

Management of root-knot nematode infection by using fly ash and *Trichoderma harzianum* in *Capsicum annum* plants by modulating growth, yield, photosynthetic pigments, biochemical substances, and secondary metabolite profiles

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Abstract

A nematicide is a type of chemical pesticide used to kill plant-parasitic nematodes. Nematicides have tended to be broad-spectrum toxicants, possessing high volatility or other properties that promote migration through the soil. In addition, the nematicides used are more expensive and have adverse effects on health and the environment, so it must use more eco-friendly and less expensive alternative methods to control root-knot nematodes (*Meloidogyne incognita*). Chili (*Capsicum annum*) suffers from nematode infestation, which reduces its quality and quantity. Therefore, the goal of this research was to assess the effect of different doses of fly ash (FA) mixed soil (5%, 10%, 15 and 20% FA) with two doses of *Trichoderma harzianum* (1 g and 2 g) for the management of root-knot nematode infection in chili crop. The results showed that significant enhancement in plant growth, yield, chlorophyll, and carotenoid content, protein, carbohydrate, amino acid, tryptophan, indole acetic acid, phenolics, flavonoids, proline, and nitrate reductase content of chili plants was recorded at 10% fly ash with 2 g of *T. harzianum* (T6). The inoculated plants registered the greatest damage with galling indexes. The lowest galling index was estimated at the T6 treatment. At higher levels of FA + combined with both doses of *T. harzianum*, nematode could not survive that's why eighter galls or egg masses were observed. Nematodes may have ceased to function, lost their activity, and hence been unable to resist the stress of fly ash and *T. harzianum* set. The application of *T. harzianum* with a lower dose (10%) of fly ash to control the nematode favored plant growth in general. In conclusion, 10% fly ash and 2 g of *T. harzianum* have the ability to operate as growth promoters and biocontrol agents for *M. incognita*.

Keywords: chili; carbohydrate; flavonoids; *Meloidogyne incognita*; phenolics; proline

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Introduction

The pathogenicity of root-knot nematodes (RKNs) is known to inflict serious economic harm to farmers worldwide and cause serious crop damage (Ahmad *et al.*, 2021a). *Meloidogyne incognita*, an endoparasite belongs to RKNs are microscopic creatures that dwell in soil and transmit to host plants, infecting and developing feeding mechanisms with styles (Costa *et al.*, 2021) that harms a variety of economically significant crops (Ahmad *et al.*, 2021b). For instance, a study on *Lycopersicon esculentum* plants found *M. incognita* caused a reduction in yield and productivity of about 27%, and also caused damage to vegetable crops that is estimated to be 50% worldwide (Kaur *et al.*, 2011). They suck the nutrients from the host roots and cause damage to entire plant thereby reducing crop productivity (Nyaku *et al.*, 2017). As a result, during the infection stage of *M. incognita* second-stage juveniles, it migrates through the root tip region and invades the plant's vascular system. However, once they've successfully colonized the vascular tissue, nematodes become immobile and spend the rest of their lives in the roots (Elhady *et al.*, 2018).

Wilting, reduction in plant length and fresh and dry weights, enlarged roots, damage to the plasma membrane, and the production of root galls are all symptoms of nematode infection, as well as a decrease in yield (Jang *et al.*, 2014). Moreover, it has been reported that nematode attacks can cause physiological and metabolic disturbances like water balance, mineral and solute transport, cytoplasmic leakage, and photosynthetic efficiency (Seid *et al.*, 2015). Besides, pathogen infections trigger ROS production in plants (Mohamed and Abd-El Hameed, 2014; Sofy *et al.*, 2021a, b). Secondary metabolites produced in plants after infection act as antioxidants and help in the plant's defences under biotic stress conditions (Mohamed *et al.*, 2018; Ashry *et al.*, 2018). Resistance to plant pathogens is partly conferred by secondary metabolites, such as flavonoids, proline, and phenols (Aly *et al.*, 2012; 2013; Yan *et al.*, 2021). To control nematodes, massive amounts of nematicides are utilized, but the synthetic chemical pesticides cause pollution to the environment, so that, eco-friendly biological control has become a major research priority in recent years (Singh *et al.*, 2020; Yan *et al.*, 2021).

An increasing human population and increased traffic and vehicular traffic have contributed to the accumulation of gaseous and particulate air pollutants over time (El-Beltagi *et al.*, 2020; El-Mahdy *et al.*, 2021). Automobiles are the largest source of air pollutants, such as oxides of nitrogen and sulphur. In addition, combustion coal residues (CCRs) such as fly ash, boiler slag, and bottom ash are produced by thermal power plants worldwide due to the use of coal for the generation of electricity (Heidrich *et al.*, 2013). By 2030, the coal consumption used in thermal power plants will rise from 29.9% in 2011 to 46% (Yao *et al.*, 2015). In India, the annual production of coal reaches 160 million tons (Yao *et al.*, 2015). Electric and steam-generating plants produce fly ash, a fine residue from the combustion of pulverized coal. After dispersing the atmosphere, a great deal of fly ash is deposited on the soil and vegetation's surface. Many macro-and micronutrients in fly ash improve soil physical and chemical properties, improving agricultural crop growth and yield (Dahiya and Budania, 2018; Ahmed *et al.*, 2021a). In many crops, the use of fly ash has resulted in an increase of up to 25% in leaves number, plant height, biomass, and yield, thereby serving as a fertilizer and source of plant nutrients for various crops (Ahmed *et al.*, 2021a). Application with sufficient amount of fly ash to soil could increase the availability of B, K, and other nutrients in soil (Ahmed *et al.*, 2021a), and ameliorate the physical and chemical properties of soil, such as improving soil structure, soil water content, and water holding capacity (Ahmed *et al.*, 2021a). Niu *et al.* (2021) found that composed of fly ash (FA) and polyacrylamide (PAM) and the interaction of FA and PAM all had significant impacts on the percentage of seedling emergence of *Artemisia ordosica* and total fresh weight.

Trichoderma and other soil-borne fungi are well-known for their capacity to control a wide range of plant diseases (Abd El- Rahman and Mohamed, 2014). Because of the effects of *Trichoderma* spp. colonization on plant physiology and metabolism, a wide range of defences and growth-promoting metabolites like growth regulators, organic acids, and siderophores are synthesized by the plant (Zhang *et al.*, 2015). Furthermore, by

creating enzymes and secondary metabolites (Bhattacharjee and Dey, 2014), *Trichoderma* species can operate as biological control agents, particularly against *Meloidogyne* nematodes (Sokhandani *et al.*, 2016) through preventing egg hatching and immobilizing nematode second-stage juveniles (J2) (Feyisa *et al.*, 2015). When it comes to managing root phytopathogen populations, however, various aspects must be considered, including the fungus's origin, compatibility with host plants, and the edaphic characteristics of its site (Al-Hazmi and Javeed, 2016).

Pepper is a Solanaceae plant that is among the major crops in the Mediterranean region, and the lack of water and increased salt stress are major constraints to productivity (Penella *et al.*, 2013, 2014). Ascorbic acid, carotenoids, and phenolic compounds are among the bioactive antioxidants and minerals found in them (Akladios and Mohamed, 2018). Root-knot nematodes can cause significant damage to this crop if they attack it (Moosavi, 2015). The initial population density of Nematoda at planting affects plant yield reduction (Moosavi, 2015). Plant cultivars and/or nematode strains influence the extent of a nematode's damage to a plant (Kihika *et al.*, 2017).

The current research was carried out to determine the potential of a combination of *Trichoderma* and fly ash to control *M. incognita* infections on chili plants. The potential for *Trichoderma* and fly ash to act as biocontrol agents for RKNs was assessed by studying the effect of combination between *Trichoderma* and fly ash on growth, yield, photosynthetic pigments, nitrate reductase, secondary metabolites, osmolytes and biocontrol against nematodes.

Materials and Methods

Experimental site and plant material

The experimental material consists of one cultivars of *C. annuum* (variety: NS 1101) provided by Agricultural University Ludhiana, Punjab, and is highly recommended for the agro-climatic zone of central India. The experimental material was planted in augmented RBD with 5 replications for each treatment at inter and intra row spacing was 45 and 15 cm, respectively. The pots established in the green house of the Botany Department of Aligarh Muslim University, Aligarh, India.

Culturing of nematodes

By collecting infected samples from several locations, the nematode cultures were kept alive in a greenhouse. *Meloidogyne incognita* was isolated, identified, and kept for research purposes. The egg masses were separated from their roots and placed in distilled water to preserve them. The second-stage juvenile nematodes were collected in double-distilled water after being incubated at 27 °C to stimulate hatching. At the time of inoculation, the nematode's second-stage juveniles were 24 hours old. These were counted and used in experiments using a light microscope (Figure 1A).

Preparation of Trichoderma culture and inoculum

T. harzianum GQ426038 was prepared, maintained in a potato dextrose broth (PDB) medium, and was used for mass culture. A Thoma hemocytometer was used to measure conidia concentrations, and the final concentration was adjusted to 4.0×10^8 conidia mL⁻¹ sterile distilled water for soil inoculation.

T. harzianum was injected into flasks containing PDB medium using a sterile inoculation needle. The inoculated flasks were incubated for about 15 days at 25 °C. The medium was filtered with filter paper when the fungus had grown sufficiently. On the filter paper, a fungal mycelia mat was obtained, which was collected (Figure 1B). The mycelia mat was blotted with blotting sheets to remove nutrients and excess water. In a flask, 100 g of fungal mycelia was blended in 1000 mL of double distilled water (DDW) in a flask so that 10 ml of suspension contained 1g of mycelia. For inoculation of fungus, different concentrations of suspension were

standardized, which contained 1 g fungus 10 mL⁻¹ of dry weight (DW) and 2 g fungus 20 mL⁻¹ of DW that were inoculated in the pots 8-10 days prior to inoculation of J2s of *M. incognita* for colonizing the rhizosphere to prevent root-knot nematode infection.

