Tropical Agricultural Research Vol. 23 (1): 61 – 69 (2011)

Effect of *Trichoderma viride* and Carbofuran (Curator®) on Management of Root Knot Nematodes and Growth Parameters of *Gotukola* (*Centella asiatica* L.)

B.V.T. Shamalie, R.M. Fonseka[1*] and R.G.A.S. Rajapaksha[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Sri Lanka

ABSTRACT. *Gotukola (Centella asiatica L) is a widely grown popular leafy vegetable in Sri Lanka. Root Knot Nematodes (RKN) (Meloidogyne spp.) infestation is one of the key problems which causes heavy losses in commercial Gotukola cultivations in warm climates. A field experiment was carried out in Chilaw (AEZ IL1a) during the wet season (Maha) 2010/2011 to evaluate the effectiveness of the bio-control agent, Trichoderma viride and chemical; Carbofuran (Curator®) on RKN and growth of Gotukola. The treatments imposed were T. viride (CFU 10^{11}/ml) incorporated manures (compost and poultry manure at the rate of 2 kg/m^2) and recommended dose of inorganic fertilizers, Curator® (1.5g/m^2) incorporated manures and recommended dose of inorganic fertilizer. Trichoderma, Curator® (1.5 g/m^2) untreated manures, inorganic fertilizers and no amendment (control). The results revealed that Curator® incorporated manures had no significant ($p>0.05$) difference on the promotion of growth parameters. The combined application of T. viride + compost had significant ($p<0.05$) impact on plant growth which attributed to increased number of roots, leaf length, stalk length and root length. Highest fresh weight of leaves of first harvest was recorded in the treatment of Trichoderma + compost treated plots. Treatments of Trichoderma + compost had significant ($p<0.05$) reduction in RKN gall formation in Gotukola. The results indicate that, the T. viride incorporated compost can be recommended as an alternative to Curator® to control RKN and to promote growth of Gotukola.*
Keywords: *Gotukola, Root Knot Nematodes, Trichoderma viride*

INTRODUCTION

Centella asiatica L. (also known as *Gotukola* and *Hydrocotyle asiatica*) is a perennial, herbaceous creeper with kidney shaped leaves, found in India, Sri Lanka, Madagascar, South Africa, Australia, China, and Japan (Kumar, 2010). Centella prefers to grow in shady, moist, or marshy areas. The plant contains some components, including volatile oils, flavonoids, tannins, phytosterols, amino acids, and sugars (Leung and Foster, 1995). It has been reported that *C. asiatica* is used as medicinal treatments for wide range of disease conditions and for memory enhancement and other neurodegenerative disorders (Mohandas, 2006).

There is a high demand for *Gotukola* as a fresh leafy vegetable in the local market. In addition, the crop is important as commercial cultivations generate high income with low

[1]Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka
[2]Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka
[*] Author for correspondence: ramyamf@gmail.com

inputs. At present, large-scale commercial cultivations are found in Colombo, Gampaha, Kalutara, Galle, and Puttalam Districts (Wahundeniya & Kurukulaarachchi, 1999). However, in the recent past, *Gotukola* production in most of the areas has suffered heavily due to the emergence of Root Knot Nematode (RKN) and has become a serious problem in profitable cultivations. Major reason for high incidence of RKN is continuous cultivation around 4-10 years in the same fields (Wahundeniya and Kurukulaarachchi, 1999). Root knot nematodes infect a wide range of crops particularly vegetables and cause losses up to 60 – 80 % in heavily infested fields (Siddiqi, 2000). They are sedentary endoparasites and infection starts with root penetration of second-stage juveniles (J2), hatched in soil from eggs stored in egg masses that have been laid by the females on the infected roots (Barker *et al.,* 1985).

The disease is manifested by the formation of galls in the root accompanied by stunted growth, chlorosis and loss of vigor of the plant (Babu *et al*., 1999). Use of conventional nematicides for several years may cause severe health hazards and environmental problems. Therefore, identification of an effective, environmental friendly cost effective nematode control method for managing RKN is considered necessary. There are lots of alternative strategies that have been reported to protect crops such as application of soil organic amendments of crop residues, animal manures, heat treatment, soil solarization and crop rotation with non hosts *etc*., for managing root-knot nematodes (Abubakar & Majeed, 2000; Singh & Khurma, 2007).

