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An Investigation of Phyllosphere Mycoflora of *Phyllanthus amarus*

Sumia Fatima and *Priya Lokare

Pesticides and Plant Protection Research Laboratory,
Dr. Rafiq Zakaria College for Women,
AURANGABAD-431001 (M.S.) INDIA
*Corresponding Author
Email- priyalokare2@gmail.com

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ABSTRACT

Most of the medicinal plants die because of disease attack. There are many reasons to cause diseases. Very few information is available about the incidence, prevalence, epidemiology and management of medicinal plants diseases. There is little information or research work on diseases of medicinal plants. *Phyllanthus amarus* is one of the significant medicinal herbs. A main herb in the Indian Ayurvedic Medicine System. Stomach, genitourinary organ, liver, kidneys, and spleen, as well as gonorrhea, menorrhagia, and other genital diseases, use the whole plant. *P. amarus*. In this study, there were a total of six fungi found; they are, *Aspergillus niger*, *A. nidulans*, *Alternaria* sp., *Fusarium* sp., *Passalora* sp.(Syn. *Cercospora* sp.), and one Unidentified. Most of the mycoflora were found on leaf surfaces. From the analysis, it is found that very negligible research has been done on phyllosphere fungal diseases. Therefore, care must be taken in this case as phyllosphere fungi also caused significant loss to the herb by damaging stem, branchlets, fruits, flowers. A total of six isolated and reported fungi have infected an insignificant degree of *Phyllanthus amarus*.

Figures : 02 References : 12 Table : 00
KEY WORDS : *Phyllanthus amarus*, Phyllosphere fungi, P.D.A. Medium,

Introduction

The term 'medicinal plant' refers to the different types of plants used in herbalism ('herbology' or 'herbal medicine'). The word "herb" was derived from the Latin word "herba" and the old French word "herbe"[1,4]. Nowadays, herb refers to any plant part, such as fruit, seed, stem, bark, vine, leaf, stigma or root. Earlier, the term "herb" was applied only to non-woody plants, including trees[4].

Phyllanthus amarus Schumach. and Thonn. is one of the most important medicinal herbs. It is an essential herb in the Indian Ayurvedic Medicine System. Used in the stomach, genitourinary system, liver, kidneys, and spleen. The entire plant is used for gonorrhea, menorrhagia, and other genital diseases [8]. *Phyllanthus amarus* or sleeping plant is also known as Bhui Amla in India. It is an annual herb, 15-45cm height.

Phyllosphere is a term used in microbiology to refer to total over-the-ground portions of plants as an environment for microorganisms [10]. In this research work, we studied the phyllosphere fungal diversity by collection, isolation and identification methods.

Phylloplane is a diverse terrestrial ecosystem dominated by a variety of microorganisms, including bacteria, fungi, and grass. Phylloplane fungi are the growing mycota on the surface of the leaf. Phylloplane fungi have been extensively studied in comparison to endophytic fungi, saprophytes, and pathogenic fungi. The phylloplane fungal diversity of *P. amarus* has also been studied; in their analysis, only one fungus (*Scopulariopsis* sp.) was found isolated from the leaves of *P. amarus* [9].

