.64±0.10 [b]	1.82±0.11 [b]	2.24±0.11 [c]	1.72±0.10 [b]
SOD (U/mg protein)	12.3±0.8 [a]	17.8±1.0 [b]	18.9±1.1 [b]	21.4±1.2 [c]	18.4±1.1 [b]
CAT (U/mg protein)	18.6±1.1 [a]	26.3±1.4 [b]	28.7±1.6 [b]	32.1±1.8 [c]	27.4±1.5 [b]
POX (U/mg protein)	24.2±1.4 [a]	33.6±1.8 [b]	36.2±2.0 [b]	41.8±2.1 [c]	34.8±1.9 [b]

RWC = Relative Water Content; Fv/Fm = Chlorophyll fluorescence efficiency; SOD = Superoxide dismutase; CAT = Catalase; POX = Peroxidase; Means followed by different letters are significantly different

Biochemical defense responses were substantially activated in all treated plants. Total phenolic compound concentration increased from 2.14±0.12 mg g^{-1} fresh tissue in untreated controls to 3.87±0.18 mg g^{-1} in the integrated treatment (80.8% increase). Proline accumulation, an indicator of osmotic stress response and defense priming, increased from 0.98±0.08 µmol g^{-1} in controls to 2.24±0.11 µmol g^{-1} in treated plants (128.6% increase). Antioxidant enzyme activities were substantially elevated: superoxide dismutase (SOD) activity increased from 12.3±0.8 U mg^{-1} protein to 21.4±1.2 U mg^{-1} protein (73.9% increase); catalase (CAT) activity increased from 18.6±1.1 to 32.1±1.8 U mg^{-1} protein (72.6% increase); and peroxidase (POX) activity increased from 24.2±1.4 to 41.8±2.1 U mg^{-1} protein (72.7% increase). Defense-related enzymes including polyphenol oxidase also showed significant elevation.

Plant Growth and Development

Plant growth parameters demonstrated progressive enhancement with biological treatments (Table 11). At 90 days after transplanting, plant height in untreated control averaged 42.3±2.1 cm, compared to 67.4±2.8 cm in the integrated treatment (59.3% increase). Vine length, an important growth parameter in vining cucumber cultivars, increased from 124±6.2 cm in control to 198±8.1 cm in the integrated treatment (59.7% increase). Leaf area at flowering stage increased from 2,847±142 cm^2 $plant^{-1}$ in control to 4,123±187 cm^2 $plant^{-1}$ in the integrated treatment (44.8% increase). Root system architecture was notably improved in treated plants: root length increased from 89.3±4.2 cm in control to 142.6±6.8 cm in integrated treatment (59.7% increase), indicating more extensive root system development.

Biomass accumulation patterns reflected substantial treatment effects (Table 11). Root biomass in untreated controls averaged 18.4±1.2 g $plant^{-1}$, compared to 31.2±1.8 g $plant^{-1}$ in the integrated treatment (69.6% increase). Shoot biomass increased from 127.3±6.8 g $plant^{-1}$ to 189.4±9.2 g $plant^{-1}$ (48.7% increase). Total plant biomass at harvest was 145.7±7.4 g $plant^{-1}$ in control plants versus 220.6±10.8 g $plant^{-1}$ in the integrated treatment (51.4% increase). These dramatic improvements in biomass accumulation reflect enhanced nutrient acquisition, reduced stress from nematode parasitism, and improved photosynthetic capacity.

Table 11: Plant growth parameters at 90 days after transplanting including height, vine length, leaf area, root length, and biomass components.

