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Endophytic Fungi and their Metabolites Isolated from Bauhinia Racemosa Lamk Plant

Venkatesan Govindan

Assistant Professor, P.G. and Research Department of Botany, Mannai Rajagopalaswami Government Arts College, Mannargudi-614001, Thiruvarur - District, Tamil Nadu, India

Abstract— *Bauhinia racemosa* family, Caesalpiniaceae is one of the precious resources of the earth. It is traditionally used in the indigenous system of medicine Ayurveda, Unani, and Sidha for the treatment of several ailments like headache, fever, skin and blood diseases, jaundice, chronic dysentery, diarrhea, and leucorrhoea, infection of malaria, boil, glandular swelling, tumors, and cancer. It is also used to cure scorpion bite, to relieve food poisoning in cattle and as a contraceptive by women. Endophytic fungi live in leafage of medicinally important plants are diverse and abundant; also little is known of their terrestrial and habitat variation. Age differences in endophyte infections on young, mature, and senescent leaves of a medicinal plant *Bauhinia racemosa* were studied. Enzyme evaluation, it was observed that the endophytic fungi species *Phyllosticta* sp., *Colletotrichum* sp., *Aspergillus* species were presented four enzymes amylase, pectinase, lipolytic, and cellulose activity. Particularly have indicated only cellulose was produced in *Aspergillus niger*. The endophytic fungus was tested against standard bacterial and fungal cultures. In vitro antimicrobial test was carried out by the agar well diffusion method. The minimum inhibitory concentration test was performed by the modified agar well diffusion method. Endophytic fungi showed a broad spectrum of antimicrobial activity as it inhibited Gram-positive bacteria in *Bacillus*, and fungi in *Colletotrichum*.

Keywords — *Bauhinia Racemosa; Bioactive Metabolites; Endophyte Fungi; Tissues Specificity.*

1. Introduction

Endophytes are microbes that colonize the internal plant tissue underneath the epidermal cell layers without causing any obvious symptom or symptomatic infection to their host [1]. Hence, it is more difficult to complete the global fungal enlist, as compared to other organisms such as animals, plants. Fungi play key roles in ecosystems as decomposers, mutualisms, and pathogens, while in most cases, the role of individual fungus in nature is still unknown [2]. In most of the cases, each tissue segment was infected by more than one fungal species [3]. Several ecological groups of plants such as mangroves [4], halophytes [5], fresh water [6] and trees of tropical forest [7, 8, 9] have been studied for their endophyte assemblages. Endophytes usually inhabit above-ground plant tissues which distinguish them from better known mycorrhizal symbionts. The endophytic fungi are ubiquitous and vastly diverse in host plants. Every plant examined to date harbors at least one species of endophytic fungus and many plants, especially woody plants, may contain literally hundreds of thousands of species [3, 10]. Currently, studies on endophytes of medicinal plants have given due attention as they oversee to produce of natural products beneficial to the human being [11]. Apart from isolating probable strains from medicinal plants, variety studies focus on different features of ecology. Endophytic fungi are reasonably unexplored producers of metabolites useful to pharmaceutical and agricultural industries. A single endophyte produces diverse bioactive metabolites. While the result, the function of endophytes in the production of diverse novel products with significant bioactivity has obtained promoted regard [12]. Address the common observation that foliar endophyte infections increase in density with leafage, assessing the relative importance of age-specific, leaf toughness, and duration of disclosure to fungal diversity. It explicitly identifies ecological factors underlying patterns of endophyte colonization within healthy leaves of this tropical tree of *Bauhinia racemosa*. A few additional investigations have focused on the antimicrobial activity of endophytic fungi against clinical isolates of Gram-positive bacteria and fungi.

