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ARTICLE

Biological Control of Root-Knot Nematode *Meloidogyne incognita* in *Psoralea corylifolia* Plant by Enhancing the Biocontrol Efficacy of *Trichoderma harzianum* Using Press Mud

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ABSTRACT

Meloidogyne incognita is a plant pathogen causing root-knot disease and loss of crop yield. The present study aimed to use *Trichoderma harzianum* as a biocontrol agent against plant-parasitic nematodes and used press mud, which is a solid waste by-product of sugarcane, as a biocontrol agent and biofertilizer. Therefore, the combined application of *T. harzianum* and press mud may enhance nematode control and plant growth. Elemental analysis of press mud using scanning electron microscopy (SEM) integrated with an Energy Dispersive X-ray (EDX) analyzer revealed the presence of different elements such as C, O, Mg, Si, P, K, Ca, Cu and Zn. In addition, a greenhouse study was conducted to investigate the combined effects of press mud and *T. harzianum on M. incognita* reproduction and growth and the biochemical features of *Psoralea corylifolia.* The results showed that plant length, dry biomass, leaf area, the number of seeds per plant, chlorophyll a, chl b, carotenoid content, nitrate reductase, carbonic anhydrase, and nitrogen content were significantly increased ($P \leq 0.05$) in the T2 plants (plants were treated with 100 g of press mud + 50 mL *T. harzianum* before one week of *M. incognita inoculation),* over inoculated plants (IC). Antioxidant enzyme activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in the foliage of *P. corylifolia* was significantly increased when plants were treated with press mud + *T. harzianum.* A significant reduction in the number of egg masses, nematode population, and root-knot index (RKI) was found in plants with T2 plants. These results suggest that the combined application of *T. harzianum* and press mud has the potential to control the *M. incognita* infection and can be used as an environmentally safe alternative to chemical nematicides and also help in the removal of sugarcane waste that causes environmental pollution.

KEYWORDS

Growth improvement; antioxidant enzymes; nitrate reductase; carbonic anhydrase; root-knot index

1 Introduction

Worldwide, traditional medicines are used by many people for therapeutic purposes [1–3]. *Psoralea corylifolia* Linn., a member of the Fabaceae family, is a critically endangered plant that has long been utilized to cure a variety of clinical symptoms [4]. It is an essential medicinal plant that is used to treat a variety of ailments in traditional medicine [5]. The plant has strong antioxidant properties and, therefore, the seeds of this plant have been used for various kinds of skin diseases such as psoriasis, leukoderma, and leprosy [6]. The plant and seed extracts contain psoralen and isopsoralen, which have antibacterial, anti-tumor, antioxidant, anti-inflammatory, anti-modulatory, and immune activity [7,8]. However, this medicinal plant was frequently attacked by phytopathogens such as nematodes (*Meloidogyne incognita*), resulting in a significant loss in plant growth and yield productivity [9]. *P. corylifolia* roots were infected with nematodes and heavily galled, and the soil samples were collected from the rhizosphere of affected plants and contained about 536–845 second juveniles (J2)/200 cm^3 of soil. Severe root galling and the presence of a high population density of J2 in the soil around symptomatic plants indicates that the root-knot nematode (RKN) can be a potentially damaging pest of *P. corylifolia* [10].

The present challenge imposed on us is to devise techniques for increasing crop yield and quality while minimizing pest damage and environmental impact. Plant-parasitic nematodes (PPNs) constitute a major agricultural limitation, causing huge losses of up to 80% of different economically important vegetable crops [11,12]. In order to control these plant parasitic nematodes, chemical nematicides have often been used. However, the continuous and indiscriminate use of these chemical compounds may be harmful to agricultural crops, soil fertility, and ultimately to human health [13]. So, there is an urgent need to find some harmless and eco-friendly solutions. In this context, some beneficial soil fungi are often referred to as plant growth promoting fungi and are used as biocontrol agents for the management of phytonematodes [14].

Trichoderma spp. is a filamentous fungus that has been employed for the biocontrol of nematodes in recent years [15]. *Trichoderma* spp. has antagonistic effects on nematodes through a wide range of techniques, including (a) direct method, which increases 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 [16] and organic volatile compounds [17], (b) generating of chitinase into the culture or rhizosphere, which may aid in egg hatching suppression [18], (c) conidia of *Trichoderm*a parasitize on nematode cuticle or/and eggshell [18], (d) conidia of *Trichoderma* enhance root growth and boost inorganic nutrient solubilization [15], (e) *Trichoderma* conidia produce different types of metabolites (mycotoxins) that inhibit egg-hatching and juveniles [18].

The interaction of numerous factors within the biological control technique represents an effective method for reducing nematode infection and plant damage [19]. Organic compost also plays an effective role in the management of nematodes. The addition of organic compounds to soil has been shown to be beneficial for soil fertility, plant growth, and disease management [20,21]. Different organic matters are used as organic compost, like animal waste, organic fertilizer, litterfall, and organic amendments that have shown nematotoxic properties against nematodes [20]. These organic amendments compete with the phytonematodes through a variety of mechanisms; (i) production of nematode-killing chemicals during decomposition, (ii) improved soil physico-chemical properties, and (iii) increased microbial activity [22].

Press mud is a solid waste and a solid fibrous residue produced during the clarification and filtering of sugarcane juice. It accounts for approximately 2.8%–4.5% (w/w) of the milled sugarcane, which is sustainably used in agriculture worldwide. The sugarcane crop produces a lot of press mud and disposing of this by-product is a big problem. Many times, press mud is burned in brick kilns, resulting in the loss and waste of millions of tonnes of nutrients and, as a result, environmental degradation in India. A

common use is for fertilizer, in both the unprocessed and processed form. Composting, microorganism treatment, and mixing with distillery effluents are some of the methods utilized to boost its fertilizer value and enhance soil health [23]. It is rich in inorganic phosphorus (P), potassium (K) and nitrogen (N) [24]. It is an eco-friendly, by-product that protects the crops from different diseases caused by phytopathogens. In this regard, Jonathan et al. [25] have reported that the single/combined application of press mud and neem cake decreases the efficacy of nematode infection and improves the growth of banana.

Very little information is known about the impact of press mud alone or in combination with *T. harzianum* on the efficacy of the root-knot nematode, *M. incognita*. As a result, the current study sought to assess the efficacy of combining press mud and *T. harzianum* in the control of nematode-infected *P. corylifolia* plants. The plant growth, yield, and biochemical characteristics were measured in response to the combined application of press mud and *T. harzianum* under the stress of *M. incognita*. Also, the present study aimed to use solid waste produced during the clarification and filtering of sugarcane juice to reduce the accumulation of pollutants in the environment.

2 Material and Methods

A pot experiment was conducted in the glass house of the Department of Botany at Aligarh Muslim University, Aligarh (27°522 N latitude, 78°512 E longitude, and 187.45 m.a.s.l.), Uttar Pradesh, India.

2.1 Preparation of Nematode Inoculum

Infected roots of eggplant (*Solanum melongena* L.) with the root-knot nematode (*M. incognita*) were collected from an eggplant field. Root-knot nematode species *M. incognita* was identified on the basis of the North Carolina differential host test and perennial pattern morphology. A single egg mass was inoculated on an eggplant to maintain the *M. incognita* race-1 population. The egg masses were collected from the galled roots using sterilized forceps, transferred in a 20 µm sieve, and kept at room temperature for hatching of eggs following the Baermann funnel technique [26]. In the second stage, juveniles were isolated from the infected plants roots for treatments [27]. The egg masses were extracted from the infected roots using sterile forceps. The egg masses were cleaned in distilled water (DW), put in 15 mesh sieves with an 8 cm diameter cross layer of tissue paper, and allowed to hatch at room temperature on Petri plates with distilled water just deep enough to cover the egg masses.