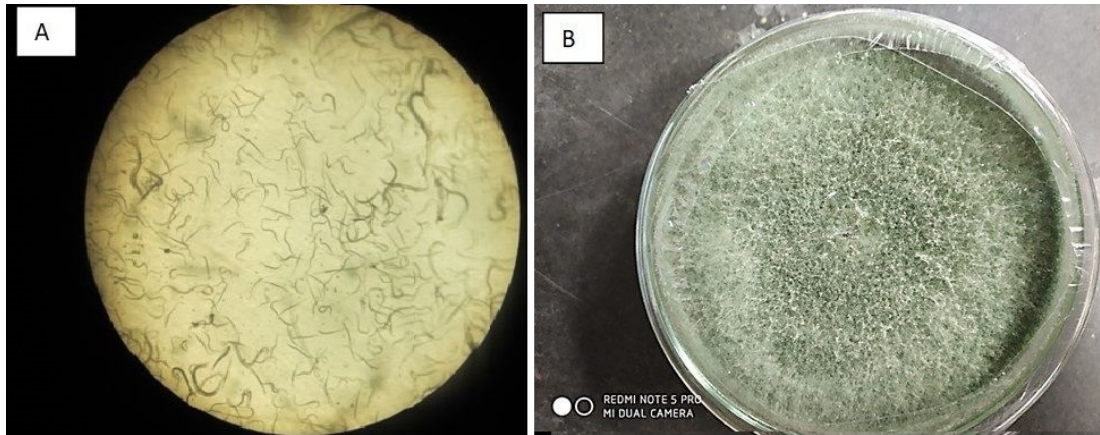


Figure 1. A: Second stage juveniles (J2s) of *M. incognita*, B: Culture of *T. harzianum*

Collection of fly ash and soil

Fresh fly ash was collected from the thermal power plant, kasimpur, Aligarh. The physical and chemical properties of soil are illustrated in Table 1. The soil was sandy loam containing 66% sand, 24% silt, 8% clay, 2% organic matter, and pH7.7. Before utilization, the soil was autoclaved.

Physico-chemical properties of soil

The soil texture was determined using the soil texture triangle method (Chopra and Kanwar, 1991). Soil Testing Kit Model 161E was used to calculate the electrical conductivity (EC) and cation exchange capacity (CEC) of the soil and fly ash (Jackson 1973). The water-holding capacity of the soil samples was calculated using the methodology given by Black *et al.* (1965). The Carter and Ball (1993) method was used to calculate porosity (St), which is the percentage of soil volume filled by pore spaces. Tensiometers were used to determine the moisture content (Nunes Instruments). One of the most essential aspects of soil that makes up a soil sample is particle density. It was accomplished using the approach previously stated by Blake (2008).

According to Jackson (1973), the soil pH was evaluated using a pH meter (PCE Instruments). Dickman and Bray (1940) method was used to estimate phosphorus (P) from sample extract using calorimeter after the addition of HClO₄. A flame photometer was used to estimate potassium (K) in soil and fly ash samples. The nitrogen content was determined using the Kjeldahl (1883) method. Chopra and Kanwar (1982) method were used to determine magnesium. The carbonates and bicarbonates in samples were determined using Richard (1954) technique. Jackson (1973) method was used to determine the chloride and sulphate content in samples. Finally, an atomic absorption spectrophotometer was used to determine Zn and Mn in soil.

Table 1. Physical and chemical properties of experimental soil

Characteristics	Soil
Colour	Light Brown
EC (mmhos cm ⁻¹)	0.050±0.11
CEC (mEq 100g ⁻¹)	2.04±0.20
Porosity (%)	38.59
Water holding capacity (%)	45.50
Moisture content (%)	2.68±0.29
Particle density (g/cm ³)	2.59±0.23
pH	6.8±0.59
Sulphate (mg L ⁻¹)	15.37±1.50
Chloride (mg L ⁻¹)	37.67±2.30
Carbonate (mg L ⁻¹)	76.23±5.44
Bicarbonate (mg L ⁻¹)	20.25±1.45
N (%)	2.65±0.38
P (%)	2.86±0.43
K (%)	18.65±1.66
Zn (mg g ⁻¹)	1.21±0.29
Mg (mg g ⁻¹)	40.02±3.16
Mn (mg g ⁻¹)	3.82±0.95

Determination of elemental status

Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX) was used to determine elements.

Autoclaving of soil and preparation of pots

The soil was sealed in gunny bags and steam sterilized for 20 minutes at 20Ib pressure in an autoclave. To achieve a varied ratio, the autoclaved soil was dried and mixed with fly ash. The test plant was chosen to be the chili. In 10-inch clay pots, the certified seeds (variety: NS 1101) were planted. Seedlings were transplanted to different pots for several studies once the four-leaf stage emerged.

Treatments

The earthen pots of 12 inches height were filled with 3 kg (3000 g) of each type of soil mixture. The treatments were 5% FA (2850 g soil + 150 g fly ash); 10% FA (2700 g soil + 3000 g fly ash); 15% FA (2550 g soil + 450 g fly ash); 20% FA (2400 g soil + 600 g fly ash). Each ration of soil and fly ash were mixed with 1 and 2 g of *T. harzianum* culture mat. The following treatment sets were taken in this experiment.

T1 – Control (only soil)

T2 – Control (Soil + 2000 J2 of *M. incognita*)

T3 – 5% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*

T4 – 5% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*

T5 – 10% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*

T6 – 10% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*

T7 – 15% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*

T8 – 15% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*

T9 – 20% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*

T10 – 20% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*

Morphological and yield parameters

All plants were harvested after 3 months on 15 February 2020, and during the experiment, plants were examined regularly for symptoms, photographs of growth, and yield. In addition, the following parameters were measured after termination. The length, fresh and dry weights (wt.) were measured. The number of branches and leaves were counted on each plant. Five average-sized leaves from each treatment were traced on tracing paper to estimate leaf area. The areas occupied by these drawings were measured with the help of a planimeter. Also, the number of flowers and fruits per plant were counted. Five average-sized fruits were collected from each treated plant, and their lengths, fresh and dry weights were measured.

Determination of biochemical substances of chili plant

The biochemical substances of the vegetative part of chili plant were determined in terms of their chlorophyll content, 0.5 g sample of fresh chili leaf was homogenized with 80% acetone. Each sample was homogenized and pestle in a 10 mL solution of acetone (80%), and then, the sample mixture was subjected to centrifugation at 12,000 rpm for 20 min. After centrifugation, the supernatants were collected for the estimation of chlorophyll and carotenoid contents by measuring the absorbance at 470, 649, and 665 nm on a UV-Vis spectrophotometer (UV-3200). Then, chlorophyll a, b (Bruinsma, 1963) and carotenoids were calculated by the formulae given by Lichtenthaler and Wellburn (1983).

Slyke *et al.* (1941), used to determine total free amino acids content in chili leaves that were pulverized in a mortar and pestle in 5 ml of 80% ethanol (v/v). The mixture was heated for 10 min and centrifuged at 2000 rpm for 10 min, and the supernatant was collected and using ninhydrin reagent to measure amino acids. Protein content was determined using Folin-Ciocalteu reagent according to Lowery *et al.* (1951) technique, the optical density read at 750 nm and bovine serum as a standard.

The quantity of carbohydrate in the chili leaf was calculated by the anthrone reagent method (Dubols *et al.*, 1956). Two hundred milligrams of dried chili leaf were homogenized in 96% v/v ethanol. For 10 min, the samples had been centrifuged at 3500 ×g. Three millilitres (150 mg anthrone + 10 mL (v) sulfuric acid) from a freshly prepared anthrone reagent was reacted with 0.5 mL of the supernatant for 10 min by heating in a bath of water. The cooling process was completed, and the mixture was absorbed using a spectrophotometer at 652 nm. Indole acetic acid was determined according to Beffa *et al.* (1990) method.

The method of Sadasivam and Manickam (1996) was used for the extraction as well as for the quantitative of amino acid tryptophan. 500 mg of chili sample was homogenized with 10 ml of 80% ethanol and centrifuged at 10,000 g for 20 minutes. The supernatant was preserved, and the extraction was repeated twice (10 ml+ 10 ml) with the residue. The pooled supernatants were collected and evaporate it to dryness on a boiling water bath and dissolve the residue in 5 ml of 0.2M citrate buffer (pH 5.0). Pipette 2 ml of the above sample preparation in a test tube. For a reagent blank, take 2.0 ml of 0.2M citrate buffer (pH 5.0) in place of the sample preparation. In another set of tubes, take graded concentrations of tryptophan (0-100 µg) and make the total volume to 2 ml with 0.2M citrate buffer (pH 5.0). Add 1ml of KCN-acetone Ninhydrin reagent and mix thoroughly. Keep the test tubes in boiling water bath for 20 min, cool under running tap water and make the volume to 10 ml with distilled water and the intensity of the purple colour developed was read using spectrophotometer (Spectronic-20, Japan) at 570 nm, with reagent blank.

Two grammes of chili leaves were extracted with 20 mL methanol (80%) and filtered through filter paper No. 1 by shaking at 150 rpm for 12 h. The methanol extract filtrate will be used to determine total phenols. 50 µL of the methanol extract was mixed with 100 µL Folin-Ciocalteu reagent, 850 µL of methanol and allowed to stand for 5 min at ambient temperature. A 500 µL of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm according to Ruanma *et al.* (2010). In addition, 0.5 mL solution of each plant extracts in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a

double beam Perkin Elmer UV/Visible spectrophotometer (USA) to determine total flavonoids according to Van acker *et al.* (1996).

Sadasivam and Manickam (1971) described the ninhydrin technique for estimating proline content. 3% sulphosalicylic acid was used to homogenize 0.3 g of fresh leaves. In a test tube held in a water bath at 100 °C for 1 hour, the filtrate was acted in response to 1 mL each of glacial acetic acid and acid ninhydrin. The reaction was stopped by putting the test tube in the freezer. The optical density of the mixture was measured at 520 nm using a spectrophotometer. The activity of nitrate reductase (NRA) was assessed using Jaworski, (1971) technique. 1.0 g of fresh leaves were milled into a powder in liquid N₂ and stored at a temperature of 80 °C. The powder was thawed in a 250 mM Tris–HCl buffer for 10 minutes at 4 °C before homogenization. The homogenate was centrifuged for 30 minutes at 10,000 ×g at 4 °C. NR activity was analysed by adopting the method of Nakagawa *et al.* (1984).

Galls, egg masses, and gall index

The roots were carefully cleaned with water and immersed in phlox in B (0.15 g L⁻¹ tap water) solution for 15 min to stain the egg masses. Then, the number of galls and egg masses of each root were counted. For the gall index, the scale of Taylor and Sasser (1978) was followed.

