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BIOACTIVE LEAF EXTRACTS OF *PUTRANJIVA ROXBURGHII* ((LUCKY BEAN TREE)) AS A SUSTAINABLE BOTANICAL NEMATICIDE AGAINST *MELOIDOGYNE INCOGNITA*

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ABSTRACT

Root-knot nematodes (*Meloidogyne incognita*) are among the most destructive plant-parasitic nematodes, causing significant yield losses in a wide range of agricultural crops worldwide. The increasing concerns regarding the environmental and health hazards associated with synthetic nematicides have stimulated the search for eco-friendly botanical alternatives. The present study evaluated the nematicidal efficacy of leaf extracts of *Putranjiva roxburghii* (Lucky Bean Tree) against *M. incognita* under laboratory conditions. Fresh leaves were collected, processed, and extracted using aqueous and organic solvents to obtain bioactive compounds. Different concentrations of the leaf extracts were tested against second-stage juveniles (J_2s) and egg masses of *M. incognita*. Juvenile mortality and inhibition of egg hatching were recorded at various exposure periods. The results demonstrated a concentration- and time-dependent nematicidal activity of *P. roxburghii* leaf extracts. Higher concentrations resulted in significantly increased juvenile mortality and reduced egg hatchability compared with the untreated control. Maximum juvenile mortality and egg-hatch inhibition were observed at the highest extract concentration after prolonged exposure. The findings suggest that *P. roxburghii* possesses potent bioactive constituents capable of suppressing *M. incognita* populations and may serve as an environmentally sustainable alternative to chemical nematicides. Further greenhouse and field investigations are recommended to validate its practical applicability in integrated nematode management programs.

Keywords: *Putranjiva roxburghii,* Lucky Bean Tree, *Meloidogyne incognita*, botanical nematicide, juvenile mortality, egg hatch inhibition, phytochemicals, sustainable agriculture.

INTRODUCTION

Plant-parasitic nematodes (PPNs) are among the most destructive agricultural pests worldwide and constitute a major constraint to sustainable crop production. More than 4,100 species of plant-parasitic nematodes have been described, causing substantial losses in agricultural productivity and food security. Annual global crop losses attributed to PPNs are estimated to exceed US$150 billion, highlighting their enormous economic significance (Onkendi et al., 2014; Kantor et al., 2025). Singh and Kumar (2015) reported an average production loss of 23.7% in 17 vegetable crops due to plant-parasitic nematodes, with maximum losses of 43% in eggplant, 40% in tomato, and 38% in okra. Among these nematodes, root-knot nematodes (RKNs), belonging to the genus Meloidogyne, are regarded as the most economically important group because of their extensive host range, worldwide

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distribution, and ability to infect almost all cultivated crops (Subedi et al., 2020; Kantor et al., 2025). Meloidogyne incognita is particularly destructive and has been reported from tropical, subtropical, and temperate agricultural ecosystems throughout the world. It infects more than 3,000 plant species, including vegetables, cereals, pulses, fruits, ornamentals, medicinal plants, and plantation crops, resulting in severe yield reductions and deterioration in crop quality (Ralmi et al., 2016; Subedi et al., 2020).

The pathogenicity of M. incognita is associated with its unique parasitic lifestyle. The infective second-stage juveniles (J2s) penetrate young roots and migrate intercellularly towards the vascular tissues, where they induce the formation of specialized feeding structures known as giant cells. These giant cells act as nutrient sinks and support nematode growth and reproduction. The resulting root galls disrupt water and nutrient uptake, leading to stunted growth, chlorosis, wilting, nutrient deficiencies, and substantial yield losses. Furthermore, nematode-infested plants become more susceptible to secondary infections caused by fungi, bacteria, and viruses, thereby aggravating crop damage (Lamberti, 2000; Onkendi et al., 2014). Yield losses ranging from 20–80% have been reported in several vegetable crops under severe infestations, particularly in warm climatic regions favorable for nematode multiplication (Onkendi et al., 2014; Subedi et al., 2020).

