PLANT SCIENCE TODAY
Vol 13(sp2) 1-8
https://doi.org/10.14719/pst.13119

eISSN 2348-1900

RESEARCH ARTICLE

Bionematicidal efficacy of *Chrysanthemum coronarium* L. against *Meloidogyne incognita*

Aashna Kumari[1], Gunjeet Kumar[1]*, Vartika Budhlakoti[1], Sharad Mohan[2], Supradeep Saha[3], Ajai Kumar Tiwari[1] & Gyan Mishra[4]

[1]Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, Pusa 110 012, New Delhi, India
[2]Division of Nematology, ICAR-Indian Agricultural Research Institute, Pusa 110 012, New Delhi, India
[3]Division of Agricultural Chemicals, ICAR-Indian Agricultural Research Institute, Pusa 110 012, New Delhi, India
[4]Division of Genetics, ICAR-Indian Agricultural Research Institute, Pusa 110 012, New Delhi, India

*Correspondence email - kumar_gunjeet@yahoo.com

Received: 08 December 2025; Accepted: 27 March 2026; Available online: Version 1.0: 30 April 2026

Cite this article: Aashna K, Gunjeet K, Vartika B, Sharad M, Supradeep S, Ajai KT, Gyan M. Bionematicidal efficacy of *Chrysanthemum coronarium* L. against *Meloidogyne incognita*. Plant Science Today. 2026; 13(sp2): 1-8. https://doi.org/10.14719/pst.13119

Abstract

Root-knot nematodes (*Meloidogyne incognita*) are major plant pathogens and the environmental hazards associated with synthetic nematicides necessitate the development of eco-friendly alternatives. This study assessed the genotype-dependent bionematicidal potential of *Chrysanthemum coronarium* L. (Pusa 15-16 White, Cream and Yellow) using aqueous extracts of fresh and lyophilised leaves and flowers under *in vitro* conditions and correlated nematicidal activity with metabolite profiling through ultra-performance liquid chromatography-time-of-flight mass spectrometry (UPLC-TOF-MS). Second-stage juveniles (J_2) obtained from a pure culture maintained on tomato *cv*. Pusa Ruby were exposed to graded extract concentrations (20–100 %) and observations on mortality, immobilisation and revival were recorded at different exposure periods. The results revealed a significant increase in nematode mortality with increasing concentration and time of exposure. Among the genotypes, Pusa 15-16 White exhibited the highest efficacy, with fresh leaf extract causing 100 % mortality at ≥80 % concentration within 24 hr and at 60 % after 96 hr, while the overall maximum mean mortality (66.32 %) was recorded at 96 hr. Lower concentrations showed nematostatic effects as evidenced by revival test, whereas marigold root extract predominantly induced reversible immobilisation. UPLC-TOF-MS analysis detected pyrethrins, cinerins, jasmolins and flavonoids such as luteolin, quercetin and myricetin, which are likely responsible for observed nematicidal activity. This study provides the first evidence of flower colour-based genotypic variation in *C. coronarium* linked to its bioactive metabolite composition and highlights Pusa 15-16 White as a promising source of eco-friendly nematicidal compounds for sustainable nematode management.

Keywords: bioassay; genotype variation; *in vitro*; juvenile; phytochemical analysis; root-knot nematode; UPLC-TOF-MS

Introduction

Chrysanthemum coronarium L. is an annual, herbaceous plant with antibacterial, antifungal, antioxidant and immunomodulatory medicinal activities (1, 2). Plants are an important source of environmentally safe biopesticides, as they synthesise a wide array of secondary metabolites with potent activity against plant-parasitic nematodes. Among these, essential oils and their bioactive constituents have attracted considerable attention due to their strong *in vitro* nematicidal effects, including inhibition of egg hatching and induction of juvenile mortality (i.e., the percentage of J_2s of *Meloidogyne incognita* that die after exposure to a treatment or factor). Owing to their biodegradability, multiple modes of action and reduced risk to non-target organisms, plant-derived products are increasingly being explored as sustainable alternatives to synthetic nematicides (3).

The root-knot nematodes (RKN) i.e., Meloidogyne spp. are among the most destructive plant-parasitic nematodes (PPNs). They infest over 2000 host plants, including ornamentals, weeds, medicinal plants and edible crops (4). Plant species belonging to the Asteraceae have been used extensively in nematode control. Their application includes use as intercrops, cover crops, in crop rotation, as green manure and as extracts or essential oils (5, 6). The advantages of employing these antagonistic plants lie in their environment friendly nature for suppressing PPNs and also offer a safer alternative compared to synthetic agro-chemicals, which pose huge risks to both human health and the environment (7).

Phytochemical constituents derived from the flowers of *C. coronarium* have demonstrated significant bioactivity against nematode populations, potentially affecting key physiological and developmental processes. These bioactive compounds may impair the mobility, survival and infectivity of second-stage juveniles (J_2), the primary infective stage responsible for host root penetration and establishment, thereby contributing to the suppression of nematode population dynamics (8).

Although synthetic chemical nematicides have been widely employed and are often effective in reducing nematode infestations,

their prolonged use is associated with adverse environmental consequences, including toxic residues and disruption of non-target soil microflora and fauna. In this context, plant-derived organic products and essential oils offer considerable potential as biopesticides due to their biodegradability, lower persistence in the environment, reduced non-target toxicity and complex modes of action that minimise the likelihood of resistance development. Collectively, these attributes support the integration of botanical nematicides and organic inputs into eco-friendly and sustainable nematode management programs (9).

Biopesticides are a safer and effective alternative to harmful chemical nematicides and found effective on *Chrysanthemum* cv. Pusa Centenary against *M. incognita* and extracted the active chemical constituents by UPLC-TOF-MS with the nematicidal activity exhibited by the aqueous extracts of both shoot and root exhibited significant mortality against J_2s and found positive corelation with concentration and exposure duration of treatment. It reached 99.2 % mortality with a 10 % shoot extract after 72 hr and chemical analysis of Pusa Centenary using UPLC-TOF-MS revealed a total of 12 bioactive compounds, including 11 flavonoids and one terpene (10).

