Plant Archives Vol. 26, No. 1, 2026 pp. 745-750 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.102

EFFECT OF INDIGENOUS ORGANIC TREATMENTS ON EGG HATCHING AND LARVAL MORTALITY OF ROOT-KNOT NEMATODES IN BASMATI RICE CULTIVATION

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(Date of Receiving-07-12-2025; Date of Revision-29-01-2026; Date of Acceptance-20-02-2026)

ABSTRACT

Rice is the most promising cereal worldwide since it provides staple food for more than half of the world's population. Several biotic and abiotic factors limit the rice productivity. Among the biotic factors, rice root knot nematode (*Meloidogyne graminicola*) is a major constraint to decreasing rice production. Present investigation was carried out in Nematology lab and field condition for the management of *Meloidogyne graminicola*. The efficacy of cow urine and neem cake extract at different concentrations @5%, 10% and 15% were tested against larval mortality and egg hatching of *M. graminicola*. At 5% concentration after 72 hours of inoculation, cow urine exhibited highly promising larval mortality of maximum (100%). At 10% and 15% concentration, after 72hrs of inoculation, cow urine and cartap hydrochloride showed maximum (100%) larval mortality. In case of egg hatching inhibition, at 5%, 10% and 15% concentration, minimum egg hatching was recorded in cow urine (43.35%), (33.35%) and (21.65%), respectively after 72hrs of inoculation.

Key words : Cow urine, Egg hatching, Larval mortality, *Meloidogyne graminicola*, Neem cake.

Introduction

Meloidogyne graminicola, also known as the rice RKN, was first identified by Golden and Birchfield, in 1965 (genus: *Meloidogyne*, Greek word means melon, apple or gourd shaped female) is one of the most important polyphagous pests in agriculture. It is an emerging threat to rice cultivation in various rice growing regions of Southeast Asia where rice is extensively cultivated (Pankaj *et al.*, 2010). In India, rice root-knot nematode has been recorded from Jammu & Kashmir, Kerala, Haryana, Himachal Pradesh, Punjab, Uttar Pradesh & Andaman Island (Pankaj *et al.*, 2011). Among different species of *Meloidogyne*, the rice root-knot nematode (*M. graminicola*) is known to infect and cause serious damage to cereals, especially rice in many countries (Pokharel *et al.*, 2007). It is considered as the most serious nematode in upland rice cultivation and causes economic losses in upland, lowland and deep-water rice and in rice nurseries. This nematode species is an obligate sedentary endoparasite that can cause extensive damage to plant growth and yield of rice crops (Jain *et al.*, 2012). The characteristic infection symptoms produced by *M. graminicola* are in the form of terminal hook shaped or spiral galls (Khan *et al.*, 2012). Juveniles enter the roots through root tips and start feeding. The second juvenile stage (J2) is an infectious stage that emerges from the egg in a hospitable environment, locates the root, moves into the meristematic zone and uses continuous feeding to cause the development of enormous galls. The galling caused by *M. graminicola* causes a modification of the root vascular system by affecting water and nutrient flow from the roots to the aboveground sections, which results in loss of plant vigor, poor development and reduced yield. Aboveground symptoms due to *M. graminicola* infection include patches in rice fields, stunted appearance, chlorotic leaves, maturation and few chaffy grains on the panicles on heavily affected root systems. The *Meloidogyne* spp. thrive in hot climates and can also survive in temperate climatic condition (Strajnar *et al.*, 2011). Root knot nematodes, predominantly *Meloidogyne graminicola*

has emerged as the main problem. Given the significant output losses caused by rice root-knot nematodes, it is imperative to reduce crop damage by implementing available environment friendly management techniques.

To control nematodes, environment friendly solutions are needed. Eco-friendly alternatives such as sanitation, soil management, organic amendments, fertilization and biological control. Biological control is one possible safe alternative to pesticides for disease management and is likely to be free from toxic residual effects. The use of botanicals possessing the anti-feedant and nematicidal properties, not only reduce the nematode population but also enhance the plant growth. New methods of control such as the use of plant extracts and cow urine studied the reduction in plant parasitic nematodes in response to application of these.

Materials and Methods

Collection of eggs

For conducting experiment on egg hatching and larval mortality of *M. graminicola*, eggs were collected from the rice plants maintained as pure culture. With the use of a dropper and a sterilized dissecting needle, roots were divided, and eggs were extracted from the galled root. The picked eggs were kept in sterilized water.

Collection of juveniles (J_2) of *M. graminicola*

For the experimentation of egg hatching and larval mortality of *M. graminicola*. The infected rice roots having galls developed by *Meloidogyne graminicola* were collected. The uprooted rice plants roots were washed under running tap water. Then galls were broken into little pieces and were grounded with the help of grinder. To confirm that the galls contain eggs or the juvenile stage of *M. graminicola*, the crushed root galls in water suspension were examined under a stereomicroscope. By applying the suspension to two layers of tissue paper laid over wire gauze and submerging them in water in petri plates, the stage of *M. graminicola* was isolated.

