COMPARATIVE EFFICACY OF ORGANIC AMENDMENTS AND CHEMICALS IN MANAGING RICE ROOT-KNOT NEMATODE (*Meloidogyne graminicola* GOLDEN AND BIRCHFIELD)

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ABSTRACT

A screen house experiment was conducted during the rainy season, 2022, Department of Plant Pathology, Rampur, Chitwan to evaluate organic amendments and chemicals, viz. chicken manure, mustard cake, titepati (*Artemisia vulgaris*) leaves, asuro (*Justicia adhatoda*) leaves, neem (*Azadirachta indica*) leaves, fosthiazate 30% SL and emamectin benzoate 5% SG against rice root-knot nematode (*Meloidogyne graminicola*) in naturally infested soils. An individual pot size of 22 cm × 18 cm was used which was filled with 4 kg nematode-infested soil and rice seeds cv. Ekle were sown in pots at three spots. Similar to that, 5 days prior to planting, emamectin benzoate 5% SG was applied at 0.5 g/l of water and fosthiazate 30% SL was applied at 300 µl/l of water. In the case of botanicals, the leaves were chopped, air-dried and mixed with soil at the rate of 15 g/kg soil 10 days before sowing. Also, mustard cake and chicken manure at 10 g/kg soil were applied to the soil 5 days before sowing. All treatments significantly reduced both nematode population and gall numbers, and increased plant growth indices compared to untreated control. Fresh shoot weight, shoot length and root fresh weight were found significantly high in the pots treated with mustard cake @10 g/kg soil. However, root length was found highest in fosthiazate 30% SL at 90 days after sowing. Maximum reduction in gall numbers per plant, root-knot severity index and *M. graminicola* population in soil was recorded in pots treated with fosthiazate 30% SL followed by mustard cake @10 g/kg soil and neem leaves @15 g/kg soil.

Key words : Emamectin benzoate, fosthiazate, *Meloidogyne graminicola*, mustard cake, root gall

INTRODUCTION

Rice root-knot nematode *(Meloidogyne graminicola* Golden and Birchfield) attacking rice and wheat, is the most serious nematode in upland, lowland and deep water rice (Prasad et al., 1987; Bridge et al., 1990) as well as in nurseries (Bridge et al., 1990). In Asia, the rice root-knot nematode *M. graminicola* has emerged as a major hindrance to rice production, particularly in light of intensified rice cropping and increasing water scarcity (Bridge et al., 2005; Soriano and Reversat, 2003). A predominant below-ground symptom is the distinctive hook-shaped galls, primarily found at the root tips, which adversely affect root development and physiology, leading to a significant reduction in yield (Win et al., 2015). High levels of *M. graminicola* populations were observed in rice fields under rice-wheat cropping systems in Chitwan, Rupandehi, Bara, Parsa, and Rautahat (Pokharel and Sharma-Poudyal, 2001).

There are several approaches to manage rice root-knot nematodes. Cultural methods, including flooding of the field and soil solarization, as well as crop rotation, have proven to be effective against *M. graminicola* (Mantelin, 2017; Pankaj et al., 2015; Rao, 1984). Experiments conducted at multiple locations have provided evidence that cultivating robust plants in solarized rice nursery soils can lead to higher rice yields (Banu et al., 2005). According to Bridge and Page (1982), *M. graminicola* has the ability to persist in typically flooded conditions.

High-nitrogen organic soil amendments offer promising alternatives for the management of *Meloidogyne* spp. and other plant parasitic nematodes (Khan et al., 1974). The efficacy of materials with a high nitrogen content and a low carbon-to-nitrogen (C:N) ratio has been demonstrated in the control of *M. arenaria* (Neal) (Mian and Rodriguez-Kabana, 1982). When these materials decompose, they release various nematicidal compounds (Akhtar and Malik, 2000; Stirling, 1989). Numerous studies have reported a reduction in *M. graminicola* infestation and an enhancement in rice plant growth following the application of diverse organic amendments such as neem leaves, neem cake, mustard cake, and poultry manures (Rahman and Miah, 1993; Roy, 1976; Sharma-Poudyal et al., 2002). These findings underscore the potential advantages of utilizing such organic amendments for nematode management and the improvement of rice production.

