Effect of Four Plant-based Soil Amendments on Root Knot Nematode Species, *Meloidogyne incognita*, Infesting Tomato Plants

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Abstract
The effect of fresh leaves of three plant species, Betel (*Piper betle*), "Keppettiya" (*Croton lacciferus*) and "Maduruthala" (*Ocimum tenuiflorum*)and their combination, as soil amendments was determined against *Meloidogyne incognita* infesting tomatoes, in pot experiments. A mixture of 600g of autoclaved sand loamy soil, 30g of chopped leaves and 500 infective juveniles of *Meloidogyne incognita* were placed in plastic pots. Two weeks later, five week-old tomato seedlings were transplanted in the pots and allowed to grow for seven weeks in a screen house. The efficacy of the amendment treatments was evaluated based on the shoot growth enhancement and the severity of root infestation compared to un-amended controls. The findings indicated that all the amendments suppressed root infestation and simultaneously enhanced the shoot growth, compared to un-amended controls. However, the efficacy significantly differed ($P<0.0001$) among the plant species. The effectiveness of amendments can be ranked as Betel (highest)>"Keppetiya">Combined amendments>"Maduruthala" (lowest). Betel and "Keppetiya" caused complete prevention of root galling while "Maduruthala" (31%) and combined amendment significantly suppressed ($P<0.0001$) the gall formation (62%) compared to controls. Suppression of egg and female production relating to combined treatment and "Maduruthala" was 75% and 71%, and 51% and 46%, respectively. In all the amendment treatments, shoot length was promoted to a lesser extent than the shoot weight. The maximum enhancement in shoot length and weight was detected as 52% and 118%, respectively, with the Betel amendment. The findings showed that Betel and "Keppetiya" are promising plant-based organic amendments in controlling *M. incognita* infestations on tomatoes.

Keywords: Amendments, Gall formation, *Meloidogyne incognita*, Shoot growth
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Introduction

Tomato (*Solanum lycopersicum* L.)Is a widely grown vegetable in Sri Lanka. Root-knot nematodes, *Meloidogyne* species, are obligate endo-parasitic nematodes and are highly destructive pests on vegetable cultivation including tomatoes. These nematodes form galls on root systems affecting water and nutrient absorption. In addition, they exploit photosynthate required for growth and fruit production. Thus, *Meloidogyne* nematodes cause great reductions in crop yield in terms of quality as well as quantity (Hussey and Williamson, 1997). In Sri Lanka, Ekanayaka and Toida, (1997) reported 59% frequency of occurrence of *Meloidogyne* nematodes on tomatoes. *Meloidogyne incognita, M. javanica, M. hapla* and *M. arenaria* have been recorded on tomatoes in Sri Lanka (Ekanayaka and Toida, 1997), among which *M. incognita* is the most prominent species (Lamberti *et al.*, 1987).

Although, application of synthetic chemicals is the commonly used control measure for *Meloidogyne* nematodes, they are hazardous to consumers, growers and the environment and cause resistance development. Therefore, it is essential to find ecologically sound alternative control strategies. One such alternative is to utilize the plant materials as soil amendments which are easily available at low price (Oka, 2010). Previous research indicated that the incorporation of plant-based organic amendments to soil can suppress *Meloidogyne* infestations (Mohammed and Mashkoor, 1989). In the present study, the effect of fresh leaves of three plant species, i.e., Betel *(Piper betle)*, "Kappettiya" (*Croton lacciferus)*, "Maduruthala" (*Ocimum tenuifloru*)and their combination, were tested as soil amendments for controlling *M.incognita* infestationon tomato plants.

Materials and Methods

Melbidogyne incognita cultures were reared on Spinach plants (*Spinacia oleracea*) (cultivar "Yodha")in outdoor pots. The inoculum was initiated with egg masses collected from field grown Spinach plants. Mature egg masses were separated by teasing off the roots under a stereoscopic microscope and set to hatch (i.e., second-stage juveniles) in sterile distilled water (SDW) in Petri dishes. Juveniles at the age of 24-72 hours were used for the experiments. Tomato (cultivar "Thilina") seeds (Agriculture Department, Matara) were sown in seedling trays containing autoclaved sandy loam soil. Five

weeks-old seedlings were used for the experiments.

Thirty grams of fresh mature leaves of Betel, "Kappettiya" and "Maduruthala" were chopped manually using a motor and pestle. Thereafter, they were mixed with 600g of autoclaved sand loamy soil, separately and in combination by adding of 100 ml of water. Subsequently, the mixture was transferred into plastic plots (500 ml) and was inoculated with 500, second-stage juveniles of *M. incognita* in 2 ml of SDW. Untreated controls were only received autoclaved soil with 500 juveniles of *M. incognita*. Thereafter, pots were placed in a screen house for two weeks and watered once in three days. All controls and treatments were arranged in completely randomized design with five replications.

Two weeks after the application of plant amendments, five week-old tomato seedlings were transplanted in the pots and kept for seven weeks in the screen house. Watering was done daily and 15 g of fertilizer (vegetable A group fertilizer, N.P.K+T.E) was applied in three weeks interval. After 7 weeks, the plants were uprooted and washed free of soil. The shoot length and weight, and number of galls, egg masses and females were counted per root system with respect to each treatment.