In the present investigation, we evaluated the nematicidal (irreversible death of J_2s after exposure to a treatment) and nematostatic (J_2s become inactive or paralysed, showing no movement, but are not necessarily dead) potential of three genotypes of *C. coronarium* Pusa 15-16 White, Pusa 15-16 Cream and Pusa 15-16 Yellow-commonly utilised and their bioefficacy was comparatively assessed against marigold under controlled *in vitro* conditions. Aqueous and dried plant extracts were tested for their activity against *M. incognita*, with particular emphasis on their effects on J_2 mortality and immobilisation.

The specific objectives of the study were: (i) to determine the nematicidal and nematostatic efficacy of different plant parts (leaves and flowers) of *C. coronarium* against *M. incognita*; and (ii) to characterise and analyse the phytochemical profile of these plant parts in order to identify bioactive constituents potentially responsible for the observed nematode suppression.

Materials and Methods

Plant material

Leaves and flowers of three genotypes of *C. coronarium* (Pusa 15-16 White, Pusa 15-16 Cream and Pusa 15-16 Yellow) were collected during the morning hours from the Research Farm of the Division of Floriculture and Landscaping, New Delhi. African marigold was also collected from the same field under similar conditions.

Fresh leaf/flower extract

Matured leaves and fully open flowers (10 g) were chopped in water and extracts were prepared using pestle and mortar. The crude extract was filtered through two-layered muslin cloth, followed by Whatman No. 1 filter paper. The clear supernatant was collected and stored at 4 °C. Six concentrations, viz. 100, 80, 60, 40, 20 and 0 % were prepared by diluting the extract with distilled water.

Dry leaf/flower extract

Lyophilisation conditions

Fresh plant samples were frozen at -40 to -80 °C and subsequently lyophilised in a freeze dryer under vacuum (<0.1 m bar). Primary drying was carried out at a condenser temperature of -50 to -55 °C for 24–48 hr, followed by secondary drying at 20–25 °C for 12–24 hr to remove bound moisture. The process was continued until a constant weight was achieved and the final moisture content of the samples was reduced to below 5 %. Mature leaves and flowers were freeze-dried using a freezone lyophiliser (Labconco, Kansas City, MO, USA) for 5–6 days to ensure complete moisture removal. The lyophilised samples were then pulverised into a fine powder, extracted with distilled water, filtered and centrifuged at 5000 rpm for 20 min.

Preparation of pure culture on tomato seedlings

Seedlings were raised in the protrays and RKN susceptible tomato var. Pusa Ruby (owing to its uniform and high level of infection, it is widely used as a standard susceptible host for screening nematicidal efficacy of botanicals, bioagents and other management practices) at 21 days old seedlings were used to culture *M. incognita* by inoculating the J_2 which were collected via modified Baermann method (11). The J_2s were inoculated directly near the roots by making small holes in the soil. The plants were irrigated at regular intervals to maintain the optimum moisture in the soil for completion of the life cycle of *M. incognita.*

Nematode inocula

A single egg-mass pure culture of *M. incognita* was maintained on tomato cv. Pusa Ruby in a greenhouse. After 30 days of inoculation with J_2, the entire root system of infected plants was uprooted, immersed in water and gently cleaned to remove any adhering dirt. The egg masses were extracted using forceps, rinsed with sterile water and placed in a 0.5 % sodium hypochlorite (NaOCl) solution, stirred for 4 min and then passed through a 26 µm sieve.

Root extracts

Thirty days old seedlings of African marigold cv. Pusa Bahar were placed in a beaker filled with distilled water. Subsequently, the active chemical compounds (nematostatic) from the roots oozed into the water. The clear supernatant was collected and stored at the 4 °C.

In vitro nematicidal/nematostatic bioassay

Polystyrene culture plates were filled with 500 µL/well of the aqueous extract of plant parts or marigold extraction was used. The bioassay was performed at room temperature. Twenty J_2 were added to each well and each treatment was replicated three times.

Revival experiment

The experiment was conducted to assess nematostatic efficacy. For this, a revival test was conducted by picking inactive nematodes from six concentrations of the aqueous extracts and transferring to polystyrene culture plates containing 1 mL of distilled water. Nematodes that regained mobility were considered indicative of nematostatic activity at the respective concentration.

UPLC-TOF-MS analysis

Ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-TOF-MS) was used for high-resolution qualitative and quantitative analysis of complex samples. Chromatographic separation and mass detection were performed using a UPLC system interfaced with a time-of-flight mass spectrometer (Waters Corporation, Milford, MA, USA). The method involves four main steps:

1.Sample preparation

Target analytes were extracted from the sample matrix using techniques such as solid-phase extraction (SPE) or liquid-liquid extraction to remove interfering substances and concentrate the analytes. Samples were then reconstituted in a suitable solvent (e.g., methanol or methanol-water mixtures) prior to injection.

2. Chromatographic and mass spectrometric conditions

Chromatographic separation was performed on an ACQUITY UPLC® BEH C_{18} column (2.1 × 100 mm, 1.7 µm) at 40 °C using water and acetonitrile (both containing 0.1 % formic acid) as the mobile phase, at a flow rate of 0.3 mL min^{-1} with a 2 µL injection volume. The linear gradient as follows: 5 % B (0–1 min), 5–95 % B (1–10 min), 95 % B (10–12 min), followed by re-equilibration to initial conditions up to 15 min.

Mass analysis was carried out using an ESI source in positive and/or negative mode with a capillary voltage of 2.5–3.0 kV, source temperature of 120 °C, desolvation temperature of 350–450 °C, cone gas flow was set at 50 L hr^{-1} and desolvation gas flow at 600–800 L hr^{-1}. Data were acquired over an m/z range of 50–1200 with a scan time of 0.2 sec.