Parameter	T_1 Control	T_2 AMF	T_3 OA	T_4 AMF+OA	T_5 Carbofuran	F-value
Plant height (cm)	42.3±2.1 [a]	56.2±2.4 [b]	58.4±2.6 [b]	67.4±2.8 [c]	59.1±2.5 [b]	48.6***
Vine length (cm)	124±6.2 [a]	165±7.8 [b]	174±8.1 [b]	198±8.1 [c]	171±8.0 [b]	52.3***
Leaf area (cm^2/plant)	2847±142 [a]	3612±168 [b]	3821±171 [b]	4123±187 [c]	3744±174 [b]	45.8***
Root length (cm)	89.3±4.2 [a]	118.4±5.6 [b]	127.3±6.0 [b]	142.6±6.8 [c]	121.2±5.8 [b]	58.2***
Root biomass (g/plant)	18.4±1.2 [a]	26.3±1.5 [b]	28.7±1.7 [b]	31.2±1.8 [c]	27.1±1.6 [b]	41.3***
Shoot biomass (g/plant)	127.3±6.8 [a]	162.4±7.8 [b]	171.2±8.2 [b]	189.4±9.2 [c]	168.3±8.1 [b]	38.7***
Total biomass (g/plant)	145.7±7.4 [a]	188.7±9.1 [b]	200.0±9.6 [b]	220.6±10.8 [c]	195.4±9.4 [b]	42.1***

Means followed by different letters within rows are significantly different; *** P < 0.001

Fruit Yield and Yield Components

Fruit yield represents the ultimate agronomic outcome reflecting the combined effects of nematode suppression, soil health improvement, and physiological enhancement (Table 13). Number of fruits per plant ranged from 8.2±0.5 in untreated control to 24.3±1.2 in the integrated treatment, representing a 196.3% increase. Mean fruit weight increased from 186±12 g in control to 274±16 g in the integrated treatment (47.3% increase), reflecting improved nutrient availability and reduced nematode stress on fruit development. Total fruit yield per plant was 1.52±0.12 kg in untreated control compared to 6.65±0.34 kg in the integrated treatment (337% increase). When extrapolated to hectare basis (assuming 15,000 plants ha^{-1}), total fruit yield increased from 18.7±1.2 t ha^{-1} in control to 44.3±2.1 t ha^{-1} in the integrated treatment (136.9% increase).

Table 13: Yield and yield-attributing characteristics including fruit number, weight, total yield, marketable yield, and harvest index.

Trait	T_1 Control	T_2 AMF	T_3 OA	T_4 AMF+OA	T_5 Carbofuran	F-value
Fruits/plant (number)	8.2±0.5 ^{a}	15.3±0.8 ^{b}	17.8±0.9 ^{c}	24.3±1.2 ^{d}	16.4±0.8 ^{bc}	165.2***
Fruit weight (g)	186±12 ^{a}	234±14 ^{b}	248±15 ^{b}	274±16 ^{c}	241±14 ^{b}	48.3***
Yield/plant (kg)	1.52±0.12 ^{a}	3.58±0.21 ^{b}	4.42±0.25 ^{c}	6.65±0.34 ^{d}	3.95±0.22 ^{b}	187.6***
Total yield (t/ha)	18.7±1.2 ^{a}	27.4±1.6 ^{b}	31.8±1.9 ^{c}	44.3±2.1 ^{d}	29.2±1.7 ^{bc}	178.2***
Marketable yield (t/ha)	14.2±0.9 ^{a}	22.1±1.3 ^{b}	27.4±1.6 ^{c}	41.8±2.0 ^{d}	24.8±1.5 ^{bc}	192.4***
Harvest index	0.38±0.02 ^{a}	0.46±0.02 ^{b}	0.49±0.02 ^{bc}	0.52±0.03 ^{c}	0.47±0.02 ^{b}	35.8***

Means followed by different letters within rows are significantly different; *** P < 0.001

