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IDENTIFICATION AND MORPHOLOGICAL CHARACTERIZATION OF PATHOGENS INFECTING *MELIA DUBIA* IN TAMIL NADU

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ABSTRACT

Melia dubia in Tamil Nadu were surveyed for the association of foliar and root fungal pathogens were recovered from infected plants. Cultural and microscopic characterization of *Fusarium moniliformis*, *Phoma* sp., *Rhizoctonia solani Lasiodiplodia theobromae* and *Pythium* sp, were obtained. *Fusarium moniliformis* produced basal cells of macro conidia with prominent and the length ranged from 19.631 µm to 20.624 µm, foot shaped and elongated micro conidia length ranged from 6.648 µm to 7.250 µm. *Phoma* sp produced grey in colour mycelium, septate branched, pycnidia is colour black and it produced the spores in concentric circle. The length of the pycnidia was 68.906 µm and breadth was 103.61 µm. *Rhizoctonia solani* showed complete full growth in Petri dish, initially the mycelium aggregated and form ball like structure later it changed brown colour sclerotia. *Pythium sp* Showed hyaline, non-septate with granular cytoplasm and oospores like bodies much in number and sporangia pores length was 7.18 µm. *Lasiodiplodia theobromae* showed a fast growth and attained full growth within five days after inoculation in 9cm diameter Petri plate. Aggregation of grey coloured mycelium and production of pycnidia in the centre of the colony were observed eight days after inoculation. Among the media tested, the potato dextrose agar medium supported the growth of all fungal pathogens in terms of mycelial extension, mycelial density and sporangia production compared to oat meal agar and malt extract agar medium.

KEYWORDS: Foliar Pathogens, *Melia Dubia*, Morphological Characterization, Root Pathogens

INTRODUCTION

Melia dubia with its multi-various uses like pulpwood, timber, fuelwood and plywood can fit as a suitable species for plantation programme. The wood is also used for packing cases cigar boxes, ceiling planks, building purpose, agricultural implements, pencils, match boxes, splints and kattamarans. It has been screened as an alternate species for pulpwood (Parthiban *et al*., 2009). The intensive increase in the area of industrial wood under forest plantations has lead to the outbreak of pests and diseases to a greater magnitude. Among the various factors, diseases rank as a prime factor causing serious losses in *M. dubia.* Tree is affected by a number of diseases at all stages of its development from nursery to harvest. The collar rot and seedling web blight caused by *Rhizoctonia solani* is the major forest nursery pathogen in Kerala and it occurs in different Anastomosis Groups of varying virulence (Mohanan, 2001). Soil-borne as well as aerial strains of *R. solani* cause web blight and the severity of the disease depends on various factors including the nursery conditions. Leaf spots caused by *Colletotrichum dematium* (Pers. *ex* Fr.) Grov and *Cylindrocladium ilicicola* (Hawley) Boedijn and Reitsma are the other diseases recorded on *M. dubia* in nursery. However, the etiology of the diseases of *M. dubia* in Tamil Nadu has not been established. The existence of different pathogenic types based on morphological and cultural characters and pathogenicity has not been studied. Therefore, the present investigation was carried out to find the major pathogens

infecting *M. dubia* in Tamil Nadu and their morphological character were studied.

MATERIALS AND METHODS

Isolation of the Pathogen

The diseased parts of affected seedlings and trees collected from different *Melia dubia* nursery and plantations were brought to the laboratory in clean polythene bags. Isolation of the causative organism was made immediately in order to avoid any saprophytic growth on the specimens. The pathogen was isolated by tissue segment method (Rangaswami, 1972) on Potato Dextrose Agar medium (PDA). PDA medium was prepared and autoclaved at 1210 C and 15 psi for 15 min. The infected parts were surface sterilized using 0.1 per cent Mercuric chloride for one min and washed in sterile water for thrice. These infected parts were then transferred on Petri plate containing Potato Dextrose Agar medium in an inoculation chamber under aseptic condition followed by incubation at 25±10 C. Pure cultures of the fungus were obtained by single spore isolation method as suggested by Tutte (1969).

