Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 06, pp.2179-2182, June, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

RHIZOPLANE AND RHIZOSPHERE MYCOFLORA OF *CYNODON DACTYLON* (L.) Pers. GRASS AND THEIR ANTAGONISTIC ACTIVITY AGAINST FUNGAL DISEASES OF MAHOGANY SEEDLINGS

***Bharathnair and Mallikarjunaswamy, G. E.**

Department of Forest Pathology, Kerala Forest Research Institute, Peechi-680653, Thrissur, Kerala, India

Received 17th April, 2018; Accepted 21st May, 2018; Published 12th June, 2018

ABSTRACT

Microorganisms found associated with plants reside in rhizosphere, rhizoplane, phylloplane and inside plant tissues. These microbes play an important role in various stages of plant growth and development. They also find application as biocontrol agent against various pathogens of plants and can be used as an alternative to chemical fungicides. Grasses and their roots are major source for microbial interaction and get rejuvenated therefore the chances of colonization of microbe is high. In view of this, the present work has been carried out to study the antagonistic activity of fungal organisms associated with rhizosphere and rhizoplane regions of grass *Cynodon dactylon* from Northern Kerala parts of Western Ghats in different seasons against fungal diseases of Mahogany seedlings of Central Nurseries of Kerala *in vitro*. Rhizosphere and rhizoplane fungi included *Aspergillus niger*, *Curvularia* sp. *Fusarium oxysporum*, *Fusarium* sp. *Mucor* sp. *Penicillium* sp. *Rhizopus* sp. *Trichoderma harzianum* and NSF. Antagonistic activity was conducted against the fungal species associated with the root rot diseases of mahogany seedlings caused by *Fusarium oxysporum* and *Fusarium monoliforme,* damping off by *Sclerotium rolfsii* and foliar diseases caused by *Colletotrichum gloeosporiodes, Alternaria alternata, Curvularia lunata,Cladosporium cladosporioides* and *Pestalotiopsis* sp. *Trichoderma harzianum* was found to be effective controlling agent *in vitro*.

Key words: *Cynodon dactylon,*Rhizosphere, Rhizoplane, *Trichoderma harzianum*, Antagonism

Citation: Bharathnair and Mallikarjunaswamy, G. E. 2018. "Rhizoplane and rhizosphere mycoflora of *Cynodon dactylon* (l.) pers. grass and their antagonistic activity against fungal diseases of mahogany seedlings" *International Journal of Current Research in Life Sciences*, 7, (06), 2179-2182

INTRODUCTION

Mahogany (*Swietenia macrophylla* King) belongs to family Meliaceae known for its timber. The out-planting success greatly depends upon their seedling health which are hindered majorly by fungal pathogens. Forest nursery diseases- collar rots, damping-off, root rots, foliar diseases and blights cause great damage to seedlings. Fungicides due to their assured results are being practised but indiscriminate use led to the development of fungicidal resistance inpathogens and toxicity to non – target organisms (Tjamos *et al.*, 1992). This led into the search for an alternative, risk free strategy for the management of diseases employing the use of biocontrol micro-organisms. The role of soil fungi is complex, helping in nutrient cycling, plant growth and development (Thorn 1997, Bridge and Spooner 2001, Martin *et al.* 2001). Rhizosphere and Rhizoplane inhabiting micro-organisms competitiveness for water, nutrients and space plays an important role in the growth and ecological fitness of their host (Hartmann *et al.* 2009). Grasses which forms an important part of ecosystem keeps rejuvenating in every growing season, homes a number of diverse microorganisms. Fungal communities of anamorphic and teleomorphic ascomycetes, zygomycetes and certain non-sporulating fungi resides in rhizosphere and rhizoplane regions (Vasanthakumari *et al.*, 2007). These microorganisms can be used for their antagonistic potential. The present work has been carried to characterize the rhizosphere and rhizoplane mycoflora associated with grass *Cynodon dactylon* and study their antagonistic activity against fungal pathogens *in vitro.*

MATERIALS AND METHODS

Isolation of pathogenic fungi: Disease survey was carried out in Central nurseries(Chettikulam, Kannavam, Kulathupuzha and Nilambur) of Kerala. Infected Mahogany seedling samples were collected, washed thoroughly in running tap water, surface disinfected with $HgCl_2$ (0.0001%), blotted and were inoculated on streptomycin amended PDA medium. Petridishes were incubated under 12/12 hr alternate light regime at 25±2° C. Fungal colonies were isolated and identified on the basis of colony morphology, mycelium, fruiting-body, spore shape and size by referring standard manuals (Arx, 1981; Ellis and Ellis, 2001; Gilman, 1994; Ramarao and Manoharachary, 1990; Subramanian, 1983).

