African Journal of Biotechnology Vol. 7 (15), pp. 2569-2572, 4 August, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Use of phylloplane fungi as biocontrol agent against *Colletotrichum* leaf disease of rubber (*Hevea brasiliensis* Muell. Arg.)

Evueh, G. A. and Ogbebor, N. O.*

Plant Protection Division, Rubber Research Institute of Nigeria, PMB 1049, Benin City.

Accepted 4 March, 2008

Phylloplane fungi were used as biocontrol agent against *Colletotrichum* leaf disease of rubber (*Hevea brasiliensis* Muell. Arg.). *Aspergillus* sp. lysed the cytoplasm of *Colletotrichum gloeosporioides* on Potato Dextrose Agar. *Trichophyton* sp. and *Gliocladium* sp. antagonised *C. gloeosporioides* by overgrowing on it. Other phylloplanes used in this study such as *Botrytis* sp., *Pleurothecium* sp. and *Staphylotrichum* sp. exhibited weak antagonism on the pathogen while *Gonatorrhodiella* sp. and *Syncephalastrum* sp. showed different levels of zones of inhibition with the pathogen. Metabolites produced by *Gonatorrhodiella* sp. and *Syncephalastrum* sp. affected the pathogen by antibiosis. This finding showed that *Trichocladium* sp. and *Trichophyton* sp. exhibited the highest antagonistic effects on *C. gloeosporioides*.

Key words: Para rubber, phylloplane fungi, biological control, disease management.

INTRODUCTION

Hevea brasiliensis (Muell. Arg.) commonly called para rubber, is an economic crop whose healthy existence is significant to its productivity (Rao, 1965). Rubber diseases are mainly caused by fungal pathogens (Igeleke, 1988; Begho, 1990). *Colletotrichum gloeosporioides* (Penz.) Sacc. is the causal agent of Colletotrichum leaf fall of rubber tree. It is one of the serious leaf diseases which affects the new flushes produced following the 'wintering effect', when the rubber tree loses its leaves during the dry season. The disease also affects young rubber plants under nursery conditions and when severe can lead to shoot die back (Rao, 1965; Webster and Baulkwill, 1989).

The successful use of chemicals in the control of rubber diseases has been extensively reported by various scientists. However, the high cost of chemical fungicides limits its availability and use by small - scale farmers.

Biological control for plant diseases is now receiving increasing attention, although the potential of biological control through the effect of phyllosphere antagonists has been realized for sometime. Several workers have investigated the use of biological control of plant diseases (Osando and Waudo, 1994; Tewari, 1995; Ogbebor and Adekunle, 2005). Osando and Waudo (1994) used various isolates of *Trichoderma* to control *Armillaria* root rot fungus of tea. They found that different isolates of *Trichoderma* sp. exhibited different level of antagonism against *Armillaria* root rot fungus. This study seeks to find possible phylloplane fungi as biocontrol agents against *Colletotrichum* leaf disease of rubber.

MATERIALS AND METHODS

Isolation of leaf pathogen

Leaves of *H. brasiliensis* infected with *C. gloeosporioides* were collected from Rubber seedlings in Rubber Research Institute of Nigeria. Bits of 1 x 1 cm cut across lesions were surfaced sterilized by submerging in 0.1% of mercuric chloride for 1 min, after which it was rinsed in five changes of sterile distilled water. Then, they were placed on PDA.

Isolation of phylloplane fungi

Phylloplane fungi were isolated from healthy leaves of rubber plant

*Corresponding author. E-mail: ogbeb06@yahoo.com.

in RRIN nursery through leaf washing technique (Blakeman, 1981). Dilution method of Pelczar and Chan (1972) was employed and dilution factor of 10^{-4} was used. Pure isolate were established on antibiotic-amended PDA.

Dual inoculation of leaf pathogen and potential antagonist on PDA

Dual inoculation of the pathogen and an antagonist was set up. A 10 mm disc of the pathogen with similar size of each potential antagonist was taken from the edge of a 5-day-old pure culture using a cork borer and plated 20 mm apart respectively on PDA medium. Potential antagonists tried are *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., *Pleurothecium* sp., *Botrytis* sp., *Staphylotrichum* sp., *Trichocladium* sp., *Gonatorrhodiella* sp., *Trichophyton* sp. and *Syncephalastrum* sp. The control plates were plated with the pathogen and antagonists separately.

Four replications per treatment were set up for each pathogen and antagonist combinations. Petri-plates were incubated at room temperature. Daily growth measurement towards and away from the opposing fungal colony was taken for 10 days. The percentage inhibition of radial growth of the pathogen was calculated using a formula by Vincent (1927) and Jacob et al. (2006). The mode of interaction was rated using the method adapted by Fokkema (1973):

0: No visible sign of inhibition of pathogenic fungi, the mycelium of which overgrew the test organism.
1: Mutual inhibitions. Both organisms stopped growing on contact at the center or close to the center of the Petri-plate.
2: Inhibition of pathogen with inhibition zone > 1cm in width.
3: Inhibition of pathogen with inhibition zone < 1cm in width.
4: Inhibition of pathogen by overgrowth or displacement of pathogen.

