

**EVALUATION OF BOTANICALS FOR THE MANAGEMENT OF ROOT-KNOT
NEMATODE (*Meloidogyne graminicola*) IN RICE UNDER POT CONDITIONS**

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ABSTRACT

Rice (*Oryza sativa* L.) production is constrained by several pests and diseases, including the rice root-knot nematode, *Meloidogyne graminicola*. This study evaluated the effectiveness of nineteen fresh green-leaf botanical amendments into soil against *M. graminicola*. A pot experiment was conducted in a completely randomized design with 20 treatments, comprising nineteen botanicals and an untreated inoculated control with three replications. The fresh leaves were chopped and incorporated into nematode-infested soil at 25 g/kg soil and allowed to decompose for 15 days before sowing rice seeds. Root gall number, shoot and root length, and fresh shoot and root weight were recorded at 25 and 50 days after sowing (DAS). At 50 DAS, all botanical treatments reduced gall formation compared with the untreated control, which recorded 24.00 galls/plant. The lowest gall numbers were recorded with *Cannabis sativa* (1.56 galls/plant) and *Jatropha curcas* (1.78 galls/plant). Several botanicals also significantly improved plant growth. *Aegle marmelos* produced the longest shoot length (49.89 cm), *Chenopodium album* the longest root length (15.78 cm), *Datura alba* the highest shoot fresh weight (3.01 g), and *Azadirachta indica* the highest root fresh weight (3.88 g). Considering both nematode suppression and plant growth, *C. album*, *D. alba*, *A. indica*, and *A. marmelos* showed promising overall performance. These botanicals, therefore, warrant further evaluation under field conditions.

Keywords: Botanical amendments, eco-friendly management, nematode suppression, root gall

INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most important cereal crop, provides staple food for more than half of the global population (FAO, 2024). Rice production is affected by various biotic and abiotic stresses. Among biotic constraints, plant-parasitic nematodes are important but often underestimated pests of agricultural crops (Akhter et al., 2024). Rice is recorded to host approximately 300 nematode species belonging to 35 genera (Khan and Haque, 2023). About 10 of these genera are considered economically important in rice production and are accounted for nearly a 10% annual yield losses globally, equivalent to an estimated US\$ 16 billion (Mo et al., 2021). These microscopic, soil-dwelling organisms can invade plant roots and disrupt normal plant growth and physiological processes, resulting in reduced plant vigor and yield (Kantor et al., 2024).

Among the plant parasitic nematodes associated with rice, the rice root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield, is considered an important pest of rice, particularly in irrigated and upland rice production systems (Rusique et al., 2021; Akhter et al., 2024). Yield losses of 20-35% have been reported in India, and the nematode is considered an important constraint to rice production in several Asian countries, including India, Bangladesh, the Philippines, Thailand, Vietnam, Cambodia, and Indonesia (Khan and Haque, 2023). Under severe infestation, yield losses may reach up to 72% (Khan & Ahamad, 2020).

Synthetic nematicides have been used to manage plant-parasitic nematodes. However, their indiscriminate and repeated use may pose serious threats to human health, the environment, and non-target organisms and may contribute to changes in biodiversity and development of resistance. Consequently, increasing attention has been given to non-chemical approaches for nematode management. Plant-derived compounds with nematicidal or nematostatic activity have received particular attention (Chitwood, 2002). Plants produce a wide range of secondary metabolites as part of their natural defense mechanisms against pests and pathogens. Some of these compounds have nematicidal properties and can be exploited for crop protection.

Plant materials used for pest management are commonly referred to as “botanicals”. Compared with many synthetic pesticides, botanical products generally degrade more rapidly in the environment and may have fewer adverse effects on non-target organisms. Consequently, botanicals are being explored as alternative components of sustainable crop protection system (Singh and Prasad, 2014). The use of locally available plant materials as soil amendments may offer an economical, environmentally friendly approach to manage *M. graminicola*.

Although several studies have demonstrated the potential of botanicals against plant-parasitic nematodes, relatively few studies have evaluated locally available fresh green leaf amendments against *M. graminicola* in rice. Therefore, the present study evaluated the effects of nineteen fresh botanical leaf amendments on nematode-induced root galling and rice plant growth under controlled pot conditions. The study focused on identifying botanical treatments that could simultaneously suppress nematode infestation and improve plant growth.