Cultural Characters of Pathogens

Cultural characters of the different isolates of pathogens such as colour, shape, texture of fungal colony, formation of fruiting bodies, growth rate and sporulation on the PDA medium were studied (Ainsworth *et al*., 1973). The pathogen was identified up to species level based on its cultural and morphological characters. A loop full of fungal culture developed on the PDA plates was taken on a glass slide and observed in a PC controlled image analyzer under 100 x magnifications. The mycelial and spore characters were observed under different microscopic fields and the characters were recorded. Conidia of the fungus were harvested from 7days old cultures by flooding plates with 10ml of sterile water and dislodging conidia by soft brushing of the colonies with aseptic glass spreader. Aqueous conidial suspensions were filtered through sterile gauze to remove hyphal fragments. The size of conidia and chlamydospores were measured through the image analyzer with specific software designed for the purpose, and compared with standard references to confirm the identity of the pathogen.

Growth of Pathogen on Different Solid Medium

Mycelial growth of fungus was compared on three medium different medium *viz* Potato Dextrose Agar (PDA), Oats Meal Agar (OMA) and Malt Extract Agar (MEA). Five millimeter culture discs were cut with a sterilized cork borer from actively growing margin of colonies of *L. theobromae, Pythium sp., R. solani, Phoma* sp., *F. moniliformis* and inoculated on three different medium separately and incubated at 25±2 °C for seven days for the growth of associated fungi. The experiment was designed in Completely Randomized Design (CRD) with three replications. The dry mycelial weights of fungal pathogens were observed after seven days of inoculation and compared.

Growth of Pathogen on Different Liquid Medium

The Potato Dextrose broth (PDB), Oat meal broth (ODB) and Malt extract broth (MEB) were prepared in 100ml conical flask and autoclaved at 1210C and 15 psi for 20 minutes. The mycelial disc of the freshly grown fungal culture (7mm diameter) was inoculated on to PDB and incubated for 8 days at room temperature. The mycelium was harvested after filtering the culture filtrates through sterile musclin cloth. The wet weight of the mycelium was taken and the samples were dried at 600 C for five days at hot air oven. The dry weight of the mycelium was taken after complete removal of

moisture (MacFaddin, 1985).

RESULTS AND DISCUSSIONS

Isolation of the Pathogen

The pathogen associated with leaf blight and leaf spot diseases were isolated from naturally infected *Melia dubia* leaves collected from three nurseries and five plantations. The three different fungal pathogens were isolated on PDA medium namely *Fusarium moniliformis, Phoma* sp. and *Rhizoctonia solani*. *Fusarium moniliformis* was isolated from Forest College and Research Institute nursery and Plantation, Farmer plantations, whereas *Phoma* sp. isolated in FC and RI nursery and *Rhizoctonia solani* was isolated except Forest College and Research Institute nursery and Farmers plantation. All the isolates of the pathogens were purified and sub cultured at frequent intervals and was identified up to species level based on cultural and morphological characters described later in this chapter. Similarly, the pathogen associated with collar and root rot symptoms were isolated on Potato Dextrose Agar medium from naturally infected *Melia dubia* parts collected from different locations. Two different fungal pathogens were isolated on Potato Dextrose Agar medium and were found belonging to *Lasiodiplodia theobromae* and *Pythium* sp. Among these, Except Farmer plantation (FT) *L. theobromae* was isolated in all the plantations and *Pythium* sp. was isolated from Eden nursery and TNPL nursery. All the isolates of these pathogens were purified and sub cultured at frequent intervals and used for further studies. Investigations on the pathogens infecting *M. dubia* were identified as *Fusarium moniliformis, Phoma* sp. and *Rhizoctonia solani* as foliar pathofgens. The pathogen associated with collar and root rot samples were identified as *L. theobromae* and *Pythium sp.* similarly, Ojha *et al.* (2010) isolated the pathogen from root and stem of the infected *Dalbergia sissoo*, the pathogen responsible for the disease was isolated and identified as *L. theobromae*. Kausar *et al*. (2009) reported the pathogen from the samples of Shisham on potato dextrose agar (PDA) medium and identified as *Fusarium solani*. Pathak (1987) recorded *L. theobromae* from the infected stem pieces of *Dalbergia sissoo.*

Cultural and Morphological Characteristics of Pathogens

Fusarium Moniliformis

Leaf spot diseases caused by *F. moniliformis* showed the cultural characteristics of medium rate of colony growth and attained 9 cm growth in Petri plate within nine days after inoculation. Initially the growth of colony was white in colour, margin was smooth in texture and topography was medium fluffy. The reverse side of the colony was light brown in colour, whereas morphological character of macro conidia were long and slender, with tapered apical cells that were elongated or even whip-like. Basal cells of macro conidia were prominent and the length ranged from 19.631 µm to 20.624 µm, foot shaped and elongated micro conidia length ranged from 6.648 µm to 7.250 µm. Similarly, Sharma and Pandey (2010) described the cultural characters of *Fusarium* sp which produce colony white in colour and sporulation was moderate. Mirhosseini *et al.* (2014) described the morphological characters of *Fusarium* sp., were initially white colony and then turned from orange - yellow to medium red with abundant aerial mycelium in Maize ears crops. The fungus was identified as *Fusarium* sp. based on morphological characteristics.