Test isolate with ratings of 2 to 4 were further observed microscopically to determine their effects of antagonism on the hyphal growth of the pathogenic fungi.

RESULTS

Antagonism between colonies of *Colletotrichum gloeosporioides* and phylloplane fungi

The mycelial growth measurement of *C. gloeosporioides* and the ten antagonists towards each other on Potato Dextrose Agar on the tenth day after inoculation and percent inhibition of *C. gloeosporioides* are summarized in Table 1. A significant interaction was exhibited by *Trichocladium* sp., in which the growth of *C. gloeosporioides* was much affected by hyphal interference. The antagonistic fungus grew over the colony of *C. gloeosporioides* and completely inhibited its growth. The interaction was rated 5. *Syncephalastrum* sp. and *Gonatorrhodiella* sp. inhibited the growth of *C. gloeosporioides* by 84.1 and 80.9% respectively. An inhibition zone of 0.16 cm with *Gonatorrhodiella* sp. and 0.43 cm with *Syncephalastrum* sp. were observed. *Trichophyton* sp. and *Gliocladium* sp. overgrew C. *gloeosporioides* and their mode of interaction was intermingling and was rated 5 and 2 respectively. *Botrytis* sp., *Pleurothecium* sp. and *Staphylotrichum* sp. Stopped growth at the center of the plate when they came in contact with *C. gloeosporioides* forming a straight line at their meeting point. Their mode of interaction were rated 5. *Trichoderma* sp. and *Aspergillus* sp. both rated as 3 inhibited the growth of *C. gloeosporioides* by 90% and did not overgrow it till the end of the experiment.

Microscopic observation of mycelial interactions

Microscopic observation was carried out on the interaction of *C. gloeosporioides* with *Trichoderma* sp., *Trichocladium* sp., *Aspergillus* sp., *Gonatorrhodiella* sp., *Syncephalastrum* sp. and *Trichophyton* sp. When *Aspergillus* species came in contact with hypha of *Colletotrichum* sp., lysis of the hypha occurred and the pathogen could not be re-isolated from the point of contact. In the case of *Trichocladium* sp., when it came in contact with that of the pathogen, lysis occurred at the point of septa formation. *Trichoderma* sp. and *Trichophyton* sp. did not show any clear pattern of hyphal interaction. In the interaction between *C. gloeosporioides* with *Syncephalastrum* sp. and *Gonatorrhodiella* sp., there were mutual inhibition of both the pathogen and antagonists.

DISCUSSION

Antagonistic effects of different phylloplane fungi indicated the importance of many such fungi as a possible biocontrol agent. *Trichocladium* sp. completely overgrew *C. gloeosporioides* in culture and eventually displaced it. This could possibly be as a result of competition between saprophytic and pathogenic microorganisms for nutrients.

Interaction between the fungal colonies of *C. gloeosporioides* with *Gonatorrhodiella* sp. and *Syncephalastrum* sp. respectively showed that they did not meet in culture. *C. gloeosporioides* was inhibited and this is as a result of the production of metabolites, possibly antibiotics, by *Gonatorrhodiella* sp. and *Syncephalastrum sp.* in the medium. Antibiotics have been shown to be involved in disease suppression (Anjaiah et al., 1995; Handelman and Stabb, 1996). Anjaiah et al. (1995) demonstrated that phenazine-1-carboxamide and anthranilate are antibiotics involved in disease suppression by *Psuedomonads*.

Trichophyton sp. and *Gliocladium* sp. antagonized *C. gloeosporioides* by overgrowth mechanism. Only *Trichophyton* sp. showed appreciable reduction in the growth of *C. gloeosporioides*. *Botrytis* sp., *Pleurothecium* sp. and *Staphylotrichum* sp. did not show appreciable antagonistic effect.

From the observation of 'direct' post contact inhibition, *Aspergillu*s sp. caused damage to *C. gloeosporioides* by coagulating its cytoplasm and also caused lysis and tip burst of the pathogen. This could be as a result of antagonism due to parasitism or and antibiosis as lytic activity has been demonstrated to be involved. Jayasu-

Table 1. The mycelial growth measurement of *Colletotrichum gloeosporioides* and ten antagonists towards each other on Potato dextrose Agar on the tenth day after inoculation and percent inhibition of *C. gloeosporioides*.