Statistical analysis

The values were entered into a one-way analysis of variance (ANOVA) using R (x64 2.14.1). Sigma Plot was used to create the graphs (11.0). Duncan's multiple range test (DMRT) at 0.05% was used to detect variations between treatments and compare them to mean values. The average mean of five replicates of the data was used to calculate each value. The experiment was carried out in a completely random manner (CRD).

Results

Colour and shape of fly ash particle

Figure 2 A-C shows images of fly ash particles taken with SEM. Some of the particles appeared solid, while others appeared hollow spheres made up of several smaller particles. The colour of the fly ash that we studied in this research can be dark grey or grey, and even black.

Element's status of fly ash

Fly ash is classified according to its elemental composition by (SEM-EDX) in Figure 2D. Elements as SiO₂ and Al₂O₃ are found in the fly ash, along with trace amounts of oxides of Al, Fe, and traces of Mg, P. According to the American Society for Testing and Materials, the fly ash used in this study is classified as class F.

*Interactive effect of different concentrations of fly ash and *T. harzianum* on growth and yield*

Figure 3, and Figure 4A, B show the effect of soil application of fly ash, *T. harzianum*, and 2000J2 of *M. incognita* on chili plants growth and yield attributes. Chili plant growth and yield attributes were significantly reduced when 2000J2 of *M. incognita* was applied in comparison with uninfected plants. In contrast, when compared to plants treated with 2000J2 of *M. incognita*, treatment with various concentrations of fly ash and *T. harzianum* resulted in a considerable increase in growth and yield parameters. The most noticeable increases were detected in plants that had been treated with 10% fly ash, 2.0 g of *T. harzianum* and 2000J2 of *M. incognita* (T6) in terms of plant length (33.68%) and (36.19%), fresh weight (23.89%), and (21.34%), dry weight of the plant (23.05%) and (23.6%), the number of leaves (32.30%) and (29.05%), leaf area (16.66%) and (22.7%), branches per plant (25.8%) and (26.13%) respectively as compared to both control like control

plants (T1) and inoculated control plants (T2). Plant yields were also maximum at 10% fly ash, 2.0 g of *T. harzianum* and 2000J2 of *M. incognita* (T6) treatment like the number of flowers (38.04%) and (40.03%), fruits number (24.17%) and (26.34%), fruit length (19.67%) and (21.05%), fresh (18.32%) and (20.76%), dry weights (17.19%) and (19.76%) as compared to untreated control (T1) and inoculated control (T2).

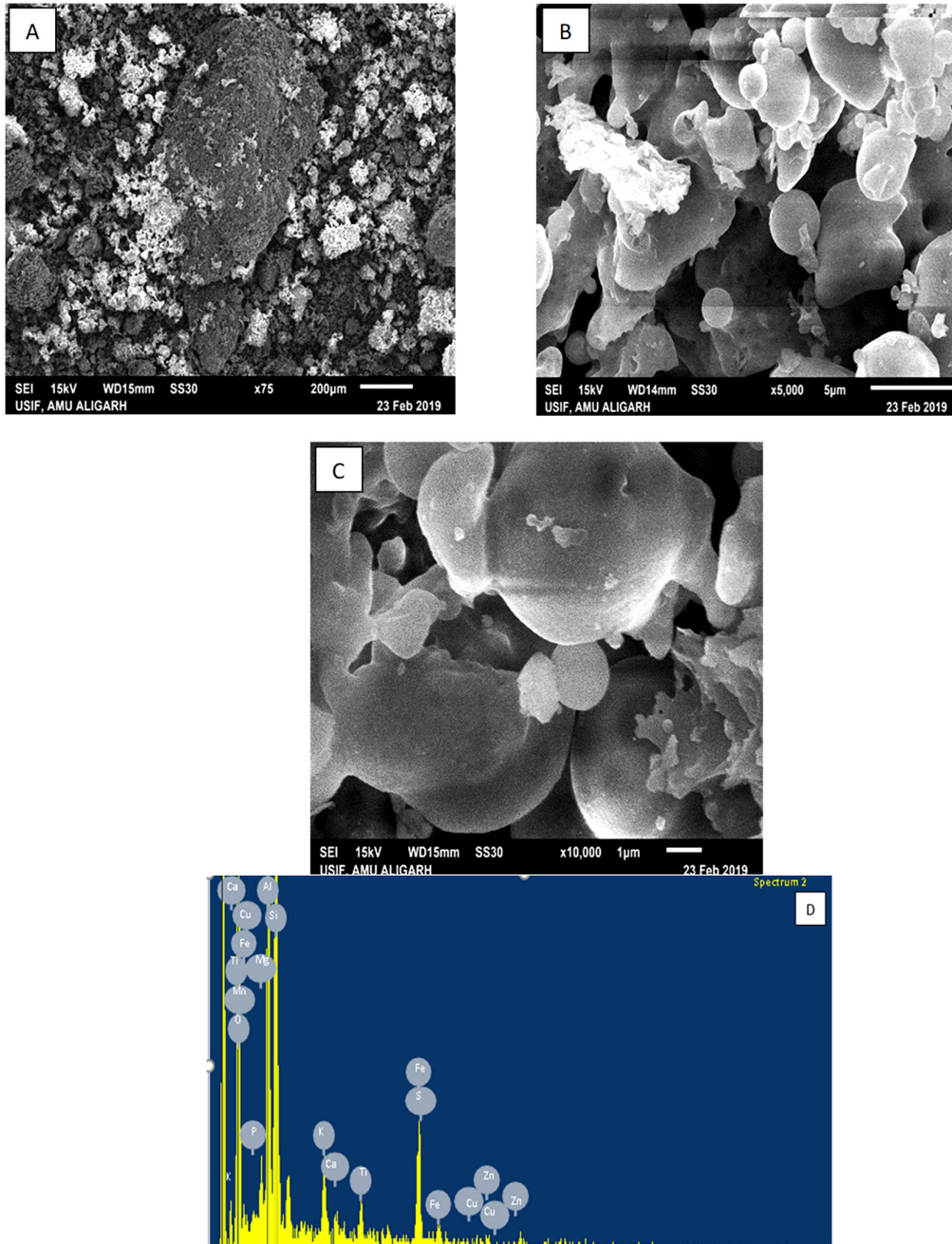


Figure 2. A-C The size, shape, and colour of fly ash by SEM images, D: SEM EDX of fly ash elemental status



Figure 3. Pot experiment illustrates the effect of different concentration of fly ash and *T. harzianum* on growth and yield of chili infected with *M. incognita*

T1 – Control (only soil), T2 – Control (Soil + 2000 J2 of *M. incognita*), T3 – 5% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*, T4 – 5% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*, T5 – 10% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*, T6 – 10% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*, T7 – 15% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*, T8 – 15% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*, T9 – 20% FA + *T. harzianum* (1.0 g) + 2000 J2 of *M. incognita*, T10 – 20% FA + *T. harzianum* (2.0 g) + 2000 J2 of *M. incognita*.

Interactive effect of different concentrations of fly ash and T. harzianum on chlorophyll content

The results in Figure 5 show that applied with 2000J2 of *M. incognita* significantly decreased chlorophyll a, b, total chlorophyll, and carotenoid contents of chili plants compared to control plants. In addition, chl a, b, total chl and carotenoids contents of Chili plants grown in T6 (10% FA + 2 g *T. harzianum* + 2000J2 of *M. incognita*) were significantly ($P \leq 0.05$) maximum by about (18.01%) and (21.13%), (20.18%) and (23.08%), (19.27%) and (20.43%), (16.55%) and (19.69%) respectively as compared to both control that is without nematode and fly ash (only soil) as well as nematode inoculated plant (nematode and soil). Above 10% level of fly ash and both levels of *T. harzianum* combination (T7, T8, T9, and T10), the chl a, b, total chl, and carotenoid contents were also greater than both control but less than the T6 treatment (Figure 5).

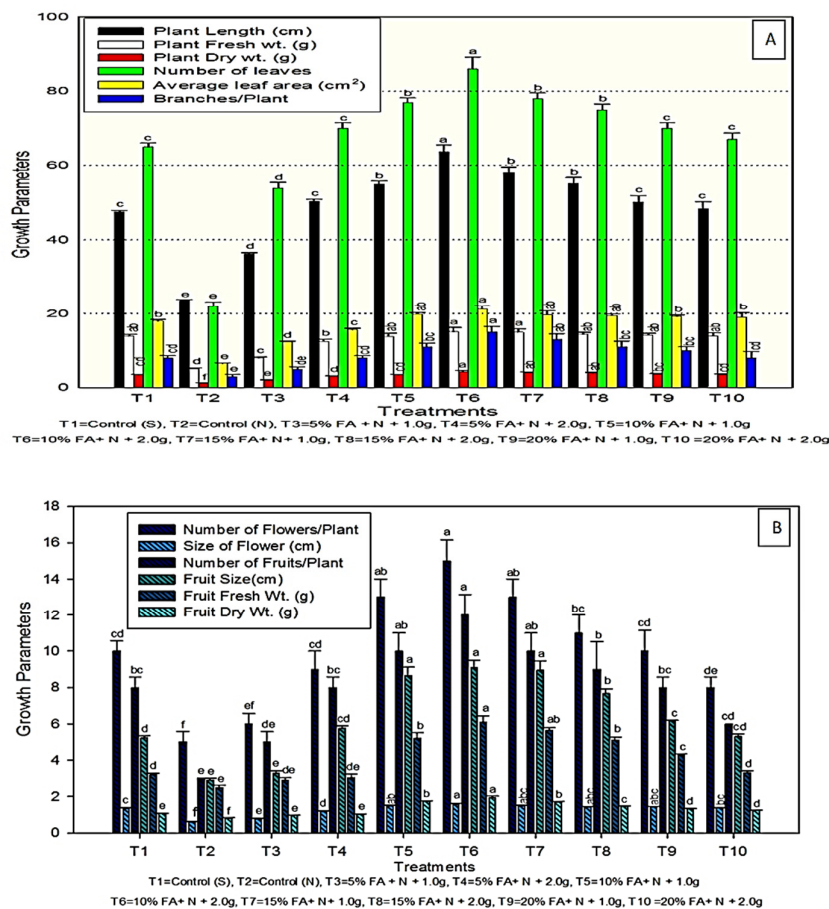


Figure 4. Interactive effect of different concentration of fly ash and *T. harzianum* on growth of chili (A) and yield (B) infected with *M. incognita*

