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Nematicidal Potential of *Brassica* Species against *Meloidogyne incognita*: An In vitro Study

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***Abstract*:** The root-knot nematode (*Meloidogyne incognita*) poses a significant threat to crop productivity, necessitating eco-friendly alternatives to synthetic nematicides. This study evaluated the *in vitro* nematicidal efficacy of aqueous leaf extracts from selected canola (Gobhi Sarson Canola 7- GSC 7, Raya Ludhiana Canola-RLC 3) and non-canola (Punjab Brassica Raya- PBR 357, Taramira Ludhiana Composite 2 - TMLC 2) *Brassica* varieties. Extracts were tested at four concentrations for their ability to inhibit egg hatching and induce second-stage juvenile (J_2) mortality over a 10-day exposure period. All varieties exhibited concentration and time-dependent nematode suppression. Among canola types, RLC 3 showed stronger inhibitory effects than GSC 7, maintaining substantial activity even at lower concentrations. Non-canola varieties outperformed the canola varieties, with TMLC 2 consistently demonstrating the highest level of inhibition and juvenile mortality. Complete egg hatch inhibition was recorded with TMLC 2 at S/2 within 24 hours, while J_2 mortality reached 100% at full-strength (S). PBR 357 also exhibited strong nematicidal activity, although slightly lower than TMLC 2. These findings suggest that non-canola *Brassica* varieties-particularly TMLC 2 harbours potent bioactive compounds and represent promising candidates for the development of plant-based nematode management strategies aimed at sustainable crop protection.

Keywords: *Brassica* spp., Canola, Egg hatching, Juvenile (J_2) mortality, *Meloidogyne incognita,* Non-canola.

1. INTRODUCTION

Plant-parasitic nematodes, particularly root-knot nematodes (*Meloidogyne* spp.), represent a major constraint to global agricultural productivity, causing estimated annual losses of over $100 billion worldwide (Jones et al., 2013). *Meloidogyne incognita*, the most widespread and economically damaging species, is notorious for its wide host range and aggressive colonization of plant roots, leading to the formation of characteristic galls that disrupt water and nutrient uptake (Sun et al., 2024).Traditional nematode management relies heavily on chemical nematicides; however, increasing environmental and health concerns, along with stricter regulatory frameworks, have prompted the search for safer and sustainable alternatives (Abd-Elgawad MMM, 2024; Desaeger et al., 2020; Chitwood, 2002).

Botanical nematicides, derived from plant metabolites, have emerged as promising candidates due to their natural origin, biodegradability, and low toxicity to non-target organisms (Mwamula et al., 2022; Akhtar and Malik, 2000). Members of the Brassicaceae family have attracted significant interest, primarily due to their production of glucosinolates and their hydrolysis products such as isothiocyanates, which exhibit broad-spectrum bioactivity, including nematicidal, antifungal, and insecticidal properties (Sharma et al., 2024; Zasada and Ferris, 2004). When the plant tissues of Brassica are disrupted, certain enzymes *viz*., endogenous myrosinase convert glucosinolates into toxic compounds, which are capable of impairing normal metabolism and reproduction of nematode (Chhajed et al., 2020; Lazzeri et al., 2004). The nematicidal potential of certain *Brassica* species is well established, variations among cultivars and extraction methods may influence efficacy.

We evaluated the *in vitro* nematicidal activity of aqueous leaf extracts from selected canola (*Brassica napus*; RLC 3 and GSC 7) and non-canola (*Brassica rapa* and *Eruca sativa*; PBR 357 and TMLC 2) varieties against root knot nematode (*M. incognita*). The findings are expected to contribute to the development of botanical-based control measures that are environmentally benign and compatible with sustainable agriculture practices.