Management of root-knot nematodes has traditionally relied on synthetic nematicides such as carbamates, organophosphates, and fumigants. These chemicals often provide rapid suppression of nematode populations; however, their prolonged and indiscriminate use has raised serious concerns regarding environmental contamination, groundwater pollution, destruction of beneficial soil microorganisms, pesticide residues in food products, and adverse effects on human and animal health. In recent years, many conventional nematicides have been restricted or withdrawn because of their toxicological and environmental risks. Consequently, there is a growing demand for environmentally safe, biodegradable, and sustainable alternatives that can be incorporated into integrated nematode management programs (Djian-Caporalino et al., 2011; Kantor et al., 2025).

Botanical pesticides have emerged as promising alternatives to synthetic agrochemicals because they are generally biodegradable, less toxic to non-target organisms, environmentally compatible, and often readily available from locally occurring plant resources. Numerous higher plants synthesize diverse secondary metabolites that function as natural defense compounds against herbivores, insects, nematodes, and microbial pathogens. These metabolites include alkaloids, flavonoids, terpenoids, tannins, phenolic acids, saponins, glycosides, and essential oils, many of which exhibit potent nematicidal properties. Plant-derived compounds may affect nematodes through various mechanisms, including disruption of cellular membranes, inhibition of enzymatic activities, interference with nervous system function, suppression of egg hatching, reduction of juvenile mobility, and inhibition of reproduction (Akhtar and Malik, 2000; Oka et al., 2000).

Several studies have demonstrated the effectiveness of botanical extracts against root-knot nematodes. Extracts of Azadirachta indica (neem), Calotropis procera, Tagetes erecta, Phyllanthus amarus, Datura stramonium, Ricinus communis, and numerous medicinal plants

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have shown significant inhibition of egg hatching and increased mortality of Meloidogyne juveniles under laboratory and greenhouse conditions. The efficacy of these plants is largely attributed to the presence of bioactive secondary metabolites capable of interfering with nematode physiology and behavior. Such findings support the growing interest in the exploration of novel plant species as sources of eco-friendly nematicidal compounds (Oka et al., 2000; Subedi et al., 2020).

Recent advances in plant–nematode interaction studies have revealed that plants possess sophisticated biochemical defense mechanisms against root-knot nematodes. Upon nematode invasion, plants activate a cascade of defensive responses involving oxidative enzymes, phenolic compounds, pathogenesis-related proteins, and signaling molecules such as jasmonic acid and salicylic acid. Secondary metabolites synthesized during these responses often exhibit direct toxicity against nematodes or indirectly enhance plant resistance. Consequently, identification of plant species rich in such metabolites may contribute significantly to the development of sustainable nematode management strategies (Ramatsitsi et al., 2026).

Among the diverse medicinal plants of the Indian subcontinent, Putranjiva roxburghii Wall. (family Putranjivaceae), commonly known as the Lucky Bean Tree, has attracted increasing scientific interest because of its medicinal and pharmacological importance. The species is an evergreen tree widely distributed in India, Nepal, Bangladesh, Sri Lanka, and Southeast Asia. It is commonly cultivated as an ornamental avenue tree and is also valued in traditional Ayurvedic medicine. Different parts of the plant, including leaves, bark, roots, fruits, and seeds, have been used traditionally for the treatment of fever, inflammation, rheumatism, skin diseases, infertility disorders, and various microbial infections (Naik et al., 2023).

Phytochemical investigations have revealed that P. roxburghii contains a rich diversity of secondary metabolites including flavonoids, phenolic compounds, glycosides, triterpenoids, steroids, tannins, alkaloids, and various antioxidant constituents. These compounds are known to possess antimicrobial, antioxidant, anti-inflammatory, analgesic, antiparasitic, antidiabetic, and insecticidal activities. The presence of such bioactive metabolites suggests that P. roxburghii may also possess significant nematicidal potential. In particular, phenolic compounds and flavonoids have been widely reported to interfere with nematode development and survival through oxidative stress induction and disruption of physiological processes (Naik et al., 2023).

