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Antifungal efficiency of Karanj seed extract

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Abstract
Pongamia pinnata is a leguminous tree, commonly known as Karanj. It is widely distributed in Western Ghats. It has various antifungal and antibacterial properties. In current study, Karanj (*Pongamia pinnata*) seeds extract is used to check the growth of *Aspergillus niger* and *Penicillium* spp. fungi on Dextrose media. The soxhlet extraction method was carried out in laboratory to obtained pongamia seed extract. The yellowish brown colour extract was obtained. The media of seed extract and PDA was prepared in petriplates and inoculated with mold fungi. The results showed that *Aspergillus niger* fungi has more inhibition than *Penicillium* spp. on treated media. The considerable amount of growth has shown by both fungi.

Keywords: *Pongamia pinnata*, antifungal, *Aspergillus niger*, *Penicillium* spp., soxhlet

1. Introduction
Pongamia pinnata commonly known as Indian Beech or Karanj, belongs to the family Fabaceae (Sunil *et al.*, 2009) [14]. The genus Pongamia has only one species which is *Pongamia pinnata* (Merra *et al.*, 2003) [5]. It is a legumimous tree having tap root system and symbiotic relationship with nitrogen-fixing bacteria which prevents soil erosion. In South-East Asia, it is widely distributed Hawaii, Philippines, India and Australia. It has been also introduced in Egypt and the United States. The diverse occurrence of *Pongamia pinnata* is reported in Western Ghats, Andhra Pradesh and Orissa. The tree is shade bearer but can also be grown under shades of other trees (Orwa *et al.*, 2009) [6]. In ayurveda, it is used as a blood purifier. Pongamia seeds had shown antifungal and antibacterial properties. Karanjin is known to be effective against a large number of insects (Rao *et al.*, 1982) [8]. *Pongamia pinnata* seed oil has been used for the protection of wood through the heating process (Sadhna *et al*., 2012) [10]. The seed cake left after extraction can be serve as a good animal feed, organic manure for agricultural and horticultural crops (Scott *et al*., 2008; Sreedevi *et al.*, 2009) [11, 13].
Mold fungus causes significant problems in building and degrades its aesthetic value. Fungi colonize in the wood and degrade cell wall components like cellulose, hemicelluloses and lignin. The hyphae of fungi penetrate the wood which can degrade the call wall constituents. The primary attacking species are *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. and *Trichoderma* spp. etc. (Chandra, 2009) [1]. *Aspergillus* species are highly aerobic, but due to high oxygen tension they commonly grow as mould on the surface of a substrate. *A. niger* is a major example of this, as it can be found growing on damp walls. *Penicillium* is a saprophytic fungi. *Penicillium species* (green mold) are known for their numerous and closely packed brush-like structures that produce spores which are called penicilli. The spores are present in clusters of flask-shapes known as phialides and are called conidiophores.
The three main factors that influence the growth of fungi in buildings are moisture, nutrients and temperature. Molds rapidly colonize on the freshly cut wood and cause discoloration of the wood surface due to the release of pigmented spores. When the dried timber regains its moisture, it provides favorable conditions for the growth of mold (Robbins *et al*., 2002) [9]. The pigmented hyphae penetrates the inner tissues and causes a blue greyish-black discoloration. The most common staining fungi is blue-staining fungi. These fungi reduce the aesthetic values final products. The conventional chemicals degrade the environment by releasing harmful compounds. Therefore, plant extracted oil or extracts are used to preserve the products from fungi
The essential oil from plant extract has inhibitory effects on wood (Yang *et al*., 2007) [16]. Oil extracted from *Pongamia pinnata* seeds is found to have antibacterial, antifungal and medicinal properties.

Singh *et al*. (2011) [17] studied the in vitro antifungal activity of 12 essential oils and two commercial fungicides against eight fungal species namely *Aspergillus flavus A. nidulans, A. niger, A. ochraceus, Mucor* sp., *Penicillium italicum, P. oxalicum* and *Rhizopus arrhizus* on agar plate as well as on blotter paper from fresh kernels of *Buchnania lanzan*. The results showed that the oil of *Ocimum canum* exhibited absolute toxicity of 100 percent against eight fungi at 500 ppm concentration and it was able to preserve kernels up to 45 and 90 days respectively.