Materials and Methods

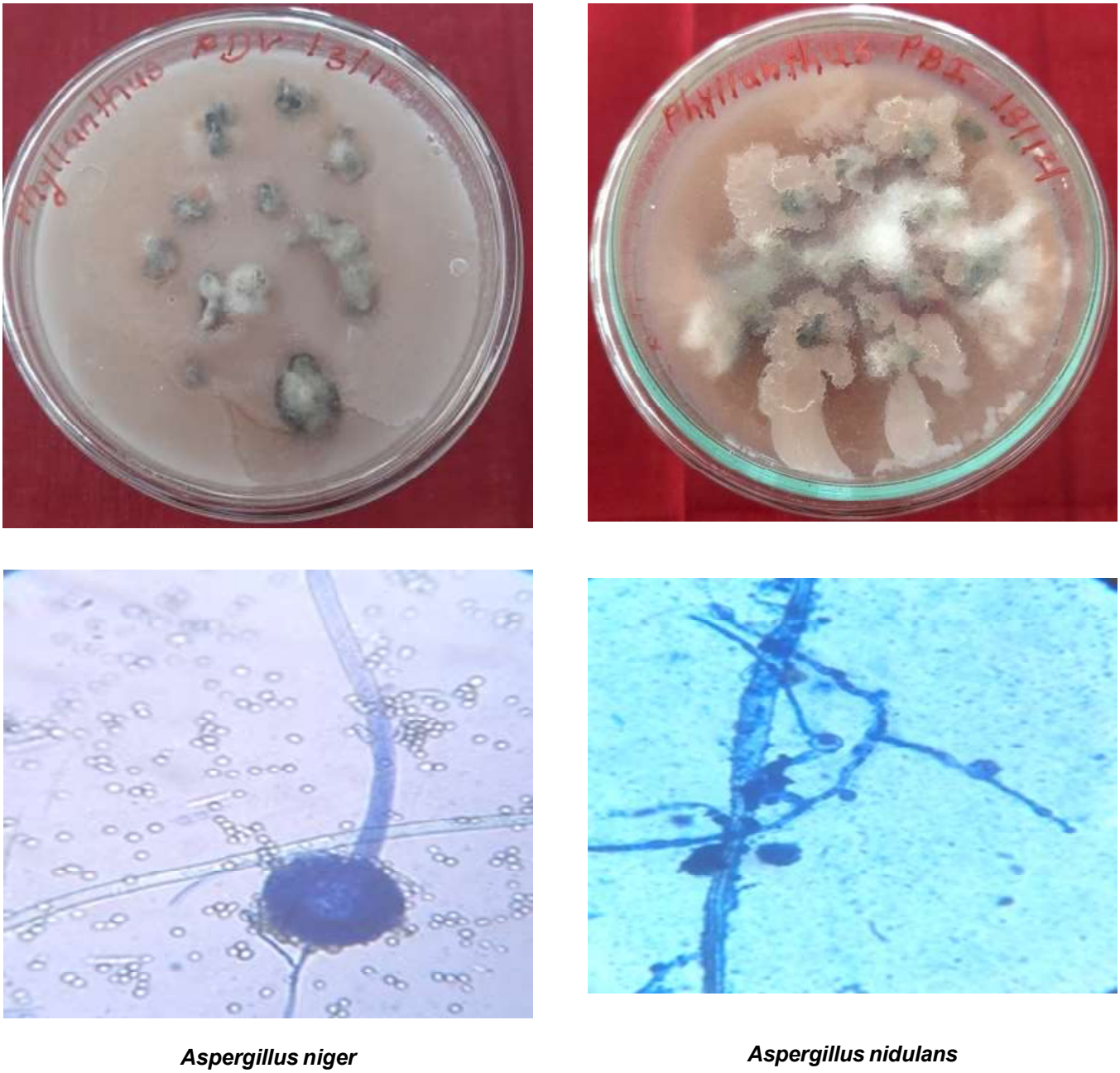
Collection of Infected *P. amarus*- Infected branchlets of *P. amarus* were collected in airtight plastic bags from Loknete Ramdas Patil Dhumal Arts, Science and Commerce College, Rahuri, District- Ahmednagar (M.S.), 413 705 and stored in the refrigerator at 5-degree Celcius for further study.

Preparation of Potato Dextrose Agar Media (P.D.A.)-

Composition –

Peeled Potatoes -200g
Dextrose sugar- 20g
Agar Powder – 20g
Distilled water – 1000ml

Procedure- The potatoes were peeled and weighed 200g and then sliced into small parts using a sterile knife. The diced potatoes were moved to a conical flask containing about 1000 ml of purified water and boiled for 20 minutes. The supernatant was purified and extracted from the muslin cloth. Dextrose (20 g) and agar (20 g) have been



