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Short Communication

In Vitro Microbial Control of Pathogenic *Sclerotium rolfsii*

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ABSTRACT

In this study, pure cultures of three antagonistic fungi, *Trichoderma, Penicillium* and *Aspergillus* species and a fungal pathogen, *Sclerotium* sp. were obtained after inoculation on potato dextrose agar (PDA) fortified with antibiotics to prevent bacterial contamination. Pathogenicity test was carried out when the antagonistic isolates were inoculated on PDA 24 h before and after *Sclerotium* inoculation. Of the three fungal antagonists evaluated for inhibitory efficacy, *Trichoderma* sp. proved to be the most effective as it exhibited the greatest inhibition to *Sclerotium* sp. ($P<0.01$) both at the initial and final tests. This was closely followed by *Aspergillus* sp. with inhibitory effect on the pathogen at both trials ($P<0.01$). However *Penicillium* sp. was slightly inhibitory against *Sclerotium*. Percentage inhibitions of the antagonists on *Sclerotium* by *Trichoderma, Aspergillus,* and *Penicillium,* were up to 81.36-80.29%, 88.35-73.12% and 56.98-46.24%, at the 6th day of inhibition at both trials, respectively. The result implied that the extent of inhibition by the fungi provides the use of potential antagonists capable of controlling the pathogenicity of *Sclerotium* sp. in crops for sustainable agriculture.

Key Words: Microbial control; Antagonist; Pathogen; Percentage inhibition

INTRODUCTION

Microbial interactions between antagonistic microorganisms and plant pathogens are widespread in nature (Fridlender *et al*., 1993). Biological control of plant pathogens can be highly effective especially with hyperparasitizing potentials of antagonists on pathogenic fungi. Species of *Trichoderma* are known to be highly efficient against *Sclerotium rolfsii* Sacc (Durrell, 1968; Barnett & Binder, 1973; Elad *et al*., 1980). *Penicillium* spp. are capable of producing volatile antibiotics in agar (Jayasuriya *et al*., 1996). Study by Omorusi *et al*. (2007) suggested that filtrate of *Penicillium* as control agents other than the organisms. Inhibitory effect of *Aspergillus* spp. in some cases may not be very efficient but can be triggered off with substantial nutrient (Jayasuriya & Deacon, 1995; Omorusi *et al*., 2007). *S. rolfsii* is a destructive soil borne pathogen, which attacks over 500 plant species and has been implicated in the enlargement of wound on the host.

In nature, the rhizosphere supplies microorganisms with suitable proliferation conditions with carbon and energy sources in form of root exudates, fragmented cells from old root parts (Cook & Baker, 1983), or the carbon sources provided by synthetic media, are involved in the germination of chlamydospore and oospore of biocontrol agents. Presence of carbon, nitrogen and iron as nutrients may interact with antagonists and pathogens. Antagonists may act against pathogens in one or more of the following mechanisms: competition, antibiosis, parasitism, predation or induce resistance in plant, hydrolytic enzymes excreted by antagonists is a well-known feature of mycoparasitism. The chitinase and β-1, 3 glucanase (Laminarinase) are especially important fungus-controlling enzymes as a result of their ability to degrade the fungal cell wall components: chitin and β-1, 3 glucan (Henis & Chet, 1975).

Biological control of plant pathogens is a potential non-chemical means for plant disease control and can serve as an alternative for costly chemical treatment (Omorusi *et al.*, 2007). The objective of the present study was to evaluate the inhibitory role of potential antagonists in the biological control of *Sclerotium* pathogen.

MATERIALS AND METHODS

Pure cultures of isolates of *Trichoderma*, *Penicillium, Aspergillus* and *Sclerotium* and species were identified using the illustration method by Barnett and Hunter (1987). The pure culture of the isolates were inoculated on potato dextrose agar (PDA), which was fortified with penicillin and streptomycin antibiotics at a concerntration of 0.1 mL stock solution. The stock solution was prepared comprising 0.5 g streptomycin and 1 million international unit (IU) penicillin in 20 mL of water (Tuite, 1969). Pathogenicity test was performed when isolates of antagonists–*Trichoderma,*

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Penicillium and *Aspergillus* species were inoculated on PDA 24 h before and after *Sclerotium* inoculation. Three replicate samples of each inoculation test (24 h before & after) were obtained. To achieve this, four 5 mm culture discs were obtained from the edge of a 5 days old pure isolates each of the antagonists were plated at equal distances on PDA. Similarly a 5 mm disc of *Sclerotium* was established at the centre of replicate sample. The samples were then incubated at ambient temperature (26 ± 2^{o}C). Mycelial growth measurements were determined for six days. Percentage inhibition was calculated using the poisoned food technique (PFT) (Schmitz, cited by Jayaratne *et al*., 2001).

RESULTS AND DISCUSSION

Results showed that of the three potential antagonists *Trichoderma* spp. proved to be the most effective biocontrol agent against *S. rolfsii.* In both inoculations of *Trichoderma* spp. (24 h before & after *Sclerotium* inoculation), *Trichoderma* completely overgrew the pathogen with percentage inhibition rates recorded in both trals were 81.36 and 80.29%, respectively (Table I), which were highly significantly (P<0.01). The mechanism with which *Trichoderma* carries its antagonism may be mainly competition for nutrients and energy (Jayasuriya *et al*., 1996). Similar findings on the antagonistic parasitism of *Trichoderma lignorum* (Tode) Harz or *T. harzanium* on *S. rolfisii* have been reported (Elad *et al*., 1980).