S/N	Pathogen/Antagonist	Mycelial growth ±SE (Treatment) cm	Mycelial growth (Control) cm	Percent inhibition (%)
1	*C. gloeosporioides*	1.13 ± 0.04	5.50	69.20
	Botrytis sp.*	0.88 ± 0.04	5.50	
2	*C. gloeosporioides*	0.55 ± 0.05	5.50	90.90
	Trichoderma sp	1.45 ± 0.05	5.50	
3	*C. gloeosporioides*	1.40 ± 0.00	5.50	74.50
	Gliocladium sp.*	2.00 ± 0.07	1.70	
4	*C. gloeosporioides*	0.93 ± 0.04	5.50	83.18
	Staphylotrichum sp.*	1.73 ± 0.04	5.50	
5	*C. gloeosporioides*	0.00 ± 0.00	5.50	100.00
	Trichocladium sp.*	3.50 ± 0.07	5.50	
6	*C. gloeosporioides*	1.05 ± 0.05	5.50	80.90
	Gonatorrhodiella sp.*	0.78 ± 0.04	1.30	
7	*C. gloeosporioides*	0.55 ± 0.05	5.50	99.09
	Aspergillus sp.*	1.48 ± 0.08	5.50	
8	*C. gloeosporioides*	0.83 ± 0.04	5.50	85.00
	Pleurothecium sp.*	1.18 ± 0.04	5.50	
9	*C. gloeosporioides*	0.93 ± 0.04	5.50	83.18
	Trichophyton sp.*	5.50 ± 0.08	5.50	
10	*C. gloeosporioides*	0.88 ± 0.04	5.50	84.09
	Syncephalastrum sp.*	0.70 ±0.00	1.50	

• = Antagonist.

riya and Deacon (1995) demonstrated *B. cinerea* to cause explosive hyphal lysis or coagulation of cytoplasm, often penetrating the hyphae of *Rigidoporus lignosus* 4 - 7 min after contact. Jacob et al. (2006) suggested that lysis of cell wall of C. *gloeosporioides* is involved in the biological control of mango anthracnose disease by *Pseudomonas fluorescence*.

This study demonstrates *Trichocladium* sp. and *Trichophyton* sp. to possess higher antagonistic effect on the pathogen. Integrated approach of disease management using the interaction of chemical means of control with a biological component is likely to be more attractive to the grower than a biological approach alone. Munnecke et al. (1981) used this approach to control *Armillaria mellea* in soil by weakening the pathogen with methyl bromide so that natural occurring antagonist could easily invade the weakened pathogen inoculum's. However, the exploitation of phylloplane fungi for the control of the pathogen is less expensive, safer and could serve as a good alternative to synthetic fungicides.

REFERENCES

Anjaiah V, Koedam N, Nowak–Thompson B, Loper JD, Hofte M, Tambong JT, Cornelius P (1995). Involvement of phenazines and anthranitate in the antagonism of *Psuedomonas aeruginoe* PNA 1 and Tn 5 derivatives towards *Fusarium* and *Pythium* sp. Mol. Plant Microbe Interact. 11: 847-854.

Begho ER (1990). Nursery diseases of *Hevea brasiliensis* in Nigeria, and their Control. Paper presented at the proceedings of a National workshop onfruit/tree crop seedling production NEARLS, Zaria, pp. 103-106.

Blakeman JP (1981). Microbial ecology of the phylloplane. Academic press London, p. 502.

Fokkema NJ (1973). The role of saprophytic fungi in antagonism against *Drechslera sorokiniana (Helminthosporium sativum*) on agar plates and on rye laves with pollen. Physiol. Plant. Pathol. 3: 195-205.

Igeleke CL (1988). Disease of Rubber (*Hevea brasiliensis*) and their control. Paper presented at the 10th Annual Conference of the Horticultural society of Nigeria, 6th - 8th November, p. 10.

Handelman J Stabb EV (1996). Biological control of soil borne plant pathogens. Plant Cell. 8: 1855-1869.

Jacob CK, Srinivas P, Roy CB (2006). A Laboratory manual for International training on strategies for management of *Corynespora* Leaf Fall Disease of *Hevea brasiliensis. Rubber Research Institute of India.* Kottayam, India. pp. 31-39.

Jayasuriya KE, Deacon JE (1995). *In Vitro* interactions between *Rigidoporus lignosus*, the cause of white root disease of Rubber and some potentially antagonistic fungi. Journal Rubber Research Institute of Sri- Lanka, 76: 36-54.

Munnecke DE, Kolbezen MJ, Ohr HD (1981). Interactions involved in controlling *Armillaria mellea*. Plant Dis. 65: 469-479.

Ogbebor NO, AT Adekunle (2005). Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* Muell. Arg.) using extracts of some plants. Afr. J. Biotechnol. 4(9): 996-1000.

Osando JM, Waudo SW (1994). Interaction between *Trichoderma* species and *Armillaria* root rot fungus of tea in Kenya. Int. J. Pest

Manage. 40: 69-74.
Pelczar MJ, Chan ECS (1972). Laboratory exercises in microbiology Mcgraw- Hill book company, pp. 197-347.
Rao BS (1965). Maladies of *Hevea* in Malaysia. Rubber Research Institute ofMalaysia; Kuala Lumpur. 108 pp.
Tewari SN (1995). *Ocimum sanctum* L., a botanical fungicide for rice blast control. Trop. Sci, 35: 263-273.
Vincent JM (1927). Distortion of fungal hyphae in the presence of certain inhibitions. Nature, 850.
Webster CC, Baulkwill WEJ (1989). Rubber Tropical Agricultural Series. Longman Scientific and Technical. U.K., p. 614.