

Journal of Biological Control, 39(1): 130-139, 2025, DOI: 10.18311/jbc/2025/45425

Research Article

Urtica dioica L.: A natural ally for eco-friendly management of *Meloidogyne incognita* in tomato

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ABSTRACT: The perennial stinging nettle, *Urtica dioica* L., is a common weed with extensive medicinal uses. Despite its reputation as a weed, *U. dioica* offers numerous benefits, including antioxidant, antimicrobial, and antihelminthic properties, which can be harnessed for agricultural purposes. This study evaluates the efficacy of the Aqueous Leaf Extract (ALE) of *U. dioica* against the root-knot nematode, *Meloidogyne incognita*, a major pest in tomato cultivation. We investigated ALE's effects on nematode egg hatching and mortality and explored greenhouse applications integrating *U. dioica* green manure with spent mushroom substrate of *Pleurotus florida* and combining it with lime and ammonium bicarbonate. Our results demonstrated that ALE significantly inhibits egg hatching and increases nematode mortality, with enhanced efficacy observed at higher concentrations and longer exposure periods. The formation of giant vacuoles in nematodes exposed to ALE further support its efficacy. This approach offers a promising, eco-friendly alternative to chemical nematicides, contributing to more sustainable agricultural practices.

KEYWORDS: Botanical, *Meloidogyne incognita*, *Pleurotus florida*, spent mushroom substrate**,** *Urtica dioica*

(Article chronicle: Received: 08-10-2024; Revised: 28-02-2025 ; Accepted: 03-03-2025)

LIST OF ABBREVIATIONS

ALE – Aqueous leaf extract
GM – Green manure
SMS – Spent Mushroom Substrate
LAB – Lime + Ammonium Bicarbonate

INTRODUCTION

The perennial stinging nettle, scientifically named *Urtica dioica* L., is a common weed found in temperate regions of the world. It is a medicinal plant falling into the family *Urticaceae*. In Uttarakhand, India, it goes by the local name 'bicchu ghas' and has been utilized for centuries due to its diverse applications and renowned medicinal qualities. This plant boasts widely spreading rhizomes that extend extensively through the surrounding soil, taking up considerable space. It synthesizes numerous chemical compounds, among which formic acid is notable (Bisht *et al.*, 2012). Despite its status as a weed, *U. dioica* possesses diverse medicinal and pesticidal properties that can be harnessed for agricultural purposes. The leaves of *U. dioica* provide a rich source of potent antioxidants and exhibit significant antimicrobial and antihelminthic properties (Taheri *et al.*, 2022). *U. dioica* has found multifaceted applications in agriculture, serving various purposes ranging from providing nutritional benefits to plants to safeguarding them against pests. Its efficacy has been noted in combating plant pests such as fungi (Hadizadeh *et al.*, 2009; Ferreira & Oliveira, 2021; Behiry *et al.*, 2022), aphids (Wulf *et al.*, 2023), and nematodes (Nasiri *et al.*, 2014; Ismail *et al.*, 2020). Furthermore, its widespread availability in Uttarakhand makes it a readily accessible and cost-effective resource for farmers seeking sustainable pest management solutions.

The second-most valuable vegetable crop globally is tomato, and its demand is escalating in the world market (FAOSTAT, 2017). Among the array of biotic stresses posing threats to tomato cultivation, the damage inflicted by nematodes is particularly significant and cannot be overlooked. *M. incognita* stands out as the most prevalent species of root-knot nematode, responsible for causing substantial yield losses in tomato cultivation (Seid *et al.*, 2015). The management of plant parasitic nematodes in tomato cultivation has traditionally relied heavily on chemical nematicides. However, due to their adverse effects on human health and the environment, there's a growing imperative to

explore alternative, integrated, and eco-friendly approaches to combat these increasingly prevalent pests.

In the eco-friendly management of nematodes, integrating various components holds paramount importance. Instead of relying solely on a single approach, employing a combination of methods offers a more effective means of nematode management. By integrating different components, synergistic effects can be achieved, resulting in enhanced control of nematode populations. Therefore, in the present work, *in vitro* and *in vivo* studies have been conducted to evaluate the efficacy of the aqueous extract of leaves of *U. dioica,* sourced from the Uttarakhand hills, against *M. incognita*. Our research extends to greenhouse conditions, where nematode management is explored through the integration of *U. dioica* green manure and the spent mushroom substrate of *Pleurotus florida*, as old mushroom compost of *Pleurotus* spp. has been used to control plant parasitic nematodes under greenhouse conditions (Palizi *et al.* 2009; Chandana *et al.*, 2023). Additionally, we investigated an innovative approach by combining two environmentally friendly chemicals, lime and ammonium bicarbonate (Su *et al.*, 2017), with the aqueous leaf extract of *U. dioica*. Top of FormThis holistic approach not only diversifies the mechanisms of action but also promotes sustainability by minimizing reliance on chemical interventions. Furthermore, combining different strategies mitigates the risk of nematodes developing resistance to specific control methods. Overall, the integration of diverse components in nematode management strategies not only improves efficacy but also aligns with environmentally conscious practices, ensuring long-term sustainability in agricultural systems.

