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Evaluation of Organic Amendments against Rice Root-Knot Nematode at Seedling Stage of Rice

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Abstract

Pot experiment was conducted during July-September 2006 to evaluate some organic amendments such as sesame (*Sesamum indicum*) biomass, buckwheat (*Fagopyrum esculentum*) biomass, neem (*Azadirachta indica*) leaves, chinaberry (*Melia azedarch*) leaves and chicken manure @ 1, 2 and 3 t ha^{-1} each against the rice root-knot nematode (*Meloidogyne graminicola* Golden & Birchfield) in direct seeded rice. The treatments were replicated five times in a randomized complete block design. The number of second stage juveniles (J2) of *M. graminicola* was significantly low in chicken manure @ 3 t ha^{-1}. The root knot severity index was significantly low in sesame @ 3 t ha^{-1}, chinaberry @ 3, 2 or 1 t ha^{-1}, neem @ 3 t ha^{-1} and chicken manure @ 2 or 3 t ha^{-1} amended soil but root lesion severity index was lower only in chicken manure @ 2 t ha^{-1} treated plots. The fresh shoot weight and length were significantly high in chicken manure amendment @ 2 or 3 t ha^{-1} at 45th day after seeding. However, the fresh root weight, length, number of leaves and number of J2 recovered from the roots were non-significant.

Key words: biomass, juveniles, *Meloidogyne graminicola*, root-knot severity index, root lesion severity index

Introduction

The rice root-knot nematode (*Meloidogyne graminicola* Golden and Birchfield) is a major soil borne pest of rice with world wide distribution including south eastern Asian countries (Bridge *et al.* 1990, Soriano & Reversat 2003). This nematode has been reported in different rice growing environments like rain-fed upland soil, shallow flooded soil and continuous flooded soils in Nepal (Pokhrel 2001, Pokharel & Sharma-Poudyal 2001, Sharma *et al.* 2001, Sharma *et al.* 2002, Sharma-Poudyal *et al.* 2002a, Pokharel 2007). It is major pest of upland rice (Panwar & Rao 1998), causes considerable economic losses in upland, lowland, deep water rice, and also in nurseries (Bridge *et al.* 1990). Rice grain yield reduction has been reported up to 40% in nematode infested farmers' fields in Chitwan (Sharma-Poudyal *et al.* 2002a). Under high nematode population density, the yield loss was incurred up to 97 percent (Sharma-Poudyal *et al.* 2004). Since this nematode is soil borne, rice seedling infected from the very beginning of their growth. Most of the commercial rice cultivars are susceptible to *M. graminicola* in Nepal (Sharma-Poudyal *et al.* 2004, Pokharel 2007). Therefore, most economic and sustainable option of nematode disease management is not available for Nepalese farmers. Similarly, other alternatives of integrated nematode management of rice are also limited in Nepal (Pokharel 2007).

Several previous studies conducted aboard concluded that chemical control of root-knot nematode is very expensive, unsustainable and affects the agro-ecosystem adversely (Ahmad & Khan 2004). Therefore, high nitrogenous organic soil amendments may be effective alternatives for the control of *Meloidogyne* spp. and other plant parasitic nematodes (Khan *et al.* 1974, Alam *et al.* 1979). High nitrogen contents and low carbon nitrogen (C:N) ratio was effective for control of *M. arenaria* (Neal) Chitwood and other plant-parasitic nematodes (Mian & Rodriguez-Kabana 1982, Rodrýguez-Kabana 1986) mainly through the release of ammonia from the amendments during their decomposition in the soil (Rodrýguez-Kabana 1986) or through increased populations of microorganism antagonistic to plant-parasitic nematodes (Rodrýguez-Kabana 1986, Stirling 1989, Akhtar & Malik 2000). Decomposition of these materials release a number of nematicidal compounds

(Stirling 1989, Akhtar & Malik 2000). Incorporation of organic residues strongly impacts physical and biological properties of soil and may promote an environment favorable to nematode-antagonistic microorganisms (Javed *et al.* 2007a). Reduction in infestation of rice by *M. graminicola* and improvement in rice plant growth by addition of different organic amendments has been reported by several workers (Roy 1976, 1978, Rahman & Miah 1993, Sharma-Poudyal *et al.* 2002b).