2. MATERIALS AND METHODS

The study was conducted in the Nematology Laboratory,

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Department of Plant Pathology, Punjab Agricultural University, Ludhiana. Healthy leaves were collected from sixty-day-old plants of *Brassica* species, comprising two canola varieties (RLC 3 and GSC 7) and two non-canola varieties (PBR 357 and TMLC 2). The leaves were washed meticulously with sterile distilled water to clear off surface residues or microbes. Aqueous leaf extracts were prepared separately for each variety by grinding 10 grams of fresh leaf tissue in 20 mL of distilled water using a sterile pestle and mortar. The homogenized sample was passed through four layers of muslin cloth to eliminate coarse debris, followed by centrifugation at 4000 rpm for 5 minutes. The supernatant was then filtered using Whatman No. 1 filter paper to obtain a clear extract. These aqueous extracts were stored in sterilized, covered flasks under refrigeration at 4°C and were used as stock solutions (denoted as S) for in vitro nematode bioassays.

2.1. Evaluation of Egg Hatching Inhibition

For the evaluation of egg hatching inhibition, egg masses of *M. incognita* were extracted from pure culture raised on susceptible brinjal cultivar 'Punjab Sadabahar' maintained in Research Farm, Department of Plant Pathology, PAU, Ludhiana. Brinjal plants were gently removed from the soil, and the galled roots were cleaned using sterilized distilled water. Uniform sized egg masses were hand pinched using a pair of forceps. The stock solutions were used to prepare four concentrations: full strength (S), half (S/2), quarter (S/4), and one-eighth (S/8). Total five treatments were tested including four concentrations and one untreated control (distilled water). Each treatment was replicated three times. Ten mL of each concentration was added to the respective Petri plates (50 mm diameter), and four egg masses were transferred into each plate. Plates containing sterilized distilled water served as the control. The Petri plates were incubated in a BOD incubator at 25 ± 1°C, and observations on egg hatching were recorded under a binocular microscope at 24, 48, 96, 144, 192 and 240 hrs after treatment. Percent egg hatching was calculated using the formula: % egg hatch = (T - C)/C × 100, where T represents the total number of juveniles in treatment and C represented the total number of juveniles in control.

2.2. Evaluation of Juvenile Mortality Inhibition

The egg masses were surface sterilized in 0.5% sodium hypochlorite (NaOCl) solution for 2-3 minutes and rinsed thoroughly with sterile distilled water. The sterilized egg masses were incubated on double-layer tissue paper supported on wire mesh in a modified Baermann funnel at room temperature (25 ± 2 °C). Freshly hatched second-stage juveniles (J_2) were collected daily from the funnel outlet into beakers, concentrated by allowing them to settle, and used immediately for juvenile mortality inhibition assays. Only active and healthy juveniles were considered for experiments. For the J_2 mortality experiment, approximately 100 freshly hatched second-stage juveniles of *M. incognita* were added to 50 mm Petri dishes containing 10 mL of each treatment solution (S, S/2, S/4, S/8). Control plates contained only sterilized distilled water. Each treatment was replicated four times. The plates were incubated at 25 ± 1°C in a BOD incubator and mortality of juveniles was assessed at 24, 48, 96, 144, 192 and 240 hrs post-treatment using a binocular microscope. Juveniles were considered dead if they failed to respond to probing with a fine needle. Percent juvenile mortality was calculated using the formula: % mortality = (T - C)/C × 100, where T represented the number of dead juveniles in the treatment and C represented the number of dead juveniles in the control.

2.3. Statistical Analysis

The experiments were laid out in a completely randomized design (CRD), and data on percent egg hatching and juvenile mortality were subjected to analysis of variance (ANOVA). Treatment means were compared to determine statistical significance at the 5% level by R Software version 4.1.1.

3. RESULTS AND DISCUSSION

3.1. Nematicidal Activity of Extracts of *Brassica* spp. (canola and non-canola) Extracts against Egg Hatching of *M. incognita*

