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ECO-FRIENDLY MANAGEMENT OF ROOT-KNOT NEMATODES (*Meloidogyne* spp.) IN OKRA AT FARMERS' FIELD IN CHITWAN, NEPAL

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ABSTRACT

The field experiment was conducted in a naturally root-knot nematode infested tomato field from March-July, 2015, in randomized complete block design with three replications using seven treatments *viz*. neem cake @ 3 t/ha, chicken manure @ 20 t/ha, yellow mustard oil cake @ 3 t/ha, Alstasan Silvox (silver hydrogen peroxide) @ 1 ml/L of water/m^2 before sowing and 5ml/L of water/plant at 30 days after sowing, Bio-Nematon (*Paecilomyces lilacinus*) @ 10 kg/ha, biogas slurry @ 20 t/ha and control. Disease parameters i.e., juvenile population of nematode in soil and root and root-knot index were recorded at 45, 75 and 122 days after sowing. Growth parameters i.e., plant height, fresh shoot weight, root length and dry root weight were recorded after final harvesting of pods. Chicken manure, yellow mustard oilcake and neem cake significantly reduce juvenile population of nematode in soil and root and root-knot index as well as increase yield as compared to control . However, Alstasan Silvox and Bio-Nematon also gives satisfactory results. Therefore, chicken manure, neem cake and yellow mustard oil cake could be better alternative to chemical nematicides for eco-friendly management of root-knot nematodes.

Keywords: alternative, eco-friendly, management, *Meloidogyne* spp., nematicides

1. INTRODUCTION

In Nepal, okra is cultivated as spring-summer season vegetable crops in kitchen garden and commercial farm in terai, inner-terai and lower hills and immature, tender pods are mainly consumed as cooked vegetable or as pickle. Several limiting factors are responsible for the low production of okra. Among biotic factors, root-knot nematodes (*Meloidogyne* spp.) are considered as the economically most important, destructive and complex genus of plant parasitic nematodes [1,2,3]. *Meloidogyne* spp. are sedentary endo-parasites, cosmopolitan in distribution and capable of attacking wide range of crops, especially vegetable crops; causing considerable yield losses throughout the world especially in developing countries independently or in association with fungi or bacteria causing wilt and root rot [4] Most of the cultivars of okra are susceptible to RKNs. Invasion of J2s of RKNs into the root system induces extensive galling in susceptible crops, alter the uptake of water and nutrients and interferes with the translocation of minerals and photosynthates [5]. The poor functioning of the root system due to RKNs' infection changes the shoot to root ratio [6] and makes plant more susceptible to *Fusarium* attack [7]. Initial buildup of the pathogen's inoculum in soil, higher temperature during growing season and continuous growing of susceptible crops in the same field are responsible for the huge losses of yield [8]. Fewer nematicides, even though very effective and give promising result, they are very expensive as well as they are highly toxic and hazardous [9]. Economically feasible and environmentally safe control measures that can be readily available and easily applicable to the farmers are in need. Therefore, the objectives of this study were to evaluate the efficacy of different eco-friendly management measures of RKNs.

2. MATERIALS AND METHODS

Naturally root-knot nematode infested tomato field was selected for eco-friendly management of root-knot nematodes of okra at Bharatpur-14, Chitwan in February, 2015. The field experiment was conducted from March-July, 2015 in completely randomized block design with three replications and seven treatments namely: neem cake @ 3 t/ha, chicken manure @ 20 t/ha, yellow mustard oil cake @ 3 t/ha, Alstasan Silvox @ 1 ml/L of water/m^2 before sowing and 5ml/L of water/plant at 30 days after sowing, Bio-nematon @ 10 kg/ha, biogas slurry @ 20 t/ha and control. The individual plot size was 3.6m^2 (2.0m×1.8m) with the total experimental area of 136.12m^2 (16.6m×8.2m). The spacing between two plots and two blocks were 0.5 m and 0.6 m respectively.

The experimental field was thoroughly ploughed three weeks prior to sowing. Layout of field was carried out and all the treatments except Alstasan Silvox was applied one day before sowing with profuse watering. Alstasan Silvox was applied at the rate of 35ml/L of water/m^2 in a saturated soil before six hours of sowing and soil drenching at the rate of 5ml/L of water at 30

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days after sowing. The overnight water soaked seeds of Arka Anamika variety of okra were sown on 20th March, 2015 at the spacing of 50 cm row to row and 30 cm plant to plant. Plants were thinned out and one plant per hill was maintained three weeks after sowing. Weeding and earthing up were carried out three times at one-month interval after sowing.

