



Study on Diversity of Fungus Associated with *Nephila pilipes*

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Abstract

The fungus has various type of association with spider. They can be pathogenic or symbiotic or commensal in relationship to spiders they live on. In the current study the diversity of fungi in association with *Nephila pilipes* spider were recorded. The fungi were isolated from its legs and saliva. The microscopic and macroscopic characters were obtained. The measurements of hyphae, conidia and conidiophore were recorded. The fungal growth was more on legs compared to saliva. The legs of the adult spider carried more fungi than saliva as they are associated with soil, carrying microflora on their legs. Further the four different fungus isolated as aspergillus, penicillium, Trichoderma and Cladosporium. The penicillium colony had abundant growth, aspergillus had moderate growth and Cladosporium had less growth compared to Trichoderma.

Keywords: Biodiversity, Conidia, Conidiophore, Fungi, Hyphae, *Nephila pilipes*

Introduction

Spiders belong to a group of animals called 'Arachnids'. Spiders are arthropods having eight legs, chelicerae with fangs. As of July 2019, at least 48,200 species of spider and 120 families have been recorded by taxonomists. All spiders are predators feeding almost entirely on insects. Spider ranges in body length from 0.5 mm to 90 mm. *Nephila pilipes* belongs to family *Nephilidae*, are abundant and widely distributed in many parts of the world. This spider is also known as Golden orb-web spider. It is second largest of the orb-weaving spiders. These spiders are sexually dimorphic, that is, the females are much larger than that of males. Females grow to a body size of 30-50 mm; whereas, males grow to a body size of 5-6 mm.

Fungi are eukaryotic, achlorophyllous organism with absorptive mode of nutrition and they bear spores. Most of the fungi reproduce by spores and their body (thallus) is composed of microscopic tubular cells known as hyphae (Carris *et al.*, 2012). Fungi are heterotrophs which obtain their energy in the form of carbon from sources like chitin, cellulose along with other complex carbohydrates. Most of the fungi also causes plant disease and acts as a pathogen but fewer than 10% of all known fungi can colonize living

plant (Knogge, 1996). Fungi are also associated with the plants as a beneficial symbiont and as cryptic plant colonizers called endophytes (Carris *et al.*, 2012). Fungi are very successful inhabitants of soil, due to their high plasticity and adaptability for various adverse or unfavorable conditions (Sun *et al.*, 2015). Most of the fungi have the ability to grow on both invertebrates and vertebrates. They attack most on insects and nematodes. Fungi which attack insects are called entomopathogens, which include wide range of fungi belonging to phyla Ascomycota, Zygomycota and Chytridiomycota. Many of the fungi can also alter insect's behavior. Soil is spiders' most common habitat; they live in associated with soil. Spider, *Nephila pilipes*, from tropical forest adhere many of soil and air borne fungi on them. Our aim is to study common genus present on them.

Materials and Methods

Specimen Collection

Five of the female spiders of species *Nephila pilipes* were collected from the area of Turahalli forest located about 20 km away from Bangalore off Kanakapura Road. The Total area of forest sums to 590 acres with 888 meters of Elevation. The Karnataka Forest Departments governs the

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forest. A wide variety of spider diversity has been found in the region hence, the area has been selected for study as described by (Jalajakshi and Usha, 2019). The spiders were collected by hand collection method into a plastic container with tiny holes for ventilation and brought to laboratory in live condition. They were identified by referring to Journal of Arachnology "Identification to Indian Spiders".

Media Preparation

The modified Rose Bengal agar media was prepared and described in "Techniques for Studying Bacteria and Fungi Manual" by Hauser (2006). The different components used for 150 ml of media included Dextrose of 1.5 g, Peptone of 0.75 g, Agar of 3.03 g, Potassium dihydrogen phosphate of 0.15 g, Magnesium sulphate of 0.075 g, Rose Bengal of 0.00045 and with 0.0045 g at pH of 6.8 ± 0.2 . (Atlas and Parks, 1997; Cooke, 1954; Jarvis, 1973; Ottow, 1972; Papavizas and Davey, 1959; Smith and Dawson, 1944; Waksman, 1922) the prepared media and poured into the five sterilized petri plates.