MATERIALS AND METHODS

Obtaining and preparing the nematode inoculum

The original population was obtained by collecting a single egg mass of *M. incognita* from infected roots of tomato plants and transferring it to Petri dishes containing a small amount of sterile distilled water. The identification of the species followed the method of the perineal pattern of adult females as described by Eisenback (1985). Petri dishes were incubated at 23 to 25°C for 3 days (Den Ouden, 1958). The population multiplication was carried out in the tomato plant's 'Lakshmi' cultivar (September 2022) under greenhouse conditions. The inoculum for the experiments was obtained from the galled roots with egg masses from the pure culture of nematodes following the Modified Baermann funnel technique. The soil for the pot experiments was collected from the polyhouse situated at Narayanpur village of the Udham Singh Nagar district in Uttarakhand, where heavily infected tomato plants were grown.

Preparation of aqueous extracts from leaves of *U. dioica*

Leaves of *U. dioica* plants were collected from KVK, Jeolikote, and Nainital (D) in February 2022. *U. dioica* leaves were chopped into small pieces and shade-dried for a week. Dried leaf material was ground into fine powder. The leaf powder was later passed through 20 mesh-size sieves to get a fine powder. A 10 per cent stock solution for *in vitro* tests was prepared and left 24 h for soaking. The solution was filtered through Whatman filter paper, centrifuged at 2000 rpm, and used in various dilutions. The pH of the botanical was checked before use and was found neutral. The temperature recorded during extract preparation was 27±*1.0ºC.*

In vitro evaluation of efficacy of aqueous leaf extract on egg hatching and mortality of *M. incognita*

Eggs and newly hatched second-stage juveniles of *M. incognita* were acquired from a maintained pure culture. Hatching and mobility experiments were conducted in gridded Petri dishes. 100 *M. incognita* eggs were suspended in varying concentrations (1%, 2%, 3%, and 5%) of ALE, along with a control group. Similarly, 100 freshly hatched second-stage juveniles were suspended in 1%, 2%, 3%, and 5% ALE of *U. dioica*. All treatments were replicated five times in a completely randomized design and maintained at 27ºC in a BOD incubator. Hatchability observations were recorded at 12, 24, 48, and 72 h by counting hatched eggs under a microscope at 4x magnification (Hussey & Barker, 1973). Mortality observations for J2 were recorded over the same periods. Nematodes that appeared straight in posture were picked and placed in distilled water to confirm larval death. Those nematodes that showed mobility or appeared in winding shapes were considered alive.

In planta evaluation of the botanical

Three experiments were conducted in April 2022 at the Department of Plant Pathology's glasshouse in G. B. Pant University of Agriculture and Technology, Pantnagar. In each experiment, previously sterilized pots were filled with 2 kg of infested soil. Tomato seedlings of the Lakshmi cultivar, aged 21 days, were transplanted into each pot, and the plants were maintained in the greenhouse at 35°C. Control pots contained only nematode-infested soil. The treatments of all three experiments were arranged in a completely randomized design in the glasshouse.

Nematicidal potential of U. dioica ALE

In the assessment of *U. dioica* ALE as a nematicide, three distinct treatments with concentrations of 5, 10 and 15 per cent were administered by drenching 250 ml of ALE per pot containing 2 kg of soil during transplanting. Conversely, another set of three treatments with identical concentrations

underwent drenching at two distinct intervals: the first application coincided with transplanting, while the second occurred 10 days thereafter. Pots without any treatment were kept as the control group. The treatments for the experiment were determined based on the results obtained from the *in vitro* test, comprising seven distinct treatments, each replicated three times. The treatments involved varying concentrations and applications of ALE of *U. dioica* against *M. incognita*. T1, T2 and T3 consisted of a single application of 5, 10 and 15% ALE to *M. incognita*-infested soil, respectively, at the time of transplanting. Whereas, T4, T5 and T6 involved double applications of 5, 10 and 15% ALE, respectively- first at the time of transplanting and second at 15 days later. T7 served as a negative control, with only *M. incognita* in the soil and no ALE applied.

Combined effect of U. dioica Green Manure (GM) and P. florida Spent Mushroom Substrate (SMS) on M. incognita population

Preparation of green manure (GM) of *U. dioica*

Fresh leaves of *U. dioica* were chopped into small pieces and added in different amounts according to treatments into sterilized plastic pots of 2 kg capacity containing 1 kg of autoclaved soil. Pots were watered sufficiently and left for decomposition under glasshouse conditions at a temperature of 30±1ºC for 30 days.

Obtaining SMS of *P. florida*

Fresh leaves of *Urtica dioica* were finely chopped and added in varying quantities, as per the treatments, to sterilized 2 kg capacity plastic pots containing 1 kg of autoclaved soil. The pots were adequately watered and left to decompose under glasshouse conditions at a temperature of 28 ± 1°C for 30 days. The SMS (spent fungal mat + wheat straw) was sourced from the oyster mushroom production unit at the Mushroom Research and Training Centre, G. B. Pant University of Agriculture and Technology, Pantnagar. It was collected after the harvest of the white oyster mushroom (*Pleurotus florida*) crop had been completed.