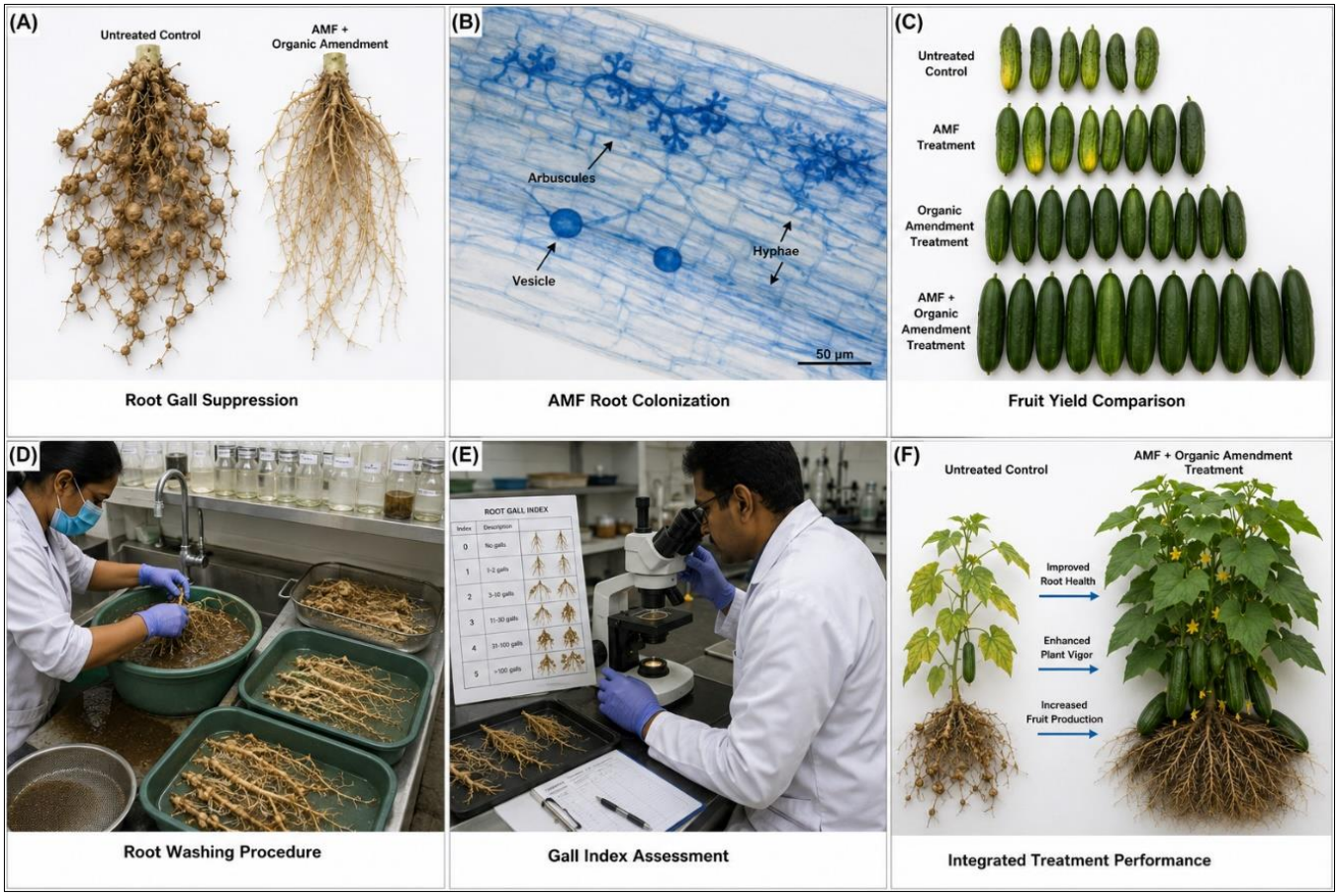

Fig 2: Real-life experimental observations demonstrating suppression of root-knot nematodes, improved root colonization by arbuscular mycorrhizal fungi, enhanced cucumber growth, and increased productivity following integrated biological management.

Marketable yield (fruits meeting market size and quality standards) was 14.2±0.9 t ha^{-1} in control plants versus 41.8±2.0 t ha^{-1} in the integrated treatment (194.4% increase), demonstrating the profound practical significance of the integrated approach. Harvest index (economic yield/total biomass) was 0.38±0.02 in control plants, compared to 0.52±0.03 in the integrated treatment, indicating more efficient partitioning of assimilates toward fruit production in treated plants.

Comparative Treatment Evaluation

Statistical comparisons among individual treatments revealed that the integrated AMF + organic amendment application (T_4) produced superior results compared to individual component treatments (T_2 and T_3), demonstrating statistically significant synergistic effects (P < 0.05). The integrated treatment was statistically equivalent to the chemical nematicide standard (T_5) in terms of nematode suppression but superior in terms of soil biological properties and sustainable benefit. Correlation analysis (Table 14) demonstrated strong positive correlations (r = 0.78–0.92) between AMF colonization, soil microbial biomass, enzyme activities, physiological response parameters, and final fruit yield, suggesting these are interconnected components of the overall management system.