2.2 Preparation of Pure Culture of Fungal Biocontrol Agents

The culture of *T. harzianum* was obtained from the Indian Type Culture Collection (ITCC) IARI, New Delhi, India. It was grown and maintained on a potato dextrose agar (PDA) culture medium. The mass production of *T. harzianum* was done on Richard's medium. In 250 mL corning flasks, the medium was prepared, filtered through muslin cloth, and sterilized in an autoclave at 15 lb for 15 min. In an aseptic room, the liquid medium was infected with a tiny quantity of fungus and kept on PDA slants with the assistance of an inoculated needle. The inoculated flask was kept in an incubator at 25°C–30°C for about 15 days to allow copious growth of the fungus and it was used throughout the crop experiments.

2.3 Mass Culture Preparation of T. harzianum and Inoculation in Pots

After enmeshing of *T. harzianum*, (100 spore mL^{-1}) after counting spore density using a haemocytomete (Neubauer-ruled Bright Line counting chambers; Hausser Scientific, Horsham, Pa.) was blended for 2 min at high speed to mix properly into 1000 mL of distilled water in a Waring blender such that 10 mL of suspension contained one gram of mycelium. The fungal suspension of *T. harzianum* was incorporated into the soil around the root of *P. corylifolia* by making holes 5–7 cm deep within a radius of 2 cm. After inoculation of the fungus, the holes were plugged with soil.

2.4 Preparation and Characterization of Press Mud

Press mud was obtained from Dwarikesh Sugar Industries Limited Bundki (Bijnor, India). The characterization of press mud was carried out using industry-standard techniques. After oven drying, press mud at 110°C for 2 h and proximate analysis of the powdered sample was performed. The morphological and elemental analysis of press mud was done by using a scanning electron microscope (SEM, JEOL-JSM6100) integrated with an Energy Dispersive X-ray (EDX) analyzer. The X-ray diffraction (XRD) examination was performed at the Department of Physics, AMU Aligarh, India, using copper as the target and nickel as the filter media, with a radiation angle of 1.542 degrees and a goniometer speed of 1/min. The presence of functional groups in the press mud was determined using Fourier Transform Infrared Spectroscopy (FTIR). This was accomplished using the pellet (pressed disk) approach. The chosen spectral range was 4000 to 500 cm^{-1}.

2.5 Treatments

Each experimental unit consisted of a *P. corylifolia* plant in a pot. Each treatment had five replications (n = 5) in a properly randomized experimental design. The amount of inoculated nematode and fungi were based on the preliminary work in our lab to determine the suitable amounts used in the experiment and also based on literature [28,29]. The treatment counted the number of infective juveniles, biocontrol agents and press mud given to the test plants by making holes of 2.5–5 cm deep near the plant bases carefully without damaging the roots. After inoculation, the holes were covered by soil as soon as possible. The moisture content in the pots was maintained by regular watering. The treatment pattern was as follows:

C = Control un-inoculated

IC = 2,000 J2 of *M. incognita*.

T1 = 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M) one week prior to 2,000 J2 inoculation.

T2 = 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M) one week prior to 2,000 J2 inoculation.

T3 = Simultaneous inoculation of 2,000 J2 inoculation and 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M).

T4 = Simultaneous inoculation of 2,000 J2 inoculation and 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M).

T5 = 2,000 J2 inoculation one week prior to 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M).

T6 = 2,000 J2 inoculation one week prior to 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M).

2.6 Harvesting

The life cycle of the plants was terminated and harvested after 4 months of sowing. Each treated plant was carefully removed from the soil system and cleaned with tap water. A meter scale was used to measure the length of the roots and shoots. Fresh and dry weights were noted, and the number of egg masses and number of galls were observed and counted visually.

2.7 Biochemical Measurements

2.7.1 Estimation of Photosynthetic Pigments

The amount of chlorophyll a, chlorophyll b, and carotenoid in the leaves of treated and *M. incognita* infected plants was determined using Maclachlan et al. [30] method.

The formula used was as follows:

$$\text{Chl a} = \frac{12.7\ (\text{D } 663) - 2.69\ (\text{D } 645) \times \text{V}}{1000\ \text{W}}\ (\text{mg g}^{-1})$$

$$\text{Chl b} = \frac{22.9\ (\text{D } 645) - 4.68\ (\text{D } 663) \times \text{V}}{1000\ \text{W}}\ (\text{mg g}^{-1})$$

$$\text{Carotenoid} = \frac{7.6\ (\text{D } 480) - 1.49\ (\text{D } 510) \times \text{V}}{1000\ \text{W}}\ (\text{mg g}^{-1})$$

V = Total volume of the solution.

W = Weight of the leaves used for extraction of the pigment.

D = Optical Density of sample.

2.7.2 Determination of Enzymatic Activity

The activity of nitrate reductase (NR, 1.6.6.1) in the leaves of treated plants was estimated by Jaworski [31]. One hundred milligrams of young leaves were cut and placed in test tubes containing 0.1 M phosphate buffer (pH 7.4), KNO3, and 5% isopropanol. This combination was kept for 2 h at 25°C. After brooding, 0.2 ml of this solution was transferred to a separate cylinder, and 0.15 mL of 1% sulphanilamide and 0.02% N-(1-naphthyl)-ethylenediamine dihydrochloride (NED-HCl) were blended and left at room temperature for 20 min to achieve the best shading results. The test solution was placed in a cuvette, and the absorbance was measured using a spectrophotometer at 540 nm against a transparent background. A typical bend was plotted using sodium nitrite's known convergence. After comparing the OD of the sample with the standard curve, the NR activity was expressed in nM NO_2 g^{-1}FW h^{-1}.

In the fresh leaves, the carbonic anhydrase activity (CA, 4.2.1.1) was determined by Dwivedi et al. [32]. In a testing tube containing 0.2 M cysteine hydrochloride solution, 100 mg of fresh leaf test were chopped into small pieces. This blend was brooded for 15–20 min at 4°C. 2 mL of phosphate support (pH 6.8), 0.2 M sodium bi carbonate, bromothymol blue, and the methyl red marker were added to each test tube. Before titrating against 0.05 N HCl, each test tube was vigorously shaken. As soon as a red-pink color developed, readings were obtained. A control test was also performed without leaf tissue and titrated against 0.05 N HCl. The CA activity was measured in μM CO_2 kg^{-1} leaf FW S^{-1}.

2.7.3 Determination of Leaf Nitrogen Content

Leaves from each treatment were dried in an oven at 80°C and ground into a fine powder using an electric grinder. A total of 500 mg of leaf powder was digested in a digestion tube containing 2 mL sulfuric acid and 0.5 mL 30% hydrogen peroxide, which was added dropwise. After digestion, the filtrate was completed to a known volume and used to determine nitrogen by using Nessler's reagent. The optical density (OD) of the solution was measured at 525 nm using a spectrophotometer according to Lindner [33].

