Studies on fungal flora collected from spider webs of Melghat forest Maharashtra India

V.P. Ughade[1*], S. S. Deshmukh[2]

1*. Department of Microbiology,S.G.B.A.U.,Amaravati (MS)
2. Principal, of Matoshri Vimlabai Deshmukh Mahavidyalaya, Amravati
Correspodence author : V.P. Ughade, Department of Microbiology, S.G.B.A.U., Amaravati (MS).

Abstract

Spider webs were shown to be effective collectors of airborne microorganisms such as bacteria and fungi. Spider webs were collected from different regions of Melghat forest such as Chikhaldara, Simadoh, Jaridhred . These spider webs were cultured on Potato Dextrose Agar plate supplemented with antibiotic to inhibit the growth of bacteria Methodology assured sterility during collection and permitted *In situ* microbial growth , observation and enumeration of fungi. These samples were then analyzed for fungi content. To be considered suitable passive collectors, webs had to satisfy some basic conditions such as collection of microorganisms without discrimination based on species or size or type, Collection under variable environmental conditions and saturation avoidance in the presence of strong microbial launching sources. Samples were collected from different height, at different temperatures, from thin to thick forest .These parameters covers major region of Melghat forest to create a database of fungi . Variety of isolates of fungi were recovered from all collected web samples.

Depending upon the pure culture isolation, morphology, microscopy , cultural characteristics , pigmentation fungi were confirmed. Dominantly constitute *Aspergillus specie, Fusarium species* and *Trichoderma species* and others are under investigation.

Keywords: *Airborne microorganisms, Aspergillus species,Fusarium species Spider webs, , Trichoderma species.*

INTRODUCTION

Forest is the ultimate habitat for spiders. A spider web or cobweb (from the obsolete word *coppe*, meaning "spider")[1] is a device created by a spider out of proteinaceous spider silk extruded from its spinnerets. Melghat is situated at the northern extreme of the Amravati district of Maharashtra, on the border of Madhya Pradesh, in the South-western Satpura mountain ranges. These constitutes forest which are part of world's fifth biologically richest heritage country. It harbours a viable population of Tiger and of the endangered Gaur. It also harbor a number of other faunal species such as Wild dog, Jackal,Sloth bear, Leopard, Caracal, and Ratel.The forests is of "Dry decidous forests" type and soil is rich in mineral and have a high water holding capacity. They have a high rate of exchangeable calcium and ph varing from 6.5 to 7.5 thus supporting the best form of teak, bamboos. Natural grassy openings are almost nonexistent.Almost 262 species of Bird, 54 species of amphibian, 96 species of Pisces occur. This topographic zone is away from the habitations and is least disturbed. Domestic cattle rarely share the water and forage. This zone is rich in floral diversity, which assumes adequate palatable forage to the wild animals.Spider webs have existed for at least 100 million years, as witnessed in a rare find of early Cretaceous amber from Sussex, southern England.[2] Insects can get trapped in spider webs, providing nutrition to the spider; however, not all spiders build webs to catch prey, and some do not build webs at all. "*Spider web*" is typically used to refer to a web that is apparently still in use (i.e. clean), whereas "*cobweb*" refers to abandoned (i.e. dusty) webs.[3] However, "cobweb" is used to describe the tangled three-dimensional web [4] of some spiders of the *theridiidae* family. Whilst this large family is also known as the tangle-web spiders, cobweb spiders and comb-footed spiders, they actually have a huge range of web architectures. Several different types of silk may be used in web construction, including a "sticky" capture silk and "fluffy" capture silk, depending on the type of spider. Webs may be in a vertical plane (most orb webs), a horizontal plane (sheet webs), or at any angle in between. It is hypothesized that these types of aerial webs co-evolved with the evolution of winged insects. As insects are

spiders' main prey, it is likely that they would impose strong selectional forces on the foraging behavior of spiders.[3][12] Most commonly found in the sheet-web spider families, some webs will have loose, irregular tangles of silk above them. These tangled obstacle courses serve to disorient and knock down flying insects, making them more vulnerable to being trapped on the web below. They may also help to protect the spider from predators such as birds and wasps.[13] Some species of spider do not use webs for capturing prey directly, instead pouncing from concealment (e.g. trapdoor spiders) or running them down in open chase (e.g. wolf spiders).