Phoma sp

Leaf blight pathogen *viz., Phoma* sp. recorded the cultural and morphological characters which includes the colony was slow growing and attained mycelial full growth on Petri dish in nine days after inoculation at room

temperature. Colour of growth is characterized with pale green-olive, flocky aerial hyphae of the air mycelium with white edge. The reverse colony was olive grey. Whereas mycelium was grey in colour, septate branched, pycnidia is colour black and it produced the spores in concentric circle. The length of the pycnidia was 68.906 µm and breadth was 103.61 µm. No variation was observed in the conidial characters of the isolates obtained from different samples. The study was proved with the colony and conidial measurements recorded by this pathogen were found comparable to those reported by earlier workers (Ismael *et al*., 2008 and Davidson *et al.,* 2012). The large, aseptate conidia produced by *Phoma koolunga* in culture are similar to those depicted in published descriptions of *Mycosphaerella phaseolina*. However the size of conidia of *Mycosphaerella phaseolina* was 3.5-10 mm is larger than that of the conidia. Kliejunas *et al.* (1985) reported the morphological characters of *Phoma glomerata* which produces conidia are of one celled, hyaline, ellipsoidal and 3-5 X 1.5-2.5 µm, colonies on PDA characterized by dense mycelial growth which is dark green at first and then becomes black.

Rhizoctonia Solani

Colony character of *Rhizoctonia solani* includes the rate of growth on PDA medium and complete full growth in Petri dish in seven DAI. The growth of colony was white in colour. Initially the mycelium aggregated and form ball like structure later it changed brown colour sclerotia. The reverse sides were brown in colour. Morphological characters having hyaline colorless, septate branched and hyphae branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction. No variation was observed in the colony and hyphae characters of the isolates obtained from all different samples. The colony and hyphae measurements recorded by this pathogen were found comparable to those reported earlier workers (Palo, 1926 and Gonzalez *et al.*, 2011). Hyphae diameter varies widely both within and among the isolates. Bracker and Butler (1963) reported that the septate are a prominent feature of *R. solani*, constriction of branch hyphae at the point of origin and formation of septum at right angle in the point of origin appear to be stable and reliable characteristics of *R. solani.*

Lasiodiplodia Theobromae

Compared to other pathogens, colonies of *Lasiodiplodia theobromae* showed a fast growth and attained full growth within five days after inoculation in 9cm diameter Petri plate. Initially the growth was feeble and white in colour. Later, the colour changed to greyish black and the colony showed a fluffy aerial growth. Aggregation of grey coloured mycelium and production of pycnidia in the centre of the colony were observed eight days after inoculation. The reverse side of the colony was black. The colony characters and conidial measurements recorded in *Lasiodiplodia theobromae* were found comparable to *Jatropha curcus* (Latha *et al.,* 2009). Mature conidia, oozing out as a black mass from mature pycnidia, were oval, 16 - 23×8 - 11 µm, dark brown, one-septate with longitudinal striations on the conidial wall. Immature conidia were hyaline, aseptate and without striations, with a similar shape and size to the mature ones, Paraphysis was septate. Gezahgne *et al.* (2014) observed cultural and morphological characters of *Lasiodiplodia theobromae* from *Boswellia papyrifera*. Colonies obtained from the different isolation procedures produced white and dense aerial mycelium, later turning black at the top and grey to black on the reverse side of the Petri dish. The brown coloured conidia were single septate, sub ovoid to ellipsoid. Rehman *et al*. (2012) identified the *L. theobromae* from Shisham (*Dalbergia Sissoo Roxb)* the mature conidia, oozing out as a black mass from mature pycnidia, were oval, dark-brown, medially one-septate with longitudinal striations on the conidial wall. Immature conidia were hyaline, aseptate and without striations, with a similar shape and size to the mature ones.

