Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
10(1): 514–523 (2020) ISSN 2229-2225
www.creamjournal.org

Article

Doi 10.5943/cream/10/1/40

Bio-control potentiality of *Penicillium multicolor* Grig.-Man. and Porad., against important root pathogens

Nair B, Abraham D, Saranya K and Mallikarjunaswamy GE

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

Nair B, Abraham D, Saranya K, Mallikarjunaswamy GE 2020 – Bio-control potentiality of *Penicillium multicolor* Grig.-Man. and Porad., against important root pathogens. Current Research in Environmental & Applied Mycology 10(1), 514–523, Doi 10.5943/cream/10/1/40

Abstract

Penicillium multicolor a soil-borne fungus was evaluated for its activity against root pathogens *Fusarium oxysporum* and *Sclerotium rolfsii*. *In vitro* plate assay exhibited mycelia inhibition of the pathogens on PDA medium. Microscopic analysis revealed conidiophores and conidia deformations and mycelia piercing in the case of *F. oxysporum* where as mycelia coiling and cytoplasmic disintegration resulted for *S. rolfsii*. Rhizosphere competence of *P. multicolor* where sterile paddy straw segments placed at different centimetres up to 7cm depth to which 40ml of 2 x 10^6 conidial suspensions were poured and determined at 21 days of incubation exhibited mycelia growth up to 6 cm depth but observed very low colonisation frequency rate, 22%. *In vitro* root colonisation of *P. multicolor* and pathogens was studied for Teak and Mahogany seedlings. 2 x 10^5 and 2 x 10^6 conidial suspensions of pathogens and the bio-agent respectively at different concentrations v/v (1pathogen:1 biological agent and 1 pathogen: 5 biological agent) were tested and 1:5 ratio was found to be effective where complete suppression of pathogens were observed. The present study exerted on the further standardisation of biological agent for their success in field application.

Key words – Bio-agent – Colonisation – *In vitro* assay – Root pathogens – Saprophytic ability

Introduction

Sustainable production of plants depends upon culture practices producing quality planting stocks, soil nutrient management and minimal chemical usage. Seedling health in nurseries determine the quality of the output which besides culture practices also influenced by biotic and abiotic factors. Biotic agents such as bacteria, fungi, nematodes, protozoans, viruses, insects, infest plants especially during seedling stage thereby hindering quality planting stocks. Forest nursery diseases- collar rots, damping-off, root rots, foliar diseases, blights, powdery mildews and rusts result either total damage to seedlings or weaken them to different degrees so that the nursery-initiated diseases are carried over to the main field resulting in disease spread and severe loss (Sharma et al. 1985). Collar rots, damping-off and root rots affect most of the forestry species in nurseries incited by fungi majorly- species of *Cylindrocladium, Fusarium, Pythium, Phytophthora, Rhizoctonia* and *Sclerotium* resulting in unpredictable damage and severely hampering the economy.

Kerala, popularly known as God's own Country, South western state of India is bestowed with nature's beauty and homes diverse wildlife with forest cover about 52.30% of the total geographical area as per Kerala forest statistics 2018. Forests contribute considerably to the

Submitted 28 May 2020, Accepted 3 December 2020, Published 18 December 2020
Corresponding Author: Bharath Nair – e-mail – bharathleo5@gmail.com

government revenue and many tribes and locals depend upon forest products for their livelihood. Forests besides other natural produce majorly serves as a source of timber and considerable area has been allocated for various plantations. Teak (*Tectona grandis* Linn.) and Mahogany (*Swietenia macrophylla* King) are important timber yielding crops and have been widely cultivated since 19th century (Ball et al. 2000). As per Kerala forest statistics report (2018), 13.51% of total forest area is occupied by different plantations of which Teak about 49.61% and Mahogany about 0.33% of total plantation area together share for major timber plantations in Kerala. On-set of Teak and Mahogany seedlings are greatly hampered by root diseases of which species of *Fusraium, Sclerotium* and *Rhizoctonia* are most devastating (Sharma et al. 1985, Mohanan 2001).

Fusarium oxysporum Schltdl. a severe fungal pathogen distributed through-out the world and inhabit different soil types (Burgess 1981). This species is normally present in the rhizosphere communities of different plant groups and upon favourable conditions invade plant roots causing tracheomycosis resulting in wilting of plants or causing root-rots (Gordon & Martyn 1997, Olivain & Alabouvette 1997).

Sclerotium rolfsii Sacc. another important soil-borne fungal pathogen has wide host range resulting collar and root rots (Aycock 1966, Bhattacharrya et al. 1977). The species produce amber-coloured hardened structures called sclerotia which help them to strive in unfavourable conditions and upon the advent of suitable conditions reactivate resulting in severe infestation on different parts of the plants (Agrios 2005).