2.7.4 Determination of Defense-Related Enzymes Activity

Using a pre-chilled pestle and mortar, 0.5 g of fresh leaf sample was homogenized in 5.0 mL of cold (40°C) extraction buffer. After centrifuging the mixture at 10,000×g for 10 min, the supernatant was collected, and enzyme activity was determined. Peroxidase (POD) (EC 1.11.1.7) activity in the enzyme extract was measured by using the method of Chance et al. [34]. Catalase (CAT) (EC 1.11.1.6) activity was measured by adding a cold sodium phosphate buffer to the enzyme extract. To start the reaction, H_2O_2 was added to the reaction mixture. The rate of decline in absorbance at 240 nm was measured at 10 s intervals for 1 min [34]. Superoxide dismutase (SOD) (EC1.15.1.1) was assayed by following the

method of Giannopolitis et al. [35], and the activity of ascorbate peroxidase (APX) (EC 1.11.1.11) was calculated using Asada et al. [36] method.

2.7.5 Number of Galls, Egg Masses and Root-Knot Index

The number of galls per plant was counted visually, and the size of each gall was recorded using a micrometer to measure its maximum length and breadth (in μm). Infected roots were immersed in phloxin-B solution for at least 20 min. The roots were cleaned thoroughly with tap water, and the red-colored egg masses on infected roots were counted per root system [37]. Disease indexes were measured based on number of gall and number of egg masses per root system (Not per individual plant root). Taylor et al. [38] technique was used to count galls and egg masses on a 0–5 scale after harvest:

0 = 0 galls/egg masses/root system.

1 = 1–2 galls/egg masses/root system.

2 = 3–10 galls/egg masses/root system.

3 = 11–30 galls/egg masses/root system.

4 = 31–100 galls/egg masses/root system.

5 => 100 galls/egg masses/root system.

2.7.6 Nematode Related Parameters

For determination of the nematode population in soil, Cobb's sieving and the Baermann funnel method were used. Each pot's soil was carefully mixed, and the juveniles were retrieved because eggs and larva inside the roots are also part of the final population, so they had to be counted. A counting dish was used to count the number of nematodes per root system and per kg of soil. The formula for calculating the reproduction factor (Rf) was:

$$\mathrm{Rf} = \frac{\mathrm{Pf}}{\mathrm{Pi}}$$

Pf denotes the final population, whereas Pi denotes the initial population.

2.8 Statistical Analysis

The data was analyzed statistically with the program SPSS (Statistics Package for Social Science 26.00) according to Snedecor et al. [39]; five replicas were used on the analysis for variance (ANOVA). The average differences were compared at 5% level of significance by the Duncan's Multiple Range Test. Correlation was calculated using Stat graphics XVII program Version 17.20 to depict the link between quantitative statistical data.

3 Results

3.1 Characterization of Press Mud

The SEM is frequently used to investigate the morphological properties and surface characteristics of adsorbent materials. It confirms the shape, surface texture, and porosity of press mud at a qualitative level. Figs. 1A–1C show that SEM image of press mud. The results indicate that the dry press mud is composed of fine particles and shows the loose distribution between them without forming large clustering blocks.

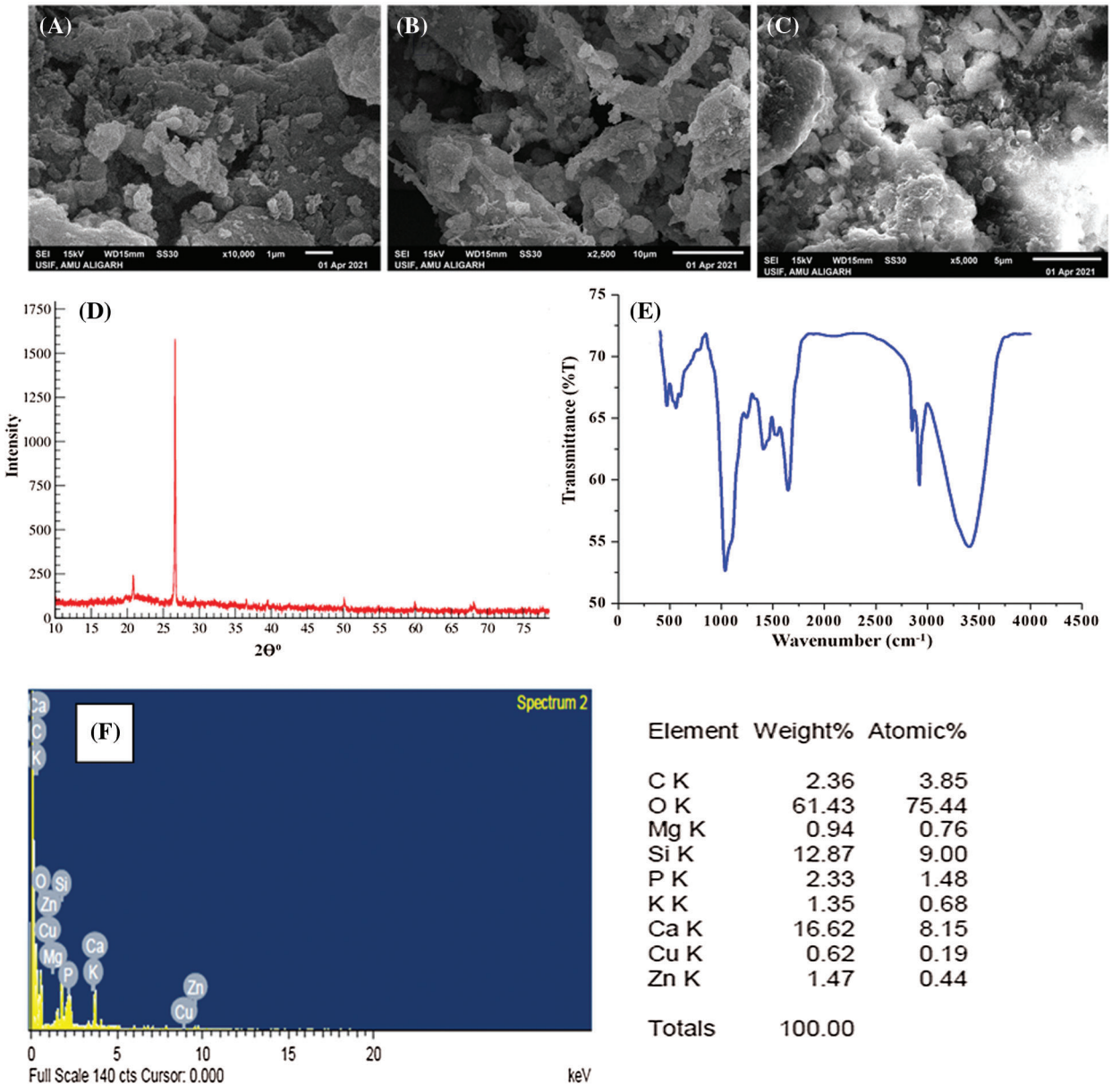

Figure 1: Characterization of press mud; scanning electron microscopic (SEM) examination of press mud representing the surface morphology (panels A, B and C), XRD pattern (D) and FTIR analysis (E). EDX analysis showing the elemental compositions of press mud (F)

The color of press mud was observed as gray or dark gray. The biomass aggregates in the press mud sample were organized into cellulose fibers, and the protein matrix was tightly bonded. The SEM image of sugarcane press mud reveals a fibrous structure with a surface porosity of 20–25 nm. Furthermore, the proximate composition of press mud has been provided in Table 1.