Biological control is an environmental friendly and economically viable alternative as it offers durable and cost effective alternatives to soil applied nematicides. Genus *Trichoderma* represents widely studied fungi that show antigonistic activity towards nematodes and soil-borne fungal pathogens. Parasitic activities of members of *Trichoderma* to nematodes had been recorded (Mujeebur *et al.,* 2004). Among the *Trichoderma* species, *T. viride* had been more effective in controlling RKN (Sharon *et al*., 2001).

The biological agent used to control nematodes, is a fungus which colonizes near plant roots and grows on roots, provide a physical barrier for nematodes to contact, and also enhance the plant's root growth and nutrient absorption (Wickramaarachchi and Ranaweera, 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 egg shell 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 mechanisms for the suppression of nematodes. Therefore, this study was undertaken to identify the efficacy of the bio-control agent, *T. viride* incorporated with organic manure for control of RKN and its affect on growth parameters in *Gotukola* cultivation.

MATERIAL AND METHODS

Collection of *Trichoderma* Isolates

The *T. viride* was obtained from the culture collection of the Plant Pathology Division at Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya. It was inoculated on to Potato Dextrose Agar (PDA) and incubated at room temperature for 7-10

days. The fungus growing on the medium was isolated and identified using morphological characters of the mycelia and spores.

Mass culturing of *Trichoderma*

Paddy soaked in water for 6 h was parboiled in a pressure cooker (1.1 kg/cm^2 pressure for 45 min). After parboiling the closed container was cooled to 15 ^{o}C for 2 h and 2 kg of parboiled paddy was filled into polyethylene bags. Mouth of the bag was passed through a polyvinyl pipe of 2 cm diameter and 0.6 cm width and the mouth was thereafter plugged with a piece of sterilized, non absorbent cotton wool. A piece of paper was wrapped over the cotton wool plug and the paper was kept intact using a rubber band. This media was inoculated with 7 - 10 days old *Trichoderma* isolate on PDA and used to prepare liquid suspension for field application.

This 10 days old *Trichoderma* mass culture was flooded with 2 l of tap water and was shaken well in a closed container. The resulting suspension was filtered through muslin cloth. The spore suspensions were then adjusted to the desired concentration after counting spore density using a Thoma haemocytometer. The filtrate was diluted with tap water to obtain conidia concentration of 10^{11} CFU/ml for field application. One liter of this conidia suspension was mixed with 1 ml surfactant (wetting agent-commercial product from Lankem Ltd.) before application. This suspension was mixed evenly with compost and well decomposed poultry manure separately and covered for 4 days to prepare *Trichoderma* incorporated manures.

Field trial establishment

Field experiment was laid out in Randomized Complete Block Design (RCBD) with three replicates in a field at Chilaw during the growing season of 2010/11 *Maha* in a soil naturally infested with RKN. The agro ecological zone was IL1a and annual average rainfall was 1100 – 1400mm. The treatments imposed were T1 – *Trichoderma* ($1x10^{11}$ CFU /ml) + compost @ 2 kg/m^2, T2 – *Trichoderma* ($1x10^{11}$ CFU/ml) + poultry manure @ 2 kg/m^2, T3 – *Trichoderma* ($1x10^{11}$ CFU/ml) + inorganic fertilizer, T4 - soil application of *Trichoderma* ($1x10^{11}$ CFU /ml), T5 - Curator® @ 1.5 g/m^2 + compost @ 2 kg/m^2, T6 – Curator® @ 1.5 g/ m^2 + poultry manure @ 2 kg/m^2 , T7 – Curator® @ 1.5 g/m^2 + inorganic fertilizer (DOA recommendation) T8 - Curator® @1.5 g/m^2 , T9 – compost @ 2 kg/m^2, T10 - poultry manure @ 2 kg/m^2, T11 - inorganic fertilizer (DOA recommendation), T12 - untreated control.

The experiments were terminated three months after initiation and observations were made on plant growth parameters *i.e.* stalk length, root length, leaf length, number of leaves per bush, number of roots per bush. Disease severity was measured by the ratio of the number of knotted roots per total number of roots.

$$\text{Root gall percentage} = \frac{\text{No. of affected roots X 100}}{\text{Total no. of roots}}$$

Laboratory analysis was done at Regional Agricultural Research and Development Centre, Makandura. The data were analyzed using the Statistical Analysis System Version 9 (SAS) for the Analysis of Variance (ANOVA) and General Linear Model (GLM).

RESULTS AND DISCUSSION

Treatment effect on growth parameters

Trichoderma incorporated compost significantly increased stalk length, root length and number of roots on *Gotukola* (Table 1). The resulted increase in growth parameters could be due to certain plant growth hormones and secondary metabolites produced by *Trichoderma* spp. Also, Altmare *et al*. (1999) have reported that in-cooperation of *Trichoderma* increase phosphate solubility and the availability of micro nutrients in the soil and it could promote growth of the plants.