The present containment strategy mostly relies on chemical pesticides and unchecked application pushed for an alternative approach so as to manage the disease economically as well as sustainably (Eziashi et al. 2007). A number of bio-agents *Trichoderma* sp. Pers. (Shabir-U-Rehman et al. 2013), *Aspergillus* sp. Micheli. (Suárez-Estrella et al. 2007) and *Penicillium* sp. Link. (De Cal et al. 2009) found success *in vitro* but on field it proves to be a failure stresses on in-depth study of various aspects of biological activity. The present study deals to understand the activity of *Penicillium multicolor* against root pathogens- *F. oxysporum* and *S. rolfsii* of Teak and Mahogany seedlings *in vitro*.

Materials & Methods

Fungal cultures

Fungal pathogens namely *Fusarium oxysporum* and *Sclerotium rolfsii* were previously identified and obtained from Forest Pathology Department, Kerala Forest Research Institute (KFRI), Thrissur, Kerala. The isolates were stored as live cultures in Potato Dextrose Agar (PDA) slants at -10°C and were then reactivated on antibiotic amended PDA medium by culturing mycelia discs at 25 ± 2°C for seven days.

The bio-agent *Penicillium multicolor* was previously isolated from rhizosphere regions of grasses and was stored as live culture in PDA slants at -10°C. The fungal culture was sub-cultured and purified as mentioned earlier.

Antagonistic ability

In vitro antagonism was analysed via dual culture method on PDA medium. Seven mm diameter agar plugs of *P. multicolor* and pathogens *F. oxysporum* and *S. rolfsii* were cut from actively growing edge of five-day-old cultures using a cork borer. The mycelia discs were paired leaving 3-4 cm gap in between. The control plates were inoculated with the pathogens and the antagonist separately. The plates were incubated for ten days at 25 ± 2°C and observed for dual culture activity. The percent inhibition of radial growth of fungal plant pathogens was calculated using formula given by (Kucuk & Kivanc 2004).

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

Where,
R1 = Radial growth of fungal pathogens in control
R2 = Radial growth of fungal pathogen in dual culture

Microscopic analysis was done via slide culture method. Water agar (WA) medium 1% was poured and allowed to solidify and 1 cm square bits were cut and placed over sterile microscopic slides. To one corner, mycelia of *P. multicolor* was placed and diagonally mycelia of pathogens. A sterile cover slip was mounted and slides were incubated for 3-5 days at 25 ± 2°C and regularly moistened so as to avoid the drying of agar discs. Slides were observed under Leica DM2000 LED microscope and photo-micrographs were taken using attached Leica DMC2900 camera on the microscope.

Rhizosphere competence of *P. multicolor*

Preparation of fungal inoculum

Penicillium multicolor was cultured in PDA medium for 7 days at 25 ± 2°C. Freshly grown cultures were inoculated in Potato Dextrose Broth (PDB) were kept at incubator shaker for 14 days at 25 ± 2°C. Mycelial mat was separated out by filtering through Whatsman No.1 filter paper, dried, grounded using mortar and pestle and centrifuged at 10000 rpm for 15 min to remove hyphal debris. Conidial suspensions thus obtained were then suspended in sterile distilled water and the concentration was adjusted to 2 x 10^6 conidia per ml.

Testing saprophytic competency

Saprophytic ability of *P. multicolor* was tested by the Cambridge method (Garret 1970). Freshly harvested paddy straw were cut in to 1-cm long segments and autoclaved. Sterile plastic cups procured from the market were perforated at the bottom and plugged with sterile cotton pads. These cups were filled with 200 g autoclaved potting medium up to 7-cm length of the cup and placed with paddy straw segments at 1cm apart from the bottom of the cup. Eighteen autoclaved straw pieces were placed in a radial fashion and sterile potting medium was over laid up to 8-cm length of the cup. Forty ml of conidial suspensions were poured over the potting medium separately and each set was placed in an individual plastic tray containing sterile distilled water. The cups were not watered from the top but the potting medium in the cup was allowed to imbibe water only through capillary action from the holes at the bottom of the cup. Colonization of paddy straw segments was determined after 21 days of incubation.

Isolation of *P. multicolor*

Paddy straw segments removed after regular intervals of incubation were washed in slow running tap water, then twice in sterile distilled water and placed on antibiotic amended PDA medium at 25 ± 2°C for 14 days. The fungal colonies developing from these segments were identified and compared with the characteristics of the original colony culture. Percent colonization by *P. multicolor* at different depth levels at given time was determined.

$$\text{Colonisation frequency (CF)} = \frac{\text{Number of fungus isolated in each bits}}{\text{Total number of bits observed}} \times 100$$

