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STUDY ON APPROACHES TO INTEGRATED CONTROL OF *MELOIDOGYNE GRAMINICOLA* IN RICE

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ABSTRACT

This study presents the results obtained from the studies carried out to find out the response of *Meloidogyne graminicola* to different rice varieties and to different soil amendments when applied with and without NPK fertilizer in young rice plants.

The use of soil amendments such as poultry manure and cow dung significantly reduced the nematode population, the total percentage of yellow leaves and dead leaves and significantly increased the plant growth.

Two varieties namely, Bg 300 and Bg 352 were found to be immune for *M. graminicola* not allowing the nematodes to penetrate the roots. The varieties Bg 350, Bg 356, Bg 357 and Bg 360 were categorized as resistant. These varieties allowed the nematodes to penetrate the roots, but the development and the reproduction of the nematodes were not permitted. The varieties Bg 11, Bg 351, and Bg 358 were found to be susceptible for the nematode, *M. graminicola* of which Bg 351 is highly susceptible host with high rate of reproduction while Bg 358 and Bg 11 are less susceptible with low rate of reproduction of the nematode. This study also revealed that the young plants of susceptible varieties were unable to compensate the nematode damage under unfertilized conditions.

Keywords: root-knot nematode, *Meloidogyne graminicola*, *Oryza sativa*, management

INTRODUCTION

Meloidogyne graminicola, the root-knot nematode is an obligate parasite of rice, *Oryza sativa*. This nematode has been reported in major paddy growing areas in Sri Lanka such as Galle, Udawalawe, Ratnapura, Polonnaruwa, Kurunegala, Moneragala, Ampara, Mahaweli system B (Ekanayake, 2001a) and Kalutara and Gampaha districts (Mohottige & Amarasinghe, 2004; Pathirana *et al.,* 2004).

Adult female of *Meloidogyne graminicola* develops within the roots and eggs are deposited to gelatinous egg sac in the cortex of the plant root. The second stage juvenile that hatches under favourable temperature and moisture conditions remain in the maternal gall or migrate inter-cellularly through the cortical tissues to new feeding sites within the same root (Bridge *et al.*, 1990). This behaviour appears to be an adaptation by *M. graminicola* to flooded conditions enabling it to continue multiplying within the host tissue even when roots are deeply covered by water. This nematode can survive in water logged soil as eggs in egg masses or as juveniles for long periods and quickly invade when infested soils are drained (Bridge *et al.*, 1990). Thus, there is a danger of dissemination through irrigated or run off water. *M. graminicola* can be spreaded through contaminated farm equipment, utensils, workers foot, seedlings and also through alternative host plants.

Once the second stage juveniles (J2) penetrate the root epidermis, they migrate intra cellularly up to vascular system. Root tissues around the nematode, such as meristem, cortex, endodermis and xylem undergo hyperplasia and hypertrophy resulting the characteristic root galls swollen and hooked. Nutrients and water absorption are greatly reduced by the damaged, galled root system. The infestation levels varied from mild through moderate to severe. Nugaliyadde *et. al.*, (2001) reported that the yield is affected only when the infestation exceeds more than 75% i.e. percent gall bearing roots per total roots.

Sri Lankan farmers traditionally use different cultural control measures to protect their crops from pests. Crop rotation, fallowing lands during dry periods after several crops, removal of crop residues, and incorporation of available plant materials or animal waste as to amend the soil condition are among them (Ekanayake, 2001b).

Juveniles of *M. graminicola* in the soil die due to solar heating of the top soil in a few weeks or months. However, there is a possibility that even if the soil is very dry, at least some of the unhatched juveniles may survive, especially inside fragments of roots (Whitehead, 1998).

Many problems are encountered in nematode management with chemicals. They involve the usage of nematicides which are costly, the development of resistance in target nematode species, and causing human health hazards and environmental hazards (Ekanayake, 2001b). Therefore, more economically potential measures should be targeted to control the pest.

Objectives of the present study are; to determine the effect of different soil amendments with and without NPK fertilization on *M. graminicola* and on the growth of infested young rice plant, to study the varietal difference of rice against the infestation and population development of *M. graminicola* and to study the effect of *M. graminicola* on unfertilized rice at seedling stage.

MATERIALS AND METHODS

Inoculum of *Meloidogyne graminicola*:

M. graminicola infested rice plants were collected from a field left after harvest in Makola area in Gampaha District. Species identification was done using perineal pattern of adult female nematodes. Nematode population for inoculum was raised in laboratory maintained rice plants. Second stage juveniles (J2) were extracted from infested rice roots using extraction tray method.