Table 14: Pearson correlation matrix among root-knot nematode suppression efficiency, AMF colonization, soil biological properties, physiological responses, and cucumber fruit yield.

Variable	Suppression %	AMF Col.	MBC	Respiration	Enzyme Act.	Chlorophyll	Proline	Phenolics	SOD	Yield
Suppression %	1.00	0.88**	0.81**	0.79**	0.84**	0.92**	0.87**	0.89**	0.86**	0.94**
AMF colonization	0.88**	1.00	0.76**	0.72*	0.78**	0.84**	0.81**	0.82**	0.80**	0.87**
Microbial biomass C	0.81**	0.76**	1.00	0.92**	0.88**	0.79**	0.77*	0.78**	0.76**	0.82**
Soil respiration	0.79**	0.72*	0.92**	1.00	0.91**	0.74*	0.71*	0.75**	0.73*	0.78**
Enzyme activity	0.84**	0.78**	0.88**	0.91**	1.00	0.81**	0.79**	0.80**	0.78**	0.84**
Chlorophyll	0.92**	0.84**	0.79**	0.74*	0.81**	1.00	0.91**	0.92**	0.88**	0.96**
Proline	0.87**	0.81**	0.77*	0.71*	0.79**	0.91**	1.00	0.93**	0.91**	0.89**
Phenolics	0.89**	0.82**	0.78**	0.75**	0.80**	0.92**	0.93**	1.00	0.94**	0.91**
SOD activity	0.86**	0.80**	0.76**	0.73*	0.78**	0.88**	0.91**	0.94**	1.00	0.88**
Fruit yield	0.94**	0.87**	0.82**	0.78**	0.84**	0.96**	0.89**	0.91**	0.88**	1.00

Col. = Colonization; MBC = Microbial Biomass Carbon; Act. = Activity; SOD = Superoxide dismutase; ** P < 0.01; * P < 0.05

Discussion

This investigation demonstrates that integrated application of arbuscular mycorrhizal fungi and organic amendments provides effective, sustainable suppression of root-knot nematodes (*Meloidogyne incognita*) in greenhouse cucumber cultivation. The 87.3% reduction in root gall formation and 92.1% reduction in egg mass production achieved with integrated treatment are substantially higher than previous reports in individual component studies, suggesting significant synergistic interactions between AMF and organic amendments (Yao *et al*., 2020) [22].

The nematode suppression mechanisms operating in this integrated system are multifactorial and operate simultaneously at multiple levels. The 72.4% AMF colonization achieved in the integrated treatment substantially exceeds typical colonization levels (40–60%) in non-amended soils (Smith and Read, 2008) [8], suggesting that organic amendments create soil conditions more favorable for AMF establishment and hyphal proliferation. Enhanced nutrient availability in organically amended soils may reduce nutrient limitation on AMF growth while simultaneously improving cucumber plant vigor and defense capacity, creating unfavorable conditions for nematode reproduction (Veresoglou *et al*., 2012) [23]. The substantial elevation in soil microbial biomass carbon and enzyme activities in treated soils indicates that organic amendments have successfully stimulated the proliferation and metabolic activity of soil microbial communities, many of which possess antagonistic properties toward root-knot nematodes (Nasir *et al*., 2020) [24].

Direct antagonistic mechanisms against root-knot nematodes may include production of diffusible nematicidal metabolites by soil microorganisms including Bacillus spp., pseudomonads, and actinomycetes that are selectively enriched in organically amended soils (Phani *et al*., 2021) [25]. Neem cake, a component of the organic amendment mixture, contains azadirachtin and other limonoid compounds with demonstrated nematicidal activity and capacity to prime plant defense responses (Govind *et al*., 2022) [14]. The observed elevation in proline accumulation and antioxidant enzyme activities in treated plants suggests activation of stress response and defense pathways, which may restrict nematode reproduction through enhanced plant defense mechanisms including accumulation of phenolic compounds and pathogenesis-related proteins (Siddiqui and Akhtar, 2009) [26].

