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Management of Root-knot Nematode, *Meloidogyne incognita* in *Psoralea corylifolia* by using Press Mud and *Glomus mosseae* at Different Time Intervals

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A B S T R A C T

A glass house experiment was conducted to investigate the efficacy of press mud and *Glomus mosseae* for the management of *Meloidogyne incognita* on *Psoralea corylifolia*. From the results it was found that all the treated plants were recorded to improve the plant length, fresh and dry biomass of shoot and root, biochemical characteristics with decrease gall formation, nematode population and root-knot index. The greatest and most significant increases were found in plants cultivated on soil drenched with press mud and treated with *G. mosseae* one week earlier to *M. incognita* inoculation. Lowest gall formation, nematode population and root-knot index were observed in the same treatment. It may be concluded that using press mud in conjunction with *G. mosseae* promotes plant growth, yield qualities, and reduce the nematode development.

***Key words*:** *Glomus mosseae, Meloidogyne incognita*, Management, Press mud, *Psoralea corylifolia*

Psoralea corylifolia (Babchi), belongs to the family fabaceae is an important medicinal plant. Different parts of the plant are used to treat a number of skin problems, including leukoderma, skin rashes, infections, and others [29]. The seed extract of *P. corylifolia* was discovered to have anti-oxidative, anti-microbial, anti-inflammatory, antitumor, anti-mutagenic, and anti-mutagenic properties, as well as to suppress insect hormonal activity [28], [8]. *P. corylifolia* has been utilised as a raw material by several Indian pharmaceutical businesses in the creation of pharmaceuticals and Ayurvedic skin care products [9]. This plant is highly susceptible towards the root knot disease caused by *Meloidogyne* spp [39], [27]. *Meloidogyne incognita* infects the roots of *P. corylifolia*, affecting its growth and yield, as well as altering its chemical qualities. The plant eventually succumbs to stress, resulting in yield decrease [13].

Among plant parasitic nematodes, *Meloidogyne incognita* is a sedentary endoparasite that causes root deterioration and polyphagous pests in a wide range of agricultural crops across the world [24], [38]. Nematode infiltration into the root reduces plant yields and pharmacological characteristics of therapeutic crops [50]. Chemical nematicides have frequently been employed to manage these plant invading pathogens [11]. However, the continued and indiscriminate use of these chemical compounds may be damaging to agricultural crops, soil fertility, and, eventually, human health [40]. As a result, there is an urgent need to identify some safe and environmentally friendly alternatives. As an alternative to regulating the root-knot nematode, beneficial microorganisms provide antagonistic action. The use of biocontrol agents and their chemical components is a promising alternative for reducing nematode populations efficiently. A diverse variety of microorganisms, including bacteria, fungus, algae, and some protozoans, create chemical compounds that have antimicrobial action and are used to combat pathogens [6].

The use of arbuscular mycorrhizae fungi (AMF) for controlling plant diseases are well documented by various workers such as [1], [17], [34], [52], [56]. Arbuscular mycorrhizae fungi (AMF) and nematodes both use plant roots to occupy space and get nourishment. AM fungus and nematode interaction is significant because plants infected with AM fungi have increased tolerance to the nematode. AM fungi are utilized as bio-fertilizers because they are obligate symbionts that form colonies in the roots of most cultivated plants. This system benefits plant development by increasing nutrient intake, hormonal activity, and growth rate [35]. Inoculation with AM fungus prior to nematode infection reduced the population of M. incognita as well as the incidence and severity of root knot disease [4].

Sugar press mud is a sugar industry waste product that is used as an organic fertiliser [7], [14], [18]. Sugar press mud contains a high concentration of nutrients, which nourishes the soil and encourages plant development [32-33]. Press mud is water soluble and serves as a favourable substrate for fungal and

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bacterial biocontrol activities, both of which stimulate plant development and soil fertility [45]. Microbes benefit from press mud as a substrate [42]. The use of press mud increases the organic carbon, nitrogen, and phosphorus content of soil [44]. There is no instances of mixed dosages of press mud and AM fungus being used to manage *M. incognita*. Based on the nematicidal potential of *Glomus mosseae* and press mud, we evaluated the combination application of press mud + *G. mosseae* at different time intervals on *P. corylifolia* plants for the management of root-knot nematode *M. incognita* in the soil system.