Aqueous extracts derived from different *Brassica* varieties significantly inhibited the hatching of *M. incognita* eggs and the egg hatch inhibition increased with both concentration of the extracts and duration. Egg hatch percentage decreased consistently with increasing extract concentration. Maximum inhibition (100%) was observed at full-strength (S) concentration across all varieties within 24 hours. The canola variety GSC 7 exhibited strong inhibition, with 97.42%, 89.89%, 86.45%, and 75.91% inhibition at S, S/2, S/4, and S/8 concentrations, respectively, on the second day. Although egg hatching gradually increased over time, higher concentrations continued to suppress it effectively even on later days. After 144 hours, inhibition at S/8 concentration was 54.60% for GSC 7 and 70.26% for RLC 3. Among the canola varieties, RLC 3 exhibited complete inhibition on the first day and maintained higher effectiveness than GSC 7 throughout the observation period. Overall, RLC 3 was found to be the most effective in reducing egg hatch of *M. incognita*, indicating its strong

nematicidal potential. Egg hatch inhibition of *M. incognita* was significantly influenced by variety (A), extract concentration (B) and duration of exposure (C). The significant A × B interaction indicated that varietal differences depended on extract concentration (Table 1). Both varieties exhibited comparable inhibition at higher concentrations. The efficiency of of RLC 3 became more pronounced even at lower concentrations. The B × C interaction showed that inhibitory effects declined more rapidly over time at lower concentrations. These interactions demonstrate that the nematicidal efficacy of canola *Brassica* extracts is jointly regulated by variety, concentration and exposure duration.

Non-canola *Brassica* cultivars (PBR 357 and TMLC 2) also exhibited a concentration and time dependent inhibition of *M. incognita* egg hatching (Table 2). Egg hatch percentage decreased with increasing extract concentration and this egg hatch inhibition increased gradually over time. Among the two, TMLC 2 showed consistently higher inhibitory effects than PBR 357. At full-strength (S)

Table 1. Effect of canola varieties (GSC 7 and RLC 3) on egg hatch inhibition of *Meloidogyne incognita*

Duration (hrs)	Concentration							
	S		S/2		S/4		S/8	
	GSC 7	RLC 3	GSC 7	RLC 3	GSC 7	RLC 3	GSC 7	RLC 3
24	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0
48	97.42±1.208	100.00±0	89.89±0.430	95.05±0.431	86.45±2.444	92.69±1.041	75.91±4.441	88.17±1.273
96	85.59±1.612	92.61±1.003	76.22±1.524	88.29±0.569	68.11±1.441	87.57±0.662	58.56±2.688	77.84±1.732
144	83.07±1.208	86.72±0.669	74.60±1.334	80.73±1.145	66.72±0.438	77.08±0.715	54.60±3.258	72.26±0.894
CD (P=0.05)								
A	1.038							
B	1.468							
C	2.075							
A×B	1.797							
B×C	2.542							

A = Variety (GSC 7 and RLC 3); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

Table 2. Effect of non-canola varieties PBR 357 and TMLC 2 on egg hatch inhibition of *Meloidogyne incognita*

Duration (hrs)	Concentration							
	S		S/2		S/4		S/8	
	PBR 357	TMLC 2	PBR 357	TMLC 2	PBR 357	TMLC 2	PBR 357	TMLC 2
24	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0
48	100.00±0	100.00±0	92.04±0.93	98.49±0.64	82.80±2.01	97.20±0.26	72.04±2.01	81.08±2.89
96	90.45±2.27	99.09±0.63	88.83±1.01	95.86±0.61	75.86±2.17	94.59±0.57	48.11±2.25	70.09±5.36
144	87.45±2.05	93.70±2.07	81.61±0.93	89.34±1.23	73.28±2.08	83.50±2.85	40.88±4.22	65.26±4.27
CD (P=0.05)								
A	0.092							
B	0.130							
A×B	0.184							
C	1.59							
A×C	0.225							
B×C	0.319							

