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Evaluation Phylloplane Fungi as Biocontrol Agent of *Corynespora* Leaf Disease of Rubber (*Hevea brasiliensis* Muell. ARG.)

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Abstract: Ten phylloplane fungi were assessed as potential biocontrol agents of *Corynespora* leaf fall disease (CLFD) of rubber (*Hevea brasiliensis* Muell. Arg.) cause by *Corynespora cassiicola* (Berk and Curtis) Wei. *Trichoderma viride* Pers inhibited the growth of *C. cassiicola* on Potato Dextrose Agar (PDA) and was observed to coagulate the cytoplasm of the target fungus at the point of contact under a light microscope. *Aspergillus* sp also showed great potential in inhibiting the vegetative growth by causing hyphal lyses of *C. cassiicola* on contact. *Trichophyton* sp did not cause any physical damage on contact with the hyphae of *C. cassiicola* but produced hyphae that coiled around it while *Gonatorrhodiella parasitica* Thaxt. and *Trichocladium* sp showed no antangonistic effect on *C. cassiicola*. *Pleurothecium* sp, *Gliocladium* sp *and Botrytis sp* exhibited weak antagonism against the pathogen. The study showed that *Trichocladium* sp *and Aspergillus niger* recorded the best antagonistic effect on *C. cassiicola*.

Key words: Phylloplane fungi % Biological control % *Corynespora* % Rubber % Antagonism

INTRODUCTION

Rubber (*Hevea brasiliensis*) is an economic crop whose healthy existence is significant to its productivity output [1,2]. In Nigeria, there is a huge dependence by industries on rubber plants which is a major economic crop for natural rubber. Sufficient production of natural rubber and efficient generation of adequate foreign exchange through the improved management and productivity of scattered rubber plantations in the country are threatened by a few biotic agents amongst which are pathogens of both fungal and bacteria origins [3]. There are many pathological problems encountered by most commercial crops in monoculture and rubber plants are not exempted [4,5].

Corynespora cassiicola (Berk and Curtis) Wei is a plant pathogenic fungus that is responsible for a variety of plant diseases which include leaf spots and rots in stem, fruits, seeds and flowers of about 500 plant species representing approximately 50 families. The pathogen has been reported in many parts of the world including Nigeria where it is responsible for the devastating *Corynespora* leaf fall disease (CLFD) of young and matured rubber trees [6-8].

Reports abound on the extensive use of chemicals in the control of CLFD of rubber plants [9]. However, the high cost of chemical fungicides, their labour intensive nature resulting from repeated application regime and the difficulty of applying chemical sprays to tall or matured rubber trees limit their sustainability in the economic management CLFD. In addition, poor accessibility to the

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chemicals by the small and medium scale plantation owners also militate against its feasible and sustainable use in combating the menace of CLFD and other fungal diseases of tree crops. This has caused an obvious shift in disease management practices to the application of biological control as the most suitable alternative to chemical control methods of plant disease causing pathogens.

Several workers have investigated the use biotic agent in the control of plant diseases [10, 11, 6]. Osando and Waudo [10] 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. Many advanced countries are now placing ban on the frequent use of synthetic chemical pesticides due to it long term effect on humans and their ability to form toxic residues on agricultural produce and now promoting biological control strategy [12]. The commercial production of biopesticides formulation and their successful field application according to Wesseling *et al*. [13] remained a challenge in many developing nations despite recorded sporadic *in vitro* laboratory success. Information are however scarce on the use of phyllosphere fungi as potential biological control agents of diseases of economic crops. This study therefore screens some phylloplane fungi for their antagonistic potentials against *Corynespora* leaf fall disease of rubber.