***In vitro* Root colonisation**

P. multicolor and root pathogens *F. oxysporum* and *S. rolfsii* were studied for their interactions with roots. Mycelial discs were incubated in 1% sucrose solution for 14 days at 25 ± 2°C and were separated out by filtering through Whatsman No.1 filter paper and centrifuged at 10000 rpm for 15 min to remove hyphal debris. Conidial suspensions thus obtained were then suspended in sterile distilled water and the concentration was adjusted to 2 x 10^5 and 2 x 10^6 conidia per ml for pathogens and the bio-agent via haemocytometer. 15 day old Teak and Mahogany

seedlings were subjected with different concentrations v/v (1pathogen:1 biological agent and 1 pathogen: 5 biological agent) and a separate set for control was also maintained. Seedlings were incubated for 21 days and were observed for possible interactions via Root clearing method. Root samples were treated with 10% KOH solution for 1 hour in a hot water bath at 60°C and were washed with distilled water and treated with 2% HCl solution. Samples were stained with 0.05% Trypan blue in lactic acid and kept in a hot water bath for 10-15 min. Samples were de-stained with lactic acid and were observed under the microscope to observe the interaction. Slides were observed under Leica DM2000 LED microscope and photo-micrographs were taken using attached Leica DMC2900 camera on the microscope.

Results

Antagonistic ability

Current *In vitro* dual culture assay resulted in the inhibition of mycelia growth of the pathogens, *F. oxysporum* and *S. rolfsii* by *P. multicolor* (Table 1). Comparatively the activity of the bio-agent varied among pathogens exerting maximum inhibition on hyphal development of *F. oxysporum* 65.71%. Microscopic studies revealed conidiophores and conidia deformations and mycelia lysis thereby inhibiting conidial germination (Fig. 1A, B).

Sclerotium rolfsii exhibited resistance to the *P. multicolor* stress to about 51% but further elongation of the hyphae was restricted. Mycelia interactions resulted in coiling of the bio-agent hyphae and subsequently disintegrating cytoplasmic content thus restricting the vegetative propagation (Fig. 1C, D).

Table 1 Antagonistic activity of *P. multicolor* against root disease causing fungal pathogens

	F. oxysporum	*S. rolfsii*
P. multicolor	65.71±2.85[1]	48.89±2.22[1]

[1]standard deviation

Rhizosphere competence and colonisation frequency

Penicillium multicolor was subjected for saprophytic and colonizing ability. The present work was to analyse the competency of the bio-agent *in vitro*. Sterile paddy straw segments placed at 1 cm apart upto a depth of 7 cm and over flowed with 40 ml of 2 x 10^6 conidial suspensions per ml were incubated for 21 days. Paddy straw segments at each depth level on isolation for *P. multicolor* resulted upto a depth of 6 cm. Similarly colonization frequency was also checked at different levels of depths and exhibited 72% CF at 2 cm but with increasing depth level a decrease in CF was observed and only exhibited 22% at 6 cm depth (Fig. 2). The ability of microbes as a successful bio-control agent relies on rhizosphere compatibility and to cope up with the plant root-soil interface.

In vitro Root colonisation

To understand the nature of interaction, Teak and Mahogany seedlings were treated with the bio-agent and pathogens. Fifteen day old seedlings were treated with 2 x 10^5 and 2 x 10^6 conidial suspensions per ml of pathogens and the bio-agent at different concentrations v/v (1pathogen:1 biological agent and 1 pathogen: 5 biological agent). Roots were then observed for possible colonisation and interactions by the fungal agents.

Penicillium multicolor was found to attach to the surface of root segments as was evidenced for Teak seedlings (Fig. 3A). But for treatments it was observed that 1:1 concentration was not sufficient from preventing the attack of the pathogen. In case of *F. oxysporum* the hyphae colonized root epidermal tissues and resulted in the damage of root cells and on the contradictory 1:5 concentrations inhibited hyphae elongation and disintegrating mycelia (Fig. 3B, C). For *S. rolfsii* on the other hand for 1:1 concentration, heavy mycelia tissues were observed on the surface of root nd

damaging root cells but for 1:5 concentrations, *S. rolfsii* though were found to present over the root surface but cytoplasmic disintegrations were observed thereby weakening mycelia activity (Fig. 3D, E).