Aspergillus niger ***Aspergillus nidulans***

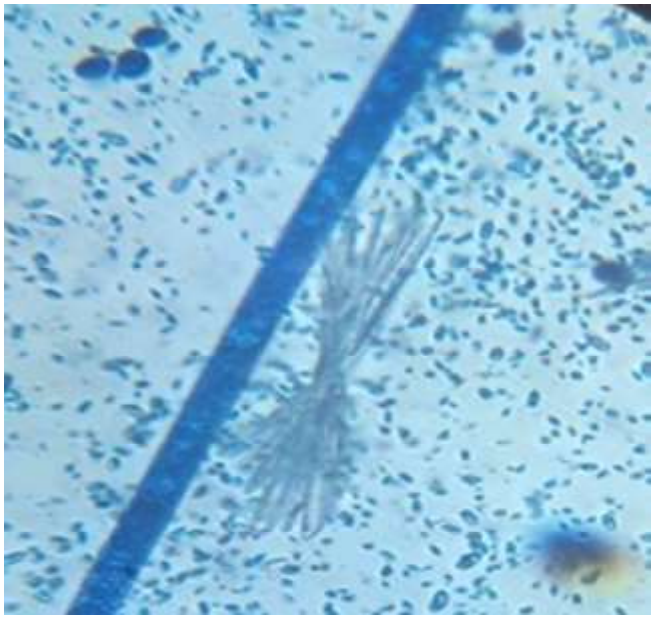
Fig. 1 : Growth of Isolated Phyllosphere fungi on Potato dextrose Agar Medium (P.D.A.)

moved to the extract for dissolution.

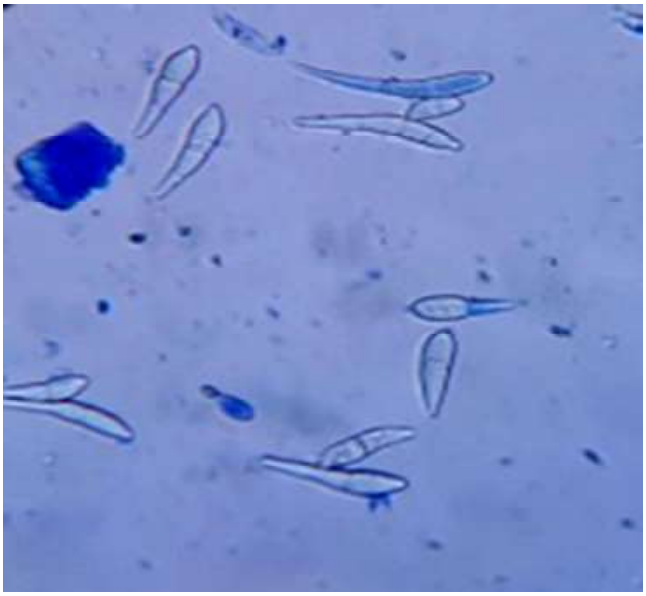
The medium was made upto 1 liter by adding distilled water. The median pH was adjusted to 5.6. Eventually, cotton was plugged and autoclaved at 121°C for 15 minutes.

Isolation of phyllosphere mycoflora- For the isolation of fungi from the leaf surface, the leaf impression method was followed, and the same method was followed to the other parts of the *P. amarus* plant. In this method, the infected plant part was gently pressed on the surface of the nutrient P.D.A. medium in Petri plates for incubation at room temperature for three days. The fungal growth was observed, and later the pure culture was maintained for further studies. The leaf impression method was followed to separate the fungi from the leaf surface, and the same method was applied to other areas of the *P. amarus* plant.

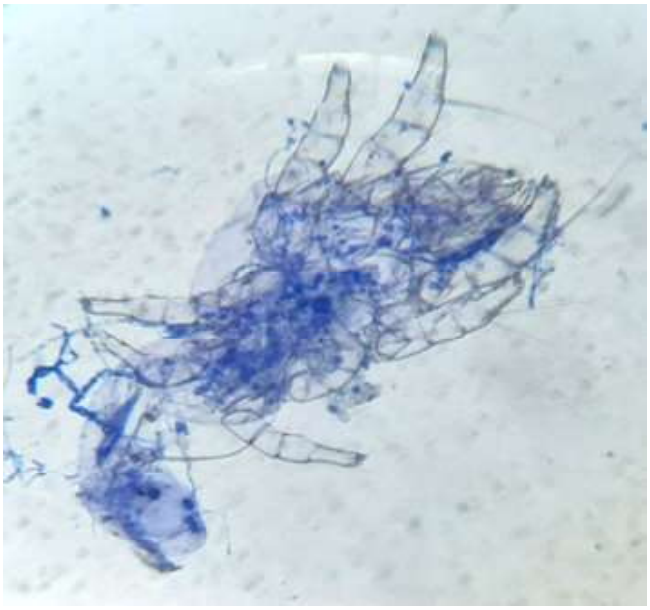
Identification of purified fungal colonies- For microscopic identification, slides were made using Lacto Phenol Cotton Blue Mounting. Purified fungal colonies were identified by reference to the standard manuals, Handbook of soil fungi [3], Dematiaceous Hyphomycetes, and More Dematiaceous Hyphomycetes [2]. Good slides were prepared and viewed under the (Besto, Model 10B) light microscope combination (10x, 45x) as well as the 'METZER-M,' Bionolocuar Microscope (100x), fungi



