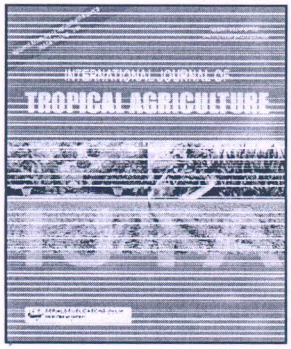

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Compatibility Studies of Antagonistic Fungus with Neem based Formulations

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Abstract: The microbial free extracts of the Neem based formulations – Wellgro samples (autoclaving and membrane filtration) were tested against the five antagonistic fungal microorganisms (*Trichoderma harzianum, T. hamatum, T. viride, and Trichoderma harzianum - 001* and *T. viride - 002).* Poison food technique was followed to test the effect of Wellgro crops and Wellgro soil at five different concentrations viz., 0.5, 1.0, 2.5, 5.0 and 10% on the growth of these microorganisms. At lower concentrations, Wellgro soil was found to have no effect on the growth of the test fungal spp. However, with increase in concentration above 2.5% there was significant reduction in the spore germination of the test fungi *in-vitro.* The study helps in fortification of beneficial microorganisms using Neem formulations (Wellgro) as medium for multiplication there by increasing the application and antagonistic efficiency by fitting in sustainable IPM programmes.

Key Words: Wellgro Soil, Wellgro Crops, *Trichoderma,* Neem, Compatibility

INTRODUCTION

Trichoderma as a potent fungal biocontrol agent against a wide range of plant pathogens has attracted considerable scientific attention (e.g., Tewari and Mukhopadhyay, 2001; Rini and Sulochana, 2007). Different organic media like neem cake, coir pith, farmyard manure, and decomposed coffee pulp also have been suggested for its multiplication (Saju et al., 2002). Yet reports on the optimum moisture levels of these substrates for high inoculum production of *Trichoderma* spp are inadequate. Therefore a study was conducted to evaluate Wellgro crops and Wellgro

soil which are powder formulations developed for Integrated Crop Management, aimed at plant growth and Integrated Pest Management (Vithal PSRVS, *et al,* 2007). These are ready to use formulations developed by Agri Business Division of ITC Limited from non-timber forest products like Neem that are water soluble and eco-friendly. These products are rich in organic matter and are good substrate for multiplication of beneficial microbes like *Trichoderma.* In the current study, these products were tested against five commonly occurring beneficial soil microorganisms for their growth promoting/ inhibition activity. The microbial free extracts of the Wellgro samples (autoclaving and membrane filtration) were tested against the five antagonistic fungal microorganisms (*Trichoderma harzianum, T. hamatum, T. viride, Trichoderma harzianum - 001* and *T. viride - 002).* Poison food technique was followed to test the effect of Wellgro crops and Wellgro soil at five different concentrations viz., 0.5, 1.0, 2.5, 5.0 and 10% on the growth of these microorganisms.

MATERIALS AND METHODS

Triplicate samples of moistened substrates were transferred to 250 ml conical flasks, sterilized by autoclaving at 121°C for 15 Min, and inoculated with 1 cm^2 of actively growing culture discs of the fungus (*Trichoderma harzianum, T. hamatum, T. viride, Trichoderma harzianum - 001* and *T. viride - 002*). The contents were incubated at room temperature and the treatments were arranged in completely randomized design with three replications. Visual observations on fungal growth were made daily and the propagule density estimated on potato dextrose agar (PDA) supplemented with Rosebengal @ 25 mg L^{-1} on the 10th day of inoculation by dilution plate technique. Colony forming units (cfu) were counted after 2 days of incubation.

In another experiment the microbial free extracts of the given samples were obtained by autoclaving the substrate with excess water (1:4) and membrane filtering the excess water with 0.2 µm PTFF syringe, this microbial free extract was used to test the survivability and compatibility of the test microorganisms. Required quantity of microbial free extracts of the substrates were added separately into molten and cooled potato dextrose agar so as to get the different concentration of the substrate. Later, 20 ml of the poisoned medium was poured into sterile Petri plates. Mycelial disc of 5 mm size from 10 day old culture was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. Then, such plates were incubated at 25°C for 10 days at the end of which redial colony growths were measured. The efficacy of fungicides was expressed as per cent inhibition of mycelia growth over control that was calculated by using the following formula [Vincent, 1947].