Despite the awareness of the detrimental effects of chemicals, pesticides remain the most effective approach for managing nematodes in rice ecosystems (Khan et al., 2012). This preference for pesticides among farmers is due to the limited availability of rice varieties resistant to *M. graminicola* (Dutta, 2012). Pesticides offer immediate results, whereas other disease management practices require a significant amount of time to demonstrate noticeable effects. Pesticides can function through direct contact and/or systemic action, and they are administered using various methods, including seed treatment (Jain and Gupta, 1990), root-dipping (Jain and Bhatti, 1991), and nursery bed treatments. However, soil application is the most commonly employed method (Jain and Bhatti, 1991). Consequently, this study aims to develop suitable nematode management strategies using both organic amendments and chemical treatments to improve rice yield.

MATERIALS AND METHODS

To determine whether chemicals, organic amendments, and botanicals were effective against the root-knot nematode, an experiment was conducted in a screen house of the Department of Plant Pathology, Rampur, Chitwan, Nepal during the rainy season of 2022. The experiment was conducted in a randomized complete block design (RCBD) with four replications. Three pots were assigned to each treatment, making a total of 96 pots for the entire experimental setup. An individual pot size of 22 cm × 18 cm was used which was filled with 4 kg of nematode-infested soil. Sowing of rice cv. Ekle was done in pots at three spots (2-3 seeds per spot) with a spacing of 8 cm. Thinning was done seven days after germination, and one rice plant per hill was maintained. The crop received irrigation two-times daily. Fertilizer application was done with $N:P_2O_5:K_2O$ 50:30:30 kg/ha as a basal dose and 50 kg/ha N was top dressed during tillering 45 days after transplanting (DAT). Simulated field conditions were created throughout the experiment period. Plants were harvested 90 days after sowing.

Collection of *M. graminicola*-Infested Soil and Count of J2

Top 15 cm soil was taken from the nematode-infested rice field of the Department of Agronomy, Agriculture, and Forestry University, Rampur, Chitwan. The collected soil was stored in a shaded area and thoroughly mixed to ensure a uniform nematode population before potting. Juveniles (J2) of *M. graminicola* in the soil were counted after extraction by the modified Baermann tray method (Schindler, 1961). The J2 population per 200 cc of soil was found to be 350. Each pot was filled with 4 kg of the nematode-infested soil.

Treatment Details

Table 1. Treatment details used during the experiment at Rampur, Chitwan, 2022

Treatments	Treatment details	Application rate
T_1	Chicken manure	10 g per kg soil
T_2	Mustard cake	10 g per kg soil
T_3	Titepati (*Artemisia vulgaris*) leaves	15 g per kg soil
T_4	Asuro (*Justicia adhatoda*) leaves	15 g per kg soil
T_5	Neem (*Azadirachta indica*) leaves	15 g per kg soil
T_6	Fosthiazate 30% SL (Nemazone)	300 µl/l of water (100 ml/ pot)
T_7	Emamectin benzoate 5% SG (Rajmate)	0.5 g/l of water (100 ml/ pot)
T_8	Untreated control (Distilled water)	100 ml per pot

Application of Treatments in Soil

Four kg of infested soil was well mixed with the different treatments (Table 1). They include chopped and air-dried (for 1 week) neem leaves, asuro leaves, titepati leaves @ 15 g per kg soil; chicken manure, and mustard cake at 10 g per kg of soil; fosthiazate 30% SL @ 300 µl/l of water; and emamectin benzoate 5% SG at 0.5 g/l of water (100 ml/pot). Nematicides, botanicals, mustard cake, and chicken manure were applied directly into the potted soil 5 days before sowing, and the botanicals (neem, asuro and titepati) 10 days before sowing. While in the untreated control pot, 100 ml distilled water was applied 5 days before sowing.

