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Meloidogyne graminicola **infestation in selected Sri Lankan rice varieties,** ***Oryza sativa*** **L. and nemato-toxic effect of** ***Trichoderma viride*** **to reduce infectivity**

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ABSTRACT

Meloidogyne graminicola is a nematode parasite of rice, *Oryza sativa* L., in some rice growing areas in Sri Lanka. *M. graminicola* infestation levels in eight rice varieties namely Bg 407, Bg 366, Bg 403, Bg 251, Bg 369, Bg 380, Bg 310, and Bg 745 and nemato-toxic effect of *Trichoderma viride* against the nematode infectivity in a susceptible rice variety were evaluated in this study. Results revealed that varieties Bg 366 and Bg 251 were susceptible; varieties Bg 407, Bg 369, Bg 380, Bg 310, and Bg 745 were moderately susceptible while Bg 403 was moderately resistant to *M. graminicola*. There was a significant reduction of root galls ($p = 0.000$) and weight of the fresh roots ($p = 0.001$) of *T. viride* treated Bg 366 susceptible rice variety compared to the untreated tillers of the same rice variety. This study concludes that none of the rice varieties tested were totally resistant to *M. graminicola* infestation, hence, *T. viride* treatment can be integrated into nematode management practices to reduce the nematode population in susceptible rice varieties.

Keywords: gall index, root galls, chlorosis, nematode management, resistant, susceptible

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INTRODUCTION

The root-knot nematode, *Meloidogyne graminicola* (Golden and Birchfield), is a major threat to Sri Lankan rice cultivation (Ravindran, *et al*., 2017). It was first reported to infest rice (*Oryza sativa* L.) fields in Sri Lanka in early 1990s and it has now dispersed in to major rice growing areas of the country (Ekanayake *et al*., 2001, Nugaliyedda *et al.*, 2001). The infestation level varies from mild through moderate to severe (Nugaliyedde *et al*., 2001). The infestation of *M. graminicola* was severe in rice fields in some parts of the dry zone of the country. Dispersion of this nematode occurs mainly through irrigated water and soil. Mature female nematodes produce a large number of eggs at once leading to a sudden increase of the pathogen population causing severe outbreaks in a very short period of time. It has been found that there is a decline of rice yield when more than 75% of the roots of an infested plant is affected by nematodes (Nugaliyedde *et al*., 2001). Experiments have proven that infestation level varies based on the rice variety (Amarasinghe, 2011; Amarasinghe *et al*., 2007).

Adult female and juvenile nematodes cause the damage by altering physical changes in the host plant. The juveniles continuously feed on cells close to the vascular system of the root enlargement, hence, vascular disruption is occurred. If the meristematic cells near the root tip are damaged by the juveniles, the root elongation will be interrupted (Norton & Niblack, 1991). Gradually, nutrient and water absorption of the root system is blocked and upward translocation is interrupted. This situation indirectly affects photosynthesis that leads to the condition known as 'chlorosis' (Bridge *et al.,* 1994)**.** *M. graminicola* affected rice plants show stunting due to the characteristic terminal swellings/galls on the root tips which ultimately result in severe reduction in growth and yield (Jaina *et al.,* 2011). Wilting of seedling occurs, when the population density of *M. graminicola* is high (Ou, 1985).