Different amounts of fresh leaves of *U. dioica* (20, 40 and 60 g) were added into 1 kg soil; these were considered 3 treatments. Another treatment with 100 g SMS per kg nematode-infested soil was maintained. The quantity of SMS applied from *P. florida* remained constant across the other three treatments, with only the amount of green manure being varied. 1 kg of nematode-infested soil was mixed with 1 kg of green manure and 50 g of SMM from different treatments. Pots containing only nematode-infested soil were taken as a control. The experiment included eight treatments, each replicated three times. T1, T2 and T3 involved 20 g, 40 g and 60 g of green manure, respectively; T4 included 100 g of SMS; T5, T6 and T7 combined 100 g of SMS with 20 g, 40 g and 60 g of green manure, respectively. T8 served as the control, with only *M. incognita* in the soil and no green manure or SMS applied.

Combined effect of Lime + Ammonium Bicarbonate (LAB) and ALE on M. incognita population

The experiment consisted of eight treatments, each replicated three times, to evaluate the combined effects of *U. dioica* ALE and LAB against *M. incognita*. T1, T2 and T3 involved the application of 5, 10 and 15% ALE of *U. dioica*, respectively, to nematode-infested soil. T4 included the application of LAB in infested soil. T5, T6 and T7 combined LAB with 5, 10 and 15 per cent ALE, respectively, in nematode infested soil. T8 served as the negative control, involving only *M. incognita* without ALE or LAB. In each pot, 1 g of ammonium bicarbonate and 2 grams of lime were introduced per kilogram of nematode-infested soil. The moisture content in the pots was adequately maintained during the application of LAB. To enhance the efficiency of soil treatment, the pots were covered with polythene and left undisturbed for 15 days to facilitate fumigation. Following this, ALE of *U. dioica* was applied by drenching 250 ml of the botanical per each LAB-treated pot at the time of transplanting according to different treatments.

Data collection and observations of pot experiments

Observations on the growth parameters of tomato plants were recorded 60 days after the treatment (Table 1).

Observations on the following reproduction parameters of *M. incognita* were recorded 60 days after the treatment.

Table 1. Observations recorded on the growth parameters of tomato

Growth parameter	Unit	Data collection
Root length	cm*	Recorded from the base of the stem to the tip of the root after harvesting
Shoot length	cm	Recorded from the tip of the plant to the base of the stem after harvesting
Fresh shoot weight	g*	Recorded immediately after harvest, using electronic balance by separating root portion
Dry shoot weight	g	Recorded after complete drying of shoot portion
Fresh root weight	g	Recorded immediately after harvest by removing adhered soil and then cleaning roots
Dry root weight	g	Recorded after complete drying of the root portion

cm-centimeters, g-grams

i. Number of knots per root system: The Total number of galls present in the root system was recorded after harvest.

ii. Nematode population in soil: The nematode population in 250 g of soil was counted using Cobb's sieving and decantation method. The initial population of J2 of the root-knot nematode was calculated, representing the J2 population in the soil before soil treatment. The final population of the root-knot nematode was recorded 60 days after the treatment, at the time of termination of the experiment.

iii. The Reproduction Factor (Rf) was calculated by using the formula , where, Pi is the initial population and Pf is the final population of J2 in soil.

Per cent increase and decrease in various parameters were calculated over control using the following formula;

$$\text{Reduction (\%)} = \frac{\text{Control}-\text{Treated}}{\text{Contol}} \times 100$$

$$\text{Increase (\%)} = \frac{\text{Treated}-\text{Control}}{\text{Contol}} \times 100$$

Statistical analysis

The data comprising the effect of aqueous extract of leaves of *U. dioica* on egg hatchability and mortality of J2 were analysed by two-factor ANOVA, whereas the data comprising pot experiments like the growth parameters of tomato and reproduction parameters of *M. incognita* were analysed by one-factor ANOVA. Pearson's correlation was calculated for egg hatching and mortality of J2 at different exposure periods. Tukey's multiple range test has been applied to assess the differences among and between the treatments. A goodness of fit was analysed between concentrations and different exposure periods on egg hatching and mortality of *M. incognita*. A unit change in egg hatching and mortality at different concentrations and exposure periods was analysed by applying regression. Data were analysed using OPSTAT software.

RESULTS

Effect of ALE on egg hatching and mortality of *M. incognita*

Aqueous Leaf Extract (ALE) of *U. dioica* at various concentrations significantly affected the egg-hatching and mortality of *M. incognita* at different exposure periods. All the treatments were significantly different from each other and the control. The interaction of concentration and exposure period on egg hatching and mortality was also highly significant.