Table 1: Proximate composition (%) of press mud

Parameters	Values
Dry matter	35.5
Moisture content	65.2
Ash content	9.38
Organic matter	75.8
Crude wax	12.4
Crude protein	10.2
Crude fiber	27.3
Sugar	11.2
Cellulose	13.02
hemicelluloses	9.5
Lignin	7.4
Total nitrogen (%)	1.3
C:N ratio	28:1

3.2 X-Ray Diffraction (XRD)

The X-ray diffraction (XRD) pattern of the press mud is shown in Fig. 1D. The XRD spectrum showed two major peaks at $2\theta = 20.91°$ and 26.63° which may be due to the presence of silica in press mud. The other small peaks may be due to the presence of other minerals like Ca, P, K, Zn, Cu.

3.3 Functional Group Analysis of Press Mud Using FTIR

The FTIR analysis of the press mud revealed the existence of several organic functional groups, indicating their respective constituents. The FTIR spectra of the press mud is shown in Fig. 1E. The peak observed at 3400.5 cm^{-1} may be due to the presence of free OH groups. The stretching vibrations at about 2910 cm^{-1} indicate the presence of silanol (Si–OH) groups. The C=O stretching frequency is represented by the peak at 1633.56 cm^{-1}. The sharp peak at 1034.01 cm^{-1} may be due to the C–O stretching vibrations in lactones. The peak at around 549.29 cm^{-1} is due to Si–H bond stretching.

3.4 Elemental Analysis of Press Mud (EDX)

In order to know the elemental composition of press mud, EDX analysis was performed. The findings revealed that activated press mud has various elemental compositions. Fig. 1F shows the elemental status of press mud (SEM-EDX). The EDX micrographs revealed that the press mud mostly contains silicon (Si) and calcium (Ca) as principal components and oxides of zinc (Zn), magnesium (Mg), copper (Cu) and iron (Fe) in trace amounts.

3.5 Changes in Growth and Yield Attributes

The data in Table 2 shows that all treatments caused a significant increase in morphological criteria and yield as compared to plants inoculated with nematodes. The most pronounced increases were detected in plants treated with 50 or 100 mL of *T. harzianum* and 50 or 100 g of press mud one week prior to *M. incognita* followed by plants having a simultaneous inoculation of 2,000 J2 inoculation and 50 or

100 mL of *T. harzianum* and 50 or 100 g of press mud. The lower values in growth and yield were detected in plants treated with 30 or 50 mL of fungal suspension and 50 or 100 g of press mud at 7 days after *M. incognita* inoculation. The plants inoculated with 50 mL of *T. harzianum* and 100 g of press mud one week prior to 2,000 J2 inoculation (T2) caused a significant increase in root length (63.6%), shoot length (93%), root fresh weight (82%), shoot fresh weight (67%), root dry biomass (60%) and shoot dry biomass (79%) over IC (inoculated with 2000 J2 of *M. incognita* only). Also, the plants inoculated with 30 mL of *T. harzianum* suspension and 50 g of press mud 7 days after *M. incognita* inoculation (T5) showed a significant increase and non-significant effect on the growth attributes of plants as compared with inoculated plants (Table 2). For instance, the most pronounced increases in the leaf area (90%) and seed yield (54%) of *P. corylifolia* plants were detected after inoculation with *T. harzianum* (50 mL) and press mud (100 g) as compared to plants inoculated with the nematode (Table 2). However, the lowest values were detected in plants treated with 30 mL of fungal suspension and 50 g of press mud at 7 days after *M. incognita* inoculation.

Table 2: Effect of *T. harzianum* and press mud on the growth and yield of *P. corylifolia* inoculated with *M. incognita*

Treatments		Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)	Number of seeds per plant
Control (C)		61.88 ± 1.52^{a}	36.95 ± 1.50^{a}	76.08 ± 2.45^{a}	17.68 ± 0.76^{a}	30.99 ± 0.64^{a}	9.1 ± 0.75^{a}	9.84 ± 0.27^{a}	221.2 ± 4.69^{a}
Inoculated control (IC)		31.49 ± 1.97^{g}	21.04 ± 1.08^{f}	44.09 ± 1.59^{f}	9.03 ± 0.41^{f}	15.71 ± 0.75^{g}	5.32 ± 0.38^{b}	5.03 ± 0.11^{h}	132.4 ± 3.23^{h}
TH and PM one week prior to *M. incognita*	30 mL TH, 50 g PM (T1)	45.3 ± 1.10^{d}	24.82 ± 1.47^{d}	50.87 ± 1.86^{d}	12.76 ± 0.50^{d}	19.4 ± 0.48^{e}	6.94 ± 0.57^{e}	6.1 ± 0.04^{e}	161.8 ± 4.43^{e}
	50 mL TH, 100 g PM (T2)	60.8 ± 1.34^{b}	34.44 ± 0.97^{b}	73.73 ± 2.42^{b}	16.5 ± 0.87^{b}	28.2 ± 0.08^{c}	8.53 ± 0.44^{b}	9.57 ± 0.08^{b}	204.4 ± 3.65^{b}
Simultaneous inoculated of *M. incognita* and TH and PM	30 mL TH, 50 g PM (T3)	41.22 ± 0.96^{e}	22.88 ± 1.04^{e}	47 ± 1.47^{e}	10.72 ± 0.49^{e}	17.52 ± 0.06^{f}	5.81 ± 0.49^{f}	5.56 ± 0.15^{f}	147.8 ± 3.74^{f}
	50 mL TH, 100 g PM (T4)	56.47 ± 0.91^{bc}	32.53 ± 1.19^{bc}	67.88 ± 1.64^{bc}	14.72 ± 0.96^{bc}	25.61 ± 0.50^{c}	8.04 ± 0.55^{c}	8.93 ± 0.31^{c}	191.8 ± 3.36^{c}
M. incognita one week prior to TH and PM	30 mL TH, 50 g PM (T5)	34.49 ± 0.78^{f}	21.33 ± 1.14^{e}	44.64 ± 1.25^{e}	9.68 ± 0.73^{e}	15.95 ± 0.91^{g}	5.43 ± 0.36^{g}	5.17 ± 0.04^{g}	139.4 ± 2.25^{g}
	50 mL TH, 100 g PM (T6)	51.66 ± 1.33^{c}	28.87 ± 0.73^{c}	62.35 ± 1.09^{c}	12.26 ± 0.68^{c}	22.67 ± 0.02^{d}	7.35 ± 0.58^{d}	7.65 ± 0.11^{d}	170.6 ± 1.86^{d}

Note: Each value is a mean (±SE) of five replicates and the different letters on the same column show significant differences according to Duncan's test at $P \leq 0.05$ TH and PM denote the *T. harzianum* and press mud, respectively.

3.6 Changes in Photosynthetic Pigments

The photosynthetic pigments in the foliage tissues of *P. corylifolia* plants were significantly decreased ($p \leq 0.05$) in plants infected by *M. incognita*. Plants inoculated with *T. harzianum* (30 or 50 mL) and press mud (50 or 100 g) press mud before, after nematode infection, and/or simultaneous inoculation reduced the effect of nematodes on photosynthetic pigments compared to control inoculated plants (IC). The higher values in Chl a (60%), Chl b (96%), and carotenoid content (40%) were detected in plants inoculated with *T. harzianum* (50 mL) and press mud (100 g) before 7 days of infestation with *M. incognita* over inoculated control (IC) (Table 3).