MATERIALS AND METHODS

Collection and preparation of pure culture of nematode inoculum

Test pathogens, *Meloidogyne incognita* was collected from roots of egg-plant's field. The North Carolina Differential host test and perennial pattern morphology were used to identify the root-knot species *M. incognita* [20]. In a greenhouse, single species populations of eggplant cultivars were maintained to obtain pure culture. The egg masses were collected using sterilized forceps from the infected brinjal root to get the requisite amount of root knot nematode inoculum. The egg masses were washed with DDW and placed in 15 mesh sieves with a serviette layer before being placed in a Petri dish with DDW, just deep enough to cover the egg masses, and left to hatch at room temperature. The second-stage juveniles (J2) were collected and counted using a nematode counting dish in a container containing DDW and used for further studies.

Biocontrol agent

Glomus mosseae, an arbuscular mycorrhizal fungus, was obtained from CMCC (Centre for Mycorrhizal Culture Collection) TERI, New Delhi India. For preparation of inoculum, this fungus was cultured on *Zea mays* grown in pure river sand mixed with the loam soil with manure in a ratio 1:3:1 (v/v) receptively. *G. mosseae* production in inoculum was determined by the method, probable number given by Porter [43]. The planting hole was treated with 50 g and 100 g of *G. mosseae* inoculum, which consisted of soil, 5 spores/g of soil, hyphae, and root pieces from infected maize plant.

Press mud

Press mud was obtained from Dwarikesh Sugar Industries Limited Bundki (Bijnor). It was used as an organic amendment agent of the soil, in different doses, according to the experimental design.

Test plant

P. corylifolia seedlings were procured from the Aligarh Muslim University Botanical Garden (Department of Botany) and planted in a completely autoclaved 38 cm clay pot filled with 2.5 kg steam sterilised soil. The seeds were placed in a sterile beaker with a 1:1 combination of NaOCl (5.25 percent) and ethanol. According to Koenning and Barker's protocol, the seeds were rinsed three times with distilled water after soaking for ten minutes [30]. Each pot contained 15 seeds, which were later thinned to one seedling per pot.

Treatments

The seedlings (two leaf stage) were treated and inoculated carefully by creating 3-5 cm deep holes around the plant bases without injuring the roots with different combinations of *Glomus mosseae* and press mud at different time intervals, as shown below:

C = Control (without *Meloidogyne incognita*)
C_1 = Inoculated control (*M. incognita* only)
T_1 = 50 g of *Glomus mosseae* + 50 g sugar press mud one week prior to *M. incognita* inoculation
T_2 = 100 g of *G. mosseae* + 100 g sugar press mud one week prior to *M. incognita* inoculation
T_3 = 50 g of *G. mosseae* + 50 g sugar press mud with simultaneous inoculation of *M. incognita*
T_4 = 100 of *G. mosseae* + 100 g sugar press mud with simultaneous inoculation of *M. incognita*
T_5 = 50 g of *G. mosseae* + 50 g sugar press mud after one week of *M. incognita* inoculation
T_6 = 100 g of *G. mosseae* + 100 g sugar press mud after one week of *M. incognita* inoculation

Harvesting and collection of data

The plant growth characteristics were examined after four months of treatment. The plant's shoot and root lengths were measured on a meter scale, and the fresh and dry weights of the shoots and roots were determined using a digital balance. A platometer was used to determine leaf area, and five mature leaves were chosen at random from each treatment. Each leaf's outline form was created and measured on butter paper. The seed yield per plant was counted visually.

Photosynthetic pigments and enzyme activities

To quantify photosynthetic pigments such as chlorophyll and carotenoid, the Arnon (1949) methods was employed. The Jaworski method was used to determine the activity of the enzyme nitrate reductase [23]. Dwivedi and Randhawa's approach was used to evaluate the activity of carbonic anhydrase (CA) in fresh leaves [15]. The nitrogen content of the leaves was determined using the method described by [36].