Healthy seedlings exhibited more vigorous growth and appeared to have greater capacity to withstand soil-borne biological stresses in the field. Healthy seedlings grown in solarized rice nursery soils, increased rice yield at many demonstration sites (Banu *et al*. 2005). Production of healthy seedlings may be a simple and low-cost method to minimize pathogen pressure in soils, reduce the chance of endoparasite's dissemination through infected seedlings like rice root knot nematode and improve plant health in transplanted field. Hence, this experiment was conducted to evaluate the effect of different organic amendments against rice root knot nematode and seedling health.

Materials and Methods

An experiment was conducted in a glass house at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan during July-September 2006. Soil was collected from naturally *M. graminicola* infested field of IAAS agronomy farm. It was filled @ 5 kg pot^{-1} in plastic pots of 19 cm diameter. Air dried (for 1 week) organic amendments were incorporated into the soil at the rate of 1, 2 and 3 t ha^{-1}. The soil amendments were as follows: sesame (*Sesamum indicum*) biomass (whole plant), buckwheat (*Fagopyrum esculentum*) biomass (whole plant), neem (*Azadirachta indica*) green leaves, chinaberry (*Melia azedarch*) green leaves and chicken manure 15 days prior to sowing of rice seed (cv. Sabitri). One set of pots were kept as control (i.e. without amendment). Five pre-germinated seeds of rice were seeded per pot. The treatments were replicated five times in a randomized complete block design. Simulated field condition was maintained throughout the experimental period.

Seedling height and number of leaves per plant were measured on 45th day of seeding. Plants were uprooted and washed gently with running tap water to remove soil. Root length and weight of root and shoot were recorded. *M. graminicola* second juveniles (J2) present in root and soil were also assessed. The cleaned roots were indexed for root lesion following 0-4 scale according to Sharma-Poudyal *et al.* (2002a):

0 = Healthy roots, without lesion,
1 = Lesion up to 25% of roots or 25% roots rotten,
2 = Lesion up to 50% of roots or 50% roots rotten,
3 = Lesion up to 75% of roots or 75% roots rotten and
4 = Lesion more than 75% of roots or more than 75% roots rotten.

Root was also indexed for root knot indexing by the use of 0 (no root swellings or galls) to 10 (all roots galled) scale according to Bridge *et al.* (2005). Root knot severity index and root lesion severity index were calculated as follows:

$$\text{Severity index} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of ratings x Maximum grade}} \text{ x } 100\%$$

Rice root was thoroughly washed and chopped into small pieces of about 10 mm length for extracting *M. graminicola* second juveniles (J2). About 2 g of chopped roots were placed in an electric blender with 100 ml of water, blended for 2 minutes and placed in a modified Baermann tray for extraction (Schinder 1961). Similarly, for extracting J2 nematodes from the rhizosphere soil, it was homogenized and 100 g working sample was taken and processed by modified Baermann tray method (Schindler 1961). After 48 hours of processing, nematode suspension was collected in plastic tubes (50 ml). After allowing standing for an hour, the final volume of suspension was reduced to 20 ml with the help of a glass pipette. Two milliliter (10%) aliquot was sampled from the 20 ml suspension in counting disc, allowed to settle for five minutes. J2 was counted under a binocular microscope (Bridge *et al.* 2000).

Nematode count was transformed in logs (x + 1) for statistical analysis (Gomez & Gomez 1984). Data was analyzed with Microsoft Excel and MSTAT-C (MSTAT, Michigan State Univ., USA). Mean comparison was done by Duncan's multiple range test.

Results

Fresh shoot weight of rice plants grown in chicken manure added soil (@ 2 t ha^{-1}) was 31% more than in the control plots. Chicken manure @ 3 t ha^{-1} was at par with chicken manure @ 2 t ha^{-1}, which was significantly higher than control. Shoot length was also highest in chicken manure @ 2 t ha^{-1} which was 13.77% higher than the control. Similarly, chicken manure @ 3 t ha^{-1} and neem leaves @ 3 t ha^{-1} were also at par with chicken manure @ 2 t ha^{-1} that was significantly more than control (Table 1). Siddiqui (2004) also found significantly higher plant length and fresh weight of tomato plant in poultry manure amendment than goat dung, horse dung, cow dong and control (no fertilizer). However, fresh root weight, root length and number of leaves were not significantly different.

