Evaluation of Effect of Antagonistic Fungi, and Arbuscular Mycorrhizal Fungi (AMF) on Incidences of Some Disease of *Hevea brasiliensis* (Muell. Arg).

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Abstract: Evaluation of the effect of the use of Arbuscular mycorrhizal fungi (AMF) and antagonistic fungi on the incidences of some diseases of *Hevea brasiliensis* (Muell.Arg.) was investigated. Saplings of RRIC45 were grown in heat sterilized soils inoculated with an AMF strains (*Glomus deserticola*) and soils not inoculated at all. In vitro assessment of the efficacy of three potential antagonists-*Trichoderma*, *Penicillum* and *Aspergillus* species against white root rot (*Rigidoperus lignosus*) fungus were also determined. Effects of AMF on Bird's eye spot incited by *Drechslera heveae*, and C*olletotrichum gloeosporioides* leaf spot showed some level of bioprotection of the seedlings ($P<0.05$).Of the three antagonists,*Trichoderma* sp appeared most outstanding with 81.85% inhibitory rating against *R. lignosus* , than *Penicillium* sp (65.27%) and *Aspergillus* sp with no trace of inhibition. The results of the study are discussed.

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1. Introduction

Fungi pathogens may infect the leaves, stem and branches, and roots of rubber plants which are usually prone to infection from the nursery stages. Rubber is plagued with a plethora of infective fungi such as *Colletotrichum gloeosporioides* (Penz) Sac., *Drechslera heveae* (Petch) Ellis, *Corynespora Cassiicola* (Berk and Curt) Wei, and *Rigidoporus Lignosus* (*Klotzsch*) Imazeki (Von Arx, 1974, Rao, 1975), among others. In Nigeria, over 65% of rubber diseases are caused by fungi (Begho, 1995), and *Colletotrichum* leaf spot, and Bird's eye leaf spot (*Drechslera heveae*) are notorious in their destructive actions and pose a great threat to the growth of rubber seedlings in the nurseries causing retardation, die back, secondary leaf fall, and eventual death of seedlings. In the plantation, they are implicated in the reduction of latex (Webster and Baulkwill, 1989, Begho, 1995, Sabu *et al*, 2000).

The arbuscular mychorrhizal fungi (AMF) occur in virtually all vascular terrestrial plants and are extremely ubiquitous in soils (Baltruschat, 1987). Their ecological category as root inhabitants is beneficial in terms of properties relating to their behaviour in the soil, host infection, effect on host, and special physiological requirements are relevant and similar in mycorrhizal association; the hyphal network formed by AMF is known to provide a greater absorptive surface and this increases the absorption of relatively immobile ions such as phosphate, zinc and copper ions (Bagyaraj and Sturmer, 2008). In particular, the low available phosphate ions limit plant development. Beneficial effect of increased uptake of P by AMF results in greater vigour of plants and this is the basis of plant resistance to disease (Okeke, 1990, Omorusi, 2005).

The AMF exhibit various inhibitory actions against pathogen infection. They are known to produce antibiotic compounds, volatile and non volatile compounds that are fungistatic to pathogen development (Schenck, 1981). They also stimulate rhizospheric mycoflora antagonistic to pathogens, as well as increased wall thickening in the cortical cells to deter pathogen penetration (Schenck, 1981). Production of higher levels of amino-acids especially argines can reduce development of chlamydospore as in the inhibition of *Thielaviopsis basicola, fusarium oxysporum, phytophthora spp.*

The effects of antagonistic fungi such as *Trichoderma, Peninillium*, and *Aspergillus* species on the reduction of incidence of pathogens and the immense contribution of AMF in the control of plant diseases are significant in the sustainability of agriculture.

