Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0503.22**

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI INHABITING *AZADIRACHTA INDICA* A. JUSS. FROM DIFFERENT REGIONS OF JABALPUR (M.P) INDIA

Abid Hussain Qureshi*, Shoket Ali, Prof. A. K. Pandey

Mycological Research lab. Department of Post graduate studies and research in biological sciences R.D. University, Jabalpur, Madhya Pradesh, India.

ABSTRACT: A total 75 plant samples of *Azadirachta indica* A. Juss. Viz., leaf, stem and root were collected from different region of Jabalpur district of Madhya Pradesh state for isolation and identification of endophytic fungi. A total 11 different species of endophytic fungi were recorded. A maximum frequency *Aspergillus flavus* showed the highest colonizing frequency (36.36%) followed by *Alternaria alternata* (28.57%), *Penicillium* (28.00%), *Aspergillus niger* (24.00%), *Aspergillus oryzae* (23.80%), *Fusarium spp.* (20.00%) *Aspergillus fumigatus* (18.75%) *Cladosporium cladosporioides* (15.78%) *Phoma spp.* (13.63%) *Chaetomium globosum* (13.33%) and *Colletotrichum spp.* (13.33%) respectively.

KEYWORDS: *Azadirachta indica*, Endophytic Fungi, Identification and Isolation.

Corresponding Author: Dr. Abid Hussain Qureshi* Ph.D.

Mycological Research lab. Department of Post graduate studies and research in biological sciences R. D. University, Jabalpur, Madhya Pradesh, India.

1. INTRODUCTION

Azadirachta indica A. Juss belong to the family (Meliaceae) having its origin from south East Asia now has worldwide presence. It is native to India and significantly contributes to the forest cover of the northern areas. All parts of this plant show an array of negative effects on insects including ovipositor deterrent, antifeedant, and other inhibitory activities [1, 8]. The word endophyte means "in the plant" (endo-within, phyton-plant) [27]. Plants may provide as a reservoir of large numbers of microorganisms known as endophytes. Endophytes are microorganisms (mostly fungi and bacteria) that inhabit plant hosts for all or part of their life cycle. They colonize the internal plant

tissues beneath the epidermal cell layers without causing much disturbance in their host plants natural system and machinery, living within the intercellular spaces of the tissues and its seems that they may penetrate the living cells [32]. More than 100 compounds have been isolated from various parts of the neem tree [3, 26] and most of the active principles (Limonoids) belong to the group of tetranortriterpinoids especially 'Azadirachtin' and its analogs [5]. The people of India have known the useful properties of neem since time immemorial, and only recently have other people in more developed countries realized the value and importance of this tree to human activity [23]. For instance, various researchers have studied the medicinal properties of *Azadirachta indica* including its antipyretic effects [15,7], antimalarial effects [32,21], anti-tumor effects [4], anti-ulcer effects [18], anti-diabetic effects [25], anti-fertility effects [29], CNS effects [28], and cardiovascular effects [34]. Preliminary investigations of leaves and bark of neem [19, 26] cited the fact that living tissues of neem can successfully harbor endophytic fungi. Several reports in the recent years show that the endophytic fungi from this host produce several bioactive compounds [38, 11, 6, 37]. Approximately endophytes are microorganisms that are present inside in living tissue of various plant parts (root, stem, leaf etc.) establishing mutual relationship without apparently any symptom of diseases. [22, 30]. These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites [2, 31, 13]. The endophytic fungi play a vital roles in physiological and ecological in their host life. Recent investigation have been intensified by the potentialities of endophytic fungal strains in production of bioactive metabolites like taxol, pestaloside, torreyanic acid and enzymes [12, 35, 20]. Endophytic fungi are a good source of antibiotics, anticancer agents and biological control agents. Endophytic fungi are also capable to produce antimicrobial metabolites. The production of Hypericin ($C^{30}H^{16}O^{8}$), a naphthodianthrone derivative and Emodin ($C^{15}H^{10}O^{5}$) believed to be the main forerunner of hypericin, by the endophytic fungus isolated from an Indian medicinal plant [2]. A compound polyketide citrinin produced by endophytic fungus *Penicillium janthinellum* from fruits of Melia azedarach, presented antibacterial activity against *Leishmania sp*. [4]. Medicinal plants are known to harbor endophytic fungi that are believed to be associated with the production of pharmaceutical commodities. [39]. Therefore, it is important to survey endophytic mycoflora in the medicinal plants. The present study was carried out to isolate, identify of endophytic fungi which are isolated from different parts of *Azadirachta indica* A. Juss (Neem).