With an increase in the concentration of ALE of *U. dioica* and an increase in the exposure period, a significant decrease in egg hatching and a significant increase in mortality of J2 was noticed, which infers that egg hatching is negatively correlated and mortality is positively correlated with concentration and exposure period as shown in Table 2. Linear models provided a good fit for the relationships between concentrations and different exposure periods on egg hatching and mortality of *M. incognita*. The individual decrease in egg hatching and increase in mortality of *M. incognita* at each exposure period is shown in Table 3 through regression equations. The values of LC_{50} were calculated at different exposure periods, as represented in Figure 1.

When J2s were exposed to ALE of *U. dioica*, they exhibited rapid vacuolation, leading to lethargy. Subsequently, these vacuoles expanded and merged to form a conspicuous giant vacuole, as shown in Figure 2. This distinct giant vacuole formation was consistently observed in all deceased nematodes, setting them apart from those that expired under normal conditions. Notably, ruptures of these giant vacuoles were also detected. Consequently, the middle and posterior sections of the deceased nematodes displayed the formation of these giant vacuoles. Rajasekharan *et al*. (2017) reported similar findings in their study, noting that methuotic or vacuolar death in pinewood nematode, *Bursaphelenchus xylophilus*, marked by vacuolization, frequently involves the formation of numerous vacuoles, which later coalesce to create giant vacuoles.

Effect of ALE of *U. dioica* on the population of *M. incognita* under glasshouse conditions

The highest increases in plant length and weight were observed in the 15% double application treatment (T6) (Table 4). Although root and shoot lengths showed significant differences compared to the control, they did not vary significantly across the treatments. Plant weight was notably higher in all double application treatments compared to others, suggesting that the second application effectively eradicated any remaining nematodes after the initial treatment.

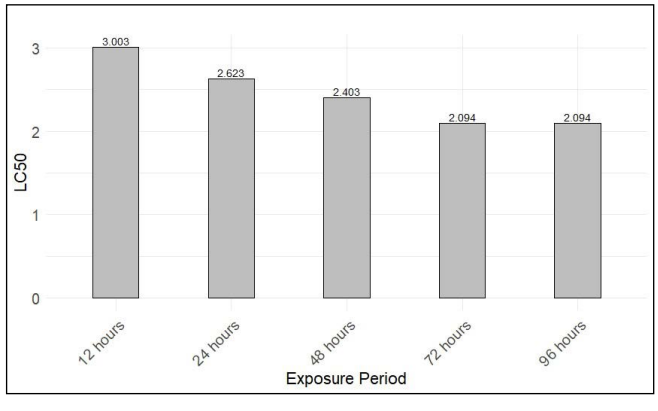


Figure 1. LC_{50} values of *M. incognita* treated with Aqueous Leaf Extract of *U. dioica.*

Table 2. Effect of aqueous leaf extract (ALE) of *U. dioica* on egg hatching and mortality of *M. incognita*

Concentration of ALE of *U. dioica* (%)	Egg hatchability at different exposure periods (in hours)				Mortality of J2 at different exposure periods (in hours)			
	12	24	48	72	12	24	48	72
1.0	9.10 (82.33)^{a}	9.51 (90)^{a}	9.57 (91)^{a}	9.57 (91)^{ab}	0.71 (0)^{d}	0.71 (0)^{d}	2.31 (5)^{d}	3.58 (12.33)^{d}
2.0	7.36 (53.67)^{b}	8.09 (65)^{b}	8.84 (77.67)^{b}	9.03 (81)^{b}	3.38 (11)^{c}	3.94 (15)^{c}	4.98 (24.33)^{c}	5.43 (29)^{c}
3.0	4.46 (19.33)^{c}	4.70 (21.67)^{c}	4.98 (24.33)^{c}	4.98 (24.33)^{c}	7.80 (60.33)^{b}	8.22 (67)^{b}	8.67 (74.67)^{b}	9.53 (90.33)^{b}
5.0	0.71 (0)^{c}	0.71 (0)^{d}	0.71 (0)^{d}	0.71 (0)^{d}	9.14 (83)^{a}	10.02 (100)^{a}	10.02 (100)^{a}	10.02 (100)^{a}
0.0	9.46 (100)^{a}	9.92 (100)^{a}	10.02 (100)^{a}	10.02 (100)^{a}	0.71 (0)^{d}	0.71 (0)^{d}	1.22 (1)^{d}	1.77 (2.67)^{e}
CV (%)	10.81	10.85	6.98	8.62	7.44	5.76	7.45	7.01
SE (d)	4.59	4.90	3.34	4.17	1.87	1.71	2.49	2.68
Regression equations	y=-21.221x +97.752	y=- 21.869x +103.446	y=-21.978x +106.950	y=-22.023x+107.716	y=18.815x -10.527	y=22.338x-12.743	y=22.072x-7.559	y=22.013x-1.563
R^2	0.95	0.95	0.92	0.91	0.89	0.91	0.93	0.88
Pearson correlation	-0.976**	-0.972**	-0.961**	-0.955*	0.94*	0.96*	0.96**	0.94*
Factor	**C.D**		**SE (d)**		**C.D**		**SE (d)**	
Treatments (A)	4.291		2.130		2.15		1.07	
Exposure period (B/C)	4.291		2.130		2.15		1.07	
Factor (A X B/C)	9.595		4.763		4.81		2.38	