Table 1. Effects of *Trichoderma* and curator® incorporated treatments on stalk length, root length and number of roots

Treatments	Stalk length (cm)	Root length (cm)	Number of roots
T1 - *Trichoderma* + compost	14.72 [a]	4.82 [b]	13.89 [a]
T2 - *Trichoderma* + poultry manure	14.12 [b]	6.55 [a]	13.10 [a]
T3 - *Trichoderma* + inorganic fertilizers	14.09 [b]	6.33 [a]	12.48 [a]
T4 - *Trichoderma* + soil	14.07 [b]	2.97 [d]	5.57 [b]
T5 - Curator + poultry manure	10.01 [c]	2.81 [d]	6.41 [b]
T6 - Curator + inorganic fertilizer	10.07 [c]	2.87 [d]	4.47 [b]
T7 - Curator + compost	13.07 [c]	2.86 [d]	5.04 [b]
T8 - Curator + soil	13.08 [c]	2.86 [d]	5.05 [b]
T9 - Compost	8.20 [d]	2.13 [d]	4.34 [b]
T10 - Poultry manure	6.98 [d]	3.58 [c]	4.18 [b]
T11 - Inorganic fertilizers	9.57 [c]	2.47 [d]	4.57 [b]
T12 – Non-treated	8.19 [d]	1.95 [e]	5.17 [b]
Pr > F	.0004	.0001	.0001

*Means followed by the same letter in a column are not significant at p = 0.05

Fig. 1. Morphology of *gotukola* roots under different treatments. T1 – *Trichoderma* + compost treatment-well developed roots with few numbers of knots. T 11 Inorganic fertilizer - deformed roots with a large number of root-knots

Gall formation on roots was significantly different ($p<0.0001$) in the treatment *Trichoderma* incorporated compost when compared to other treatments. The lowest percentage of gall formation was observed in *Trichoderma* incorporated compost (T1) (Table 2).

It was reported that, the number of nematodes in soil and the severity of root galling can be reduced by incorporation of compost into the soil (Akhtar and Malik 2000; Cayuela *et al*., 2008). *Trichoderma* as a bio-control agent has been extensively tested for controlling plant parasitic nematodes (Sikora, 2008) and Windhum *et al*. (1989) showed that *Trichoderma spp*. significantly suppressed the root-knot disease in maize.

Even though, the application of curator (carbofuran) is found as one of the most effective methods in controlling nematodes associated diseases in plants (Adegbite & Agbaje, 2007), it could not be conceded as a suitable method as far as environmental, health and economical issues are concerned. Therefore, biological control can be applied as a safer method for controlling nematode in crop plants.

Table 2. Root gall (%) in *Gotukola* with different treatments

Treatments	Root gall (%)
T1 - *Trichoderma* + compost	7.19 [a]
T2 - *Trichoderma* + poultry manure	11.08 [b]
T3 - *Trichoderma* + inorganic fertilizers	16.70 [c]
T4 - *Trichoderma* + soil	13.32 [b]
T5 - Curator + poultry manure	33.43 [g]
T6 - Curator + inorganic fertilizer	20.73 [d]

Table continued on next page

T7 - Curator + compost	21.43 [e]
T8 - Curator + soil	21.41 [e]
T9 - Compost	27.62 [f]
T10 - Poultry manure	15.21 [c]
T11 - Inorganic fertilizers	21.44 [e]
T12 – Non-treated	70.07 [h]
Pr > F	.0001

*Means followed by same letters in a column are not significant at p = 0.0001

Effect on top fresh weight

Trichoderma incorporated compost showed significant increase of top fresh weight. (Fig. 2) *Trichoderma viride* in combination with organic amendments had been known to produce growth hormones, which eventually boost the plant vigor (Chang *et al*., 1986). It had been also found that *Trichoderma spp* stimulated growth of floricultural and horticultural plants (Barker *et al*., 1985).