Disease Assessment

Three recordings were made during the experiment period at 30-day intervals (30, 60, and 90 days after seeding). One plant per pot was uprooted for each observation and kept submerged in water to prevent them from sticking to soil particles. Root and shoot length (cm), fresh root and shoot weight (g), the number of galls per plant were recorded and galling was measured using a 0-10 scale (Bridge et al., 2005). The root-knot index was calculated using the 0-10 scale.

$$\text{Root-knot severity index} = \frac{\text{Sum of all numerical rating}}{\text{Total number of ratings x maximum grade}} \text{ x } 100$$

At the end, 200 cc of soil samples were taken from each of the pots and subsequently processed by the modified Baermann tray method (Schindler, 1961) for nematode extraction. Nematode population was estimated - an average of 5 counts from 1 ml out of the 100 ml suspensions.

Statistical Analysis

The collected data were processed in Microsoft Excel 2013. Statistical evaluations were carried out using R-studio 4.2.1. The significant mean comparison was done using Duncan's multiple range test ($\alpha = 0.05$).

RESULTS

Effect of Treatments on Root Length

Table 2 presents the effect of treatments against root-knot nematodes on the root length of rice plants at various planting dates. At 30 DAS, no significant reactions were observed between the treatments. Root length increased substantially over untreated control for all treatments at both 60 and 90 DAS. The longest root length was recorded in pots treated with fosthiazate 30% SL, which was statistically similar to mustard cake, neem leaves, and titepati leaves. The treated control pot had the minimum root length.

Table 2. Effect of treatments on root length of rice (cv. Ekle) inoculated with root-knot nematode (*Meloidogyne graminicola*) in screenhouse, Rampur, Chitwan, 2022

Treatments	Root length (cm)		
	30 DAS	60 DAS	90 DAS
Chicken manure	6.91 ± 0.55	10.22^{cd} ± 0.29	17.305^{bc} ± 2.88
Mustard cake	8.18 ± 0.15	12.48^{a} ± 0.25	21.33^{ab} ± 0.75
Titepati leaves	7.83 ± 0.96	11.55^{abc} ± 0.77	17.21^{bc} ± 0.79
Asuro leaves	7.29 ± 0.38	10.09^{cd} ± 0.04	16.05^{c} ± 1.12
Neem leaves	7.93 ± 0.44	12.11^{ab} ± 0.82	18.66^{abc} ± 1.30
Fosthiazate 30% SL	8.01 ± 0.75	12.64^{a} ± 0.94	21.92^{a} ± 1.08
Emamectin benzoate 5% SG	7.70 ± 0.29	10.64^{bc} ± 0.39	15.09^{c} ± 0.19
Untreated control	6.13 ± 0.48	8.63^{d} ± 0.27	9.31^{d} ± 0.51
$LSD_{0.05}$	1.67	1.69	4.15
CV (%)	12.91	8.86	14.03
P value	NS	**	***
Grand mean	7.49	11.05	17.25

Effect of Different Treatments on Shoot Length

The effect of treatments on the shoot length of rice was significantly different (Table 3, Fig. 1). For all observations, significantly higher shoot length was observed in the pots treated with mustard cake, followed by neem leaves, chicken manure, and fosthiazate 30% SL, which were statistically at par. The untreated control had minimum shoot length (Table 3).

Table 3. Effect of treatments on shoot length of rice (cv. Ekle) inoculated with root-knot nematode (*Meloidogyne graminicola*) in screenhouse, Rampur, Chitwan, 2022