Experiment I: Effects of soil amendments with and without NPK fertilization on young rice plants inoculated with *M. graminicola*

The following soil treatments were tested according to the ratio of soil: soil amendment 2:1 v/v in 15 cm diameter and 10 cm height pots.

T1 Poultry manure +Soil (PM)

T2 Hay material +Soil (HM)

T3 Cow dung + Soil (CD)

T4 Poultry manure + Soil + NPK (PMNPK)

T5 Hay material +Soil + NPK (HMNPK)

T6 Cow dung + Soil +NPK (CDNPK)

T7 Soil + NPK (SNPK)

T8 Soil (S)

From each soil treatment, eight pots were made of which four pots continued with regular NPK fertilization calculated amounts at the rate recommended by the Department of Agriculture and other four without. One germinating seed paddy (Bg 351) was planted in each pot. On the fourth day five active J2 stages were introduced from the extraction tray to the base of the plant in each plant. The experiment was arranged in Completely Randomized Design. The pots were watered daily. After four weeks % green leaves, % yellow leaves and % dead leaves were counted. Plants were then fully removed and wet weight of the plant and the root system, root length and number of galls in the root system was recorded. Gall Index was calculated by dividing the number of galls from the wet weight of each root system. The roots and galls in each plant were teased separately using entomological pins in a Petri dish with little amount of water to expose the nematode population. Total number of eggs, J2, J3/J4, adult females and adult males were counted separately for each plant under the low power (10×4) of the light microscope.

<u>Experiment II: Screening the resistance/susceptibility of different rice varieties to different doses of *M. graminicola*</u>

Nine rice varieties (*Oryza sativa*) Bg 11, Bg 300, Bg 350, Bg 351, Bg 352, Bg 356, Bg 357, Bg 358, and Bg 360 were tested for their resistance/susceptibility to *M. graminicola* in 15 cm x 10 cm size soil pots with six replicates (seeds were obtained from the Rice Research and Development

Institute at Bathalagoda, Sri Lanka). Single rice plant was maintained in a single soil pot. When the plants were two weeks old, three second stage juveniles were inoculated at the base of the plants of three replicates of each variety and other three replicates remained free of nematodes. The pots were arranged in complete randomized design. Plants were maintained until harvesting time, 3 - 3 ½ months depending on the life span of the variety. All the plants were watered daily and fertilized regularly to a calculated amount as per recommended by the Department of Agriculture. At the time of harvest the plants were carefully removed from the pots and following observations were recorded; shoot length (cm), diameter of the stem (cm), number of total leaves, number of seeds, number of dry leaves, root length (cm), dry weight of the root system (g), number of galls. Randomly selected 5 galls were removed from each gall containing root systems separately and total population (adults, eggs, juvenile stages) in each gall was recorded, and the population per plant was calculated.

This experiment was repeated for selected five rice varieties at the rate of fifteen nematodes per plant.

Experiment III: Effect of *M. graminicola* in 3 days old seedling stage of different rice varieties in unfertilized condition (without NPK fertilizer).

Varieties, Bg 11, Bg 351, Bg 352, Bg 356 and Bg 357 were tested. Three germinated seeds were planted in each soil pot. Experiment was set as paired plot design with six replicates. After three days, three sets of replicates inoculated with two *M. graminicola* second stage juveniles. The other three sets of replicates were left without nematodes. All the pots were watered daily. After two weeks, inoculated plants were observed separately for above ground symptoms, and the gall formation and nematode population within the root system.

Data analysis

The statistical analysis was performed using Minitab 11.12 (Windows)

RESULTS

Experiment I:

The gall index and number of nematodes found inside the root tissues of live plants and the total number of dead plants are shown in Table 1.