2. MATERIALS AND METHODS

In the present study endophytic fungal species were isolated from different parts of the medicinal plant *Azadirachta indica* A. Juss. commonly called neem were collected from different regions of Jabalpur, Madhya Pradesh (India). Healthy and mature plant parts were carefully chosen for sampling. The plant parts were brought to the laboratory in sterilized polythene bags and processed

within a few hours after sampling was done.

Isolation of Endophytic fungi

Isolation of endophytic fungi from different parts of *Azadirachta indica* A. Juss. was done according to the method described by [17, 30]. First the plant parts material was rinsed in tap water to remove the dust and debris then cut into the small pieces by a sterilized blade under aseptic conditions. Each sample was surface sterilized by 70% ethanol for 1 minute and after that plant parts were immersed in sodium hypochlorite (NaOCl) solution for 30 seconds to 1 minute. The plant samples were rinsed in sterile distilled water for 1 minute and then allowed to surface dry on filter paper. After proper drying 4 pieces of plant parts were inoculated in PDA plate supplemented with antibiotic (Tetracycline) and incubated at 28 $\pm 1^0$C for 5 to 7 days. Pure colonies were transferred on PDA slant. The fungal strains in the pure culture were preserved on potato dextrose agar (PDA) slant at 4 to 5^{o}C with proper labeling and were subcultured from time to time. The microscopic observation of Endophytic fungi was carried out by using cotton blue staining technique [14]. The rate of colonization percentage of endophytes was calculated by following formula.

$$\text{Rate of colonization (\%)} = \frac{\text{Number of segments colonized by single fungus} \times 100}{\text{Total number of segment observed}}$$

3. RESULTS AND DISCUSSION

Table 1: List of endophytic fungi isolated from different parts of *Azadirachta indica* A. Juss.

S. No.	Plant parts	Name of endophytic fungi	Class
1	Leaf	*Aspergillus fumigatus*	Hyphomycetes
2	Leaf	*Aspergillus oryzae*	Hyphomycetes
3	Stem	*Aspergillus niger*	Ascomycetes
4	Stem	*Fusarium semitectum*	Hyphomycetes
5	Leaf	*Cladosporium cladosporioides*	Hyphomycetes
6	Root	*Phoma sp.*	Hyphomycetes
7	Leaf	*Alternaria alternata*	Hyphomycetes
8	Root	*Chaetomium globosum*	Ascomycetes
9	Root	*Penicillium*	Hyphomycetes
10	Leaf	*Colletotrichum sp.*	Coelomycetes
11	Root	*Aspergillus flavus*	Hyphomycetes

Table 2: Colonizing frequency (%) of Endophytic fungi isolate from different parts of *Azadirachta indica* A. Juss.

S. No.	Name of Endophytic Fungi	Isolate from	% Frequency of colonization	No. of isolates
1	*Aspergillus flavus*	Root	36.36%	4
2	*Alternaria alternata*	Leaf	28.57%	6
3	*Penicillium*	Root	28.00%	7
4	*Aspergillus niger*	Stem	24.00%	6
5	*Aspergillus oryzae*	Leaf	23.80%	5
6	*Fusarium spp.*	Stem	20.00%	4
7	*Aspergillus fumigatus*	Leaf	18.75%	3
8	*Cladosporium cladosporioides*	Leaf	15.78%	3
9	*Phoma spp.*	Root	13.63%	3
10	*Chaetomium globosum*	Root	13.33%	2
11	*Colletotrichum sp.*	Leaf	13.33%	2