Isolation of Fungi

The *Nephila pilipes* spider was made to amble on the media poured petri plates for a minute and was collected back a numbering was given to each plate. Further it anesthetized with Chloroform, saliva from the mouth of the spiders were collected with the help of sterile loop and was inoculated on to the respectively numbered by continuous streaking (Benson, 2005; Cappuccino and Welsh, 2017). Further the plates were kept for incubation for 7 days at room temperature in inverted position for microscopic and macroscopic study.

Identification Methods

The macroscopic observation of fungal morphology was studied by observing colony characteristics. The characteristics included were colony forms, color of surface pigment and color of the sub-surface. The method of Microscopic study from each culture included, a small amount of mycelium stained with Lacto phenol cotton blue was mounted on the sterile glass slide by referring (Gaddeyya et al., 2012). The fungi were observed under 45X objective of Menzel Research Microscope and the preparation were recorded and photographed using Asus Phone Camera with 15 mp. By referring to the manual "The Identification of Fungi: An Illustrated Introduction with Keys, Glossary and Guide to Literature" by Dugan (2017), genus of each fungi was identified. Further, using ocular Micrometer in 45X objective of light microscope diameter of conidia, conidiophore and hypha was recorded and photographed by Asus Phone Camera with 15 mp.

Results and Discussion

In this study the four fungal forms were isolated from the saliva and the outer body surface of *Nephila pilipes* spider. The isolated fungi were identified and examined based on the macroscopic and microscopic characteristics. The four forms identified were *Aspergillus*, *Penicillium*, *Trichoderma* and *Cladosporium*. *Aspergillus* colony was powdery form, with black surface pigment and pink subsurface pigment. *Penicillium* was cottony form with light green surface

pigment and black subsurface pigment. *Trichoderma* and *Cladosporium* were both powdery forms. *Trichoderma* had green surface pigment and pink subsurface pigment. *Cladosporium* had white surface pigment and black sub surface pigment (Plate 1).

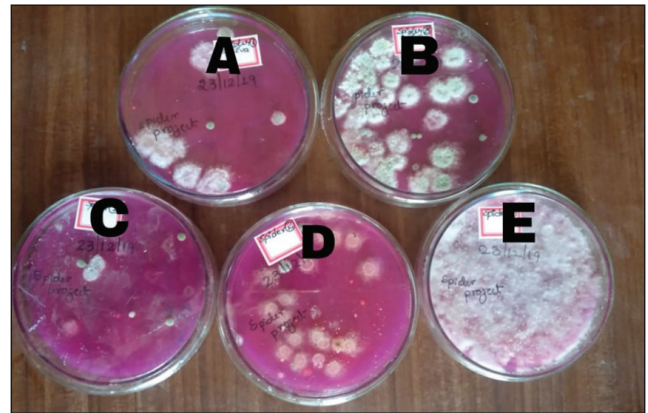


Plate 1: Microscopic Characters of isolated fungi; A, B, C- fungal growth isolated by ambulating spider; D and E - fungal growth isolated from saliva of spider

Aspergillus

It belongs to the Class: Eurotiomycetes, Order: Eurotiales, Family: Trichocomaceae and Genus: *Aspergillus*. The colony morphology reveals the black colour on the surface and pink colour on the sub-surface. It is commonly called as 'black mold' and it is saprophytic fungus commonly found on the fruits, vegetables and in soil, air and water. It has three structures - a somatic hyphal cell known as foot cell measured 96 μm in length. The hyphae projects out in to a long elongated filamentous structure called conidiophore measured 79 μm . Conidiophore becomes globular and give rise to a vesicle measured 2.4 μm in diameter. The vesicle give rise to the bottle shaped projection known as sterigmata. Each sterigmata bears a chain of conidia. Asexual reproduction is by the means of conidia and sexual reproduction is by the formation of ascospores. Some species may cause allergies and produce aflatoxins (Figure 1).

