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AGRO-INDUSTRIAL BY-PRODUCTS FOR MASS CULTURING OF *Trichoderma koningii*, THEIR EFFECT ON *Meloidogyne incognita* AND THE GROWTH OF TOMATO *Lycopersicon esculentum*

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ABSTRACT

Investigations were carried out under laboratory and plant house conditions to select cost effective and suitable materials. The study included testing sixteen different culture media for mass culturing of the fungus, *Trichoderma koningii* using boiled paddy, glucose and five kinds of agro-industrial by-products (molasses, tea waste, paddy husk, coconut poonac and poultry manure). The effect of the fungus grown on these culture media on *Meloidogyne incognita* and growth of tomato cv T-245 was also determined. Laboratory investigations revealed that the growth rate of the fungus was significantly higher ($p \geq 0.05$) in five culture media; molasses + tea waste, molasses + boiled paddy, molasses + soil, glucose + tea waste and distilled water + tea waste than other media used. Spore production was significantly ($p \geq 0.05$) higher in boiled paddy with glucose than other treatments. However, considering the cost and the availability, tea waste and molasses mixed with soil and glucose were selected as the best media for mass culturing of the fungus. Investigations under plant house conditions with different levels of selected culturing media revealed that 20g of molasses with 7.5g of tea waste significantly ($p \geq 0.05$) increased the fresh and dry shoot weight 50 days after inoculation (DAI) of the nematode. Fungal Spore production was significantly ($p \geq 0.05$) higher in all three culture media containing boiled paddy. The multiplication of the nematode 50 DAI in root systems of plants grown in six different culture media containing molasses or tea waste was significantly ($p \geq 0.05$) lower than the inoculated control treatment. It was found that tea waste and molasses can be used with soil/ glucose to prepare effective culture media for mass scale production of the fungus *T. koningii* and these media can be used to incorporate the fungus into *M. incognita* infested soil in integrated management programme of the nematode.

KEYWORDS: Ago-industrial by-products, biological control, nematodes.

INTRODUCTION

The root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitw. is widespread and attack many crop plants in Sri Lanka causing substantial economic losses (Lamberti *et al.*, 1987). Unlike other pesticides, higher dosages of nematicides are needed for effective management of nematodes on many crops. As nematicides are expensive besides their harmful effects on human and the

environment, efforts are being made to deploy biological control agents in integrated nematode management programmes.

Soils are rich in micro-organisms, which contain many fungi and bacteria antagonistic to soil nematodes. Among these micro-organisms, the fungi, *Trichoderma* spp. have been identified as potential biological control agents of *Meloidogyne* spp. (Rao *et al.*, 1996). The *Trichoderma* spp. which are present in substantial numbers in all agricultural soils can be an effective bio control, agent for the management of *Meloidogyne* spp. Several *Trichoderma* spp. have been identified from soil samples collected from the vegetable growing areas of the Central Region of Sri Lanka (HORDI Annual Report, 1999). Among these, a local isolate of *T. koningii* has been identified as an effective bio control agent in controlling *Meloidogyne* spp. under laboratory and plant house conditions (Dr. R. Ekanayake, Personal communications).

Although several fungi and bacteria are known to reduce nematode populations under laboratory and plant house conditions, results of field trials have been inconclusive, because of the difficulties encountered in incorporation of these bio control agents into the soil. Selection of suitable and cost effective media for large-scale production of these bio control agents is essential requirement to overcome this problem. Hence, Laboratory and plant house investigations were carried out to select suitable media for mass culturing of the fungus. The effect of *T. koningii* grown on selected cultures from experiment I on *Meloidogyne incognita* and the growth of tomato variety T-245 was also determined.

MATERIALS AND METHODS

The experiments were conducted at the Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya. Fungus *T. koningii* was cultured on Potato Dextrose Agar (PDA) medium and a pure culture of the nematode *M. incognita* was maintained on tomato variety KWR in the plant house. The eggs and juveniles of the nematode were extracted using Sodium hypochlorite (Hussey and Barker, 1973).

Experiment I – Laboratory investigation

Five kinds of agro-industrial waste (molasses, poultry manure, tea waste, Coconut poonac and paddy husk) boiled paddy, soil and glucose were used to

prepare 15 different culture media as indicated in table 1. PDA was used as the control. A thin layer of each sterilized media was spread in petridishes under sterilized conditions with eight replicates per each treatment. One cm diameter disk of the fungus cultured on PDA was introduced into each petridish, sealed and kept for the fungal growth under sterile condition. Growth of the fungus on area basis was recorded 96 hrs after inoculation using mm mesh and mean growth area (rate) per hour was calculated. In addition, the fungus suspensions were prepared and the number of spores per each suspension was recorded using counting chamber method under the microscope. The results were subjected to an analysis of variance and treatment means were compared using least significant difference test at ($p \geq 0.05$) level.