2. Material and Methods

The following investigations were carried out in Wood Preservation Discipline, Forest Research Institute, Dehradun (30°31'65'' N, 78°03'22"E). The overview of the experiments carried out is given in schematic representation.

2.1 Preparation of Extractive

Pongamia pinnata seeds without pods were purchased from the local vendor of Dehradun. The seeds of *Pongamia pinnata* were crushed in a machine. Weigh the desired amount of crushed pongamia seed powder in a weighing machine and placed in a "thimble" made of strong filter paper, which is placed inside a soxhlet chamber. The soxhlet apparatus is connected to the condenser which is connected to the inlet and outlet water pipe. The round bottom flask is filled half level with petroleum ether and heated at 60-80 °C for distillation. After extraction the solvent in the extract is removed, by distillation process. The extraction was carried out till no more extractive was isolated. The extract obtained was viscous and yellowish brown in colour.

2.2 Procurement of Fungi

Pure culture of *Penicillium* spp. (green mold) *and Aspergillus niger* (black mold) fungi were procured from the Forest Pathology Division, Forest Research Institute, Dehradun.

2.3 Preparation of PDA medium

A nutrient medium of potato-dextrose (rose bengal) agar was weighed in a weighing machine. 39 g of PDA powder was dissolved in 500 ml of distilled water in a 1000 ml beaker (Pant, 2010) [7]. It was stirred for 15-20 minutes on a machine by using a magnetic stirrer, till dissolved completely. The solution was then poured into conical flasks of 250 ml and plugged with cotton. The conical flasks were wrapped with aluminium foil and kept in autoclave at 15 lbs pressure and 120 temperature for 20 minutes to sterilize the medium (Datar, 1995) [3]. It was then kept in laminar flow for 15 minutes. The Petri plates were also cleaned with spirit and sterilized in autoclave. 30 ml of the PDA medium was poured in each Petri plate in laminar flow. The Petri plates were allowed to cool for 1h till the medium solidified.

2.4 Inoculation of Fungi

Under aseptic conditions of laminar flow, *Penicillium* spp.(green mold) *and Aspergillus niger* (black mold) fungi were inoculated on prepared culture medium in Petri plates using sterilised inoculation needle**.** The fungus inoculated Petri plates were sealed with paraffin tapes. The Petri plates were kept for 14-17 days in the BOD incubator at 25±2^{0}C temperature and 75±5% RH for the growth of fungal mycelia (Thakur, 2017) [15]. The Plates should be monitored regularly to check the growth of mycelia.

Grading of surface coverage of the test fungi on PDA medium

Growth type	Surface coverage of mycelium on the medium (%)
None	0
Sporadic	0-5
Little	5-25
Moderate	25-50
Considerable	50-75
Complete	75

(Source: Pant, 2010) [7]

3. Results and Discussion

The mold growth of *Aspergillus niger* and *Penicillium species* on petri plates were measured after two weeks. The petri plates were treated with *Pongamia pinnata* extract. The surface area covered by the two mold fungi on petri plates were calculated by assuming the covered area as ellipse as follows in Plate 1. The *Aspergillus niger* covered 51.85 percent area and *Penicillium species* covered 56.9 percent area in petri plates. The *Aspergillus niger* shows more inhibition than *Penicillium species* in petri plates after 14 days of incubation. Therefore, it has been concluded that both molds have considerable growth on medium in the range of 55–75 percent and *Penicillium species* make a number of small colonies on available medium.