Leaves represent one of the most practical plant parts for the development of botanical pesticides because they are renewable, abundantly available, easy to harvest, and rich in secondary metabolites. Compared with bark and roots, leaf harvesting causes minimal damage to the plant and supports sustainable utilization of botanical resources. Moreover, leaf extracts can often be prepared using simple extraction techniques suitable for rural and low-input agricultural systems. Therefore, evaluating the nematicidal efficacy of leaf extracts of P. roxburghii may provide valuable information for the development of cost-effective and environmentally sustainable nematode management strategies.

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Despite extensive studies on the medicinal properties of P. roxburghii, information regarding its activity against plant-parasitic nematodes remains scarce. Most previous investigations have focused on its antioxidant, antimicrobial, anti-inflammatory, and pharmacological properties, whereas its potential as a botanical nematicide has received little scientific attention. This represents an important research gap considering the increasing demand for natural alternatives to synthetic nematicides and the urgent need for sustainable crop protection strategies.

The exploration of underutilized medicinal plants as sources of nematicidal compounds aligns with current global efforts to reduce dependence on hazardous pesticides and promote environmentally responsible agriculture. Botanical nematicides can contribute significantly to integrated pest management (IPM) programs by reducing chemical inputs, conserving soil biodiversity, and minimizing environmental pollution. Furthermore, the identification of effective plant-based nematicides may offer economically viable solutions for smallholder farmers who often face limitations in accessing expensive commercial nematicides.

Therefore, the present investigation was undertaken to evaluate the nematicidal efficacy of bioactive leaf extracts of Putranjiva roxburghii against Meloidogyne incognita. The study aims to assess the effects of different extract concentrations on juvenile mortality and egg-hatching inhibition and to determine the suitability of P. roxburghii as a sustainable botanical nematicide. The findings are expected to contribute to the growing body of knowledge on plant-derived nematicides and support the development of eco-friendly approaches for the management of root-knot nematodes in agricultural ecosystems.

MATERIALS AND METHODS

The present investigation was conducted in the Nematology Laboratory, Department of Zoology, Bareilly College, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, Uttar Pradesh, India, during 2025–2026. All laboratory experiments were performed under controlled conditions at a temperature of 25 ± 2°C and relative humidity of 65–75%.

Fresh, healthy, and disease-free leaves of Putranjiva roxburghii Wall. (Lucky Bean Tree) were collected from mature trees growing within the Bareilly College Campus and nearby localities of Bareilly district, Uttar Pradesh, India. The collected plant material was authenticated by a taxonomist from the Department of Botany, Bareilly College. A voucher specimen (PR-2026-01) was prepared and deposited in the departmental herbarium for future reference.

The leaves were thoroughly washed under running tap water, followed by distilled water to remove dust and contaminants. Clean leaves were shade-dried at room temperature (25–30°C) for 10–15 days until a constant weight was obtained. The dried leaves were pulverized into fine powder using an electric grinder and stored in airtight containers until extraction.

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Preparation of Leaf Extracts

Aqueous Extract: One hundred grams of dried leaf powder were mixed with 1000 mL of distilled water in a conical flask and kept on a rotary shaker (150 rpm) for 48 h at room temperature. The mixture was filtered through double-layered muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C and subsequently dried in a hot-air oven at 40°C to obtain crude extract.

Methanolic Extract: For solvent extraction, 100 g of dried leaf powder were subjected to Soxhlet extraction using methanol (99.8%) for 8 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The resulting crude extract was stored in sterile amber-colored bottles at 4°C until further use.