Elimination of the favorable environmental conditions that is required for completing the life cycle of *M. graminicola* is an efficient way to control the nematode. Use of resistant rice varieties can also be implemented to keep the nematode population under control. Bg 352, Bg 300 and Bg 357 are some of the existing rice varieties identified as resistance to the nematode infestation in Sri Lanka (Amarasinghe *et al*., 2011). Several indices have been developed to identify the level of resistance of different crop varieties. Some indices were based on the ratio between final nematode population and the initial nematode population in an infested rice plant and some were based on the galling index, the number of galls produced in an infested rice plant by the nematode (Bridge *et al.*, 1994). Apart from varying susceptibility levels, several authors have described the successful use of biological control agents against *M. graminicola*. Fungal species such as *Paecilomyces lilacinus*, *Trichoderma harzianum* and other *Trichoderma* sp. (Huong *et al*. 2009) the bacterium, *Bacillus subtilis* (Narasimhamurthy *et al*., 2017) and rhizobacterium, *Pseudomonas fluorescence* (Seenivasan *et al*., 2012) have shown promising results as biocontrol agents against *M. graminicola*. *Trichoderma viride* causes direct and indirect effects on nematode reproduction and host responses such as the growth of the host and gall formation in the root system. When the density of spores of *Trichoderma viride* is up to 1×10^4, nematode reproduction and the root galling are suppressed while productivity of the plant is enhanced (Al-Hazmi and Tariq Javeed, 2015). This study was conducted to determine the levels of susceptibility of some of the new rice varieties to *M. graminicola* and to evaluate the potential of *T. viride* as a bio-control measure to reduce *M. graminicola* infestation in Sri Lanka.

METHODOLOGY

Rising up the inoculum of *Meloidogyne graminicola*

According to the approval and guidance of the officers at the Regional Office of the Department of Agriculture at Dehiaththakandiya, Sri Lanka, a minimum number of infested rice plants (n = 6) were collected from infested rice fields. The infested plants were kept inside polythene bags (30 cm×15 cm) to avoid the exposure of the plants to the outside environment and brought to the laboratory. Dry soil from apparently uninfested, tilled paddy fields in Kurunegala district was collected into transparent polythene bags. Soil bags were exposed to direct sunlight for five hours continuously each day for two weeks to defaunation. De-faunated soil was filled into plastic basins (25 dia. × 22hei. cm) with adequate water and germinated seed paddy were sown in these containers. Infested rice plants were transplanted in them. The plants were kept under *in-situ* conditions. NPK fertilizer was added according to the recommended rate where necessary and watered regularly. Prior to the experiments, required amount of J_2 juveniles were collected from root-galls. The roots were washed and root galls were separated into a watch glass. The galls were crushed using a glass rod to expose the nematodes. The suspension of right stage of the nematode (J_2 juveniles) was prepared in a 100 mL beaker.

Rice varieties

Seeds of ten rice varieties namely Bg 407, Bg 366, Bg 455, Bg 38, Bg 403, Bg 251, Bg 369, Bg 380, Bg 310, Bg 745 were obtained from the Rice Research and Development Institute at Bathalagoda, Sri Lanka and Plant Genetic Resources Centre, Gannoruwa, Sri Lanka. Bg 38 and Bg 455 rice varieties died at seedling stage before the beginning of the experiment hence, did not use in the experiment.

Preparation of the inoculum of *Trichoderma viride*

Ten number of Potato Dextrose Agar (PDA) plates (9 cm × 1.5 cm) were prepared. A loop of fungal hyphae from the laboratory maintained *Trichoderma viride* cultures originally prepared by (Amarasinghe & Madurusinghe 2012) were placed on the agar plates under sterilized conditions. The pure cultures were identified as *T. viride* as described by (Amarasinghe & Madurusinghe 2012) and maintained in the incubator at 10 °C until they were used for the experiment.

Levels of susceptibility of rice varieties to *Meloidogyne graminicola*

About 50 seeds each of rice varieties of Bg 407, Bg 366, Bg 403, Bg 251, Bg 369, Bg 380, Bg 310, Bg 745 were soaked in glass beakers (100 mL) each containing 50 mL of water for 24 hours. They were transferred to a moistened filter paper and were covered with a transparent polythene sheet until seed germination. Square shaped plastic pots (7.5 L × 7.5 W × 11.5 H cm) (n = 96) were filled with 250 g of de-faunated soil and arranged in 12 rows. Twelve seedlings each from each rice variety (V_1-V_8) were planted in randomly selected pots. Six of the randomly selected plants of each variety were assigned as control pots (C_1-C_6) and the other six were assigned for nematode inoculum (T_1-T_6). After two weeks, 1 mL suspension containing five J2 juveniles was injected to the rhizosphere of each test plant using a pasture pipette. The plants that were maintained as controls (C_1-C_6) were treated with distilled water. Experiment was arranged in fully randomized design. This experiment was continued for two month. All plants were watered daily. The water level was maintained about 2 mm above the soil layer in the pots for three weeks. Thereafter, adequate amount of water was added only to retain the moisture of the soil throughout the remaining period of the experiment. A known amount of NPK fertilizer was added once in two weeks. The plant growth was monitored daily. After eight weeks, number of yellow leaves, root galls per plant and wet weight of the root system were recorded by destructive sampling. The gall index (percent gall bearing roots/total roots) for each variety was calculated. The final nematode population (Pf) in each plant was counted. Reproduction factor (Pf/Pi) was calculated (Pi = initial population).