Mortality test

To study the effect of cow urine and neem cake extract on larval mortality. Cow urine was collected from L.R.C of Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U.P. and neem cake was collected from local market and was tested against rice root knot nematode. 100gms of neem cake was required for 1 litre of water. The neem cake was put in a muslin cloth and soaked overnight in water and then filtered extract was used. In case of 100% concentration, 5ml, 10 ml and 15ml cow urine and neem cake extracts were separately poured in 5 cm size petri dishes. The three replications were maintained for each treatment. Control petri plates were maintained by adding 10 ml of sterilized distilled water. In all these petri plates 1 ml suspension containing freshly hatched 20 juveniles of *M. graminicola* were added with the help of flat tipped picking dropper. Petri plates were incubated at room temperature. Observations on larval mortality were recorded after 24, 48 and 72 hours of inoculation with the help of stereomicroscope. Per cent of larval mortality was calculated by following formula given by Ahmad *et al*. (2004).

Per cent larval mortality = Total no. of larvae killed/ Total no. of larvae inoculated × 100

Egg hatching test

To study the effect of cow urine and neem cake extract on egg hatching, cow urine and neem cake extract of 5ml, 10ml and 15ml were separately poured in 5 cm size petri dishes. The three replications were maintained for each treatment. Control petri plates were maintained by adding 10 ml of sterilized distilled water. All these petri plates were poured by 1 ml suspension containing 20 freshly eggs of *M. graminicola* with the help of a flat tipped picking dropper. Petri plates were incubated at room temperature. Observations on egg hatching were recorded after 24, 48 and 72 hours of inoculation under stereo binocular microscope. Per cent of egg hatched was calculated by following formula given by Ravichandra *et al.* (2010).

Hatching percentage = No. of hatched juveniles = No. of hatched + Unhatched egg × 100

Treatment details

The experiment consisted of ten treatments designed to evaluate the effects of various concentrations of cow urine, neem cake extract, and cartap hydrochloride 50SP. The treatments were as follows: T_1 – Effect of cow urine at 5% concentration, T_2 – Effect of cow urine at 10% concentration, T_3 – Effect of cow urine at 15% concentration, T_4 – Effect of neem cake extract at 5% concentration, T_5 – Effect of neem cake extract at 10% concentration, T_6 – Effect of neem cake extract at 15% concentration, T_7 – Effect of cartap hydrochloride 50SP at 5% concentration, T_8 – Effect of cartap hydrochloride 50SP at 10% concentration, T_9 – Effect of cartap hydrochloride 50SP at 15% concentration and T_{10} – Control (no treatment applied).

Results and Discussion

Different concentrations @5%, 10% and 15% of cow urine and neem cake extract was tested against larval

mortality and egg hatching of root knot nematode. Also, for positive control cartap hydrochloride was taken under in vitro conditions.

Larval mortality

Results from Tables 1, 2 and 3 indicates that cow urine increased larval mortality at 5%, 10% and 15% concentration over control.

At 5% concentration, after 24hrs inoculation, maximum (88.35%) larval mortality was recorded in cow urine followed by cartap hydrochloride (36.65%) whereas (10.00%) larval mortality was recorded in neem cake extract. At 48hrs, maximum (91.65%) larval mortality was recorded in cow urine followed by cartap hydrochloride (58.35%) and minimum (26.65%) larval mortality was recorded in neem cake extract. At 72hrs after inoculation, maximum (100%) larval mortality was found in cow urine, followed by cartap hydrochloride (83.35%) and minimum (50.00%) larval mortality was found in neem cake extract. At 10% concentration, after 24hrs inoculation, maximum (98.35%) larval mortality was recorded in cow urine followed by cartap hydrochloride (63.25%) whereas minimum (11.65%) larval mortality was recorded in neem cake extract. Similar findings with Gupta *et al*. (2020) cow urine and Agni Astra were evaluated for their effect on juvenile mortality and egg hatching inhibition of root-knot nematode, *Meloidogyne incognita*. Cow urine (93.76%) @ 10% concentration was most effective for the juvenile mortality of *M. incognita* followed by agniastra (91.81%) at 2% concentration. Cow urine (75.00%) was found to be most effective followed by agniastra at 2% and NSKE (66.67%) at 10% concentration for the egg hatching inhibition of *M. incognita*. Whereas aqueous DSKE at 2% concentration was found least effective for juvenile mortality as well as egg hatching inhibition of *M. incognita*. Similarly, Dongre and Simon (2013) reported that nematicidal activity of different plant extracts namely Neem (*Azadirachta indica*), Bael (*Aegle marmelos*), Jatropha (*Jatropha curcas*), Eucalyptus (*Eucalyptus*

Table 1 : Effect of cow urine and neem cake extract on Larval (J_2) mortality of *M. graminicola* at 5% concentration.