3. UPLC separation

The sample is introduced into a UPLC system, where high pressure pushes it through a chromatographic column (typically C18). A gradient elution of mobile phases (commonly water and acetonitrile or methanol) is used to achieve optimal separation based on compound polarity.

4. TOF-MS detection

Eluting compounds are ionised, typically via electrospray ionization (ESI) and introduced into the TOF mass analyser. Ions are separated based on their mass-to-charge (m/z) ratios by measuring their flight time in a field-free region.

Data analysis

The mass spectrometer generates spectra for each compound, enabling identification based on accurate m/z values. Quantification is performed by comparing peak areas with those of calibration standards.

Chemical profiling of the active compounds

Leaves and petals (5 g) of *C. coronarium* were placed in a 100 mL beaker and extracted with 80 mL solvent (methanol) by sonication for 30 min. The methanolic extract was filtered using filter paper and subsequently concentrated using a rotary evaporator. A small quantity of the concentrated sample was then filtered through a 0.45 µm syringe filter, transferred to a vial and analysed for active chemical compounds using UPLC-TOF-MS.

Statistical analysis

The bioassay was laid out in a factorial completely randomised design with three independent replicates per treatment. Each replicate consisted of a separate cavity block containing approximately 100 freshly hatched J_2 of *M. incognita*. Percent mortality data were subjected to arcsine square-root transformation to stabilise the variance and analysed by ANOVA using the SAS statistical package (IASRI, New Delhi). For ease of interpretation, non-transformed mean values are presented in the tables.

To study the effect of genotypes, plant parts, concentration and their interactions on various parameters following general linear model was used.

$$Y_{ijkl} = \mu + G_i + P_j + (GP)_{ij} + C_k + (GC)_{ik} + (PC)_{jk} + (GPC)_{ijk} + e_{ijkl}$$

μ = General mean effect

Y_{ijkl} = l-th Observed value of i-th Genotype in j-th plant part of k-th concentration

G_i = Effect of i-th genotype

P_j = Effect of j-th plant part

C_k = Effect of k-th concentration

$(GP)_{ij}$ = Interaction of i-th genotype and j-th plant part

$(GC)_{ik}$ = Interaction of i-th genotype and k-th concentration

$(GPC)_{ijk}$ = Interaction of i-th genotype, j-th plant part and k-th concentration

e_{ijkl} = Error term

Multiple comparisons between genotypes, plant parts, concentration and their interactions for various parameters were performed using Tukey's HSD test (Honestly significant difference) at 5 % level of significance. The analysis was conducted using JMP 9.0 software.

Results and Discussion

Nematicidal evaluation for juvenile mortality through *in vitro*

Effect of genotypes

In the laboratory condition during *in vitro* bioassay among the three genotypes, Pusa 15-16 White was significantly superior (43.13 %) and followed by genotype Pusa 15-16 Cream (39.93 %) at 24 hr (Table 1). The genotype Pusa 15-16 Yellow was found significantly inferior. All three genotypes differed significantly from each other. The highest mortality (66.32 %) observed at 96 hr. The genotype Pusa 15-16 Yellow was found to be least effective even at 96 hr with 47.08 % mortality (Table 1). The genotypic differences among five *Chrysanthemum* species in terms of J_2 survival have been attributed to variations in the essential oil content of flower heads, which confer differing degrees of juvenile susceptibility (12).

Effect of extract concentration

The effect of aqueous extracts of *C. coronarium* at six different concentrations on the mortality of *M. incognita* J_2 was observed overtime. At 100 % concentration maximum 98.61 % mortality was recorded and followed by 85.42 % at 80 % concentration at 96 hr (Table 1). Whereas at the lower time duration of 24 hr the highest mortality 85.97 % was noted at 100 % concentration followed by

Table 1. Mean nematicidal effect of marigold on J_2 of *Meloidogyne incognita*

Concentration (%)	Time intervals (hours)			
	24	48	72	96
0	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)
20	10.00 (18.43)	13.33 (21.15)	16.67 (23.74)	18.33 (25.19)
40	15.00 (22.60)	20.00 (26.45)	21.67 (27.60)	21.67 (27.60)
60	18.33 (25.31)	25.00 (29.93)	21.67 (27.71)	30.00 (33.16)
80	18.33 (25.31)	25.00 (29.93)	26.67 (31.07)	30.00 (33.21)
100	20.00 (26.45)	25.00 (30.00)	30.00 (33.21)	40.00 (39.23)

*Values in parentheses indicate arcsine-transformed data.

55.69 % at 80 % (Table 1).

Variation in concentration and exposure duration has also been reported in *Catharanthus roseus*, where extracts of dried leaves at the highest dose (2000 ppm) caused 21.4, 46.8, 88.5 and 100 % mortality after 3, 6, 24 and 48 hr, respectively, while a 1000 ppm extract also resulted in 100 % mortality after 72 hr (13). Similar findings have been reported, with J_2 mortality increasing significantly with increasing plant extract concentration (10)

Effect of plant parts

Aqueous extract of fresh and lyophilised leaves and flowers of *C. coronarium* were found to be effective against mortality of J_2. Among the plant parts tested, fresh leaf were the most effective and showed maximum mortality of J_2s at both the time intervals whereas the dry flower, found least effective. The fresh leaf showed maximum (64.91 %) mortality where lesser mortality of 51.02 % by dry flower at 96 hr (Table 1). The nematicidal effect of leaf extract was earlier reported in *Chrysanthemum indicum l*eaf extract on RKN egg hatching and found *in vitro* effect on nematicidal activity (14). *In vitro* experiment indicated that the essential oil compounds from *C. coronarium* flower heads directly affect the nematode biology by interfering with nematode hatching and J_2 viability, as reported for other phytochemical compounds (15, 16).