A = Variety (PBR 357 and TMLC 2); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

concentration, both varieties recorded nearly complete inhibition (76%-100%) within the first 48 hours. Egg hatch inhibition in PBR 357 at S and S/2 concentrations remained statistically similar at both 24 and 48 hours. At the lowest concentration (S/8), TMLC 2 achieved 42.57% inhibition, while PBR 357 showed 33.11%, indicating a relatively lower efficacy. Interestingly, TMLC 2 exhibited complete (100%) inhibition at the S/2 concentration after 24 hours, which decreased to 66.76% by 240 hours, indicating an approximate 33% decline over time. Overall, TMLC 2 demonstrated greater efficacy than PBR 357 in reducing *M. incognita* egg hatching, particularly at higher concentrations and shorter exposure periods. Egg hatch inhibition of *M. incognita* by non-canola *Brassica* varieties was significantly affected by variety (A), extract concentration (B), and duration of exposure (C) (Table 2). The significant A × B interaction indicated that varietal differences were concentration dependent with both varieties showing comparable inhibition at higher concentrations but TMLC 2 maintained greater inhibition at lower concentrations. The A × C interaction showed that TMLC 2 retained inhibitory activity for a longer duration than PBR 357. The B × C interaction further demonstrated that egg hatch inhibition declined more rapidly over time at lower concentrations whereas higher concentrations sustained inhibitory effects for longer durations.

Among all the *Brassica* varieties tested, the non-canola cultivars demonstrated superior efficacy in inhibiting egg hatching of *M. incognita* compared to the canola varieties. Notably, the TMLC 2 extract emerged as the most potent, consistently exhibiting the highest level of egg hatch inhibition across all concentrations and time intervals, outperforming not only its non-canola counterpart PBR 357 but also all tested canola varieties. The present study clearly demonstrated that aqueous extracts of both canola and non-canola *Brassica* cultivars possess strong nematicidal activity against the egg hatching of *M. incognita*. Across all tested varieties, egg hatch inhibition increased consistently with extract concentration and exposure duration, with near-complete or complete inhibition observed at higher concentrations during early exposure periods. This uniform response across *Brassica* types indicates that egg hatching represents a highly sensitive developmental stage of *M. incognita* and can be effectively targeted using *Brassica*-derived bioactive compounds such as glucosinolates and their enzymatic hydrolysis products, particularly isothiocyanates which are released upon disruption of *Brassica* tissues. This enhanced activity can be attributed to their higher glucosinolate content and hydrolysis products such as isothiocyanates, which interfere with nematode development and physiology (Eugui et al., 2022). In contrast, canola varieties typically possess reduced glucosinolate levels due to selective breeding for improved palatability and oil quality, which likely accounts for their comparatively lower efficacy. Kumar et al (2019) demonstrated that dilution of botanical extracts led to reduced inhibition of *M. incognita* egg hatching. Their study, which included treatments with neem, cabbage, and cauliflower leaf extracts, highlighted that the most potent inhibition occurred at the highest extract concentration, particularly during early exposure, aligning with the trends recorded in the present work. These compounds are known to interfere with nematode embryonic development by penetrating the egg shell and disrupting essential metabolic processes. Recent studies have consistently reported that *Brassica*-based materials exert pronounced toxic effects on root-knot nematode eggs, supporting the rapid and substantial egg hatch suppression recorded in the present study (Dutta et al., 2019; Bui et al., 2021).

The clear concentration-dependent decline in egg hatching observed for both canola and non-canola cultivars aligns well with previous in vitro studies using botanical extracts. Higher extract concentrations likely release greater quantities of active metabolites, resulting in enhanced toxicity and prolonged inhibition of egg development. Similar dose-dependent suppression of *Meloidogyne* egg hatching has been reported by Das et al. (2021), who emphasized that reduced efficacy at lower concentrations is often associated with insufficient levels of nematicidal compounds to fully arrest embryogenesis. A gradual decline in inhibitory activity over time, particularly at diluted concentrations, was evident across both *Brassica* groups. This time-dependent reduction in efficacy is consistent with the transient nature of *Brassica*-derived volatiles. Isothiocyanates and related compounds are known to degrade or volatilize over extended exposure periods, especially in aqueous systems, leading to partial recovery of egg hatching. Such patterns have been well documented in recent studies, which reported strong initial nematicidal activity followed by reduced persistence over time (Bui et al., 2021; Petrikovszki et al., 2023).