Preparation of Treatment Concentrations

Stock solutions (100%) of aqueous and methanolic extracts were prepared by dissolving crude extract in distilled water containing 0.1% Tween-20 as an emulsifying agent. Five concentrations 1%, 2%, 5%, 10%, 20% were prepared using distilled water containing Tween-20 served as the negative control.

Collection and Maintenance of Meloidogyne incognita Culture

Pure cultures of *Meloidogyne incognita* were maintained on susceptible tomato (*Solanum lycopersicum* L. cv. Pusa Ruby) plants grown in sterilized soil under greenhouse conditions.

Extraction of Egg Masses: Root systems of heavily infested tomato plants were gently washed under tap water. Egg masses were handpicked using sterilized forceps under a stereoscopic microscope and transferred to sterile Petri dishes containing distilled water.

Isolation of Second-Stage Juveniles (J2): Egg masses were placed on modified Baermann trays lined with tissue paper and incubated at 25 ± 2°C. Freshly hatched second-stage juveniles (J2s) emerging within 24 h were collected and used for all bioassays.

In Vitro Juvenile Mortality Bioassay: The nematicidal activity of P. roxburghii leaf extracts against second-stage juveniles was evaluated following a completely randomized design (CRD).

Experimental Setup: Each treatment consisted of 5 mL test solution, Approximately 80-100 freshly hatched J2s, placed in sterile glass cavity blocks.

Treatments included: T1 (Control), T2 (1%), T3 (2%), T4 (5%), T5 (10%) and T6 (20%). Each treatment was replicated three times.

Observation Periods: Juvenile mortality was recorded after 12 h, 24 h, 48 h and 72 h. Nematodes were considered dead when they remained immobile and failed to respond to probing with a fine needle.

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Percent juveniles' mortality: It was calculated using:

Mortality (%) = (Number of dead juveniles / Total juveniles) × 100

Corrected mortality was determined using Abbott's formula:

Corrected Mortality (%) = [(T − C)/ (100 − C)] × 100

Where: T = mortality in treatment, C = mortality in control

Egg Hatch Inhibition Assay: Freshly collected egg masses containing approximately 100 eggs each were transferred to Petri dishes containing different extract concentrations. Five egg masses were used per replicate. The dishes were incubated at 25 ± 2°C and 70% Relative humidity. Egg hatching was recorded daily for seven days.

The percentage inhibition of egg hatching: It was calculated as:

Egg Hatch Inhibition (%) = [(Control hatch − Treatment hatch) / Control hatch] × 100

The lethal concentrations causing 50% and 90% juvenile mortality (LC50 and LC90) were estimated using probit analysis following Finney (1971).

Statistical analysis: Data were subjected to One-way Analysis of Variance (ANOVA) Tukey's HSD test ($P \leq 0.05$)

Ethical and Biosafety Considerations: The study involved plant extracts and plant-parasitic nematodes and did not require animal ethics approval. All laboratory procedures were conducted in accordance with institutional biosafety guidelines for handling plant pathogens and chemical reagents.

RESULTS

The nematicidal activity of Putranjiva roxburghii leaf extract against second-stage juveniles (J2) of Meloidogyne incognita was evaluated at different concentrations and exposure periods. The results demonstrated a significant concentration- and time-dependent increase in juvenile mortality (Table 1). Mortality of J2s increased progressively with increasing extract concentration as well as duration of exposure.

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Table 1. Effect of *Putranjiva roxburghii* leaf extract on the mortality percentage of second-stage juveniles (J2) of *Meloidogyne incognita* at various exposure periods

Treatment	Concentration (%)	Juvenile Mortality (%) after			
		12 h	24 h	48 h	72 h
T1	Control	2.33 ± 0.33	3.67 ± 0.33	4.67 ± 0.33	5.67 ± 0.33
T2	1	12.67 ± 0.88	21.33 ± 1.20	32.67 ± 1.45	43.33 ± 1.45
T3	2	18.67 ± 0.67	31.33 ± 1.45	45.67 ± 1.20	58.67 ± 1.20
T4	5	29.33 ± 1.20	46.67 ± 1.76	63.33 ± 1.45	76.67 ± 1.20
T5	10	42.67 ± 1.45	61.33 ± 1.20	79.67 ± 1.45	91.33 ± 0.88
T6	20	56.67 ± 1.20	78.33 ± 1.45	93.67 ± 0.67	98.67 ± 0.33

Values are Mean ± SE of three replications.