Treatments	24 hrs		48 hrs		72 hrs	
	No. of dead J_2	% mortality	No. of dead J_2	% mortality	No. of dead J_2	% mortality
Cow urine	17.67	88.35	18.33	91.65	20.00	100.00
Neem cake extract	2.00	10.00	5.33	26.65	10.00	50.00
Cartap Hydrochloride	7.33	36.65	11.67	58.35	16.67	83.35
Control	0.00	0.00	0.00	0.00	0.00	0.00
CD at 5%	**0.781**		**1.912**		**1.104**	

Table 2 : Effect of cow urine and neem cake extract Larval (J_2) mortality *M. graminicola* at 10% concentration.

Treatments	24 hrs		48 hrs		72 hrs	
	No. of dead J_2	% mortality	No. of dead J_2	% mortality	No. of dead J_2	% mortality
Cow urine	19.67	98.35	20.00	100.00	20.00	100.00
Neem cake extract	2.33	11.65	7.33	36.65	14.00	70.00
Cartap Hydrochloride	12.67	63.25	19.00	95.00	20.00	100.00
Control	0.00	0.00	0.00	0.00	0.00	0.00
CD at 5%	**1.104**		**1.745**		**0.956**	

Table 3 : Effect of cow urine and neem cake extract Larval (J_2) mortality of *M. graminicola* at 15% concentration.

Treatments	24 hrs		48 hrs		72 hrs	
	No. of dead J_2	% mortality	No. of dead J_2	% mortality	No. of dead J_2	% mortality
Cow urine	20.00	100.00	20.00	100.00	20.00	100.00
Neem cake extract	5.33	26.65	12.00	60.00	16.67	83.35
Cartap Hydrochloride	17.33	86.65	20.00	100.00	20.00	100.00
Control	0.00	0.00	0.00	0.00	0.00	0.00
CD at 5%	**1.561**		**0.956**		**0.552**	

globus), Sahjan (*Moringa oleifera*), Ber (*Ziziphus mauritiana*), Sarifa (*Annona reticulate*), Congress grass (*Parthenium argentatum*) against *Meloidogyne graminicola*.

Egg hatching

Result from Tables 4, 5, 6 indicates that all treatments inhibit the egg hatching of *M. graminicola* at the tested concentrations (5, 10 and 15%) over control.

At 5% concentration, minimum (6.65%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (23.35%) and maximum (35.00%) in neem cake extract. In case of control, (61.65%) egg hatching was recorded after 24 hrs of inoculation. After 48hrs of inoculation, minimum (13.35%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (36.65%) and maximum (46.65%) in neem cake extract. In case of control, (76.65%) egg hatching was recorded. Whereas, after 72 hrs of inoculation, minimum (43.35%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (70.00%) and maximum (75.00%) in neem cake extract. In case of control, (88.35%) egg hatching was recorded.

At 10% concentration, minimum (13.35%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (16.65%) and maximum (23.35%) in neem cake extract. In case of control, (61.65%) egg hatching was recorded after 24 hrs of inoculation. After 48hrs of inoculation, minimum (31.65%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (35.00%) and maximum (36.65%) in neem cake extract. In case of control, (76.65%) egg hatching was recorded. Whereas, after 72 hrs of inoculation, minimum (33.35%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (60.00%) and maximum (63.35%) in neem cake extract. In case of control, (88.35%) egg hatching was recorded.

At 15% concentration, minimum (11.35%) egg hatching was recorded in cow urine, followed by cartap

Table 4 : Effect of cow urine and neem cake extract on Egg hatching of *M. graminicola* at 5% concentration.

Treatments	24 hrs		48 hrs		72 hrs	
	No. of dead J_2	% mortality	No. of dead J_2	% mortality	No. of dead J_2	% mortality
Cow urine	2.67	13.35	2.67	13.35	8.67	43.35
Neem cake extract	7.00	35.00	9.33	46.65	15.00	75.00
Cartap Hydrochloride	4.67	23.50	7.33	36.65	14.00	70.00
Control	12.33	61.65	15.33	76.65	17.67	88.35
CD at 5%	**2.529**		**3.312**		**1.912**	

Table 5 : Effect of cow urine and neem cake extract on Egg hatching of *M. graminicola* at 10% concentration.