2. Materials and Methods

2.1 Botanical description

Bauhinia racemosa (Lamk), belonging to the family Caesalpiniaceae is a small deciduous tree used in the primordial treatment of medicine (Fig.1), the tree growing throughout India and Srilanka. Its importance played a significant role in human civilization since ancient times. It has universally one of the major roles in the treatment of human Injury and diseases worldwide. Currently, the demand for medicinal plants is increasing for both developed and developing countries due to the growing recognition of natural products. It is traditionally used for the treatment of various ailments like vermicides, urinary discharges, thirst headache, quatrain fever, fistula, tuberculoses glands, skin diseases, throat troubles, tumors, diseases of the blood, etc. It is having antifilarial activity, anthelmintic, antimicrobial, antihistaminic, anti-

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inflammatory, analgesic, antimalarial, anti-oxidant, and various other pharmacological activities. The leaves of *B. racemosa* are used for bidis production like so the plant is called as bidi leaf tree, and also used for good fodder in sheep, goats, and cattle.

2.2 Surface Sterilization and Culture Protocols

The host plant studied was collected from Mannargudi, Tamil Nadu, and South India. These stages of plant leaves were collected for the investigation. Leaf samples were collected from healthy plants. In these plants from Young, mature, and senescent leaves were randomly collect fifty leaves a few plants and their one hundred and fifty tissue segments were cut from fifty leaves. However, sterilization techniques were followed before cutting these segments. The plant leaves are washed thoroughly with running water and then the leaves have sterilized as followed. After the surface sterilization, the leaves were cut into 0.5 cm^2 bits (segments) each leaf. The samples were washed in running water, dipped in 70% ethanol for 60 seconds, immersed in 2.5% NaoC1 for 90 seconds, and then washed in sterile water for 60 seconds or three times. The sterilized samples were placed on the PDA medium amended with antibiotics contained in Petri dishes. The Petri dish was sealed with Parafilm^{TM} and incubated in a light chamber at 26+1ºC for 7 to 21 days [13, 14]. The light regimen given was 12 hours light followed by 12 hours of darkness. Fungi that grew from the segments were periodically observed and the endophytes were identified.

2.3 Statistical Analysis

Colonization frequency (CF %) is the number of colonies/ number of total X 100. The number of colonies and the number of totals is the number of segments colonized by each endophyte and the total number of segments observed respectively [15].

2.4 Biological Active Analytical Evaluation

The isolated endophytic fungi from these aquatic plants of *Bauhinia racemosa* and selected dominant and common hyphomycetes and Coleomycetes group of endophytes were screened extracellular enzyme activity. The enzyme examine was made on prepared by both a spread- plate technique with spores and mycelium [16].

2.5 Cellulase Actitivity

Yeast peptone medium containing Na-Carboxy-methylcellulose (0.5%) was used for Cellulase activity. Later than 3-5 days of fungal colony growth, the Petric plates were streamed with 0.2% aqueous Congo red strain compound and destained with 1M NaCl (15 min each). The appearance of the yellow zone around the fungal colonization in red medium indicates cellulase activity.

3. Results and Discussion

Fungal endophytes were isolated from the tissues of the *Bauhinia racemosa*. The leafage has found in dissimilar fungal species were isolated. The tissues of the young, mature, and senescence leaves reveal 8, 9, and 24 species, respectively, and also the number of isolates is disguised from age of leaf such as young (20), mature (109), senescence (119) of leaves (Fig. 2). The fungal species were constantly isolated. Hyphomycetes were the abundant group followed by Coelomycetes, Ascomycetes, and unidentified forms.

Fig. 1: Photo is shown on the plant of *Bauhinia racemosa*

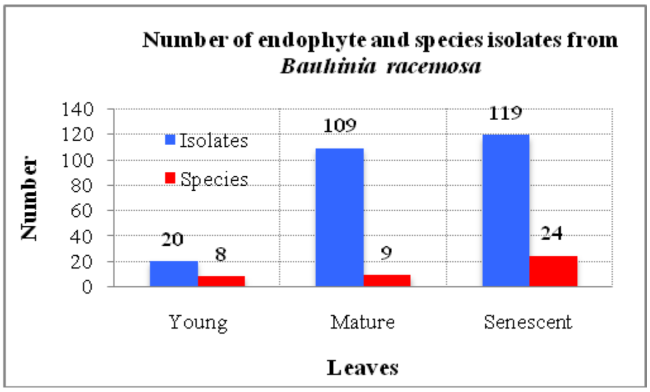

Fig. 2: The number of fungal endophytes and species isolated from Young, Mature, and Senescence leaves