Treatments	Shoot length (cm)		
	30 DAS	60 DAS	90 DAS
Chicken manure	56.22^{abc} ± 2.10	93.27^{abc} ± 0.61	138.28^{ab} ± 1.02
Mustard cake	61.014^{a} ± 1.72	100.55^{a} ± 5.99	147.40^{a} ± 1.80
Titepati leaves	53.5^{bc} ± 3.41	88.98^{bc} ± 2.97	135.15^{bc} ± 3.83
Asuro leaves	49.83^{cd} ± 0.69	85.36^{cd} ± 3.53	134.24^{bc} ± 5.99
Neem leaves	59.04^{ab} ± 3.62	98.77^{ab} ± 1.00	138.57^{ab} ± 1.40
Fosthiazate 30% SL	59.58^{ab} ± 1.13	95.66^{abc} ± 4.42	142.42^{ab} ± 3.46
Emamectin benzoate 5% SG	50.72^{cd} ± 0.65	77.95^{de} ± 2.31	134.31^{bc} ± 2.46
Untreated control	44.73^{d} ± 1.25	71.05^{e} ± 2.41	128.05^{c} ± 0.763
LSD $_{0.05}$	6.36	10.02	9.22
CV (%)	6.76	6.51	7.88
P value	***	***	*
Grand mean	54.33	88.95	137.30

Fig. 1. Effect of different treatments on root character of rice (cv. Ekle) inoculated with root-knot nematode (*Meloidogyne graminicola*) in screenhouse recorded at 90 DAS

Effect of treatments on fresh root weight and shoot weight

The effects of treatments on both fresh root and shoot weight at 90 DAS were significantly different (Table 4). Pots treated with mustard cake, followed by fosthiazate 30% SL had the highest fresh root and shoot weight. The untreated control had the least fresh root weight.

Table 4. Effect of different treatments on fresh root and shoot weight of rice (cv. Ekle) inoculated with root-knot nematode (*Meloidogyne graminicola*) plants 90 days after seeding in screenhouse, Rampur, Chitwan, 2022

Treatments	Fresh root weight (g)	Fresh shoot weight (g)
Chicken manure	$11.89^{c} \pm 1.69$	$54.09^{bcd} \pm 4.37$
Mustard cake	$23.35^{a} \pm 1.40$	$68.50^{a} \pm 3.23$
Titepati leaves	$14.95^{c} \pm 0.99$	$46.34^{cd} \pm 4.73$
Asuro leaves	$11.39^{c} \pm 0.29$	$43.13^{d} \pm 2.75$
Neem leaves	$19.03^{b} \pm 0.93$	$56.07^{bc} \pm 4.11$
Fosthiazate 30% SL	$20.90^{ab} \pm 1.45$	$60.55^{ab} \pm 2.77$
Emamectin benzoate 5% SG	$13.32^{c} \pm 1.19$	$49.3^{cd} \pm 1.50$
Untreated control	$7.04^{d} \pm 0.72$	$28.9^{e} \pm 1.80$
$LSD_{0.05}$	3.49	11.48
CV (%)	13.24	11.08
P value	***	***
Grand mean	15.23896	50.86

Same letters followed in the columns are not significantly different (P=0.05) by DMRT. SEm (±) indicates standard error of mean; LSD: Least Significant Difference; CV: Coefficient of Variation

Effect of Treatments on Number of Galls/Plant

The treatments resulted in a statistically significant decrease in the number of galls per plant at both 30 DAS and 60 DAS (Table 5). Pots treated with fosthiazate 30% SL and mustard cake had least number of galls, which were statistically at par with neem leaves and emamectin benzoate 5% SG. The untreated control had the highest number of galls per plant.

Effect of Treatments on Population of Juveniles in Soil

All treatments at 90 DAS significantly reduced the juvenile's population in the soil as compared to the untreated control (Table 5). Fosthiazate 30% SL followed by mustard cake had the least population of juveniles. The untreated control had the highest number of juveniles.

Effect of Treatments on Root-Knot Severity Index

The treatments resulted in a highly significant reduction in the root-knot severity index for all three observations (Table 6). Pots treated with fosthiazate 30% SL and mustard cake had the least severity index and the untreated control had the highest severity index.