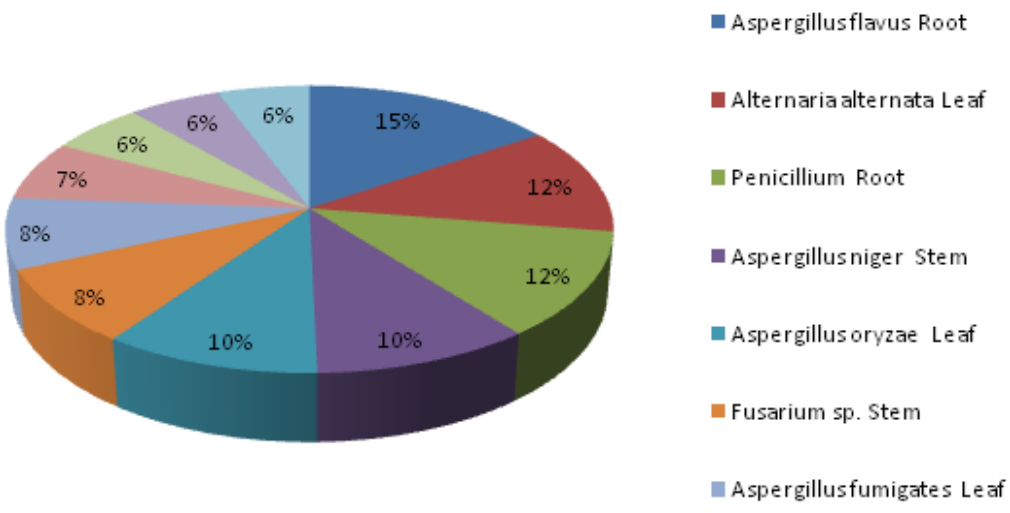

Figure 1: Percentage of endophytic fungi isolated from different parts of *Azadirachta indica* A. Juss.

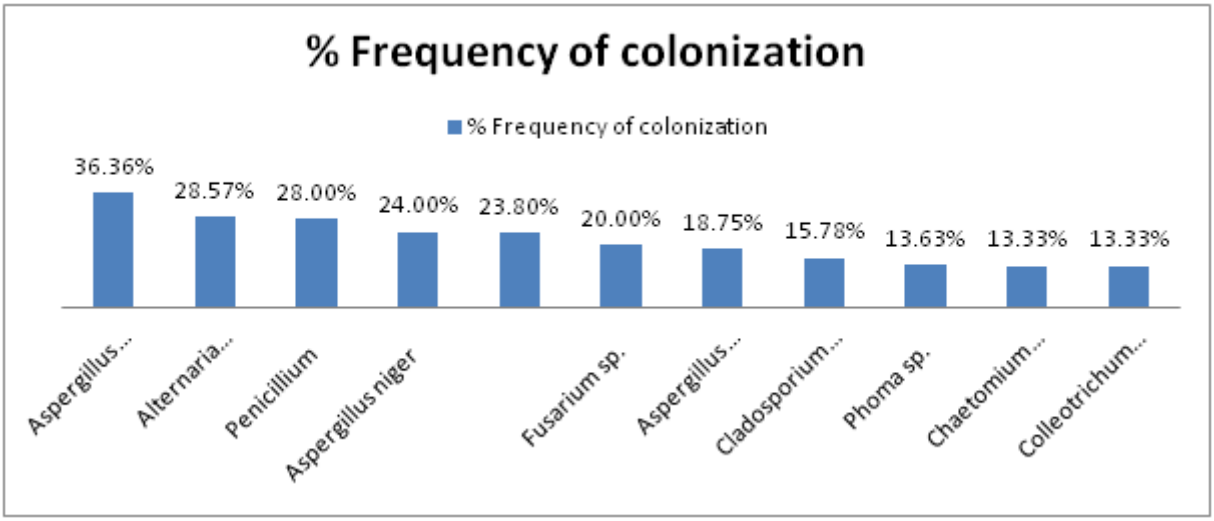

Figure 2: Colonizing Frequency (%) of endophytic Fungi isolated from from different parts of *Azadirachta indica* A. Juss.