Nematode's parameters

Galls were counted visually, and their sizes were determined by measuring their maximum length and breadth (in mm^2) using a micrometer. The egg mass was determined by staining egg masses with phloxin B solution and counting them visually. Cobb's sieving and the Baermann funnel technique were used to determine the nematode population in soil [53]. The gall index was rated on Taylor and Sasser scale [54].

The reproduction factor was calculated using the following formula:

$$(Rf).Rf = \frac{Pf}{Pi}$$

Where, initial population is Pi and Pf represent the final population.

Statistical analysis

Using SPSS 17.0, the data were analyzed to analysis of variance (ANOVA). At a probability threshold of $P \leq 0.05$, the least significant differences (LSD) were determined.

RESULTS AND DISCUSSION

From the results it was evident that the application of AM fungus *Glomus mosseae* and press mud enhances shoot and root length, fresh and dry weight, leaf area, and seed yield over the inoculated control plants (C_1). The greatest and most significant increases were recorded in plants (T_2) treated with *Glomus mosseae* in conjunction with press mud one week before to nematode inoculation, followed by T_4 plants treated with *Glomus mosseae* and press mud concurrently with nematode. However, when T_2 plants were compared to control plants, a non-significant decline was observed (C). When compared to control plants, C_1 plants (inoculated control plants)

showed a significant decrease in all growth and yield variables (Table 1). There was a significant reduction in the physiological parameters of the inoculated control plants (C_1) when compare with the control plants (C). Highest and significant improvement in the physiological characteristic such as chlorophyll 'a', 'b' and carotenoid, nitrate reductase, carbonic anhydrase and nitrogen contents was encountered in T_2 plants followed by T_4 plants. where the T_2 plants treated by *Glomus mosseae* and press mud, seven days prior to *M. incognita*, and the T_4 plants, which was simultaneously inoculated by *G. mosseae* and press mud with nematode. Minimum but significant improvement was observed in T_5 plant treated with *Glomus mosseae* and press mud after one week of nematode inoculation (Fig 1-2).

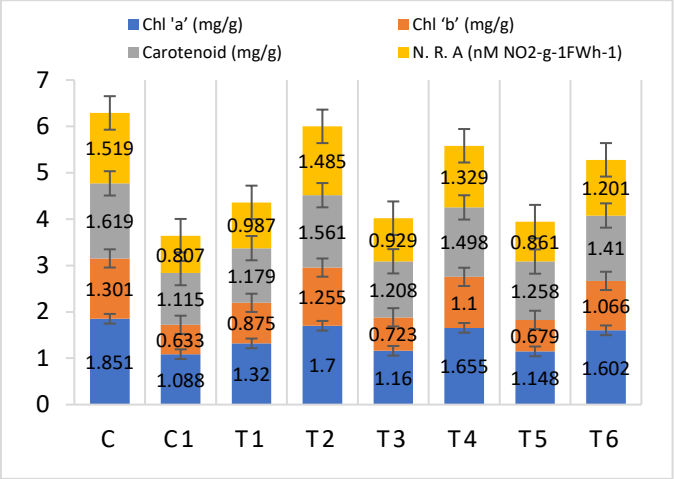

Fig 1 Effect of *G. mosseae* and press mud with different time inoculation of *M. incognita* on chl a, chl b, carotenoid content, and nitrate reductase activity of *P. corilyolia*