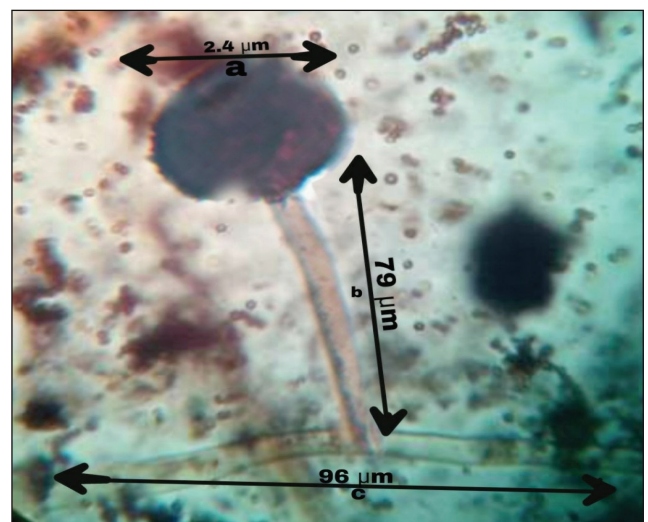


Figure 1: *Aspergillus* [a] Conidia, b) Conidiophore and c) Hyphae]

Table 1: Micrometric Measurements of Conidia, Conidiophore and Hyphae of each fungal form

Fungal Forms	Conidia (Diameter, μm)	Conidiophore (Length, μm)	Hyphae (Length, μm)
Aspergillus	2.4	79	96
Penicillium	2.0	46	66
Trichoderma	0.3	1.1	52
Cladosporium	0.1	47	80

Penicillium

It belongs to the Class: Eurotiomycetes, Order: Eurotiales, Family: Trichocomaceae and Genus: penicillium. The colony morphology reveals the light green colour on the surface and black colour on the sub-surface. It is commonly known as ‘green or blue mold’. It is found in air, water and soil and it is saprophytic in nature. The aerial hyphae measured 66 μm and develop in to conidiophores 46 μm which branches and have brush like heads bearing spores. The ultimate branches of conidiophores are called ‘Metulae’. They produce a bottle shaped substance known as sterigmata or phialide, which in turn give rise to single celled globular and ovoid chain of conidia. Conidia measured 2.0 μm . Asexual reproduction takes place by the allospores and sexual reproduction takes place by the formation of conidia. Some species may produce highly toxic mycotoxins (Figure 2).

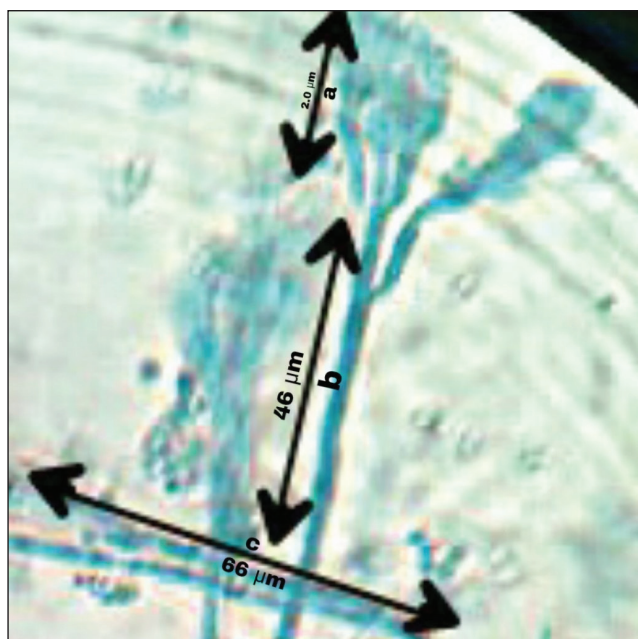


Figure 2: Penicillium [a) Conidia, b) Conidiophore and c) Hyphae]

Trichoderma

It belongs to the Class: Sordariomycetes, Order: Hypocreales, Family: Hypocreaceae and Genus: Trichoderma. The colony morphology reveals the green colour on the surface and pink colour on the sub-surface. It is commonly found in all type of soils. They are saprophytic fungus and can be characterised as opportunistic avirulent plant symbionts. The hyphae measured 52 μm . Conidiophores measured 1.1 μm are highly branched and loosely or compactly tufted. Main

branches of the conidiophores produce lateral side branches and often phialides arising directly from the main axis. The branches may rebranch with the secondary branching. All primary and secondary branches arise at or near 90° with respect to main axis. Conidia typically appear dry and are 0.3 μm in diameter, ellipsoidal or globose in appearance. Reproduction is by the means of asexual spores (Figure 3).