Figure: (a) Isolation of endophytic fungi, (b and c) Pure fungal isolates, (d) Preservation of pure fungal isolates

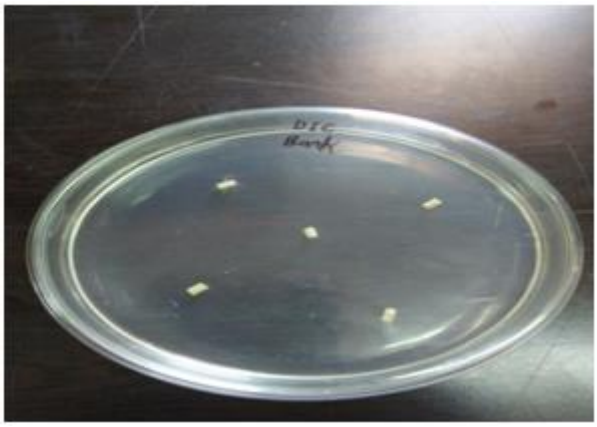

Fig.(a) Isolation of endophytic fungi

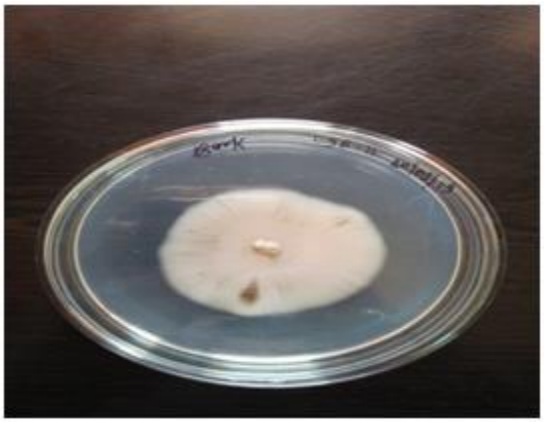

Fig.(b) pure culture of endophytes

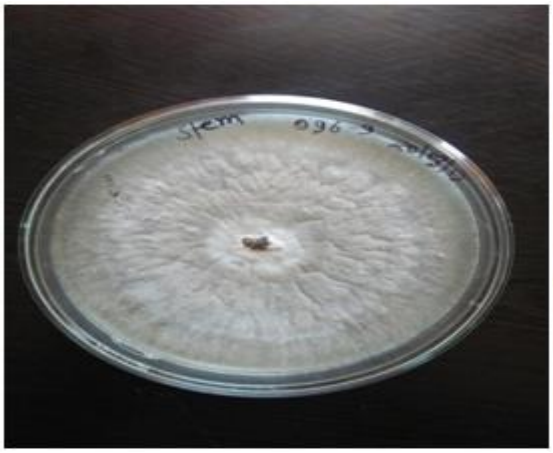

Fig.(c) pure cultures of endophytes

Fig.(d) Preservation of pure fungal isolate**.**

It was observed that more endophytic fungi were isolated from stem (22.22%) as compared to root (21.91%) and leaves (20.65). During this study a total 11 different endophytic fungi were found. Among these *Aspergillus flavus* showed the highest colonizing frequency (36.36%) followed by *Alternaria alternata* (28.57%), *Penicillium* (28.00%) ,*Aspergillus niger* (24.00%), *Aspergillus oryzae* (23.80%), *Fusarium sp.* (20.00%) *Aspergillus fumigatus* (18.75%) *Cladosporium cladosporioides (*15.78%) *Phoma sp. (*13.63%) *Chaetomium globosum* (13.33%) and *Colletotrichum sp.*

4. CONCLUSION

There is a symbiotic relationship between endophytic fungi and host plants. Plants provide nutrition and shelter to endophytes and in returns endophytic fungi excrete functional products and increase their resistance to different stresses. Therefore, endophytic fungi, producer of a wide array of secondary bioactive metabolites with their peculiar potential compounds, namely, melanin, antimicrobials, antioxidant, anti-inflammatory, insecticides, nematicides, etc. These bioactive compounds have arrange of potential to combat etiologic agents of plants and animals disease.