2. Materials and Method

2.1 Effect of Test Antagonists

In particular reference to white root rot (WRR) disease of *Hevea Brasiliensis,* three antagonistic fungi, namely *Trichoderma*, *Aspergillus* and *Penicillium* species were tested against the development of *Rigidoporus lignosus,* the causal agent of WRR. Pure cultures of the antagonists and pathogens were prepared from isolation from the rhizosphere, and infective rhizomorph of *Hevea brasiliensis,* respectively. All pure cultures obtained were devoid of bacterial contamination achieved by fortifying the potato dextrose agar (PDA) with penicillin and streptomycin antibiotics at the rate of 0.2 ml of the stock solution per 20 ml PDA (Tuite, 1969). The composition of the stock solution was 0.1 g of streptomycin and 10^6 international units (I.U). of penicillin. For antagonistic and pathogen interaction, a 5 mm mycelial disc was incised from the edge of their pure cultures. Discs for interaction test between *R. lignosus, Trichoderma, Penicillin,* and *Aspergillus* were plated 25 mm apart on PDA in a 9cm Petri plates with three replications for each pairing between the pathogen, and the antagonists and subsequently incubated at 26 $\pm$ 2 ^{0}C. Radial growth measurement between the pathogen and the antagonist were taken for five days. Percentage inhibition of growth in each pair treatment was determined (Jayaratne *et al*., 2000).

2.2 Use of Arbuscular Mycorrhizal Fungal Isolate

Monoclonal *Hevea Brasiliensis* RRIC 45 seedlings were grown in heat sterilized soils and inoculated with an exotic strain of AMF *Glomus* deserticola after multiplication in pot cultures in heat sterilized soil (pH 5.9, P 3.62 ppm and OC 0.94%) using trap plant (*Zea mays)* (Sieverding, 1991). The seedlings, in the heat sterilized soils was amended with four levels of P_2O_5 (SSP as source) (0, 7, 14.8, and 21 kg/ha) around, below and above optimum (Onuwaje and Uzu, 1982) at the rates of 0, 0.28, 0.59 and 0.83g SSP/8kg soil, respectively, with five replications per treatment combination, were established outdoors in a screen house and in surrounding vicinity, of a rubber nursery, which served as source of foliar infection.

Effect of *G. deserticola* on C. gloeosporioides, and *D. heveae* leaf spots, height and girth, root colonization of test seedlings and elemental concentrations – N, P, K, Ca, mg and Zn were evaluated. Disease assessment of infected leaves was based on Parry (1990) formula.

3. Results and Discussion

Inhibitory effects of antagonistic against the pathogen were observed. Of the three inhibitory antagonists, *Trichoderma* species proved to be the most effective against *R. lignosus.* The percentage inhibition recorded was 81.85%. This was followed by *penicillium* species with 65.27% inhibition rating. Inhibitory effect of *Aspergillus* species on *R. lignosus* was less effective as no visible inhibition was obtained.

The mechanism of inhibition is not clearly understood, however, Jayasuriya et al. (1996) stated that *Trichoderma* species exhibit varied antagonistic actions as it is able antagonize basidiomycetes by a complex mechanisms such as the production of soluble metabolites, inhibitory volatiles and lytic enzymes. Hashim (1990) has known that a combination of *Trichoderma* and appropriate concentration of fungicide is efficacious against pathogens. Further investigation of this combination against *R. lignosus* is necessary.

The result obtained for the inhibitory effect of *Pencillium* is consistent with similar result of Jayasuriya *et al.* (1996), and is suspected to be due to production of antibiotics diffused into the medium inhibiting the growth of *R. lignosus.*

The failure of *Aspergillus* to impact inhibitory effect on *R. lignosus* was unexpected. It is suggested that the use of an appropriate medium to initiate inhibitory action from wood blocks may be required (Jayasuriya and Deacon, 1996).

Effect of AMF – *G. deserticola* produced taller and bigger inoculated seedlings at 3, 4 and 6, months after planting (MAP) than those from the uninoculated. Percentage AMF root colonization of the inoculated seedlings was significantly higher (62.92%) ($P<0.01$) than the uninoculated plants (Table 1).