MATERIALS AND METHODS

Location of the study

A pot experiment was conducted at the Department of Plant Pathology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India.

Experimental design, season and growing conditions

The experiment was laid out in a completely randomized design (CRD) with 20 treatments and three replications and conducted during the Kharif season of 2015-2016 in a naturally ventilated net-house. A commonly grown rice variety, PB-1121, in the region, was used. The soil was sandy loam in texture and steam-sterilized at 121°C for 1 hour before use to eliminate the native nematode population. Earthen pots of 5 kg capacity and dimensions of approximately 25 cm in diameter × 25 cm in height were filled with 3 kg of sterilized soil.

Incorporation of botanicals leaf amendments

Fresh, healthy green leaves of nineteen plant species were collected, washed, chopped, and incorporated into the soil at the rate of 25 g/kg soil. Each pot received 3 kg soil and 75 g chopped leaf material. The plant species evaluated include *Achyranthes aspera*, *Aegle marmelos*, *Azadirachta indica*, *Calotropis procera*, *Cannabis sativa*, *Catharanthus roseus*, *Chenopodium album*, *Curcuma domestica*, *Cymbopogon citrullus*, *Datura alba*, *Jatropha curcas*, *Lantana camara*, *Ocimum tenuiflorum*, *Parthenium hysterophorus*, *Prunus persica*, *Syzygium cumini*, *Thevetia peruviana*, *Tinospora cordifolia*, and *Withania somnifera*. Following incorporation of the botanical leaf material, pots were incubated for 15 days under natural net-house conditions at the College of Agriculture, SVPUAT, Meerut. Ambient temperatures ranged from 21-25 °C (night) to 34-42 °C (day), typical of the Kharif season, favoring microbial decomposition of the plant material. Soil moisture was maintained near field capacity through daily watering. This period allowed partial decomposition of the botanical amendments before sowing, consistent with incubation periods used in similar studies (Lopes et al., 2011).

Sowing rice seeds

Ten rice seeds were sown in each pot, and the seedlings were subsequently thinned to five plants per pot. The pots were irrigated daily to maintain adequate soil moisture without flooding, thereby simulating upland rice conditions.

Inoculation with *M. graminicola*

The plants were inoculated with second-stage juveniles (J2) of *M. graminicola* at the three-leaf stage. A total of 1,000 J2 were inoculated per pot. The concentration of second-stage juveniles (J2) was determined by averaging five counts of 1 mL aliquots under a stereobinocular microscope using a nematode counting dish (Syracuse watch glass). The nematode inoculum used was obtained from a pure culture of identified *M. graminicola* maintained in rice plants grown in pots in the department.

Observations taken

Root gall number, shoot length, root length, fresh shoot weight, and fresh root weight were recorded at 25 and 50 days after sowing (DAS). Observations for each growth and nematode parameter were recorded from the five plants within each pot and averaged to obtain a single value for each experimental unit. The experiment was terminated at 50 DAS.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 20.0, (IBM Corp., Armonk, NY, USA). Gall-count data were transformed using the square-root transformation, $\sqrt{(X + 0.5)}$, following Gomez and Gomez (1984), to stabilize variance before statistical analysis. The standard error of the mean [SE(m)±], and critical difference (CD) at $p = 0.05$ are presented with the treatment means in Table 1 and 2. Treatment means differing by a value equal to or greater than the corresponding CD were considered significantly different.

RESULTS

Effect of botanicals on root galling and plant growth at 25 DAS

The tested botanicals differed considerably in their effects on root galling and plant growth (Table 1). No galls were recorded in plants grown in soil amended with *Azadirachta indica*, *Calotropis procera*, *Chenopodium album*, *Jatropha curcas*, *Parthenium hysterophorus*, *Thevetia peruviana*, and *Tinospora cordifolia*. Among the treatments, the highest number of galls was recorded with *Curcuma domestica* (2.56 galls/plant), followed by *Cymbopogon citrullus* (0.56 galls/plant), whereas the untreated control recorded 4.22 galls/plant).

Several treatments significantly increased shoot length compared with the untreated control (21.89 cm). The longest shoot length was recorded in *Datura alba* (33.11 cm), followed by *Chenopodium album* (32.89 cm), *Cannabis sativa* (30.33 cm), and *Aegle marmelos* (29.67 cm). The shortest shoots were recorded in plants treated with *Cymbopogon citrullus* (15.67 cm).