Table 1. Mean nematode population, Gall Index and number of dead plants after four weeks exposure of nematodes in Experiment I

Treatment	Gall Index	Nematode Population	No of dead plants
PM	1	1	0
PMNPK	4	2	0
HM	76	116	0
HMNPK	14	64	0
CD	7	37	0
CDNPK	24	218	0
SNPK	80	267	4
S	110	535	1

PM=poultry manure, HM= hay material without NPK, HMNPK= hay material with NPK, CD= cow-dung without NPK, CDNPK= cow-dung with NPK, SNPK= soil with NPK, S= Soil without NPK

The highest nematode population (535) and gall index (110) were recorded in the soil treatments in absence of both amendments and NPK fertilization (S). This was followed by the same treatment but with NPK fertilization (SNPK), cow-dung with NPK (CDNPK), hay material without NPK (HM), hay material with NPK (HMNPK) and cow-dung without NPK (CD). The lowest nematode population and gall index were recorded in the poultry manure treatments with and without NPK fertilization (PM and PMNPK). The nematode population was significantly differed among the treatments (p= 0.000, f= 1766.85, pooled standard deviation=12.24) except for poultry manure with and without NPK fertilization. Highest value for chlorosis was recorded for the treatments where there were no soil amendments (S and SNPK) while the lowest value was for poultry manure treatments (Fig.1). There are significant positive correlations between the nematode population and gall index (p = 0.000, R^2 = 71.4%) (Fig. 2) and between the mean percentage of yellow leaves and mean nematode population ($p = 0.002$ and R-sq = 62%) (Fig. 3).

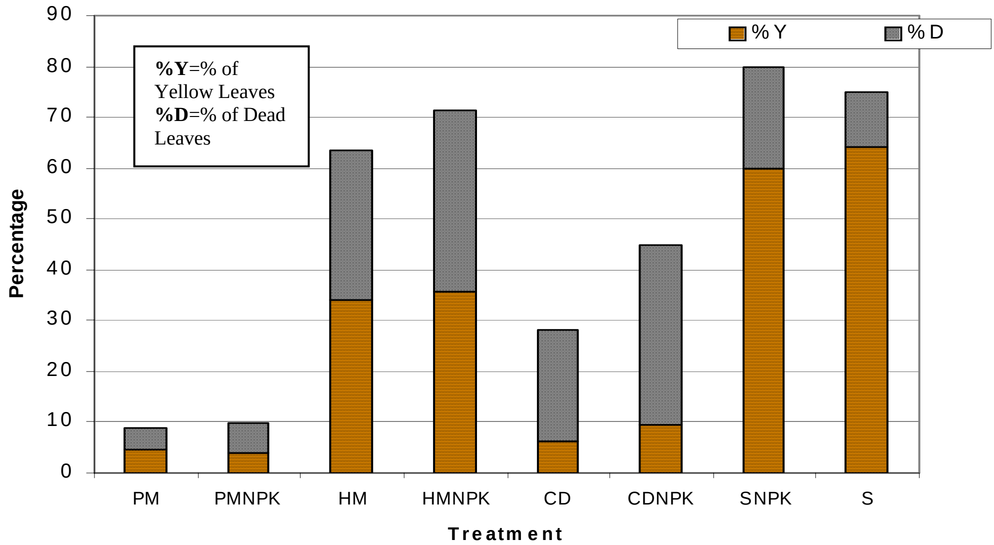


Figure 1. Graph of the Total of Percentage of Yellow Leaves and Dead Percentage Leaves Vs Treatment in Experiment I