Distinct varietal differences were evident within both canola and non-canola groups. Among canola cultivars, RLC 3 consistently exhibited higher egg hatch inhibition than GSC 7, while among non-canola cultivars, TMLC 2 outperformed PBR 357 across most concentrations and

exposure periods. These differences are likely linked to variation in glucosinolate content and composition among *Brassica* genotypes. Previous studies have highlighted that plant genotype is a critical determinant of nematicidal efficacy, with certain *Brassica* cultivars maintaining higher activity even at lower concentrations or longer exposure durations (Dutta et al., 2019; Mwamula et al., 2022). Notably, the non-canola Brassica cultivars demonstrated overall superior efficacy compared to canola varieties, with TMLC 2 emerging as the most potent among all tested genotypes. This observation suggests that non-canola *Brassica* types may possess more favorable biochemical profiles for nematode suppression. Similar findings have been reported in earlier investigations, where non-canola *Brassica* species exhibited stronger biofumigation potential due to higher or more diverse glucosinolate concentrations (Dutta et al., 2019; Mwamula et al., 2022).

The significant interactions observed among variety, extract concentration, and exposure duration further indicate that nematicidal efficacy is governed by the combined influence of biochemical composition, dose, and persistence of active compounds. Higher concentrations sustained inhibitory effects for longer durations, while varietal differences became more pronounced under sub-optimal concentrations and prolonged exposure. Overall, the integrated results strongly support the potential of *Brassica* extracts, particularly from highly effective non-canola cultivars such as TMLC 2 and canola cultivar RLC 3, as promising botanical agents for suppressing *M. incognita* egg hatching under in vitro conditions.

3.2. Nematicidal Activity of Extract of *Brassica* spp. (canola and non-canola) on Second Stage Juveniles (J_2) of *M. incognita*

This experiment evaluated the effect of aqueous leaf extracts from various *Brassica* varieties-canola (GSC 7 and RLC 3) and non-canola (PBR 357 and TMLC 2)-on the mortality of second-stage juveniles (J_2) of *M. incognita* under *in vitro* conditions. All evaluated extracts caused a significant rise in juvenile mortality, which varied according to concentration and exposure duration. Among the canola varieties, RLC 3 was found to be the most effective, causing up to 98% mortality at full-strength (S) concentration after 10 days of exposure. At the lowest concentration (S/8), juvenile mortality was 58% in RLC 3 and 38.4% in GSC 7. Mortality at S/2 concentration increased from 45.2% to nearly 90% in GSC 7 and from 60 to over 90% in RLC 3 over the 10-day period. Similarly, at S/4 concentration, maximum mortality reached 77.2% for RLC 3 and 66% for GSC 7. A significant positive correlation was observed between concentration, exposure duration, and juvenile mortality. A significant A × B interaction indicated that varietal differences in juvenile mortality were concentration dependent. While both varieties caused comparatively high mortality at higher concentrations, the superiority of RLC 3 over GSC 7 became more pronounced even at lower concentrations particularly S/4 and S/8. The A × B × C interaction further demonstrated that these varietal differences across concentrations were influenced by exposure duration with RLC 3 maintaining higher mortality at lower concentrations over longer exposure periods. In contrast, the B × C interaction was non-significant, indicating that the relative effect of concentration on juvenile mortality remained consistent across exposure durations (Table 3).

In the non-canola group, both varieties were effective in reducing J_2 numbers with TMLC 2 showing stronger nematicidal activity. Mortality of second-stage juveniles (J2) of *M. incognita* was significantly affected by variety (A), extract concentration (B), and duration of exposure (C), with clear interaction effects. Across all concentrations and observation periods, TMLC 2 consistently induced higher juvenile mortality than PBR 357. At full-strength concentration (S), mortality in PBR 357 increased from 82.40% at 24 h to 97.20% at 240 h, while TMLC 2 increased from 84.00% to complete mortality (100%) over the same period. At S/2 concentration, mortality ranged from 50.40% to 89.60% in PBR 357 and from 74.40% to 98.80% in TMLC 2 confirming the superior efficacy of TMLC 2. At S/4 concentration, mortality in PBR 357 increased from 17.20% at 24 h to 64.40% at 240 h, whereas TMLC 2 showed higher values ranging from 42.80% to 86.40%. Similarly, at the lowest concentration (S/8), mortality increased from 8.00% to 59.20% in PBR 357 and from 18.40% to 68.80% in TMLC 2 over the exposure period. The significant A × B interaction indicated that varietal differences were concentration dependent, with differences between TMLC 2 and PBR 357 becoming more pronounced at lower concentrations (Table 4). The A × C interaction further showed that TMLC 2 maintained higher juvenile mortality over longer exposure periods compared to PBR 357. The B × C interaction demonstrated that mortality increased more rapidly at higher concentrations, while lower concentrations required longer exposure to achieve comparable effects. The significant A × B × C interaction confirmed that varietal differences in juvenile mortality across concentrations were further