The longest root length was recorded in *Chenopodium album* (9.56 cm), followed by *Azadirachta indica* (8.44 cm), *Aegle marmelos* (7.89 cm), and *Thevetia peruviana* (7.77 cm). These treatments recorded significantly longer root length than the untreated control (4.44 cm). In contrast, *Withania somnifera* (3.83 cm) and *Lantana camara* (4.11 cm) did not improve root length relative to the control.

The highest fresh shoot weight was recorded in *Aegle marmelos* (1.22 g), followed by *Catharanthus roseus* (1.07 g), *Prunus persica* (1.06 g), and *Chenopodium album* (1.05 g). The shoot weight of *A. marmelos* and *C. roseus* was statistically comparable. The lowest shoot weight was recorded in *Syzygium cumini* (0.15 g), lower than that of the untreated control (0.22 g).

For fresh root weight, *Catharanthus roseus* (0.87 g) and *Chenopodium C. album* (0.86 g) recorded the highest and were statistically at par. These were followed by *Thevetia peruviana* (0.69 g) and *A. marmelos* (0.65 g). The lowest root weight was recorded in *Lantana camara* (0.06 g), compared with 0.15 g in the untreated control.

Table 1. Effect of botanical amendments on root galling caused by *Meloidogyne graminicola* and growth of rice plant cv. PB-1121 at 25 days after sowing in pots at Meerut, India during 2015-16

Botanicals*	Number of root galls/plant		Shoot length (cm)		Root length (cm)		Shoot weight/plant (g)		Root weight/plant (g)	
	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±
<i>Withania somnifera</i>	0.33	0.011	28.33	0.327	3.83	0.066	0.43	0.012	0.14	0.004
<i>Lantana camara</i>	0.33	0.011	23.67	0.273	4.11	0.071	0.25	0.007	0.06	0.002
<i>Curcuma domestica</i>	2.56	0.089	25.00	0.289	5.39	0.093	0.54	0.016	0.28	0.008
<i>Aegle marmelos</i>	0.33	0.011	29.67	0.343	7.89	0.137	1.22	0.035	0.65	0.019
<i>Chenopodium album</i>	0.00	0.002	32.89	0.380	9.56	0.166	1.05	0.030	0.86	0.025
<i>Datura alba</i>	0.22	0.008	33.11	0.382	8.22	0.142	0.89	0.026	0.53	0.015

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Botanicals*	Number of root galls/plant		Shoot length (cm)		Root length (cm)		Shoot weight/plant (g)		Root weight/plant (g)	
	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±
<i>Azadirachta indica</i>	0.00	0.002	29.44	0.340	8.44	0.146	0.82	0.024	0.50	0.014
<i>Achyranthes aspera</i>	0.11	0.004	25.78	0.298	6.56	0.114	0.51	0.015	0.25	0.007
<i>Cannabis sativa</i>	0.44	0.015	30.33	0.350	6.78	0.117	0.48	0.014	0.17	0.005
<i>Jatropha curcas</i>	0.00	0.002	27.56	0.318	5.78	0.100	0.42	0.012	0.14	0.004
<i>Prunus persica</i>	0.11	0.004	29.56	0.341	6.89	0.119	1.06	0.031	0.31	0.009
<i>Catharanthus roseus</i>	0.33	0.011	27.00	0.312	6.78	0.117	1.07	0.031	0.87	0.025
<i>Syzygium cumini</i>	0.11	0.004	16.67	0.192	4.78	0.083	0.15	0.004	0.43	0.012
<i>Cymbopogon citrullus</i>	0.56	0.019	15.67	0.181	5.00	0.087	0.16	0.005	0.15	0.004
<i>Thevetia peruviana</i>	0.00	0.002	29.33	0.339	7.77	0.135	0.88	0.025	0.69	0.020
<i>Calotropis procera</i>	0.00	0.002	27.33	0.316	5.22	0.090	0.51	0.015	0.27	0.008
<i>Tinospora cordifolia</i>	0.00	0.002	29.11	0.336	6.56	0.114	0.77	0.022	0.47	0.014
<i>Ocimum tenuiflorum</i>	0.22	0.008	20.67	0.239	5.22	0.090	0.47	0.014	0.56	0.016
<i>Parthenium hysterophorus</i>	0.00	0.002	26.67	0.308	4.83	0.084	0.51	0.015	0.40	0.012
Untreated control	4.22	0.146	21.89	0.253	4.44	0.077	0.22	0.006	0.15	0.004
SE(m)±	0.17	—	0.76	—	0.52	—	0.02	—	0.01	—
C.D. (p = 5%)	0.491	—	2.184	—	1.506	—	0.066	—	0.021	—

*Each treatment received 75 g green leaf amendment per 3 kg soil.