It is attributed to the mortality that shoot extract inflicted the highest J_2 mortality (99.2 %) at 10 % concentration after 72 hr, while the root extract showed less mortality (73.3 %) even after 72 hr exposure time at 10 % concentration (10).

Effect of time duration

It was observed that the mortality of J_2 was directly proportional to the longer stages of time duration. The highest mortality across all factors (genotype, plant parts concentration and time duration) at longer duration indicating a stronger effect and maximum (66.32 %) mortality was noted in Pusa 15-16 White, 64.91 % by fresh leaf and 98.61 % at 100 % concentration (Table 1).

Dandelion extract has also shown a clear concentration and time-dependent increase in J_2 mortality, with low mortality at 250 ppm, 53.3 % at 1000 ppm after 24 hr and 100 % mortality at 1000 ppm after 72 hr, while 2000 ppm achieved complete mortality within 48 hr (17). Similar findings have been reported, wherein the LC_{50} of *Foeniculum vulgare* was determined for achieving maximum J_2 mortality of *Meloidogyne javanica* after 48 hr (18).

Nematicidal effect of interaction of genotype, plant part used and extract concentration

The mean data pertaining to interaction effects of three genotypes, plant parts and concentration of aqueous extracts at different stages of time durations (Tables 2 and 3) indicated that the fresh leaf of all three genotypes was superior. At 24 hr, the fresh leaf extract of Pusa 15-16 White showed 100 % mortality at both 80 and 100 % concentrations which was higher than the other plant parts used while at 96 hr, 100 % mortality was found even at 60 % concentration (Tables 2 and 3). The dry leaf extract showed highest (83.33 %) mortality at 24 hr of 100 % concentration (Table 2). The fresh flower extract of Pusa 15-16 White showed 100 % mortality at 80 and 100 % concentration at 24 hr (Table 2). At 96 hr, maximum mortality was observed in Pusa 15-16 White and it was lowest in Pusa 15-16 Yellow. The minimum mortality was observed in dry flower extract in Pusa 15-16 Yellow (33.89 %) and Pusa 15-16 Cream (54.44 %) while in Pusa 15-16 White, the least mortality (61.39 %)

Table 2. Effect of three-way interaction among the genotypes, plant parts and concentrations at 24 hr

Concentration (%)	Genotypes														
	Pusa 15-16 White					Pusa 15-16 Cream					Pusa 15-16 Yellow				
	FL	FF	DL	DF	Mean	FL	FF	DL	DF	Mean	FL	FF	DL	DF	Mean
0	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.28)	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.28)	0.00 (1.28) ^{U}	0.00 (1.28)	0.00 (1.28) ^{U}	0.00 (1.28) ^{U}	0.00 (1.281)
20	16.67 (20.42) ^{PQRS}	6.67 (12.71) ^{RSTU}	5.00 (10.87) ^{STU}	8.33 (14.16) ^{QRST}	9.16 (14.54)	23.33 (28.07) ^{NOP}	6.67 (12.32) ^{RSTU}	26.67 (30.94) ^{LMNOP}	1.67 (5.16) ^{TU}	16.67 (20.42) ^{PQRS}	6.67 (12.71) ^{RSTU}	5.00 (10.87) ^{STU}	8.33 (14.16) ^{QRST}	9.16 (14.54)	23.33 (28.07) ^{NOP}
40	41.67 (40.00) ^{IJKLM}	43.33 (41.15) ^{HIJKL}	18.33 (25.00) ^{OPQ}	25.00 (30.00) ^{LMNOP}	32.08 (34.03)	36.67 (37.09) ^{IJKLMN}	15.00 (22.59) ^{OPQR}	46.67 (43.07) ^{GHIJK}	21.67 (27.21) ^{NOP}	30.00 (32.49)	16.67 (23.74) ^{OPQR}	20.00 (26.56) ^{NOP}	11.67 (19.88) ^{PQRS}	13.33 (21.14) ^{PQRS}	15.41 (22.83)
60	61.67 (51.92) ^{DEFGH}	68.33 (56.14) ^{DE}	26.67 (31.07) ^{LMNOP}	46.67 (43.07) ^{GHIJK}	50.83 (45.55)	38.33 (38.07) ^{IJKLMN}	16.67 (24.04) ^{OPQR}	58.33 (50.00) ^{DEFGHI}	43.33 (41.15) ^{HIJKL}	39.16 (38.31)	50.00 (45.00) ^{EFGHIJK}	21.67 (27.59) ^{NOP}	18.33 (25.00) ^{OPQ}	16.67 (23.85) ^{OPQR}	26.66 (30.36)
80	100 (88.71) ^{A}	68.33 (55.81) ^{DEF}	58.33 (50.00) ^{DEFGHI}	65.00 (53.92) ^{DEFG}	72.91 (62.11)	61.67 (51.92) ^{DEFGH}	66.67 (54.83) ^{DEFG}	75.00 (60.77) ^{CD}	36.67 (37.22) ^{JKLMN}	60.00 (51.18)	55.00 (48.07) ^{EFGHIJ}	23.33 (28.66) ^{MNOP}	33.33 (35.25) ^{KLMNO}	25.00 (29.92) ^{LMNOP}	34.16 (35.48)
100	100 (88.71) ^{A}	81.67 (68.90) ^{BC}	96.67 (83.00) ^{A}	96.67 (83.00) ^{A}	93.75 (80.90)	100.00 (88.71) ^{A}	91.67 (79.14) ^{AB}	91.67 (79.14) ^{AB}	100.00 (88.71) ^{A}	95.83 (83.93)	75.00 (60.00) ^{CD}	66.67 (55.00) ^{DEF}	83.33 (69.57) ^{BC}	48.33 (44.03) ^{FGHIJK}	68.58 (57.15)
Mean	53.33 (48.51)	44.72 (39.33)	47.49 (33.53)	40.27 (37.57)	43.13 (39.74)	43.33 (40.86)	32.78 (32.37)	49.72 (44.20)	33.89 (33.45)	39.93 (37.72)	33.33 (30.85)	22.50 (24.35)	25.56 (27.28)	17.22 (20.25)	24.65 (25.68)
C.D ($p \leq 0.01$)								11.83							

* Values in parentheses indicate arcsine-transformed data. [FL: Fresh leaf; FF: Fresh flower; DL: Dry leaf; DF: Dry flower]. Means followed by the same letter(s) are not significantly different at 5 % level according to Tukey's HSD test.