Appearance of symptoms of *colletotrichum* and bird's eye spots was noticed within two months of exposure to natural infection. In general, percentage reduction of the foliar disease incidence by *G. deserticola* was greater in the inoculated than uninoculated seedlings, however, greater disease reduction effects were recorded on colletotrichum incidence in both the screen house and exposed seedlings (Table 1). Reduction of the foliar diseases in the inoculated plants was significant ($P<0.05$) both in the screen house and exposed seedlings than uninoculated plants. This reduction of incidences of the two major foliar diseases in Hevea is reported for the first time in Nigeria. The ability of *G. deserticola* to confer bio-protection to the seedlings may be attributed to the improved plant nutrition brought about by AMF root colonization and nutrient uptake (Rhodes, 1980).

Elemental concentrations of N, P, K, Ca, Mg and Zn in the lamina tissues of 6 months old seedling are shown in table 3. Available N and P were lower than other elements. Available Mg Ca, K and Zn were high but gave no significant effects in the inoculated and uninoculated screened and exposed seedlings (Table 2). It is expected that roots of inoculated plants by AMF will respond to lower fertilizer application especially P in the inoculated than the uninoculated plants (1kram *et al.,* 1996).

Table 1. Effect of VAM root colonization of 6 mo-old 45 rubber seedlings in soil inoculated with Glomus deserticola fungus on foliar growth and disease reactions.

	Growth Parameter			Disease infection score			
Soil Treatment	Average VAM root Colonization %	Leaf z (CM)2	Number of Leaflets z	Indices y, z		Percentage (%)	
				BES*	CLS**	BES*	CLS**
		Screened	Seedlings				
Inoculated	66,94	98.05 (20.62)	36.25 (1.96)	8,76 (12.73)	1.86 (9.33)	30.06	5,94
Uninoculated	38,33	78.60 (16.53)	26.75 (1.84)	8.54 (1077)	2.64 911.72	29.86	9.92
		Exposed	Seedling				
Inoculated	58,89	91.90 (16.02)	28.50 91.75)	12.43 (9.04)	5.87 (11.23)	42,73	20,41
Uninoculated	26,94	69,12 (25.22)	26.50 (1.87)	22.48 (7.04)	10.59 (8.11)	59,07	29,23

* Bird's eye spot. ** Colletotrichum leaf spot. Y Disease infection rating as proposed by Parry (1980).
Z means (with standard error in parenthesis) of 5 replications.

Table 2. Concentrations of N, P, K, Ca, Mg and in lamina tissue of 6 mo-old rubber seedlings.

	Screened		Exposed	
Elemental Conc. (ppm)	**Inoculated (mycorrhizal)**	**Uninoculated (Nonmycorrhizal)**	**Inoculated (mycorrhizal)**	**Uninoculated (Nonmycorrhizal)**
N	1.79 $\pm$ 0.02a	1.97 $\pm$ 0.72a	1.95 $\pm$ 0.01a	2.0 $\pm$ 0.03a
P	1.51 $\pm$ 0.01a	1.63 $\pm$ 0.03b	1.94 $\pm$ 0.17a	1.71 $\pm$ 0.07a
K	15.89 $\pm$ 0.46a	11.71 $\pm$ 0.11b	13.89 $\pm$ 0.14a	14.00 $\pm$ 0.29a
Ca	15.21 $\pm$ 0.30c	11.89 $\pm$ 0.82c	18.82 $\pm$ 0.31c	18.68 $\pm$ 0.42b
Mg	7.48 $\pm$ 0.30c	6.99 $\pm$ 0.31b	9.40 $\pm$ 0.2b	7.96 $\pm$ 0.22c
Zn	8.35 $\pm$ 0.24c	15.24 $\pm$ 41a	12.96 $\pm$ 0.25c	13.85 $\pm$ 0.32a

* Means (with standard error in parenthesis) of three replicate treatment of plants. Means with same letter within a column are not significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

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REFERENCES

[1.] Bagyaraj, J.D. and Stűrmer, S.L. 2008: Arbuscular Mycorrhizal Fungi, (AMF) in: A handbook of Tropical Soil Biology/Eds. F.M.S. Moreira, E.J. Huising, D.E. Bignell. Earthscan publishers, London. (pp 131-143).