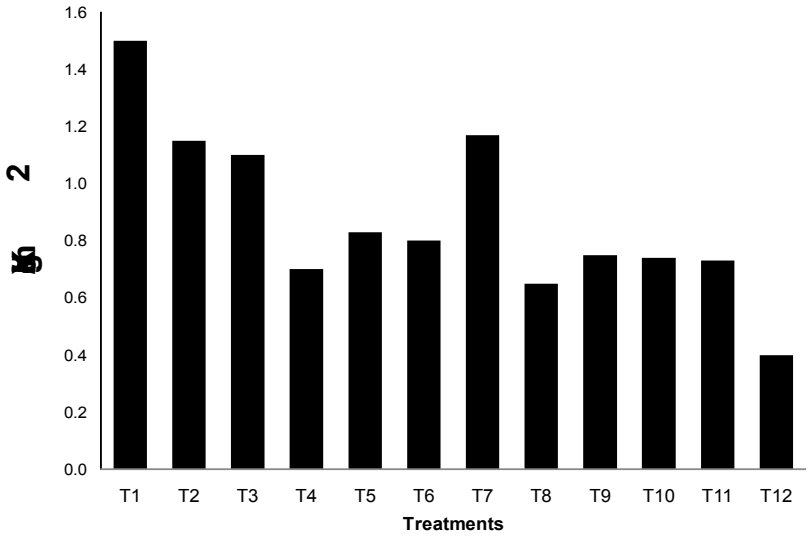

Fig. 2. Top fresh weight of *Gotukola* under different treatments

Formation of root galls

Gall formation of RKN on roots was observed at 30 days after crop establishment. Plants from un-treated control plot showed the greatest number of root galls from the initial crop growth stage. Plants from Inorganic fertilizer + Curator treated plots showed a few number of root galls at the initial crop growth stage and it was able to control gall formation up to 50 days after planting (Fig. 3)

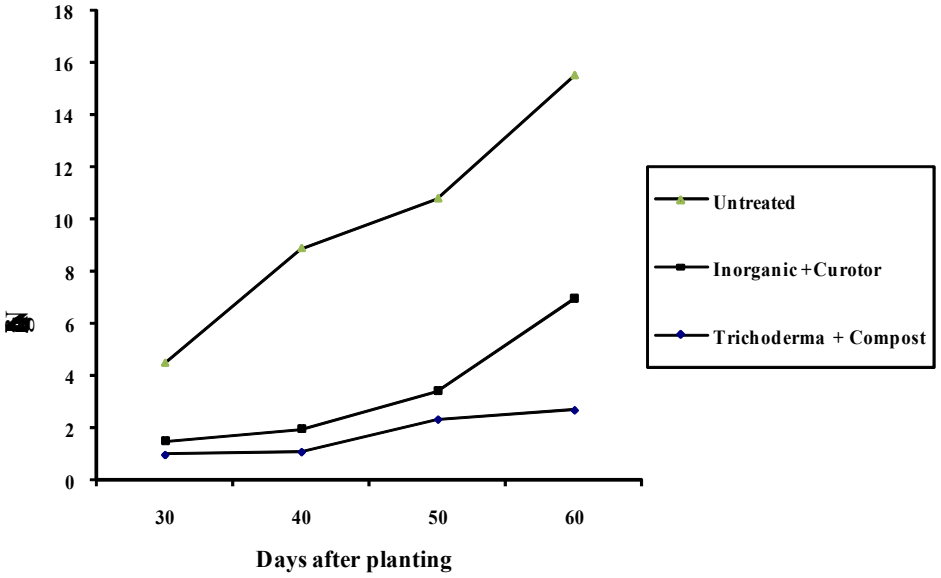

Fig. 3. Mean number of root galls under different treatments

The nematicidal properties of Curator® impaired nematode neuromuscular activity by inhibiting the function of the enzyme acetyl cholinesterase resulting in reduced movement and ability of invasion and multiplication (Evans, 1973). After 50 days it was unable to control RKN successfully, as the chemical effect was limited to a certain period of time. However, *Trichoderma* incorporated compost treated plot showed suppression of RKN during the cropping period. Liu *et al.* (2007), showed that, *T. viride* self-propagate and survive remarkably in the soil with compost and plant rhizosphere therefore, may remain in the soil for a long period and it had very high nematicidal action to the nematodes such as southern root-knot nematodes.

CONCLUSIONS

Trichoderma incorporated organic manures promote the growth of G*otukola* and had the ability to suppress Root knot nematodes effectively. Therefore, *T. viride* incorporated compost can be recommended as an alternative to Curator® to control Root knot nematodes in commercial scale *Gotukola* cultivations.

ACKNOWLEDGEMENT

Authors acknowledge the assistance extended by Dr. M. Faahim, Research Officer and the laboratory staff of the Regional Agriculture Research and Development Centre, Makandura. Assistance given by Dr. Rupika Abenayake, Senior Lecturer, Faculty of Agriculture, Wayamba University and Mr. C. Hettiarachchi, Director, Maxies Pvt. LTD. are also appreciated.