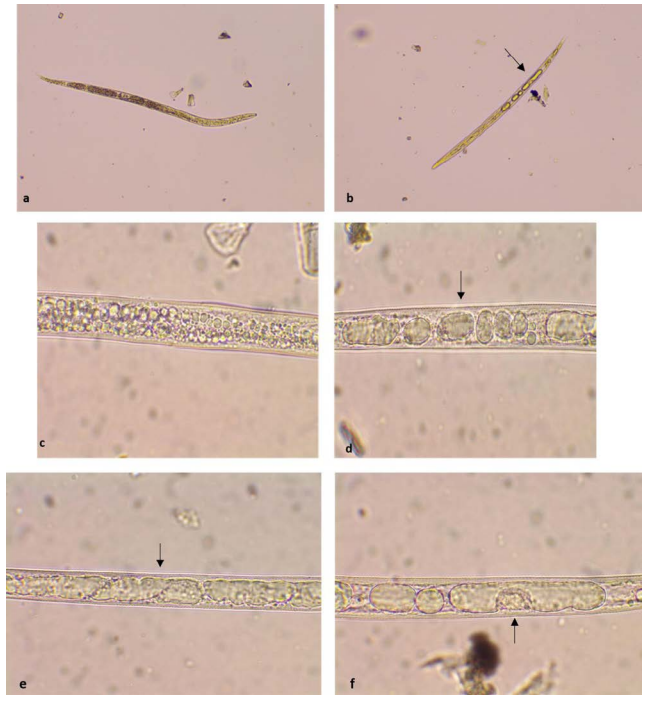


Figure 2. Nematode that has **(a)** normally been dead (control) **(b)** killed by *U. dioica* botanical **(c)** no vacuole formation in control **(d)** vacuole formation in nematodes treated with botanical **(e)** swelling and fusion of vacuoles into giant vacuoles **(f)** giant vacuolation, rupture of vacuoles and death of nematodes.

Table 3. Nematicidal potential of ALE of *U. dioica* on *M. incognita* under glasshouse conditions

Treatments	Growth Parameters							Reproduction parameters		
	Plant length (cm)		Plant weight (g)				Chlorophyll SPAD value			
	Root length	Shoot length	Fresh root weight	Dry root weight	Fresh shoot weight	Dry shoot weight		No. of knots/ root system	Final population	Reproduction factor
T1	5.38 (28.00)^{c}	8.91 (78.33)^{b}	5.45 (28.66)^{c}	1.99 (2.94)^{c}	10.63 (112.00)^{d}	4.97 (23.66)^{cd}	4.88 (22.78)^{e}	29.17 (850.00)^{b}	40.67 (1,653.66)^{b}	1.75
T2	5.42 (28.33)^{c}	9.18 (83.33)^{ab}	5.60 (30.33)^{c}	2.13 (3.53)^{bc}	12.12 (145.83)^{c}	5.13 (25.33)^{bc}	7.03 (48.47)^{c}	23.22 (538.00)^{c}	38.41 (1,474.66)^{b}	1.56
T3	5.59 (30.33)^{bc}	9.35 (86.33)^{a}	6.05 (35.66)^{b}	2.38 (4.68)^{b}	12.96 (167.00)^{b}	5.32 (27.33)^{b}	7.35 (53.05)^{b}	19.02 (360.66)^{e}	27.44 (752.66)^{d}	0.79
T4	5.59 (30.33)^{bc}	9.16 (83.00)^{ab}	6.07 (35.83)^{b}	2.21 (3.82)^{bc}	12.09 (145.16)^{c}	5.13 (25.33)^{bc}	7.07 (49.06)^{c}	22.26 (494.66)^{d}	33.11 (1,097.00)^{c}	1.16
T5	5.77 (32.33)^{ab}	9.36 (86.66)^{a}	6.27 (38.33)^{b}	2.38 (4.66)^{b}	12.96 (166.83)^{b}	5.59 (30.33)^{a}	7.73 (58.74)^{a}	17.66 (311.00)^{f}	23.12 (533.66)^{e}	0.57
T6	5.91 (34.00)^{a}	9.47 (88.66)^{a}	6.53 (41.66)^{a}	2.66 (6.05)^{a}	13.52 (181.66)^{a}	5.18 (25.83)^{bc}	7.82 (60.08)^{a}	14.46 (208.33)^{g}	17.39 (301.66)^{f}	0.32
T7	4.89 (23.00)^{d}	8.54 (72.00)^{c}	4.86 (22.66)^{d}	1.93 (2.73)^{c}	9.94 (97.83)^{e}	4.78 (21.83)^{d}	5.37 (27.79)^{d}	36.14 (1,305.00)^{a}	44.26 (1,826.66)^{a}	1.93
C.D.	0.204	0.207	0.171	0.195	0.239	0.149	0.203	0.503	2.124	*n.d*
C.V (%)	2.054	1.258	1.625	4.849	1.103	1.610	1.676	1.210	3.684	*n.d*
SE (d)	0.092	0.094	0.077	0.089	0.108	0.068	0.092	0.228	0.964	*n.d*

Initial population = 942.33/ 200 cc of soil. **n d*- not defined
Data in parentheses are the mean of nontransformed values. Data outside the parentheses are square root transformed $(x + 0.5)^{1/2}$ values.
Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Tukey's multiple range test.