Each value is a mean (\pm SD) of five replicates. Different letters on the same bars show significant differences according to Duncan's multiple range test at $p < 0.05$ using R-software

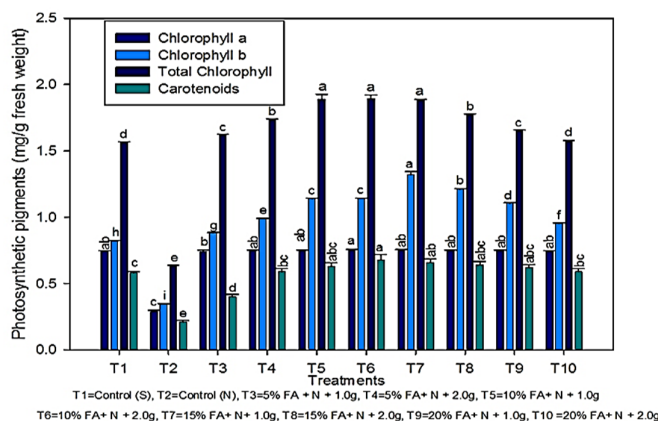


Figure 5. Interactive effect of different concentration of fly ash and *T. harzianum* on photosynthetic pigments of chili infected with *M. incognita*

Each value is a mean (\pm SD) of five replicates. Different letters on the same bars show significant differences according to Duncan's multiple range test at $p < 0.05$ using R-software

Interactive effect of different concentrations of fly ash and T. harzianum on amino acids, protein, and carbohydrates

The data in Figure 6A shows that inoculation with 2000J2 of *M. incognita* caused a significant decrease in amino acids (13.4%), protein (14.3%), and carbohydrates (25.6 content in chili plants as compared to uninfected plants. In comparison to both control (T1) and inoculated plants, amino acid (21.23%, 13.45%), protein (16.23%, 18.19%), and carbohydrate content (17.34%, 20.39%) were found to be significantly higher in plants grown in T6 combination. At higher levels of fly ash, *T. harzianum*, and *M. incognita* combinations (T7, T8, T9, and T10), the amino acid, protein, and carbohydrate content were also more significant than the control but less than the contents of the T6 combination.

Interactive effect of different concentrations of fly ash and *T. harzianum* on tryptophan and indole acetic acid (IAA) content. The tryptophan and indole acetic acid (IAA) content of chili plants grown in soils inoculated with 2000J2 of *M. incognita* was significantly decreased as compared to uninoculated control plants (Figure 6B). In addition, all concentrations of fly ash, *T. harzianum*, and *M. incognita* caused significant enhancement in tryptophan and indole acetic acid contents of chili plants as compared to inoculated plants. Plants grown in T6 produced the greatest amounts of tryptophan (12.05% and 14.19%) and IAA content (13.20% and 15.45%) as compared with plants cultivated in T1 and T2.

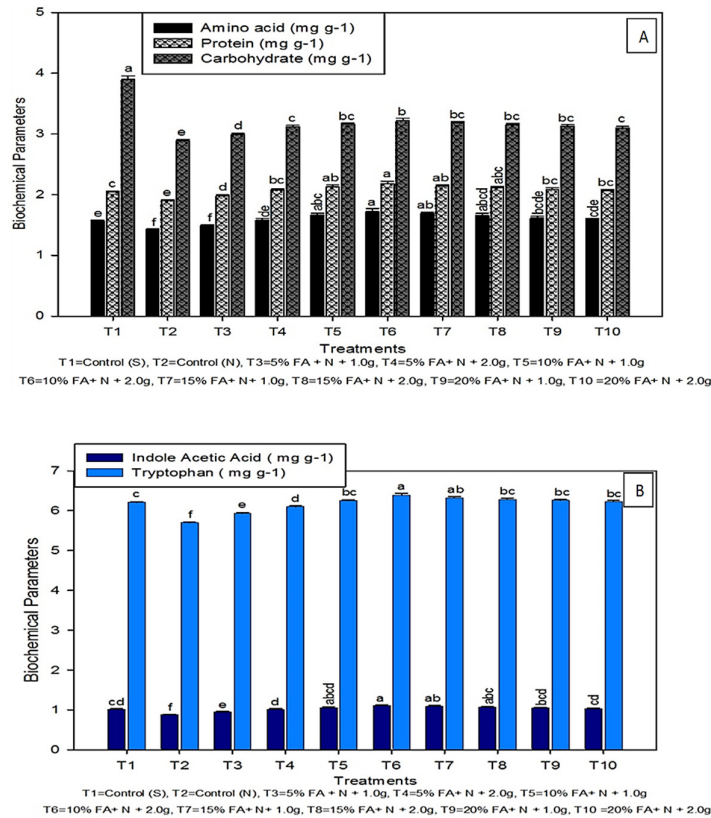


Figure 6. Interactive effect of different concentration of fly ash and *T. harzianum* on amino acid, protein, carbohydrates content (A), indole acetic acid and tryptophan content (B) of chili infected with *M. incognita*

Each value is a mean (\pm SD) of five replicates. Different letters on the same bars show significant differences according to Duncan's multiple range test at $p < 0.05$ using R-software

*Interactive effect of different concentrations of fly ash and *T. harzianum* on phenolics and flavonoids content*

The phenolic and flavonoids content of chili plants grown in soil inoculated with 2000J2 of *M. incognita* was significantly inhibited as compared to control plants (Figure 7). Furthermore, when compared to T1 and T2, the concentration of phenolic and flavonoids was considerably ($P \leq 0.05$) higher in plants grown in the T10 combination by about (16.89%, 18.67%) and (21.90%, 32.22%) respectively. On the other hand, treatment with T4, and T5 had a non-significant effect on total phenolic and flavonoid contents in chili plants compared to control plants, and T3 and T4 show a non-significant effect on flavonoid content compared with inoculated plants. In addition, T6 caused a significant increase in phenolic content (10.7, 24%) and flavonoids content (18.8, 33.3%) as compared to T1 and T2 respectively.

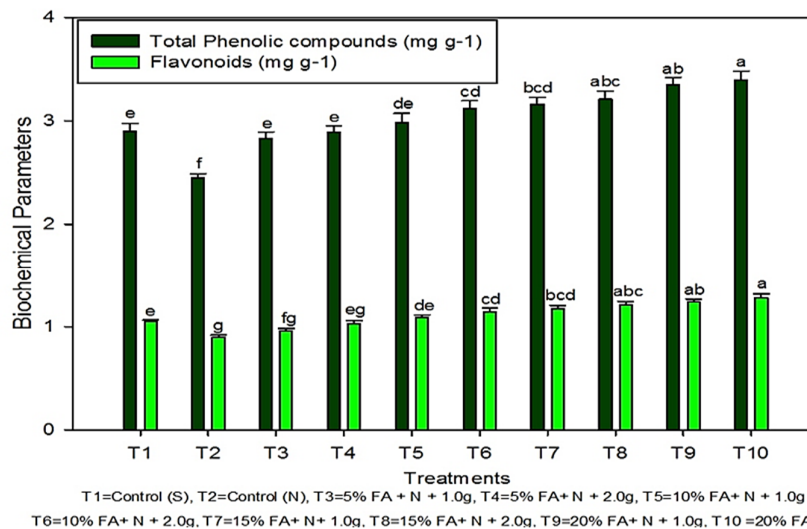


Figure 7. Interactive effect of different concentration of fly ash and *T. harzianum* on total phenol and flavonoids content of chili infected with *M. incognita*

Each value is a mean (\pm SD) of five replicates. Different letters on the same bars show significant differences according to Duncan's multiple range test at $p < 0.05$ using R-software

*Interactive effect of different concentrations of fly ash and *T. harzianum* on proline and NRA content*

The impact of various doses of fly ash, *T. harzianum*, and 2000J2 of *M. incognita* on proline and NRA content of the chili plant is shown in Figure 8 A-B. The proline and NRA content were significantly higher in plants grown in T10 by about (20.60%, 33.16) and (14.07%, 16.16) as compared to both controls, T1 and T2. On the other hand, T5 shows a non-significant effect on chili plants proline and NRA content compared to control plants.

*Interactive effect of different concentrations of fly ash and *T. harzianum* on root-knot disease*

The level of 10% FA + in combination with 2g *T. harzianum* was significantly inhibited the hatching ability of *M. incognita*. At this level, only some galls appeared, but none of the egg mass and eggs was observed. At higher levels of FA + both doses of *T. harzianum*, nematode could not survive. That's why neither galls nor egg mass was observed. As a result, nematodes may have ceased to function and so been unable to tolerate the stress of fly ash. Thus, soil application of fly ash at 10% level + 2.0 g *T. harzianum* is useful for chili crops, and it will also manage the root-knot disease on the host plant (Table 2).