Table 3. Effect of three-way interaction among the genotypes, plant parts and concentrations at 96 hr

Concentration (%)	Genotypes												
	Pusa 15-16 White				Pusa 15-16 Cream				Pusa 15-16 Yellow				
	FL	FF	DL	DF	Mean	FL	FF	DL	DF	Mean	FL	FF	DL	DF	Mean
0	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28)	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28)	0.00 (1.28) ^{X}	0.00 (1.28)	0.00 (1.28) ^{X}	0.00 (1.28) ^{X}	0.00 (1.28)
20	53.33 (46.92) ^{MNOPQR}	53.33 (46.93) ^{MNOPQR}	45.00 (41.92) ^{QRSTU}	60.00 (50.98) ^{IJKLMNO}	52.92 (46.69)	53.33 (46.92) ^{MNOPQR}	40.00 (39.14) ^{RSTUV}	50.00 (45.00) ^{NOPQR}	23.33 (28.85) ^{W}	41.67 (39.98)	25.00 (30.00) ^{UVW}	31.67 (34.14) ^{STUVW}	23.33 (28.85) ^{W}	20.00 (26.45) ^{W}	25.00 (29.86)
40	71.67 (58.06) ^{HIJKL}	63.33 (53.06) ^{IJKLMNO}	56.67 (48.84) ^{KLMNOPQR}	70.00 (56.92) ^{HIJKLM}	65.42 (54.22)	56.67 (48.84) ^{KLMNOPQR}	50.00 (45.00) ^{OPQRS}	58.00 (50.00) ^{JKLMNOPQ}	58.33 (49.83) ^{JKLMNOPQ}	55.83 (48.42)	55.00 (47.87) ^{LMNOPQR}	41.67 (40.16) ^{PQRST}	26.67 (31.07) ^{UVW}	25.00 (29.92) ^{VW}	37.08 (37.26)
60	100.00 (88.71) ^{A}	93.33 (80.29) ^{AB}	73.33 (58.92) ^{GHIJK}	75.00 (60.00) ^{FGHIJ}	85.42 (71.98)	83.33 (73.42) ^{BCD}	66.67 (54.99) ^{HIJKLMN}	68.33 (55.85) ^{HIJKL}	63.33 (52.77) ^{IJKLMNO}	71.67 (59.62)	78.33 (62.40) ^{EFGHI}	63.33 (52.87) ^{IJKLMNO}	46.67 (43.07) ^{PQRST}	28.33 (32.09) ^{TUVW}	54.17 (47.61)
80	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	93.33 (77.28) ^{BC}	83.33 (69.57) ^{CDEF}	94.17 (81.07)	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	85.00 (70.71) ^{BCDE}	81.67 (65.00) ^{DEFGH}	91.67 (78.28)	86.67 (71.97) ^{BCDE}	66.67 (54.99) ^{HIJKLMN}	81.67 (68.50) ^{CDEFG}	46.67 (43.07) ^{OPQRS}	70.42 (59.63)
100	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71)	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71)	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	100.00 (88.71) ^{A}	83.33 (69.57) ^{CDEF}	95.83 (83.93)
Mean	70.83 (59.83)	68.33 (59.83)	61.39 (52.83)	64.72 (54.58)	66.32 (57.32)	66.39 (57.98)	59.44 (52.97)	60.28 (51.92)	54.44 (47.74)	60.14 (52.65)	57.50 (50.37)	50.56 (45.36)	46.39 (43.58)	33.89 (33.73)	47.08 (43.26)
C.D ($p \leq 0.01$)	10.20														

* Values in parentheses indicate arcsine-transformed data. [FL: Fresh leaf; FF: Fresh flower; DL: Dry leaf; DF: Dry flower]. Means followed by the same letter(s) are not significantly different at 5% level according to Tukey's HSD test.

recorded in dry leaf extract (Table 3).

Previous studies have demonstrated significant interaction effects of plant species, extract concentration and exposure duration on nematicidal efficacy, with J_2 mortality increasing progressively with higher doses and longer exposure periods. In this context, palmarosa oil has been reported to cause very high mortality (70–98.75 %) across 31.12–500 ppm within 24–96 hr, confirming the dose and time-dependent response of essential oil-based nematicides (19).

Nematostatic/nematicidal effect of *Chrysanthemum coronarium* and marigold aqueous extract on *Meloidogyne incognita*

J_2 Revival test

At 20 % concentration, marigold root extract caused only 18.33 % J_2 mortality after 96 hr and showed complete revival upon transfer to distilled water, indicating predominantly nematostatic activity (Tables 1 and 4). In contrast, in *C. coronarium* (Pusa 15-16 White), fresh flower extract recorded the highest revival (50 %) at the same concentration, whereas dry leaf extract showed the least revival (25 %), reflecting comparatively greater nematicidal efficacy (Table 4). It showed that the marigold having more nematostatic potential than the nematicidal. Nematicidal effects were observed by probing the J_2 with a feather pick. Live J_{2s} moved while the dead J_{2s} lay straight and did not show any movement. Some J_2 showed nematostatic activity as they became unconscious but not dead. The highest nematostatic response was recorded at lower concentrations, as evidenced by nematode revival upon transfer to

Table 4. Revival of *Meloidogyne incognita* J_2s post-exposure to aqueous extracts of different plant parts of the three genotypes of *Chrysanthemum coronarium* and marigold

Genotypes	Plant parts	Concentrations (%)				
		20	40	60	80	100
		Revival rate (%)				
Pusa 15-16 White	Fresh leaf	25	50	0	0	0
	Fresh flower	50	50	0	0	0
	Dry leaf	25	0	0	0	0
	Dry flower	0	0	0	0	0
Pusa 15-16 Cream	Fresh leaf	25	0	0	0	0
	Fresh flower	25	0	0	0	0
	Dry leaf	25	0	0	0	0
	Dry flower	0	0	0	0	0
Pusa 15-16 Yellow	Fresh leaf	25	25	0	0	0
	Fresh flower	25	25	0	0	0
	Dry leaf	0	25	0	0	0
	Dry flower	0	0	0	0	0
Marigold	Root extracts	100	100	0	0	0

fresh medium. Earlier reports on the nematostatic action of *C. coronarium* against various nematode species corroborate the results of the present study (20).