Isolation of rhizosphere andrhizoplane fungi: Rhizosphere and rhizoplane samples of *Cynodon dactylon* were collected from Northern Kerala parts of Western Ghats. Root samples were washed thoroughly in slow running tap water, surface disinfected with $HgCl_2$ (0.0001%), blotted and were cut into 1 cm segments.

Table 1. Disease symptomology and fungal pathogens associated with diseased parts of Mahogany seedlings from central nurseries of Kerala

Sl. No.	Fungal pathogens	Disease symptoms and description	Central Nurseries of Kerala			
			KNM	NBR	CKM	KPZ
1	*Alternaria alternata*	Leaf blight: greyish spot which coalesced to form necrotic lesions	+	-	+	-
2	*Cladosporium cladosporioides*	Leaf spot: brown to black colour spot	+	-	-	+
3	*Colletotrichum gloeosporioides*	Leaf spot and blight: irregular spot, light to dark brown in colour, with a pale margin	+	+	+	+
4	*Curvularia lunata*	Leaf spot: olivaceous brown spot	+	+	+	+
5	*Fusarium monoliforme*	Root rot and Leaf spot	+	+	-	-
6	*Fusarium oxysporum*	Root rot	+	+	+	+
7	*Pestalotiopsis* sp.	Leaf spot: brown to dark brown spots, occasional fructifications developed	+	+	+	+
8	*Sclerotium rolfsii*	Damping off	-	+	-	+

KNM – Kannavam, NBR – Nilambur, CKM – Chattikulam, KPZ – Kulathu[puzha; '+'presence, '-'absence

Table 2. Fungal species from rhizosphere and rhizoplane regions of *Cynodon dactylon* grass

Sl. No.	Fungal Organisms	*Cynodon dactylon* grass	
		Rhizoplane region	Rhizosphere region
1	*Aspergillus niger*	+	+
2	*Curvularia* lunata	+	-
3	*Fusarium oxysporum*	+	+
4	*Fusarium monoliforme*	-	+
5	*Fusarium* sp. (3)	+	-
6	*Mucor* sp.	+	+
7	*Penicilliumchrysogenum*	-	+
8	*Penicilliumoxalicum*	+	+
9	*Rhizopus* sp.	+	+
10	*Trichoderma harzianum*	+	-
11	NSF-1	+	-
12	NSF-2	-	+

'+'presence, '-'absence

Table 3. Rhizosphere and rhizoplane fungi of *Cynodon dactylon* showing antagonism to fungal pathogens isolated from Mahogany seedlings *in vitro*

Sl. NO.	Fungal organisms isolated from *Cynodon dactylon*	Antagonistic activity of fungal isolates against fungal pathogens isolated from mahogany seedlings (%)[1]							
		F.o	*F. m*	*S. c*	*A. a*	*C. g*	*C. c*	*C. l*	*P.* sp.
1	*Aspergillus niger*	30.54±0.5[2]f[3]	33.23±0.7c	35.87±0.9e	36.76±0.5f	40.42±0.1g	37.14±0.2h	32.56±0.9f	39.87±0.1f
2	*Curvularialunata*	20.59±0.8b	32.56±0.8b	33.24±0.3c	17.98±0.1b	16.67±0.1b	11.98±0.0e	23.57±0.4c	22.70±0.2b
3	*Fusarium oxysporum*	23.53±1.1c	51.16±0.6f	32.45±0.4c	24.51±1.2e	29.87±1.1e	12.50±0.4e	21.43±0.2b	29.50±0.8d
4	*Fusarium monoliforme*	32.35±0.5g	39.53±1.0de	34.53±0.2d	24.65±0.2e	19.97±0.0c	6.00±0.08b	29.91±0.8e	25.00±0.4c
5	*Fusarium* sp. (3)	17.65±0.3a	41.86±0.5e	42.01±0.3f	19.87±0.0c	23.54±1.1d	8.17±0.06d	24.58±0.4d	34.09±0.5e
6	*Penicilliumchrysogenum*	27.86±1.2d	32.43±0.5b	35.67±0.9de	39.76±0.2h	33.56±0.0f	28.76±0.3g	36.98±0.2h	43.64±0.7h
7	*Penicilliumoxalicum*	29.14±0.4e	37.32±0.1cd	33.29±0.6c	38.74±0.2g	34.42±0.6f	25.44±0.0f	33.57±0.6g	41.77±0.5g
8	*Trichoderma harzianum*	73.20±0.2h	77.50±0.9g	72.09±0.1g	70.96±0.4i	82.14±0.2h	72.50±0.3i	71.16±0.5i	71.00±0.3i
9	NSF-1	17.65±0.7a	23.26±0.2a	6.78±1.16a	13.21±0.9a	12.87±0.2a	7.14±0.87 c	16.73±0.4 a	13.64±0.6 a
10	NSF-2	17.63±0.7a	32.56±0.4b	27.89±0.8b	21.86±0.1d	19.87±1.1c	5.04±0.77a	21.32±0.4b	29.55±0.3d