Effect of botanicals on root galling and plant growth at 50 DAS

At 50 DAS, significant differences were observed among the botanical treatments (Table 2). The lowest number of galls/plant was recorded in *Cannabis sativa* (1.56), followed by *Jatropha curcas* (1.78), which were statistically at par. The maximum number of galls was recorded in *Syzygium cumini*, (6.67) followed by *Curcuma domestica* (5.22) which differed significantly from each other. The untreated control recorded the highest gall count overall (24 galls/plant).

All botanical treatments produced a significantly greater shoot length than the untreated control (29.78 cm). The greatest shoot length was recorded in *Aegle marmelos* (49.89 cm), followed by *Chenopodium album* (49.00 cm), *Datura alba* (46.89 cm), and *Achyranthes aspera* (45.45 cm). The shortest shoot length was recorded in *Cymbopogon citrullus* (21.11 cm).

The longest root length was recorded in *Chenopodium album* (15.78 cm), followed by *Catharanthus roseus* (14.56 cm), *Achyranthes aspera* (14.00 cm), and *Datura alba* (13.78 cm). The shortest root length was recorded in *Lantana camara* (6.44 cm), lower than (7.33 cm) the untreated control).

All botanical treatments significantly increased fresh shoot weight compared with the untreated control. The highest shoot weight was recorded in *Datura alba* (3.01 g), followed by *Chenopodium album* (2.96 g), *Achyranthes aspera* (2.33 g), and *Withania somnifera* (1.87 g). The lowest shoot weight was recorded in *Syzygium cumini* (0.23 g), compared with (0.53 g) in the untreated control.

For root fresh weight, *Azadirachta indica* recorded the highest value (3.88 g), followed by *Chenopodium album* (3.03 g), *Datura alba* (2.54 g), and *Jatropha curcas* (1.98 g). The lowest root weights were recorded in *Lantana camara* (0.30 g) and *Cymbopogon citrullus* (0.31 g), both of which were comparable with the untreated control (0.32 g).

Table 2. Effect of botanical amendments in soil on root galling caused by *Meloidogyne graminicola* and growth of rice plant cv. PB-1121 at 50 days after sowing in pot experiment at Meerut, India during 2015-16

Botanicals*	No. of galls/ plant		Shoot length (cm)		Root length (cm)		Shoot weight/ plant (g)		Root weight/ plant (g)	
	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±	Mean	SE(m)±
<i>Withania somnifera</i>	1.89	0.18	40.56	4.39	10.34	0.56	1.87	0.12	0.99	0.13
<i>Lantana camara</i>	2.11	0.23	32.22	3.21	6.44	0.36	0.92	0.09	0.30	0.13
<i>Curcuma domestica</i>	5.22	0.56	33.55	2.93	10.44	0.64	0.84	0.05	0.68	0.10
<i>Aegle marmelos</i>	1.89	0.22	49.89	3.94	13.11	0.86	1.53	0.07	1.10	0.13
<i>Chenopodium album</i>	2.33	0.20	49.00	2.78	15.78	1.56	2.96	0.24	3.03	0.19
<i>Datura alba</i>	3.11	0.21	46.89	4.43	13.78	1.57	3.01	0.35	2.54	0.29
<i>Azadirachta indica</i>	2.00	0.14	38.67	2.66	13.11	1.17	1.12	0.05	3.88	0.45
<i>Achyranthes aspera</i>	2.44	0.24	45.45	3.71	14.00	0.70	2.33	0.20	1.52	0.13
<i>Cannabis sativa</i>	1.56	0.20	36.89	2.43	10.11	0.64	0.97	0.10	0.50	0.15
<i>Jatropha curcas</i>	1.78	0.19	27.58	1.76	13.28	1.27	1.52	0.16	1.98	0.13
<i>Prunus persica</i>	2.33	0.25	40.56	4.78	12.67	1.23	1.70	0.10	1.67	0.14
<i>Catharanthus roseus</i>	3.11	0.21	36.11	3.26	14.56	0.96	1.46	0.10	1.90	0.21
<i>Syzygium cumini</i>	6.67	0.27	21.78	1.98	10.39	1.17	0.23	0.03	0.87	0.10
<i>Cymbopogon citrullus</i>	4.89	0.47	21.11	1.34	10.00	0.67	0.35	0.04	0.31	0.16
<i>Thevetia peruviana</i>	2.44	0.16	36.00	3.06	10.94	0.78	1.17	0.09	1.08	0.12
<i>Calotropis procera</i>	4.00	0.45	36.33	2.89	11.22	0.94	1.14	0.07	1.54	0.12
<i>Tinospora cordifolia</i>	3.33	0.31	35.33	1.89	13.50	0.88	1.45	0.09	1.25	0.11
<i>Ocimum tenuiflorum</i>	4.11	0.32	30.67	2.97	9.33	0.98	0.68	0.04	0.76	0.15
<i>Parthenium hysterophorus</i>	2.11	0.24	31.89	1.65	9.89	1.03	0.76	0.05	0.60	0.13
Untreated control	24.00	2.61	29.78	2.96	7.33	0.33	0.53	0.04	0.32	0.16
SE(m)±	0.40	-	0.41	-	0.53	-	0.07	-	0.27	-
C.D. (p = 5%)	1.144	-	1.169	-	1.506	-	0.192	-	0.765	-