MATERIALS AND METHODS

Isolation of Leaf Pathogen: Leaves of *Hevea brasiliensis* infected with *C. cassiicola* (Berk and Curtis) Wei were collected from the crown region of rubber seedlings in the nursery at the Rubber Research Institute of Nigeria (R.R.I, N), Iyanomo, Edo State, Nigeria. Bits (pieces) of approximately 1cm x1cm cut across lesions were made with sterile blade and surfaced sterilized by submerging in 0.1% of Mercuric Chloride for 1 minute after which they were rinsed in 10mL of sterile distilled water. This was the water was later decanted and the washing repeated five times before inoculated into previously prepared PDA media.

Isolation of Phylloplane Fungi: Phylloplane fungi were isolated from healthy leaves of 7 to 11 years old rubber plants at R.R.I.N nursery using the leaf washing technique of Blakeman [14] and the dilution method of Pelczar and Chan [15]. A 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 10mm disc of the pathogen with similar size of each potential antagonist was taken from the edge of a 5-days-old pure culture using a sterile cork borer. This was plated 20 mm apart respectively on PDA medium. Potential antagonists tried were identified based on their cultural and microscopic characteristics as *Trichoderma viride* Pers, *Aspergillus niger* kirk, *Gliocladium* sp, *Pleurothecium* sp, *Botrytis* sp, *Staphylotrichum coccosporum* May and Nicot. *Trichocladium* sp, *Gonatorrhodiella parasitica* Thaxt. *Trichophyton* sp and *Syncephalastrum* sp. The control plates were inoculated with the pathogen and antagonists separately.

Four replications per treatment were set up for each dual inoculation (pathogen and antagonist). The inoculated Petri-plates were incubated in the laboratory at room temperature. Daily growth measurement towards and away from the opposing fungal colony was recorded for a period of 10 days. The percentage inhibition of radial growth of the pathogen was calculated according to Vincent [16] and Jacob [17]. The mode of interaction was rated following the method of Fokkema [18] .i.e. No visible sign of inhibition of pathogenic fungi, the mycelium of which overgrew the test organism (0), Mutual inhibitions. Both organisms stopped growing on contact at the center or close to the center of the Petri-plate (1), Pathogen with inhibition zone < 1cm in width (2), Pathogen with inhibition zone > 1cm in width. Test fungus had grown across the center line of the Petri dish (3),Pathogen by overgrowth or displacement of pathogen by the fungus (4).

RESULTS AND DISCUSSION

Table 1 enumerated the antagonistic ratings of ten phylloplane fungi tested on *C. cassiicola.* The mycelial growth measurement (inhibition zone) of *C. cassiicola* against each of the ten antagonists on PDA after ten days incubation period and the percentage inhibition of *C. cassiicola* were summarized in Table 2. A significant inhibitory activity was observed for *A. niger* and *T. viride. Trichocladium* sp inhibited the growth of *C. cassiicola* by 76%. The inhibition of the growth of *C. cassiicola* in the dual cultures may be due to differences in nutrient resource utilization capacity between both target and test fungi, growth rate and metabolic waste product interference by *Trichocladium* sp. *A. niger* recorded 79% inhibition until the sixth day after the dual inoculation

Table 1: Antagonistic rating of ten phylloplane fungi tested on *C. cassiicola.*

	Antagonistic rating			
Phyllolane Fungi	1	2	3	4
Aspergillus niger	-	-	-	+
Botrytis sp.	-	+	-	+
Gliocladium sp.	+	-	-	-
Gonatorrhodiella parasitica	-	-	+	-
Pleurothecium sp.	-	+	-	-
Staphylotrichum coccosporum	-	+	-	-
Synecephalastrum	+	-	-	-
Trichoderma viride	-	-	+	-
Trichocladium sp.	-	-	-	+
Trichophyton sp.	-	-	+	-

Table 2: Mycelial growth measurement of dual culture of both test (phylloplane) fungi and target fungus (Corynespora cassiicola) on PDA and their percent inhibition 10 days after inoculation.