[1]Data is an average of three replicates, [2] Standard deviationand [3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference. Among the fungal isolates tested against various pathogens, *Trichoderma harzianum* exhibited higher antagonistic activity.
F.o- Fusarium oxysporum, F. m- Fusarium monoliforme, S. c- Sclerotium rolfsii, C. g- Colletotrichum gloeosporioides, C. c- Cladosporium cladosporioides, C. l- Curvuaria lunata, P. sp.- *Pestalotiopsis* sp.

Root segments were inoculated on streptomycin amended PDA medium maintaining equal distance. Fungal colonies were identified as described earlier. Rhizosphere samples were subjected for dilution plate technique. The samples of desired dilution 10^{-3} and 10^{-4} were inoculated on PDA medium and were incubated for 5-7 days. Fungal colonies were isolated and identified as described earlier.

***In vitro* antagonism by dual culture technique:** Pathogenic fungi isolated from mahogany seedlings, and the test fungi from rhizosphere and rhizoplane of *Cynodon dactylon* grass were cultured on their respective medium under 12/12 hr light and dark cycle at 25±2°C for five days. Five mm diameter disc of selected fungi from grass and test pathogenwere taken from the growing edge of a five-day-old pure culture using a cork borer. The control plates wereinoculated with the pathogen and antagonists separately. Petri-plates were incubated at 25±2°C and daily growth measurements of fungal colonies were recorded for seven days.

The percentage inhibition of radial growth of the pathogen was calculated using formula (Vincent, 1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control
R_2 – Test organism in Dual culture

Statistical analysis: Antagonistic ability of fungal isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

RESULTS AND DISCUSSION

Disease survey of mahogany seedlings conducted in Central nurseries of Kerala yielded seven genera and eight species of fungi and showed the disease symptoms were root rot, damping off and foliar infections (Table 1).