Table 3: Effect of *T. harzianum* and press mud on physiological parameter of *P. corylifolia* inoculated with *M. incognita*

Treatments		Chlorophyll a (mg g^{-1}FW)	Chlorophyll b (mg g^{-1}FW)	Carotenoid (mg g^{1}FW)	Nitrate reductase activity (nM NO_2^- g^{-1} FW h^{-1})	Carbonic anhydrase activity (μM CO_2 kg^{-1} FW S^{-1})	Leaf nitrogen (%)
Control (C)		1.850 ± 0.05^{a}	1.305 ± 0.03^{a}	1.63 ± 0.03^{a}	1.514 ± 0.06^{a}	157.27 ± 3.60^{a}	14.76 ± 0.84^{a}
Inoculated control (IC)		1.091 ± 0.03^{d}	0.624 ± 0.02^{f}	1.12 ± 0.01^{d}	0.816 ± 0.02^{f}	86.67 ± 3.18^{g}	8.63 ± 0.49^{g}
TH and PM one week prior to *M. incognita*	30 mL TH, 50 g PM (T1)	1.221 ± 0.02^{c}	0.820 ± 0.03^{d}	1.19 ± 0.04^{d}	0.952 ± 0.03^{d}	101.34 ± 2.74^{e}	10.21 ± 0.37^{e}
	50 mL TH, 100 g PM (T2)	1.751 ± 0.04^{a}	1.230 ± 0.03^{b}	1.58 ± 0.03^{b}	1.444 ± 0.04^{b}	151.64 ± 3.45^{b}	13.39 ± 0.64^{b}
Simultaneous inoculated of *M. incognita* and TH and PM	30 mL TH, 50 g PM (T3)	1.173 ± 0.01^{ab}	0.771 ± 0.01^{de}	1.17 ± 0.04^{d}	0.892 ± 0.03^{e}	94.13 ± 2.90^{f}	9.24 ± 0.43^{f}
	50 mL TH, 100 g PM (T4)	1.720 ± 0.04^{ab}	1.113 ± 0.04^{c}	1.54 ± 0.05^{b}	1.352 ± 0.04^{c}	143.47 ± 2.28^{c}	12.33 ± 0.32^{c}
M. incognita one week prior to TH and PM	30 mL TH, 50 g PM (T5)	1.151 ± 0.03^{d}	0.641 ± 0.02^{f}	1.15 ± 0.03^{d}	0.852 ± 0.02^{e}	89.99 ± 2.57^{g}	8.91 ± 0.24^{g}
	50 mL TH, 100 g PM (T6)	1.657 ± 0.05^{b}	1.071 ± 0.02^{c}	1.45 ± 0.03^{c}	1.214 ± 0.04^{c}	134.47 ± 2.03^{d}	11.72 ± 0.32^{d}

Note: Each value is a mean (±SE) of five replicates and the different letters on the same column show significant differences according to Duncan's test at $P \leq 0.05$. TH and PM denote the *T. harzianum* and press mud, respectively

3.7 Changes in Enzymatic Activity and Leaf Nitrogen Content

In order to assess the impact of fungal biocontrol agents and organic press mud on enzymatic activity, the nitrate reductase (NR) and carbonic anhydrase (CA) in the foliage of *M. incognita* infected plants were assessed (Table 3). NR, CA, and N content were significantly decreased by about 46.1%, 44.9%, and 41.5%, respectively, in *P. corylifolia* plants inoculated with nematodes. In addition, all treatments caused a significant increase in the same content as compared with control inoculated plants, while the maximum increase in NR (76%), CA (74%), and leaf N (55%), content was recorded when *P. corylifolia* plants were detached from soil supplemented with 50 mL of *T. harzianum* and 100 g of press mud before 7 days of infestation by *M. incognita* as compared to control inoculated plants.

3.8 Changes in the Activity of Defense-Related Enzymes

The data in Figs. 2A–2D illustrated that inoculation with *M. incognita* caused a significant boost in APX, CAT, POD and SOD activity in plants as compared to control plants. All treatments caused a significant increase in defense enzymes activity as compared with plants inoculated with nematodes. In addition, treatment with 50 mL of *T. harzianum* with press mud (100 g) one week prior to nematode inoculation was exhibited as the best treatment, where it enhanced the activity of APX, CAT, POD and SOD by about 150%, 85.2%, 92.3%, and 58.3% respectively over plants inoculated with nematode only.

3.9 Changes in the Number of Galls and Number of Egg Masses

In this study, the number of galls, root-knot index, RKI, and reproduction factors (number of egg masses on roots) were significantly increased ($P \leq 0.05$) when plants were detached from soil infected only with *M. incognita*. Besides, inoculation with *T. harzianum* suspension (50 mL) and press mud (100 g) before nematode infection, exhibited a maximum reduction of 88% and 85% in the number of galls and number of egg masses, respectively (Figs. 3A and 3B). Similarly, gall formation and egg mass production were significantly reduced accordingly by all other treatments. Contrarily, T5 (2,000 J2 inoculation one week prior to 30 mL of *T. harzianum* and 50 g of press mud) had the lowest reduction in all nematode related parameters.

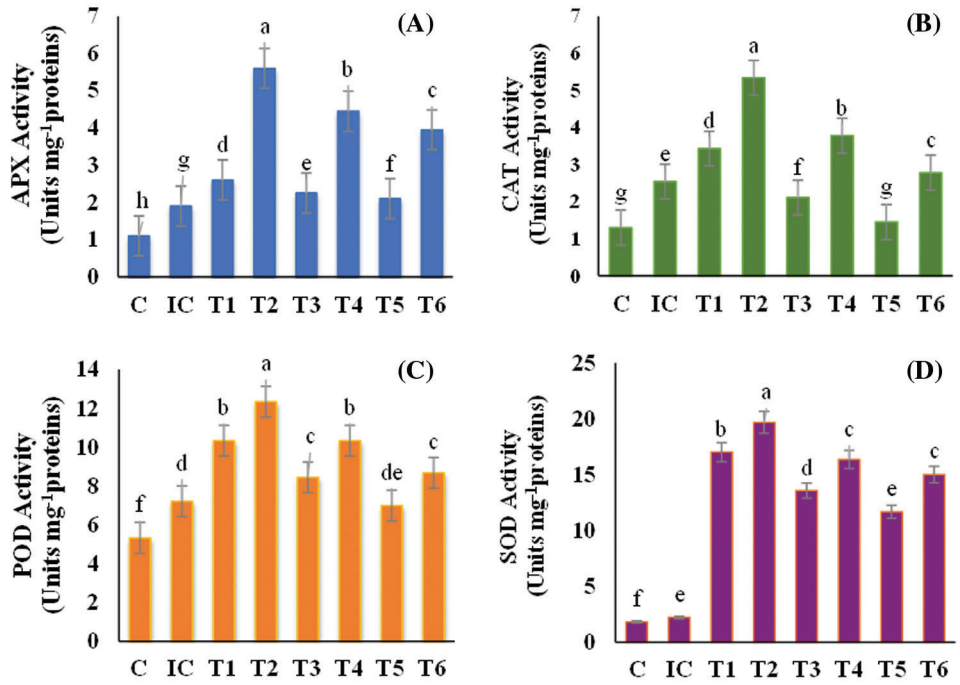

Figure 2: Combined effect of press mud and *T. harzianum* on APX (A), CAT (B) POD (C) and SOD activities (D) extracted from fresh foliage of *M. incognita* inoculated *P. corylifolia* plant raised under green-house conditions. Each value is a mean (±SE) of five replicates and the different letters on the same bar show significant differences according to Duncan's test at $P \leq 0.05$. C = Control un-inoculated; IC = 2,000 J2 of *M. incognita*; T1 = 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M) one week prior to 2,000 J2 inoculation. T2 = 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M) one week prior to 2,000 J2 inoculation; T3 = Simultaneous inoculation of 2,000 J2 inoculation and 30 mL *T. harzianum* (T. H) + 50 g press mud (P. M); T4 = Simultaneous inoculation of 2,000 J2 inoculation and 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M); T5 = 2,000 J2 inoculation one week prior to 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M); T6 = 2,000 J2 inoculation one week prior to 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M)