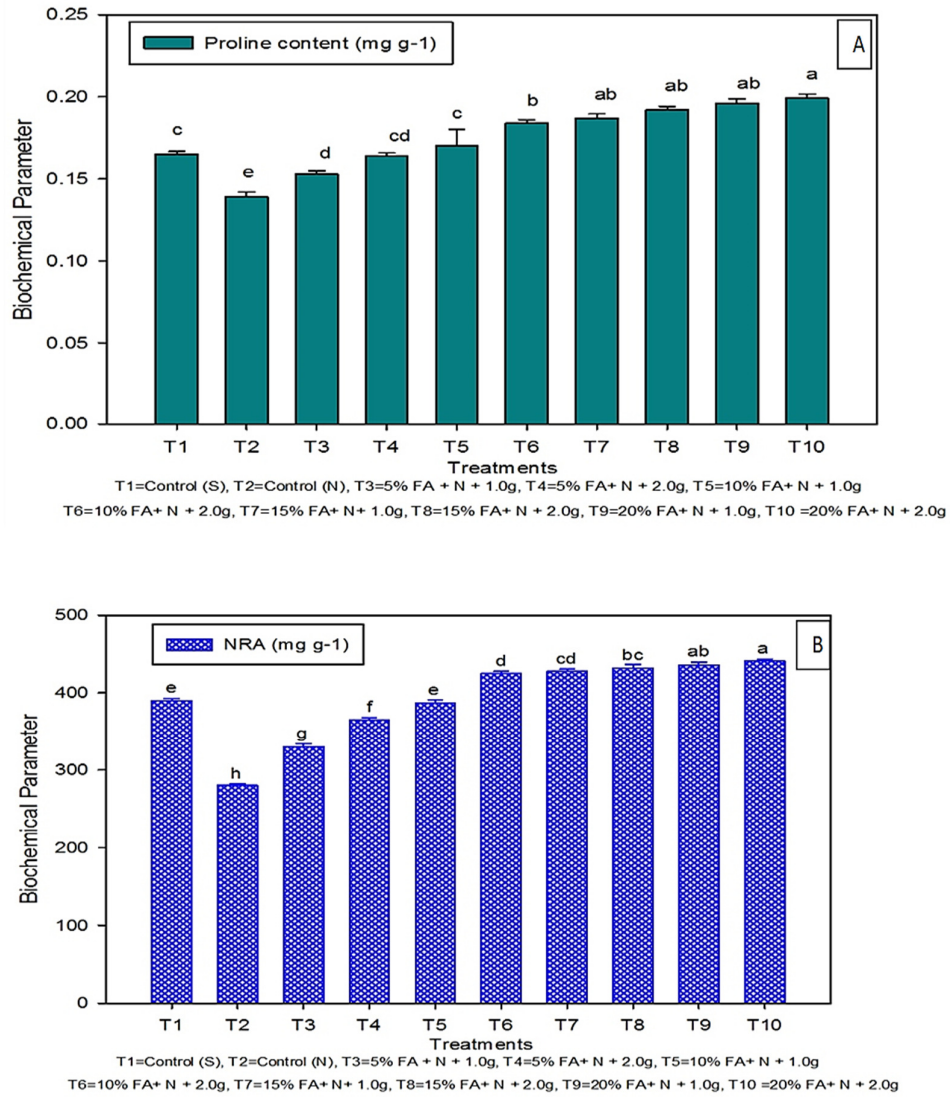


Figure 8. Interactive effect of different concentration of fly ash and *T. harzianum* on proline content (A) and nitrate reductase (B) of chili infected with *M. incognita*. Each value is a mean (\pm SD) of five replicates. Different letters on the same bars show significant differences according to Duncan's multiple range test at $p < 0.05$ using R-software

Table 2. Interactive effect of different concentration of fly ash and *T. harzianum* on root-knot disease

Treatments (FA % + N + <i>T. harzianum</i>)	Parameter			
	No. of galls plant ⁻¹	No. of egg masses plant ⁻¹	No. of eggs mass ⁻¹	Gall index
Control (N)	180.32 ^a	144.44 ^a	190.45 ^a	5 ^a
5% FA + N + 1.0 g	138.15 ^b	90.63 ^b	117.32 ^b	5 ^a
5% FA+ N + 2.0 g	80.09 ^c	32.03 ^c	25.17 ^c	4 ^{ab}
10% FA+ N + 1.0 g	15.19 ^d	5.98 ^d	0	3 ^{bc}
10% FA+ N + 2.0 g	3.27 ^c	0	0	2 ^c
15% FA+ N + 1.0 g	0	0	0	0
15% FA+ N + 2.0 g	0	0	0	0
20% FA+ N + 1.0 g	0	0	0	0
20% FA + N + 2.0 g	0	0	0	0

caused *M. incognita* in chili

Discussion

In the present investigation, chili plant growth and production increased significantly at the lower levels of fly ash (T6 = 10% FA + 2 g *T. harzianum* + 2000J2 of *M. incognita*). There have been similar beneficial effects on various crops such as pumpkins and carrots (Ahmad *et al.*, 2017; Haris *et al.*, 2019). Due to its high K, Mg, and S content, fly ash is a valuable source of essential plant nutrients and is used as fertilizer to boost crop growth (Ahmed *et al.*, 2021a). Fly ash alone had higher physical properties like water-holding capacity, moisture content, CEC, EC, and porosity than soil (Table 1). Inorganic components in fly ash and increased soluble macro- and micronutrients and metals released with the ash to the soil may be responsible for the increase in EC (Ahmed *et al.*, 2021a). Because it is rich in salts and trace elements, fly ash affects soil properties, increasing its water-holding capacity (Ahmed *et al.*, 2021a). Fly ash boosts the soil's ability to retain water because it alters its texture and structure. Soil can retain water depending on the surface area, pore space, and pore space continuity (Panda and Biswal, 2018). As well as phosphorus and potassium, the amounts of Mg, Mn, and Zn in the soil were also steadily boosted when fly ash was added to the soil. Because fly ash contains enough of these elements (Ahmed *et al.*, 2021b). Besides enriching nutrients, it also improves soil pH and makes it more conducive to plant growth (Haris *et al.*, 2019).

Trichoderma species can generate secondary metabolites such as indole acetic acid, a hormone that induces growth in meristematic tissues and improves the efficiency of cysteine, which alters root architecture and improves root growth (Samolski *et al.*, 2012). Plant metabolism depends on the production of organic acids, such as gluconic acid and fumaric acid, which decrease the pH of soil solution and enable the dissolution and solubilization of phosphates and micronutrients and minerals cations such as Fe, Mn, and Mg (Hermosa *et al.*, 2013). *Trichoderma* strain effectively suppressed root-knot nematodes (RKNs) in tomatoes (Yan *et al.*, 2021). Fly ash contains elements such as Ca, Fe, Mg, and K, which are essential for plant growth, and other minerals such as B, Se, and Mo, which are toxic to plants. Yu *et al.* (2019) showed that plant biomass boosted about 11.6-29% when fly ash was applied at low concentrations (< 25% of soil mass) but decreased by about 44.8% at high concentrations (50-100%) due to heavy metal toxicity (Mohamed, 2011; El-Beltagi *et al.*, 2020; Moustafa-Farag *et al.*, 2020). Soil fungi *Trichoderma* spp. form symbioses with many plants and are commonly found in the natural environment (Druzhinina *et al.*, 2011). Plant development, yield, nutrient cycling, and energy conversion in soil are all dependent on the relationship between the plant rhizosphere and soil microbes (De *et al.*, 2015). Plant root exudates promote the colonization of rhizosphere microorganisms, while soil microorganisms improve plant growth and increase soil nutrient levels by utilizing plant photosynthates to the fullest extent possible (De *et al.*, 2015). *Trichoderma* applied led to a dramatic boost in the soil's N and P

content due to the ability of *Trichoderma* to degrade soil macromolecular nutrients into a form that plants can use, thereby stimulates soil nutrient cycling and energy flow (Halifu *et al.*, 2019). Adding *T. harzianum* to fly ash was the main cause of the nitrogen overabundance in the soil because fly ash itself does not contain nitrogen (Ahmad *et al.*, 2021a).

For plants to absorb light energy, they need chlorophyll (Katz *et al.*, 1978). Neals (1956) found that plants need minerals to produce chlorophyll molecules. Nematodes infected chili plants reduced the photosynthetic pigments content. The findings of our study are validated by Khanna *et al.* (2019) who reported that the total chlorophyll content in *Lycopersicon esculentum* was reduced after infection with *M. incognita*. The reduction of photosynthetic pigments may be due to a decrease in enzymatic activities involved in the violaxanthin cycle, which disrupted the photosynthetic apparatus's stabilization, as well as the development of galls in the plant roots, which hindered the water balance of the entire plant due to gall formation in the roots, which effected on the chlorophyll synthesis (Khanna *et al.*, 2019).

Treatment with 10% FA + 2 g *T. harzianum* + 2000J2 of *M. incognita* caused a significant increase in photosynthetic pigments as compared to control and inoculated plants. Fly ash is a good source of macro and microelements like sulfate, magnesium, and zinc, and its application to the soil increases the absorption of these metals by plants and thereby increases the photosynthetic pigments (Shakeel *et al.*, 2019; Ahmed *et al.*, 2021a). As a consequence of a higher amount of chlorophyll, more carbohydrates and other chemicals are synthesized, which improves fruit output (Paradikovic *et al.*, 2011). Likewise, Abd El- Rahman and Mohamed (2014) found that using *T. harzianum* to control faba bean chocolate spot disease caused by *Botrytis fabae* caused enhancement in both chlorophyll production and photosynthetic activity by boosting water absorption, which eventually resulted in an enhanced of the photosynthetic pigments. *Trichoderma* and fly ash may up-regulate enzymes related to photosynthetic pigments in plants during nematode attack, resulting in increased photosynthetic activity. In addition, *Trichoderma* increases soil organic matter and essential minerals, such as nitrogen, potassium, phosphorus, and magnesium, which stimulates the development of photosynthetic pigments in plants (Sofy *et al.*, 2021b).

The present study showed that levels of osmoprotectants such as amino acids (13.4%), protein (14.3%), carbohydrates (25.6%), and proline (23.5%) were significantly decreased in chili plants under nematode treatment. On the other hand, treatment with fly ash, *T. harzianum*, and 2000J2 of *M. incognita* caused a significant increase in amino acids, protein, carbohydrates, and proline compared to plants applied with the nematode. Those findings are similar to those in treating nematode-infected tomato plants with *T. harzianum* and *Serratia marcescens* induced systemic resistance in the plants. In addition, the polyphenol oxidase and -1,3-glucanase activity triggered the production of sugar and amino acids, which increased the expression of defence genes in the plants (Abd-Elgawad and Kabeil, 2012). The current study also observed a rise in the secondary metabolite's contents, which can be related to an increase in the protein synthesis of enzymes that participate in secondary metabolism. As a result, *Trichoderma*-induced creation of defence metabolites and antioxidant compounds may provide physical and chemical protection against RKNs (Ji *et al.*, 2019).

Furthermore, *de novo* synthesis decreased utilization and decreased degradation hydrolysis of proteins could also contribute to proline accumulation. In addition to acting as an outstanding osmolyte, proline also acts as a metal chelator and an antioxidant molecule under stressful conditions, including stabilization of subcellular structures, membranes, and proteins (Sofy *et al.*, 2021a). As reported by several researchers, plants exposed to biotic and abiotic conditions may develop stress tolerance by maintaining osmotic stability or cell viability and protecting cell functions by neutralizing ROS, which would reduce the risk of the oxidative burst in plants (Sofy *et al.*, 2021 b).