Disease parameters i.e., juvenile population in soil and root and root-knot index were recorded at 45, 75 and 122 days after sowing. Growth parameters i.e., plant height, fresh shoot weight, root length and dry root weight were recorded after final harvesting of pods. Second stage juveniles (J2s) of *Meloidogyne* spp. per 100 gm of rhizosphere soil was extracted by using Modified Baermann Tray method and J2s per 10 gm of secondary root was extracted by using root maceration in domestic blender, followed by Modified Baermann Tray method [10]. Root galling was scored using a root gall index scale (0-10) [11]. The data were subjected to ANOVA with the help of RStudio [12]. Juvenile population were statistically analyzed after converting them into log transformation and mean comparison among significant variables was done by Tukey's HSD test at 5% level of significance.

3. RESULTS

3.1 Juvenile population in soil

There was highly significant difference ($p \leq 0.01$) among treatments in second stage juveniles (J2s) population in soil at all three dates of observation, i.e. 45, 75 and 122 days after sowing (DAS) (Table 1). At 45 DAS, the lowest J2s per 100 gm of soil was recorded in Alstasan Silvox (69.3±5.36), followed by neem cake (79.3±3.18) and yellow mustard oil cake (82.0±4.36) treated plots. At 75 DAS, the lowest J2s was recorded in neem cake (169.3±6.17), followed by yellow mustard oil cake (186.0±7.94), chicken manure (192.7±5.24) and Alstasan Silvox (206.3±9.53) treated plots. After final harvesting of the pods (at 122 DAS), the lowest juveniles was recorded in chicken manure (311.7±12.91) treated plots, followed by neem cake (329.7±10.93) and yellow mustard oil cake (337.7±11.22). The effect of Alstasan Silvox was the best at the first observation, but it reduced drastically afterwards. The mean J2s varied significantly among treatments. The lowest mean no. of J2s was recorded in neem cake (192.8±6.52), followed by chicken manure (198.4±6.45) and yellow mustard oil cake (201.9±7.25) treated plots, but they were significantly at par.

Table 1: Effect of treatments on juvenile population of *Meloidogyne* spp. in soil at three observation dates at Bharatpur, Chitwan, during 2015.

Treatment	Juvenile population (No./100 gm soil)			
	45 DAS	75 DAS	122 DAS	Mean
1. Neem cake	79.3^{de}±3.18 (1.8988)	169.3^{d}±6.17 (2.2282)	329.7^{d}±10.93 (2.5176)	192.8^{e}±6.52 (2.2846)
2. Chicken manure	91.0^{cd}±2.31 (1.9588)	192.7^{cd}±5.24 (2.2845)	311.7^{d}±12.91 (2.4929)	198.4^{e}±6.45 (2.2972)
3. Yellow mustard oil cake	82.0^{cde}±4.36 (1.9126)	186.0^{cd}±7.94 (2.2687)	337.7^{d}±11.22 (2.5280)	201.9^{de}±7.25 (2.3046)
4. Alstasan Silvox	69.3^{e}±5.36 (1.8382)	206.3^{cd}±9.53 (2.3136)	427.0^{c}±8.72 (2.6302)	234.2^{cd}±6.55 (2.3693)
5. Bio-Nematon	105.7^{bc}±6.39 (2.0223)	225.0^{c}±9.87 (2.3513)	403.0^{c}±9.17 (2.6051)	244.6^{c}±8.42 (2.3879)
6. Bio-gas slurry	135.7^{b}±6.89 (2.1314)	309.7^{b}±8.84 (2.4905)	512.3^{b}±13.48 (2.7092)	319.2^{b}±9.46 (2.5037)
7. Control	248.3^{a}±19.1 (2.392415)	596.0^{a}±32.45 (2.7740)	975.7^{a}±47.25 (2.9883)	606.7^{a}±26.62 (2.7821)
Mean	115.9	269.3	471.0	285.4
$HSD_{0.05}$	0.1142	0.0914	0.0670	0.0702
CV (%)	1.98	1.34	0.89	1.02
F-Test	**	**	**	**

DAS: Days after sowing; CV: Coefficient of variation; **: Significant at 0.01 level of significance; HSD: Honestly Significant Difference; Values with same letters in a column are not significantly different at 5% level of significance by Tukey's test; Figures after ± indicate standard error and figures in parentheses indicate log transformation values.