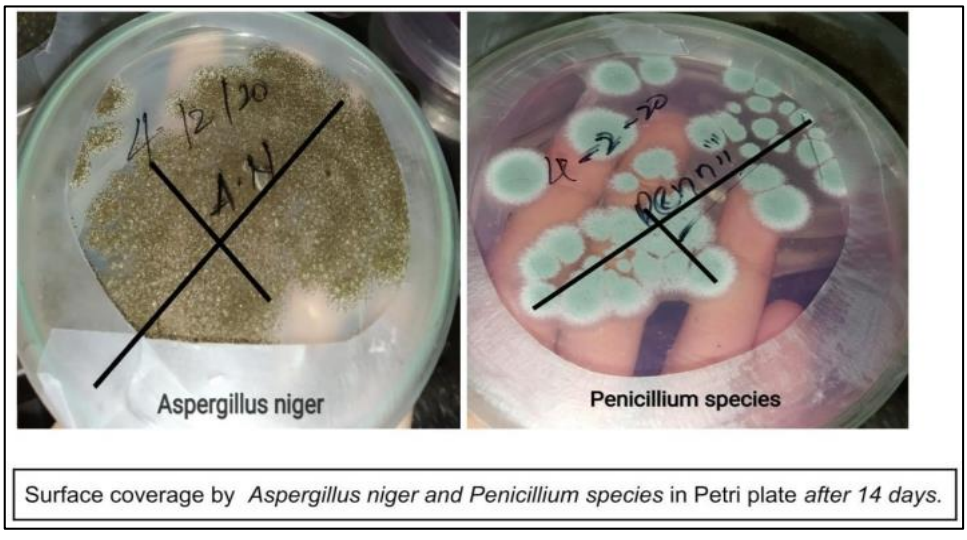

Fig 1: Mold growth on PDA medium after two weeks

The yield of the extractives obtained from various plants is affected by many factors. These factors will depend upon extraction conditions such as extraction method, solvents used, extraction period and different temperatures during the extraction, and other factors like genetic structure, growth stage, parts of plant and environment conditions for plants. It has been observed that the extraction of oil in the range of 60–70^{0}C temperature can give higher yield. There are a number of secondary metabolites present in pongamia oil and one of themajor constituent of pongamia is Karanjin. Karanjin has antifeedant or repellent properties. In the traditional system of medicines, such as Ayurveda and Unani, the *P. pinnata* plant is used for anti-inflammatory, antiplasmodial, anti-diarrheal, anti-ulcer, and antioxidant activity (Chopade *et al.*, 2008) [2]. *Aspergillus niger* is able to grow in the temperature range of 6–47°C. Lak *et al*. (1993) [4] found that mold growth can be controlled with zinc borate where zinc acts as a co-biocide. But, the insolubility of zinc borates in water restricts its commercialization. The harmful effect of these chemicals leads to the development of more environment friendly preservatives. The results showed that the antibacterial properties of Rose Bengal Agar medium restricted or slowed down the rate of growth of mold in the petri plates under B.O.D incubator at 25±2^{0}C temperature and 75% R.H. From the above discussion it can be concluded that the antifungal properties of Pongamia can be used to minimize these fungal growth.

4. Conclusion

Pongamia pinnata, a versatile resource of essential oil can be obtained through soxhlet extraction method. The higher yield of seed extract can be achieved in the temperature range of 60–70^{0}C. It has antifungal, antimicrobial properties which restrict the growth of microorganisms as discussed in literature. Potato Dextrose Rose Bengal Agar with antibacterial properties, were used in an experiment to prevent the further infestation of mold by other fungi or microorganisms in petri plates kept inside the B.O.D incubator at 25±2 ^{0}C temperature and 75% R.H. But, it showed some negative impact on the growth of mold. The rate of growth of *Aspergillus niger* and *Penicillium* spp. *w*ere delayed or slowed down. The fungal growth in the form of colonies has been shown by *Penicillium species.* From the above investigations, it has been concluded that the antifungal or antibacterial properties of Rose Bengal Agar and seed extract have a combined effect on restricting the growth of molds in the ongoing experiment. These fungal spores cause health problems like asthma, allergy to human beings. The concern towards environment and human health lead to the development of more environmentally friendly preservatives to restrict fungi growth.

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