Identification of chemical constituents found in *Chrysanthemum coronarium*

UPLC-TOF-MS analysis revealed clear genotype and tissue-specific variation in bioactive metabolites, which was consistent with the observed nematicidal activity. In Pusa 15-16 White, leaf extracts showed the highest toxicity (70.83 % mean mortality at 96 hr) and were enriched with luteolin, myricetin, quercetin, pyrethrin II, jasmolin I, jaceidin and lignoceric acid, while the flowers contained

Table 5. List of chemical compounds identified in the leaf and flower of genotype Pusa 15-16 White

Sl.No.	Leaf				Flower			
	Compound	(M+H) mass		Mass error (ppm)	Compound	(M+H) mass		Mass error (ppm)
		Theoretical mass	Observed mass			Theoretical mass	Observed mass	
1	Cinerin I	317.21	317.21	2.20	Cinerin I	317.21	317.21	-3.46
2	Linoleic acid	281.24	281.24	0.35	Parthenolide	249.14	249.14	-1.60
3	Luteolin	287.05	287.05	0.34	Luteolin-7-O-b-D-glucopyranoside	449.10	449.10	-0.06
4	Myricetin	319.04	319.04	-4.07	Hesperidin	611.19	611.19	-1.79
5	Quercetin	303.05	303.05	1.64	Luteolin/ Fisetin	287.05	287.05	0.69
6	Luteolin/ Fisetin	287.05	287.05	0.34	Heptadecanoic acid	271.26	271.26	-1.47
7	4,5,7-trihydroxylflavanone	273.07	273.07	-2.56	Apigenin/ Galangin	271.06	271.06	7.00
8	Pyrethrin II	373.20	373.20	-1.07	Campesterol	401.37	401.37	-1.24
9	Linolenic acid	279.23	279.23	-2.86	β-Cyclopyrethrosin/ Arteglasin-A	305.13	305.13	-1.96
10	Jasmolin I	331.22	331.22	-1.81	Vitexin/ Apigenin-7-O-b-D-galactopyranoside/ Apigetrin	433.11	433.11	-0.23
11	Jaceidin	361.09	361.09	-0.55	4,5,7-trihydroxylflavanone	273.07	273.07	-4.76
12	Cinerin II	361.20	361.20	-1.10	Octacosanoic acid	425.43	425.43	-2.35
13	β-Cyclopyrethrosin / Arteglasin-A	305.13	305.13	0.32	Pyrethrin I	329.21	329.21	2.12
14	Pyrethrin I	329.21	329.21	1.82	Fucosterol/ Isofucosterol/ Stigmasterol	413.37	413.37	1.45
15	Octacosanoic acid	425.43	425.43	-1.17	Cinerin II	361.20	361.20	0.00
16	Campesterol	401.37	401.37	-0.74	Handelin	553.28	553.28	1.26
17	Fucosterol/ Isofucosterol	413.37	413.37	-1.20	Linoleic acid	281.24	281.24	-12.08
18	Lignoceric acid	369.37	369.37	-0.54	Tricosanoic acid	355.35	355.35	2.25
19	Tricosanoic acid	355.35	355.35	0.00	(+)-Sesamin	355.11	355.11	2.25
20	(+)-Sesamin	355.11	355.11	0.00				

Table 6. List of chemical compounds identified in the leaf and flower of Pusa 15-16 Cream genotype

Sl.No.	Leaf				Flower			
	Compound	(M+H) mass		Mass error (ppm)	Compound	(M+H) mass		Mass error (ppm)
		Theoretical mass	Observed mass			Theoretical mass	Observed mass	
1	3-hydroxyflavone	239.07	239.07	-0.41	Linoleic acid	281.24	281.24	-1.06
2	Tricosanoic acid	355.35	355.35	-1.40	Luteolin	287.05	287.05	13.58
3	Caryolane-1,9-b-diol/ Clovanediol/ Oplopanone	239.20	239.20	2.50	Fisetin	287.05		
4	Oleic acid	283.26	283.26	1.05	Hesperidin	611.19	611.19	-1.14
5	Myricetin	319.04	319.04	0.62	Vitexin/ Apigenin-7-O-b-D-galactopyranoside / Apigetrin	433.11	433.11	2.30
6	Hesperidin	611.19	611.19	-1.79	Fucosterol	271.26	271.26	-2.21
7	Flavanone	225.09	225.09	-2.22	Apigenin/ Galangin	271.06	271.06	-2.21
8	Quercetin	303.05	303.05	2.30	Luteolin-7-O-b-D-glucopyranoside	449.10	449.10	1.33
9	Luteolin/ Fisetin	287.05	287.05	2.78	Linolenic acid	279.23	279.23	-1.07
10	4,5,7-trihydroxylflavanone	273.07	273.07	-0.73	Campesterol	401.37	401.37	-1.49
11	Pyrethrin II	373.20	373.20	2.67	β -Cyclopyrethrosin / Arteglasin-A	305.13	305.13	0.32
12	Linolenic acid	279.23	279.23	2.14	Jasmolin II	375.21	375.21	-0.53
13	Jaceidin	361.09	361.09	-1.10	Cinerin I	317.21	317.17	-111.91
14	Cinerin II	361.20			Octacosanoic acid	425.43	425.43	3.76
15	β -Cyclopyrethrosin /Arteglasin-A	305.13	305.13	-1.96	Pyrethrin I	329.21	329.21	1.51
16	Octacosanoic acid	425.43	425.43	-0.47	3-hydroxyflavone	253.18	253.18	1.18
17	Isofucosterol/ Fucosterol	413.37	413.37	-0.96	Handelin	253.35	253.35	23.28