Data were subjected to square root (X+1) transformation prior to the statistical analysis. Dunnett's test was used to compare different treatments with the untreated control while one way ANOVA was performed to ascertain the effects among the different treatments. Means were compared using Tukey's Honest significant test. All analyses were performed using SAS (SAS Institute, 1999) at the significance level of 0.05.

Results and Discussion

All the three plant amendments and their combination caused significant increase ($P<0.0001$) in shoot length and weight of tomato plants compared to un-amendment controls (Table 1). The highest increase in shoot length (52%) and weight (118.6%) were recorded in the plants amended with Betel leaves followed by "Keppetiya" (% increase: shoot length -37%; shoot weight-91%). "Madurathala" and combination of three amendments showed the similar effect (16% increases) on shoot length. However, the combined treatment caused significantly higher increase ($P<0.0001$) in the shoot weight (44%) compared to "Maduruthala". Significant differences in shoot growth parameters were detected among the four amendment treatments (shoot length $P<0.0001$). The effect of Betel on shoot growth, i.e., length and weight, was significantly greater ($P<0.0001$) in comparison to all the other treatments (Table 1). However, "Maduruthala" and combined treatment had a similar effect on shoot growth.

Gall formation was not detected on tomato plants amended with Betel and "Keppetiya" leaves (Table 1) and thus neither egg masses nor females were found inside the root tissues. The plants received two remaining treatments showed gall formation, egg and female production, however, with a significantly lesser extent (% reduction : gall formation combined treatment 61.5%, "Maduruthala" 31%; egg production combined treatment 75%; "Maduruthala" 50.8%; female production: combined treatment 70.8; "Maduruthala" 46.4%) ($P<0.001$) compared to un-amended plants. The effect of combined treatment on gall, egg and female production was significantly greater ($P<0.0001$) than that of "Maduruthala" (Table 1).

Table 1: Effect of four plant-based soil amendments against *Meloidogyne incognita* infesting tomatoes

Treatment	Shoot length (cm)	Shoot weight (g)	Number of galls/root system	Number of egg masses/root system	Number of females/root system
Betel	81.48 [a]	22.96 [a]	0.00 [a]	0.00 [a]	0.00 [a]
Keppetiya	73.46 [b]	20.06 [b]	0.00 [a]	0.00 [a]	0.00 [a]
Maduruthala	62.10 [c]	12.25 [d]	55.57 [c]	18.20 [c]	24.20 [c]
Betel+Keppetiya+Maduruthala	62.18 [c]	15.15 [c]	30.80 [b]	09.06 [b]	13.20 [b]
Untreated control	53.50 [d]	10.50 [e]	80.00 [d]	37.00 [d]	45.20 [d]

Means followed by same letter in columns indicate no significant difference (P>0.05, Tukey Honest significant test, after square root (X+1) transformation, SAS institute, 1999).

In Sri Lanka, previous investigations Ekanayaka (1990) have shown that incorporation of leaves of various plant species as soil amendments significantly reduced the *M. incognita* multiplication on tomato plants. However, there were no published reports on Betel, "Keppetiya" and "Maduruthala". The findings of this clearly indicated that all the four plant amendments suppressed root infestation caused by *M. incognita* on tomato plants as indicated by reduced/no gall formation, egg and female production on root systems. In addition, these treatments promoted the top growth of tomato plants in particular shoot weight. However, the efficacy varied with the plant species incorporated to the soil. It has been reported that the application of plant based soil amendments cause suppression of nematode infestations by directly impacting nematodes via releasing toxic compounds through decomposition process (Warnke *et al.*, 2008; Oka, 2010). In the present study, plant material was incorporated to the soil containing juveniles of *M. incognita* and kept for two weeks. During this period it was expected that the decomposition of leaf matter occurred and active ingredients contained in the cells were released into the soil affecting survival of juveniles which in turn prevented the entry to the roots. In case, if some of the juveniles survived and entered the root system, systemic action of the chemicals might have reduced the establishment in the root tissues. The findings of this trial showed that tomato plants treated with "Keppettiya" and Betel leaves totally stopped formation of galls indicating that they were more effective for controlling *M incognita* compared to "Maduruthala" and the combined treatment. Moreover, Betel treatment showed the highest shoot growth confirming the higher efficacy compared other treatments. The suppression of root infestation might cause higher shoot growth of plants. The combined treatment showed higher efficacy than "Maduruthala" in controlling root infestation. It has been reported that Betel and "Maduruthala" contain eugenol as one of the main chemical component. The potential nematicidal action could partly be due to the presence of eugenol which has been proved as a potential nematicide (Tsao and Yu, 2000). In "Keppetiya", Diterpenes, Terpenoids are commonly found chemical constituents which have been reported to contain nematicidal properties (Chitwood, 2002). Thus, the suppression of M. incognita infestation could be associated with the activity of these chemicals.

In conclusion, for the first time this study highlighted nematicidal potential of three plant species, Betel, "Keppetiya" and "Maduruthala", as soil amendments. Thus, they offer possibilities as non-chemical control of measures of root-knot nematode infestations. The effectiveness of Betel and "Keppetiya" was higher compared to "Maduruthala" and the combined treatment. The combination of three plants species seemed to be moderately effective. However, field studies are needed for the confirmation of the effectiveness.

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