The number of *M. graminicola* second stage juveniles (J2) in roots was not statistically different among organic amendments and control. However, J2 number in soil was lower in most amendmended soil, but only the chicken manure amendment at 3 t ha^{-1} was significantly lower than the control (Table 2). Lower number of J2 was observed with increase in dose of most amendments from 1 t ha^{-1} to 3 t ha^{-1} (Figure 1). Similarly, decrease in number of *M. incognita* was reported by Riegel *et al.* (1996) and Riegel and Noe (2000) in cotton with increase in rate of chicken litter amendment. Mian and Rodriguez-Kabana (1986) also found nematode control with chicken litter directly proportional to the amount of the litter added within the range of 0-5 percent . Similarly, total number of *M. arenaria* in the roots of tomato decreased with increase in rate of chicken litter (0.5, 1.0, 1.5, 2.0, and 2.5% w/w) (Kaplan & Noe 1993).

Root lesion severity index was significantly lower (P= 0.05) only in chicken manure amendment at 2 t ha^{-1}. However, root knot severity index was lower in chinaberry leaves @ 3 or 2 t ha^{-1} and sesame @ 3 t ha^{-1} which were not significantly different themselves but significantly lower than the control. Neem leaves @ 3 t ha^{-1}, chinaberry leaves @ 1 t ha^{-1}, chicken manure @ 2 or 3 t ha^{-1} amendments also resulted lower root knot severity index (Table 2). Javed *et al.* (2007b) also reported significantly reduced root galling index caused by *M. javanica* in tomato by soil amendments with neem leaves at 3% w/w. Similarly, Akhtar and Mahmood (1993) found significant reduction in root gall index in chilly caused by *M. incognita* amendmended with fresh chopped neem leaves (1% or 5 % w/w) in the presence of predatory nematode *Mononchus aquaticus*. Neem leaves of 5% (w/w) resulted in maximum reduction of root-galls. Similarly, Agyarko *et al.* (2006) recorded significantly reduced root knot index on the carrot roots by poultry manure (air dried for 3 week) application at 5g kg^{-1} soil and found suppressive effect on root knot nematodes.

Table 1. Effect of organic amendments on growth of rice seedlings on 45th day after seeding

Treatments	Fresh shoot weight (g)	Fresh root weight(g)	Number of leaves	Shootlength (cm)	Root length (cm)
Neem leaves (3 t ha^{-1})	2.56 abc†	0.48	6.40	40.33 ab	6.87
Neem leaves (2 t ha^{-1})	2.27 a-e	0.35	6.16	38.19 a-e	5.80
Neem leaves (1 t ha^{-1})	1.94 de	0.32	6.44	35.56 cde	5.75
Chinaberry leaves (3 t ha^{-1})	2.45 a-d	0.39	6.56	38.94 abc	6.37
Chinaberry leaves (2 t ha^{-1})	2.36 a-e	0.35	6.48	37.91 a-e	5.82
Chinaberry leaves (1 t ha^{-1})	2.12 b-e	0.33	6.52	36.72 cde	6.19
Buckwheat biomass (3 t ha^{-1})	2.44 a-d	0.41	6.48	38.01 a-e	6.53
Buckwheat biomass (2 t ha^{-1})	2.26 a-e	0.38	6.52	37.32 b-e	6.02
Buckwheat biomass (1 t ha^{-1})	2.39 a-e	0.40	6.60	38.84 a-d	6.39
Sesame biomass (3 t ha^{-1})	2.32 a-e	0.37	6.48	38.39 a-e	6.74
Sesame biomass (2 t ha^{-1})	2.31 a-e	0.37	6.52	38.40 a-e	6.90
Sesame biomass (1 t ha^{-1})	1.91 e	0.36	6.44	35.44 de	6.04
Chicken manure (3 t ha^{-1})	2.60 ab	0.45	6.40	40.88 a	6.72
Chicken manure (2 t ha^{-1})	2.73 a	0.47	6.64	40.90 a	6.08
Chicken manure (1 t ha^{-1})	2.07 cde	0.36	6.32	35.22 e	6.21
Control	2.07 cde	0.36	6.52	35.95 cde	5.81
LSD (d” 0.05)	0.43	ns	ns	2.89	ns
CV (%)	14.74	21.70	5.00	6.03	11.70

† Mean of 5 replications. Same letters followed in the columns are not significantly different (P= 0.05) by Duncan’s multiple range test. ns = not significantly different.