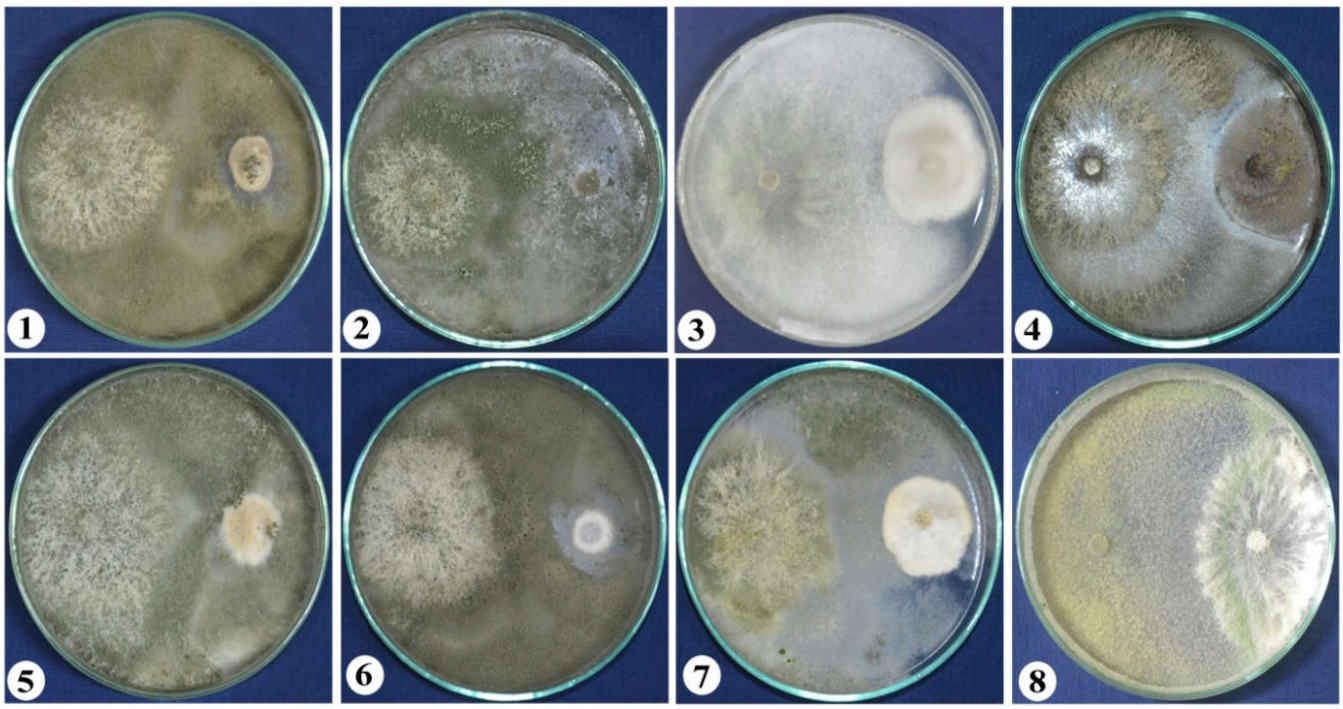

Fig 1-8. Antagonistic activity of *Trichoderma harzianum* against fungal pathogens (1) *Alternaria alternata*, (2)*Cladosporiumcladosporioides*, (3) *Colletotrichum gloeosporioides*, (4) *Curvularia lunata*, (5) *Fusarium monoliforme*, (6) *Fusraium oxysporum*, (7) *Pestalotiopsis* sp. *and* (8) *Sclerotium rolfsii*

Root rot caused by *Fusarium oxysporum* and foliar diseases caused by *Alternaria alternata*, *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides, Curvularaia lunata*, *Pestalotiopsis* sp., damping-off by *Sclerotium rolfsii* and root rot and leaf spot caused by *F. monoliforme*were found to be major disease causing fungal pathogens in Central nurseries of Kerala. Various diseases in forest crops, Teak (Mohanan, 2001, 2011; Sharma *et al.*, 1985), *Acacia* (Sharma and Florence, 1996), *Albizia* (Sharma and Sankaran, 1987) have been reported. Various bio-control approaches have been practised in forest nurseries. Mohanan (2007) reported that *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* were effective against damping-off pathogens *Rhizoctonia solani* and *Cylindrocladium quinqueseptatum*. Rhizosphere and rhizoplane regions of *Cynodon dactylon* were associated with diverse mycoflora (Table 2). The antagonistic interaction of *Trichoderma harzianum* (Fig 1- 8) among the fungal isolates showed maximum inhibition activity (70 – 83%), other isolates tested were shown to be moderate to low activity against the pathogens (Table 3). Species of *Trichoderma* namely *T. koningii*, *T. harzianum* and *T. viride*, respectively have been studied for their antagonistic activity *in vitro* (Mathew and Gupta, 1998; Prasad *et al.*, 1999; Bunker and Mathur, 2001; Pandey *et al.*, 2005; Grosch *et al.*, 2007). The present work showed the potentiality of rhizosphere and rhizoplane mycoflora against various forest plant pathogens and can be used as an alternative to chemical fungicides.

Conclusion

Upsurge in the use of chemical agents and its potential threat to the ecosystem has led to foresee an alternate and eco-friendly strategy. Biological control has been practised with such an aim. Rhizosphere and rhizoplane regions of grasses homes diverse fungal organisms and can be used as biological weapons against various plant pathogens.

Acknowledgement: The authors are grateful to the Director, KFRI and to the field staffs at various central nurseries in the state.

REFERENCES

Arx, V. J. A. 1981. The genera of fungi sporulating in pure culture, J. Cramer. In der A.R. Gantner Verlag Kommanditgesellschaft. FL – 9490, Vaduz. 424.

Barnett, H. L. and Hunter, B. B. 1972. Illustrated genera of imperfect fungi, Third edition, Burgess Publishing Company, Minneapolis 15, Minn. USA. 241.