Pythium sp

A medium rate of mycelial growth was noticed on PDA medium and completed full growth in Petri plate within six days after inoculation, initially hyaline and turned light brown in colour. Reverse side was brown in colour. The color of the matured colony of *Phythium* sp was initially hyaline and turned light brown in color. Reverse colour was brown in colour. Morphological character includes hyaline, non-septate with granular cytoplasm and oospores like bodies much in number and sporangia pores length was 7.181 µm. No variation was observed in the conidial characters of the isolates obtained from two different locations. The colony and sporangia pore measurements recorded by this pathogen were found comparable to those reported by earlier workers in *Ailanthus triphysa* (Paul *et al*., 2008 and Yan long *et al*., 2012). Wen Hsiung *et al.* (2004) reported that *Pythium sukuiense* is a new species from undisturbed natural forest which produces sporangia indistinguishable from hyphae and very small oogonia and oospores. Oogonia were smooth and terminal or intercalary and attached with a single antheridium. Oospores were aplerotic, with an average size of only 11µm. The position, shape and size of sporangia, the formation of zoospores, and the position, shape and size of antheridia, oogonia and oospores were determined in grass blade culture in *Pythium senticosum* were isolated from forest soil in Japan (Waterhouse, 1967). Koffie Clovis *et al.* (2010) observed that the colonies of *Pythium* produced white aerial mycelium on Potato Dextrose Agar Medium. The mycelium was hyaline and non-septate was length between 20-28µm, sizes of sporangia varied from 20-36 µm, oospores some time reaching 40 µm in Papaya (*Carica papaya*).

Growth on Different Solid and Liquid Medium

The effect of growth of foliar and soil borne pathogens of *Melia dubia* was tested under *in vitro* condition. Soil borne pathogens of *L. theobromae* and *Pythium* sp recorded the maximum colony growth of 90 mm in all the three different medium tested. Among the foliar pathogens, *Fusarium moniliformis* recorded maximum colony growth of 85 mm in PDA medium followed by Oat Meal Agar medium, Similarly the *Phoma* sp. and *Rhizoctonia solani* were also recorded a maximum growth in PDA medium (84 mm and 88 mm respectively) (Table 1). Similarly, Suleiman *et al.* (2011) reported that Potato Dextrose Agar (PDA) was found to be the best medium in terms of mycelial extension, mycelial density and sporangia production compared to Malt extract and Czapeck-Dox agar in Cowpea. Latha *et al.* (2013) reported that among the growth media tested Potato Dextrose Agar supported the highest mycelial growth (8.96) followed by Potato Sucrose Agar and Corn Meal Agar for *L. theobromae* in *Jatropha curcus.* The growth characters of *F. oxysporum* f. sp. *gerberae* studied on different solid media indicated that the growth was maximum on Oat meal agar followed by Richards's agar, Czapek's Dox agar and Potato Dextrose agar supported maximum growth of fungal colony (Kishore and Srikant, 2008).

Among the different liquid media tested, potato dextrose medium recorded the highest mycelial dry weight of 3.567 g for *L. theobromae* followed by oat meal agar medium (3.102 g) and malt extract medium (2.972 g). *Phythium* sp recorded least mycelial dry weight of 0.968 g, 0.862 g and 0.938 g in PDA, oat meal agar and malt extract medium respectively. In the case of foliar pathogens, the maximum mycelial dry weight was recorded in all the three medium tested for *Phoma* sp. (Table 2). Sharma *et al.* (2002) also reported that Potato Dextrose Agar was best for the growth of Metarhizium isolates. Potato Dextrose Agar medium was found to be suitable for the growth of *Fusarium pallidoroseum* in *Aphis craccivora* (Hareendranath *et al*., 1986). Gupta *et al.* (2010) reported that liquid Malt extract broth recorded 1.385 mg of mycelia dry weight for *Fusarium oxysporum* and 1.491 mg for *F. solani* in *Psidium guajava.*

FIGURES AND TABLES

Table 1: Growth Characters of Pathogens Infecting *M. Dubia* on Solid Media

S. No	Pathogens	Mycelial Growth (mm)*		
		Potato Dextrose Agar Medium	Oat Meal Agar Medium	Malt Extract Agar Medium
1	*Lasiodiplodia theobromae*	90.00[a] (71.61)	90.00[a] (71.61)	90.00[a] (71.61)
2	*Pythium* sp.	90.00[a] (71.61)	90.00[a] (71.61)	90.00[a] (71.61)
3	*Fusarium moniliformis*	85.00[c] (67.24)	82.00[c] (64.92)	76.50[b] (61.02)
4	*Phoma* sp.	84.00[c] (66.45)	66.50[d] (54.65)	68.00[d] (55.57)
5	*Rhizoctonia solani*	88.00[b] (69.44)	84.00[b] (66.45)	73.00[c] (58.71)

