

Diversity of *Aspergillus* species from Nagpur University Campus

Mala Lanjewar¹, Ankush Kayarkar*², Nitin Dongarwar³

PG Student, Department of Botany, RTM Nagpur university Nagpur, Nagpur, India¹

Assistant Professor, Department of Botany, RMG Arts & Science College, Nagbhid, India²

Professor & Head, Department of Botany, RTM Nagpur University Nagpur, Nagpur, India³

Abstract: Aspergilli are cosmopolitan group of mould first described by Pier Antonio. Members of the genus *Aspergillus* are highly opportunistic growing easily on carbon rich substrates with monosaccharide and polysaccharides throughout the year. The present study evaluates the diversity of *Aspergillus* present in the Rashtrasant Tukadoji Maharaj Nagpur University Campus. A total of 14 different species of *Aspergillus* were isolated from the sampling area from the three different medium viz. Air, soil and leaf litter. *Aspergillus niger* was found to be the dominant one among others. The growth response of the isolated species of *Aspergillus* was tested over three different media viz. PDA, CzA and MEA.

Keywords: *Aspergillus*, Air, Soil, Leaf litter.

I. INTRODUCTION

Aspergillus is a cosmopolitan fungus whose spore are present in the air whose characteristics are of high pathological, agricultural, industrial, pharmaceutical, scientific and cultural importance and play important role in the degradation of organic substrate, particularly plant material [1, 2, 3]. *Aspergillus* are not only very well known fungus in the world of mycology but also known for their ability to secrete a variety of biologically active chemical compounds including antibiotics, mycotoxins, immunosuppressant and cholesterol lowering agents [2].

The Aspergilli have become increasingly important as responsible agents in a number of industrial fermentations. Many of them are being found capable of producing antibiotic substances and their possible use in this field will undoubtedly be exhaustively explored. *Aspergillus flavi* (*Aspergillus oryzae*, *Aspergillus sojae* and *Aspergillus tamarii*) were used in oriental food fermentation process [4]. Up till now More than 250 species of *Aspergillus* have been reported from different parts of the world. Generally, basic essential tool for identification of *Aspergillus* species are macroscopic characteristics such as colony diameter, conidial color, exudates, colony reverse and microscopic characteristics including conidiophore, vesicle, metulae, phialides and conidia [5]. *Aspergillus* species is cosmopolitan, filamentous, saprobic fungus in nature, especially during the autumn and winter months in the Northern hemisphere. It is primarily isolated from soils, especially indoor soil, decomposing plant material and indoor air environment.

There are around one hundred eighty-five species under the genus *Aspergillus*. Around twenty species have been reported so far as causative agents of opportunistic infection in humans. Among these, *Aspergillus fumigatus* is the most frequently isolated species followed by *Aspergillus flavus* and *Aspergillus niger*. Other species not often isolated opportunistic pathogens are *Aspergillus clavatus*, *Aspergillus glaucous*. Invasive fungal infections, particularly "Aspergillosis" is an increasing problem in immune-compromised patients.

The Aspergilli has been found cosmopolitan in nature and have become increasingly important as responsible agents in a number of industrial fermentations. Many of them are being found capable of producing antibiotic substances and their possible use in this field will undoubtedly be exhaustively explored. It also hazardously affects the human health. For these reasons, the need for the study of diversity of *Aspergillus* present in the Mahatma Jyotiba Phule Educational Campus of RTM Nagpur University area was undertaken.

II. MATERIALS AND METHODS

For the present study following methodology were used.

A. Selection of sample site

Mahatma Jyotiba Phule Educational Campus of the Rashtrasant Tukadoji Maharaj Nagpur University, Amravati road, Nagpur (Maharashtra State) India has been selected as sampling site as it is one of the oldest said campus established in

1966. The said sampling site is situated at hilly area, 5 km away from zero mile stone. Nagpur (21°45' N - 20°30' N and 78°15' E - 79°45' E) is center of Indian Peninsula and second largest green city in India after Chandigarh, lies on the Deccan Plateau at an altitude of 310.5 meters above mean sea level. It is characterized by a tropical wet and dry climate with an annual rainfall 1,166.3 mm (45.917 inches) during monsoon rain from June to September. Summer is extremely hot lasting from March June with maximum temperature 47.80C occurring in May. Winter lasts from November to January during which temperature can drop below 12.60C [6].

B. Collection of sample

From the selected site different species of *Aspergillus* were isolated through different components like by Air (Aeromycoflora) of the campus, from the different soil samples in the campus and from the different leaf litter sample in the campus. Different soil samples were collected from the different sites in the campus for isolation of *Aspergillus*, the samples of leaf litter were also collected from the different localities in the campus and some species were isolated from the indoor and outdoor environment of the campus by the method used by researchers [7, 8, 9, 10, 11].

B. Isolation and Identification of *Aspergillus* species

For the isolation of fungi from Air, soil and leaf litter, three different fungal media were used viz. Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek Dox Agar. From the isolated fungi, *Aspergillus* species were purified separately over these media. The identification was done with the help of colony character, Color of colony, reverse of colony. They were identified with the help of manual of *Aspergillus* [12] and also from the book of Handbook of soil fungi [13].