* Each treatment received 75 g green leaf amendment per 3 kg soil.

Correlation between the gall numbers and different plant growth parameters

Correlation analysis revealed a negative relationship between gall number and all measured growth parameters, indicating that increasing nematode infestation progressively suppressed plant growth (Fig. 1). The strongest association was observed for root length ($r = -0.44$, $p = 0.050$), followed by shoot weight ($r = -0.36$), shoot length ($r = -0.33$), and root weight ($r = -0.32$). Overall, this supports the inference that botanicals effective in suppressing gall formation also favored better plant growth,

consistent with the reduced root damage and improved nutrient and water uptake associated with lower nematode infestation.

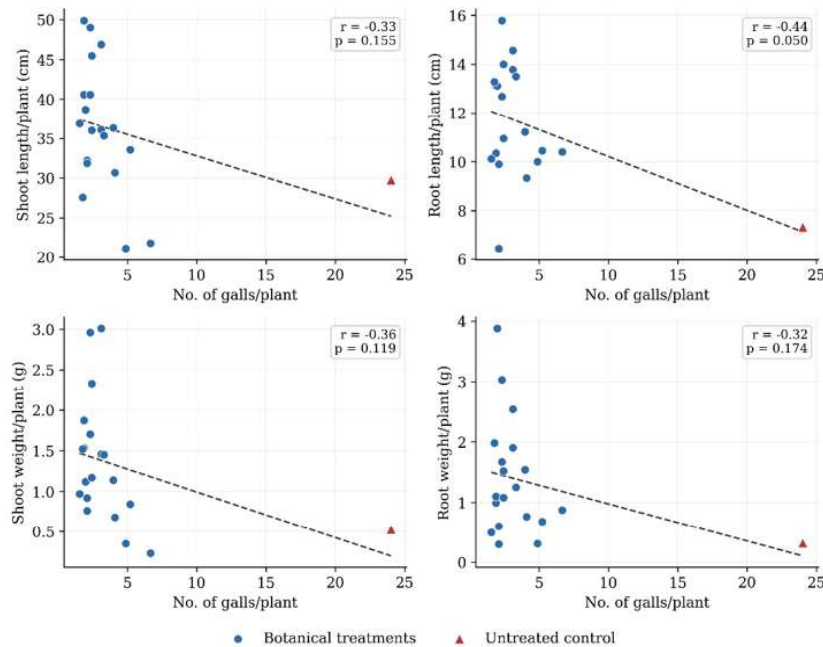


Fig. 1. Relationship between the number of galls caused by *Meloidogyne graminicola* and growth parameters of rice cv. PB1121.

DISCUSSION

The present study demonstrated considerable variation among the botanical leaf amendments in their effects on root gall formation and rice plant growth. This finding is consistent with earlier reports showing the potential of plant-derived materials as soil amendments for suppressing root-knot nematodes.

