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Full Length Article

Potential Antagonist Organisms against *Poria hypolateritia* of Red Root Disease in Tea Plantation

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ABSTRACT

A dual culture method was practiced in order to observe the interaction between *Poria* and the soil isolates. This method was slightly modified by allocating *Poria* in the middle and the soil isolate was inoculated 2 cm from the periphery of the plate; opposite to each other. The soil isolates was labeled as Noor Fazila (NF)-1, NF- 2, NF- 3, NF- 4, NF- 5, NF- 6, NF- 7, NF- 8, NF- 9, NF- 10, NF- 11 and NF- 12. After twelve days of interaction, two isolates; NF-1 and NF-5 were identified as potential antagonist organisms and suspected to be *Cunninghamella sp*. Other successfully isolated fungi are *Trichoderma, Stylopage, Verticillium, Blastomyces, Ovulariopsis, Periconia* and *Ustilago*. The rest of the fungi showed either agonism or mutualism interaction against *Poria*.

Key Words: *Poria*; Dual culture; *Cunninghamella*

INTRODUCTION

Pytopathologists have neglected for a long time the role of the microbial population of the soil, considering only the parasite and the disease. Many decades ago, researchers had begun the study of the relationship between soil microorganisms and the pathogen host complex (Chandniwala, 1995). However, the seminal work that crystallized biocontrol research into coherent discipline was 'Ecology of Soil-Borne Plant Pathogens: Prelude to Biological Control', based on the meeting in 1963 at Berkeley, California (Paulitz, 2000). The distribution of antagonist organisms varied widely according to the different samples of the soil. Among the many antagonistic bacteria were *Bacillus subtilis* and the most frequently occurring antagonistic fungi were species of *Penicillium*, *Aspergillus*, *Trichoderma* and *Trichothecium* (Chandiwala, 1995). One of the species in *Trichoderma*; *Trichoderma pseudokoningii* found to be able to impede the spread over of *Poria hypolateritia*; the causal agent of red root disease in tea plantation. *Aspergillus* also can hamper this pathogen but merely as pre-pathogen (Cooray & Balasuriya, 2002). The infection of *Poria* on tea bushes not only means a gradual loss of yield and income during the period of infection, but also a loss of capital because of death of the entire bush (Fuchs, 1989). Recently, Sabah Tea Plantation found to be infected by this disease. Countless methods have been applied including planting *Guatemala* grass, uprooting, drenching and sanitation by burning the infected area. All the mentioned methods did not reach the target to fully eradicate the disease severity. In fact, methyl bromide had been sprayed over to eradicate *Poria*. But, this merely jeopardized Sabah Tea Plantation as one of the few organic tea producers. Therefore, application of methyl bromide has been discontinued. Thus, biological control may be the best alternative to overcome this problem.

MATERIALS AND METHODS

Root and soil sampling: The infected tea root from Sabah Tea Plantations was uprooted and placed in a clean plastic bag together with the soil swiftly. Fifteen samples were taken representing the infected tea's roots. Whereas, 10 soil samples were dug surrounding the healthy tea root which is far from the infected tea bushes. The samples were kept in autoclaved bottles and transferred to Laboratory in University Malaysia Sabah.

Plate inoculation with infected root: The tea's root surface was washed properly before sterilization by immersing into 75% of alcohol for several minutes and rinsed in three changes of sterile distilled water. In a sterile dish, a small portion of infected root was transferred to a PDA plate. Besides, another method described by Chong *et al*. (2004) also was applied to detect the existence of *Poria* colony.

Preparation of serial dilution and plate inoculation: 1 g of soil sample was diluted in 10 mL of distilled water in a test tube. One mL of the diluted soil was dropped in another test tube for further dilutions (Tortora *et al*., 2004). The dilution factor was 1×10^{-5}. Then, the sample was inoculated on three PDA plates using a pipette, streaked using a hockey stick rode. The steps were repeated for other soil samples.

Inoculation of isolated organisms: *In vitro* comparisons consisted of removing four mm diameter disks from the

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edge of expanding colonies grown. Both *Poria* and soil-borne fungus were placed in a new Petri dish; the soil-borne fungus colony was placed two cm from the periphery of the plate, whereas *Poria* was placed in the middle of the plate. The paired culture was incubated at ambient room temperature (25±2ºC) for one week. All pairings were replicated three times (Bell *et al*., 1982).

Measurement of the radius: The calculation method was extracted from the text written by Kasturi (1999). The inoculated plates were observed and the radius of *Poria* colony towards the soilborne organisms was measured. The percent inhibition of radius growth (PIRG) was calculated using the followed equation:

$$PIRG = \frac{R_1 - R_2}{R_1} \times 100$$

R1–Radius of *Poria* towards two cm from the periphery of the control plate.

R2–Radius of *Poria* towards the potential antagonism organism colony.