3.10 Changes in Nematode Population in Root and Soil System

Nematode development in terms of root and soil population was considerably reduced in all the treatments. The inoculation of *T. harzianum* suspension (50 mL) and press mud (100 g) before nematode infection had the best effect where it reduced the nematode population and reproduction factor by 80% and 81%, respectively over plants solely infected with nematodes (Figs. 3C–3E). Correlation matrix results are presented between the different parameters studied in Fig. 4.

4 Discussion

Naturalists, biologists, environmentalists, and hydrologists are continuously pressuring farmers to use fewer pesticides (nematicides) and synthetic fertilizers. They must, however, preserve crop profitability and crop quality. Plant-parasitic nematode damage is projected to cost the global agriculture industry $100 billion per year. Because pesticides (nematicides) and synthetic fertilizers are expensive, they can contribute to higher crop production costs and, as a result, considerable rises in food prices, especially during low harvest years (in the dry or very rainy). Furthermore, pesticides are usually selective, eliminating only the species that are targeted. They react fast and vigorously [40], but plant protection is

limited because their effect is brief and has no other beneficial effects on plant growth or soil quality. As a result, organic fertilizer as a tool for controlling parasitic nematodes and other soil pathogens is advantageous because it is a natural, low-cost material (usually made by growers), from which nutrients and nematicidal substances are gradually released throughout the entire vegetation period [40]. In this context, press mud, a waste product material of sugar industry is used as bio-fertilizers. Here, we had assessed the combined application of *T. harzianum* (biocontrol agent) and press mud against *M. incognita*.

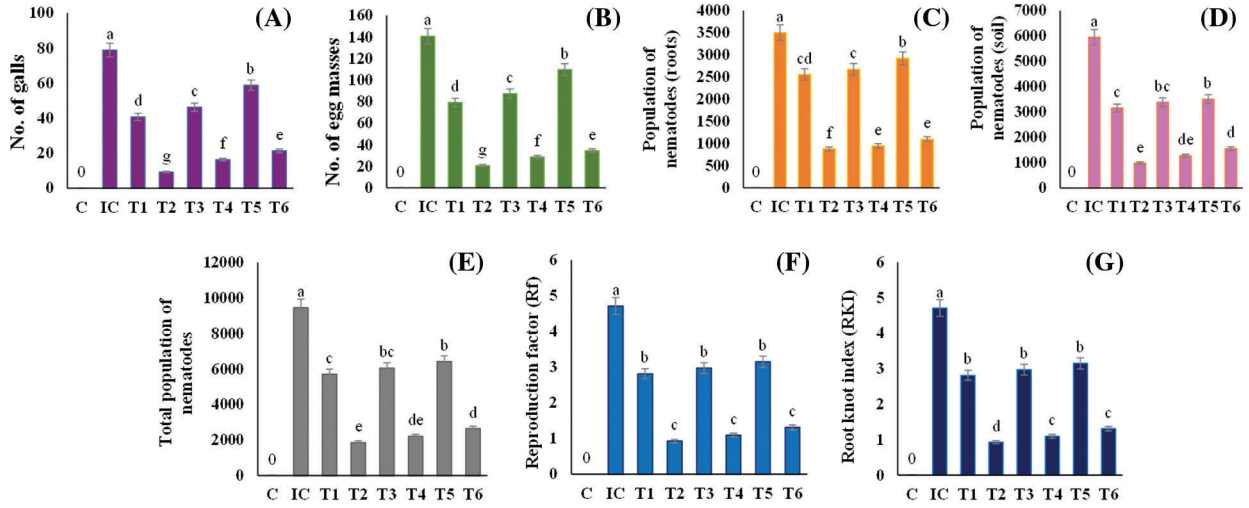

Figure 3: Combined effect of press mud and *T. harzianum* on number of galls (A), number of egg masses/plants (B) population of nematodes in roots (C), population of nematodes in soils (D), total nematode population (E), reproduction factor (F) and root knot index (G) of *M. incognita* inoculated *P. corylifolia* plants raised under green-house condition. Each value is a mean (±SE) of five replicates and the different letters on the same bar show significant differences according to Duncan's test at $P \leq 0.05$. C = Control un-inoculated; IC = 2,000 J2 of *M. incognita*; T1 = 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M) one week prior to 2,000 J2 inoculation. T2 = 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M) one week prior to 2,000 J2 inoculation; T3 = Simultaneous inoculation of 2,000 J2 inoculation and 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M); T4 = Simultaneous inoculation of 2,000 J2 inoculation and 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M); T5 = 2,000 J2 inoculation one week prior to 30 mL *T. harzianum* (T.H) + 50 g press mud (P. M); T6 = 2,000 J2 inoculation one week prior to 50 mL *T. harzianum* (T.H) + 100 g press mud (P. M)

The press mud was morphologically characterized using SEM as recently described by Rondina et al. [41]. The XRD spectrum showed two major peaks at $2\theta = 20.91°$ and 26.63°. The FTIR method is a useful tool for identifying the functional groups that distinguish a chemical/compound. The adsorption process also requires an understanding of the chemical structure of adsorbents. By comparing the absorption frequencies of various organic functional groups. The peak at around 549.29 cm^{-1} is due to Si–H bond stretching [42]. Likewise, Rout et al. [43] investigated a similar pattern when they analyzed the press mud using FTIR spectroscopy.