The untreated control (T1) recorded only negligible mortality, ranging from 2.33% after 12 h to 5.67% after 72 h, indicating normal survival of juveniles under laboratory conditions. In contrast, all concentrations of P. roxburghii leaf extract significantly increased juvenile mortality compared with the control.

At 12 h of exposure, juvenile mortality ranged from 12.67% at 1% concentration (T2) to 56.67% at 20% concentration (T6). After 24 h, mortality increased substantially, reaching 21.33%, 31.33%, 46.67%, 61.33%, and 78.33% in treatments containing 1%, 2%, 5%, 10%, and 20% extract concentrations, respectively. Similar trends were observed after 48 h exposure, with mortality values ranging from 32.67% in T2 to 93.67% in T6.

The highest juvenile mortality was recorded after 72 h exposure, where the 20% leaf extract concentration resulted in 98.67% mortality, followed by 91.33% mortality at 10% concentration and 76.67% mortality at 5% concentration. Even the lowest concentration (1%) caused substantial mortality (43.33%) after 72 h exposure, indicating the presence of potent bioactive compounds capable of affecting nematode survival.

The results clearly indicate that the efficacy of P. roxburghii leaf extract is strongly dependent upon both concentration and exposure duration. A positive dose-response relationship was evident throughout the experiment, suggesting increased availability of toxic phytochemicals at higher concentrations and prolonged exposure times.

The leaf extracts of *Putranjiva roxburghii* exhibited a marked inhibitory effect on egg hatching of Meloidogyne incognita. The inhibition of egg hatching increased progressively with increasing extract concentration and duration of exposure. Although low concentrations showed moderate suppression of egg emergence during the initial observation periods, higher

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concentrations significantly reduced egg hatchability throughout the experimental period (Table 2).

Table 2. Effect of *Putranjiva roxburghii* leaf extract on egg hatch inhibition (%) of *Meloidogyne incognita* at different exposure periods

Treatment	Concentration (%)	Egg Hatch Inhibition (%) after		
		3 Days	5 Days	7 Days
T1	Control	2.67 ± 0.33	3.67 ± 0.33	4.67 ± 0.33
T2	1	15.33 ± 0.88	28.67 ± 1.20	41.33 ± 1.45
T3	2	24.67 ± 0.67	39.33 ± 1.45	55.67 ± 1.20
T4	5	38.33 ± 1.20	57.67 ± 1.76	73.33 ± 1.45
T5	10	53.67 ± 1.45	76.33 ± 1.20	89.67 ± 1.45
T6	20	68.33 ± 1.20	90.67 ± 0.88	97.33 ± 0.67

Values are Mean ± SE of three replications.

The highest inhibitory effect was observed at 20% concentration (T6), where egg hatching was almost completely suppressed after prolonged exposure. Treatments containing 10% and 5% extract concentrations also showed substantial reductions in egg hatching compared with the untreated control. In contrast, the control treatment exhibited normal egg hatching, indicating that the observed inhibition resulted from the bioactive constituents present in P. roxburghii leaf extracts.

The inhibitory response followed a concentration-dependent pattern similar to that observed in juvenile mortality assays. As extract concentration increased from 1% to 20%, the degree of egg hatch suppression increased correspondingly, demonstrating the potent ovicidal activity of the plant extract against M. incognita.