Pathogen/ Antagonist	Mycelial growth ±SE (Treatment) cm	Mycelial growth (Control) cm	Percent inhibition (%)
C. cassiicola	0.825 ± 0.04	2.50	67.00
Botrytis sp*	1.200 ± 0.00	5.50	
C. cassiicola	0.800 ± 0.00	2.50	68.00
T.viride*	1.200 ± 0.00	5.50	
C. cassiicola	1.250 ± 0.05	2.50	50.00
Gliocladium sp*	0.750 ± 0.05	1.70	
C. cassiicola	0.825 ± 0.04	2.50	67.00
S. coccosporum *	2.500 ± 0.00	5.50	
C. cassiicola	0.600 ± 0.00	2.50	76.00
Trichocladium sp*	19.00 ± 0.00	5.50	
C. cassiicola	0.950 ± 0.04	2.50	62.00
G. parasitica *	0.775 ± 0.04	1.30	
Gonatorrhodiella sp*	0.525 ± 0.04	2.50	79.00
C. cassiicola	1.500 ± 0.00	5.50	
A. niger*	0.800 ± 0.00	2.50	68.00
C. cassiicola	1.175. ± 0.04	5.50	
C. cassiicola Pleurothecium sp*	0.775 ± 0.04	2.50	69.00
Trichophyton sp*	1.925 ± 0.04	5.50	
C. cassiicola	0.900 ± 0.04	2.50	64.00
Syncephalastrum sp*	0.800 ± 0.00	1.50	

when *C. cassiicola* was observed to overgrew *A. niger* in culture by 0.25mm through the ten-days period of the experiment. The temporary growth suppression behaviour of *C. cassiicola* by *A. niger* is not yet understood but it may not be unconnected with innate systematic acclimatization of *C. cassiicola* to the *A. niger* medium-altered environment [12]. Induced resistance due to the production of cassiicolin may have also accounted for the observation rather than direct parasitism [19,20]. Further investigation is however necessary for full understanding the intricate nature of the pathogen-test fungi interface-interaction and antagonistic activities.

Trichoderma sp did not overgrew *C. cassiicola* in culture but grew steadily past the centerline and stopped the growth of the pathogen on contact with a percent inhibition of 68% while *Trichophyton* sp inhibited the growth of *C. cassiicola* by 69% and observed to stop the growth of the pathogen after its mycelium ran over it by

0.65cm. The two phylloplane fungi exhibited moderate antagonistic activity which according to Sobia *et al*. [21] may be attributed to their individual ability to out-compete the pathogen. This is in accordance with their potentially fast growth rate.

Staphylotrichum sp showed slight inhibitory effect on *C. cassiicola.* It grew past the centerline of the Petri dish and ran over the pathogen by 1.27cm. The antagonist was able to stop the growth of the pathogen up till the end of the experiment. *Synecephalastrum* sp and *Gonatorrhodiella* sp also showed zones of inhibition of 0.30cm and 0.28cm respectively at their advancing interface with *C. cassiicola.* Although the use of these set of phylloplane fungi as potential biocontrol agent of plant pathogens is more recent and less reported in literature compared to soil fungi, the principle of action remains consistent and analogous [21,22] The inhibitory actions *Synecephalastrum* and *Gonatorrhodiella* against *C. cassiicola* may be connected to the production of potent fungicidal secondary metabolites that suppresses pathogen resistance capacity. This may also have accounted for the kind of interaction observed between *C. cassiicola* and *Gonatorrhodiella* sp *in vitro*. *Gonatorrhodiella* was observed to be slow growing with white fringe at its edge which faded out as the fungus advances *C. cassiicola* in the dual culture. Although, this ran contrary to expected result, it nevertheless suggests that even the pathogen (target fungus) under extraneous conditions showed a mild mycofungicidal resistance. The nature of resistance and its extent can however not be presently ascertained by this study.