$$I = \frac{(C - T)}{C} \times 100$$

Where,

I = Per cent inhibition

C = Radial growth in Control

T = Radial growth in treatment

RESULTS AND DISCUSSIONS

The fungal bio-agents grown in sterile Wellgro crops recoded significant increase in the production of colony forming units (CFU) while no significant increase in production of CFUs in Wellgro soil (Table 1). Of all the five fungal bio agents tested, *T. harzianum* and *T. viride* recorded more colony forming units (Table 2). The two fungal species i.e. *T. viride* & *T. harzianum* - 001 exhibited enhanced spore germination at 0.5% concentration in a medium infused with extract of Wellgro crops. The soil fungi *T. harzianum* recorded increased spore germination at 0.5% and 1.0% of Wellgro soil while *T. viride* recorded increased spore germination only at 0.5% (Table 3). Of all the fungi tested, *T. hamatum* was

found to be highly sensitive to Wellgro soil (Table 4). The study helps in fortification of beneficial microorganisms using Wellgro formulations as medium for multiplication there by increasing the application and antagonistic efficiency by fitting in sustainable IPM programmes. When there is a need to apply any of the beneficial fungi, the probable use of either 'Wellgro crops' or 'Wellgro soil' as medium will offer the combined benefits of growth promotion and pest/disease management.

Table 1
Effect of Wellgro crops at different concentrations on spore concentrations of selected fungi

Fungal antagonists	*Concentration (%)*				
	Increase/ reduction in CFU x 10^6				
	0.5	*1.0*	*2.5*	*5.0*	*10.0*
T. harzianum	-6.05	-18.05	-30.66	-37.64	-46.87
T. hamatum	+1.53	-6.15	-31.18	-37.12	-44.10
T. viride	+8.39	-2.64	-33.33	-31.61	-50.34
T. harzianum-001	+4.43	-3.44	-14.28	-23.64	-38.42
T. viride -002	-3.56	-18.99	-26.30	-36.71	-49.95
SEM	2.57	1.77	3.19	4.59	1.36
CD (P=0.05)	8.21	5.65	10.20	NS	4.34

Table 2
Effect of Wellgro soil at different concentrations on the spore concentrations of selected soil fungi

Fungal antagonists	*Concentration (%)*				
	Increase/ reduction in CFU x 10^6				
	0.5	*1.0*	*2.5*	*5.0*	*10.0*
T. harzianum	-4.10	-11.90	-24.51	-66.56	-38.87
T. hamatum	-5.12	-12.51	-16.92	-29.74	-21.12
T. viride	+3.90	-16.55	-36.66	-41.49-	45.63
T. harzianum-001	-30.54	-35.46	-43.02	-48.44	-53.53
T. viride -002	-18.90	-33.97	-31.51	-39.73	-49.95
SEM	2.54	1.91	5.06	2.14	1.45
CD (P=0.05)	7.99	6.09	16.17	6.85	4.65

Table 3
Effect of Wellgro crops at different concentrations on spore germination of selected soil fungi

Fungal antagonists	*Concentration (%)*				
	Reduction/ Increase in spore germination over Control %				
	0.5	*1.0*	*2.5*	*5.0*	*10.0*
T. harizanum	-1.66	-7.22	-11.66	-13.33	-19.44
T. hamatum	0.00	-1.66	-11.30	-16.39	-20.81
T. viride	+1.13	-4.39	-7.67	-14.32	-20.25
T. harzianum-001	+2.53	-0.01	-2.80	-8.67	-16.65
T. viride -002	-2.53	-4.73	-11.03	-16.53.	-22.72
SEM	0.94	1.94	2.77	1.97	1.39
CD (P=0.05)	3.52	NS	NS	NS	NS

Table 4
Effect of Wellgro soil at different concentrations on spore germination of some soil microorganisms

Fungal antagonists	*Concentration (%)*				
	Reduction/ Increase in spore germination over Control %				
	0.5	*1.0*	*2.5*	*5.0*	*10.0*
T. harzianum	+8.42	+7.09	-3.44	-9.57	-19.62
T. hamatum	-12.51	-18.05	-19.82	-27.26	-32.51
T. viride	+10.81	-4.21	-6.87	-15.99	-22.65
T. harzianum-001	-4.33	-6.44	-8.77	-19.72	-27.29
T. viride -002	-0.19	-3.90	-13.10	-27.17	-32.52
SEM	0.61	1.23	2.21	8.87	1.57
CD (P=0.05)	1.97	3.93	7.05	5.97	5.01

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