Regarding *M. incognita* reproduction, there were significant differences in the number of knots per root system and the final nematode population among treatments. Both parameters decreased with higher botanical concentrations and more applications (Table 4). The control (T7) had the highest number of knots (1,305) and final population (1,826.66), while the 15% double drench (T6) had the lowest number of knots (208.33) and final population (301.66). The initial nematode population was 942.33 per 200 cc of soil. The control had a final population of 1,826.66 and a reproductive factor (Rf) of 1.93, indicating high nematode reproduction. In contrast, the 15% double application had a final population of 301.66 and the lowest Rf of 0.32, demonstrating the effectiveness of this treatment in managing nematodes.

Combined effect of *U. dioica* GM and SMS of *P. florida* on the population of *M. incognita* under greenhouse conditions

The greatest increases in plant length, weight, and chlorophyll content were observed in the 60g GM + 100g Spent Mushroom Substrate (SMS) treatment (T7), with minimal effects seen in the control (T8), as shown in Figure 3. Significant deviations from the control were noted in plant weight, with all growth parameters showing exponential increases in T7 compared to the control. Green manure alone (T1, T2, and T3) and SMS alone (T4) did not significantly manage nematodes. However, combining green manure and SMS (T5, T6, and T7) resulted in a notable increase in plant weight compared to the control and the treatments using only green manure or SMS.

The number of knots per root system and the final nematode population varied significantly among treatments compared to the control. The control (T8) had the highest number of knots (863.3) and final population (3,066.6), while the 60g GM + 100g SMS treatment (T7) had the lowest number of knots (180) and final population (465.3). Combining SMS with green manure led to reductions in both parameters. The reproductive factor (Rf) was highest in the control (1.75) and lowest in T7 (0.27) (Supplementary Table 1), demonstrating that the combined use of GM and SMS managed nematodes more effectively than either component alone.

Combined effect of ALE of *U. dioica* and lime + ammonium bicarbonate (LAB) on *M. incognita* under glasshouse conditions

The most significant increases in plant length, weight, and chlorophyll content were observed in T7 (15% ALE of *Urtica dioica* + LAB), as shown in Figure 4. While root length did not vary significantly compared to the control or among treatments, shoot length varied significantly, with the maximum measurements recorded in T7. This combination of T3 (15% ALE of *Urtica dioica*) and T4 (LAB) resulted in higher plant weight compared to each treatment individually, indicating the effectiveness of combining botanicals with