Determination of antagonist organisms: The plate with the highest PIRG value was sifted. The morphology of the colonies was observed through microscope. The morphology and the spores' structure were observed to identify the potential antagonist by referring to the fungi, which were identified to the method of Barnett and Hunter (1972).

RESULTS AND DISCUSSION

Table I showed the different diameter measured, morphology and color of the soil isolates on petri dishes after one week.

A-Appearance	**M-Margin**	**E-Elevation**
I-Irregular	F-Filamentous	R-Raised
C-Circular	E-Entire	F-Flat
	U-Undulate	X-Convex
		U-Umbonate

Interspecific interaction and PIRG measurement: From a serial of repeated experiments (at least three times) the interactions between soil isolates and *Poria* are summarized in Table II. Isolate NF 1 and Isolate NF 5 showed an antagonistic interaction against, whereas NF 3 is suspected to be agonist organism based on the condition observed after the interaction in dual culture (Fig. 1). Other soil isolates showed an interaction, which can be classified as either agonism or mutualism (Fig. 2). Mean of percentage of inhibition in radius growth (PIRG) of twelve soil isolates against *Poria* showing that only NF1 and NF5 are significant, which the mean values are 86.65% for NF 1 and 85.33% for NF 5, respectively. No PIRG observed in the other isolate.

Classification of isolated fungi: The structures of NF 1 (Fig. 3a & b) and other soil isolates were observed under the microscope to identify their genera based on the previous research and finally listed in Table III.

Table I: Traits of twelve fungi colonies isolated from the soil samples

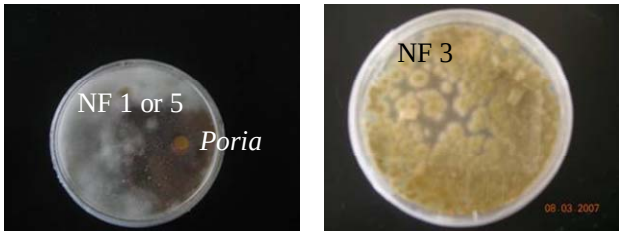
	Diameter (mm)	A	M	E	Color
NF 1	90	I	F	R	White
NF 2	45	I	E	F	White greenish
NF 3	10	I	E	X	Green brownish
NF 4	5	I	U	X	Grey
NF 5	45	I	F	R	White
NF 6	90	C	F	U	White yellowish
NF 7	90	C	F	R	White
NF 8	90	I	F	R	White
NF 9	90	C	F	U	white
NF 10	90	C	E	F	Yellow greenish
NF 11	90	C	E	F	White yellowish
NF 12	90	C	F	R	Green greyish

Table II: Interspecific interaction between *Poria* and soil isolates (NF 1-NF 12)

Soil isolates	Interspecific interaction
NF 1	Antagonism
NF 2	Agonism/mutualism
NF 3	Agonism
NF 4	Agonism/mutualism
NF 5	Antagonism
NF 6	Agonism/ mutualism
NF 7	Agonism/mutualism
NF 8	Agonism/mutualism
NF 9	Agonism/mutualism
NF 10	Agonism/mutualism
NF 11	Agonism/mutualism
NF 12	Agonism/mutualism

Fig. 1: Interspecific interaction between *Poria* and (a) NF 1 or NF 5 (b) NF 3

Note: NF 3 conquered the whole plate



(a) (b)

NF 1 is suspected to be *Cunnighamella* based on the morphology structures observes under the microscope. *Cunninghamella*, which belongs to class Zygomycetes is able in metabolizing chlorpromazine (CPZ) and methdilazine (MDZ) to produce essential anti-stress compounds for humans. *Cunninghamella* is able to secrete P-450 enzymes. These enzymes are also secreted in human body. Thus, it is suspected that this fungus has the capability to secrete complex enzymes to degrade *Poria*'s mycelia (Zhang *et al.*, 1996).

Besides *Cunninghamella*, NF 3, which is suspected to be *Periconia*, has a distinctive interaction against *Poria* compared to other isolates. Thus, *Periconia* can be pointed

