Isolation of Antagonistic Organisms against *Rigidoporus microporus* from Soils of Main Rubber Growing Areas in Sri Lanka

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Introduction

White root disease is the most destructive root disease in Sri Lankan rubber plantations. It is caused by the fungal pathogen *Rigidoporus microporus* which spreads through infected roots and mycelial aggregates (rhizomorphs) through the soil (Jayasinghe *et al*., 2010). Soil characters are important for the disease progress. Soil microorganisms may reflect changes in soil quality since the dynamics of their populations describe the status and trends of soil conditions (Lu *et al*., 2012). Soil habited micro-organisms play a critical role in rubber plantations, for example in biological control of soil borne fungal diseases. Present study was carried out to isolate the potential antagonistic micro-organisms from different rubber growing soils, against *Rigidoporus microporus* the causative agent of white root disease in Sri Lanka.

Methodology

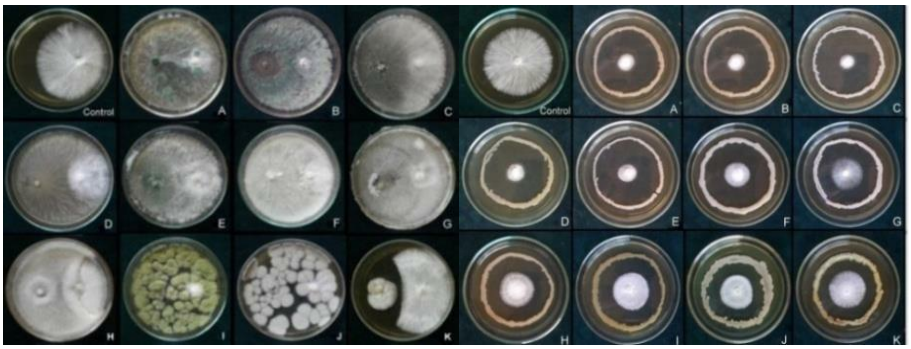
Isolation of the pathogen was under taken from rubber (*Hevea brasiliensis*) roots after sterilization using 0.01% Hg_2Cl_2 for 2 minutes. The resulted fungal culture was maintained on Malt Extract Agar (MEA). Microorganisms were isolated from twelve soil samples collected from main rubber growing districts in Sri Lanka *viz,* Kaluthara, Rathnapura, Kegalle and Monaragala. Chemical properties of the soil such as pH, organic matter content and moisture content of each sample were measured. Dilution plate technique was used to isolate the microorganisms in Potato Dextrose Agar medium (PDA). The dishes were then incubated with three replicates and colony forming units were counted after 4 days. Direct opposition method was used for isolating the potential antagonistic microorganisms against the pathogen in the MEA medium for six days. Selected antagonistic fungi were introduced onto PDA media and bacteria were cultured in nutrient agar (NA) while actinomycetes were grown in starch-casine agar. Growth inhibition of the pathogen's colony was measured the presence of different antagonistic microorganisms using dual culture plates to investigate the most promising antagonistic organisms that would be useful in controlling the growth of *R. microporus*. The variation of soil properties and microbial populations among sites and districts were analyzed by Nested ANOVA procedure by using SAS (SAS Institute Inc., 2009) software programme. The growth inhibition of pathogen was analyzed with analysis of variance (ANOVA) procedure of SAS (SAS Institute Inc., 2009). Significant means of treatments were separated using the Least Significant Difference ($p<0.05$) test (LSD).

Results and Discussion

Fungi and bacteria were isolated from the four rubber growing districts under investigation. The results demonstrated that both the antagonistic fungi and bacteria effective against *R. microporus* are available in the soils of rubber plantations in Sri Lanka. The distribution varied with the agro-climatic region and the environmental conditions. The number of bacterial colonies was more compared to the fungal colonies (Table 1). Among the micro-organisms isolated 11 bacteria ssp. and 20 fungi were effective based on the mean growth inhibition (Plate 1 a and b). Variation of growth inhibition of the pathogen by different antagonistic organisms was significantly different ($p<0.05$) at 95% confidence level ($\alpha = 0.05$).