comparatively less potent constituents (Tables 3 and 5). In Pusa 15-16 Cream, leaf extracts possessing oleic acid, myricetin, flavanone, pyrethrin II and cinerin II resulted in 66.39 % mean mortality (Tables 3 and 6). Similarly, in Pusa 15-16 Yellow, leaf-specific compounds such as cinerin I, linoleic and arachidic acids, β-cyclopyrethrosin, jasmolin, (+)-catechin, pyrethrin I and fucosterol were associated with higher toxicity (57.50 % mean mortality) (Table 3). Overall, leaf extracts of all genotypes exhibited greater nematicidal efficacy than flowers, indicating a strong positive relationship between metabolite composition and J_2 mortality.

UPLC-TOF-MS analysis enabled the reliable identification of metabolites based on molecular ions $[M+H]^+$ (Theoretical mass and Observed mass) with mass error (ppm) below 3 ppm (Table 7). In Pusa 15-16 White, a higher number of compounds were detected in fresh leaf (20) than in flower (19). The major flavonoids identified included luteolin-7-O-β-D-glucopyranoside, luteolin and apigenin, indicating a rich flavonoid profile associated with the observed bioactivity (Tables 1–3). Compounds separated based on their m/z ratio include in cinerin I, cinerin II, pyrethrin I, pyrethrin II, jasmolin I, campesterol and Myricetin which are known for the synthesis of bio -pesticides or insecticides.

A wide spectrum of phenolics and flavonoids has been

Table 7. List of chemical compounds identified in the leaf and flower of Pusa 15-16 Yellow genotype

Sl.No.	Leaf				Flower			
	Compound	(M+H) mass		Mass error (ppm)	Compound	(M+H) mass		Mass error (ppm)
		Theoretical mass	Observed mass			Theoretical mass	Observed mass	
1	Cinerin I	317.21	317.21	17.02	Luteolin-7-O-b-D-glucopyranoside	449.10	449.10	0.00
2	Linoleic acid	281.24	281.24	0.00	Heptadecanoic	271.26	271.26	-0.36
3	Arachidic acid	313.31	313.31	-1.91	Apigenin/ Galangin	271.06	271.06	0.36
4	Arteglasin-A/ b-	305.13	305.13	-1.96	Myricetin	319.04	319.04	7.20
5	Jasmolin I	331.22	331.22	-1.20	Luteolin/ Fisetin	287.05	287.05	6.61
6	(+)-Catechin	291.08	291.08	-1.71	Quercetin	303.05	303.05	0.65
7	Octacosanoic acid	425.43	425.43	0.47	Campesterol	401.37	401.37	-2.49
8	Pyrethrin I	329.21	329.21	0.00	Cinerin II	361.20	361.20	0.55
9	Jaceidin	361.09	361.09	-0.83	Jaceidin	361.09	361.09	-0.27
10	Cinerin II	361.20	361.20	0.55	Octacosanoic acid	425.43	425.43	-0.47
11	Fucosterol /	413.37	413.37	-0.24	Heptacosanoic acid	411.42	411.42	0.48
12					Tricosanoic acid	355.35	355.35	-0.84

reported from single tissues of *Chrysanthemum*, including luteolin and its glycosides, apigenin 7-O-glucoside and acacetin derivatives, indicating a rich flavonoid profile that may contribute to the observed biological activity (21). Flavonoids are associated with enhanced resistance to pathogens, including nematodes (22). Previously it was reported that the antibacterial, antifungal, antiviral and anti-inflammatory activities were found in flowers and leaf of Compositae plants (23). Fresh leaves and flowers contained higher levels of pyrethrins I and II and cinerins I and II; pyrethrin I is primarily responsible for mortality, whereas pyrethrin II induces rapid knockdown. Their occurrence corroborates the strong insecticidal as well as nematicidal activity of *Chrysanthemum* extracts (24). These compounds, known for their insect-control properties, may also play a significant role in nematode suppression, highlighting the bionematicidal potential of the plant.

Conclusion

The present study demonstrated that aqueous extracts of *C. coronarium* possess significant nematicidal activity against *M. incognita*. Among the tested genotypes, Pusa 15-16 White was the most effective, with fresh leaf extract causing the highest mortality, which increased with concentration and exposure period. UPLC-TOF-MS profiling revealed the presence of pyrethrins, cinerins and bioactive flavonoids, which are likely responsible for the observed activity. These findings highlight the potential of *C. coronarium* as an eco-friendly bionematicidal resource. Further validation under greenhouse and field conditions, along with the development of suitable formulations, is needed. The use of this plant as a botanical amendment, intercropping, or as a green biomass amendment or in crop rotations may provide a cost-effective and sustainable nematode management option for farmers.

Acknowledgements

The authors express their sincere gratitude to the Director, ICAR-Indian Agricultural Research Institute (IARI), New Delhi and the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi, for providing the necessary facilities and support for the successful conduct of the research. The first author gratefully acknowledges the ICAR-JRF fellowship received during the M.Sc. programme.