Table 5. Effect of treatments on number of galls per rice plant (cv. Ekle) and population of root-knot nematode (*Meloidogyne graminicola*) per 200 cc soil in a screenhouse, Rampur, Chitwan, 2022

Treatments	Number of galls per plant				Population of juveniles per 200 cc soil	Reduction (%)
	30 DAS	Reduction (%)	60 DAS	Reduction (%)		
Chicken manure	14.44^{abc} ± 3.65 (1.16)	38.97	50.13^{ab} ± 11.38 (1.67)	25.13	463.33^{ab} ± 74.71 (2.65)	26.33
Mustard cake	8.22^{cd} ± 0.87 (0.96)	65.25	27.63^{de} ± 3.79 (1.43)	58.73	252.67^{c} ± 46.18 (2.38)	59.83
Titepati leaves	19.5^{ab} ± 3.76 (1.29)	17.59	45.72^{abc} ± 3.20 (1.65)	31.73	478.33^{ab} ± 55.54 (2.67)	23.95
Asuro leaves	20.83^{a} ± 0.83 (1.33)	11.98	56.28^{a} ± 6.99 (1.74)	15.95	557.33^{a} ± 60.91 (2.74)	11.44
Neem leaves	11.83^{bc} ± 2.45 (1.093)	49.99	30.02^{cd} ± 4.706 (1.46)	55.16	323.67^{bc} ± 35.69 (2.50)	48.54
Fosthiazate 30% SL	6.31^{d} ± 1.52 (0.84)	73.35	18.11^{e} ± 2.89 (1.24)	72.95	239.67^{c} ± 43.07 (2.36)	61.89
Emamectin benzoate 5% SG	11.48^{bc} ± 14 (1.08)	51.46	32.51^{bcd} ± 3.17 (1.51)	51.45	421.67^{ab} ± 23.24 (2.62)	32.96
Untreated control	23.66^{a} ± 1.07 (1.39)		71.64^{a} ± 3.50 (1.854)		629.33^{a} ± 50.20 (2.79)	
$LSD_{0.05}$	0.21		0.18		0.17	
CV (%)	10.76		6.79		3.80	
P value	***		***		***	
Grand mean	14.53		42.01		420.67	

Table 6. Effect of treatments on root-knot severity index of root-knot nematode (*Meloidogyne graminicola*) in rice (cv. Ekle), in a screenhouse, Rampur, Chitwan, 2022

Treatments	**Root-knot severity index (%)**					
	30 DAS	**Reduction (%)**	**60 DAS**	**Reduction (%)**	**90 DAS**	**Reduction (%)**
Chicken manure	$58.33^{b} \pm 3.88$	27.94	$54.26^{bcd} \pm 5.20$	33.34	$58.36^{bcd} \pm 1.53$	31.01
Mustard cake	$34.66^{cd} \pm 3.33$	57.17	43.04^{de} 2.60	47.12	$43.86^{ef} \pm 2.23$	48.14
Titepati leaves	$46.33^{bc} \pm 7.31$	42.76	$57.25^{bc} \pm 4.02$	29.67	$59.67^{bc} \pm 3.78$	29.46
Asuro leaves	$62.56^{b} \pm 8.18$	22.71	$64.52^{b} \pm 3.91$	20.74	$66.25^{b} \pm 2.69$	21.69
Neem leaves	$46^{bc} \pm 9.45$	43.17	$50.19^{cd} \pm 2.20$	38.34	$49.46^{de} \pm 3.70$	41.52
Fosthiazate 30% SL	$22.33^{d} \pm 2.33$	72.41	$33.89^{e} \pm 6.11$	58.37	$36.65^{f} \pm 2.29$	56.66
Emamectin benzoate 5% SG	$49.33^{bc} \pm 5.20$	39.06	$51.17^{cd} \pm 2.26$	37.14	$55.34^{cd} \pm 2.95$	34.58
Untreated control	$80.95^{a} \pm 2.63$		$81.41^{a} \pm 2.47$		$84.6^{a} \pm 3.09$	
SEm(±)	6.16		3.85		2.87	
LSD (α=0.05)	17.67		11.58		8.63	
P value	**		***		***	
Grand mean	50.06		54.46		56.77	

Same letters followed in the columns are not significantly different (P=0.05) by DMRT. SEm (±) indicates standard error of mean; LSD: Least Significant Difference; CV: Coefficient of Variation