ACKNOWLEDGEMENT

The authors are thankful to Prof A.K. Pandey Mycological research lab. Department of post graduate studies and research in Biological science R.D. University, Jabalpur, (M.P.) for his moral support and suggestions made by him.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Butterworth JH, Morgan ED, Percy GR.The structure of azadirachtin; the functional groups. J Chem Soc Perkin Trans. 1972; 1:2445–2450
2. Carroll GC, Carroll FE. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest: Can J Bot. 1978; 56:3032-3043.
3. Champagne DE, Koul O, Isman MB, Scudder GGE, Towers GHN. Biological activity of limonoids from the Rutales. Phytochem.1992; 31:377–394.
4. Fujiwara T, Takeka TO, Gihara Y, Shimizu M, Nomura T, Tomuta Y. Studies on the structure of polysaccharides from the bark of Melia azadirachta. Chem Pharm Bull. 1992; 30:4025–4030.
5. Kaushik B, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activity and medicinal properties of neem (*Azadirachta indica*). Curr Sci. 2002; 82:1336–1345.
6. Kharwar RN, Verma VC, Kumar A, Gond SK, Harper JK, Hess WM, Lobkovsky E, Ma C, Ren Y, Strobel GA. Javanicin, an antibacterial naphthoquinone from an endophytic fungus of neem, Chloridium sp. Curr Microbiol. 2009; 58(3):233–238.
7. Khattak SG, Gilani SN, Ikram M. Antipyretic studies on some indigenous Pakistani medicinal

plants. J Ethnopharmacol. 1985; 14:45–51.

8. Koul O, Isman MB, Ketkar CM. Properties and uses of neem, *Azadirachta indica*. Can J Bot. 1990; 68:1–11.
9. Kusari S, Spiteller M. Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes: Nat Prod Rep. 2011; 28:1203-1207.
10. Ley SV, Denholm AA, Wood A. The chemistry of azadirachtin. Nat Prod Rep. 1993; 10:109–157.
11. Li GH, Yu ZF, Li X, Wang XB, Zheng LJ, Zhang KQ. Nematicidal metabolites produced by the endophytic fungus Geotrichum sp. AL4. Chem Biodivers. 2007; 4:1520–1524.
12. Malinowski DP, Zuo H, Beleskey DP, Alloush GA. Evidence for copper binding by extracellular root exudates of tall fescue but not perennial ryegrass infected with Neotyphodium spp. endophytes: Plant Soil. 2004; 267:1-12.
13. Marinho MR, Rodrigues-Filho E, Moitinho R, Santos LS. Biologically active polyketides produced by Penicillium janthinellum isolated as an endophytic fungus from fruits of Melia azedarach: J Brazil Chem Soc. 2005; 16: 280-283.
14. Nagamani A, Kunwar IK, Manoharachary C. Handbook of Soil Fungi:I K International (P) Publication Ltd., New Delhi. 2005.
15. Okpanyi SN, Ezeukwu GC. Anti-inflammatory and antipyretic activities of *Azadirachta indica*. Planta Med. 1981; 41:34–49.
16. Patil MG, Pagare J, Patil SN and Sidhu AK. Extracellular Enzymatic Activities of Endophytic Fungi Isolated from Various Medicinal Plants.International Journal of Current Microbiology and Applied Sciences. 2015; 4: 1035-1042.
17. Petrini O. Taxonomy of endophytic fungi of aerial plant tissues in Fokkema, N.J and van den UK. 1986; pp. 175–187.
18. Pillai NR, Santhakumari G. Effects of Nimbidin in acute and chronic gastroduodenal ulcer models in experimental animals. Planta Med. 1984; 50:143–146.
19. Rajagopal R, Suryanarayanan TS. Isolation of endophytic fungi from leaves of neem (*Azadirachta indica*). Curr Sci. 2000; 78:1375–1378.
20. Red “et al.”Isolation of fungal endophytes from Garcinia mangostana and their antibacterial activity. Afri J Biotechnol. 2011; 10(1):103-107.
21. Rochankij S, Thebraranonth Y, Yenjal CY. Nimbolide a constituents of *Azadirachta indica* inhibits Plasmodium falciparum in culture. SEA J Trop Med Pub Health. 1985; 16:66–72.
22. Sandhu SS, Suneel K, Aharwal RP, Shukla H and Rajak R C. Endophytic Fungi: As A Source of Antimicrobials Bioactive Compounds. World J Pharm Sci. 2014a; 3 (2):1179-1197.
23. Schmutterer H. Properties and potential of natural pesticides from the neem tree. *Azadirachta indica*-Rev Entomol 1990; 35:271–297.