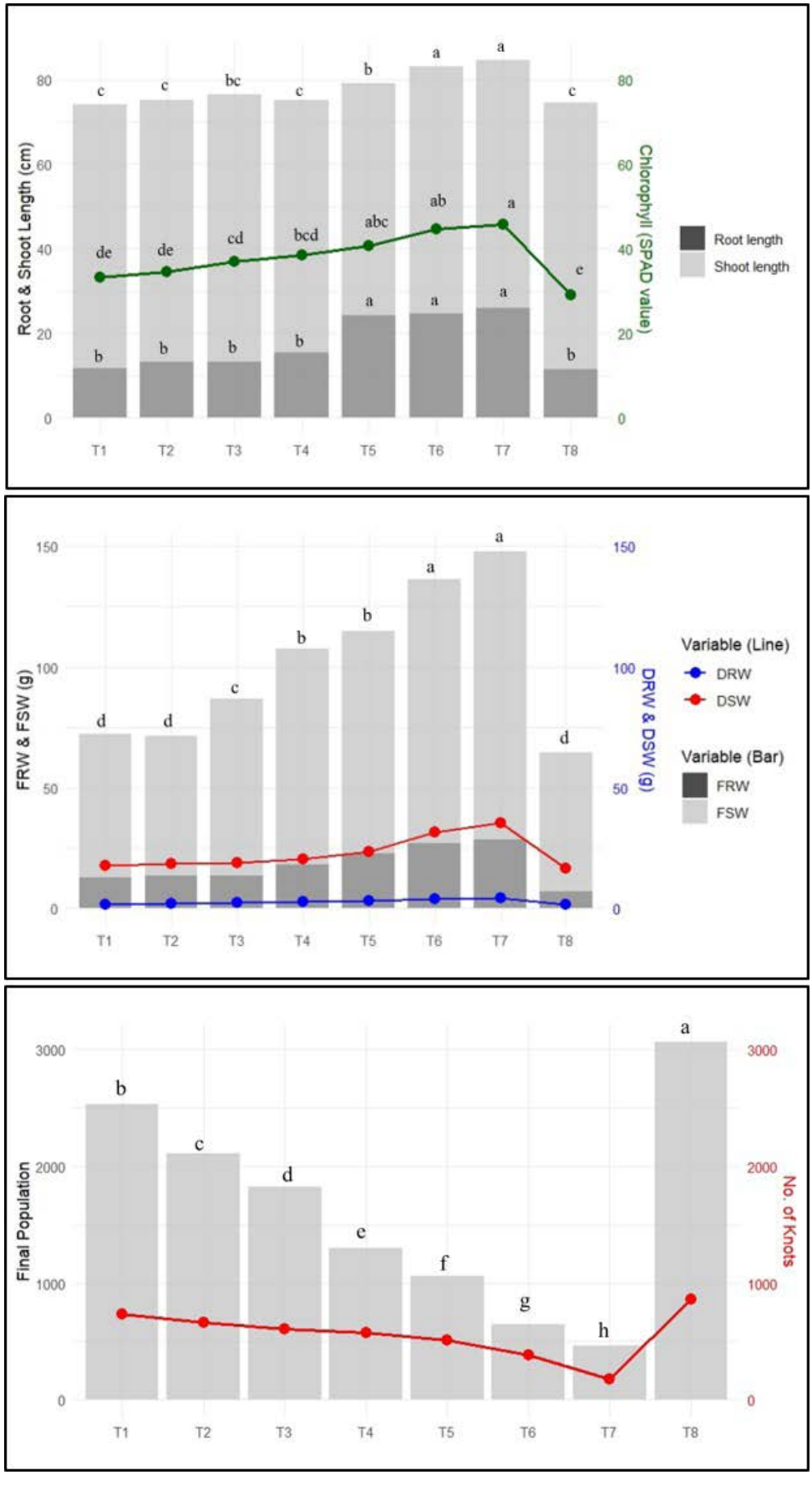


Figure 3. The combined effect of *U. dioica* GM and SMS of *Pleurotus florida* on growth parameters of tomato and reproduction parameters of *M. incognita* under glasshouse conditions.