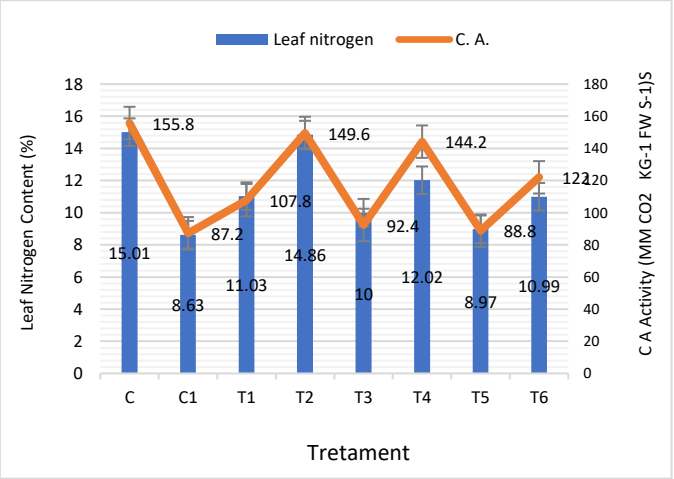

Fig 2 Effect of *G. mosseae* and press mud with different time inoculation of *M. incognita* on leaf nitrogen content and carbonic anhydrase activity of *P. corilyolia*

Table 1 Effect of *Glomus mosseae* and press mud on the growth and yields of *Psoralea 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 mm^2	No. of seeds per plant
Control	C	65.01±1.22	37.21±0.44	79.71±2.56	18.54±0.37	31.08±0.63	10.41±0.20	10.22±0.16	246.60±7.80
Inoculated control	C_1	32.82±1.03	20.83±0.48	46.76±1.11	9.32±0.18	15.86±0.39	5.31±0.07	5.10±0.02	137.20±4.30
GM and PM one	T_1	47.91±1.50	27.38±1.35	53.60±0.89	13.83±0.34	22.37±0.28	7.21±0.08	7.79±0.19	183.20±6.35
week prior to *M. incognita*	T_2	61.32±1.99	36.22±0.92	76.49±0.96	17.1±0.20	29.91±0.25	9.31±0.06	9.48±0.04	215.20±6.10
GM and PM and	T_3	44.78±0.99	25.01±0.64	49.42±1.07	11.14±0.06	19.09±0.12	6.01±0.05	6.97±0.16	168.80±6.78
Mi Simultaneous inoculation	T_4	58.88±2.24	33.36±1.03	70.88±0.73	14.73±0.50	25.11±0.30	7.88±0.28	8.95±0.20	184.80±5.30
M. incognita one	T_5	33.42±1.16	22.31±0.77	47.80±1.27	9.95±0.07	15.96±0.25	5.80±0.25	6.06±0.07	148.60±4.33
week prior to GM and PM	T_6	53.03±1.00	26.15±0.50	61.23±0.83	11.85±0.10	19.97±0.48	6.95±0.18	7.08±0.11	149.40±8.82
L.S.D. (P≤ 0.05)		4.24	2.38	3.76	0.79	1.08	0.50	0.40	18.52

Each value is a mean of five replicates. GM - *Glomus mosseae,* and PM – Press mud

Table 2 Effect of *Glomus mosseae* and press mud on the number of galls, number of egg masses, number of eggs per egg mass, root and soil population and reproduction factor of *Psoralea corylifolia* inoculated with *M. incognita*

Treatments		No. of galls	No. of egg masses/plant	Root population	Soil population	Total population	Reproduction factor	RKI
Control	C	0	0	0	0	0	0	0
Inoculated control	C_1	77.8±1.88	136.8±3.05	3356.6±96.14	5804±10.23	9160.6±106.25	4.58	4
GM and PM one week	T_1	44.2±1.71	83.4±3.07	2321±72.02	3342.2±35.92	5663.2±107.09	2.83	4
prior to *M. incognita*	T_2	10.2±0.73	24.0±1.34	892.6±23.64	1022.4±13.69	1915.0±37.11	0.95	2
GM and PM and Mi	T_3	51.2±2.22	89.6±3.50	2584.8±39.31	3510.4±48.29	6095.2±83.18	3.04	4
Simultaneous inoculation	T_4	14.6±1.32	31.6±1.28	1071±12.13	1248.2±16.80	2321.2±27.69	1.06	3
M. incognita one week	T_5	61.4±2.04	115.4±3.42	2881.8±38.40	4125.2±40.88	7007.0±79.12	3.50	4
prior to GM and PM	T_6	24.4±1.53	38.0±1.92	1268.4±10.57	1766.8±17.96	3035.2±28.33	1.51	3
L.S.D. (P≤ 0.05)		4.60	7.23	138.29	80.50	201.74	0	0