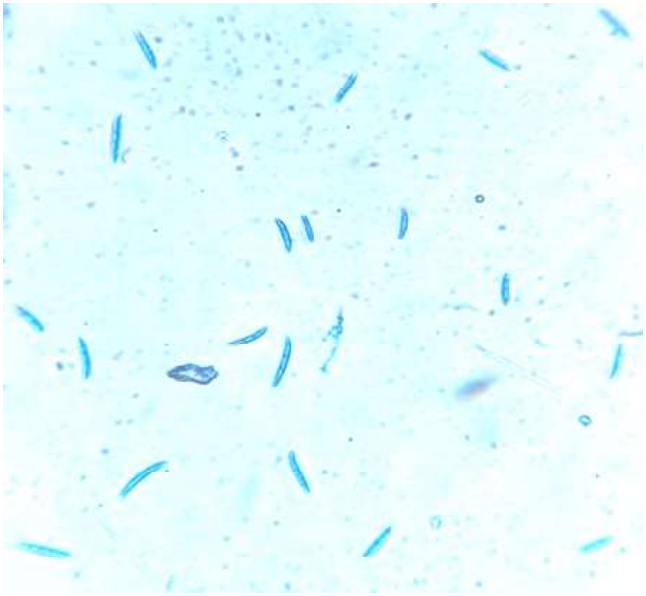
Unidentified



***Alternaria* sp.**



***Passalora* sp.(Syn. *Cercospora* sp.)**



***Fusarium* sp.**

Fig. 2 : Microscopic images of fungi observed under a compound microscope

images by the 'Metzer-M Biowizard program' with the Computer Imaging System.

Results and Discussion

Phyllanthus amarus Schum. and Thonn. is a small herb, well-known for its medicinal qualities and commonly used worldwide. In this study, there were a total of six fungi found; they are, *Aspergillus niger*, *A. nidulans*, *Alternaria* sp., *Fusarium* sp., *Passalora* sp.(Syn. *Cercospora* sp.), and one Unidentified. Much of the plant-fungal diversity was found on *P. amarus* leaves. *P. amarus* is given a range of therapeutic principles to treat various disorders; the crop is susceptible to several harmful factors affecting the quality of medicinal products and their quantity [11].

With the rising demand for plant-based drugs on the global market, the commercial production of *P. amarus* has received attention in the last decade as the occurrence of stem blight has induced a full crop failure. As an export-oriented crop, recent work on the etiology and management of stem blight disease will support the

agricultural community. Different parts of the plant like root, stem, leaflets, rachis, petiole, and fruits inoculated in both P.D.A. (Potato Dextrose Agar) and PPDA (*Phyllanthus* amended Potato Dextrose Agar) yielded good responses in terms of several endophytes [7].

In developing countries such as India, they rely on the traditional herbal medicine program, particularly *P. amarus* [12]. It is generally referred to as Bhuiamla belonging to the family Euphorbiaceae, which occupies a prime place among medicinal plants grown commercially [11]. Workers reported isolated from *Phyllanthus amarus*; *Fusarium oxysporum*, *Alternaria* sp., *Gibberella moniliformis*, and *Edenia gomezpompae*[5]. Other workers also found *P. amarus* fungal endophytes, isolated fungal species representing Ascomycete, Coelomycete, Hyphomycete, Zygomycete, and Sterile types [6].

Conclusion

From the above study, it is noticed that there is very negligible work that has been done regarding the phyllosphere fungal diseases. Hence, it is essential to pay attention in this case because phyllosphere fungi also caused significant loss to the herb by damaging stem, branchlets, fruits, flowers. There are a total of six fungi isolated and identified, which have infected the *Phyllanthus amarus* to significant extent.

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