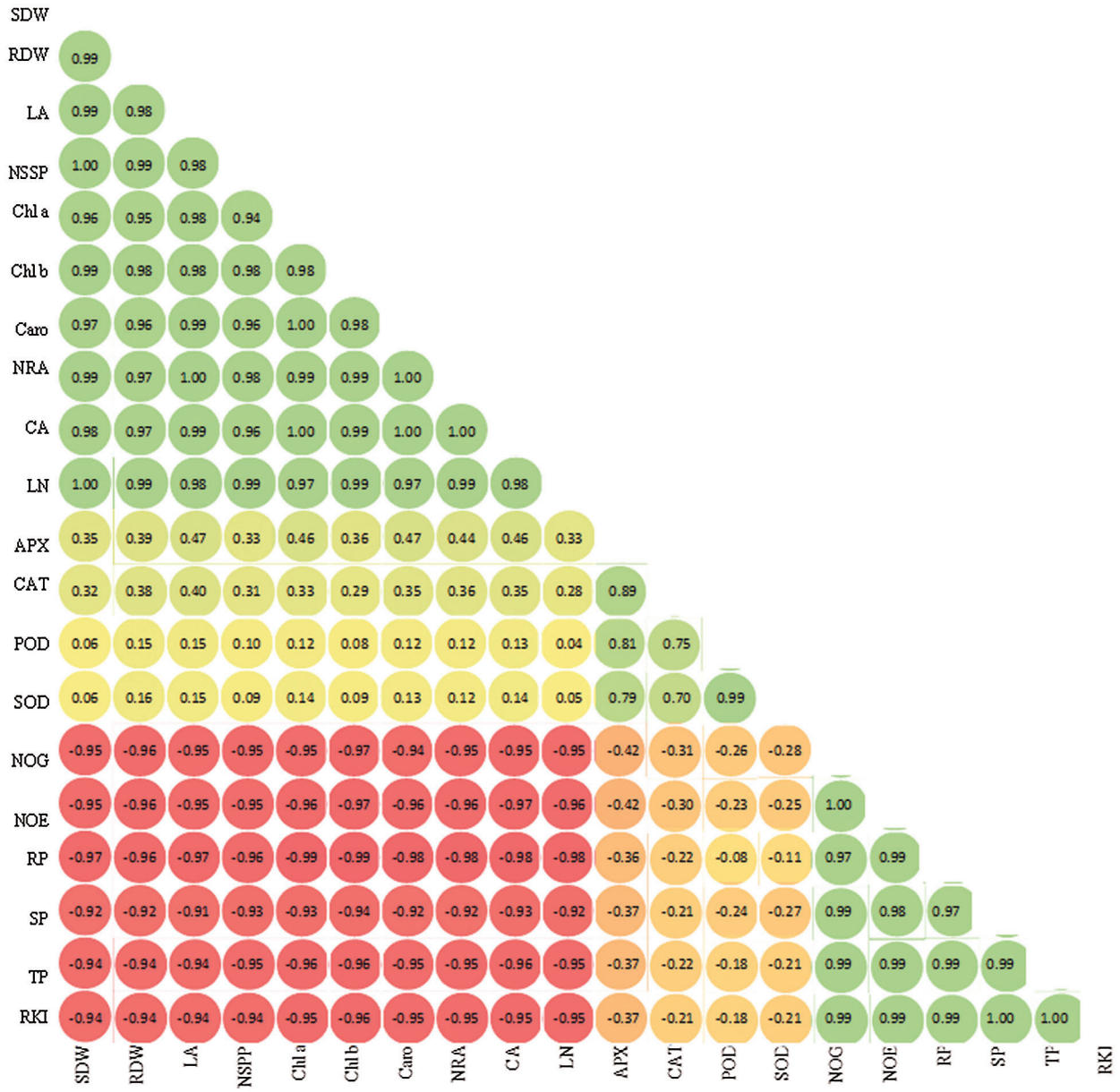

Figure 4: Correlation matrix results between some studied parameters of *Psoralea corylifolia* at different combined treatments of press mud and *T. harzianum* before, simultaneously, and after 1 week of *M. incognita* inoculation. SDW, shoot dry weight; RDW, root dry weight; LA, leaf area; NSPP, Number of seed yield per plant (g); Chl a, chlorophyll a; Chl b, chlorophyll b, NRA, nitrate reductase activity; CA, anhydrase activity; LN, leaf nitrogen concentration; APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; NOG, number of galls per root system; and NOE, number of egg masses per root system; RP, root population; SP, soil population; TP, total population; RKI, root-knot index

The data in the present study showed that treatment with press mud and *Trichoderma* caused a significant boost on morphological criteria and yield as compared to plants inoculated with nematodes. Similar results are recorded by Lakshman et al. [44] who found that treatment with arbuscular mycorrhizal fungi, Press mud and indole acetic acid caused a significant increase in tomato growth, biomass and yield as compared to control plants. It is widely known that press mud nourishes and promotes the growth and yield of plants by assisting in the retention of soil moisture and increasing root multiplication [45]. In agronomic practices, press mud might be used as an organic matter supply, a source of agricultural nutrients, manure, and as a soil ameliorant [46]. Press mud comprises of fiber, crude protein, sugar, crude wax, lipids, ash including oxides of Si, Ca, P, Mg, and K that may play a crucial role in the development of plants [47]. This organic matter is highly soluble, making it easily available for the activity of microbiota and, as a result, easily taken up by the soil [46]. As a valuable source of plant nutrients, press mud may have an impact on the physical, chemical, and biological aspects of a soil [48]. Also, press mud contains a high amount of potassium so that, the involvement of K in nutrient and sugar translocation in plants, as well as turgor pressure in plant cells, may be responsible for the increased plant growth and yield. It also plays a role in cell expansion and meristematic growth [49]. In addition, *Trichoderma* spp. increased agricultural productivity by improving shoot and root growth [50]. As a result, it is possible that press mud enriches the soil with nutrients that aid *T. harzianum* development. Enhanced root area allows them to explore larger volumes of soil for nutrients, improve the solubility of insoluble substances, as well as increase the availability of micronutrients, which may also contribute to an increased plant development by *Trichoderma* spp. [45]. Many researchers have noticed that soil application of *Trichoderma* spp. to various plants infected with root-knot nematode, resulted in improved growth and biochemical features of plants [45]. Similar to our study, various workers have reported that *Trichoderma* significantly improved the leaf area and yield of plants [51].

Inoculation with nematodes caused suppression of photosynthetic pigments in leaves of *P. corylifolia*. Infection with nematodes caused the production of reactive oxygen species (ROS) that could decompose the photosynthetic pigments (i.e., chlorophyll), as well as cause the impairment of the photosynthetic equipment, reduction in electron transport, carbon fixation capacity, and photophosphorylation [52]. On the other hand, *T. harzianum* and press mud treatment resulted in an increase in photosynthetic pigments (chl a, chl b and carotenoid). Besides, nitrogen (N) is a key component of chlorophyll molecules; nematode inoculation reduces its bioavailability and thus lowers the chlorophyll concentration in the foliage [9]. This might be one of the primary and main reasons that plants infected with phytonematodes including *M. incognita* often had a lower photosynthetic rate. Our findings showed that *T. harzianum* along with press mud could possibly increase photosynthetic performance. Likewise, in comparison to plants infected with the nematode (alone), inoculation of tomato roots with *T. harzianum* UBSTH-501 dramatically increased total chlorophyll content and chitinase activity by increasing the absorption of water, which ultimately resulted in an increase in photosynthetic pigments [53]. The use of a 40% sugarcane press mud treatment increased the chlorophyll content of eggplant, which is likely owed to Fe, Mg, and Mn concentrations in the sugarcane press mud, which are related to chlorophyll synthesis [54]. As a result, the press mud after a 40% treatment includes the optimal levels of nutrients essential for *S. melongena* to reach its maximum vegetative growth [54].

Infection of plants with nematode caused a significant decrease in NR, CA and N content as compared to control plants. Similar results were recorded by Danish et al. [55] who found that infestation with *M. incognita* to *Trachyspermum ammi* (L.) caused a significant decrease in the same contents. On the other hand, treatment with 50 mL of *T. harzianum* and 100 g of press mud before 7 days of infestation by *M. incognita* significantly increased NR, CA and N contents. *Trichoderma* spp. have the ability to invade the root systems of a variety of plants and thus coordinate the host plant's defense mechanisms [56]. This might have been attributed to an increased water and mineral absorption by the root systems of plants.