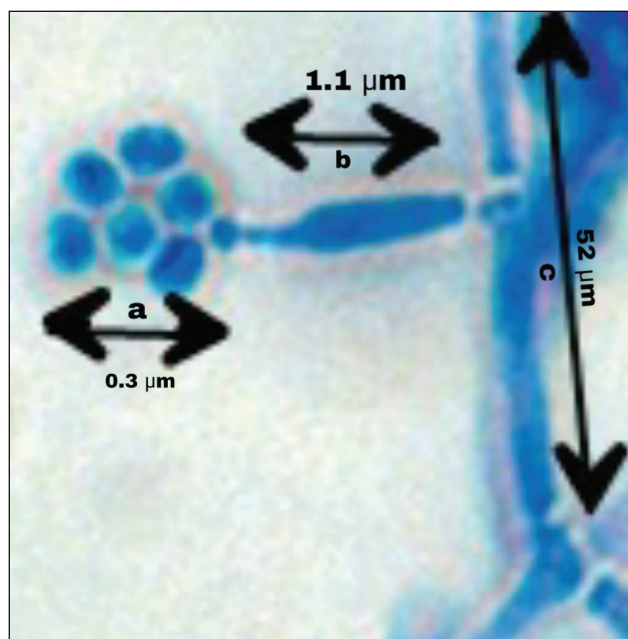


Figure 3: Trichoderma [a) Conidia, b) Conidiophore and c) Hyphae]

Cladosporium

It belongs to the Class: Dothideomycetes, Order: Capnodiales, Family: Davidiellaceae and Genus: Cladosporium. The colony morphology reveals the white colour on the surface and black colour on the sub-surface. The colonies are slow growing and often powdery due to the production of abundant conidia. They are found in soil and air; they are saprophytic in nature. It is most commonly indoor and outdoor mold. The hyphae measured 80 μm in length. Conidiophores more or less distant from the vegetative hyphae are erect, straight, unbranched or branched only in the apical region (Ezz, 2012), conidiophore measured 47 μm in length. Conidia are one to four celled, smooth and shield shaped and chains of conidia that readily disarticulate are diagnostic for the genus Cladosporium (Ezz, 2012), conidia are 0.1 μm in diameter. Reproduction is by the means of asexual spores (Figure 4).

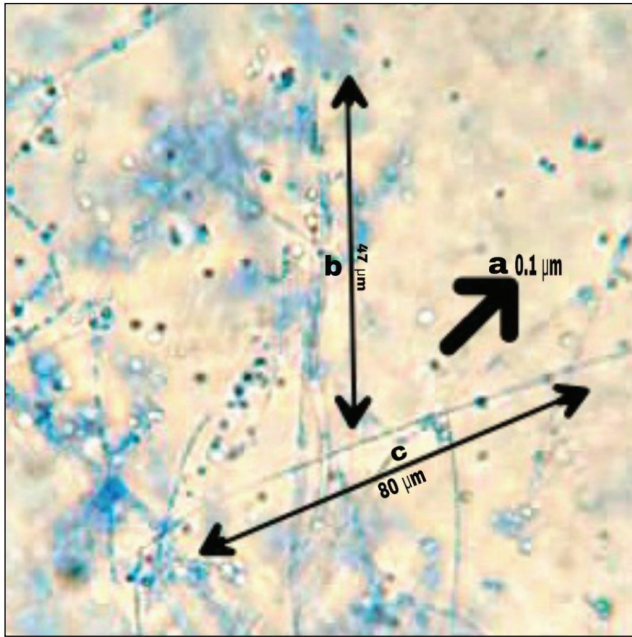


Figure 4: Cladosporium [a) Conidia, b) Conidiophore and c) Hyphae]

Conclusion

In this study, the fungi can be commensal, symbiotic or pathogenic to the spider *Nephila pilipes*. Majority of the microorganisms are commensal, where the organisms that reside on the skin, deriving benefit from the host, but the host do not benefit from them. In some case, the organisms may be symbiotic, where the microorganisms and the host are mutually beneficial or may or may not be pathogenic, where the microorganisms are benefited but they cause disease to the host. In this case the relationship between the microorganisms and the spider may or may not be commensal. The commensal microorganisms can prevent the colonisation of the pathogenic microorganisms and it depletes the nutrient and produce toxin metabolites thus preventing the adherence of the harmful bacteria to skin cells of the host.

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