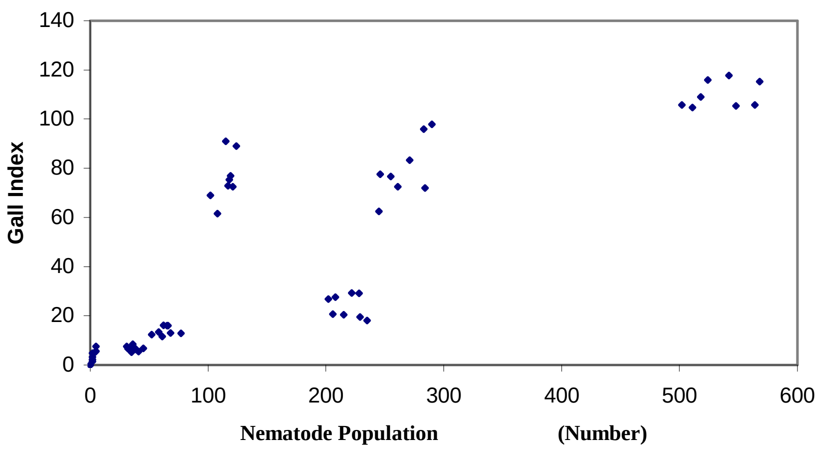


Figure 2. Regression Plot of Gall index Vs Nematode Population in Experiment I

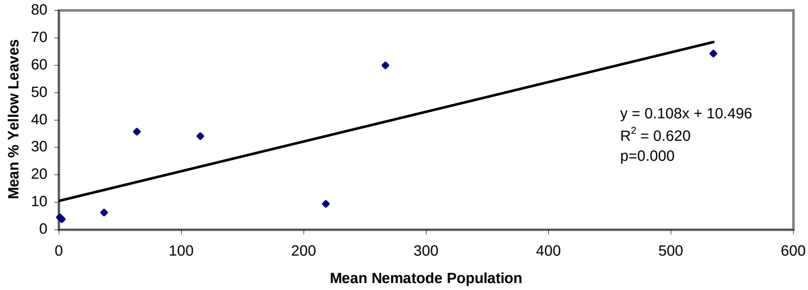


Figure 3. Regression plot of % mean yellow leaves vs mean nematode population in experiment I

The mean values for percentage green leaves, stem height, root index, wet weight of the plant, and length of the root system taken after four weeks of exposure to each treatment are given in Table 2. The highest values were recorded in poultry manure without NPK (PM) and for poultry manure with NPK (PMNPK) treatments. This was followed by cow dung treatments, hay material treatments respectively. The lowest values were recorded in treatments without soil amendments that are soil with NPK (SNPK) and in the pots with only soil (S).

Table 2. Mean values of measurements taken for plants after four weeks of exposure in Experiment I

Treatment	%G	Stem Height (cm)	Root Index	Plant Weight (g)	Root Length (cm)
PM	91.13	13.34	5.0	1.91	21.89
PMNPK	92.07	13.48	4.8	1.89	18.39
HM	36.67	6.38	1.5	0.75	12.01
HMNPK	28.57	9.12	2.1	0.83	12.08
CD	71.88	9.02	2.9	1.2	17.15
CDNPK	55.00	11.75	3.6	1.28	16.89
SNPK	10.00	5.79	1.0	0.56	8.96
S	21.88	5.70	1.0	0.39	9.89