Number and size of galls, number of egg masses and reproduction were all highest in the plants inoculated with nematode only. All the plants that were treated and inoculated with *G. mosseae* and press mud at different time intervals had the lowest gall development, smallest galls, lowest number of egg masses, and lowest nematode population with reproduction factor. When compared to the inoculated control plants, T_2 plants treated with *G. Mosseae* and press one week prior to

nematode inoculation demonstrate the greatest and most substantial reduction in all nematode developments (C_1). Additionally, highest reduction in root-knot index was observed in T_2 plants over the inoculated control plants (Table 2).

In the nematode management strategies Arbuscular mycorrhizal fungi (AMF) play an effective role to suppress nematode population and enhance the plant growth parameters. Symbiotic relationship of AMF renders several advantages like plant growth by secretion of hormones [49]. Natural symbionts, AM fungus supply necessary plant nutrients as well as better growth and yield [3], [10], [37]. AM fungi have induced tolerance against root-knot nematode, in several economic crop plants [2]. The results of the present experiment exhibited that the infection of root-knot nematode, *M. incognita* suppressed the growth of *Psoralea corylifolia*. Combined application of *G. mosseae* and press mud improved plant growth parameters, such as shoot and root length, shoot and root fresh and dry weight and leaf area and seed yield at different time intervals. Growth characteristics and physiological parameters are affected by nematode infection [13], [41, [46, [48].

The use of mycorrhizal fungi before to nematode inoculation inhibited the nematodes more than the use of mycorrhizal fungi after the nematodes [48]. This might be connected to the time required for mycorrhizal formation in the root cortex. Similarly, results of the present study clearly indicated that the plants treated with *G. mosseae* with press mud prior to *M. incognita* had significantly enhanced plant growth and development, as well as improved physiological parameters. Delaying the application of mycorrhizal fungi after nematode inoculation resulted in increased root galling and nematode population in the soil, as well as reduction of spore population and mycorrhizal fungi colonization. Changes in colonization of mycorrhizal fungi and their spore population have previously been documented in the presence of nematodes [12] and have been attributed mostly to competition between mycorrhizal fungi and *Meloidogyne* spp. for feeding locations and carbon substrates from host photosynthesis [21]. The reduction in the number and size of galls, egg masses, and nematode population may have resulted from *G. mosseae* root colonization prior to nematode invasion. Pathogen tolerance in plants was boosted by mycorrhizal fungus via physiological, biochemical, and molecular pathways [16], [55], [51].

Different studies have discovered that *G. mosseae* inoculation increased plant growth and nutrient intake, decreased nematode population, and inhibited gall development [26], [47], [48]. Our findings are corroborated by previous research by Gupta [19], who found that applying press mud increased maize growth and yield variables, as well as the absorption of key micronutrients and soil quality. Joshi and Sharma discovered that sugar press mud increased crop yield and soil quality because it is high in macro and micro plant nutrients [25]. Press mud application decreased gall development and nematode population, improved soil fertility, eliminated several plant diseases, and increased crop output [31-32]. Thus, it might be concluded that combining different doses of press mud (organic fertiliser) and *G. mosseae* (AM fungus) prior to *Meloidogyne incognita* invasion is highly successful in treating root-knot disease and improving growth and yield of *Psoralea corylifolia.*

CONCLUSION

Thus, it might be concluded that soil amendment with sugar press mud with *G. mosseae* increased *P. corylifolia* growth and reduced *M. incognita* population. The application of the 100 gm SPM with 600 propagules of *G. mosseae* prior to root-knot nematode inoculation was the best dose for the nematode management, on comparing with simultaneous and after inoculation of root-knot nematode. Thus, this type of approach may create an alternative for greenhouses, small areas, or for the use of patches. To know the best concentration of the mycorrhizal fungi (*G. mosseae*) and SPM can be performed in further studies to adjust the attention regarding the capability of nematode management at lower costs for farmers.

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