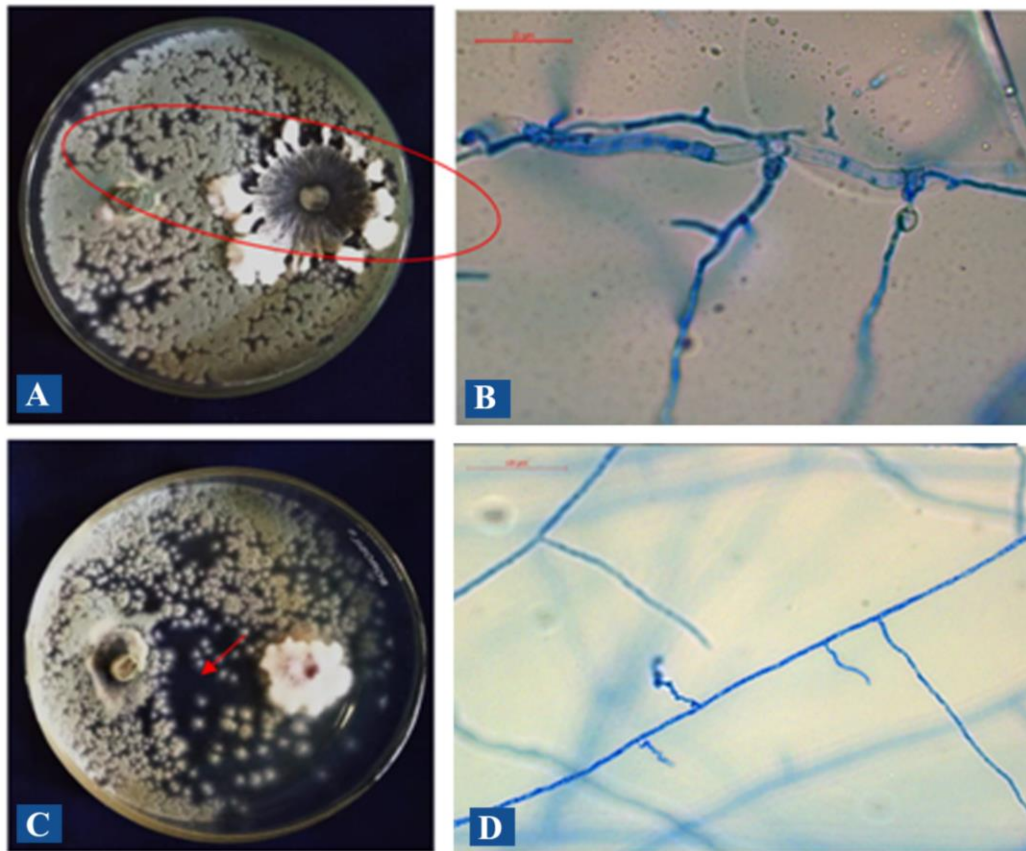

Fig. 1 – A-D Dual culture assay and Microscopic observations. *P. multicolor* and *S. rolfsii* exhibiting mycoparasitic interactions, hyphal coiling and cytoplasmic disintegration (A, B). *P. multicolor* and *F. oxysporum* exhibiting mycoparasitic interactions, conidiophore deformations (C, D). Photomicrographs taken at 100X.