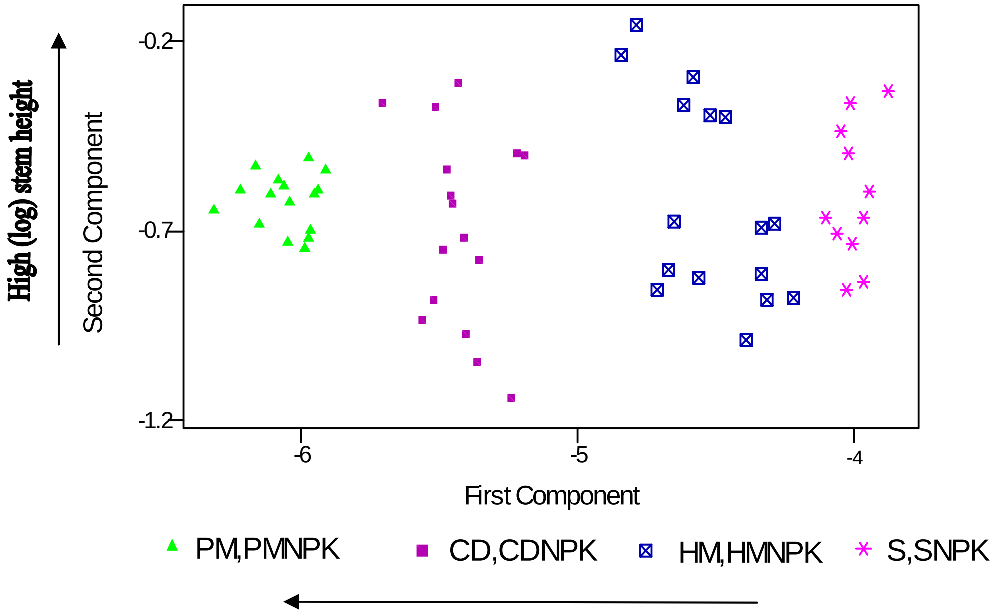
%G= Percentage of green leaves

Principal components analysis (PCA) was carried out for the log transformed data of percentage green leaves, stem height, root index, wet weight of plant, and length of roots. Table 3 shows that the data was described to a proportion of 88% in principal component 1 axes (PC1), 6% in the PC2 and 4% in PC3. Thus, it was sufficient to use only the first two principal components to plot the score plot (Fig. 4) and describe data, as these two describe 94% of variance in this analysis. Percentage of green leaves scored the highest negative value on PC 1 (Table 3, Fig. 4). Plants with more green leaves were aggregated at one extreme (left hand side in Fig. 4) and less green leaves at the other extreme of the score plot. The same pattern was followed by other variables (log stem height, log root index, log wet weight of plant, log length of roots). The one way ANOVA for PC1 scores (in which the higher negative values indicated higher growth) recorded p value 0.000, F= 255.37 and pooled standard deviation 0.1399. The regression analysis for nematode population against PC1 score (Fig. 5) indicated that the plants with highest growth (plants in the highest negative extreme) consisted the lowest nematode population in their root systems (p=0.000 and R^2 = 0.481).

Table 3. Principal component analysis for data of each treatment pot in Experiment I (N=64)

Eigenvalue	Proportion	Cumulative
PC1 0.62079	0.876	0.876
PC2 0.04357	0.062	0.938
PC3 0.02907	0.041	0.979

Variable	PC1	PC2	PC3
log Green leaves	-0.649	-0.632	0.300
log Stem Height	-0.309	0.640	0.015
log Root Index	-0.520	0.416	0.200
log Length(Root)	-0.341	-0.099	-0.932
log Wet Weight(Plant)	-0.311	0.096	0.044



High (log) percentage of green leaves

Figure 4. PCA ordination score plot of the soil pots in Experiment I

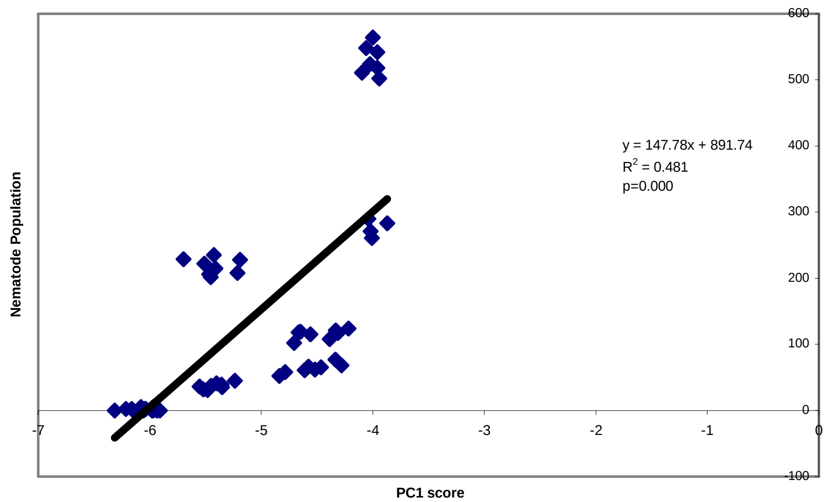


Figure 5. Regression plot of nematode population vs PC1 scores in Experiment I

<u>Experiment II:</u>

Above and below ground plant measurements

The mean values for the above ground plant measurements such as height of the plant, % of dry leaves, number of seeds harvested did not have any significant difference between control and test plants of any varieties tested in experiment II which was done with three nematodes per plant.

When tested using 15 nematodes per plant, two varieties Bg 350 and Bg 352, continuously showed non significant difference between control and test plants. However, the variety Bg 351 showed a significant height difference (P=0.037, f=9.42) between test plants (mean height 47.8 ± 3.01 cm) and control plants (mean height 54.1 ± 1.88 cm) and also a yield difference (P=0.035, f=9.86) between test plants (mean seeds 102 ± 15) and control plants (mean seeds 148 ± 21). The varieties Bg 356 and Bg 358 showed only a significant height difference between control and test plants (P=0.036, f= 9.6 where mean height of test plants of Bg 356 is 64.5 ± 1.8 cm and mean height of control plants is 68.5 ± 1.3 cm) and P=0.017, f= 15.5 where mean height of test plants of Bg 358 is 58.00 ± 2.18 cm and mean height of control plants is 65.17 ± 2.27 cm).