Table 2. Effect of organic amendments on development of rice root-knot and population of *M. graminicola* in rice seedlings on 45th day after seeding

Treatments	Root lesion severity index (%)	Root-knot severity index (%)	Number of J2 per	
			100g soil	2g root
Neem leaves (3 t ha^{-1})	15 abc†	68.00 bcd	1.30 (22) abc	3.85 (7413)
Neem leaves (2 t ha^{-1})	14 abc	83.60 a	1.48 (32) ab	3.88 (7930)
Neem leaves (1 t ha^{-1})	13 abc	77.60 ab	1.51 (34) a	4.10 (15302)
Chinaberry leaves (3 t ha^{-1})	14 abc	60.00 d	1.42 (28) ab	3.89 (8251)
Chinaberry leaves (2 t ha^{-1})	12 bc	60.00 d	1.42 (28) ab	3.90 (9222)
Chinaberry leaves (1 t ha^{-1})	18 abc	72.80 bc	1.53 (36) a	4.01 (10740)
Buckwheat biomass (3 t ha^{-1})	19 abc	68.80 bcd	1.24 (18) bc	3.96 (9732)
Buckwheat biomass (2 t ha^{-1})	19 abc	76.40 ab	1.33 (22) abc	4.15 (14583)
Buckwheat biomass (1 t ha^{-1})	24 a	78.80 ab	1.39 (24) abc	4.13 (13577)
Sesame biomass (3 t ha^{-1})	13 abc	60.40 d	1.45 (28) ab	3.89 (10483)
Sesame biomass (2 t ha^{-1})	24 a	84.00 a	1.52 (34) a	4.06 (12325)
Sesame biomass (1 t ha^{-1})	23 ab	86.80 a	1.53 (34) a	4.02 (12256)
Chicken manure (3 t ha^{-1})	17 abc	69.20 bcd	1.15 (14) c	3.92 (10177)
Chicken manure (2 t ha^{-1})	10 c	65.60 cd	1.49 (32) ab	3.83 (6976)
Chicken manure (1 t ha^{-1})	13 abc	73.20 bc	1.48 (30) ab	4.01 (10445)
Control	23 ab	84.00 a	1.49 (32) ab	4.05 (11768)
LSD (d” 0.05)	9.31	9.31	0.22	ns
CV (%)	43.47	17.25	12.30	5.08

† Mean of 5 replications. Values without and with parenthesis are log (x+1) and original values, respectively. Same letters followed in the columns are not significantly different (P= 0.05) by Duncan’s multiple range test. ns = not significantly different.

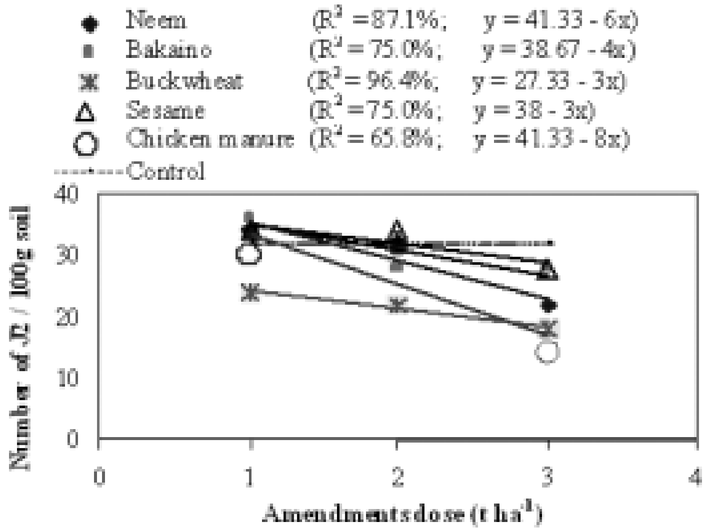

Fig. 1. Effect of different doses of organic amendments on number of J2 per 100g soil