Similar to our finding, Sofy et al. [50] found that treatment with *T. harzianum* noticed an increase in nitrogen absorption efficiency of plants. In addition, photosynthetic molecules, comprised of N, Mg, and other essential nutrients are very helpful in the absorption of water and minerals. These minerals are crucial for the metabolic activities and the growth of plants [57]. The increment of nitrogen accumulation by press mud was probably due to mineralization of the organic matter containing proteins and conversion of ammonium-nitrogen into nitrate [13].

Antioxidant enzymes like ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) are often associated with biotic and abiotic factors [58–62]. In contrast, *T. harzianum* colonization in soil drenched with press mud substantially enhanced the activity of antioxidant enzymes. *Trichoderma* spp. has been found to boost the activity of plant defense enzymes and the concentration of defense metabolites such as phenolic compounds and flavonoids [61]. In a similar way, Yan et al. [63] observed that pre-soil-inoculation of *T. harzianum* to tomato plants infected with *M. incognita* resulted in improved defense-related enzymes and pathogenesis-related (PR) proteins including chitinases, 1,3-glucanase, protease, and amylase.

As a consequence of our findings, *T. harzianum* mixed with press mud exhibited the greatest nematicidal impact against *M. incognita*. These findings corroborate those of Olabiyi et al. [64], who found that combining *T. harzanium* with composted wastes reduced nematode population and gall index in sesame. Additionally, the combined inoculation of the fungal agent *Trichoderma vierns* with forest debris obtained from oak plants considerably reduced the number of galls in roots which were already infected with *M. javanica* [65]. It has been hypothesized that reduced galling was due to better proliferation of the fungus *T. harzianum* in soil drenched with press mud. During planting time, 15 t/ha of cured press mud is applied to help control plant parasitic nematodes. Because it is high in nutrients and organic matter, press mud is an excellent substrate for the growth of nematode antagonistic fungi and bacteria in soil [66]. *Trichoderma* spp. has been shown to reduce the root gall formation by nematode and worm populations as it has been reported by several workers [67].

The reduction in the nematode population might have been caused by the fungal mycelia colonizing the surface of the roots prior to nematode infestation and by producing various lytic and cell wall degrading enzymes. *Trichoderma* spp. inhibits the plant-parasitic nematodes in a variety of ways [68] *T. harzianum* may colonize the roots very fast and effectively decrease the number of feeding sites for RKNs in the rhizosphere [69]. *Trichoderma* spp. strains have been shown to impact J2 motility, nematode development, egg hatching, nematode reproduction, and disease severity as nematode antagonists [61]. Some biocontrol organisms, such as *T. harzianum*, colonize soils rich in organic matter, which improves biocontrol activity. Their inhibitory effects against plant nematodes have also been discovered in recent years [70]. Also, Osman et al. [71] observed that *T. harzianum* had a good effect on root-knot nematode *M. incognita* management and a moderate improvement in eggplant yield production. They attributed their findings to the fungus's chitinolytic activity, which induces a chitin layer disintegration in worm eggs. *T. harzianum* isolates induced systemic resistance in tomato plants against the root-knot nematode, *M. javanica*, by increasing the accumulation of hydrolytic enzymes that affect nematode invasion [72,73].

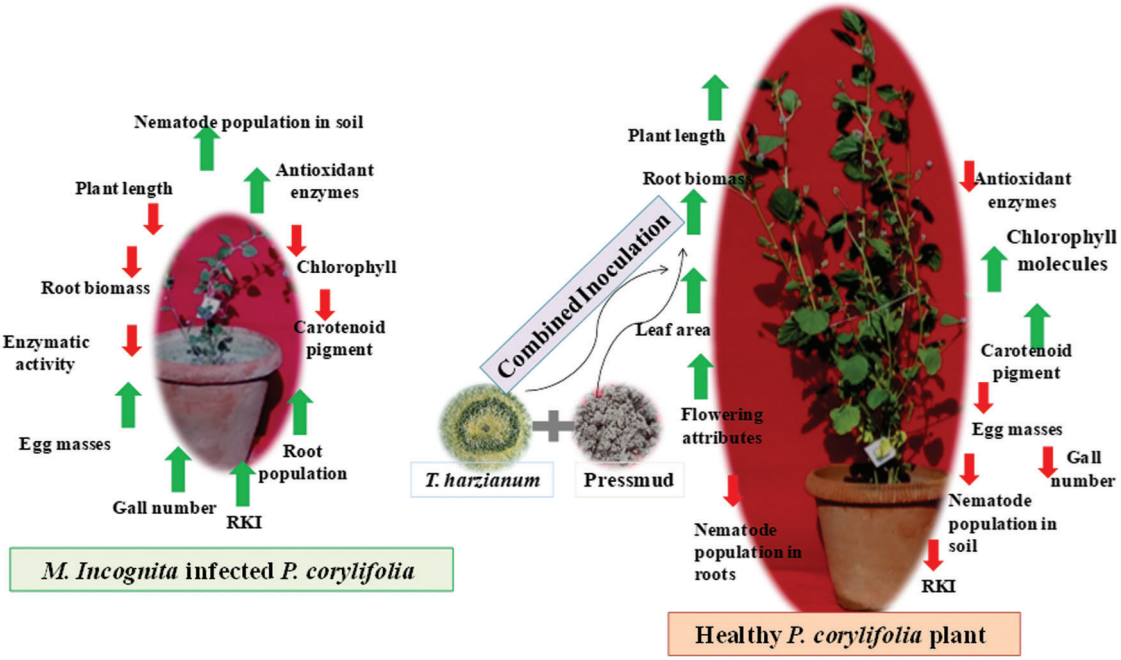

Figure 5: Diagram depicts the (1) reducing effects of *M. incognita* and (2) combined application of *T. harzianum* and press mud improving the growth, biomass, photosynthetic pigments, antioxidants, and enzymatic activity on *P. corylifolia* plants

5 Conclusion

The findings of this study indicate that the combination of *T. harzianum* and press mud is an environmentally friendly and effective treatment for *M. incognita* root-knot disease. Thus, it might be concluded that the application of soil amendment with press mud and *T. harzianum* increases plant growth and reduces the nematode population (Fig. 5). Application of 100 g press mud in combination with 50 mL of *T. harzianum* prior to one week of *M. incognita* inoculation proved to be the best concentration in comparison to the simultaneous and after inoculation of *M. incognita*. We discovered that treatment of *Trichoderma* and press mud prior to one week of *M. incognita* infestation induced systemic resistance or numerous possible defence mechanisms were responsible for the increase in plant growth and reduction in nematode infestation. The study provides new insights into the enhanced bio-control efficacy of *T. harzianum* induced by soil drenching with press mud which are considered eco-friendlily methods against the *M. incognita* infection and also help in the removal of sugarcane wastes that cause pollution to the environment.

Authorship: The authors confirm contribution to the paper as follows: study conception and design: Y. N., M. D. and H. S.; data collection: Y. N., M. D. and H. S.; analysis and interpretation of results: Y. N., M. D., H. I. M. and H. S.; draft manuscript preparation: Y. N., M. D., H. I. M., H. S., A. E. All authors reviewed the results and approved the final version of the manuscript.

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