Fungal endophytes reside in healthy tissues of all tropical and temperate terrestrial plant taxa studied to date. These fungal were diverse and abundant in leaves of tropical woody angiosperms. Although, the fungal diversity was varied from leaves (ages) tissue specificity. Studies have illustrated that plant location and leaf age impact the density of endophyte infection in leaves of *Bauhinia racemosa*. However, ecological factors have not been investigated in detail. In order to understand the status of

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endophytes assemblage in different stages of the leaf, the young, mature and senescent leaves were screened. Bright green, thin, tender, young leaves; dark green, thick mature leaves and yellow, old senescent leaves of *B. racemosa* tree were collected and screened. The yellow senescent leaves were collected by gently tapping the petioles. Only those senescent leaves that could be detached by this method were included for the study. This indicated that they were in the late stage of senescence and would have fallen in a few days. The procedure for inoculation of endophytes from leaves of these all ages of leaves was standard sterilization. The senescent leaves were more densely colonized by endophytes when compared with young leaves in the host of *B. rasemosa*. The senescent leaves were found to assume that to be more species of fungi as endophytes. The fungal spores can be transmitted from air/soil to leaf in aerial tissues/seedling of the surface the wetting leaves. However, these conceptions cannot be proclaimed for a single host studied. The endophytes diversity and richness is determined the importance of several factors such as canopy, leafage, leaf toughness, leaf chemistry and duration of exposure to air etc. In this studies have demonstrated that plant leaves environments and leaf ages influence the diversity of endophytes present in leaves of *B. racemosa* (Fig. 3). For this tree species, we recorded varied these endophytic fungi have been found to study in different leaf ages (young, mature, senescent). Among 20 (13.3%) colonies isolates, 8 fungal species be held in young leaves, 109 (72.6%) colonies isolates, 9 fungal species be held in mature leaves and 119 (79. 3%) colonies isolates, 24 species held in senescent leaves from 450 leaves (each age 150 bits) segments leaves were isolated (Fig. 2).

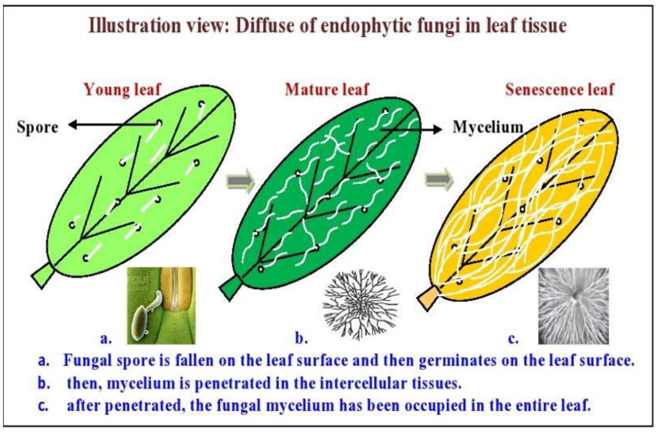

Fig. 3: Imaginary diagram show on penetrate and increase of endophytic fungi in leaf

Nevertheless of age at genesis, all mature leaves of plant seedling contained fungal endophytes. It is detected in many diverse differences in morphology between leaves that matured in the plant and leaves that were mature at outset. The endophyte species had a higher colonization frequency, as well as fungal species, increased in senescent leaves. *Phomopsis* sp. *Colletotrichum* sp. and *Phyllosticta* sp were obtained from all the ages of leaves. The fungal endophyte colonization is high richness becoming to occupy in leaf tissues from young to mature leaves. Endophytes isolated from mature *B. racemosa* more rapidly on rich diversity mature leaf. Also, we believe this information allows us to discover underlying patterns of endophytic colonization within healthy leaves of this tropical tree [5, 17].

