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Investigation On the Larvicidal Potential of Endophytic Fungi Isolates from *Psoralea corylifolia* And *Leptadenia reticulata* Against Aedes Aegypti

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Abstract

Globally, several million peoples are infected with epidemic-prone mosquito-borne diseases like yellow fever and malaria that is transmitted to humans by the bites of infected mosquitoes. Psoralea corylifolia, often known as babchi, is a well-liked herb that has been utilised in traditional Ayurvedic and Chinese medicine for many years for its miraculous abilities to treat a variety of skin conditions and Leptadenia reticulata, also known as Jivanti is an herb that is traditionally used in treating the conditions like malaria, diabetes, and liver disorders. The present study investigated the larvicidal properties of endophytic fungi isolated from Psoralea corylifolia and Leptadenia reticulata against Aedes aegypti. The highest larvicidal activity was seen in Trichodermata viride and Candida albicans species from leaves of L. reticulata was found to be 85.71% and 90.47 at 20 ppm. Likewise, Piriformospora indica and Aspergillus niger species from the leaves of P. corylifolia was found to be 66.66% and 83.33 % respectively. Candida albicans from leaves of L. reticulata and Aspergillus niger from the leaves of Psoralea corylifolia showed the highest mortality percentage was found to be 90.47 and 83.33 at 20 ppm respectively. The present study reveals the determination of optimal, eco-friendly, and selective mosquito larvicidal agents. The development of innovative and affordable medications to combat diseases spread by mosquitoes will be aided by more research on the isolation, characterisation, and determination of the mechanism of action against the selective mosquitos.

Keywords: *Endophytic fungi, Psoralea corylifolia, Leptadenia reticulata, Aedes aegypti, Larvicidal activity*

Introduction

Mosquitoes are considered a major source of infection and serve as a vector for the spread of diseases such as dengue fever, yellow fever, malaria, rift valley, and filariasis, which have a significant global impact on human health, especially in tropical and subtropical regions (WHO, 2018). The *Aedes aegypti* mosquito is a vector for a number of diseases, including dengue (Omondi *et al*., 2019), chikungunya fever (Aragão *et al*., 2018), and the Zika virus (Singh *et al*., 2019). As such, it is a public health concern and this species of mosquito is found globally (Braga and Valle, 2007). The World Health Organisation (WHO) estimated that 500,000 persons have suffers with dengue fever; 90% of those cases are in children, and the death rate is 2.5% (Azeem *et al*., 2019). Numerous chemical pesticides have been successfully employed to manage mosquito populations during the last few decades. However, ongoing use of synthetic pesticides results in biologically amplified toxicity in the food chain, development of resistance in vector species, and negative impacts on human health and the environment (Pitasawat *et al*., 2007). Due to these reasons, there is a growing need to determine the repellents against the mosquito species, that are biodegradable, economical, and beneficial to the environment (Nazar *et al*., 2009). Due to their importance in disease transmission, mosquitoes, as opposed to other infectious disease vectors, have been identified as the most significant vector (Becker *et al*. 2020). In addition to the diseases that mosquitoes spread, their bites can occasionally result in an accompanying syndrome with swellings, sore,

reddish areas that are itchy and painful (Ojianwuna 2022). In underdeveloped nations, these diseases not only have high rates of mortality and morbidity but also have a significant negative social and economic impact. The world's known mosquito species number about 3500, with the exception of the polar areas.

Endophytic fungi are a phylogenetically varied collection of fungi that colonise plant tissue asymptomatically and sporadically, promoting saprophytic, commensalistic, or mutualistic interactions with their host plant (Schouten, 2019). The endophytic fungal association is a sophisticated chemical interkingdom connection that controls the host plant's defence systems, controls fungal virulence factors, and interacts both antagonistically and cooperatively with other microbes (Chagas *et al*., 2018). Endophytic fungus creates a range of secondary metabolites as a result of these interactions, which may be used biotechnologically. Basappa *et al*. (2023) analysed that endophytic fungi were widely used in the sectors of pharmacology, agriculture, and medicine. Endophytic fungi were special because they could live inside plant tissues. The key role of endophytic fungi in producing novel bioactive substances with a wide range of biological activities was the main topic of that review. The antibacterial and antiviral properties of metabolites produced by endophytic fungus were of particular importance and show promise in preventing human illnesses.