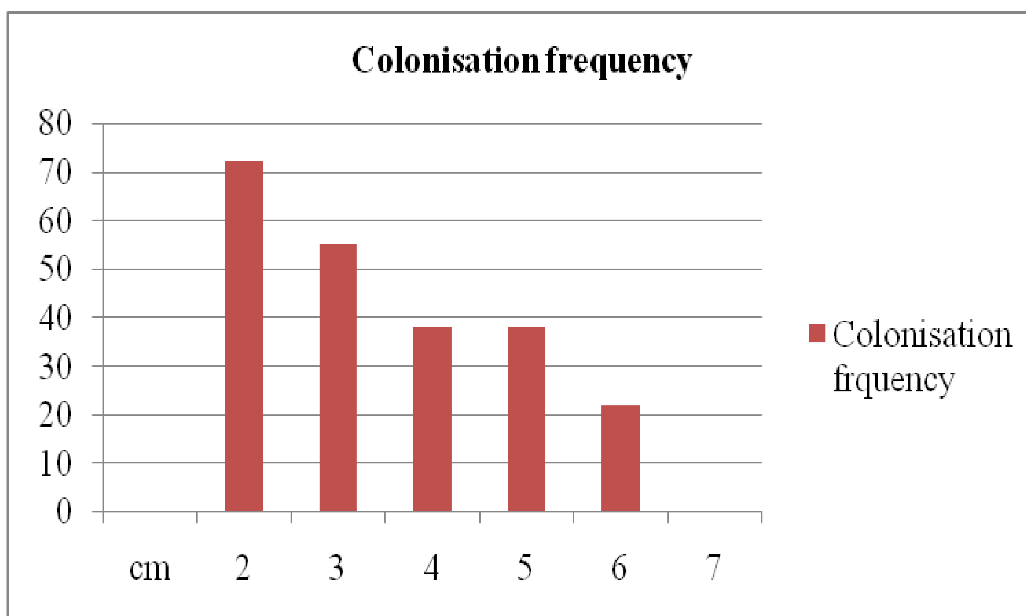

Fig. 2 – Colonization frequency of *P. multicolor* 21 days of incubat

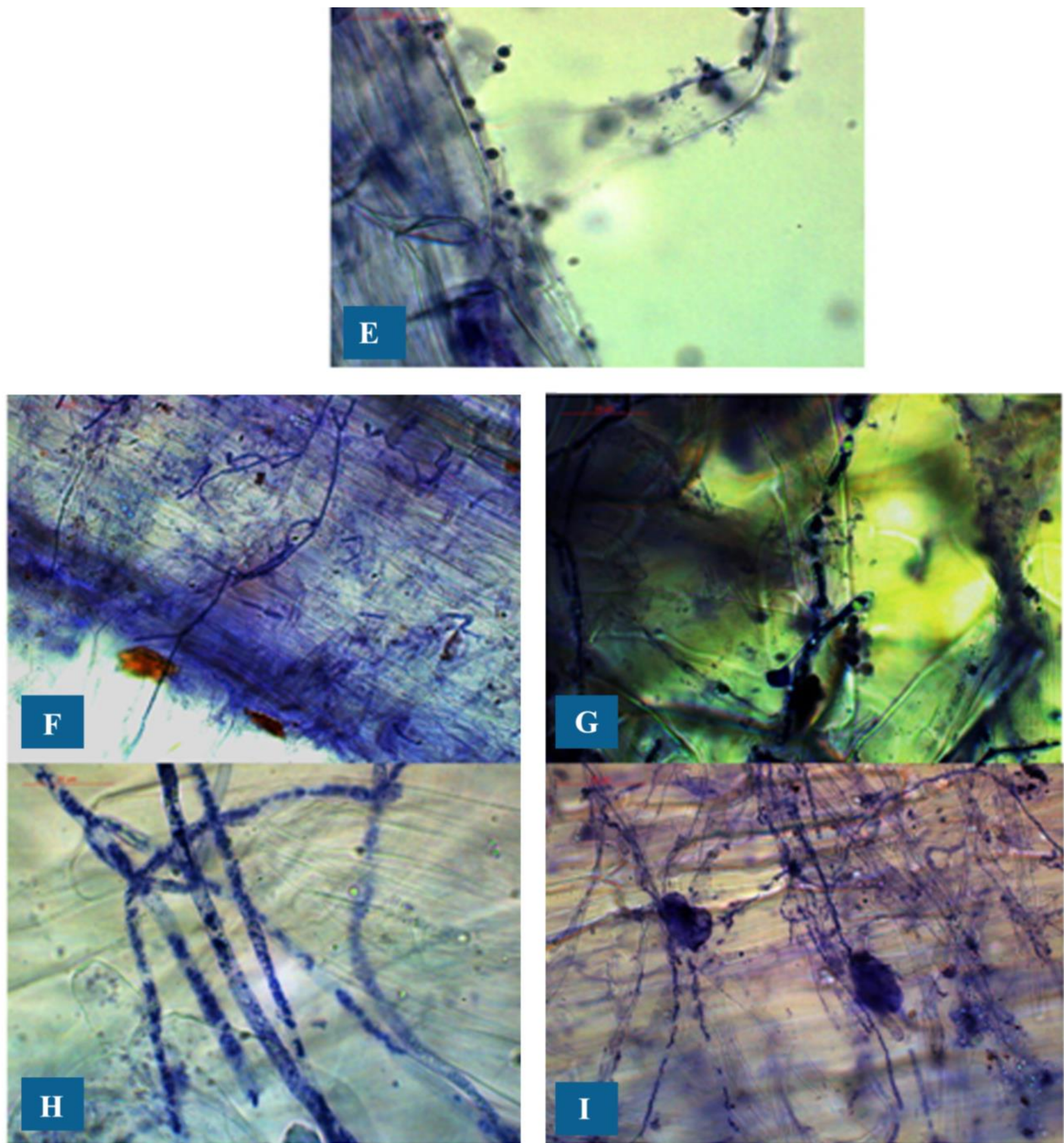

Fig. 3 – E-I Root colonisation of fungi in Teak seedlings. *Penicillium multicolor* colonising over root surface (E). Interactions of *F. oxysporum* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (F, G). Interactions of *S. rolfsii* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (H, I). Photomicrographs taken at 100X.

In the case of Mahogany seedlings heavy conidia concentration and hyphal strands of *P. multicolor* were seen on the root surface and no signs of any attachments clearly indicating the bio-agent acting as a shield over the surface of the root (Fig. 4A). 1:1 concentrations of pathogens and bio-agent were found ineffective in the management of pathogens. *Fusarium oxysporum* hyphae invaded root tissues and subsequently damaging root cells. When 1:5 concentration was applied heavy conidial mass were seen over the root surface appeared to be acting as a shield (Fig. 3B, C). Again for *S. rolfsii* when 1:1 concentration was analysed root surface were found to be colonized by *Sclerotium* mycelia on the other hand when subjected to 1:5 concentration, numerous *P. multicolor* conidia mass was observed and *Sclerotium* hyphae with cytoplasmic disintegrations were observed (Fig. 4D, E