Endophytic fungi are rich sources of bioactive natural products that can be used to gratify of Pharmaceutical, medicinal, agriculture and industries. These ubiquitous fungi of *Aspergillus niger* were screened for four enzymes tested. This endophyte showed that produce amylase, lipolytic, cellulase, laccase enzymes such as cellulase enzyme are rich produce (Fig. 4) and also similar recorded [18, 19, 20]. Cellulose, the substrate of cellulose, is the most plenty of polysaccharide present on earth. It is the main substance in fungi and plant materials. In the present study, pretreated lignocellulose was used for the maximum production of cellulase from *Aspergillus niger*. The cellulose resource of *Aspergillus niger* was commonly screened by plate assay method. For this, Yeast peptone (YP) medium amended with 0.5% carboxymethylcellulose (CMC) and the plates were incubated for 3 days. After incubation plates were stained with 0.2% Congo-red solution, followed by de-staining with 1M NaCl solution for 15 minutes. This test has gained; the cellulases are a complex group of enzymes which are secreted by a broad range of microorganisms including fungi, bacteria, and actinomycetes. Cellulose-degrading fungi can be directly used in industrial production, but the optimum conditions for enzyme production and utilization must be considered simultaneously, because their environmental requirements are not uniform. Filamentous fungi are outward hyphae, that is, their major mode of obtaining nutrients is to secrete hydrolytic enzymes and then absorb the products through the plasma membrane (Fig. 5).

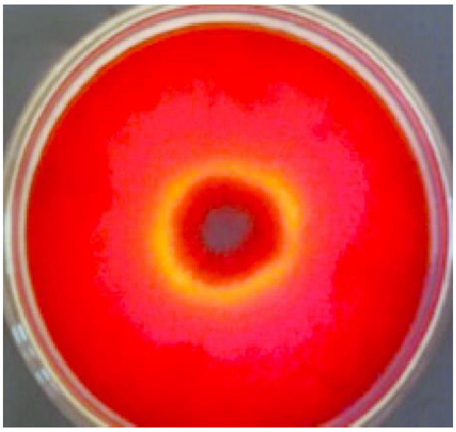

Fig. 4: Screening of cellulolytic activity of *Aspergillus niger*

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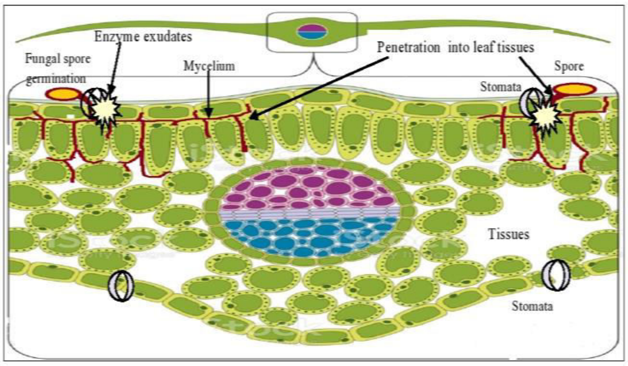

Fig. 5: Diagram showing on the diffusion of endophytic fungi in leaf tissue

The endophytic fungi of *Colletotrichum* species of *Bauhinia racemosa* were showing good antimicrobial activity against standard *Bacillus subtilis* bacterial cultures. In vitro antimicrobial test was produced by agar well diffusion method on Mueller Hinton agar and Sabouraud dextrose agar for bacterial and fungal cultures respectively. The minimum inhibitory concentration test was performed by a modified agar well diffusion method. The endophytic fungi showed a broad spectrum of antimicrobial activity as it inhibited Gram-positive bacteria (*Bacillus subtilis*) verses fungi (*Colletotrichum* species).

4. Conclusion

Endophytic fungi of woody plants proceed between hosts as spores, germinating epiphytically, and penetrating leaf cuticles to grow intercellularly within healthy tissues. Therefore, successful colonization of host tissues by foliar endophytes likely is correlated with factors influencing the local abundance of aerial and epiphytic propagules, and with diverse aspects of host plant suitability, including host genotype and leaf characteristics. This study denotes refer that high inoculums volume, beneficial conditions for the survival of epiphytic propagules, and multiple applications throughout the leaf and plant lifetimes should increase colonization of healthy tissues by foliar endophytes under field conditions. Hence, in this investigation finding can be further developed by performing the young, mature, and senescent leaves of endophytic fungal diversity, identification, and utilizing these species to produce secondary metabolites and antibacterial biological activity for various applications.

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