Leptadenia reticulata (Retz.) Wight. & Arn. (Family Asclepiadaceae), also known as Jivanti, Swarnjivanti, or Dodi, is a member of the genus *Leptadenia*. In ethanobotanical investigations, *Leptadenia reticulata* is discovered to be a significant plant. According to ayurveda, it is a tonic that offers the body overall vigour and has been utilised historically for a number of purposes. Since 4500 BC, it has been regarded as a significant medication in Ayurveda. This plant is referred to be a source of life and vigour in the Atharva Veda (Shekhawat *et al*., 2006; Rai *et al*., 2023). Economic importance is attributed to the medicinal qualities of the majority of these *Leptadenia* species. *L. reticulata* is one of the maximum significant therapeutic plants used in Ayurveda to support vigour and life among them.

P. corylifolia (Bakuchi), the medicinal plant is rich in natural sources of various alkaloids and chemical elements, including those from the "*Leguminosae"* family. It is also known as Babchi (Bakuchi) (Belge and Jeurkar, 2023), and it is a huge, widely dispersed genus of shrubs and plants with glandular compound leaves and spicate or breadroot-see racemose purple or white flowers (Bachmeier *et al*., 2013; Rai *et al*., 2023). The most mentionable aspect is that every part of the plant has the phytochemicals that may heal types of skin issues, skin rashes, such as infections, leucoderma, and *etc.* The present study investigates the larvicidal properties of endophytic fungi isolated from *Psoralea corylifolia and Leptadenia reticulata* against *Aedes aegypti*.

Materials and Methods

Collections of plant material

Fresh plant components of *L. reticulata* (RUBL211619) and *P. Corylifolia* (RUBL10309) were procured from Naharhgarh Biological Park, Kukas, Jaipur, Rajasthan, India. The collected plant's authenticity was validated by assigning a voucher specimen number (RUBL No.), which was recorded in the herbarium of the Department of Botany at the University of Rajasthan in Jaipur. Sterile bags were used to transport plant species to the lab, where they were treated right away to lower the chance of infection.

Isolation of endophytic fungi

Most significantly for isolating endophytic fungi residing within the internal plant tissues, the plant parts were surface sterilized. To streamline the sterilization and isolation process, the plant parts under investigation were finely dissected into small pieces. The incubated petri dishes with tissue samples were observed on a daily basis to check for fungal growth. Actively growing fungal tips of morphologically different endophytic fungi, such as different colours, growth patterns, colony colours, *etc*., were transferred to fresh PDA slants for the pure culturing of endophytic fungi. Continuous transfer of fungal hyphae was carried out for 3-4 weeks after the appearance of pure culture, and its sustainability was further confirmed by transferring the fungal culture 2-3 times to fresh plates. The growth pattern of endophytic fungi was observed for at least one week, and for the confirmation of pure endophytic fungi, they were all subcultured.

Identification of endophytic fungi

The identification of isolated cultures of endophytic fungi was based on morphological characteristics such as growth patterns, characteristics of spores, the presence of mycelium, and the production and characteristics of spores. Microscopic observation of endophytic fungi isolated in the present study was performed by preparing temporary mounts stained with lactophenol cotton blue. Prepared slides were observed under a light

microscope at 40x and, in some cases, at 100x magnification, and photographs were taken. The identification of isolated fungi was based on their shape, size, colour, and arrangement of hyphae.

Larvicidal Assay

Twenty-five batches of larvae in their third or fourth instar were arranged in small, disposable test cups or containers, with a capacity of 100–200 cc apiece, using strainers, screen loops, or droppers. Starting with the lowest concentration, the required amount of thinning was added to 100 *ml* or 200 *ml* of H_2O in the cups to achieve the intended target dose. For every concentration, four or more replicates were put up, and at the same time, an equivalent number of controls were established up using water and one millilitre of alcohol. Every test was conducted three times on their whole larval phase. Larval food was provided to every test cup for extended revelations. A photoperiod of 12 hours of light and 12 hours of darkness was used to keep the temperature of the test tubes between 25 ˚C and 28˚C. Larval mortality after a 24-hour exposure was recorded. It could be required to read for 48 hours while using slow-acting insecticides. Morbid larvae were counted and added to dead larvae in order to calculate the percentage of mortality. The given form was used to document the findings, together with the LC_{50} values and the examination of slope and heterogeneity. Three independent experiments, each with four replicates and six concentrations, may be accommodated on the form. The mortality rate is measured in different concentration *viz*. 1.25ppm, 2.25ppm, 5.00ppm, 10ppm and 20ppm. During the test time, larvae that had pupated were investigated.