Nemato-toxic effect of *Trichoderma viride* on *Meloidogyne graminicola*

A 100 mL of water containing pre-determined concentration of the *Trichoderma viride*, 1×10^4 spores/mL (Amarasinghe & Madurusinghe 2012) was prepared from a stock suspension (4.05×10^5 spores/mL). Bg 366 variety rice plants were raised individually in sixty plastic pots (7.5 L × 7.5 W × 11.5 H cm) filled with un-infested soil (250 g). The tillers were allowed to grow for two weeks. Following three treatments (T1 to T3) were applied randomly to 20 pots each as follows; T1 - distilled water (10 mL) spread around the rice tillers, T2 – 10 mL of 1×10^4 spores/mL *Trichoderma viride* applied to the soil around the tiller and T3-10 mL of 1×10^4spores/mL *T.viride* injected at the root level. Each tiller was inoculated with J_2 juveniles at the rate of five individuals per rice tiller as described above. The plants were allowed to grow for eight weeks in a screen house. During the experimental period, the plants were monitored daily. NPK fertilizers were added once in two weeks. Post-treatment assessment was done after uprooting the plants. The number of yellow leaves was recorded. Root system was carefully washed and the number of root galls per plant and wet weight of the roots were recorded.

Statistical analysis

Two sample t-test was carried out to determine whether there were significant differences among control and test plants. One-way ANOVA was performed to ascertain the effects among the different treatments. Tukey’s test was carried out to compare the means (Minitab Version 14)

RESULTS

Symptoms of *M. graminicola* infestation on rice varieties:

Chlorosis: The percentage of yellow leaves increased in nematode infested rice tillers compared to uninfested control rice plants in all the varieties (Table 1). The percentage of yellow leaves in test tillers increased significantly in Bg 366 ($p = 0.019$), Bg 251 ($p = 0.021$), and Bg 369 ($p = 0.031$) compared to control tillers.

Length of the total leaves: The mean length of the total leaves among nematode infested test tillers and uninfested control tillers in all the varieties were not significantly different ($p>0.05$). Nevertheless, a diminutive reduction of the mean length of the total leaves was observed in control plants of most of the varieties.

Wet root weight: Fresh root weight of test rice tillers of Bg 407 ($p = 0.001$), Bg 366 ($p = 0.003$), Bg 251 ($p = 0.02$, $T = -2.91$), and Bg 369 ($p = 0.046$, $T = -2.35$) significantly increased compared to that of uninfested control plants. Fresh root weight of the test tillers of other varieties such as Bg 403 ($p = 0.366$, $T = -0.96$), Bg 380 ($p =0.116$, $T = -1.76$), Bg 310 ($p =0.064$, $T = -2.14$), and Bg 745 ($p = 0.549$, $T = -0.55$) was not significantly different from that of control tillers.

Table 1. Mean percentage of yellow leaves, length of total leaves and root wet weight. Mean values in a column with different superscript letters were significantly different from each other (Two sample t-test p< 0.05).