[2.] Baltruschat, H. 1987: Field Inoculation of maize with vesicular -arbuscular mycorrhizal fungi by using expanded clay as carrier material for mycorrhizal. *Journal of Plant Diseases and Protection,* 94 (4), 419-430

[3.] Begho, E.R. 1995: Hevea Plantation Establishment. *Proceedings of Training Workshop on the Hevea Plantation Establishment.* Rubber Research Institute of Nigeria, Iyanomo, Benin City, 2nd – 4th August, 1995, 55 pp.

[4.] Hashim, I. 1994: Effect of fungicides defoliaton and manuring on Corynespora leaf fall of Hevea rubber. IRRDB *Symposium on Diseases of the Hevea, Cochin, India.*

[5.] Ikram, A., M.N. Sudin and D. Napi 1996: Phosphate response curves of mycorrhizal *Hevea brasiliensis* in two sterilized soils. *Journal Natural Rubber Research* 11 (1): 59-68.

[6.] Jayaratne, R., P.C. Wettasinghe, D. Siriwardene and D. Peiris. 2001: Systemic fungicides as a drench application to control white root disease rubber. *Journal Rubber Research Institute of Sri Lanka*, 84:1-7.

[7.] Jayasuriya, K.E. and J.E. Deacon. 1996: Possible role of 2-Furaldehyde in the biological control of white root rot disease of rubber. *Journal*

Rubber Research Institute of Sri Lanka, 77:15-77

[8.] Jayasuriya, K.E., J.E. Deacon and T.H.P.S. Fernando 1996: Weakening effect of 2-furaldehyde on Rigidoporus *lignosus. Journal Rubber Research Institute of Sri Lanka*, 77:15-77

[9.] Okeke, J.E. 1990 Status of the cultural management component in an integrated control of the cassava mealybug and cassava green spider mite. *Proceedings of the Workshop on the global status and prospects for integrated pest management of root and tuber crops in the Tropics*. 20-30th October, 1987, Ibadan, Nigeria, 235 pp.

[10]. Omorusi, V.I. 2005: Benomyl and NPK soil treatment effects on cassava response to diseases, pests and mycorrhizal symbiosis. Ph.D Thesis, University of Benin, Benin City, Nigeria, 219 pp.

[11.] Onuwaje, O.U and F.O. Uzu. 1982: Growth response of rubber seedlings to N,P, and K fertilizer in Nigeria. *Fertilizer research* 3: 169-175.

[12.] Parry, D. 1990: Plant pathogen in Agriculture. Cambridge Press. UK.

[13.] Rao, B.S. 1975: Maladies of Hevea in Malaysia, Rubber Research Institute of Malaysia, Kuala Lumpur, 108 pp

[14.] Rhodes, L.H. 1980: The use of mycorrhizae in crop production systems. *Outlook Agriculture* 10: 275-281.

[15.] Sabu, P.I., Kuruvila, C.J., Manju, M.J. and Kothandaraman, R. 2000: Current status of Corynespora Leaf fall disease in India. Presented at the International Rubber Research and Development Board (IRRDB) workshop on Corynespora leaf fall of Rubber in Kuala Lumpur and Medan from 6th – 14th.

[16.] Schenck, N.C. 1981. Can Mycorrhizae Control Root Disease? *Plant Disease* 65 (3): 230-234

[17.] Sieverding, E. 1991: Vesicular-arbuscular mycorrhizal management in tropical agro system. Deutsch Gesellshft fur technische zusammenarbeit (GTZ). Dag Hammarskjdd weg. Germany, 371 Pp.

[18.] Tuite, J. 1969: Plant Pathological Methods. Burgess Publishing Co. Minn. USA. 239 pp

[19.] Von Arx, J.A. 1974: The genera of fungi sporulating in pure culture. (2ndEd.) In der A.R. Gantner verlag Kommenditg esellschaft. 167-221 pp.

[20.] Webster, C.C. and Baulkwill, W.E.J. 1989. Rubber. Tropical Agricultural Series. *Longman Scientific and Technical*. U.K. 614 pp.

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