Organic nitrogen compounds, amino acids have traditionally been regarded as precursors and components of proteins (Azher *et al.*, 2011; El-Beltagi *et al.*, 2019). As a growth regulator and a cell differentiation modulator, it may impact general metabolism and morphogenesis (Basu *et al.*, 1989; Mohamed *et al.*, 2016). The amino acids also play an important role in developing multiple shoots (Vasanth *et al.*, 2006).

In every aspect of plant growth and development, proteins play a key role. These processes include chemical reactions, membrane transport, intracellular structure, and energy-generating reactions requiring electron transport. Low protein levels in plants result from structural carbohydrates that make up the majority of plant structure (Vierstra, 1993). In-plant growth, development, and stress response, carbohydrates play a critical role. A wide range of factors influences species, organs, growth conditions. However, carbohydrates are indicative of the relationship between photosynthesis and growth. For many crops, biomass and yield are also heavily dependent on photosynthetic carbon fixation and carbohydrate metabolism (Azher *et al.*, 2011).

Chili plants tryptophan and indole acetic acid (IAA) contents significantly increased after treatment with *T. harzianum* with fly ash. A precursor to IAA, tryptophan is one of the most studied amino acids. As a result of higher levels of tryptophan, the synthesis of IAA (auxin) increases, resulting in increased plant growth (Wurtman and Anton-Tay, 1969; Ghonaim *et al.*, 2021). As a precursor to auxin and cytokinin, tryptophan plays an essential role in the plant metabolic process (Law, 1987). Therefore, higher levels of tryptophan in plants may aid in phytohormone synthesis, resulting in increased plant growth. *Trichoderma* strains generated considerable amounts of IAA and boosted the fresh weight of *Arabidopsis* seedling shoots (Nieto-Jacobo *et al.*, 2017).

Treatment with fly ash, *T. harzianum*, and 2000J2 of *M. incognita* boost phenolic and flavonoids content in chili plants. As fly ash contains various toxic elements, it becomes detrimental for root-knot nematodes to survive thus results in their death. The substantial decrease in the egg masses and galling shows that the fly ash has caused a direct inhibitory response on the survival and multiplication of *M. incognita* (Ahmad and Khan, 2016). Similarly, Ahmad and Khan (2016) observed that the soil amendment with different levels of fly ash inhibits the penetration of the *M. incognita* juveniles in the roots of pumpkin. Root-knot disease in terms of root gall index, no. of egg masses and eggs/egg mass was highest in untreated inoculated control. In fly ash + nematode combinations, galls were formed but less than inoculated control which gradually decreased to 10%, 20% and 30% levels of fly ash. However, galls were completely absent at 50% level of fly ash (Haris *et al.*, 2019).

The phenolic compounds have a positive effect on nematode infection (Patel *et al.*, 2017) through impacts on gall production, egg hatching, and second-stage nematode proliferation (Patel *et al.*, 2017). Flavonoids have also been shown to diminish the nematode's negative effects by slowing worm mobility, reducing nematode activity, limiting nematode migration, and eventually killing nematodes. Using *T. harzianum* in soil improved growth and yields and significantly reduced *M. incognita* galling, egg production, and soil population when applied to the soil before planting. *Trichoderma* can inhibit egg hatching and create host resistance in plants (Khan, 2016). *Trichoderma* produced chemical metabolites that can also inhibit nematode feeding, penetration and increased crop yield (Khan *et al.*, 2020). In addition, Yan *et al.* (2021) found that tomato plants treated with *T. harzianum* have significantly reduced root-knot disease and boosted plant growth.

Egg formation was reduced by 60% in *C. annuum* after treatment with *T. virens* (Meyer *et al.*, 2001), and egg production was repressed by 86% and 84% in *S. lycopersicum* treated with *T. brevicompactum* and *T. longibrachiatum*, respectively (Zhang *et al.*, 2015). *Trichoderma* biocontrol mechanisms against nematodes may be based on their ability to produce lysis enzymes that affect the cuticle and the viability of nematode eggs (Zhang *et al.*, 2015). The importance that the nematode did not reach the mature stage and, as a result, no crop loss is critical from an agricultural standpoint. Recently, Haris *et al.* (2019) also observed that all the levels of fly ash (10-50%) significantly reduced *M. incognita* on carrots and hatching, mortality, and penetration on pumpkin (Ahmad and Khan 2016). It was found that the maximum inhibition occurred at 50% fly ash content. Juvenile *M. incognita* larvae were significantly suppressed in the pumpkin roots in all fly ash and soil mixtures. *Trichoderma* increase the level of extracellular enzymes like chitinase and protease, which allow the penetration of the fungus into the eggs by directly affecting very abundant structural components of the eggshell, thus reducing the number of eggs capable of hatching and therefore, the number of infective J2. Specifically, *T.*

longibrachiatum has a strong inhibitory effect on the hatching of cysts produced by *Heterodera avenae*, since the spores completely cover the surface of these structures, causing their destruction, which is probably due to the production of enzymes (e.g., chitinases) that caused physiological alterations to the cysts (Zhang *et al.*, 2014). *T. longibrachiatum* also showed an effect on females and on the development of both eggs and J2s of *H. avenae* (Zhang *et al.*, 2017), hence *T. longibrachiatum* can be used as a BCA for the management of *H. avenae* in selected crop species. Within the cyst-forming nematodes, the specie *Globodera pallida* has also a high agronomic impact. Figure 9. represents the possible mechanisms of the effects of *Trichoderma* and fly ash on chili plants.

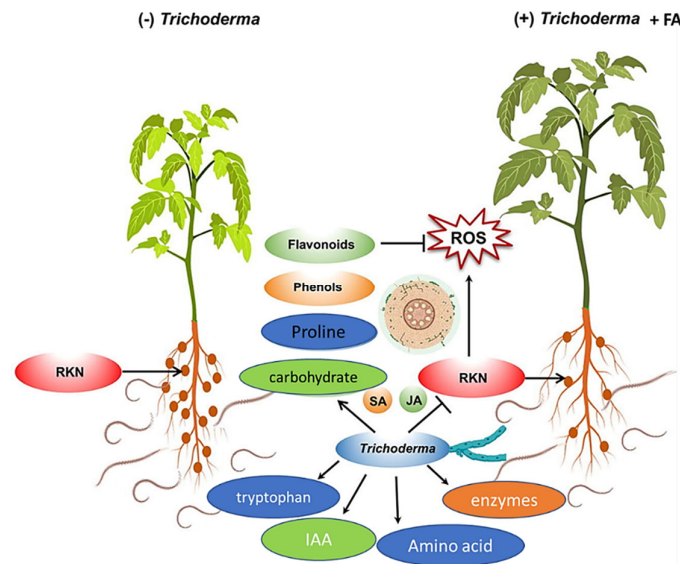


Figure 9. Diagram to illustrate effect of *Trichoderma* and fly ash on chili plants

Conclusions

Nematode infection of chili plants altered their physiological and metabolic characteristics in the current study. The inoculation of nematode-infected plants with *Trichoderma* and fly ash, on the other hand, increased plant photosynthetic pigments, phenolic compounds, and osmolyte levels (Figure 9). After being exposed to nematode infection and then treated with *Trichoderma* or fly ash, plants accumulate metabolites, showing that this is an effective strategy to combat the nematode infection. Therefore, 2 g of *Trichoderma* and 10% of fly ash play an important role in nematode-stressed chili plant stress reduction. Using *Trichoderma* and fly ash is a cost-effective and promising way to improve the growth and productivity of plants infected with nematode pests. But more research is needed to understand the mechanisms behind *Trichoderma* and fly ash nematicidal activity.

Authors' Contributions

Conceptualization, G.A., A.K., S.A., A.A.K., A.El., R.S., and H.I.M.; methodology, G.A., A.K., S.A.; software, X.X.; validation, G.A., A.K., S.A., A.A.K., A.El., R.S., and H.I.M.; formal analysis, H.I.M.; investigation, G.A., A.K., S.A., A.A.K., A.El., R.S and H.I.M.; resources, G.A., A.K., S.A.; data curation, G.A., A.K., S.A.; writing—original draft preparation, G.A., A.K., S.A., A.A.K., A.El., R.S., and H.I.M.; writing—

review and editing, G.A., A.K., S.A., A.A.K., A.El., R.S., and H.I.M.; visualization, X.X.; supervision, H.I.M.; project administration, G.A., A.K., S.A., A.A.K., A.El., R.S., and H.I.M.; funding acquisition, A.El. and R.S. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abd El- Rahman SS, Mohamed HI (2014). Application of benzothiadiazole and *Trichoderma harzianum* to control faba bean chocolate spot disease and their effect on some physiological and biochemical traits. *Acta Physiologia Plantarum* 36(2):343-354. <https://doi.org/10.1007/s11738-013-1416-5>
- Abd-Elgawad MM, Kabeil SS (2012). Biological control of *Meloidogyne incognita* by *Trichoderma harzianum* and *Serratia marcescens* and their related enzymatic changes in tomato roots. *African Journal of Biotechnology* 11:16247-16252. <https://doi.org/10.5897/AJB12.233>
- Abu-Shahba MS, Mansour MM, Mohamed HI, Sofy MR (2021). Comparative cultivation and biochemical analysis of iceberg lettuce grown in sand soil and hydroponics with or without microbubble and microbubble. *Journal of Soil Science and Plant Nutrition* 21:389-403. <https://doi.org/10.1007/s42729-020-00368-x>
- Ahmad G, Khan AA, Ansari S (2017). Interaction of a fly ash and root-knot nematode pathogens on pumpkin (*Cucurbita moschata* Duch. ex Lam.). *Tropical Plant Research* 4(3):449-455. <https://doi.org/10.22271/tpr.2017.v4.i3.059>
- Ahmad G, Khan AA (2016). Fly ash effect on hatching, mortality and penetration of root-knot nematode (*Meloidogyne incognita*) in pumpkin roots. *International Journal of Environment* 5(3):66-73. <https://doi.org/10.3126/ije.v5i3.15705>
- Ahmad G, Khan A, Khan AA, Ali A, Mohamed HI (2021a). Biological control: a novel strategy for the control of the plant parasitic nematodes. *Antonie van Leeuwenhoek* 114:885-912. <https://doi.org/10.1007/s10482-021-01577-9>
- Ahmad G, Khan AA, Mohamed HI (2021b). Impact of the low and high concentrations of fly ash amended soil on growth, physiological response, and yield of pumpkin (*Cucurbita moschata* Duch. Ex Poiret L.). *Environmental Science and Pollution Research* 28(14):17068-17083. <https://doi.org/10.1007/s11356-020-12029-8>
- Akladios SA, Mohamed HI (2018). Ameliorative effects of calcium nitrate and humic acid on the growth, yield component and biochemical attribute of pepper (*Capsicum annuum*) plants grown under salt stress. *Scientia Horticulturae* 236:244-250. <https://doi.org/10.1016/j.scienta.2018.03.047>
- Al-Hazmi AS, TariqJaveed M (2016). Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato. *Saudi Journal of Biological Science* 23(2):288-292. <https://doi.org/10.1016/j.sjbs.2015.04.007>