modulated by exposure duration.

From all the four varieties tested for mortality against J_2 of *M. incognita*, TMLC 2 was the most effective treatment across all concentrations and time points. The present study demonstrated that aqueous extracts from various *Brassica* species significantly increased the mortality of second-stage juveniles (J_2) of *M. incognita*, with effectiveness strongly influenced by both extract concentration and duration of exposure. All tested varieties exhibited nematicidal activity, confirming the presence of bioactive compounds capable of disrupting nematode viability. A clear dose and time dependent trend was observed, wherein juvenile mortality increased progressively with higher concentrations and prolonged exposure. Aqueous leaf extracts of both canola and non-canola *Brassica* varieties caused significant mortality of second-stage juveniles (J2) of *M. incognita* under in vitro conditions. Mortality increased consistently with extract concentration and duration of exposure. This clear dose- and time-dependent response indicates that *Brassica*-derived metabolites exert direct toxic effects on the infective juvenile stage of the nematode. High juvenile mortality observed at full-strength and half-strength concentrations suggests rapid penetration of toxic compounds through the nematode cuticle resulting in disruption of neuromuscular coordination and essential metabolic functions. Plant-derived sulfur-containing compounds, including glucosinolate degradation products, have been reported to induce paralysis and mortality in plant-parasitic nematodes by interfering with respiration and nervous system activity (Ntalli et al., 2017). Such mechanisms explain the rapid and high mortality recorded at higher concentrations in the present study. At lower concentrations, juvenile mortality increased gradually with prolonged exposure, indicating cumulative toxic effects rather than immediate lethality. Similar delayed but irreversible effects of botanical nematicides on *Meloidogyne* juveniles have been reported earlier where extended exposure compensated for reduced dosage (Oka, 2020). This supports the observed requirement for longer exposure periods to achieve substantial mortality at diluted extract concentrations. Marked varietal differences were evident among the tested *Brassica* cultivars. The consistently higher mortality induced by RLC 3 among canola varieties and by TMLC 2 among non-canola varieties suggests genotype-specific variation in the quantity and composition of nematicidal metabolites. Previous studies have demonstrated that *Brassica* genotypes differ significantly in their toxicity toward *Meloidogyne* juveniles due to variation in bioactive compound profiles (López-González et al., 2019). The superior and sustained efficacy of TMLC 2 across concentrations and time intervals highlights its strong nematicidal potential. Overall, the present findings confirm that aqueous *Brassica* extracts possess strong direct toxicity against *M. incognita* juveniles and that nematicidal efficacy

Table 3. Effect of canola varieties (GSC 7 and RLC 3) on mortality of second stage juveniles (J2) of *Meloidogyne incognita*

Duration (hrs)	Concentration							
	S		S/2		S/4		S/8	
	GSC 7	RLC 3	GSC 7	RLC 3	GSC 7	RLC 3	GSC 7	RLC 3
24	60.00±2.60	72.80±3.72	45.20±1.62	60.00±1.87	30.80±1.35	28.00±2.75	5.20±1.20	11.20±0.80
48	74.80±2.57	80.80±2.87	58.80±2.87	68.00±3.40	36.40±0.98	35.60±3.70	12.00±1.67	18.40±0.98
96	81.60±2.35	86.80±2.80	66.00±2.75	73.20±3.13	42.40±3.86	44.40±2.85	14.80±3.34	32.80±1.35
144	86.80±2.87	89.20±2.04	77.20±3.26	80.00±2.89	49.20±3.26	57.20±2.33	24.40±2.04	41.60±0.98
CD (P=0.05)								
A	0.103							
B	0.146							
C	0.207							
A×B	0.179							
B×C	NS							
A×B×C	0.358							

