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EFFECT OF BIOLOGICAL AGENTS AND BOTANICALS IN CONTROLLING ROOT-KNOT NEMATODES, *MELOIDOGYNE* SPP., IN *NICOTIANA TABACUM*

KF Motha[1], R Abeysekara[2] and NS Kottearachchi[1]
[1]Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka. Makandura
[2]Ceylon Tobacco Company (PLC), Mawilmada, Kandy

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ABSTRACT

A study was conducted to investigate the effectiveness of *Pseudomonas fluorescens and Trichoderma viride* and two botanicals; ground neem seed and tobacco waste dust for the control of Root-Knot-Nematodes (RKN) in Tobacco. Suppression of RKN was examined under field condition by estimating root knots and parasitic nematode counts. Seedling density, fresh weight, dry weight, shoot length and root length of tobacco also were measured to evaluate the growth performance. Results indicated that of all bio-preparations, ground neem seed treatment has suppressed the RKN to the best level expressing least number of root-knots and parasitic nematodes. Tobacco waste treatment found to be the second best followed by the treatment with *P. fluorescens.* Although *T. viride* has not controlled the infection, it has significantly improved the seedling density and vegetative growth. Further experiments should be conducted to determine the combined effect of these bio-preparations.

Key words: Biopesticides*, Meloidogyne,* Neem*, Nicotiana tabacum, Pseudomonas fluorescens,* Root Knot Nematodes*, Trichoderma viride,* Tobacco waste.

INTRODUCTION

The root knot nematodes (RKN), *Meloidogyne* spp., are a group of endoparasitic nematodes attacking a wide range of crops including tobacco, tomato, potato, chilli, okra, mung-bean, rice and tea (Akhtar 2000). In Sri Lanka tobacco is one of the most widely grown commercial non-food crop which contributes to the government income through taxes and export earnings. Southern RKN (*M. incognita*), Javanese RKN (*M. javanica*) and peanut RKN (*M. arenaria*) are the most important soil-borne pests in tobacco in Sri Lanka (Ekanayake 2001 and Ekanayake and Torida 1997). They are ubiquitous in tobacco lands, both in nurseries and fields and often more than one species is present in an infected land.

In most cases nematode infestation occurs in the nurseries and plants show symptoms after transplantation. RKN attack the roots of host plant in the nurseries and sets up feeding locations where it deforms the normal root cells and establishes giant cells leading to nodule or gall formation. Early symptoms of nematode damage include plant stunting and poor growth in oval patterns in the field. After transplantation leaves of infected plants turn pale-green, then yellow and at severe stages the condition known as 'rim firing' occurs that includes necrosis of leaf tips and leaf margins (Jimmy and Robert 2005). These symptoms can be misdiagnosed as caused by lack of water and nutrient uptake. Examination of roots for nematode-induced galling (knots) is an easy and accurate method to diagnose the disease. In addition to the direct effects, RKN can make the crop more susceptible to other wilt diseases such as bacterial wilt and *Fusarium* wilt (Tharshani and Sivapalan 2009).

The RKN is usually controlled through the application of carbofuran 3%G or fipronil 0.3%G. At present there is an increasing demand for organically grown tobacco, hence, efforts were made to test the efficacy of locally available bio-pesticides for the control of RKN in tobacco. Bio pesticides are various natural substances including natural enemies and plant compounds that act against the pests and they do not cause toxicities to the crop or to the harvest, thereby considering as a solution for nematode control in tobacco.

Pseudomonas fluorescens, a biological control agent, has been studied widely and formulations containing this bacterium are used as a biopesticide against nematodes in many crops. The bacteria might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide (Vanloon and Bakker 2005). *Trichoderma viride,* another biological agent used widely to control nematodes, is a fungus which colonizes near the plant roots and grows on roots, provide resistance to wilt and rot diseases and cause a physical barrier for nematodes to contact, and also enhance the plant's root growth and nutrient absorption (Wickramaarachchi and Rana-

*Corresponding author: *kottearachchins@yahoo.com*
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weera 2008). Sharon et al. (2001) have suggested that nematicidal activity of *T. viride* may be due to the eggs and larvae being infected through the increase in chitinase and protease activity. As chitin is a major component of eggshell of nematodes, nematophagous egg parasitic fungus can penetrate the eggs leading to the reduction in population. According to Shebani *et al.* (2008), direct parasitism of eggs through increase in extra cellular chitinase activity as indicated by egg infection capability and inducing plant defense mechanism leading to systemic resistance are the two possible mechanism for the suppression of nematodes.

