Isolation and Data Analysis of Fungal Endophytes

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Abstract

Plant-fungal associations have been known for millions of years. Mutual associations provide immense benefits; endophytic fungi get shelter, whereas plants get protected from foreign pathogens and help develop resistance against environmental stress. Endophytic fungi produce numerous kinds of secondary metabolites that are highly important in various sectors, including medicinal, industrial, and cosmetics. More research on colonisation of various endophytic fungi is required. In this study, endophytic fungi were isolated from *Azadirachta indica*, *Emblica officinialis*, and *Ocimum* species, and their colonisation and isolation rates were determined. Initially, plant samples were collected and surface sterilised, followed by inoculation. Then colonisation and isolation rates were recorded. From *Azadirachta indica*, *Emblica officinialis*, and *Ocimum species*, the total colonisation rate was recorded as 93.60%, and the isolation rate was 45.34%. The colonisation rate of individual plants indicates the fungal richness carried by plants.

Keywords: Fungal endophyte, Colonisation frequency, Isolation rate**.**

1. INTRODUCTION

Plants are inhabited by various kinds of microbes, and microbes that survive within the plant tissues are termed endophytes. Endophytes and plants coexist and mutually benefit each other rather than harming each other (Kumari et al.,2023; Khare et al., 2018). Microorganisms that are predominantly found in internal tissues of plants are fungi (Dastogeer et al., 2020). Fungal endophyte-plant interactions are known to have occurred for more than millions of years. Research over approximately 100 years reveals that, for endophytic fungi, plants act as a natural habitat, and they share a symbiotic association. De Barry (a German researcher in the 1990s) described the presence of microorganisms in the internal tissues of plants; since then, more studies on endophytic fungi have been conducted. Plants act as macroscopic hosts, and endophytic fungi act as microscopic symbionts. Fungal endophytes can potentially resist foreign pathogens and abiotic and biotic stress but also promote plant growth. Fungal endophytes can produce secondary metabolites, thereby carrying vast applications in various fields, including industrial, medical and agricultural sectors. Secondary metabolites influence the colonisation frequency within the plant tissues (Taheri et al., 2022).

2. Method

2.1 Plant sample collection.

Uninfected plant samples of *Ocimum* sp., *Azadirachta indica* and *Emblica officinialis* were collected from Jabalpur, Madhya Pradesh, India. In a sterile plastic bag, plant samples were kept, then moved to the laboratory and stored at 4°C. Within 24 hours, isolation should be conducted (Kanjana et al. 2019).

2.2 Isolation of Fungal Endophyte.

Wash plant samples under running tap water and dry them at room temperature. Prepare small slices of plant samples. According to Dobranic et al. (1995), plant samples were surface sterilised. Plant slices were immersed in 70% ethanol (5sec), followed by immersing the tissue in 4% sodium hypochlorite (90 sec), and finally dipping the tissues into deionised water (10 sec). Excess water adhered to the plant tissue was removed using tissue paper. Various plant parts were used for fungal isolation, including flowers, stems, and leaves. Antibiotic (chlorophenicol) supplemented potato dextrose agar plates were inoculated with plant tissues. Plates were then incubated at 27±2°C for 7 days. Observe the fungal emergence and then subculture fungal colonies into other PDA plates. Subculture the colonies till a pure fungal

isolate is obtained. Preserve the pure fungal isolates on PDA slants and store them at 4°C (Kurandawad and Lakshman, 2014; Yang et al., 2014).

2.3 Determining colonisation frequency (CF) and Isolation rate of fungal endophytes

Fungal endophytes were isolated from three different plant specimens. The number of segments that fungal endophytes have colonised can be calculated by dividing the total number of segments colonised by the total number of segments incubated (Paul et al., 2017).

$$\text{Colonising frequency (CF)} = \frac{\text{Total number of segments colonised}}{\text{Total number of segments incubated}} \times 100 \quad (1)$$

Plant specimens were used to harvest fungal endophytes. The richness of fungal endophytes that inhabit the segments of plant specimens can be determined using the isolation rate (IR). IR can be expressed as the total number of fungal endophytes harvested from a segment of plant tissue divided by the total number of plant segments that exhibit fungal endophytes (Vemireddy et al., 2020).

$$\text{Isolation rate (IR)} = \frac{\text{Total number of fungal endophytes harvested}}{\text{Total number of plant segments}} \times 100 \quad (2)$$

3. Results and discussion

In this study, numerous fungal endophytes were isolated from *Azadirachta indica*, *Emblica officinialis*, and *Ocimum* species. A total of seventy-eight (78) fungal strains were isolated out of these fourty six strains belongs to *Azadirachta indica*. From the tissues of leaves (27) and from the stem (19), fungal strains were isolated. From the *Ocimum* species (24), fungal cultures were isolated. From leaves (14), stems (5) and flowers (5), fungal strains were isolated. From *Embilica officinialis* (8), fungal strains were isolated. From tissues of leaves (5), petiole (1) and stem (2), fungal strains were isolated. Similarly, Gond et al., 2012 reported that 19 fungal endophytes were isolated from the Nyctanthes arbor-tristis plant. For isolation, healthy stem and leaf were segmented into 400 pieces. From the stem, they isolated 126 endophytic fungi, whereas from the leaf, 281 fungal endophytes were isolated.