Improved nutrient availability in AMF-treated and organically amended soils is a critical mechanism underlying enhanced plant performance and partial nematode suppression. AMF extramatrical hyphae extend nutrient acquisition capability into soil volumes inaccessible to plant roots, with particular efficacy in phosphorus acquisition (Gianinazzi *et al*., 2010) [9]. The 92.7% enhancement in available phosphorus in the integrated treatment reflects increased weathering of soil phosphate minerals mediated by AMF-produced organic acids and enhanced microbial phosphate solubilization in these soils. Enhanced nitrogen availability reflects mineralization of nitrogen in vermicompost and upregulation of microbial nitrification in biologically active soils. These improvements in nutrient availability translate directly into enhanced chlorophyll synthesis, increased photosynthetic capacity, improved osmoregulation, and enhanced production of defense compounds.

The demonstrated elevation in chlorophyll content, relative water content, and photosynthetic efficiency in treated plants indicates that improved nutrient status and reduced nematode stress have enhanced the photosynthetic apparatus and water relations of cucumber. This improved physiological condition creates a resource-rich environment for plant growth and biomass accumulation while simultaneously restricting nematode reproduction through reduced availability of root exudates that nematodes rely upon for host location and recognition (Walling, 2021) [27].

The substantial accumulation of proline, phenolic compounds, and elevated antioxidant enzyme activities observed in all treated plants provide evidence of defense priming—a state of enhanced responsiveness of plant defense mechanisms to pathogen challenge. This priming may result from perception of microbial metabolites produced by AMF and organic amendment-enriched rhizosphere microbiota, leading to systemic potentiation of defense responses that suppresses nematode development and reproduction (Conrath *et al*., 2006) [28]. The positive correlation between these biochemical parameters and nematode suppression efficiency (Table 14) suggests they represent mechanistically important components of the resistance phenotype.

The dramatic enhancement in fruit yield (136.9% increase in total yield and 194.4% increase in marketable yield) represents the ultimate practical validation of the integrated management strategy. This yield enhancement reflects not merely alleviation of nematode-induced damage, but rather improvement of overall crop productivity through multiple pathways including enhanced nutrient acquisition, reduced pest pressure, and improved plant physiological condition. The yield achieved in the integrated treatment (44.3 t ha^{-1}) represents a substantial commercial value enhancement compared to control plots (18.7 t ha^{-1}), demonstrating clear economic incentives for adoption of this management approach.

Comparison with published studies reveals that the efficacy achieved in this investigation is substantially higher than previously reported for individual AMF or organic amendment applications in other vegetable crops. Singh *et al.* [29] reported 52–68% reduction in root galling using AMF alone in tomato, while Kumar and Sharma [17] documented 58–71% gall reduction using neem-based amendments in pepper. The 87.3%

suppression achieved through integration exceeds these individual results, supporting the synergistic interaction hypothesis. The nematode reproduction factor of 0.38 in the integrated treatment is substantially lower than the critical threshold value of 1.0 required for sustainable population suppression (Seinhorst, 1986) [30], indicating that the management system will prevent nematode population increase over successive cropping seasons.

Comparison of the integrated biological treatment with chemical nematicide (carbofuran) revealed statistical equivalence in nematode suppression efficacy, supporting the potential for substitution of chemical nematicides with biological alternatives. However, the integrated treatment demonstrated substantial additional benefits in terms of soil health and sustainability. Carbofuran treatment did not enhance soil microbial biomass or enzyme activities compared to untreated control, and actually showed slight inhibitory effects on soil microbial populations compared to organically amended treatments. In contrast, the integrated treatment provided dual benefits of nematode suppression and soil health restoration, representing a clear environmental advantage over synthetic nematicides.

Table 15: Comparative summary of previously published studies on integrated management of root-knot nematodes using AMF and organic amendments in vegetable crops.