Experiment II – Plant house investigation in mini plots

Ten and twenty grams each of Molasses, tea waste, soil and glucose were used to prepare three different culture media for the fungus were used as treatments (table 2). Forty-eight sterilized clay pots were filled with steam sterilized potting mixture (sand: clay: compost: silt, 80: 15: 5: 5). Experiment had eight treatments with six replicates. The two controls included one potting mixture without fungus and inoculated with the nematode (T7) and un-inoculated. Thirty-six clay pots were filled with the above soil mixture. One set of 18 pots were inoculated with 10 g and other set was inoculated with 20 g of each of the fungus culture by making a hole in the centre of the potting mixture. All pots were transplanted with a single 20-day-old tomato seedling of the variety cv T - 245. Four days after transplanting 42 pots were inoculated with 2000 eggs and juveniles of the nematode obtained from the pure culture maintained in the plant house. The pots were arranged on a bench in the plant house according to a completely randomised design where temperature varied between 25-27 $^{\circ}$ C in the day and 18-21° C in the night. The fresh and dry weights of shoot and roots of plants were recorded at 50 Days after inoculation (DAI) of the nematode. Each root system was rated according to a 0 – 5 scale (Taylor and Sasser, 1978), where 0 represents no galling and 5, heavy galling. These results were subjected to an analysis of variance and treatments means were compared using least significant difference test at ($p \geq 0.05$) level.

RESULTS AND DISCUSSION

Experiment I- Laboratory investigation

Table 1 indicates the effect of sixteen different culture media on growth rate and spore production of *T. koningii*.

Highest growth rate of 0.99 cm^2 /hr was observed on PDA (T16). Growth rate of the fungus cultured on tea waste + molasses (T2) was significantly higher ($p \geq 0.05$) than that of on other treatments. Fungus culture media T3, T4, T7 and T12 showed significantly higher growth rates ($p \geq 0.05$) as compared to media T1, T5, T6, T8, T9, T10, T11, T13, T14 and T15. The fungus did not grow in the treatment T15. This may be due to insufficient nutrient content of the poultry manure.

Table 1. Effect of sixteen different culture media on the growth and spore production of *Trichoderma koningii*.

Treatment	*Growth Rate* cm^2*/hour*	*No. spores* $x\ 10^3$
T1-5.5-ml molasses + 15g Poonac + Paddy husk	0.25 gh	1.03 c
T2-5.5-ml molasses + 7.5g Tea waste	0.83 b	1.24 b
T3-5.5-ml molasses + 15g Boiled paddy	0.55 c	1.55 b
T4-5.5-ml molasses + 5g soil	0.54 c	0.94 cd
T5-5.5-ml molasses + 15 g Poultry manure	0.12 j	0.01 g
T6-5.5-ml Glucose + 15 g Poonac + Paddy husk	0.30 fgh	1.84 b
T7-5.5-ml Glucose + 7.5g Tea waste	0.54 c	0.52 def
T8-5.5-ml Glucose + 5g Boiled Paddy	0.43 de	2.46 a
T9-5.5-ml Glucose + 15g soil	0.22 hi	0.28 fg
T10-5.5-ml Glucose + 15 g Poultry manure	0.02 j	0.01 g
T11-5.5-ml Distilled water + 15g Poonac + Paddy husk	0.34 efg	0.33 efg
T12-5.5-ml Distilled water + 7.5g Tea waste	0.55 c	0.12 fg
T13-5.5-ml Distilled water + 15g Boiled Paddy	0.41 ef	1.73 b
T14-5.5-ml Distilled water + 15g soil	0.36 efg	0.09 fg
T15-5.5-ml Distilled water + 15 g Poultry manure	0.0 j	0.01 g
T16-PDA	0.99 a	2.39 a

Values followed by the same letter in each column are not significantly different at $p \geq 0.05$

The highest number of spores of 2.46 $x\ 10^3$ was observed in T8 (glucose + boiled paddy) which was not significantly different to that of PDA (T16). No significant difference was observed in spore production in treatments; Molasses + Tea waste (T2), Molasses + Boiled paddy (T3), Glucose + Coconut Poonac + Paddy husk (T6) and distilled water + Boiled paddy (T13). They were significantly higher than other treatments. All the culture media containing boiled paddy have shown considerable increase in spore production. Considering the effectiveness, cost and the availability, tea waste and molasses with soil or glucose were selected to prepare cultures for the mass production of the fungus *T. koningii* for the second experiment.

Experiment II – Plant house investigation in mini plots

There was no significant difference in fresh and dry weights of roots among the treatments (table 2). Formation of root galls in infested plants and growth of root systems in control plants could have nullified the effects.