Statistical analysis

For statistical analysis, table creation, and graph generation, we utilized software tools including Microsoft Excel 365 and SPSS 27. We conducted Two-way ANOVA at a significance level of 5%, followed by the least significant difference test to assess variations among the means.

Results and Discussion

The current study clarified the larvicidal action of endophytic fungi isolated from a few medicinal plants, including *Psoralea corylifolia* and *L. reticulata* leaves, roots, and stems, on *Aedes aegypti*. In the current investigation, endophytic fungi were isolated and identified based on their microscopic and macroscopic properties. *Aspergillus sp.* was frequently isolated from *L. reticulata* and *Psoralea corylifolia*. The endophytic extracts in this investigation exhibited a noteworthy degree of larvicidal activity. Table 1 and 2 represents the larvicidal activity of isolated endophytes from *L. reticulata* and *Psoralea corylifolia* against *Aedes aegypti* following 24 exposures. Throughout the entire larvae bioassay test using endophytic fungal extracts, the mortality percentage increased significantly ($P<0.05$) as the concentration increased. It has been reported that insecticides derived from plants are useful for controlling mosquitoes specifically.

In the present work, third and fourth instar *Aedes aegypti* larvae were shown to exhibit a considerable concentration-dependent larvicidal activity of fungal extracts from *Leptadenia reticulata* and *Psoralea corylifolia.* In *L. reticulata*, at 20 ppm, *Candida albicans, Trichodermata viride* showed more that 80% of mortality rate. In *P. corylifolia*, at 20 ppm, only *Aspergillus niger* Showed more than 80% of mortality rate. Thus, the fungal extract of all the endophytic fungi exhibits moderate to high mortality rate.

Table 1. Larvicidal activities of endophytic fungi isolated from *L. reticulata* on *Aedes aegypti*.

Concentrations	Control	*Aspergillus flavus*	*Aspergillus ruantti*	*Candida tropicalis*	*Candida albicans*	*Trichodermata viride*
1.25ppm	0	0	0	0	0	0
2.25ppm	0	0	5.5 ± 0.08	11.11± 0.11	0	19.04±0.26
5.00ppm	0	11.11±0.12	16.66±0.14	26.66± 0.13	50 ± 0.21	20.0 ±0.11
10ppm	0	27.77±0.18	33.33± 0.16	6.84±0.17.	66.66± 0.16	42.85±0.18
20ppm	0	55.55± 0.21	61.11 ± 0.53	44.44±0.23	90.47±0.72	85.71±0.57