DISCUSSION

Effect on Plant Growth

In the present study, all treatments exhibited higher performance with longer roots and shoots. Mustard cake and fosthiazate 30% SL had higher effect compared to other treatments (neem leaves, poultry manure, titepati leaves, and emamectin benzoate 5% SG). Similar findings were reported by Sharan et al. (2018), who found that mustard cake followed neem leaves had the maximum plant heights. Our findings are also in line with Faruk (2019). Kumar et al. (2022) also reported that mustard cake applied at 50 g/kg of soil and neem cake at the same rate showed effective control of the nematode and gave good growth of rice plants.

In Nepal, Dangal et al. (2009) examined the efficacy of several organic amendments against the rice root-knot nematode in direct-seeded rice pots. They found better fresh root and shoot weight in the treatment with chicken manure at 2 or 3 t/ha followed by neem cake at 3 t/ha. The improvement of the beneficial microflora of the soil rhizosphere, which serves as growth-promoting agents, may be the reason for this positive correlation and improvement in plant growth parameters during the decomposition of the organic amendment (Akthar and Malik, 2000).

Nematode Multiplication and Population

In the present study, fosthiazate 30% SL and mustard cake gave highest reduction in the number of galls per plant and nematode population in soil. Neem leaves, emamectin benzoate 5% SG, and titepati leaves also showed promising results in reducing the number of nematode galls per plant and nematode population. Sherbiny and Allah (2014) found fosthiazate 10% G at a rate of 2.85 g per m^2 effective for the control of *Meloidogyne incognita* in tomato plants. Pullen and Fortnum (1999) reported the effectiveness of fosthiazate against root-knot nematodes in tobacco.

Devi et al. (2019) reported organic amendments, such as neem, mustard, and caster cake (10 g per kg soil), most effective in plant development and lowering nematode galls in rice nursery. In plots treated with mustard cake at 2% following addition of neem leaves at 2%, Sharan et al. (2018) found effective in reducing the number of galls in rice and nematode population in soil. Agyarko et al. (2006) found poultry manure at a rate of 5 g/kg of soil, effective in decreasing root-knot index in carrot roots. These studies show how organic amendments can be used to control nematode infestations and foster healthy plant growth.

The application of fosthiazate 30% SL and mustard cake significantly reduced the root-knot severity index compared to untreated controls. The highest effectiveness of fosthiazate was observed at 30 days after sowing (DAS), but its nematicidal effect declined at 60 and 90 DAS, consistent with findings by Wang et al. (2023), who reported a similar reduction in its effectiveness over time. Neem leaves and chicken manure also resulted in lower root-knot severity which are consistent with the findings of Dangal et al. (2008), likely due to the presence of pesticidal compounds in neem and the conversion of uric acid in manure to ammonia, which is toxic to plant parasitic nematodes (Agu, 2008).

Moreover, Soil treated with fosthiazate 30% SL and mustard cake showed the greatest reduction in *Meloidogyne graminicola* populations, followed by neem leaves. Mustard cake, rich in glucosinolates, was particularly effective due to the production of bioactive compounds like isothiocyanates during enzymatic hydrolysis, which are highly effective in controlling nematodes population in soil (Zukalová and Vašák, 2002). These findings are consistent with previous studies (Sharan et al., 2018; Devi et al., 2019) that reported mustard cake and neem leaves significantly reduced nematode multiplication.

CONCLUSIONS

From the present study, it can be concluded that both organic amendments and chemical treatments significantly reduced root-knot nematode (*Meloidogyne graminicola*) infestation and enhanced rice plant growth under screen house conditions. Fosthiazate 30% SL and mustard cake were most effective in controlling root knot nematode, showing the highest reduction in gall number, root-knot severity index, and nematode population. Neem leaves and chicken manure also showed beneficial effect in controlling nematode. Thus, it can be inferred that these organic amendments and chemical treatments can be included in managing rice root-knot nematode along with enhancing the plant growth.

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