Rice variety	Mean percentage of yellow leaves ± SE		Mean length of total leaves (cm) ± SE		Mean weight (wet) of the root (g) ±SE	
	Control	Test	Control	Test	Control	Test
Bg 407 (V1)	28.2 ± 7.0 [a]	41.8 ± 9.2 [a]	23.96 ± 1.10 [a]	21.80 ± 1.20 [a]	0.640 ± 0.040 [a]	0.876 ± 0.021 [b]
Bg 366 (V2)	27.0 ± 4.2 [a]	42.6 ± 3.3 [b]	27.84 ± 0.57 [a]	26.72 ± 0.33 [a]	0.384 ± 0.068 [a]	0.832 ± 0.130 [b]
Bg 403 (V3)	24.6 ± 3.5 [a]	27.4 ± 5.2 [a]	25.60 ± 1.20 [a]	25.70 ± 0.80 [a]	0.462 ± 0.057 [a]	0.538 ± 0.055 [a]
Bg 251 (V4)	21.2 ± 2.0 [a]	36.6 ± 5.0 [b]	23.60 ± 0.68 [a]	21.60 ± 0.73 [a]	0.370 ± 0.084 [a]	0.670 ± 0.060 [b]
Bg 369 (V5)	29.2 ± 4.0 [a]	42.6 ± 3.3 [b]	27.00 ± 1.50 [a]	26.80 ± 2.50 [a]	0.382 ± 0.067 [a]	0.554 ± 0.030 [b]
Bg 380 (V6)	36.4 ± 6.7 [a]	40.0 ± 6.5 [a]	21.10 ± 2.20 [a]	24.98 ± 1.20 [a]	0.544 ± 0.040 [a]	0.634 ± 0.032 [a]
Bg 310 (V7)	26.8 ± 8.3 [a]	44.0 ± 2.4 [a]	28.64 ± 0.76 [a]	28.12 ± 0.79 [a]	0.436 ± 0.044 [a]	0.630 ± 0.079 [a]
Bg 745 (V8)	23.0 ± 6.8 [a]	27.6 ± 3.9 [a]	25.10 ± 1.20 [a]	20.76 ± 2.00 [a]	0.442 ± 0.059 [a]	0.492 ± 0.068 [a]

Reproduction factor (Pf/Pi)*:* The highest reproduction factor (Pf/Pi) was observed in Bg 366 (151.8). Bg 251 also possessed a higher reproduction factor (127.2). The lowest reproduction factor (Pf/Pi) was observed in Bg 403 (Table 2).

Susceptibility level of rice varieties: The levels of susceptibility in tested rice varieties were determined by using the index prepared by Ravindra *et al*., (2015) according to the number of galls per root system *i.e.* zero galls – Immune; 1-2 galls – resistant; 3-10 galls – moderately resistant ; 11-30 galls – moderately susceptible; 31-100 galls – susceptible; >100 galls – highly susceptible.

Root galls: There were no galls in control tillers (Plate 1a, Plate 2a). According to the gall index, the eight rice varieties were categorized as either susceptible, moderately susceptible or moderately resistant to the *M. graminicola* infestation (Table 2). Bg 403 rice variety had the lowest number of galls among the rice varieties tested and hence categorized as moderately resistant (Plate 1b, Table 2). Bg 366 and Bg 251 rice varieties were categorized as susceptible because the average number of galls exceeded thirty one, which demarcate lower level of the susceptible category. Out of these two varieties, the highest number of galls (39) was observed in Bg 366 rice variety (Plate 2b). Bg 407, Bg 369, Bg 380, Bg 310, Bg 745 rice varieties with 28, 29, 27, 24, 26 mean number of galls respectively were categorized as moderately susceptible. Accordingly, the lowest gall index was observed in Bg 403 (27%) rice variety while the highest gall indices were observed in Bg 366 (92%) and Bg 251 (86%) rice varieties. Approximately similar gall indices were observed in Bg 407, Bg 369, Bg 380, Bg 310, Bg 745 rice varieties (78.3%, 81.6%, 77.8%, 76.5% and 77% respectively) (Table 2). The highest reproduction factor was also observed in Bg 366 rice variety. The lowest reproduction factor was observed in Bg 403 rice variety that had the lowest number of galls.