Neem, *Azadirachta indica*, is known to possess potential nematicidal compounds (Abib 1996). Various neem products, oils, cakes, extracts, powder *etc* prepared from leaves and seeds are used as seed treatment and root-dips for the control of nematode pests. According to the previous studies azadirachtin is the major nematotoxic compound in neem and all other nematotoxic compounds are released through volatilization, exudation, leaching and decomposing of the plant parts (Akhta 2000 and Ntalli *et al.* 2009). Tobacco plant contains nicotine, a powerful neurotoxin that is particularly harmful to insects and other toxic compounds such as germacrene, anabasine, piperidine and alkaloids (Panter *et al*. 1990). Recently it was found that tobacco dust, which is produced as a waste in tobacco processing has nematicidal activity when it was tested in tea plantations (Personal communication with K. Mohotti). Therefore the tobacco waste dust could be a beneficial bio-pesticide for RKN in tobacco as well.

This study was conducted to examine the possibility of using several bio-preparations including biological agents and botanicals for the control of RKN in tobacco plantations aiming at obtaining nematode free healthy nursery plants through this eco-friendly pest control methods.

MATERIALS AND METHODS

Experimental location

The field study was carried out in a tobacco field severely infested by RKN at Handapanagala, Wellawaya from December 2008 to May 2009. Bed preparation was started one month before sowing of the seed. Five 1m X 1m raised beds were prepared in 1m X 6m sized four blocks to carryout a Randomized Complete Block Design (RCBD) minimizing the effect of the slope. The samples were analyzed at the Agricultural Research Institutes at Kahagolla, Bandarawela and Madurukatiya, Monaragala.

Treatments

Four bio-preparations comprising of *P. fluorescens* (BioGold, T2), *T. viride* (BioVaccine, T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) were selected as treatments in comparison with a control (T1) which was null treatment. A 10% diluted solution of BioGold and BioVaccine (products of Bio Power Lanka Pvt. Ltd.) was applied at the rate of 2.5 l per bed together with compost. Neem seed and tobacco waste dust were incorporated at the rate of 250g per bed and 1kg per bed respectively up to the depth of 7cm from the top. All treatments were applied 15 days before the seeds were sown. The blocks were covered by a polythene 0.5m height and maintained till the data were collected.

Nursery management

Seeds of tobacco variety K326 were sown 15 days after the applications of bio-preparations and seeded beds were provided with a polythene shade. Routine management practices such as watering, weeding, fungicide application etc. were carried out as recommendations. Acclimatization of plants was done by gradual exposure to the sun after seedlings were emerged.

Data collection

Twenty one days after seed germination seedling density of three random locations from each bed were taken by quadrant method using 15cm x15cm eekel. Disease incidence was measured by the root knot count in the plant from 5th week to 9th week and by parasitic nematode count in the soil at 8th and 9th week. Five randomly selected plants from each bed in all blocks were uprooted and root knots were countered. Composite soil samples were taken from the root zone of five randomly selected plants of each bed and tested for *Meloidogyne* spp, which exhibit stylet, using the basic Baermann funnel technique. As vegetative parameters, plant fresh weight, dry weigh, shoot length and root length also were measured using five randomly selected plants from each bed. The data were analyzed by General Linear Model using SAS version 9.0 (SAS Institute, 2009).

RESULTS AND DISCUSSION

Influence of bio-preparations on seedlings density

Significantly higher seedling densities were observed in plots treated with *T. viride* and neem as compared to the untreated control. However, tobacco dust and *P. fluorescens* treated plots did not show any difference as compared to the control (Table 1). It is reported that J2 juvenile stage of the nematode has the ability to infect seeds and that

Table 1. Comparison of seedling densities in plots treated with different bio-preparations and untreated control: *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) and untreated control (T1)

Treatments	Chi-Square value
T1 vs T2	3.30^{ns}
T1 vs T3	16.74***
T1 vs T4	1.05*
T1 vs T5	0.59^{ns}
T3 vs T4	9.55**

^{ns}, *, **, ***: non significant, significant at 5%, 1% and 0.1% probability levels respectively

may be the reason for obtaining low germination percentage in plots treated with *P. fluorescens*, tobacco waste dust and control. Highest seedling density was observed in plots treated with *T. viride* and it could probably be due to the direct parasitism of eggs and lava through the increase of extracellular chitinase activity as reported by Sharon et al. (2001) and Sahebani et al. (2008).