3.2 Juvenile population in root

The number of second stage juveniles (J2s) in 10 gm of secondary roots differed highly significantly ($p \leq 0.01$) among the treatments in all three dates of observation (i.e., 45 DAS, 75 DAS and 122 DAS) (Table 2). At 45 DAS, the least J2s per 10 gm of secondary roots was recorded in Alstasan Silvox (23.7±1.20) treated plots, followed by neem cake (26.3±3.18), chicken manure (30.7±5.55), yellow mustard oil cake (33.0±5.20) and Bio-Nematon (44.0±4.04). At 75 DAS, the lowest J2s was recorded in chicken manure (74.7±4.91), followed by neem cake (77.3±3.53), yellow mustard oil cake (83.0±4.93) and Alstasan Silvox (86.7±6.17) treated plots.

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At 122 DAS, the least J2s was recorded in neem cake (114.3±4.67), followed by yellow mustard oil cake (120.3±6.36) and chicken manure (126.3±7.62) treated plots. The effect of Alstasan Silvox reduced sharply from the second observation. The mean J2s in root varied significantly among treatments. The least mean J2s was recorded in neem cake (72.7±3.56), followed by chicken manure (77.2±5.38), yellow mustard oil cake (78.8±5.49) and Alstasan Silvox (96.4±3.72) treated plots.

Table 2: Effect of treatments on juvenile population of *Meloidogyne* spp. in roots of okra at three observation dates at Bharatpur, Chitwan, during 2015.

Treatments	Juvenile population (No./10 gm root)			
	45 DAS	75 DAS	122 DAS	Mean
1. Neem cake	26.3^{c}±3.18 (1.4141)	77.3^{d}±3.53 (1.8875)	114.3^{c}±4.67 (2.0575)	72.7^{c}± 3.56 (1.8603)
2. Chicken manure	30.7^{bc}±5.55 (1.4725)	74.7^{d}± 4.91 (1.8712)	126.3^{c}±7.62 (2.0999)	77.2^{c}±5.38 (1.8855)
3. Yellow mustard oil cake	33.0^{bc}±5.20 (1.5073)	83.0^{cd}±4.93 (1.9176)	120.3^{c}±6.36 (2.0792)	78.8^{c}±5.49 (1.8943)
4. Alstasan Silvox	23.7^{c}±1.20 (1.3730)	86.7^{bcd}±6.17 (1.9357)	179.0^{b}±9.64 (2.2516)	96.4^{bc}±3.72 (1.9836)
5. Bio-Nematon	44.0^{bc}±4.04 (1.6395)	109.3^{bc}±1.86 (2.0386)	163.0^{b}±7.21 (2.2113)	105.4^{b}±4.06 (2.0224)
6. Bio-gas slurry	56.3^{ab}±7.51 (1.7427)	116.7^{b}±10.49 (2.0635)	191.0^{b}±7.02 (2.2804)	121.3^{b}±6.50 (2.0828)
7. Control	99.3^{a}±8.41 (1.9939)	182.0^{a}±14.15 (2.2574)	325.0^{a}±20.13 (2.5103)	202.1^{a}±10.68 (2.3044)
Mean	44.8	104.2	174.1	107.7
$HSD_{0.05}$	0.2896	0.1459	0.1004	0.1250
CV (%)	6.37	2.56	1.59	2.18
F-Test	**	**	**	**

DAS: Days after sowing; CV: Coefficient of variation; **: Significant at 0.01 level of significance; HSD: Honestly Significant Difference; Values with same letters in a column are not significantly different at 5% level of significance by Tukey's test; Figures after ± indicate standard error and figures in parentheses indicate log transformation values.