Table III: Taxonomy of isolated soil fungi based on the microscopic features

Isolate	Classification				
	Phylum	Class	Order	Family	Genus
NF 1	Zygomycota	Zygomycetes	Mucorales	Cunninghamellaceae	*Cunninghamella*
NF 2	Deutromycota	Hyphomycetes	Moniliales	Moniliaceae	*Verticilium*
NF 3	Deutromycota	Hyphomycetes	Moniliales	Dematiaceae	*Pericornia*
NF 4	Zygomycota	Zygomycetes	Zoopagales	Zoopagaceae	*Stylopage*
NF 5	Zygomycota	Zygomycetes	Mucorales	Cunninghamellaceae	*Cunninghamella*
NF 6	Deutromycota	Hypomycetes	Moniliales	Moniliaceae	*Trichoderma*
NF 7	Ascomycota	Eauscomycetes	Onygenales	Onygenaceae	*Blastomyces*
NF 8	Ascomycota	Eauscomycetes	Onygenales	Onygenaceae	*Blastomyces*
NF 9	Ascomycota	Ascomycetes	Erysiphales	Erysiphaceae	*Ovulariopsis*
NF 10	Deutromycota	Hypomycetes	Moniliales	Moniliaceae	*Trichoderma*
NF 11	Deutromycota	Hypomycetes	Moniliales	Moniliaceae	*Trichoderma*
NF 12	Basidiomycota	Basidiomycetes	Ustilaginales	Ustilaginaceae	*Ustilago*

Fig. 2: Condition observed on NF 2, NF 4, NF 6, NF 7, NF 8, NF 9, NF 10, NF 11 and NF 12 against *Poria*

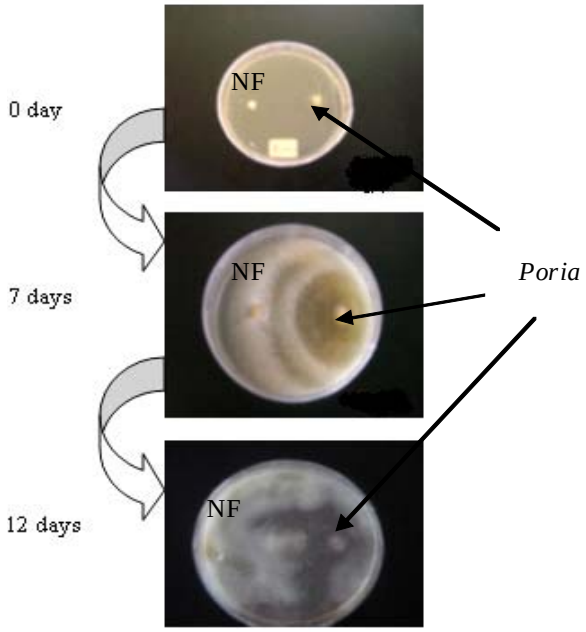
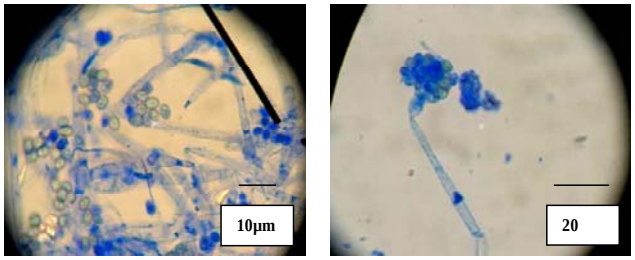
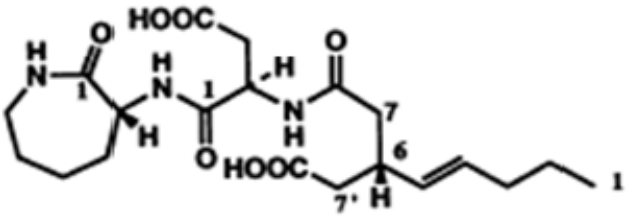


Fig. 3: The morphological structures observed under the microscope for the potential antagonist organism, NF 1



(a) (b)

Fig. 4: Precursor for the toxins synthesis in *Periconia* (Macko *et al*., 1992)



1 Circinatin

as agonist organism. *Periconia* is able to secrete toxins as a pathogenic fungus, which causes root and brown rot of the grain sorghum *Sorghum bicolor* (Macko *et al*., 1992).

The toxins are called peritoxins and periconins. These toxins are actually host specific toxins; solely excreted to cause disease in sorghum. But, the main concern is on the precursor of these compounds to be synthesized. The precursor is known as circinatin (Fig. 4). When the fungus was grown under suppressive conditions, it is suspected that circinatin acts as biosynthetic precursor of the unknown toxins (Macko *et al*., 1992). Thus, there is a potential for the circinatin to be the precursor to synthesize toxin, which can inhibit the growth of *Poria*.

CONCLUSION

The soil isolates were *Cunninghamella* (Isolates NF 1 & NF 5), *Verticillium* (Isolate NF 2), *Periconia* (Isolate NF 3), *Stylopage* (Isolate NF 4), *Trichoderma* (Isolates NF 6, NF 10 & NF 11), *Blastomyces* (Isolate NF 7 & NF 8), *Ovulariopsis* (Isolate NF 9) and *Ustilago* (Isolate NF 12). From twelve soil isolates, only one was interacted as antagonist organism, whereas the rest showed either agonism or mutualism interaction against *Poria*. Further insight into the potential antagonist of the soil isolate will aid in the knowledge for sourcing possible future of antifungal compounds.

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