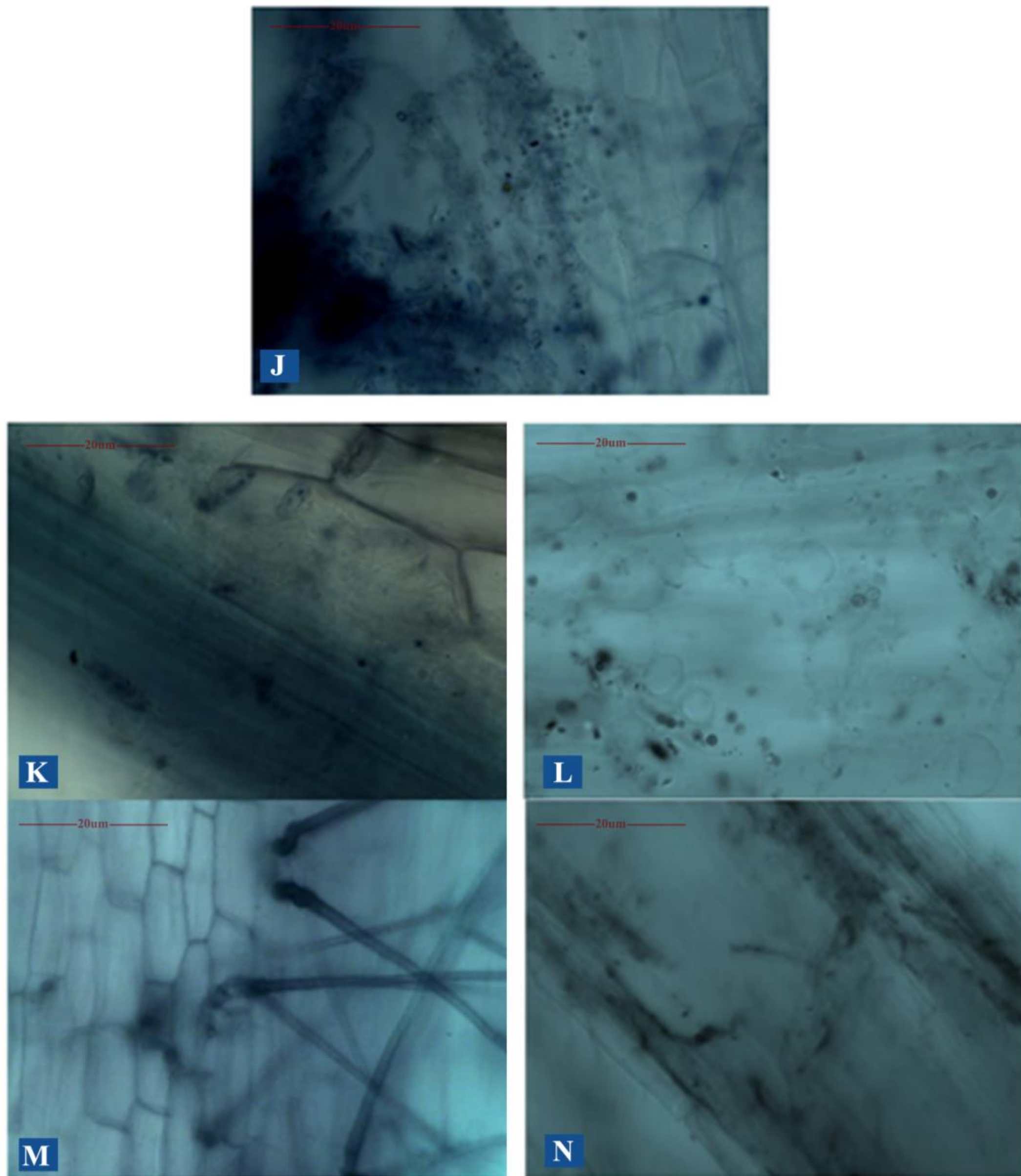

Fig. 4 – J-N Root colonisation of fungi in Mahogany seedlings. *P. multicolor* colonising over root surface (J). Interactions of *F. oxysporum* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (K, L). Interactions of *S. rolfsii* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (M, N). Photomicrographs taken at 100X.

Discussions

Penicillium multicolor against root pathogens *Fusarium oxysporum* and *Sclerotium rolfsii* exhibited parasitic activity inhibiting mycelium growth by lysing and coiling of hyphae and disintegrating cytoplasmic contents. Many bio-control agents against *Fusarium* wilt such as *T. harzianum*, *Penicillium oxalicum* Currie & Thom, and non-pathogenic *F. oxysporum* having ability to inhibit conidia production over 90% have been demonstrated (El-Sheshtawi et al. 2014). Ability of *P. oxalicum* inhibiting *F. oxysporum* mycelium and subsequent reduction in wilt incidence in Tomato plants are also reported by various researchers (Duijff et al. 1998, Larena et al. 2003, Shishido et al. 2005). There are limited studies on the activity of *Penicillium* sp. over *S. rolfsii*. Hadar & Gorodecki (1991) observed a 90% reduction in the production of sclerotia by *Sclerotium rolfsii* within seven days of mixing of *Penicillium* sp. in peat thus effecting the germination of sclerotia and further mycelia progression. Further evaluation of the bio-agent for rhizosphere competency revealed good saprophytic ability but a low colonising frequency. Numerous studies

on microbial density and rhizosphere competence with respect to plant species have been evaluated by various researchers (Papavizas 1967, Wells et al. 1972, Newman & Bowen 1974, Chao et al. 1986). A low percent of colonisation by *P. multicolor* with successive depth levels highlighting the importance in field success stresses on application modifications so as to efficiently establish in the natural conditions.