Authors' contributions

AK conducted the investigation, methodology, data curation, writing-original draft and writing-review and editing. GK contributed to conceptualisation, project administration, funding acquisition, methodology, resources, supervision and writing-review and editing. VB contributed to data curation and visualisation. SM managed resources, supervision and editing. SS, AKT and GM provided supervision and assisted with writing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. El-Esawi MA, Elkelish A, Soliman M, Elansary HO. Phytochemical and biological activities of *Chrysanthemum coronarium* L.: A review. Plants (Basel). 2020;9(7):907. https://doi.org/10.3390/antiox9010043
2. Bendiabdellah A, Mami IR, Dib MEA, Djabou N, Muselli A. Analysis of the chemical composition and evaluation of the antioxidant, anti-inflammatory and hemolytic activities of the essential oil and its oxygenated fraction of *Chrysanthemum coronarium*. J Appl Biotechnol Rep. 2024;11(3):1378–85.
3. Dababat A, Ulas F, Yuksel E, Aasim M, Sameeullah M, Imren M. Essential oils as sustainable alternatives for managing plant-parasitic nematodes: A comprehensive review. Sustainability. 2025;17(22):10189. https://doi.org/10.3390/su172210189
4. Bernard GC, Egnin M, Bonsi C. The impact of plant-parasitic nematodes on agriculture and methods of control. In: Shah MM, Mahamood M, editors. Nematology: concepts, diagnosis and control. London: Intech Open; 2017. p. 121–51. https://doi.org/10.5772/intechopen.68958
5. Grimme E, Zidack NK, Sikora RA, Strobel GA, Jacobsen BJ. Comparison of *Muscodor albus* volatiles with a biorational mixture for control of seedling diseases of sugar beet and root-knot nematode on tomato. Plant Dis. 2007;91(2):220–5. https://doi.org/10.1094/PDIS-91-2-0220
6. Khalaj MA, Amiri M, Azimi MH. Allelopathy: Physiological and sustainable agriculture important aspects. Int J Agron Plant Prod. 2013;4(5):950–62.
7. Osei K, Osei MK, Mochiah MB, Lamptey JNL, Bolfrey-Arku G, Berchie JN. Plant parasitic nematodes associated with tomato in Ghana.

Nematol Mediterr. 2012;40:33–7.

8. Ibrahim H, Nchiozem-Ngnitedem VA, Dandurand LM, Popova I. Naturally-occurring nematicides of plant origin: Two decades of novel chemistries. Pest Manag Sci. 2025;81(2):540–71. https://doi.org/10.1002/ps.8504
9. Kamaraj N, Nagachandrabose S, Pon S, Kandasamy D, Kuppusamy S, Kalimuthu R, et al. Advancing plant-origin bioactive compounds as nematicides-present insights and future directions. Physiol Mol Plant Pathol. 2025;136:102543. https://doi.org/10.1016/j.pmpp.2024.102543
10. Jayanthi M, Singh K, Chaudhary S. Detection of nematicidal compounds and development of an *in vitro* mass multiplication protocol in Pusa Centenary *chrysanthemum*. Indian J Hortic. 2020;77(4):704–12. https://doi.org/10.5958/0974-0112.2020.00102.4
11. Chen SY, Dickson DW. A technique for determining live second-stage juveniles of *Heterodera glycines*. J Nematol. 2000;32(1):117–21.
12. Pérez MP, Navas-Cortés JA, Pascual-Villalobos MJ, Castillo P. Nematicidal activity of essential oils and organic amendments from Asteraceae against root-knot nematodes. Plant Pathol. 2003;52 (3):395–401. https://doi.org/10.1046/j.1365-3059.2003.00859.x
13. Sosamma VK, Jayasree D. Effect of leaf extracts on the mortality of root-knot nematodes, *Meloidogyne incognita* juveniles. Indian J Nematol. 2002;32:201–3.
14. Sharma R, Trivedi PC. Nematicidal properties of some leaf extracts against *Meloidogyne incognita*. J Phytol Res. 1991;4(2):131–7.
15. Chitwood DJ. Phytochemical based strategies for nematode control. Annu Rev Phytopathol. 2002;40:221–49. https://doi.org/10.1146/annurev.phyto.40.032602.130045
16. Chandravadana MV, Nidiry ESJ, Khan RM, Rao MS. Nematicidal activity of serpentine against *Meloidogyne incognita*. Fundam Appl Nematol. 1994;17(2):185–92.
17. Kotova VV, Shesteperov AA, Konowkov PF, Kozar EG. Nematicidal properties of plants. Zashchita Rastenii. 1994;(9):25.
18. Ayazpour K, Hasanzadeh H, Arabzadegan MS. Evaluation of the control of citrus nematode (*Tylenchulus semipenetrans*) by leaf extracts of many plants and their effects on plant growth. Afr J Agric Res. 2010;5(14):1876–80.
19. Ajith M, Pankaj, Shakil NA, Kaushik P, Rana VS. Chemical composition and nematicidal activity of essential oils and their major compounds against *Meloidogyne graminicola*. J Essent Oil Res. 2020;32(6):526–35. https://doi.org/10.1080/10412905.2020.1804469
20. Bar-Eyal M, Sharon E, Spiegel Y. Nematicidal activity of *Chrysanthemum coronarium*. Eur J Plant Pathol. 2006;114:427–33. https://doi.org/10.1007/s10658-006-0011-7
21. Wang Y, Yang X, Guo Q. Studies on chemical constituents in Huangjuhua (flowers of *Chrysanthemum morifolium*). Zhongguo Zhong Yao Za Zhi. 2008;33:526–30.
22. Chin S, Behm CA, Mathesius U. Functions of flavonoids in plant-nematode interactions. Plants. 2018;7:85. https://doi.org/10.3390/plants7040085
23. Ribeiro GdJG, de Souza EE, Palmisano G, Durigon EL, Liebau E, Wrenger C. Plant-derived extracts and natural products with antiviral activity. Front Virol. 2025;5:1632734. https://doi.org/10.3389/fviro.2025.1632734
24. Cho KH, Kim HJ, Lee JH, Kim YC. Evaluation of insecticidal ingredients and efficacy of extracts from pyrethrum flowers cultivated in South Korea. Korean J Appl Entomol. 2024;63(3):253–63.

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.