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3.3 Root-knot index

All the treatments except control were effective ($p \leq 0.01$) in reducing root-knot index (RKI) at all three dates of observation and differed significantly from control (Table 3). Control had highest RKI in all three observations. RKI was lower in all treatments than control at 45 DAS; however, the least RKI was in Alstasan Silvox treated plots (2.89±0.22). At 75 DAS, the least RKI was observed in neem cake (4.39±0.20), followed by yellow mustard oil cake (4.50±0.66) and chicken manure (4.67±0.38) treated plots. At 122 DAS, the RKI was least in chicken manure (5.33±0.19) treated plots, followed by neem cake (5.44±0.11) and yellow mustard oil cake (5.67±0.19). The mean RKI varied significantly among treatments. The lowest mean RKI was recorded in neem cake (4.35±0.18), followed by yellow mustard oil cake (4.48±0.30) and chicken manure (4.54±.26) treated plots, but they were not significantly different from each other.

Table 3: Effect of treatments on root-knot index at three observation dates in field at Bharatpur, Chitwan, during 2015.

Treatments	Root-Knot Index (0-10 scale)			
	45 DAS	75 DAS	122 DAS	Mean RKI
1. Neem cake	3.22^{b}±0.29	4.39^{b}±0.20	5.44^{c}±0.11	4.35^{c}±0.18
2. Chicken manure	3.45^{b}±0.40	4.67^{b}±0.38	5.33^{c}±0.19	4.48^{c}±0.30
3. Yellow mustard oil cake	3.44^{b}±0.11	4.50^{b}±0.66	5.67^{c}±0.19	4.54^{c}±0.26
4. Alstasan Silvox	2.89^{b}±0.22	4.78^{b}±0.45	$6.11b^{c}$±0.22	4.59^{c}±0.20
5. Bio-Nematon	3.83^{b}±0.25	5.11^{b}±0.11	5.89^{c}±0.22	4.94^{bc}±0.12
6. Bio-gas slurry	3.56^{b}±0.06	5.78^{b}±0.11	7.17^{ab}±0.25	5.50^{b}±0.11
7. Control	5.78^{a}±0.11	7.61^{a}±0.06	8.34^{a}±0.33	7.24^{a}±0.07
Mean	3.74	5.26	6.28	5.09
$HSD_{0.05}$	1.2476	1.5088	1.2024	0.8870
CV (%)	11.68	10.03	6.70	6.09
F-Test	**	**	**	**

DAS: Days after sowing; RKI: Root-Knot Index; CV: Coefficient of variation; **: Significant at 0.01 level of significance; HSD: Honestly Significant Difference; Values with same letters in a column are not significantly different at 5% level of significance by Tukey's test; Figures after ± indicate standard error and figures in parentheses indicate log transformation values.

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3.4 Vegetative growth and yield

There was highly significant difference ($p \leq 0.01$) in plant height, fresh shoot weight, root weight and yield/plant and significant difference ($p \leq 0.05$) in root length among treatments (Table 4). Highest height was observed in chicken manure (145.3±4.47 cm) treated plots, followed by neem cake (141.8±3.13 cm) and yellow mustard oil cake (140.2±1.89 cm). Highest fresh shoot weight was recorded on chicken manure (504.1±19.96 gm) treated plots, followed by neem cake (495.5±3.83 gm) and yellow mustard oilcake (492.0±4.73 gm). Highest root length per plant was observed on chicken manure (27.5±0.73 cm) treated plots, followed by neem cake (26.8±2.48 cm) and yellow mustard oil cake (26.3±0.69 cm). Highest dry root weight per plant was observed in chicken manure (31.8±1.02 gm) treated plots, followed by yellow mustard oilcake (30.6±0.64 gm) and neem cake (29.2±0.18 gm). Highest plant yield (gm/plant) was recorded in chicken manure treated plots (179.40±16.78), followed by neem cake (171.50±11.75) and yellow mustard oil cake (165.67±17.95).

Table 4: Effect of treatments on vegetative growth and yield of okra in field at Bharatpur, Chitwan, during 2015.