The present work gave an overview on the interactions and rhizosphere compatibility of *P. multicolor*. Hossain et al. (2007) studied root colonising ability of PGPF *Penicillium simplicissimum* GP17-2 and observed resistance induction in host plants. In tomato plants infested with *Fusarium oxysporum* f. sp. *lycopersici* on prior treatment with *Penicillium oxalicum* observed to induce resistance against the pathogen (Sabuquillo et al. 2005, 2006). Shishido et al. (2005) for non-pathogenic strains of *Fusarium oxysporum* reported the bio-agent was more effective when applied under sterile seedbeds, compared with non-sterile soil, as a decrease in soil microbes competition enhanced bio-agent activity. Furthermore, the bio-control agents were more effective when antagonist propagules exceeded that of pathogens. Similar observations were observed in the present analysis when the magnitude of the bio-agent was increased five times that of pathogen suppressed pathogen activity near the root surface.

Antagonistic affectivity of members of *Penicillium* genus against several plant pathogens have been reported (Ma et al. 2008, Sabuquillo et al. 2010, Sempere & Santamarina 2010). The activity of *Penicillium* members performs different actions against various pathogens which involve hyphal interactions, production of various metabolites and in some cases inducing resistance on host plants (Samson 2004, Samson et al. 2009, Houbraken et al. 2010, Kim et al. 2012). However, proper identification of *Penicillium* sp. and management strategies need to standardised for its effectiveness in field conditions (Peterson et al. 2011, Varga et al. 2011). Hence, understanding antagonism mechanism and modifying development protocols need to be aimed before application in natural ecosystems (Heydari & Pessarakali 2010, Oliveira et al. 2015).

Acknowledgements

The authors are grateful to the Director, KSCSTE-KFRI and to all the lab members of Forest Pathology Department, KFRI.

References

Agrios GN. 2005 – Plant Pathology. 4th ed. Academic Press. London. 395–397.

Aycock R. 1966 – Stem rot and other diseases caused by *S. rolfsii*. Tech. Bull. 174. Agric. Expt. Station, North Carolina State University, Raleigh. 202.

Ball JB, Pandey D, Hirai S. 2000 – Global overview of Teak plantations. In: Enters, T. and Nair CTS. (Eds.). Site Technology and Productivity of Teak Plantations. FORSPA Publication (FAO, Bangkok) 24, 11–33.

Bhattacharrya SK, Phadtare SG, Sharma VC. 1977 – Fungal diseases of potato. Recent Technology in potato improvement and production. CPRI. Simpla. India. 209–237.

Burgess LW. 1981 – General Ecology of the Fusaria. In: Nelson PE, Toussoun TA, Cook RJ, eds. Fusarium: diseases, biology and taxonomy. University Park, PA, USA: The Pennsylvania State University Press, 225–235.

Chao WL, Nelson EB, Harman GE, Hoch HC. 1986 – Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76, 60–65.

De Cal A, Sztejnberg A, Sabuquillo P, Melgarejo P. 2009 – Management *Fusarium* wilt on melon and watermelon by *Penicillium oxalicum*. Biol Control 51, 480–486.

Duijff BJ, Pouhair D, Olivain C, Alabouvette C, Lemanceau P. 1998 – Implication of systemic induced resistance in the suppression of *Fusarium* wilt of tomato by *Pseudomonas fluorescens* WCS417r and by non-pathogenic *Fusarium oxysporum* Fo47. Eur. J. of Plant Pathol., 104, 903–910.