Berg, G. 2007. Analysis of antagonistic interactions between *Trichoderma* isolates from Brazilian weeds and the soil-borne pathogen *Rhizoctonia solani*. *J. Plant Dis. Protec.,* 114:167-175.

Bridge P, Spooner BM. 2001 – Soil fungi: diversity and detection. Plant and Soil 232, 147–154.

Bunker, R. N., and Mathur, K. 2001. Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root rot of chilli. *J. Mycol. Pl. Pathol.,* 31:50-53.

Domesch, K. H. and Gams, W. 1972. Fungi in Agricultural soils, Longman Groups Ltd., London. 290.

Ellis, M. B. and Ellis, J. P. 2001. Microfungi on land plants an identification handbook. Croom helm Australia Pvt. Ltd., Sydney, Australia. 818.

evolution of pathogen aggressiveness. New Phytologist 129, 371–387.

fungal community at the interphase between soil and roots (eds R Pinton, Z Varanini, P Nannipieri. New York, Marcel Dekker 263–296.

Gilman, J. C. 1994. A manual of soil fungi. The Iowa state college Press-Ames, Iowa, USA. 450.

Grosch, R., Lottmann, J., Rehn, V. N. C., Rehn, K. G., Mendonca-Hagler, L., Smalla, K., and

Hartmann A., Schmid M., van Tuinen D. and Berg G., 2009. Plant-driven selection of microbes. *PlantSoil*, 321, 235-257.

Jarosz AM, Davelos AL. 1995. Effects of disease in wild plant populations and the

Martin F.M., Perotto S., Bonfante P. 2001. Mycorrhizal fungi. In: The Rhizosphere – a

Mathew, A. K., and Gupta, S. K. 1998. Biological control of root rot of french bean caused by *Rhizoctonia solani*. *J. Mycol. Pl. Pathol.,* 28:202-205.

Mohanan, C. 2001. Biological control of Damping-off in forest nurseries in Kerala. Kerala Forest Research Institute Research Report NO. 214, India 65.

Mohanan, C. 2007. Biological control of seedling diseases in forest nurseries in Kerala. *J. Biol. Control.,* 21(2):189-195.

Mohanan, C. 2011. Pink Disease in Teak Plantations and its Management. Kerala Forest Research Institute Research Report NO. 386, India 25.

Pandey, K. K., Pandey, P. K., and Upadhyay, J. P. 2005. Mycoparasitism of *Trichoderma* spp. on *Fusarium* and *Rhizoctonia*. *J. Mycol. Pl. Pathol.,* 35:174-176.

Prasad, R. D., Rangeshwaran, R., and Kumar, S. P. 1999. Biological control of root and collar rot of sunflower. *J. Mycol. Pl. pathol.,* 29:184-188.

Ramarao, P. and Manoharachary, C. 1990. Soil fungi from Andhra Pradesh. Department of Botany, Osmania University. 164.

Sharma, J. K., and Florence, E. J. M. 1996. Fungal Pathogens as Potential Threat To Tropical Acacias, A case study of India. Kerala Forest Research Institute Research Report NO. 113, India 44.

Sharma, J. K., and Shankaran, K.V. 1987. Diseases of *Albizia falcataria* in Kerala and their Possible Control Measures. Kerala Forest Research Institute Research Report NO. 47, India 50.

Sharma, J. K., Mohanan, C., and Florence, E. J. M. 1985).Disease Survey in Nurseries and Plantations of Forest Tree Species Grown in Kerala. Kerala Forest Research lnstitute, Research Report No. 36, India, 268.

Subramanian, C. V. 1983. Hyphomycetes. Taxonomy and Biology. *Academic Press*, London, Vol. I and II. 930.

Thorn G. 1997) – The fungi in soil. In: Modern Soil Microbiology (eds JD van Elsas, JT

Tjamos EC, Papavizas GC, Cook RJ. eds) Biological control of plant diseases. Progress and challenges for the future. *Plenum Press, New York,* 1992.

Trevors, EMH Wellington. New York, Marcel Dekker 63–127.

Vasanthakumari MM, Mallikarjunaswamy GE, Shivanna MB. 2007 – Root mycoflora of certain grass species of Eragrosteae tribe in forests near Bhadra reservoir of Shimoga dist, Karnataka. Paper presented at 2nd Asian Congress of Mycology and Plant Pathology,Osmania University, Hyderabad, A.P., India.

Vincent, J.H.(1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 15: 850.

****