*** Values are mean of three replications**
In a column, means followed by a common letter is not significantly different at 5% level by DMRT
Values in parentheses are arcsine transformed values

Table 2: Growth Characters of Pathogens Infecting *M. Dubia* on Liquid Media

S. No	Pathogens	Mycelial dry weight(g)*		
		Potato Dextrose Agar Medium	Oat Meal Agar Medium	Malt Extract Agar Medium
1.	*Lasiodiplodia theobromae*	3.567[a] (10.872)	3.102[a] (10.018)	2.972[a] (9.886)
2.	*Pythium* sp.	0.968[c] (4.877)	0.862[c] (4.421)	0.938[c] (4.557)
3.	*Fusarium moniliformis*	1.022[c] (5.194)	1.120[c] (5.636)	1.043[c] (5.294)
4.	*Phoma* sp.	2.572[b] (9.074)	2.134[b] (8.300)	1.972[b] (7.957)
5.	*Rhizoctonia solani*	1.460[c] (6.717)	1.240[c] (6.061)	0.980[c] (4.955)

*** Values are mean of three replications**
In a column, means followed by a common letter is not significantly different at 5% level by DMRT
Values in parentheses are arcsine transformed values

REFERENCES

1. G.C. Ainsworth, F.K. Sparrow and A.S. Sussman. *The fungi - An advanced treatise: A taxonomic review with keys. Ascomycetes and Fungi Imperfecti*. (Academic Press, 4: 1973, 621).
2. C.E. Bracker and E.E. Butler, The ultra structure development of septa in hyphae of *Rhizoctonia solani*. *Mycologia*, 55: 1963, 35-58.
3. J.A. Davidson, K.M. Krysinska and H.A. McKay, Comparison of cultural growth and in planta quantification of *Didymella pinodes*, *Phoma koolunga* and *Phoma medicaginis* var. *pinodella*, causal agents of ascochyta blight on field pea (*Pisum sativum*). *Mycologia*, 104(1): 2012, 93-101.

4. A.Gezahgne, Y. Abraham and K. Habtemaria, First report of *Lasiodiplodia theobromae* causing canker on tapped *Boswellia papyrifera* trees in Ethiopia. *New Disease Reports*, 29: 2014, 11.
5. V. Gonzalez and T. Maria Luisa, The endophyticmycota associated with *Vitis inifera* in Central Spain. *Fungal Diversity*, 47(1): 2011, 29-42.
6. V.K. Gupta, A.K. Misra and K.G. Rajarshi, Growth characteristics of *Fusarium* spp. causing wilt disease in *Psidium guajava* L. in India. *Journal of Plant Protection Research*, 50(4): 2010, 452-462.
7. K.P. Hareendranath, V. Nair and P. Suma, *Fusarium pallidoroseum* (Cooke) Sacc. as a fungal pathogen of *Aphis craccivora* Koch. *Entomon.*, 12: 1986, 392-399.
8. E.B.V. Ismael, I.R.V. Lydia and C.B. Juan, Morphological, pathogenic and molecular characterization of *Phoma* sp. isolated from onion field soils in Puerto Rico. *J. Agric. Univ. P.R.*, 92(1-2): 2008, 73-86.
9. P. Kausar, C. Sobia and P. Rashida, Physiological studies on *Lasiodiplodia theobromae* and *Fusarium solani* the cause of Shesham decline. *Mycopath*., 7(1): 2009, 35-38.
10. C. Kishore and K. Srikant, Effect of Media on the Growth of *Fusarium oxysporum* f. sp. *gerberae* and *Fusarium oxysporum* f. sp *dianthi*. *Karnataka J. Agric. Sci*., 21 (2): 2008, 303-304.
11. J.T. Kliejunas, J.R. Allison, A.H. McCain and R.S. Smith, Phoma blight of fir and Douglas-fir seedling in a California nursery. *Plant Disease*, 69: 1985, 773-775.
12. N.B. Koffie Clovis, A.D. Hortense, Y.K. Justin K. Paula, G.B. Alan and M.T. Lukasz, Occurrence of *Phythium aphanidermatum* root and collar rot of Papaya (*Carica papaya* L.) in Cote d Ivoire. *Fruit, Vegetables and Cereal Science and Biotechnology*, 4(1): 2010, 62-67.
13. P. Latha, V. Prakasam, E.I. Jonathan, R. Samiyappan and C. Natarajan, Effect of culture media and environmental factors on mycelial growth and pycnidial production of *Lasiodiplodia theobromae* in physic nut (Jatropha curcas). *Journal of Environmental Biology*, 34: 2013, 683-687.
14. P. Latha, V. Prakasam, A. Kamalakannan, C. Gopalakrishnan, T. Raguchander, M. Paramathma and R. Samiyappan, First report of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl causing root rot and collar rot disease of physic nut (*Jatropha curcas* L.) in India. *Australasian Plant Disease Notes*, 4: 2009, 19-20.
15. MacFaddin, *Media for isolation-cultivation-identification-maintenance of medical bacteria*. (Williams and Wilkins, Baltimore, Md. 1985, 1.)
16. H.A. Mirhosseini, V. Babaeizad, and L. Hashemi, First report of *Fusarium brachygibbosum* causing leaf spot on Oleander in Iran. *Journal of Plant Pathology*, 25: 2014, 103-125.
17. C. Mohanan, Introduction of root-trainer technology in forestry - impact on nursery disease management. In: Lilyia and Sutherlan (Ed.). *Diseases and insects in forest nurseries*. (Finnish Forest Research Institute, Finland, 2001).
18. S. Ojha, S. Khatun, M.R. Chakraborty and N.C. Chatterjee, Occurrence of die-back of *Dalbergia sissoo* in West