El-Sheshtawi M, Bahkali AH, Al-Taisan WA, Elgorban AM. 2014 – Pathogenicity of *Fusarium oxysporum* f. sp. melonis to melon genotypes (*Cucumis melo* L.) and its biocontrol. J P A M 8(Spl. Edn. 1), 317–324.
Eziashi EI, Omamor IB, Odigie EE. 2007 – Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. Afr J Biotechnol 6(4), 388–392.
Garret SD. 1970 – Pathogenic Root-Infecting Fungi. Cambridge mutants not only produced greater amounts of 8-1, 4-glucanases University Press, London. 294.
Gordon TR, Martyn RD. 1997 – The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35, 111–128.
Hadar Y, Gorodecki B. 1991 – Suppression of germination of sclerotia of *Sclerotium rolfsii* in compost. Soil Biology and Biochemistry 23, 303–306.
Heydari A, Pessarakli M. 2010 – A Review on Biological Control of Fungal Plant Pathogens Using Microbial Antagonists. Journal of Biological Sciences, 10, 273–290.
Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M. 2007 – The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiol 48, 1724–1736.
Houbraken JA, Frisvad JC, Samson RA. 2010 – Taxonomy of *Penicillium citrinum* and related species. Fungal Diversity, 44, 117–133.
Kim HY, Park HM, Lee CH 2012 – Mass spectrometry based chemotaxonomic classification of *Penicillium* species (*P. echinulatum*, *P. expansum*, *P. solitum*, and *P. oxalicum)* and its correlation. The Journal of Microbiological Methods, 90, 327–335.
Kucuk C, Kivanc M. 2004 – *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turk. J. Biol., 28, 111–115.
Larena I, Pascaual S, Melgarejo P, De Cal A. 2003 – Biocontrol of *Fusarium* and *Verticillium* wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. J. Phytopathol., 151, 507–512.
Ma Y, Chang ZZ, Zhao JT, Zhou MG. 2008 – Antifungal activity of *Penicillium striatisporum* Pst10and its bio-control effect on *Phytophthora* root rot of chilli pepper. Biological Control, 44, 24–31.
Mohanan C. 2001 – Biological control of Damping-off in forest nurseries in Kerala. Kerala Forest Research Institute Research Report NO. 214, India 65.
Newman EI, Bowen GD. 1974 – Patterns of distribution of bacteria on root surfaces. Soil Biol 6, 205–209.
Olivain C, Alabouvette C. 1997 – Colonization of tomato root by a non-pathogenic strain of *Fusarium oxysporum*. New Phytologist 137, 481–494.
Oliveira TAS, Duarte EAA, Silva RM, Moreira ZPM et al. 2015 – Biocontrole de doenças pós-colheita em frutas. Revisão Anual de Patologia de Plantas, 23, 293–325.
Papavizas GC. 1967 – Survival of root-infecting fungi in soil I. A quantitative propagule assay method of observation. Phytopathology 57, 1242–1246.
Peterson SW, Orchard SS, Menon S. 2011 – *Penicillium menonorum*, a new species related to *P. pimiteouiense*. IMA Fungus, 2, 121–125.
Sabuquillo P, De Cal A, Megarejo P. 2005 – Dispersal improvement of a powder formulation of *Penicillium oxalicum*, a bio-control agent of tomato wilt. Plant disease 89, 1317–1323.
Sabuquillo P, De Cal A, Megarejo P. 2006 – Bio-control of tomato wilt by *Penicillium oxalicum* formulations in different crop conditions. Biological control 37, 256–265.
Sabuquillo P, De Cal A, Melgarejo P. 2010 – Development of a dried *Penicillium oxalicum* conidial formulation for use as a biological agent against *Fusarium* wilt of tomato: Selection of optimal additives and storage conditions for maintaining conidial viability. Biological Control, 54, 221–229.

Samson RA, Seifert KA, Kuijpers AF, Houbraken JAMP, Frisvad JC. 2004 – Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β-tubulin sequences. Studies in Mycology, 49, 175–200.

Samson RA, Houbraken J, Varga J, Frisvad JC. 2009 – Polyphasic taxonomy of the heat resistant Ascomycete genus *Byssochlamys* and its *Paecilomyces* anamorphs. Persoonia, 22, 14–27.

Sempere F, Santamarina MP. 2010 – Study of the interactions between *Penicillium oxalicum* Currie & Thom and *Alternaria alternata* (fr.) Keissler. Brazilian Journal of Microbiology, 41, 700–706.

Shabir-U-Rehman WA, Ganie DSA, Bhat JA, Mir GH et al. 2013 – Comparative efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *ciceris* causing wilt of chickpea. Afr J Microbiol Res 7(50): 5731–5736.

Sharma JK, Mohanan C, Florence EJM. 1985 – Disease Survey in Nurseries and Plantations of Forest Tree Species Grown in Kerala. Kerala Forest Research lnstitute, Research Report No. 36, India, 268.

Shishido M, Miwa C, Usami T, Amemiya Y, Johnson KB. 2005 – Biological control efficacy of *Fusarium* wilt of tomato by nonpathogenic *Fusarium oxysporum* Fo-B2 in different environments. Phytopathology, 95, 1072–1080.

Suárez-Estrella F, Vargas-García MC, López MJ, Capel C, Moreno J. 2007 – Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. melonis. Crop Prot 26, 46–53.

Wells HD, Bell DK, Jaworski CA. 1972 – Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii*. Phytopathology 62, 442–447.

Varga J, Frisvad JC, Kocsubé S, Brankovics B et al. 2011 – New and revisited species in *Aspergillus* section *Nigri*. Studies in Mycology, 69, 1–17.