Inhibitory effect of *Aspergillus* spp. on *Sclerotium* spp. was also highly significant (P<0.01) in both trials (24 h before & after inoculation of *Sclerotium*). Inhibition recorded in the initial and final tests were 88.35 and 73.12%, respectively (Table II). Initially, inhibitory effect of *Aspergillus* was slow but picked up rapidly from the 2nd day and completely inhibited the pathogen. Result of this tends to agree with the work of Jayasuriya and Deacon (1996), suggesting the need for a substantial nutrient amendment to trigger off antagonistic activity against a pathogen for a complete inhibition.

In this study, *Penicillium* spp. failed to produce obvious significant inhibitory effect on the *Sclerotium*. The pathogen even outgrew the growth of *Penicillium* on the 4th day however, mycelial growth of *Penicillium* picked up lately and eventually overcome the growth of the pathogen with inhibition rates of 56.98 and 46.24%, respectively for two trials (Table 111). The late antagonistic activity of *Penicillium* could be attributed to the late production of antibiotics. Study by Omorusi *et al.* (2007) suggested the use of filtrates of *Penicillium* as a biocontrol substance instead of the organism to obtain better results.

CONCLUSION

The study evaluated the inhibitory effectiveness of test antagonists-*Trichoderma, Aspergillus* and *Penicillium*

Table I: Inhibitory effect of *Trichoderma* sp on the growth of *Sclerotium rolfsii*

Growth (Mycelia) diameter (CM)					
Inoculation (days)	***Trichoderma* sp.**	**Control**	***Sclerotia* sp.**	**Control**	**Percentage inhibition**
Initial Inoculation[a]					
2	1.03	1.85	0.80	3.21	75.08
3	1.48	2.57	0.99	3.75	73.60
4	1.65	3.20	1.03	4.25	75.76
5	1.95	4.00	1.03	4.75	78.32
6	2.00	4.85	1.04	5.58	81.36
P<0.01					
Final Inoculation[b]					
2	1.23	1.85	0.53	2.91	81.79
3	2.00	2.57	0.80	3.75	78.67
4	2.10	3.20	1.03	4.25	75.76
5	2.20	4.00	1.10	4.75	76.84
6	2.40	4.85	1.10	5.58	80.29
P<0.01					

[a]*Trichoderma* inoculation on PDA 24 h before *Sclerotium* inoculation
[b]*Trichoderma* inoculation on PDA 24 h after *Sclerotium* inoculation

Table II: Inhibitory effect of *Aspergillus* sp on the growth of *Sclerotium rolfsii*

Growth (Mycelia) diameter (CM)					
Inoculation (days)	***Trichoderma* sp.**	**Control**	***Sclerotia* sp.**	**Control**	**Percentage inhibition**
Initial Inoculation [a]					
2	1.49	1.68	0.52	2.21	76.47
3	2.87	3.43	0.55	3.75	85.33
4	3.17	4.25	0.65	4.23	84.63
5	3.35	4.55	0.65	4.75	86.32
6	6.35	5.75	0.65	5.58	88.35
P<0.01					
Final Inoculation [b]					
2	1.09	1.68	0.70	2.61	73.18
3	2.40	3.43	1.28	3.75	65.87
4	2.58	4.25	1.45	4.25	65.88
5	3.60	4.55	1.50	4.75	68.42
6	3.28	5.57	1.50	5.58	73.12
P<0.01					

[a]*Aspergillus* inoculation on PDA 24 h before *Sclerotium* inoculation
[b]*Aspergillus* inoculation on PDA 24 h after *Sclerotium* inoculation

Table III: Inhibitory effect of *Penicillium* sp on the growth of *Sclerotium rolfsii*

Growth (Mycelia) diameter (CM)					
Inoculation (days)	***Trichoderma* sp.**	**Control**	***Sclerotia* sp.**	**Control**	**Percentage inhibition**
Initial Inoculation[a]					
2	0.80	0.60	0.40	2.16	81.48
3	1.00	1.20	0.95	3.75	74.67
4	1.40	2.05	1.85	4.25	56.47
5	1.45	3.00	2.35	4.75	50.52
6	1.50	3.45	2.40	5.58	56.98
Final Inoculation[b]					
2	0.73	0.60	0.93	2.67	65.17
3	0.90	1.20	1.51	3.75	59.73
4	1.10	2.05	2.50	4.25	41.18
5	2.00	3.00	2.95	4.75	37.89
6	2.15	3.45	3.00	5.58	46.24

[a]*Penicillium* inoculation on PDA 24 h before *Sclerotium* inoculation
[b]*Penicillium* inoculation on PDA 24 h after *Sclerotium* inoculation

species against the pathogenic *Sclerotium*. Results revealed that *Trichoderma* and *Aspergillus* species were identified as significantly and potentially effective antagonists against the pathogen known to be destructive to most agricultural crops. Thus the relevance of this study contributes to the sustainability of agriculture. Mesike *et al.* (2008) asserted that in Nigeria, agriculture provides about 40% of the GDP and contributes substantially to food security, employment and income generation and availability of raw materials for the industrial and manufacturing sectors.

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