Pleurothecium and *Botrytis* species showed weak antagonism while *Gliocladium* sp did not record any antagonistic or inhibitory effect on the pathogen. The pathogen grew past the centerline of the dual culture and stopped on contact with *Gliocladium* sp reflecting the possibility of intricate-biochemically induced resistance. This result is contrary to the report by Viterbo *et al*. [23] that listed *Gliocladium virens* as effective biocontrol agents of soil-borne pathogens.

Microscopic study of the interaction between *C. cassiicola* (pathogen) and a set of phylloplane fungi (*Trichoderma, Trichocladium, Aspergillus* sp, *Gonatorrhodiella* and *Trichophyton*) showed significant variation in the *in vitro* pathogen-test fungus interaction. The hyphae of *Trichoderma* was observed to coagulate the cytoplasm of *C. cassiicola* hyphaeat the interface in dual culture while *Trichophyton* developed hyphae that coiled loosely around those of the pathogen with no visible damage to the integrated of the hyphal walls. *Aspergillus* sp was observed to invade and lyze the hyphae of *C. cassiicola* on contact causing hyphal tip burst. This was in concordance with its mycoparasitic and/or proteolytic activities resulting in the production of differing levels of potent lytic enzymes (acid phosphatase, polygalacturonidase, alpha-amylase, invertase, esterase, chitinase etc.) and presented by Lahoz *et al*. [24]. The microscopy of the interaction between the pathogen, *Gonatorrhodiella* and *Trichocladium* showed that the hyphae *C. cassiicola* were bigger at the center of the Petri-dish than at the edge. The reason for this is not yet fully understood but it may be connected to the fungus physiochemical mechanism and nutrient distribution.

Aspergillus sp and *Trichoderma* sp showed the strongest antagonism by suppressing the growth of *C. cassiicola* 79% and 68% inhibition while *Trichophyton, Staphylotrichum* and *Gonatorrhodiella* mildly antagonized *C. cassiicola* in culture. *Synecephalastrum* sp inhibited the growth of *C. cassiicola* up to the 8th day of dual inoculation after which it overgrew *Synecephalastrum* by 0.75cm at the end of the experiment. *C. cassiicola* resistance may have been stimulated by the presence of cassiicolin in amounts (yet unknown) that could have reduced the growth vigor of the antagonist.

The search for sustainable alternatives to chemical management of plant disease continues to be a long term goal of agricultural research and development yet biological methods of reducing disease levels and increasing yields already exist in many traditional methods of crop management. However, integrated approach to disease management using a combination of disease control strategies is of recent a more attractive options adopted by crop production managers. Munnecke *et al*. [19] demonstrated this first weakening *Armillaria mellea* in soil by methyl bromide to all naturally occurring antagonistic fungi evade the pathogen. The exploitation of phylloplane fungi for the control of pathogens as demonstrated in this study has therefore further expanded the frontier of research into finding affordable, easy to mass produced in culture, non-toxic to humans, non-pathohenic to host and effective at low concentration mycofungicides [25]. This is besides contributing to baseline knowledge on locating biological control tools that occur in an easily distributed form and can be easily formulated as a mycofungicide.