A = Variety (GSC 7 and RLC 3); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

Table 4. Effect of non-canola varieties (PBR 357 and TMLC 2) on mortality of second stage juveniles of *Meloiodogyne incognita*

Duration (hrs)	Concentration							
	S		S/2		S/4		S/8	
	PBR 357	TMLC 2	PBR 357	TMLC 2	PBR 357	TMLC 2	PBR 357	TMLC 2
24	82.40±1.47	84.00±1.09	50.40±3.70	74.40±1.93	17.20±2.33	42.80±2.32	8.00 ±1.26	18.40±1.60
48	84.40±2.40	88.80±1.20	56.00±4.33	82.80±1.20	24.00±1.89	51.60±3.81	16.40±1.32	23.60±2.40
96	88.40±2.48	94.40±1.16	65.20±2.24	86.40±1.16	33.20±2.05	58.80±3.61	28.40±2.27	29.60±1.93
144	94.00±2.19	98.40±1.16	71.60±4.11	91.20±1.20	44.80±2.57	68.40±2.40	37.20±2.05	39.20±1.62
CD (p=0.05)								
A	0.092							
B	0.130							
C	0.159							
A × B	0.183							
A × C	0.225							
B × C	0.318							
A × B × C	0.449							

A = Variety (PBR 357 and TMLC 2); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

is governed by cultivar, extract concentration, and exposure duration. These results support the potential use of highly effective *Brassica* cultivars as botanical sources for nematode management under in vitro conditions. These findings are also consistent with those reported by Feyisa et al. (2016), who evaluated botanical extracts including rapeseed (*Brassica napus*), *Lantana camara*, *Tagetes erecta*, and *Azadirachta indica* and reported maximum mortality (100%) in neem extract at the highest concentration after three days highlighting a similar pattern of increasing efficacy with higher dosage and longer exposure periods.

Overall, the data support the conclusion that *Brassica* extracts-particularly those from non-canola varieties like TMLC 2-hold considerable promise as bio-based alternatives to chemical nematicides. Their efficacy at higher concentrations and over extended periods suggests their potential for incorporation into sustainable nematode management strategies.

4. CONCLUSION

The present study clearly established the nematicidal potential of aqueous leaf extracts from various *Brassica* species against *M. incognita* under *in vitro* conditions. All tested extracts significantly inhibited egg hatching and increased juvenile mortality in a dose and time-dependent. Among the canola varieties, Raya Ludhiana Canola (RLC 3) was the most effective and Taramira Ludhiana composite 2 (TMLC 2) from the non-canola group consistently outperformed all other varieties to achieve complete juvenile mortality and maximum egg hatch suppression at higher concentrations and longer exposure durations. The superior efficacy of non-canola varieties can be attributed to their higher glucosinolates content and associated bioactive compounds. These findings confirm the potential of *Brassica* species, particularly TMLC 2, as effective botanical agents for the biological control of root-knot nematodes. This eco-friendly approach holds significant promise as a viable alternative to synthetic nematicides, contributing to sustainable agriculture and improved soil health.

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Conflict of Interest

The authors declare they have no conflict of interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use generative AI or AI-assisted technologies in the writing of this manuscript.

Credit Authorship Contribution Statement

Harkanwal Pal Singh and Narpinderjeet Kaur Dhillon jointly planned and executed the research work. Harkanwal

Pal Singh and Anupam Sekhon carried out data collection for juvenile mortality, egg hatch inhibition and statistical analysis. Narpinderjeet Kaur Dhillon and Sukhjeet Kaur supervised the entire study and critically reviewed the manuscript. All authors contributed to manuscript writing and approved the final version.

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