The highest shoot fresh weight of 64.45g was observed in un-inoculated control treatment (T8). This was not significantly different ($p \geq 0.05$) from plants treated with the fungus cultured on 20g of 5.5 ml Molasses + 7.5 g Tea waste (T5). Treatments 10g of 5.5ml glucose + 7.5g Tea waste (T1), 10g of 5.5 ml Molasses + 7.5 Tea waste (T2), 10g of 5.5 ml Molasses + 15 g soil (T3) and 20g of 5.5ml glucose + 7.5g Tea waste (T4), 20g of 5.5 ml Molasses + 15 g soil (T6) showed similar results. The lowest fresh shoot weight was observed in inoculated control plants (T7).

Table 2. Effect of *Trichoderma koningii* cultured on different media on growth of roots and shoots of tomato inoculated with *Meloidogyne incognita* after 50 days of inoculation.

Treatments	*Root*		*Shoot*	
	Fresh Weight (g)	*Dry Weight (g)*	*Fresh Weight (g)*	*Dry Weight (g)*
T1 – 10g (5.5ml glucose + 7.5g Tea waste)	3.21a	0.21a	32.28bc	2.56bc
T2 – 10g (5.5 ml Molasses + 7.5 Tea waste)	2.71a	0.21a	32.27bc	2.81bc
T3 – 10g (5.5 ml Molasses + 15 g soil)	2.25a	0.21a	25.47bc	2.16c
T4 – 20g (5.5ml glucose + 7.5g Tea waste)	1.61a	0.17a	32.83bc	2.93bc
T5 – 20 g (5.5 ml Molasses + 7.5 Tea waste)	3.53a	0.31a	57.93a	5.26a
T6 – 20 g (5.5 ml Molasses + 15 g soil)	3.66a	0.30a	38.27b	3.13bc
T7 – Control- (Nematode + No fungus)	3.16a	0.31a	16.68c	2.11c
T8 – Control I (No fungus or nematode)	3.75a	0.43a	64.45a	4.60ab

Values followed by the same letter in each column are not significantly different at $p \geq 0.05$

Highest shoot dry weight was observed in plants treated with 20g of 5.5 ml Molasses + 7.5 g Tea waste (T5) than of which the effect was greater in un-inoculated control plants (T8). No significant ($p \geq 0.05$) differences of shoot dry weights were seen among treatments 10g of 5.5ml glucose + 7.5g Tea waste (T1), 10g of 5.5 ml Molasses + 7.5 Tea waste (T2), 10g of 5.5 ml Molasses + 15 g soil

(T3), 20g of 5.5ml glucose + 7.5g Tea waste (T4), 20g of 5.5 ml Molasses + 15 g soil (T6) and the inoculated control T7.

The highest galling index of 4.16 was observed on roots in inoculated control plants (T7) (figure 1). No galls were observed in roots of un-inoculated control plants (T8). Significant differences in mean galling indices were not observed among the treatments except in T7 and T8. This showed that agro-industrial by-products tested have significantly ($p \geq 0.05$) reduced the nematode multiplication and helped increase the plant growth.

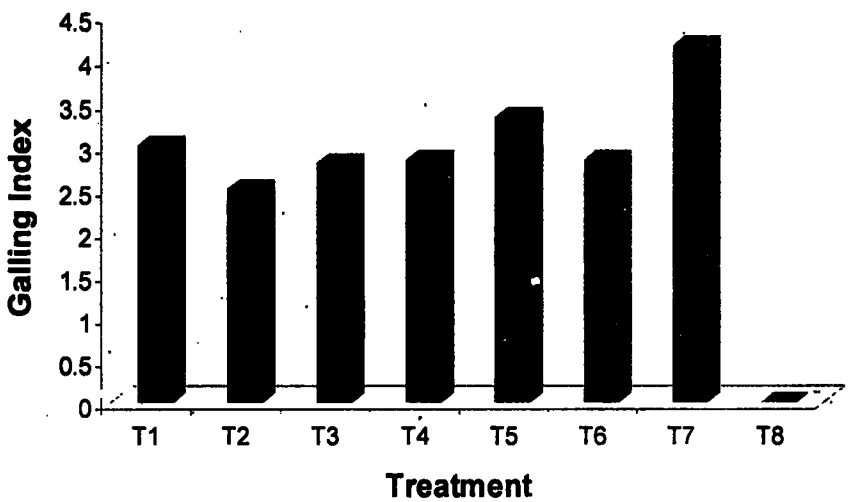

Figure 1. Effect of *Trichoderma koningii* cultured on different media on galling index of tomato roots after 50 days of inoculation of *Meloidogyne incognita*.

CONCLUSION

The results of these investigations revealed that agro-industrial by-products like molasses and tea waste can be used in the preparation of cultures for the mass production of *T. koningii*. These materials are both locally available and economically feasible. The fungus grown in these culture media can be incorporated at tested levels infested with *M. incognita* to obtain better plant growth while controlling the nematode population.

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