Table 2. Nematode reproduction factor, root gall formation and susceptibility level of each variety (Pi = 5)

Rice variety	Final population (Pf)	Pf/Pi	Total No. of root galls	Gall index	Resistant level
Bg 407	285	57	28	78.3	Moderately susceptible (MS)
Bg 366	759	151.8	39	92	Susceptible (S)
Bg 403	35	7	8	36	Moderately resistant (MR)
Bg 251	636	127.2	36	86	Susceptible (S)
Bg 369	356	71.2	29	81.6	Moderately susceptible (MS)
Bg 380	241	48.2	27	77.8	Moderately susceptible (MS)
Bg 310	125	25	24	76.5	Moderately susceptible (MS)
Bg 745	187	37.4	26	77	Moderately susceptible (MS)

Plate 1a: Root system of the control plant of Bg 403

Plate 1b: Root system of the test plant (inoculated) of Bg 403

Plate 2 a: Root system of the control plant of Bg 366

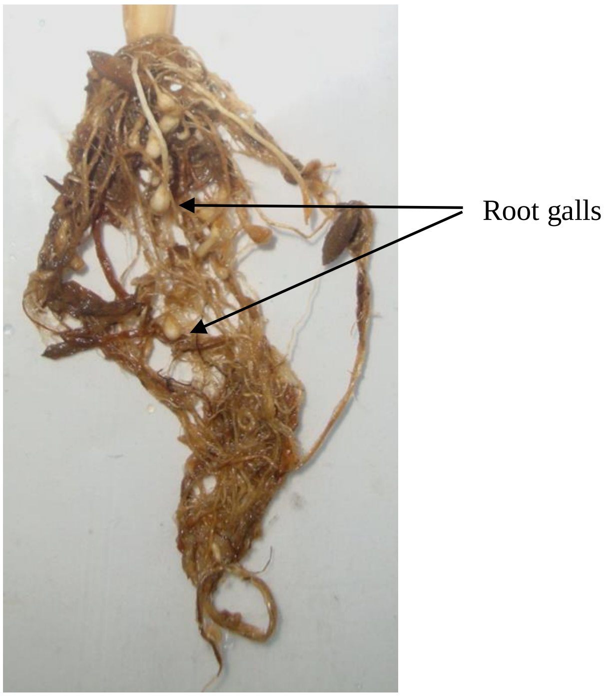

Plate 2 b: Root system of the test plant (inoculated) of Bg 366

Nemato-toxic effect of *Trichodermaviride* on *Meloidogyne graminicola*

There was a reduction in mean percentage of yellow leaves in T2 and T3 compared to T1 untreated control (Table 3). The percentage of yellow leaves in treatment 02 was lower than that of treatment 03. However, they were not significantly different (p= 0.731, F=0.32, DF = 2). The mean weight of the fresh root of tillers in treatment 1 was significantly higher than that of treatment 2 and treatment 3 (p = 0.001,F = 12.89, DF = 2). The mean fresh root weight of the T2 tillers dipped in *T. viride* (1×10^4 spores/mL) suspension was lower than that of T3, but it was not significant. The number of root galls were significantly higher in untreated plants (p = 0.000, F =68.92 DF = 2) compared to treated plants. However, there was no significant difference between T2 and T3. Though they were not significant, the number of galls was lower in treatment 2 than that of treatment 3.

Table 3: Above and below ground post treatment measurements ± SE. The different superscript letters in a column indicate that they were significantly different from each other (One-way ANOVA, Tukey's test, p< 0.05).

Treatment	Mean percentage of yellow leaves ± SE	Fresh root weight (g) ± SE	Mean number of galls ± SE
T_1 – untreated control	23.00 ± 2.81^{a}	0.290 ± 0.022^{a}	15 ± 0.8^{a}
T_2 - *T. viride* (drenched)	16.40 ± 4.30^{a}	0.164 ± 0.020^{b}	1 ± 0.58^{b}
T_3 - *T. viride* (injected)	18.00 ± 9.17^{a}	0.210 ± 0.008^{b}	3 ± 1.17^{b}