24. Selim KA, El-Beih AA, AbdEl RTM, Diwany AI. Biology of Endophytic Fungi.Current Research in Environmental & Applied Mycology. 2012; 2:31-82.
25. Shukla R, Singh S, Bhandari CR. Preliminary clinical trials on anti diabetic actions of *Azadirachta indica*. Med Surg. 1973; 13:11–12.
26. Siddiqui S, Faizi S, Siddiqui BS, Mahmood J Tetracyclic triterpenoides and their derivatives from *Azadirachta indica*. J Nat Prod. 1988; 51:30–43.
27. Singh D, Rathod V, Singh AK, Joshi R, Nagaratna H, Azmath U, Avinash B. Antibacterial Activity and Phytochemical Analysis of the Crude Extracts of Endophytic Fungus, Alternaria sp. from the Medicinal Plant Euphorbia hirta (L). International Journal of Green Chemistry and Bioprocess. 2015; 5:14-20.
28. Singh PP, Junnarkar AY, Reddi GS, Singh KV. *Azadirachta indica* neuro psychopharmacological and anti-microbial studies. Fitoterapia. 1987; 58:233–238.
29. Sinha KC, Riar SS, Tiwary RS, Dhawan AK, Bhadhan J, Thomas P, Kain AK, Jain PK Neem oil as a vaginal contraceptive. Ind J Med Res. 1984; 79:131–136.
30. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Molec Biol Rev. 2003; 67:491-502.
31. Strobel GA. Microbial gifts from rainforests. Can J Plant Pathol. 2002; 24:1420.
32. Tella A. The effects of *Azadirachta indica* in acute Plasmodium berghei malaria. Nig Med J. 1977; 7:258–263.
33. Theantana T, Hyde KD, Lumyong S. Asparaginase production by Endophytic fungi isolated from some Thai medicinal plants. KMITL Sci Tech J. 2007; (S1):1318.
34. Thomson EB, Anderson CC. Cardiovascular effects of *Azadirachta indica* extract. J Pharma Sci. 1978; 67:1476–1478.
35. Tintjer T, Rudgers JA. Grass herbivore interaction altered by strains of a native endophyte. New phytol.2006; 170(3):513-21.
36. Verma VC, Gond SK, Kumar A, Kharwar RN, Strobel G. The endophytic mycoflora of bark, leaf and stem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). Microb Ecol. 2007; 54:119–125.
37. Wu SH, Chen You-Wei, Shao Shi-Cheng, Wang Li-Dong, Ying Yu, Zhi-Ying Li, Yang Li-Yuan, Shao-Lan Li, Huang Rong. Two new solanapyrone analogues from the endophytic fungus Nigrospo sp. YB-141 of *Azadirachta indica*. Chem Biodivers. 2009; 6:79–85.
38. Wu SH, Chen YW, Shao SC, Wang LD, Li ZY, Yang LY, Li SL, Huang R. Ten-membered lactones from Phomopsis sp., an endophytic fungus of *Azadirachta indica*. J Nat Prod. 2008 ; 71(4):731–734.
39. Zhang H W Song Y C and Tan RX. Biology and chemistry of endophytes. Nat Prod Rep. 2006; 23:753.