Treatments	Plant height (cm)	Fresh shoot weight (gm/plant)	Root length (cm/plant)	Dry root weight (gm/plant)	Yield (gm/plant)
1. Chicken manure	145.3^{a}±4.47	504.1^{a}±19.96	27.5^{a}±0.73	31.8^{a}±1.02	179.40^{a}±16.78
2. Neem cake	141.8^{ab}±3.13	495.5^{a}±3.83	26.8^{a}±2.48	29.2^{abc}±0.18	171.50^{a}±11.75
3.Yellow mustard oil cake	140.2^{ab}±1.89	492.0^{a}±4.73	26.3^{ab}±0.69	30.6^{ab}±0.64	165.67^{ab}±17.95
4. Bio-Nematon	130.8^{abc}±1.54	396.6^{b}±7.23	25.4^{ab}±1.39	26.2^{abc}±1.94	136.50^{ab}±8.31
5. Alstasan Silvox	128.7^{bc}±1.63	371.4^{b}±4.18	23.7^{ab}±1.16	24.5^{bcd}±1.68	126.43^{ab}±11.91
6. Biogas slurry	125.3^{c}±2.16	316.5^{c}±5.25	23.1^{ab}±0.92	22.5^{cd}±0.99	106.67^{bc}±16.67
7. Control	101.4^{d}±3.61	265.8^{d}±8.06	20.2^{b}±1.03	18.1^{d}±2.19	62.47^{c}±7.75
Mean	130.5	406.0	24.7	26.1	135.52
$HSD_{0.05}$	14.7099	47.4302	6.3807	6.7343	62.0102
CV (%)	3.94	4.09	9.03	9.02	16.01
F-Test	**	**	*	**	

CV: Coefficient of variation; **: Significant at 0.01 level of significance; *: Significant at 0.05 level of significance; HSD: Honestly Significant Difference; Values with same letters in a column are not significantly different at 5% level of significance by Tukey's test and figures after ± indicate standard error values.

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4. DISCUSSION

Decrease in disease parameter and suppression of nematodes multiplication after the application of organic amendment might be attributed to production of nematicidal compound after decomposition and increased antagonists' microbial activity in soil. The enhancement in vegetative growth and yield through the application of chicken manure, neem cake and yellow mustard oil cake might be due to the addition of nutrients to the soil resulting from the decomposition of organic matter, direct killing or inhibiting effect of decomposed material to nematodes and ability of root to uptake water and nutrients required for photosynthesis. Higher yield by chicken manure might be due to higher dose than other treatments, however lower nematode population in neem cake and yellow mustard oil cake might be due to its active phenolic compound content to the nematodes. The Alstasan Silvox controls the root-knot nematode disease of okra very well at first observation. However, it's effect drastically reduced from second observation. It might be due to the short life of the chemical in soil after application. Therefore, regular application of Alstasan Silvox during cropping season might be effective to control RKN disease in okra. *Paecilomyces lilacinus* was less effective than other three treatments, which might be due to slower effect of it in reducing the nematode population in comparison to chicken manure, neem cake and yellow mustard oil cake or might be due to adverse soil environmental condition for its multiplication during research period.

Khan and Saxena (1997) [13] reported that the decomposition of organic matter released nematicidal chemical and the residual organic matter improved antagonist's microbial activity, survival and persistence Poultry manures release ammonia gas during decomposition, which has nematicidal properties causing toxic to nematodes [14]. Khan *et al*. (1973) [15] reported that from neem extracts, two active components, nimbidin and thionimone, showed toxicity to adults of *H. indicus*, *R. reniformis*, *T. brassicae* and *M. incognita*, and inhibited the growth of their larvae. Volatile sulfur-containing compounds, glucosinolates, present in the plant parts of brassicae family undergoes enzymatic degradation in soil to produce several kinds of active volatile compounds including isothiocynates which has nematicidal properties [16]. *P. lilacinus* contains protease and chitinase which play an important role in the degradation of the egg shell [17]. The reduction in disease by hydrogen peroxide (H_2O_2) might be due to its effect on nematodes directly by its toxicity and/or indirectly as an elicitor triggering the host-plant defense as suggested by Karajeh (2008) [18] who studied the effect of H_2O_2 on *Meloidogyne javanica* of tomato and observed that the nematode reproduction rate (eggs/gm of fresh root) was significantly reduced due to exogenous application of H_2O_2.

5. CONCLUSION

The treatments varied significantly in disease parameters, vegetative growth and yield. However, all treatments did not reduce the nematode population below economic threshold level i.e., 50-200 juveniles/ 100 gm of soil [19] which might be to high level of inoculum present in soil in previous season crop. The study showed that maximum root-knot nematode disease reduction and increment in vegetative growth and yield of okra was brought by chicken manure, neem cake and yellow mustard oil cake without significant difference among themselves. Therefore, regular and continuous use of effective, ecofriendly treatments based on availability and price might be effective in long run and could be suggested as an alternatives approach to the chemical nematicides in an integrated disease management for conserving healthy environment.

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