Treatments	24 hrs		48 hrs		72 hrs	
	No. of dead J_2	% mortality	No. of dead J_2	% mortality	No. of dead J_2	% mortality
Cow urine	2.33	11.35	6.33	31.65	6.67	33.35
Neem cake extract	5.67	28.35	7.33	48.35	12.67	63.35
Cartap Hydrochloride	3.67	18.35	7.00	35.00	12.00	60.00
Control	12.33	61.65	15.33	76.65	17.67	88.35
CD at 5%	**2.406**		**3.073**		**1.831**	

Table 6 : Effect of cow urine and neem cake extract on Egg hatching of *M. graminicola* at 15% concentration.

Treatments	24 hrs		48 hrs		72 hrs	
	No. of dead J_2	% mortality	No. of dead J_2	% mortality	No. of dead J_2	% mortality
Cow urine	1.33	6.65	3.67	18.35	4.33	21.65
Neem cake extract	4.67	23.35	7.33	36.65	10.67	53.35
Cartap Hydrochloride	3.33	16.65	6.67	33.35	10.00	50.00
Control	12.33	61.65	15.33	76.65	17.67	88.35
CD at 5%	**2.208**		**3.447**		**1.352**	

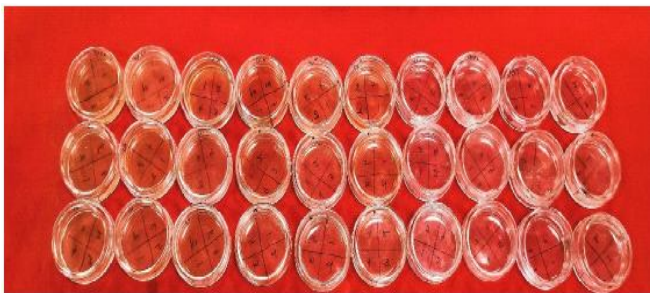

Fig. 1 : Experimental view of egg hatching and Larval mortality.

hydrochloride (18.35%) and maximum (28.35%) in neem cake extract. In case of control, (61.65%) egg hatching was recorded after 24 hrs of inoculation. After 48hrs of inoculation, minimum (30.00%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (33.35%) and maximum (48.35%) in neem cake extract. In case of control, (76.65%) egg hatching was recorded. Whereas, after 72 hrs of inoculation, minimum (21.65%) egg hatching was recorded in cow urine, followed by cartap hydrochloride (50.00%) and maximum (48.35%) in neem cake extract. In case of control, (88.35%) egg hatching was recorded. Similar findings with Haroon *et al.* (2024) investigated the effect of neem (*Azadirachta indica*) leaf extract on the egg hatching of root-knot nematodes (*Meloidogyne* spp.). The study found that egg hatching decreased progressively with higher concentrations of neem extract and longer exposure times. The extract showed strong nematicidal activity, with an IC_{50} value of 397.1 ppm. At 72 hours, neem extract in 2000, 4000 and 6000 ppm inhibited egg hatching by 75%, 79% and 81%, respectively. The highest concentration (6000 ppm) was most effective, while 2000 ppm was the least. The findings support neem extract as a potential natural alternative for controlling root-knot nematodes. Also, Javed *et al*. (2007) reported that aqueous extracts of crude neem formulations used as a seedling dip treatment significantly reduced the number of females and egg masses in roots whereas the refined one did not. When applied to the root portion, all formulations significantly reduced the number of egg masses and eggs per egg mass. Whereas on the untreated root portion, neem cake at 3% w/w and aza at 0.1% w/w significantly reduced the number of egg masses as compared with neem leaves at 3% w/w, aza at 0.05% and control. All the neem formulations significantly reduced the number of eggs per egg mass on the untreated root portion.

Conclusion

Based on the findings, cow urine demonstrated the highest efficacy in controlling *Meloidogyne graminicola*, achieving complete larval mortality (100%) and significantly reducing egg hatching (43.35%) at 5% concentration within 72 hours. In contrast, the control group showed no larval mortality and a higher rate of egg hatching (88.35%). This suggests that cow urine, even at a lower concentration, is a potent natural treatment for managing *M. graminicola*. Further studies could explore its long-term impact and optimal application methods for practical field use.

Acknowledgement

I sincerely express my gratitude to Head of Department, Department of Plant Pathology, Sardar Vallabh bhai Patel University of Agriculture and Technology, Modipuram, Meerut, for providing all the necessary facilities to carry out this research work.

Author's contribution

H.Y conducted the trial as suggested by his guide K.K. K.K. designed all the technical programs and provide all the necessary glassware and chemicals. A.Y., S.L. helped in data analysis and interpretation of result.

Funding

Funding support for carrying out the research work was supported by University's fund.

Conflict of interest

All the authors have read and checked the manuscript and have given their consent for publication. The authors have no conflict of interest.

Ethics approval and consent to participate

The authors confirms that there are no ethical issues in publication of the manuscript.

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