A total of 172 plant tissues were inoculated on PDA plates and incubated. Of these, 161 tissues showed fungal emergence. From these, 78 fungal strains were isolated. Therefore, the total isolation rate of all three plants combined was 45.34%. The colonisation rate was 93.60%. A total of 101 *Azadirachta indica* plant tissues were inoculated on PDA plates. Fungi emerged from 95 tissues, and only 46 fungal strains were isolated. Therefore, the colonisation rate of *Azadirachta indica* was 94.0%, and the isolation rate was 45.54%. On PDA, 46 tissues of *Ocimum* species were inoculated. From 46 tissues, fungi emerged, of which 24 fungal strains were isolated. Therefore, the colonisation rate of *Ocimum* species was 97.8%, and the isolation rate was 51.06%. A total of 24 tissues of *Embilica officinialis* were inoculated on PDA. From eighteen (18) tissues, fungi emerged, of which 9 fungal strains were isolated. Therefore, the colonisation rate of *Embilica officinialis* species was 75.0%, and the isolation rate was 33.33% (Table 1, Fig.1). Similarly, Choudhury et al., 2023 reported that *Aspergillus* sp. exhibited a 15% CF, whereas Fusarium exhibited 20% CF and Penicillium exhibited 10% CF.

TABLE 1 - Colonisation rate and isolation rate of endophytic fungi isolated from plants

Item	*Azadirachta indica*	*Ocimum* sp.	*Emblica officinalis*	Total	Average
Number of inoculated tissue blocks	101	47	24	172	57.3
The number of infected tissue blocks	95	46	18	161	53.6
Number of isolated strains	46	24	9	78	26
Colonisation rate (%)	94.0	97.8	75	93.60	30.78
Isolation rate (%)	45.54	51.06	33.33	45.34	15.11
Proportion of isolated strains to	58.97	30.7	10.25	99.92	33.30

total strains					

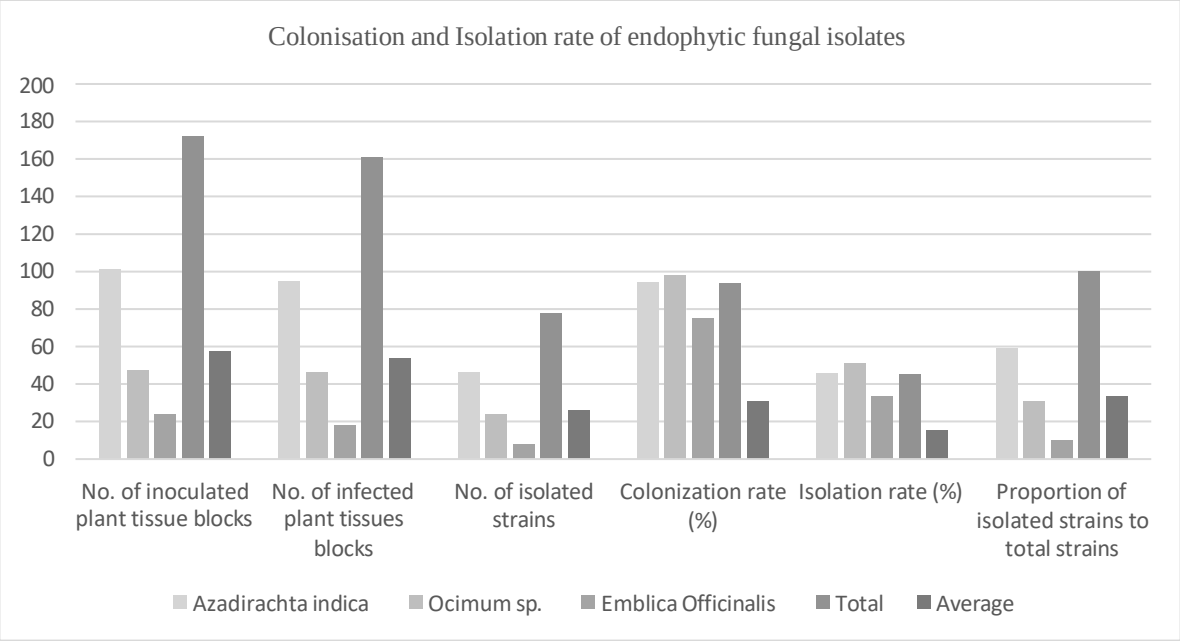


Fig. 1 – Colonisation and isolation rate of fungal endophytes

4. Conclusion

Plants can be an excellent reservoir of endophytic fungi. In the present study, colonisation and isolation rates of endophytic fungal strains were studied. It was recorded that the total colonisation rate was 93.60% and the isolation rate was 45.34%. These results indicate that plants are highly colonised by fungi, and these fungi can be isolated and utilised in various sectors such as medicinal, industrial, biofuel, cosmetics, food, etc.

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Availability of data and materials

All data generated or analysed during this study are included in this article.

Authors' contributions

M.Kothari, Conceptualisation, methodology, investigation, writing—original draft preparation; S.S.Sandhu, supervision. All authors have read and agreed to the published version of the manuscript.

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