- Aly AA, Mansour MTM, Mohamed HI, Abd-Elsalam KA (2012). Examination of correlations between several biochemical components and powdery mildew resistance of flax cultivars. *Plant Pathology Journal* 28(2):149-155. <https://doi.org/10.5423/PPJ.2012.28.2.149>
- Aly AA, Mohamed HI, Mansour MTM, Omar MR (2013). Suppression of powdery mildew on flax by foliar application of essential oils. *Journal of Phytopathology* 161:376-381.
- Ashry NA, Ghonaim MM, Mohamed HI, Mogazy AM (2018). Physiological and molecular genetic studies on two elicitors for improving the tolerance of six Egyptian soybean cultivars to cotton leaf worm. *Plant Physiology and Biochemistry* 130:224-234. <https://doi.org/10.1016/j.plaphy.2018.07.010>
- Azher AA Zaghoul AM, Mahmoud SA, Siam HS (2011). Stimulatory effect of kinetin, ascorbic acid and glutamic acid on growth and chemical constituents of *Codiaeum variegatum* L. plants. *American-Eurasian Journal of Agricultural and Environmental Sciences* 10:318-323.
- Basu A, Sethi U, Mukherjee SP (1989). Regulation of cell proliferation and morphogenesis by amino acids in *Brassica* tissue cultures and its correlation with threonine deaminase. *Plant Cell Report* 08:333-335.
- Beffa R, Martin HV, Pilet PE (1990). *In vitro* oxidation of indoleacetic acid by soluble auxin-oxidases and peroxidases from maize roots. *Plant Physiology* 94:485-491.
- Bhattacharjee R, Dey U (2014). An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. *African Journal of Microbiological Research* 8(17):1749-1762. <https://doi.org/10.5897/AJMR2013.6356>
- Black CA, Evans DD, White JL, Ensminger LE, Clarke FE, Dinauer RC (1965). Methods of soil analysis. Part I- Physical and mineralogical properties including statistics of measurement and sampling (9 Series Agronomy). Madison: American Society of Agronomy.
- Blake GR (2008). Particle density. In: Chesworth W (Ed). *Encyclopedia of Soil Science*. Encyclopedia of Earth Sciences Series. Springer, Dordrecht.
- Bruinsma J (1963) The quantitative analysis of chlorophylls a and b in plant extracts. *Photochemistry and Photobiology* 2(2):241-249. <https://doi.org/10.1111/j.1751-1097.1963.tb08220.x>
- Carter MR, Ball B (1993). Soil porosity. In: Carter MR (Ed). *Soil sampling and Methods of Analysis*. Canadian Society of Soil Science. Lewis Publishers, CRC Press, Boca Raton, pp 581-588.
- Chopra SL, Kanwar JS (1991). *Analytical agricultural chemistry*. Kalyani Publishers, New Delhi-Ludhiana.
- Chopra SL, Kanwar JS (1982). *Analytical agricultural chemistry*. Kalyani publishers, New Delhi, pp 162.
- Costa SR, Ng JLP, Mathesius U (2021). Interaction of symbiotic rhizobia and parasitic root-knot nematodes in legume roots-from molecular regulation to field application. *Molecular Plant-Microbe Interactions* <https://doi.org/10.1094/MPMI-12-20-0350-FI>
- Dahiya HS, Budania YK (2018). Prospects of fly ash application in agriculture: a global review. *International Journal of Current Microbiology and Applied Sciences* 7:397-409. <https://doi.org/10.20546/ijcmas.2018.710.043>
- De SR, Adriana A, Passaglia LMP (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology* 38:401-419. <https://doi.org/10.1590/S1415-475738420150053>
- Dickman SR, Bray RH (1940). Colorimetric determination of phosphate. *Industrial Engineering Chemistry and Analytical Edition* 12:665-668. <https://doi.org/10.1021/ac50151a013>
- Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Kubicek CP (2011). *Trichoderma*: The genomics of opportunistic success. *Nature Reviews Microbiology* 9:749-759.
- Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28(3):350-356. <https://doi.org/10.1021/ac60111a017>
- El-Beltagi HS, Sofy MR, Aldaej MI, Mohamed HI (2020). Silicon alleviates copper toxicity in flax plants by up-regulating antioxidant defense and secondary metabolites and decreasing oxidative damage. *Sustainability* 12:4732. <https://doi.org/10.3390/su12114732>
- El-Beltagi HS, Mohamed HI, Elmelegy AA, Eldesoky SE, Safwat G (2019). Phytochemical screening, antimicrobial, antioxidant, anticancer activities and nutritional values of cactus (*Opuntia ficus* Indicia) pulp and peel. *Fresenius Environmental Bulletin* 28(2A):1534-1551.
- Elhady A, Adss S, Hallmann J, Heuer H (2018). Rhizosphere microbiomes modulated by pre-crops assisted plants in defense against plant-parasitic nematodes. *Frontiers in Microbiology* 9:1133. <https://doi.org/10.3389/fmicb.2018.01133>

- El-Mahdy OM, Mohamed HI, Mogazy AM (2021). Biosorption effect of *Aspergillus niger* and *Penicillium chrysosporium* for Cd and Pb contaminated soil and their physiological effects on *Vicia faba* L. Environmental Science and Pollution Research 28:67608-67631. <https://doi.org/10.1007/s11356-021-15382-4>
- Feyisa B, Lencho A, Selvaraj T, Amb PBN, Getaneh G (2016). Evaluation of some botanicals and *Trichoderma harzianum* for the management of tomato root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chit Wood). Advances in Crop Science and Technology 4:201. <https://doi.org/10.4172/2329-8863.1000201>
- Ghonaim MM, Mohamed HI, Omran AAA (2021). Evaluation of wheat salt stress tolerance using physiological parameters and retrotransposon-based markers. Genetic Resources and Crop Evolution 68:227-242. <https://doi.org/10.1007/s10722-020-00981-w>
- Halifu S, Deng X, Song X, Song R (2019). Effects of two *Trichoderma* strains on plant growth, rhizosphere soil nutrients, and fungal community of *Pinus sylvestris* var. *mongolica* annual seedlings. Forests 10(9):758. <https://doi.org/10.3390/f10090758>
- Haris M, Ahmad G, Shakeel A, Khan AA (2019). Utilization of fly ash to improve the growth and the management of root-knot nematode on carrot. Haya Saudi Journal of Life Science 4(7):221-226. <https://doi.org/10.21276/haya.2019.4.7.1>
- Heidrich C, Feuerborn HJ, Weir A (2013). Coal combustion products: a global perspective. In: 'World of Coal Ash' Conference, pp 22-25.
- Hermosa R, Rubio MB, Cardoza RE, Nicolás C, Monte E, Gutiérrez S (2013). The contribution of *Trichoderma* to balancing the costs of plant growth and defense. International Microbiology 16(2):69-80. <https://doi.org/10.2436/20.1501.01.181>
- Jackson ML (1973). Soil chemistry analysis. Prentice Hall, New Delhi.
- Jang JY, Dang QL, Choi YH, Choi GJ, Jang KS, Cha B, Luu NH, Kim JC (2014). Nematicidal activities of 4-quinolone alkaloids isolated from the aerial part of *Triumfetta grandidens* against *Meloidogyne incognita*. Journal of Agricultural and Food Chemistry 63:68-74. <https://dx.doi.org/10.1021/jf504572h>
- Jaworski EG (1971). Nitrate reductase assay in intact plant tissues. Biochemical and Biophysical Research Communications 43(6):1274-1279. [https://doi.org/10.1016/S0006-291X\(71\)80010-4](https://doi.org/10.1016/S0006-291X(71)80010-4)
- Ji X, Li J, Meng Z, Dong S, Zhang S, Qiao K (2019). Inhibitory effect of allicin against *Meloidogyne incognita* and *Botrytis cinerea* in tomato. Scientia Horticulture 253:203-208. <https://doi.org/10.1016/j.scienta.2019.04.046>
- Kaur DN, Sharma SK, Sultan MS (2011). Effect of different chemicals on root knot nematode in seed beds of tomato. Plant Disease Research 26:170-170.
- Katz JJ, Norris JR, Shipman LL, Thurnauer MC, Wasielewski MR (1978). Chlorophyll function in the photosynthetic reaction center. Annual Review of Biophysics and Bioengineering 7:393-434.
- Khan MR, Khan MW (1996). Effect of fly ash on plant growth and yield of tomato. Environmental Pollution 92:105-111. [https://doi.org/10.1016/0269-7491\(95\)00098-4](https://doi.org/10.1016/0269-7491(95)00098-4)
- Khan RAA, Najeeb S, Hussain S, Xie BY, Li Y (2020). Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms 8:817. <https://doi.org/10.3390/microorganisms8060817>
- Khanna K, Jamwal VL, Kohli SK, Gandhi SG, Ohri P, Bhardwaj R, Wijaya L, Alyemeni MN, Ahmad P (2019). Role of plant growth promoting bacteria (PGPR) as biocontrol agents of *Meloidogyne incognita* through improved plant defense of *Lycopersicon esculentum*. Plant Soil 436:325-345. <https://doi.org/10.1007/s11104-019-03932-2>
- Kihika R, Murungi LK, Coyne D, Ng'ang'a M, Hassanali A, Teal PEA, Torto B (2017). Parasitic nematode *Meloidogyne incognita* interactions with different *Capsicum annum* cultivars reveal the chemical constituents modulating root herbivory. Scientific Reports 7: 2903. <https://doi.org/10.1038/s41598-017-02379-8>
- Kjeldahl J (1883). New method for the determination of nitrogen. Chemistry News 48:101-102.
- Law DM (1987). Gibberellin enhanced indole-3-acetic acid biosynthesis: D-tryptophan as the precursor of indole-3-acetic acid. Plant Physiology 70:626-632.
- Lichtenthaler HK, Wellburn AR (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Portland Press Limited, London.
- Menon MP, Ghuman GS, James J, Chandra K (1990). Physicochemical characterization of water extracts of different coal fly ashes and fly ash amended composts. Water, Air Soil Pollution 50:343-353. <https://doi.org/10.1007/bf00280634>