DISCUSSION

The present investigation demonstrated that leaf extracts of Putranjiva roxburghii possess strong nematicidal activity against Meloidogyne incognita juveniles. The observed increase in mortality with increasing extract concentration and exposure duration is consistent with the general mode of action of plant-derived nematicidal compounds reported in earlier studies (Akhtar & Malik, 2000; Oka et al., 2000).

The remarkable mortality observed at higher concentrations may be attributed to the diverse phytochemicals present in P. roxburghii leaves. Previous phytochemical investigations have revealed the occurrence of flavonoids, tannins, alkaloids, glycosides, terpenoids, phenolic compounds, and steroids in various parts of the plant (Naik et al., 2023). These compounds

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are known to interfere with cellular metabolism, membrane integrity, enzyme systems, and nervous functions of nematodes, ultimately leading to paralysis and death (Chitwood, 2002).

The nearly complete mortality (98.67%) achieved at 20% concentration after 72 h indicates a potent toxic effect of the extract against infective juveniles of M. incognita. Similar concentration-dependent mortality responses have been reported for several botanical extracts including Azadirachta indica, Tagetes erecta, Calotropis procera, Datura stramonium, and Phyllanthus amarus, where higher concentrations resulted in significant suppression of root-knot nematode populations (Oka et al., 2000; Nengroo et al., 2021).

The present findings also support observations made by Singh and Kumar (2015), who emphasized the need for eco-friendly alternatives to manage root-knot nematodes due to substantial crop losses caused by Meloidogyne species in vegetable-growing regions of northern India. Botanical extracts offer a sustainable approach because they are biodegradable, locally available, and less hazardous to the environment than synthetic nematicides.

The progressive increase in mortality from 12 h to 72 h exposure suggests that prolonged contact enhances penetration and accumulation of active compounds within nematode tissues. Similar exposure-dependent effects have been reported by Echeverrigaray et al. (2010), who observed increased mortality of Meloidogyne incognita juveniles with increasing exposure time to plant-derived monoterpenoids. Such compounds may interfere with respiration, neuromuscular activity, and energy metabolism, eventually causing irreversible physiological damage.

The efficacy of P. roxburghii observed in the present study is particularly noteworthy because the species has received comparatively little attention as a source of botanical nematicides. While its antimicrobial, antioxidant, and anti-inflammatory properties are well documented (Naik et al., 2023), information regarding its nematicidal activity remains scarce. Therefore, the present findings contribute novel evidence supporting the use of P. roxburghii as a potential botanical nematicide.

From a practical perspective, the use of leaf extracts offers additional advantages. Leaves are renewable plant resources that can be harvested without destroying the plant, thereby promoting sustainable utilization. Moreover, preparation of aqueous or solvent-based extracts requires relatively simple technology, making them suitable for adoption by smallholder farmers and organic production systems.

Overall, the results indicate that Putranjiva roxburghii leaf extract possesses considerable nematicidal potential against Meloidogyne incognita. The high mortality observed at elevated concentrations suggests that the plant may serve as an effective source of bioactive compounds for the development of environmentally safe nematode management strategies. Further studies involving phytochemical characterization, greenhouse validation, and field evaluation are recommended to identify the active principles responsible for nematicidal activity and to determine their practical applicability under agricultural conditions.

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Juvenile mortality increased significantly ($P \leq 0.05$) with increasing extract concentration and exposure period. The highest mortality (98.67%) was observed in the 20% leaf extract treatment after 72 h exposure. The lowest mortality among treated groups was recorded in the 1% concentration after 12 h exposure (12.67%). Control treatments exhibited negligible mortality (≤5.67%) throughout the experimental period (Table 1). A strong positive correlation ($R^2 > 0.95$) was observed between extract concentration and juvenile mortality. Probit analysis may yield approximate LC_{50} values of 6.8%, 4.5%, 2.9%, and 1.7% at 12, 24, 48, and 72 h exposure periods, respectively.

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