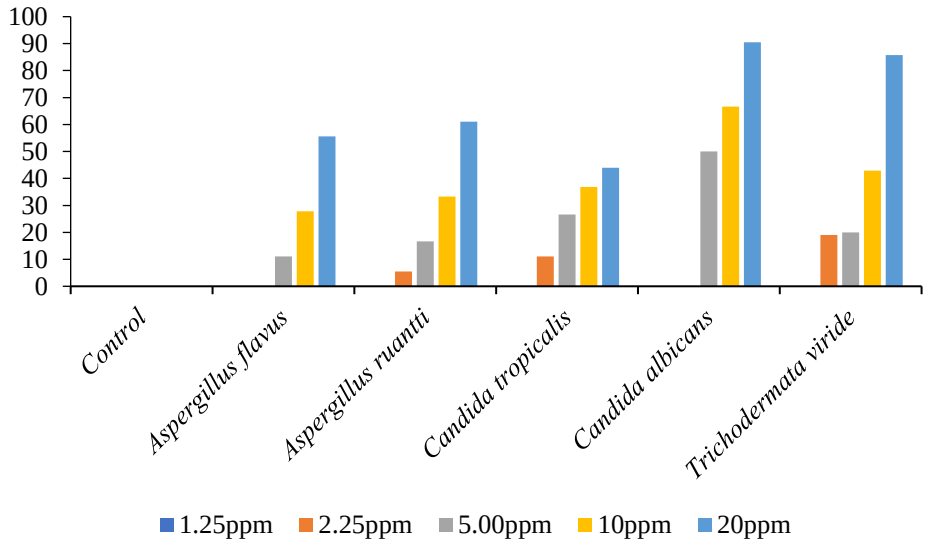

Figure 1: Larvicidal activities of endophytic fungi isolated from *L. reticulata* on *Aedes aegypti* in 24 hours.

Table 2: Larvicidal activities of endophytic fungi isolated from *Psoralea corylifolia* on *Aedes aegypti.*

Concentrations	Control	*Piriformospora i ndica*	*Alternaria alternate*	*Aspergillus niger*	*Penicillium citrinum*
1.25ppm	0	0	0	0	0
2.25ppm	0	0	8.3 ± 0.02	11.11 ± 0.11	0
5.00ppm	0	22.22 ± 0.13	16.66 ± 0.14	26.66 ± 0.13	21.02 ±0.12
10ppm	0	50.00 ± 0.21	33.33 ± 0.16	52.38 ± 0.21	38.09 ± 0.21
20ppm	0	66.66 ± 0.32	53.33 ± 0.35	83.33 ± 0.34	61.90 ± 0.23

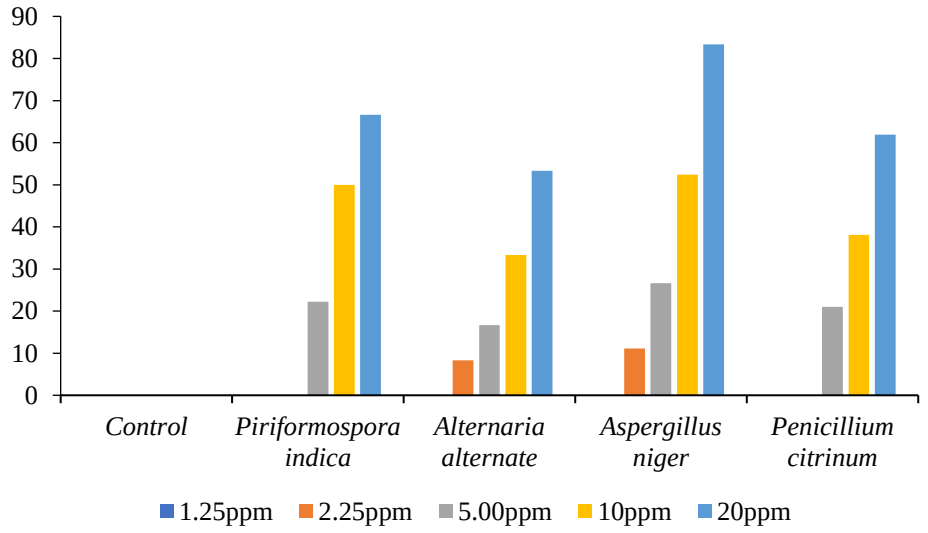

Figure 2: Larvicidal activities of endophytic fungi isolated from *Psoralea corylifolia* on *Aedes aegypti* in 24 hours.

Conclusion

In this study, all the fungal extracts of *L. reticulata* and *P. corylifolia* showed larvicidal activity. In *L. reticulata*, *candida albicans* exhibited higher mortality as compared to other four extracts. The fungal isolate *Trichodermata viride* showed less mortality rate as compared with *candida albicans*. *Aspergillus flavus, Aspergillus ruantti* and *Candida tropicalis*. In *P. corylifolia*, highest mortality was shown in *Aspergillus niger isolate,* whereas *Piriformospora indica* showed moderated mortality rate. Hence, the present study reveals endophytic fungi isolates from *L. reticulata* and *P. corylifolia* contains the potential mosquito larvicidal property and could be explored for the development of eco-friendly larvicidal product.

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Conflicts of Interest
The authors declare that there are no conflicts of interest.

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