Incidences of root knots

All bio-preparations found to suppress the gall formation in roots as compared to the untreated control from 5th week up to 9th week (Table 2 & Table 3). Contrast analysis indicated that coarsely ground neem as the best treatment out of all four bio-preparations as it showed significantly low root knots as compared with other treatments.

Plate 1 illustrates root knot infected roots in the untreated control plots and healthy roots in the neem treated plots. The infected roots in the control had more than 20 tiny knots and stunted and branched roots. Once the root was infected with knots more fibrous roots emerged and give hairy appearance.

Table 2. Root knots (as percentages) in tobacco plants in plots treated with *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) and untreated control (T1)

Treatment	*% root knots				
	Week 5	Week6	Week7	Week8	Week9
T1	100	100	100	100	100
T2	18.9	33.5	46.0	47.8	23.5
T3	13.9	30.31	58.6	52.6	64.9
T4	8.3	19.4	33.6	21.75	21.3
T5	5.0	37.0	28.28	29.0	25.9

% root knots were calculated based on the assumption that root knot number in T1 is equal to 100

Nematotoxic compounds especially the azadirachtins released through gradual decomposition of the neem seeds (Akhtar 2000) and suppress nematode populations through out the whole period of the nursery stage. Gradual decomposition of the tobacco dust may have released toxic compounds that exhibit repellent activity. Therefore, both neem and the tobacco treatments demonstrated better nematode suppression towards the end of the nursery period.

Soil nematode population

Significantly less number of parasitic nematodes was observed in the soil samples obtained from plots treated with bio-preparation at 8th and 9th week as compared to the control. Plots treated with coarsely ground neem had the lowest percentage of parasitic nematodes (Table 4). This was followed by tobacco dust. Contrast analysis confirmed coarsely ground neem treated plots had the significant lower nematodes than those received other treatments (Table 5).

Both neem and the tobacco waste treated soils showed better suppression of the parasitic nema-

Table 3. Comparison of root knots in plots treated with *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) and untreated control (T1)

Treatment comparison	Chi-square values				
	Week5	Week6	Week7	Week8	Week9
T1 vs T2	8.02**	9.05**	4.78*	16.95***	4.78*
T1 vs T3	8.24**	12.95***	20.19***	37.37***	20.19***
T1 vs T4	8.83**	10.04**	1.561*	17.78***	1.051*
T1 vs T5	8.24**	8.10**	9.90**	26.84***	9.90**
T2 vs T3	0.14^{ns}	0.05^{ns}	3.5^{ns}	0.01^{ns}	3.5^{ns}
T2 vs T4	1.54^{ns}	0.98^{ns}	11.01***	8.99**	11.01***
T2 vs T5	1.54^{ns}	0.05^{ns}	1.18^{ns}	1.6^{ns}	1.18^{ns}
T3 vs T4	0.91^{ns}	0.59^{ns}	18.81***	8.4**	18.81***
T3 vs T5	0.91^{ns}	0.2^{ns}	8.16**	1.32^{ns}	8.16**
T4 vs T5	0^{ns}	1.43^{ns}	6.78**	3.49^{ns}	6.78**

^{ns}, *, **, *** : not significant, significant at 5%, 1% and 0.1% probability levels respectively

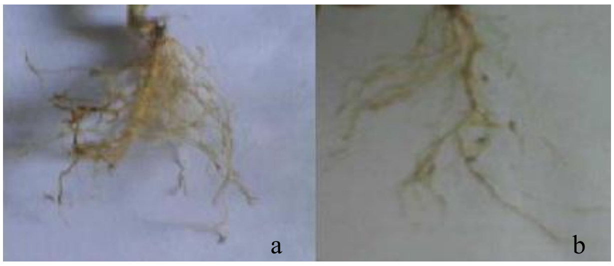

Plate 1 Root morphology of the root knot nematode controlled (a) and neem treated (b) tobacco plants (a. stunted hairy roots with large number of knots; b. long root with a few root-knots

todes towards the 9th week and it may be due to the fact that gradual decomposition of the neem and tobacco waste have resulted in releasing compounds with toxic or repellent activities through out the nursery period.

Fresh and dry weight of tobacco plants

The treatments did not show any negative effects on plant growth (Fig 1). At the time of seedling uprooting for transplanting (on the 9th week), plants in the treated plots had higher fresh and dry weight. Plant in plots treated with *T. viride* had significantly higher fresh weight than other plant samples. A good dry mater accumulation requires to withstand the transplant stress.