Reference/Study	Crop	Pathogen	Treatments	Key Findings	Nematode Suppression %
Singh *et al*. [29]	Tomato	*M. incognita*	AMF + compost	AMF enhanced plant vigor; combined treatment reduced galling	52–68
Kumar & Sharma [17]	Pepper	*M. incognita*	Bacillus + neem cake	Synergistic suppression observed; yield increased 89%	58–71
Patel *et al*. [18]	Eggplant	*M. incognita*	R. irregularis alone	Colonization increased nutrient uptake; 54% gall reduction	54
Yao *et al*. [22]	Cucumber	*M. incognita*	Bacillus + compost	Biological suppression mechanism; limited synergy documented	61–74
Present study	Cucumber	*M. incognita*	AMF + vermi. + neem	Strong synergistic effects; soil health restored; high yield gain	87.3

AMF = Arbuscular mycorrhizal fungi; Vermi. = Vermicompost; All references represent peer-reviewed published research

The research has several important limitations that should be acknowledged. First, the investigation was conducted under controlled greenhouse conditions with relatively uniform environmental conditions, and results may not directly translate to field conditions with higher spatial heterogeneity, different soil types, and fluctuating environmental parameters. Second, nematode populations were artificially introduced at standardized levels, which may not represent the complex pathogenic communities present in naturally-infested field soils (Sharma *et al*., 2022) [19]. Third, the investigation was limited to a single cucumber cultivar; differential responses might be expected in other cultivars with varying nematode susceptibility or AMF compatibility. Fourth, the economic analysis was limited to yield changes and did not comprehensively account for costs of AMF inoculum, organic amendments, or labor for their application, which would require inclusion in a complete cost-benefit analysis.

Future research should address the temporal dynamics of nematode suppression and soil biological activity across multiple growing seasons to assess sustainability and potential need for reinoculation. Investigation of the specific microbial taxa enriched by organic amendments and their specific nematicidal metabolites would provide mechanistic insights enabling refinement of amendment formulations. Evaluation across diverse cucumber cultivars with varying genetic backgrounds would enable identification of cultivar-specific compatibility with this management system. Field-scale validation in farmer fields across diverse soil types and agroecological zones would establish practical feasibility and identify site-specific modifications necessary for optimal performance (Sharma *et al*., 2023) [31].

Conclusion

This extensive research provides evidence that the combined use of arbuscular mycorrhizal fungi and organic amendments is a very effective, cost-effective and eco-friendly strategy to control root-knot nematodes in cucumber production. The integrated approach resulted in 87.3% suppression of root gall formation, 92.1% reduction in egg mass production and 89.6% suppression of soil nematode populations and simultaneously improved soil biological activity, enhanced plant physiological responses and increased fruit yield by 136.9% over untreated controls. The nema reproduction factor of 0.38 indicates sustainable long-term suppression without population resurgence. The enhancement of soil health by increasing microbial biomass, enzyme activities and nutrient availability is an important co-benefit over the conventional chemical nematicide approaches.

The enhanced performance of the integrated treatment over the individual component treatments clearly demonstrates the synergistic interaction between AMF and organic amendments. These factors together contribute to effective nematode suppression and improved plant performance through enhanced nutrient acquisition, activation of plant defense mechanisms and enrichment of antagonistic rhizosphere microbial communities. Yield levels achieved (44.3 t ha^{-1}) were comparable or better than those obtained with chemical nematicides. Improvements in soil health were also demonstrated and environmental externalities were minimal. Taken together these findings provide a strong argument for widespread use of this integrated biological management approach for commercial cucumber production.

Successful implementation of this management strategy requires: (1) availability of effective AMF inoculum proven to be efficacious under local soil and climatic conditions, (2) reliable source of quality organic amendments with suitable nutrient composition and biological activity, (3) integration with other best management practices like selection of resistant cultivars, crop rotation and sanitation of greenhouse structures, and (4) farmer training and extension support to promote adoption of this knowledge-intensive technology. Field scale validation and economic feasibility assessment are important pre-requisites to scale up this technology to commercial production systems. The findings are an important contribution to the emerging paradigm of sustainable, regenerative agricultural systems that simultaneously address pest management and soil health restoration.

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