Gall formation and nematode population development

The mean number of galls and total number of nematodes found inside galls that are developed from the initial number taken at the time of harvest are tabulated in Table 4. There was neither nematode infection nor population development in the root systems of Bg 300 and Bg 352 (Table 4). The nematodes were detected inside the roots of varieties Bg 350, Bg 357, Bg 356 and Bg 360 but no further development was observed in the plant. J2 stages were remained in the roots at the same stage as they were inoculated. The P_f/P_i value shows that (P_f = final nematode population, P_i = initial nematode population) all these varieties are resistant to the nematode *M. graminicola*. The varieties Bg 11, Bg 351, Bg 358 had varying rates of gall formation and nematode development in the galled root systems (Table 4). Bg 350 and Bg 352 were confirmed as resistant for nematode *M. graminicola* by the experiment conducted using 15 nematodes per plant (Table 5). The highly susceptible variety was Bg 351 which was resulted with larger galls (about 0.5cm in diameter) each occupied by average of three adult females. About 250 nematodes were developed within a plant of this variety when tested with 15 nematodes per plant (Table 5). The varieties Bg 358 and Bg 11 were rated as less susceptible hosts which showed different levels of nematode development within the root system.

Table 4. Mean number of galls formed, mean number of nematodes developed and P_f/P_i value for each variety inoculated with 3 nematodes per plant in experiment II (P_f=final nematode population, P_i= initial nematode population)

variety	Mean number of galls	Mean number of nematodes	P_f/P_i
Bg 11	38	38	12.7
Bg 300	0	0	0
Bg 350	0	0	0
Bg 351	60	60	20
Bg 352	0	0	0
Bg 356	0	0	0
Bg 357	0	0	0
Bg 358	45	45	15
Bg 360	0	0	0

Table 5. Mean number of galls formed, mean number of nematodes developed and P_f/P_i value for each variety inoculated with 15 nematodes per plant in experiment II (P_f=final nematode population, P_i= initial nematode population)

variety	Mean no. of galls	Mean no. of nematodes	P_f/P_i
Bg 350	0	0	0
Bg 351	87	262	17.5
Bg 352	0	0	0
Bg 356	0	0	0
Bg 358	77	77	5.2

Experiment III

Two average mature females were found developed within two galls formed in the root system per plant of variety Bg 351. Although plants were survived, the leaves were yellowish and became chlorotic and wilted (40%), comparatively to the control plants.

No any nematode development was found in the root systems of any of the test plants of varieties Bg 356 and Bg 352. Plants did not symptomize with chlorosis.

One average mature female nematode was found within a gall formed in the root system per plant of variety Bg 11 with many yellowish leaves (40%) compared to control plants.

DISCUSSION

After the chemical pesticides came into play in pest control presently they have been identified as substances that can cause human health hazards and environmental hazards and even some chemical pesticides are not economical. Thus, the present approach was to find out more economical and environmental friendly methods in controlling one of the major nematode pests of rice, *M. graminicola*.

Soil amendments of different kinds used as nutrient sources for crop production have been found effective in control of root diseases of plants. The materials, green manure, cow-dung, poultry droppings, dried crop residue, industrial by products have been successfully used. Remarkable reductions have been achieved in nematode population in both green house and field conditions with concomitant increase in growth and yield of the plants. Such increase in growth have been attributed to either improvement in soil condition resulting in greater root growth, thereby enhancing the utilization of soil nutrients or to changes in the biotic and abiotic environment of plants. These ultimately alter the host parasite relationship thereby minimizing the nematode damage (Abubakar *et al*., 2004).

In the experiment I where only the soil amendments were used, all treatments with amendments showed significantly lower nematode population than that of the treatments with no amendments (S, SNPK). This was similar in the total of percentage yellow leaves and percentage dead leaves. This envisaged that the nematode population was adversely affected by the soil amendments such as cow-dung, hay material and poultry manure.

The nematode population and the percentage yellow leaves, which is a damage symptom of root-knot nematodes, had shown a significant correlation. Thus, the nematode population has adversely affected on the plant. Considering the NPK fertilization, in each soil amendment, the NPK treated ones had shown higher nematode population than the same treatment without NPK. However, the treatments with hay material (HM, HMNPK), soil (S, SNPK) had shown exceptions. In these treatments, although the NPK treated ones had lower nematode population (Table 1) the total of percentage yellow leaves and percentage dead leaves were higher than the same treatment without NPK (Fig. 6).

The growth of plants was also shown significant negative correlation with the nematode population. There was a decrease in the plant growth when the nematode population in the plant was high. Thus, the plant growth was affected by the nematode population. The plants with soil amendments had shown significantly higher growth than that of the treatments without amendments (S and SNPK)

Generally this study shows that, the nutrients available in the soil amendments, promoted the growth of plants and the soil amendments by any means have significantly inhibited the nematode population.