Shoot and root length

Root of the plants grown in treated plots were significantly longer those in untreated control plots. It may probably be due to the fact that once the roots are infected by nematodes root elongation stops and become stunted. However, we did not observe any relationship between shoot length and root length among the plants grown in treated and control plots. This phenomenon indicates that aerial plant growth is not influenced by the nematode infestation during the nursery period. However, shoot growth is drastically influenced only after transplanting.

Table 4. Nematodes in soil samples treated with *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) tobacco waste dust (T5) and untreated control (T1) at 8 and 9 weeks after seed sowing

Treatment	*% of parasitic nematodes	
	Week8	Week9
T1	100	100
T2	38.9	40.7
T3	37.8	96.6
T4	24.4	14.5
T5	29.7	32.1

* % was calculated based on the assumption that the nematode population in T1 is equal to 100

Table 5. Comparison of *Meloidogyne* populations in soils treated with *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) and untreated control (T1) at 8 and 9 weeks after seed sowing

Treatment comparison	Week8	Week9
	Chi-square	Chi-square
T1 vs. T2	22.04***	29.08***
T1 vs. T3	22.04***	56.56***
T1 vs. T4	62.51***	3.34***
T1 vs. T5	44.14***	35.58***
T2 vs. T3	0^{ns}	4.1*
T2 vs. T4	18.89***	12.21***
T2 vs. T5	5.63*	1.56^{ns}
T3 vs. T4	18.89**	18.74***
T3 vs. T5	5.63*	8.16**
T4 vs. T5	4.83*	6.78**

ns, *, **, *** : non significant, significant at 5%, 1% and 0.1% probability levels respectively

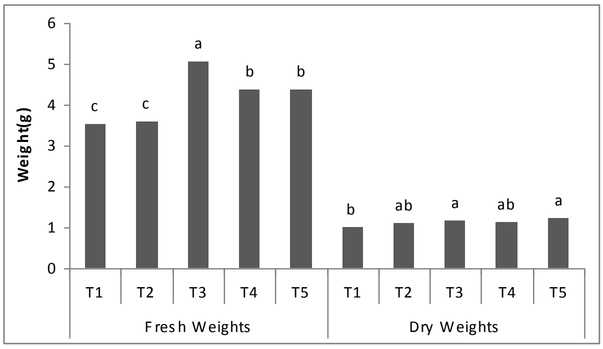

Figure 1. Fresh and dry weight of 9-week-old tobacco plants grown in plots treated with *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) and untreated control (T1)

CONCLUSION

This study was planned to obtain nematodes free healthy nursery plants through an eco-friendly method. Coarsely ground neem seed can be considered as the best treatment for the control of RKN, *Meloidogyne* spp., in tobacco followed by the tobacco waste and *P. fluorescens* as far as the diseases root knot count and parasitic nematode count are considered. From the assessment of seedling density and vegetative parameters *T. viride* treated plants exhibited higher seedling density, fresh weight, dry weight and shoot length. However, *T. viride* treatment has not performed well in controlling the infection which might ad-

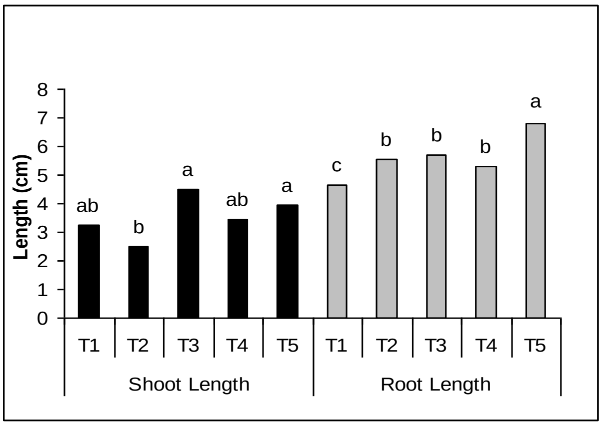

Figure 2. Shoot and root length of 9 week old tobacco plants grown in plots treated with *P. fluorescens* (T2), *T. viride* (T3), coarsely ground neem seeds (T4) and tobacco waste dust (T5) and untreated control

versely affect later for the plant growth after transplanting. Therefore the assessment should be continued also after the transplantation of the treated nursery plants to give an exact conclusion on each bio-preparation. No deleterious effect was observed from the neem seeds and tobacco waste on seeding growth at nursery stage.

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