The net-casting spider balances the two methods of running and web spinning in its feeding habits. This spider weaves a small net which it attaches to its front legs. It then lurks in wait for potential prey and, when such prey arrives, lunges forward to wrap its victim in the net, bite and paralyze it. Hence, this spider expends less energy catching prey than a primitive hunter such as the wolf spider. It also avoids the energy loss of weaving a large orb web. Some spiders manage to use the signaling-snare technique of a web without spinning a web at all. Several types of water-dwelling spiders rest their feet on the water's surface in much the same manner as an orb-web user. When an insect falls onto the water and is ensnared by surface tension, the spider can detect the vibrations and run out to capture the prey.In traditional European medicine, cobwebs are used on wounds and cuts and seem to help healing and reduce bleeding.[15] Spider webs are rich in vitamin K, which can be effective in clotting blood. Webs were used several hundred years ago as gauze pads to stop an injured person's bleeding.[16]According to Hawksworth (2002), fungi are a major component of biodiversity,essential for the survival of other organisms and are crucial in global ecological processes. Fungi being ubiquitous organisms occur in all types of habitats and are the most adaptable organisms. The soil is one of the most important habitats for microorganisms like bacteria, fungi, yeasts, nematodes, etc. The filamentous fungi are the major contributors to the soil biomass (Alexander 1977). They form the major group of organotrophic organisms responsible for the decomposition of organic compounds. Their activity participates in the biodeterioration and biodegradation of toxic substances in the soil (Rangaswami et al, 1998). It has been found that more number of genera and species of fungi exist in soil than in any other environment (Nagmani et al, 2005). Contributing to the nutrient cycle and maintenance of ecosystem fungi play an important role in soil formation, soil fertility, soil structure and soil improvement (Hao-quin et al, 2008). Fungi take a very important position in structure and function of ecosystem. They decompose organic matter from humus, release nutrients, assimilate soil carbon and fix organic nutrients. An intense study of abundance and diversity of soil microorganisms can divulge their role in nutrient recycling in the ecosystem.The present study was undertaken to throw a light on the diversity and abundance of fungal species to reveal the characteristic distribution and diversity.The study involves isolation, identification and enumeration of fungal species associated with spider web from Melghat region of Maharashtra, India.

MATERIALS AND METHODS

Spider Web collection and fungal isolation

1. **Selection of sites:** 10 different sites from Melghat forest were selected 6 sites representing the barren land and 7 gardens were selected for the present study. The agricultural and barren lands were selected on all the directions along the outskirts of the city of Aurangabad while the gardens were situated in the city.

2. **Collection of soil samples**: Samples were collected from 10 – 15 cm deep pits dug in the area to be sampled. The samples were collected with a surface sterilized trowel. Soil was scraped along the walls of the pits and collected in polythene bags. Soil from 8 – 10 pits was pooled

Spider webs were collected from 20 sites of Melghat. With sterilized scissors and forceps webs were cut at four corners and transferred on PDA plates and incubate for two weeks at 27 ^{0}C During incubation plates were observed after three days interval. After two weeks of incubation total 90 different colonies were observed from 40 web samples.

Identification of Fungi

Isolation of fungi from spider webs inoculated on PDA plates were carried out by serial diluttion method (Warcup 1950, 1960) using different synthetic and semi synthetic media. The fungi were identified studying cultural characteristics, staining ,pigment formation, spore formation, and doing microscopy of 90 colonies

in pure cultures incubating for 5 days, 7 days and 2 weeks at 27^0C and P^H 5.6 ± 2 . Fungi were confirmed with the help of literature (Barnett1998, Ellis 1993, Gilman 2001, Raper & Fennel 1965, Thom & Raper 1941, Subramanian 1971).