Bengal and evaluation of fungicidal control of its pathogen. *International Journal of Plant Protection*, 3(1): 2010, 17-19.

19. M.A. Palo, Rhizoctonia diseases of rice I. A study of the disease and of the influence of certain conditions upon viability of the sclerotial bodies of the causal fungus. *Philippine Agric*., 15: 1926, 361-376.
20. K.T. Parthiban, A.K. Bharathi, R. Seenivasan, K. Kamala and M. Govinda Rao, Integrating *Melia dubia* in agroforestry farms as an alternate pulpwood species. *Asia-Pacific Agroforestry Newletter*, 34: 2009, 3-4.
21. V.N. Pathak, *Laboratory manual of plant pathology* (Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, 1987).
22. B. Paul, R. Mathew, B. Kanak, A. Paul, M. Henry, F. Lefort and L. Belbahri, Morphology, taxonomy and phylogenetic analysis of a new species of *Pythium* isolated from France. *Fungal Diversity*, 28: 2008, 55-63.
23. G. Rangaswami, *Diseases of crop plants in India.* (Prentice Hall of India Pvt. Ltd. New Delhi. 1972).
24. A.Rehman, S.T. Sahi, M.A. Khan and S. Mehboob, Fungi associated with bark, twigs and roots of decline Shisham (*Dalbergia sissoo* Roxb.) trees in Punjab Pakistan. *Pak. J. Phytopathol*., 24(2): 2012, 152-158.
25. G. Sharma and R.R. Pandey, Influence of culture media on growth colony characters and sporulation of fungi isolation from decaying vegetable waste. *Journal of Yeast and Fungal Research*, 1(8): 2010, 157-164.
26. S. Sharma, R.B.L. Gupta and C.P.S. Yadava, Selection of a suitable medium for mass multiplication of entomofungal pathogens. *Indian Journal of Entomology*, 64(3): 2002, 2254-2261.
27. M.N. Suleiman, S.A. Emua and S.M. Ayodele, Growth and physiological studies on root rot fungus of cowpea. *European Journal of Experimental Biology*, 1(4): 2011, 181-188.
28. J. Tutte, *Plant pathological methods. Fungi and Bacteria*. (Burgass Publishing Company, USA, 1969).
29. G.E. Waterhouse, Key to *Pythium pringsheim*. *Mycol. Pap*., 109: 1967, 1-15.
30. K. Wen Hsiung, W. Shin-Yuan and A. Pao-Jen, *Pythium sukuiense*, a new species from undisturbed natural forest in Taiwan. *Mycologia*, 96(3): 2004, 647-649.
31. Y. Yan Long, J. Guang Wei, X. Sun, Y. Qiu He, J. Tong Luo and L. Dong Gu, Two new *Pythium* species from China based on the morphology and DNA sequence data. *Mycological Progress*. 11: 2012, 689.