Lopes et al. (2011) reported that soil amendments reduced gall formation and egg production of *Meloidogyne javanica* in tomato, although the level of suppression varied with the type of plant material used. Neem leaves reduced gall formation by 61%, whereas ground castor leaves resulted egg production by 69%. The relatively strong performance of *Azadirachta indica* in the present study is in agreement with the reported nematicidal potential of neem-based plant materials. The differences observed among the botanical treatments also suggest that the type and form of plant material may influence their effectiveness against root-knot nematodes.

The improvement in shoot and root growth observed with several botanical treatments is also consistent with the findings of Dongre and Simon (2013), who reported increased shoot and root growth in rice treated with botanical leaf and root extracts and challenged with *M. graminicola*. In the

present study, *Chenopodium album*, *Azadirachta indica*, *Aegle marmelos*, and *Thevetia peruviana* produced relatively better growth responses, although the magnitude of response varied among plant species and between the two observation periods.

At 50 DAS, *Cannabis sativa* and *Jatropha curcas* recorded the lowest numbers of galls, whereas some other botanical treatments produced greater improvements in plant growth. Parihar et al. (2012) reported that fresh chopped leaves of *Datura stramonium* reduced root-knot nematode reproduction while improving plant growth. The relatively good performance of *Datura alba* in the present study, particularly in terms of shoot length and shoot fresh weight, is therefore consistent with the reported potential of *Datura* species for suppressing root-knot nematodes and plant growth.

The reductions in gall numbers accompanied by improved plant growth may be due to both nematode suppression and improved soil conditions. Decomposing botanical materials can add organic matter and release of macro- and micro-nutrients thereby improving nutrient availability and supporting plant growth. Organic amendments may also increase microbial activity and nutrient cycling in the soil, which can benefit plant growth when their effect on nematodes is limited (Thoden et al., 2011). Similar observations were reported by Tiwari et al. (2024), who found that organic amendments were effective against *M. graminicola* and contributed to improved soil health.

In addition to their nutritional effects, decomposing plant materials may produce compounds such as fatty acids or ammonia that can adversely affect nematodes. Some plant residues may also contain secondary metabolites with nematocidal properties. These effects could reduce nematode penetration, feeding and subsequent gall development. Reduced nematode infection would help maintain root function and improve the uptake of water and nutrients, which may explain the higher shoot and root biomass recorded in treatments such as *Chenopodium album* and *Azadirachta indica*.

The relationship between reduced galling and improved plant growth was not consistent among all treatments. For example, *Chenopodium album* produced the highest biomass despite showing only a moderate reduction in galling. This suggests that its effect on plant growth may have been influenced not only by nematode suppression but also by the nutritional and soil-improving effects of the incorporated plant material. In contrast, treatments with greater reductions in galling but relatively modest plant growth may have had stronger effects on the nematode without providing the same benefit to the plant growth.

The present findings are also comparable with those of Hasan et al. (2021), who reported that *Calotropis procera*, *Lantana camara* and *Achyranthes aspera* reduced root-knot nematode galling and increased plant length, biomass and chlorophyll content in spinach. These results suggest that botanical amendments can influence both nematode development and plant growth, although the extent of these effects may differ among plant species.

Overall, the effectiveness of the botanical amendments varied in their effects on nematode suppression and plant growth. *Chenopodium album* and *Aegle marmelos* performed relatively well for both parameters, whereas some other treatments showed a stronger effect on either gall suppression or plant growth. For example, *Syzygium cumini* had relatively high gall numbers at 50 DAS and produced poor shoot growth. These differences indicate that botanical amendments should

be selected not only for their ability to suppress *Meloidogyne* spp. but also for their effects on crop growth and soil conditions.

CONCLUSIONS

Among the nineteen botanical leaf amendments evaluated under controlled pot conditions, several treatments showed potential for reducing root gall formation and improving rice plant growth. At 50 DAS, *Cannabis sativa* and *Jatropha curcas* recorded the lowest numbers of galls. Considering both nematode suppression and plant growth, *Chenopodium album*, *Datura alba*, *Azadirachta indica*, and *Aegle marmelos* showed comparatively better overall performance. These botanical amendments may therefore have potential for managing root-knot nematodes in rice, although their effectiveness needs to be further evaluated under field conditions.

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