RESULTS AND DISCUSSION

Enumeration of fungi:

Total90 colonies were isolated from the 40 web samples collected from different site of Melghat ecosystem namely devi point ,chikhaldara . The colonies are distributed in15 different species belonging to 11 genera. Maximum variation was observed in colonies from rare to dense forest cover .281 colonies were isolated from 40 samples. by dilution plate method (Warcup 1950, 1960) using different synthetic and semi synthetic media. The fungi were identified with the help of literature (Barnett1998, Ellis 1993, Gilman 2001, Raper & Fennel 1965, Thom & Raper 1941, Subramanian 1971). Cultural characteristics, Microscopy, Pigment formation, Spore formation, Spore haracteristics etc was studied in detail by inoculating all 90 colonies in pure culture and incubating for 5 days, 7 days, and 2 weeks at 27 ^{0}C at pH 5.6 ± 2. On the basis of this study and photographs of microscopy, the genus was confirmed with the help of std. references. Isolation of fungi from the web samples were carried out by streak plate method

Observations and Results:-

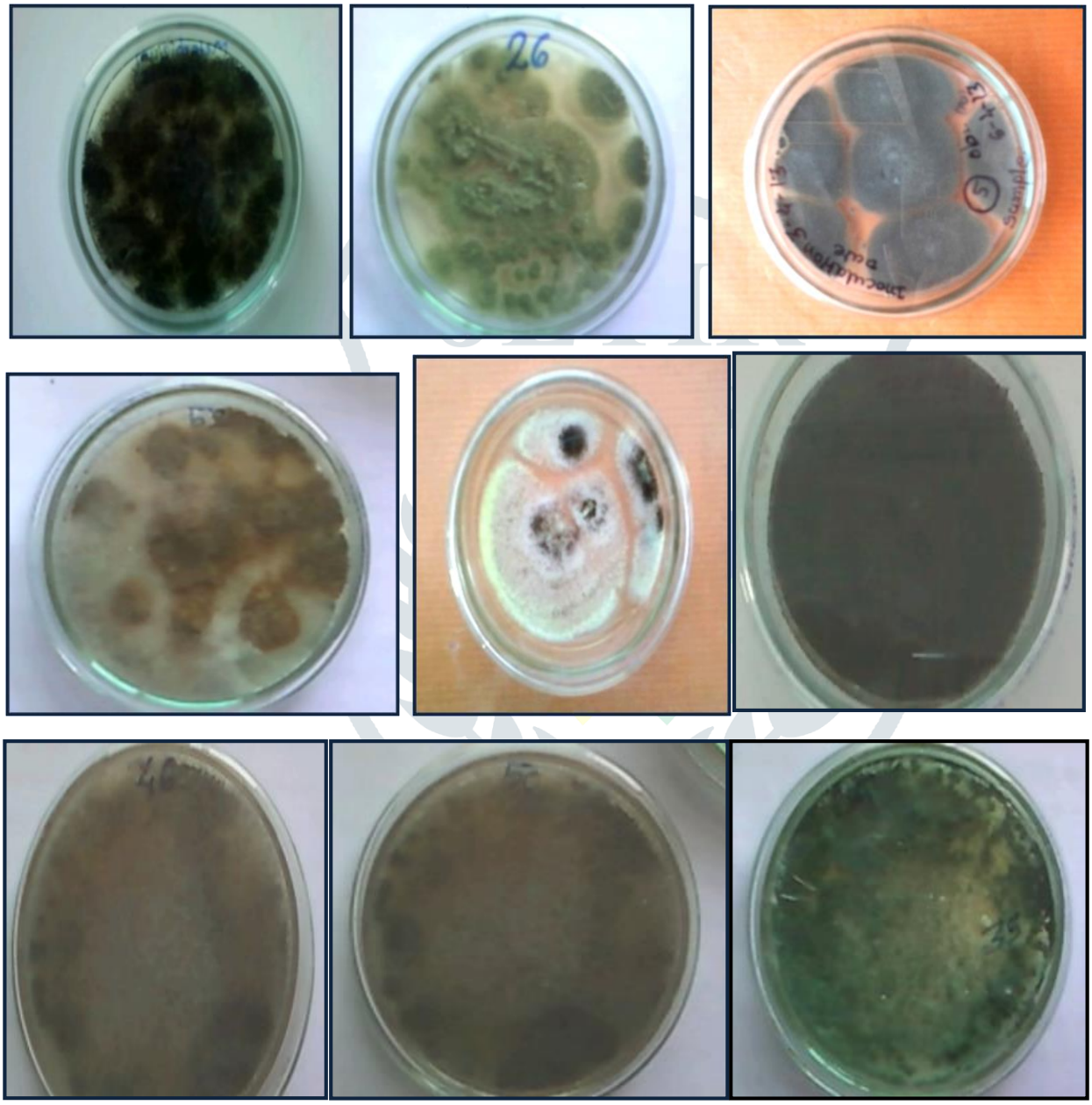
Identification of fungi:-

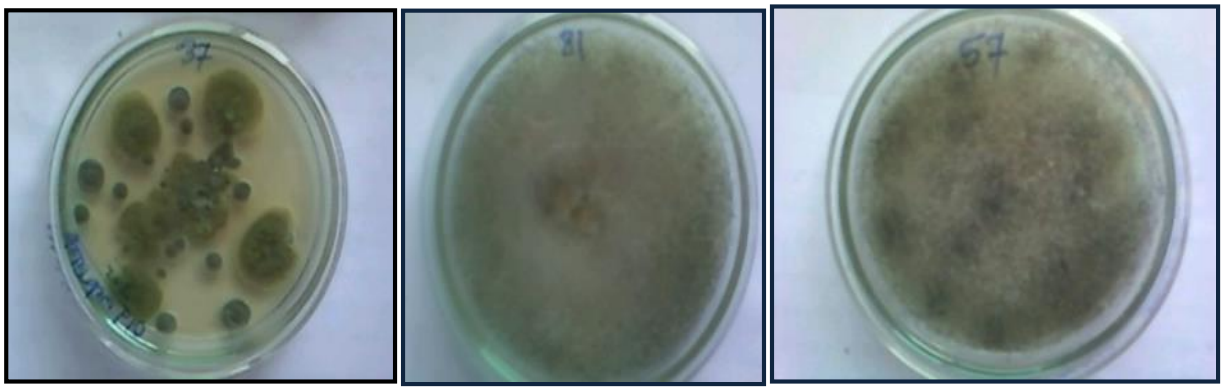
Cultural characteristics, Microscopy, Pigment formation, Spore formation etc was studied in detail by inoculating all 90 colonies in pure culture on PDA medium from 40 web samples a nd incubating for 5days,7days,and 2weeks at 27^0C. On the basis of study and microscopy, the genus and speci es was confirmed with the help of std. references, (Dubey, R.C.Practical ofMicrobiology, A.Nagamani, Handbook of soil fungi, Anantnarayan and Panikar, Alcamo's fundamental of microbiology, www.Dr.fungus.com, www.fungi online .com)

Sr no.	Surface	Reverse	Colony Texture.	No. of pure culture tube
1	Black like charcoal	White to yellow	Granular	21
2	Yellowish green	Golden to red brown	Granular	8
3	Smoky gray green	Yellow to brown	Velvety	5
4	Cinnamon to brown	White to brown	Granular	5
5	Carbon black+ Dry yeast like crystals	Yellow	Granular	3
6	Dry brownish black.	Yellow to dark brown	Granular	16
7	Whitish	Red/Sunset	Waxy	3
8	Dusty whitish	Pink	Waxy	3
9	Green	Lemon color	Velvety	5
10	Olive dark green	Pale yellow	Rough, sticky.	5