REFERENCES

Abubakar, U. and Majeed, Q. (2000).Use of animal manure for the control of root knot nematodes of cowpea. J. Agric. Environ. *12*, 29 -33.

Adegbite A.A.and Agbaje, G.O. (2007). Efficacy of Furadan (Carbofuran) in control of root-knot nematode in hybrid yam varieties in south –west Nigeria. World J. Agric. Sci. *3(2)*, 256-258.

Akhtar, M.and Malik A. (2000). Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresource Technology. *74*, 35-41.

Altmare, C., Norvell, W.A., Bjorkman, T and Harman, G.E. (1999). Solubilization of Phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Appl. Environ. Microbe. 65:2926–2928. Babu,A.M.,Kumar,V and Philip, P. (1999). Root Knot Nematodes. Indian Silk *38*, 11-12.

Baker, K.R., Carter, C.C. and Sasser, J.N. (1985). Screening for Root Knot Nematode (*Meloidogyne hapla*) Using Lettuce [on line]. [Accessed on 11.03.2011].Available at http://www.uwlax.edu/urc/JUR-online/PDF/2003/appleman.pdf

Cayuela, M.L., Bernal, M.P., Roig .A. (2004) Composting olive mill waste and sheep manure for orchard use. Compost Sci Util; *12*, 130.

Chang, Y.C., Baker, R., Kleifeld, O. and Chet, I. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harcianum*. Plant Dis. *70*, 145–148.

Evans, A.A.F. (1973) .Mode of action of nematodes. Annals of Applied Biology.; *75*, 469

Kumar Pati, (2010). CEO Best Nutrition Products, Hayward, CA 94544, USA. [online]. [Accessed on 06.10.2010]. Available at http://www.prlog.org/10732717-gotu-kola-ayurvedic-plant-botanical-centella-asiatica-drabhay-kumar-pati-ceo-best-nutrition.htm

Leung, A.Y and Foster, S. (1995). *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. 2nd ed. pp. 177-178.

Liu, T., Wang. L., Duan. Y.X., Chen, L.J., Wang. X.(2007). Effects of *Trichoderma viride* on egg hatching and juvenile mortality of *Meloidogyne halpa*. *Acta Agriculturae Universitatis Jiangxiensis* (Natural Sciences Edition), *4*, 566-569.

Mohandas, R. (2006). *Centella asiatica*: Wikipedia, the free encyclopedia [on line]. [Accessed on 07.04.2011]. Available at http://en.wikipedia.org/wiki/Centella_asiatica.

Mujeebur, K., Khan,.R., Shahana, M. and F.A. Mohiddin, (2004). Biological control of Fusarium wilts of chickpea through seed treatment with the commercial formulation of Trichoderma harzianum and/or Pseudomonas fluorescens. Phytopathol. Mediterr., *43*, 20-23

Sharon, E., Bar-Eyal,M., Chet, I., Herrera,A., Kleifeld,O. and Spiegel,Y.(2001). Biological control of root knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology, *91*, 687-690.

Shebani, N. and Hadavi, N. (2008). Biological control of the root-knot nematode *Meloidogyne javanic*a by *Trichoderma harzianum*. Journal of Soil Biology and Bio Chemistry *40*, 2016-2020.

Siddiqi, M.R (2000) Parasites of Plants and Insects (2nd edition). CABI Publishing, CAB International, Wallingford.pp.43-44.

Sikora, R.A (2008). Mutualistic endophytic fungi and *in planta* suppressiveness to plant parasitic nematodes. Biol. Control *46(1)*, 15-23.

Singh, S.K. and R.K. Khurma.(2007). Susceptibility of six tomato cultivars to the root knot nematode, *Meloidogyne incognita.* The South Pacific J. Natural Sci., *13*, 73-74

Wahundeniya, K.B. and Kurukulaarachci, N. (1999). Cultivation of Leafy Vegetable. Dept of Agriculture, Peradeniya. pp. 5-15.

Wickramaarachchi, W.A.D.A and Ranaweera. B (2008) Effect of *Trichoderma viride* in Combination with Soil Sterilization and Poultry Manure on the Growth of *Capsicum annum* Seedlings. Proceedings of 8th Agricultural Symposium, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka. pp. 255 -257.

Windhum, G.L. Windhum, M.T. and Williams, W.P. (1989). Effect of Trichoderma species on maize growth and Meloidogyne arenaria reproduction. plant Disease., *73*, 493-495.