Considering the NPK fertilization in each soil amendment, the plant growth between the NPK treated ones and not treated ones had no significant difference (Table 2). Therefore, this study reveals that if using soil amendments in order to reduce nematode population in an infested rice field, NPK fertilization is not necessary.

Out of the three types of soil amendments used in this study, poultry manure had resulted the significant higher plant growth and significant lower nematode population and generally lower chlorosis than all the other amendments (Table 2, Fig. 6). Thus, poultry manure has enhanced the natural defense against nematodes leading to a higher growth.

Arrestment of the development of nematodes in varieties Bg 300, Bg 350, Bg 352, Bg 356, Bg 360 and Bg 357 in the experiment II tested using 3 nematodes per plant may be either due to death of nematodes before or after the penetration of root epidermis or they have been rejected by the plant owing to its resistance. However, in the same experiment tested using 15 nematodes per plant and also in the experiment III, where the varieties Bg 352 and Bg 356 were tested confirm that the above varieties are resistant hosts to *M. graminicola*. In addition, these varieties have not shown any above and below ground plant measurements to be significant compared to control plants of the same variety. Hence this further confirms that there is no effect of nematodes on these rice varieties. This study revealed that the susceptible varieties to *M. graminicola* are Bg 351, Bg 358 and Bg 11. However, variety Bg 11 did not show significantly low plant growth compared to control plants. This shows that the variety Bg 11 though susceptible to *M. graminicola* can compensate the damage caused by three initial numbers of nematodes per plant. However, it was envisaged that the three days old seedlings of the same variety, Bg 11 could not compensate for the damage caused by initial two numbers of nematodes per plant at unfertilized condition. Susceptibility of variety Bg 358 was expressed through significant height difference where test plants were shown retarded growth in presence of initial fifteen numbers of nematodes per plant. However, this has not caused any yield reduction. Hence the economic loss is somewhat less in this variety. The variety Bg 351 was the highly susceptible among the varieties tested. This variety was affected at both lower and higher number of nematodes. Significant difference was obtained for both plant height and yield (experiment II). This shows that the plant cannot compensate the damage caused by fifteen number of nematodes per plant, hence considerable yield loss was occurred (102 seeds per plant P=0.03, F=9.86).

According to the experiment I and experiment II the damage to the plant has always positively correlated with number of female nematodes or number of galls per plant. For example, the variety Bg 351 when tested with initial three nematodes per plant ended up with 60 final nematodes per plant with no symptoms. Same variety has shown growth retardation and yield loss respectively when tested with fifteen nematodes per plant. Nematode females continue to feed on plant sap for longtime. They absorb high amount of nutrients for their egg production. Males leave the plant without long periods of feeding, and also very rarely they developed in to males. Inoculated second stage juveniles feed on roots, but they soon molt in to non-feeding third and fourth stages of juveniles. Therefore greater damage is done by female nematodes. Therefore in calculating $P_{(f)} / P_{(i)}$ only the females were considered.

Higher number of galls per plant and larger size of the gall directly reduce the growth of the plant and the yield owing to the disturbance to nutrient uptake from the root system. This can be proved from the data obtained for variety Bg 351 when tested with initial fifteen nematodes per plant ended up with 87 galls per plant and the gall size was 0.5 cm diameter containing three females per gall. The usual gall size was 0.1 cm diameter containing single female per gall. It agrees with Nugaliyadde *et al.*, (2001) who state when 75% of the root system is infected with *M. graminicola*, will lead to a yield loss.

According to the results of experiment III the varieties Bg 11 and Bg 351 could not compensate the nematode damage even under the rate of two nematodes per plant. They were shown yellowish and wilted leaves compared to the control plants. Therefore, farmers have to apply fertilizer in presence of nematodes to compensate their damage at the very early stage of the plant.

CONCLUSIONS

Use of soil amendments such as poultry manure, cow dung and hay material reduced the growth of *M. graminicola* population and enhanced the growth of rice plant.

When using soil amendment use of NPK fertilization is not essential at early stage of rice plant.

Poultry manure could be used at least occasionally as a nematicide cum fertilizer to improve soil fertility and to reduce nematode infestation level.

Bg 300, Bg 353, are immune hosts to *M. graminicola* while Bg 350, Bg 356 and Bg 357 and Bg 360 are resistant; Bg 11 and Bg 358 less susceptible and Bg 351 is highly susceptible.

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