11	Black colony	Yellow to dark brown	Mat like	14
12	Green colony with orange spore	Yellow	Mat like	2

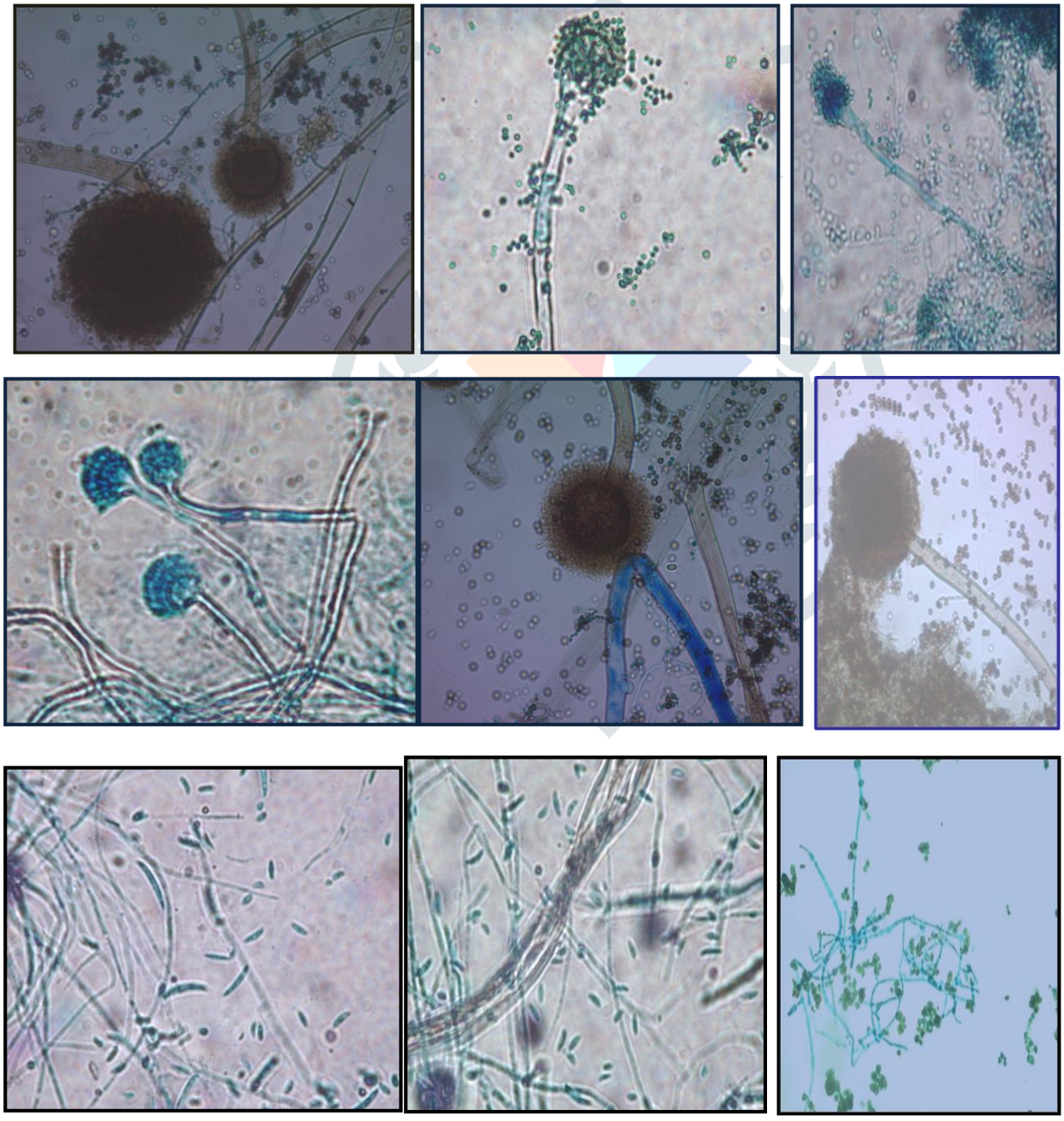
Table NO 1 : Showing Cultural Characteristic Of Fungi On PDA Medium.

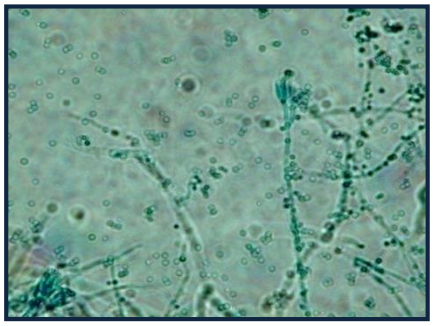




Macroscopic Observations

Microscopic observation





6. Discussion:

In the present study the web samples were screened (tested) for the isolation of fungal flora as a biological filter of air of that particular environment. Hence spider web was studied in relation with microbial aspect. It was found to be most effective biological filter to know the microbial flora of the region under study.In the present study the fungi isolates obtained from spider web of different places from Wardha Region were identified:

- *Aspergillus niger*
- *Aspergillus flavus*
- *Aspergiillus fumigatus.*
- *Aspergillus terrus.*
- *Aspergillus species.*
- *Fusarium species.*
- *Trichoderma species.*
- *Penicillium species.*
- *Rhizopus species.*

The predominent forms were *Aspergillus niger* and *Rhizopus species*. Also *Penicillium species, Trichoderma species, fusarium species* were obtained .