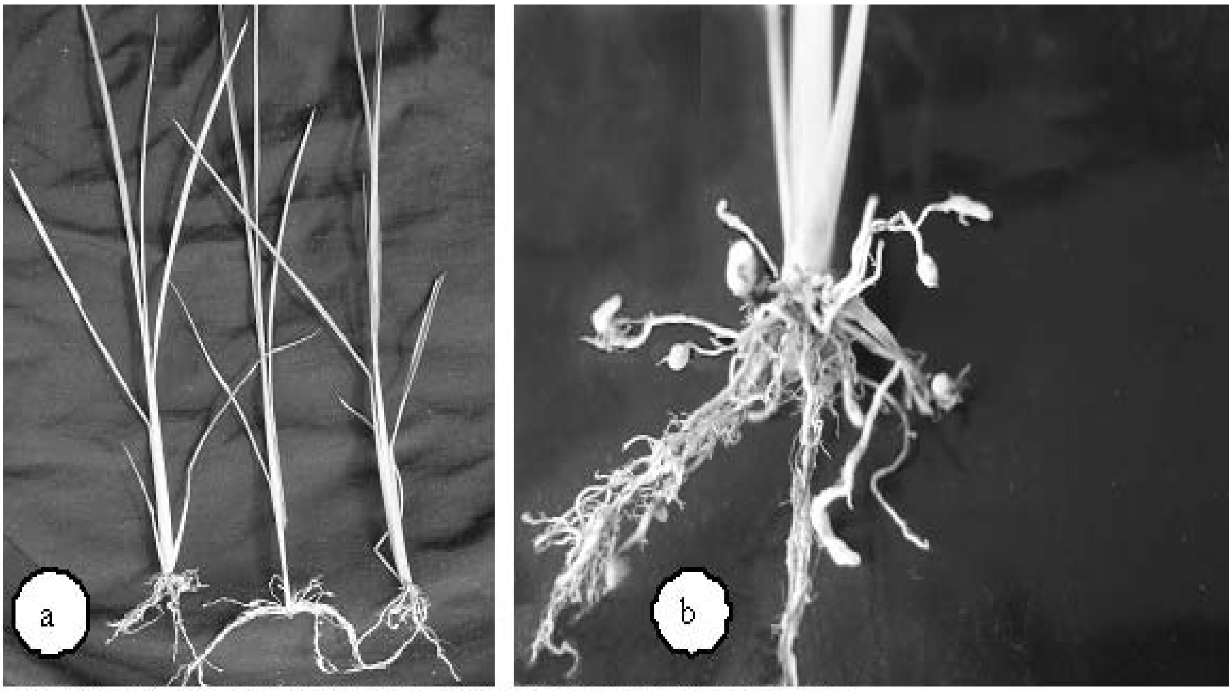

Figure 2a *M. graminicola* infected seedlings, b. root-knots in rice seedlings

Discussion

Organic amendments, especially chicken manure at higher dose suppressed the root knot nematode. The nematode population levels under organic amendment treatments might have changed due to changes in soil properties, nutrients released to plants, increase in predators and parasitic microorganisms, toxic metabolites released from organic amendments breakdown, or health of the host crop (Akhtar & Malik 2000). Ammonia released during decomposition of the amendments is toxic to plant parasitic nematodes including root knot nematodes (Khan *et al.* 1974, Mian *et al.* 1982, Mian & Rodriguez-Kabana 1982) which may be partially involved in suppressing *M. graminicola* and reducing root knot development in rice roots. Suppression of plant-parasitic nematode populations might enhance plant growth in treated soil (Akhtar & Mahmood 1997). Moreover, organic amendments might have improved the soil structure, fertility and water holding capacity of soil so that general plant health and tolerance to nematode attack was improved (Stirling 1989, Akhtar & Malik 2000). The test amendments suppressed the *M. graminicola* population in soil considerably and allowed better plant establishment. The difference in composition and amount of by-products or nematicidal chemicals and nutrients released during decomposition may be responsible for such variable results among the amendments. Therefore, incorporation of chicken manure (2 or 3 t ha^{-1}) can improve rice seedling health in *M. graminicola* infested soil. Since chicken manure would be a low cost nematode management alternative for resource poor rice growing Nepalese farmers. However, further detail research is necessary to understand the mechanism of nematode suppression and to verify the results under natural field conditions.

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