DISCUSSION

Biological control of plant parasitic nematodes plays an important role in integrated nematode management system and it can be regarded as an alternative to various chemical pesticides. The present study was carried out to determine *Meloidogyne graminicola* resistant rice varieties, the effect of *Trichoderma viride* as a bio control agent of *M. graminicola* and the efficacy of method of application of *Trichoderma viride.* However, control of plant parasitic nematodes using bio control agents alone is not an easy target. On the other hand, chemical nematicides are known to cause a remarkable rapid reduction of nematode populations. But many of these chemicals are considered as environmental and human health hazards (Wachira, *et al*., 2009). In addition to that, nematicides are relatively expensive and many small scale farmers cannot afford to use them. The resistant varieties of rice were developed for several purposes, the main purpose is for a high yield. In addition, it aids in controlling common pests which affect rice yield.

The reproduction factor (RF) for susceptible varieties namely, Bg 366 and Bg 251 rice varieties have a relatively higher and more or less similar values compared to that of moderately susceptible varieties such as, Bg 407, Bg 369, Bg 380, Bg 310, and Bg 745. Moderately resistant variety, Bg 403, possessed the lowest reproduction factor. However, nematodes were able of reproduce and multiply inside the root system at a lower rate in this rice variety.

The mean percentage of yellow leaves significantly increased in the susceptible varieties such as Bg 366 and Bg 251 and moderately susceptible variety, Bg 369. The highest reproduction factor (Pf/Pi) was shown by Bg 369, out of the five moderately susceptible varieties. This may be the reason for the significant increase in yellow leaves in Bg 369. The degree of damage caused by the nematode is proportional to the percentage of yellow leaves (Amarasinghe *et al*., 2007).

Moderately susceptible varieties, Bg 310, Bg 380, Bg 745, and Bg 407, and moderately resistant variety, Bg 403, did not show chlorosis or any deviation to plant morphometric in this study. Pokharel (2009) reported that the balanced dose of Nitrogen (N) and Phosphorous (P) might provide a degree of tolerance to the nematode infested rice plants. Thus, the expected symptoms may not be visible from the outside. This can also be practiced by farmers whose paddies are vulnerable to rice root knot infestation. The gall index of Bg 403 was 36% and hence,, this variety can be recommended for cultivation where the nematode disease outbreaks are reported.

The significant increase of the fresh root weight was observed in nematode infested Bg 407, Bg 366, Bg 251, Bg 380 and Bg 310. The root galls act as nutrient sinkers, and as shown by Endo (1975) this study reveals that the intensity of root galling is directly proportional to the increment of root weight. The number of galls per root system increased with the increment of *M. graminicola* population and the number of feeder roots.

Kumar *et al*., (2012) reported that *T.viride* reduces gall formation and production of egg masses of *Meloidogyne incognita*, which infects okra. In the present study, the application of *T. viride* against *M. graminicola,* leads to a similar outcome. The present study also revealed that *T. viride* activity is not specific either to the crop or *Meloidogyne* spp. Jegathambigai, *et al*., (2011) stated that *Trichoderma* spp parasitize and prevent hatching of the eggs and the growth of the second stage juveniles of *M. incognita* . According to this author, fungal hyphae penetrate the parasite eggs and larval cuticle by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites leading to the reduction of the nematode population, which explains the reduced number of galls.

According to the results obtained, susceptible and moderately susceptible varieties can be recommended for the integration of *T.viride* to reduce nematode infection. Goswami *et al*. (2004) proposed that the plant growth promoting hormonal property of *T. viride* increases plant vigor making it tolerant to *M. incognita* infestation in tomato plants. Similarly in the present study *T. viride* might have increased the tolerance in susceptible and moderately susceptible rice varieties against *M. graminicola* infestation.

CONCLUSIONS

This study concludes that rice varieties, Bg 366 and Bg 251 are susceptible (S); Bg 407, Bg 369, Bg 380, Bg 310, and Bg 745 are moderately susceptible (MS); and Bg 403 is moderately resistant (MR) to *M. graminicola* infestation. The gall formation reduces when the infested plants are treated with *T. viride*. Both drenching and injecting of *T. viride* are effective in reducing nematode infestation.

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