- Meyer SL, Roberts DP, Chitwood DJ, Carta LK, Lumsden RD, Mao W (2001). Application of *Burkholderia cepacia* and *Trichoderma virens*, alone and in combinations, against *Meloidogyne incognita* on bell pepper. *Nematropica* 75-86.
- Mohamed HI, El-Beltagi HS, Aly AA, Latif HH (2018). The role of systemic and non systemic fungicides on the physiological and biochemical parameters in *Gossypium hirsutum* plant, implications for defense responses. *Fresenius Environmental Bulletin* 27(12):8585-8593.
- Mohamed HI, Abd-El Hameed AG (2014). Molecular and biochemical markers of some *Vicia faba* L. genotype in response to storage insect pests infestation. *Journal of Plant Interactions* 9(1):618-626. <https://doi.org/10.1080/17429145.2013.879678>
- Mohamed HI (2011). Molecular and biochemical studies on the effect of gamma rays on lead toxicity in cowpea (*Vigna sinensis*) plants. *Biological Trace Element Research* 144:1205-1218. <https://doi.org/10.1007/s12011-011-9058-1>
- Moustafa-Farag M, Mohamed HI, Mahmoud A, Elkelish A, Misra AN, Guy KM, ... Zhang M (2020). Salicylic acid stimulates antioxidant defense and osmolyte metabolism to alleviate oxidative stress in watermelons under excess boron. *Plants* 9:724. <https://doi.org/10.3390/plants9060724>
- Mohamed HI, Elsherbiny EA, Abdelhamid MT (2016). Physiological and biochemical responses of *Vicia faba* plants to foliar application with zinc and iron. *Gesunde Pflanzen* 68:201-212. <https://doi.org/10.1007/s10343-016-0378-0>
- Moosavi MR (2015). Damage of the root-knot nematode *Meloidogyne javanica* to bell pepper, *Capsicum annuum*. *Journal of Plant Diseases and Protection* 122(5-6):244-249.
- Nakagawa H, Poulle M, Oaks A (1984). Characterization of nitrate reductase from corn leaves (*Zea mays* cv W64A* W182E): two molecular forms of the enzyme. *Plant Physiology* 75:285-289
- Neals TF (1956). Components of total magnesium content within the leaves of white clover and perennial rye grass. *Nature* 177:388-389.
- Nieto-Jacobo MF, Steyaert JM, Salazar-Badillo FB, Nguyen DV, Rostás M, Braithwaite M, ... Mendoza-Mendoza A (2017). Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. *Frontiers in Plant Science* 8:102. <https://doi.org/10.3389/fpls.2017.00102>
- Niu J, Su X, Tang Z (2021). Influence of fly ash and polyacrylamide mixtures on growth properties of *Artemisia ordosica* in the desert region of north China. *Agronomy* 11:590. <https://doi.org/10.3390/agronomy11030590>
- Nyaku ST, Affokpon A, Danquah A, Brentu FC (2017). Harnessing useful rhizosphere microorganisms for nematode control. *Nematology-Concepts, Diagnosis and Control* 153-182. <https://doi.org/10.5772/intechopen.69164>
- Panda RB, Biswal T (2018). Impact of fly ash on soil properties and productivity. *International Journal of Agriculture Environment and Biotechnology* 11:275-283. <https://doi.org/10.30954/0974-1712.04.2018.8>
- Patel VS, Shukla YM, Dhruve JJ (2017). Influence of root knot nematode (*Meloidogyne* spp.) on phenolic acid profile in root of tomato (*Solanum lycopersicum* L.). *International Journal of Current Microbiology and Applied Sciences* 6:840-848. <https://doi.org/10.20546/ijcmas.2017.610.100>
- Paradikovic N, Vinkovic T, Vrcek IV, Zuntar I, Bojic M, Medic- Saric M (2011). Effect of natural biostimulants on yield and nutritional quality: as example of sweet yellow pepper (*Capsicum annuum* L.) plants. *Journal of the Science of Food and Agriculture* 91:2146-2152. <https://doi.org/10.1002/jsfa.4431>
- Penella C, González Nebauer S, López Galarza SV, San Bautista Primo A, Gorbe E, Calatayud A (2013). Evaluation for salt stress tolerance of pepper genotypes to be used as rootstocks. *Journal of Food, Agriculture and Environment* 11(3):1101-1107.
- Penella C, Nebauer SG, López-Galarza S, San Bautista A, Rodríguez-Burruezo A, Calatayud Á (2014). Evaluation of some pepper genotypes as rootstocks in water stress conditions. *Horticulture Research* 41(4):192-200.
- Richard LA (1954). Diagnosis and improvement of saline and alkali soils. *Agric. Handbook*, 60, U.S. Dept, Agric. Washington DC, pp 160.
- Ruanma K, Shank L, Chairote G (2010). Phenolic content and antioxidant properties of green Chili paste and its ingredients. *Maejo International Journal of Science and Technology* 4(2):193-200.
- Sadasivam S, Manickam A (1996). *Biochemical Methods for Agricultural Science*. Wiley Eastern Limited, New Delhi, pp 1.

- Samolski I, Rincon AM, Pinzon LM, Viterbo A, Monte E (2012). The *gid74* gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. *Microbiology* 158(1):129-138. <https://doi.org/10.1099/mic.0.053140-0>
- Seid A, Fininsa C, Mekete T, Decraemer W, Wesemael WM (2015). Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.)—a century-old battle. *Nematology* 17(9):995-1009. <https://doi.org/10.1163/15685411-00002935>
- Shakeel A, Khan AA, Ahmad G (2019). The potential of thermal power plant fly ash to promote the growth of Indian mustard (*Brassica juncea*) in agricultural soils. *SN Applied Science* 1(4):1-5. <https://doi.org/10.1007/s42452-019-0404-9>
- Slyke V, Dillon DD, MacFadyen DA, Hamilton P (1941). Determination of carboxyl groups in free amino acids. *Journal of Biological Chemistry* 142:627-668.
- Sofy MR, Mohamed HI, Dawood MFA, Abu-Elsaoud AM, Soliman MH (2021a). Integrated usage of arbuscular mycorrhizal fungi and chicken waste biochar as economic potential tools to ameliorate antioxidant activity, osmolyte accumulation and salt endogenous hormone-stressed spinach plants. *Archives of Agronomy and Soil Science*. <https://doi.org/10.1080/03650340.2021.1949709>
- Sofy AR, Sofy MR, Hmed AA, Dawoud RA, Refaey EE, Mohamed HI, El-Dougoudg NK (2021b). Molecular characterization of the Alfalfa mosaic virus infecting *Solanum melongena* in Egypt and control of its deleterious effects with melatonin and salicylic acid. *Plants* 10(3):459. <https://doi.org/10.3390/plants10030459>
- Sokhandani Z, Moosavi MR, Basirnia T (2016) Optimum concentrations of *Trichoderma longibrachiatum* and *cadusafos* for controlling *Meloidogyne javanica* on zucchini plants. *Journal of Nematology* 48(1):54.
- Taylor AL, Sasser JN (1978). Biology, identification and control of root-knot nematodes. North Carolina State University Graphics, pp 111.
- Van Acker SA, Tromp MN, Griffioen DH, Van Bennekom WP, Van Der Vijgh WJ, Bast A (1996). Structural aspects of antioxidant activity of flavonoids. *Free Radical Biology and Medicine* 20(3):331-342. [https://doi.org/10.1016/0891-5849\(95\)02047-0](https://doi.org/10.1016/0891-5849(95)02047-0)
- Vasanth K, Lakshmi Prabha A, Jayabalan N (2006). Amino acids enhancing plant regeneration from cotyledon and embryonal axis of peanut (*Arachis hypogaea*). *Indian Journal of Crop Science* 01:79-83.
- Vierstra RD (1993). Protein degradation in plants. *Annual Review in Plant Physiology and Plant Molecular Biology* 44:385-410. <https://doi.org/10.1146/annurev.pp.44.060193.002125>
- Wurtman RJ, Anton-Tay F (1969). The mammalian pineal as a neuroendocrine transducer. *Recent Progress in Hormone Research* 25:493-522.
- Yan Y, Mao Q, Wang Y, Zhao J, Fu Y, Yang Z, ... Ahammed GJ (2021). *Trichoderma harzianum* induces resistance to root-knot nematodes by increasing secondary metabolite synthesis and defense-related enzyme activity in *Solanum lycopersicum* L. *Biological Control* 158:104609. <https://doi.org/10.1016/j.biocontrol.2021.104609>
- Yao ZT, Ji XS, Sarker PK, Tang JH, Ge LQ, Xia MS, Xi YQ (2015). A comprehensive review on the applications of coal fly ash. *Earth-Science Reviews* 141:105-121. <https://doi.org/10.1016/j.earscirev.2014.11.016>
- Zhang S, Gan Y, Xu B (2015). Biocontrol potential of a native species of *Trichoderma longibrachiatum* against *Meloidogyne incognita*. *Applied Soil Ecology* 94:21-29. <https://doi.org/10.1016/j.apsoil.2015.04.010>
- Zhang S, Gan Y, Ji W, Xu B, Hou B, Liu J (2017). Mechanisms and characterization of *Trichoderma longibrachiatum* T6 in suppressing nematodes (*Heterodera avenae*) in wheat. *Frontiers in Plant Science* 8:1491. <https://doi.org/10.3389/fpls.2017.01491>
- Zhang S, Gan Y, Xu B, Xue Y (2014). The parasitic and lethal effects of *Trichoderma longibrachiatum* against *Heterodera avenae*. *Biological Control* 72:1-8. <https://doi.org/10.1016/j.biocontrol.2014.01.009>



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