REFERENCES

1. Rao, B.S., 1975. Maladies of *Hevea* in Malaysia. Rubber Research Institute of Malaysia; Kuala Lumpur., pp: 108.

2. Fernando, T.H.P.S., C.K. Jayasinghe, R.L.C. Wijesundera, W.P.K Silva and E.A.D.N. Nishantha, 2010. Evaluation of creening methods against *Corynespora* leaf fall disease of rubber (*Hevea brasiliensis*). J. Plant Diseases and Protection, 117(1): 24-29.
3. Igeleke, C.L., 1988. Disease of Rubber (*Hevea brasiliensis*) and their control. 10th Annual Conference of the Horticultural society of Nigeria, 6th - 8th November. pp:10.
4. Chee, K.H., 1990. Present status of rubber diseases and their control. Review of plant Pathol., 69(7): 430 B 443.
5. Begho, E.R., 1995. *Hevea* plantation establishment. In: Proceeding of training workshop on the *Hevea* plantation establishment held at RRIN Iyanomo 2nd B 4th August, pp: 55.
6. Ogbebor, N.O. and A.T. 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. African J. Biotechnol., 4(9): 996 B 100.
7. Manju, M.J., C. Jacob Kuruvilla, Idicula, P. Sabu and K.K. Vinod, 2002. *Corynespora* leaf fall disease management in *Hevea* using oil-dispersible and dust fungicide formulations. Indian J. Natural Rubber Res., 15(1): 44 B 48.
8. **MISSING**
9. Jaysinghe, C.K., K.E. Jaaysuriya and H.P.S. Fernando, 1995. Pentachlorophenol B An effective and economic fungicide for the management of white root disease. Journal of Rubber Research Institute Sri-Lank, 76: 61-70.
10. Osando, J.M. and S.W. Waudo, 1994. Interaction between *Trichoderma* species and *Armillaria* root rot fungus of tea in Kenya. International J. Pest Manage., 40: 69-74.
11. Tewari, S.N., 1995. *Ocimum sanctum* L. a botanical fungicide for rice blast control. Tropical Sci., 35: 263 - 273.
12. Kaewchai, S., K. Soytong and K.D. Hyde, 2009. Mycofungicides and biofertilizers. Fungal Diversity, 38: 25-50.
13. Wesseling, C., M. Corriols and V. Bravo, 2005. Acute pesticide poisoning and pesticide registration in Central America.
14. Blakeman, J.P., 1981. Microbial ecology of the phylloplane. Academic Press London. pp: 502.
15. Pelczar, M.J. and E.C.S. Chan, 1981. Laboratory exercises in microbiology. Mcgraw-Hill Book Company, pp: 197-347.
16. Vincent, J.M., 1927. Distortion of fungal hyphae in the presence of certain inhibitions. Nature, pp: 850.
17. Jacob, C.K., P. Srinivas and C.B. Roy, 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.
18. Fokkema, N.J., 1973. The role of saprophytic fungi in antagonism against *Drechslera sorokiniana (Helminthosporium sativum*) on agar plates and on rye laves with pollen. Physiology of Plant Pathol., 3: 195-B 205.
19. Munnecke, D.E., M.J. Kolbezen and H.D. Ohr, 1981. Interactions involved in controlling *Armillaria mellea*. Plant Dis., 65: 469-479.
20. El-Kassas, H.Y. and H.M. Khairy, 2009. A trial for biological control of a pathogenic fungus (*Fusarium solani*) by some marine microorganisms. America-Euroasian J. Agriculture and Environmental Sci., 5(3): 434 B 440.
21. Sobia, M., A. Amna, K. Ibatsam and M. Irum, 2010. Antagonistic potential of soil bacteria against food borne fungi. World Applied Sciences J., 11(8): 966-B 969.
22. Gomathinayagam, S., M. Rekha, M.S Sakthivel and S. Jagessar, 2010. The biological control of paddy disease brown spot (*Bipolaris oryzae*) by using *Trichoderma viride* in *in vitro* condition. J. Biopesticides, 3(1): 093-B 095.
23. Viterbo, A., J. Inbar, Y. Hadar and I. Chet, 2007. Plant disease biocontrol and induced resistance via fungal mycoparasites. In: Environmental and microbial relationships. 2nd edn. The mycota IV. Kubicet,C.P and Druzhinina I.S. eds. Springer-Verlag. Berlin Heidelberg. pp: 127-B186
24. Lahoz, R., F. Reyes, P. Gümez and M.J. Martinez, 1983. Lytic enzyme activity in autolysing mycelium of *Aspergillus niger*. Zeitschrift Für allgemeine Mikrobiologie, 23(1): 17-B 25.
25. Irtwange, V.S., 2006. Application of biological control agents in pre- and postharvest operations. Agricultural Engineering International: CIGR Ejournal. Invited Overview, 3: 1-12.