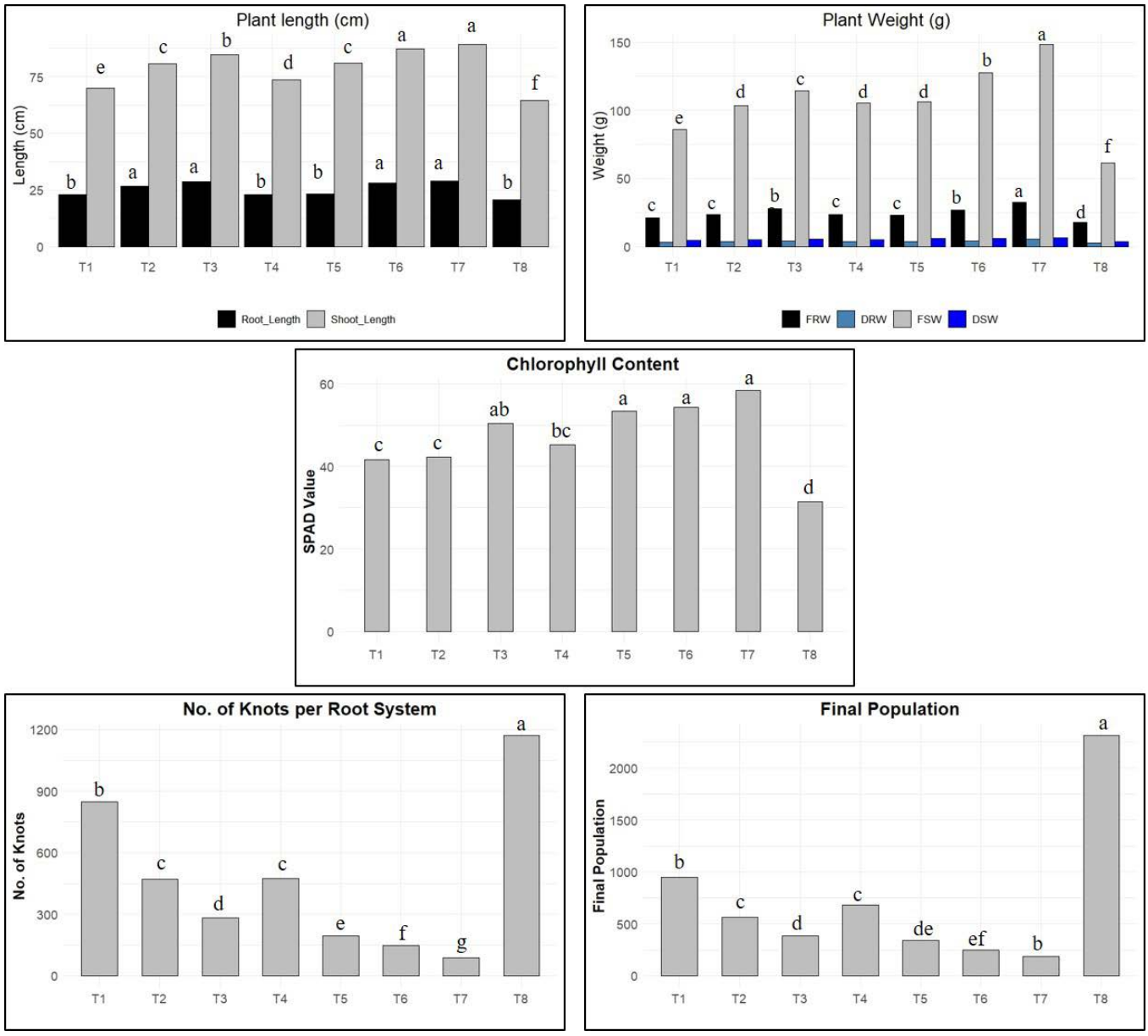


Figure 4. The combined effect of ALE of *U. dioica* and LAB on growth parameters of tomato and reproduction parameters of *M. incognita* under greenhouse conditions.

eco-friendly chemicals to enhance plant growth by managing nematodes.

Regarding *M. incognita* reproduction, significant variations were observed in the number of knots per root system and final nematode population among treatments compared to the control (Figure 4). Both parameters decreased with increasing concentrations of botanicals combined with LAB. The control (T8) had the highest number of knots (1,170) and final nematode population (2,312), while the lowest number of knots (88.33) and final population (185) were recorded in T7. The initial nematode population was 1,050 per 200 cc of soil, with a significant decrease in the final population in T7, demonstrating the lowest nematode population among treatments. The control had a reproductive factor (Rf) of 2.20, while T7 had the lowest Rf of 0.18 (Supplementary Table 2), reflecting the effectiveness of the combination in reducing nematode reproduction.

DISCUSSION

The present study showed the positive effect of the *U. dioica* weed plant and organic amendments in managing the nematode population in a sustainable and eco-friendly way. The study conducted by Hasan *et al.* (2021**)** provides considerable data for the use of weed plants such as *Calotropis procera, Ricinus communis, Lantana camara, Achyranthes aspera, Wedelia chinensis,* and *Colocasia esculenta* to manage the root-gall disease in spinach and the present study supports their findings by showing that the weed plants can

bring a significant increase in plant growth and physiological parameter by reducing root-knot development and nematode population. Ismail *et al.* (2020) evaluated the nematicidal nature of the aqueous extracts of *Allium sativum, Urtica dioica, Sophora mollis, Ephedra intermedia,* and *Tanacetum baltistanicum* and they reported that the leaf extract of *U. dioica* showed maximum mortality response (40-47 %) after 24 h of exposure as compared to the rest of the extracts studied during this study. Nassar (2016) prepared leaf extracts of *U. urens* through successive extraction and synthesized the Ag-nanoparticles of extracts and the reference nematicide. Results revealed that Ag-nano formulations of *U. urens* extracts were effective in the management of *M. incognita*. This shows the possibility of using *U. dioica* for the synthesis of green nanoparticles for the management of *M. incognita*.

Compost amendments suppress nematodes by direct inhibition of nematode growth or indirectly by enhancing beneficial microbes and improving soil quality. According to Naz *et al.* (2014), the application of organic amendments like crop residues, animal manure, green manure, and plant extract might help to reduce root-knot nematode. The results of the experiment agree with the above facts. The results agree with the work of Su *et al.* (2017), who evaluated Lime and Ammonium Bicarbonate (LAB) on tomatoes against RKN and proved the efficacy of their application. Under this study, LAB-treated soil was again treated with the botanical of *U. dioica* and evaluated for their efficiency in combination. The results revealed that the two components in combination have yielded good results in managing the nematode population at different concentrations. *U. dioica* has been used as an active pesticide against Aphids **(**Kaberia, 2007), and it also shows antifungal activity against *Alternaria solani*, *A. zinnia*, *Curvularia lunatas*, *Rhizoctonia solani* and *Fusarium oxysporum* (Tapwal *et al.*, 2011). The aqueous extract of the leaves, roots, and stems of *U. dioica* effectively controlled the larvae of *M. incognita* (Ismail *et al.,* 2020), which supports the present study. The present finding agrees with those studies carried out earlier on *U. dioica*.

CONCLUSION

The findings of this study demonstrated that *Urtica dioica* aqueous leaf extracts can be utilized in conjunction with other methods to achieve effective root-knot nematode management. This integrated approach involves the use of a weed plant from Uttarakhand, spent compost from the cultivation of edible oyster mushrooms, and two eco-friendly chemicals: lime and ammonium bicarbonate. The results indicate that the individual components, such as spent compost, green manure, LAB, or aqueous leaf extract of *U. dioica*, are not as effective as when they are combined. This highlights the significant role that botanicals play in reducing the reliance on chemical pesticides and promoting the reduction of chemical usage. Integrating *U. dioica* into agricultural practices not only offers an environmentally friendly alternative to chemical pesticides but also promotes the utilization of local flora for pest control, aligning with principles of ecological sustainability and traditional agricultural knowledge.

ACKNOWLEDGEMENT

We acknowledge the support of the Indian Council of Agricultural Research (ICAR) for providing a junior research fellowship, which contributed to the execution of this study.

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