Aspergillus niger produces amylase enzyme, citric acid etc. *Penicillium species* produces antibiotic. *Trichoderma species* use as a biopesticide and biofertilizer. Economically important fungi were isolated from spider web having great applications in Medicinal, Agricultural and Industrial sector.Other recent biotechnological applications of spider silk includes the use of recombinant spider silk particles as drug delivery vehicles, Lammel A,*et al*., (2011). Spider silk has also been suggested to be used as a load bearing biomaterial, Brown C.P. *et al*.,(2011) because of its strength toughness than manmade polymer Kevlar. Spider silk has been suggested as a suitable replacement material for many existing products such as clothing, ropes, seat belts, body armour, parachutes and biodegradable bottles, all of which could show both cost and environmental benefits if made from spider silk rather than current manmade materials, D. Sarawanan,(2006) Further research will be directed towards the study of symbiotic association of fungi and spider web. Antifungal activity of spider web protein could be further studied for its broad spectrum of activity against variety of fungi causing damage to the humans, plants etc.

REFERENCES

Mohamed E. S. Mirghani *(2012)*,Investigation Of The Spider Web For Antibacterial activity, *Malaysian International Conference on Trends in Bioprocess Engineering (MICOTriBE).*

Chakraborty, D. Das (May 2009), Antibacterial activity of cobweb proteins on different bacteria,19th European Congress of Clinical Microbiology and Infectious Diseases.

David Kaplan and colleagues (Aug. 11, 2011) Genetically Engineered Spider Silk for Gene Therapy, ACS' journal *Bioconjugate Chemistry*,Science Daily

Kokare, Dr. Chandrakant (2010) Classification of fungi, Pharmaceutical Microbiology-Principle And Application Seventh Edition Pp- 10.2 to 10.

Kristie J. Koski, Paul Akhenblit, Keri McKiernan, Jeffery L. Yarger(2013), Non-invasive determination of the complete elastic moduli of spider silks. *Nature Materials*.Daily Science.

Porter, D., Vollrath, F., and Shao, Z. (2005). Predicting the mechanical properties of spider silk as a model nanostructured polymer. *European Physical Journal E*, 16, 199–206.

Saravanan, D. (2006). Spider Silk - Structure, Properties and Spinning. *Journal of Textile and Apparel,Technology and Management* , 5 (1), 1-20

Yves Termonia(1994)Molecular Modeling Of Spider Silk Elastisity,Macromolecules ACS publication.

Aneja, K. R. (2001) Experiment in Microbiology Plant Pathology and Biotechnology Fourth Edition New age international Ltd New Delhi.Pp171,Pp 594

Sunita Bansod and Mahendra Rai (2008) Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic *Aspergillus fumigatus* and *A. niger*, IDOSI Publications

Simon Wright, BSc (Hons), (February 2011),The antimicrobial properties of spider silk, University of Nottingham for the degree of Master of Research.Pp 37.

Dubey, R. C.(2002) Practical Microbiology First Edition S. CHAND and Company LTD.Pp 71-72 www.Dr. fungus,Identification of fungi.

Nagamani, A. (2005) Identification of fungi, Handbook of Soil Fungi, I.k. International Pvt Ltd New Delhi.Pp136-141, Pp229-231,Pp305,Pp367,Pp409.

Anantnarayan and Panikar() Medical Mycology, Textbook of Microbiology, Seventh Edition Pp.611.

Molitoris H.P. (1995): Fungi in biotechnology. Past, present, future.- Czech Mycol. 48:53-65

Upadhyay and Nath() Centrifugation, Biophysical Technique, Fourth Edition Pp.301

Simon Wright and Sara L. Goodacre(2012) Evidence for antimicrobial activity associated with common house spider silk BMC Research Notes.

Varaprasad Bobbarala (Apr. 2009) Antifungal activity of selected plant extracts against phytopathogenic fungi Aspergillus niger F2723 Indian Journal of Science and Technology Vol.2 No 4.

HiMedia Laboratories pvt. Ltd 23Vadhaniind.Est.LBS Marg,Mumbai,400 086.

Spider web,Wikipedia, the free encyclopedia.

L.Rypstra(1982),Building a Better Insect Trap,208 Life Science www.fungus images.com

Jeffrey C. Pommerville(2004),Characteristics and classification of fungi, Fundamentals Of Microbiology. Jones and Bartlett. Publication.Pp556-567.

Andrew H. Limper(MAY 2010) Treatment of Fungal Infections in Adult Pulmonaryand Critical Care Patients, American Thoracic Society Documents.