Table 1. The microbial populations (colony forming units per gram of soil) from the four rubber growing agro-climatic regions and soil properties.

Site	Bacteria ($\times 10^4$ cfu g^{-1})	Fungi ($\times 10^4$ cfu g^{-1})	pH	OMC (g kg^{-1})	MC (%)
Sapumalkande	10.6±4.2	1.6±0.2	5.7±0.09	12.7±0.2	22.9±1.4
Dewalakande	11.8±1.3	1.9±0.8	5.4±0.02	11.6±0.2	23.4±5.7
Mahaoya	21.0±2.3	1.8±0.7	5.4±0.01	13.8±0.5	30.1±1.9
Kuruvita	2.3±0.6	0.1±0.1	5.4±0.06	4.2±0.2	33.2±0.6
Matuwagala	2.3±0.5	2.5±0.4	5.2±0.02	14.5±0.1	34.3±2.1
Kiriella	2.7±0.8	3.6±1.2	5.3±0.02	10.2±0.1	33.6±2.6
N.kale	15.6±0.5	1.4±0.5	5.7±0.01	12.6±0.3	24.6±1.7
Galewatta	12.7±4.3	1.1±0.4	5.5±0.02	11.4±0.1	27.9±0.0
D. field	16.0±4.0	2.2±0.4	5.7±0.03	15.9±0.5	30.7±2.3
Badalkumbura	12.3±1.4	0.6±0.3	6.8±0.02	12.0±0.1	17.0±0.4
Vakumbura	3.1±1.2	0.8±0.1	6.7±0.01	11.5±0.0	14.0±0.9
Karambagama	3.5±4.9	0.3±0.1	6.5±0.02	7.5±0.1	14.4±0.5



(a) **(b)**

Plate 1. Antagonistic (a) fungi, (b) bacteria against *R. micropores.*

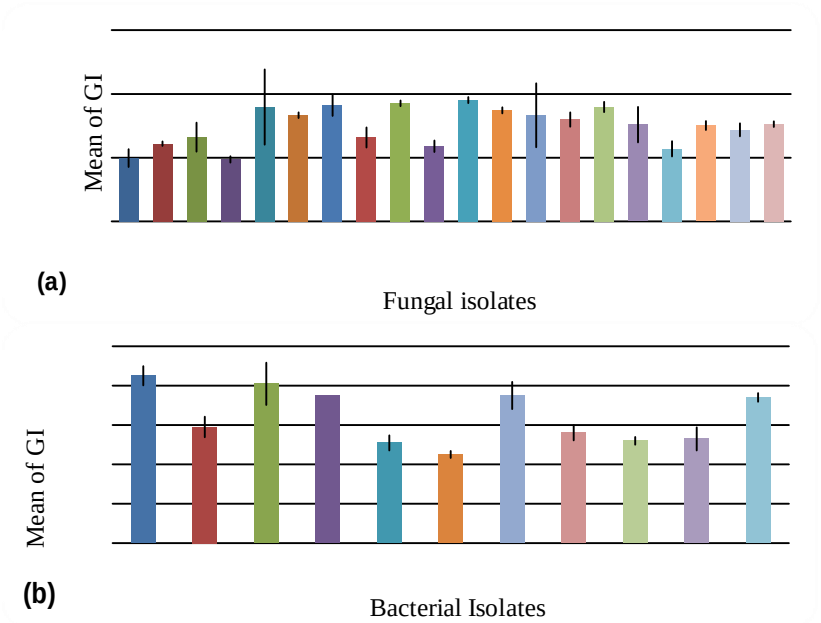


Figure 1. Mean growth inhibition (GI) of antagonistic (a) fungi and (b) bacteria isolates.

Conclusions

Abundance of the microbial population varied with their agro-climatic environment, as such the presence of antagonistic microorganisms in different soils and their antagonism were varied. Among the selected fungal colonies, *Trichoderma* species showed the highest antagonism against the fungal pathogen. These results indicate that both selected fungal and bacterial isolates were antagonistic against the white root disease pathogen. Therefore, they could be used as biological control agents for white root disease of rubber plantations.

References

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