III. RESULT AND DISCUSSION

The isolation of the different species of *Aspergillus* was taken from the different localities of the campus area under study by employing different methods. The total 1242 colonies of only *Aspergillus* species were recorded from Air, Soil and leaf litter (Table 1). A total of 14 species were identified. The identification of *Aspergillus* species was done with the help of macro and micro character such as colony color, colony diameter, reverse color of colony, structure of conidiophores, sterigmata, phialids, spores etc. a total 425 colonies were recorded from the Soil and Leaf litter whereas 392 from the Air.

Colony restricted to fast growing varying from, blue, blue green, green yellowish, green brown, and ochraceous to black, zonate or azonate, velvety, floccose. Mycelium mostly submerged consisting of separate branching hyphae, colourless, reverse in various shades, exudates present or absent. Conidial heads radiate globose or splitting in Column. Columnar, conidiophores erect or suberect rarely synnematomous frequently originating from a well developed foot cell. Hyaline to subhyaline, smooth or rough becoming pale to brown in age in some species; septate or aseptate, terminating in a fertile bulbous cell. The vesicle is globose, subglobose, hemispherical, spatulate or clavate, bearing phialids over the entire or upper half or one third of the surface. Phialids in one series (unicriate) or two series (biserial) one above the other the lower one termed as metulae, which bear a cluster of two several phialids at the apex. Conidia varying greatly in color, shape and markings, successively come out from the tips of the phialids.

Total 392 colonies were recorded from air, where total 10 different species of *Aspergillus* were recorded. Where, *Aspergillus niger* dominated in colony count i.e. 142 colonies (Table 1) followed by *Aspergillus flavus*, *Aspergillus phoenisis*, *Aspergillus japonicus*, *Aspergillus sulphureus* with colony count range from 30-70, least colony count was observed by *Aspergillus awamori* and *Aspergillus violaceofuscus* with two colonies each (Table 1).

Total 425 colonies were recorded from soil, where total 9 different species of *Aspergillus* were recorded in which *Aspergillus niger* topped in colony count 105 colonies, followed by *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus awamori*, *Aspergillus phoenisis*, *Aspergillus parasiticus*, *Aspergillus sulphureus*, *Aspergillus oryzae* has least colony count (Table 1).

In the leaf litter, 425 colonies were recorded. Total 9 different species of *Aspergillus* were recorded in which *Aspergillus niger* topped in colony count with 132 total colonies followed by *Aspergillus phoenisis*, *Aspergillus flavus*, *Aspergillus japonicus*, *Aspergillus sulphureus*, *Aspergillus candidus*, *Aspergillus parasiticus*, least colony count was observed by *Aspergillus oryzae* (Table 1).

Fungal colony colors on petri plates play an important role in species identification. All the fourteen species of *Aspergillus* were grown on three different media viz, PDA, MEA, CzA. Diameter of the every colony was recorded on the each media from day 1 to day 7 (Fig. 1, 2, 3).

On the PDA, *Aspergillus niger* showed rapid growth with 4 cm in diameter in 7 days showing carbon black color followed by *Aspergillus ochraceous* 3.89 cm in diameter, yellow at first turned ochre color at maturity, *Aspergillus phoenisis* 3.40 cm in diameter, dark carbon black in color, *Aspergillus oryzae* 3.77 cm in diameter light yellow green to deep yellow green in color, *Aspergillus wentii* 3.37 cm in diameter yellowish to olive in color, *Aspergillus flavus* 3.34 cm deep gray green in color, *Aspergillus japonicus* 3.34 cm in diameter chocolate brown color, *Aspergillus candidus*, *Aspergillus awamori* *Aspergillus sulphureus*, *Aspergillus versicolor*, *Aspergillus violaceofuscus* showed very slow growth on the PDA media (Fig. 1).

Aspergillus wentii showed rapid growth on the MEA, 3.60 cm in diameter in 7 days followed by *Aspergillus oryzae* 3.29 cm in diameter, *Aspergillus niger* 3.26 in diameter, *Aspergillus violaceofuscus* 3.06 cm in diameter, *Aspergillus parasiticus* 3.03 cm in diameter, *Aspergillus flavus* 3.01 cm in diameter, *Aspergillus awamori*, *Aspergillus japonicus*, *Aspergillus candidus* 2.74 cm in diameter showed same growth, *Aspergillus sulphureus* and *A. versicolor* were observed as slow growing species (Fig. 2).

All the species were showing slow growth on the CzA media. *Aspergillus wentii* 3.14 cm in diameter was rapidly growing species on the CzA followed by *Aspergillus flavus* 3.03 cm in diameter, *Aspergillus niger* 2.71 cm in diameter, *Aspergillus candidus* 2.51 cm in diameter, *Aspergillus oryzae* 2.49 cm in diameter, *Aspergillus japonicus* 2.17 cm in diameter *Aspergillus violaceofuscus*, *Aspergillus phoenisis*, *Aspergillus parasiticus*, *Aspergillus versicolor* were very slow growing species on the CzA media (Fig. 3).

IV. CONCLUSION

A total 14 different species of *Aspergillus* were recorded from the sampling site out of which 10 species recorded from the air, 9 from soil and 9 from leaf litter. *Aspergillus niger* was found to be the dominant species present in all the sampling modes followed by *Aspergillus flavus*, *Aspergillus phoenisis*, *Aspergillus sulphureus* which has very high frequency, *Aspergillus awamori*, *Aspergillus candidus*, *Aspergillus oryzae*, *Aspergillus ochraceous*, *Aspergillus parasiticus*, *Aspergillus sydowii*, *Aspergillus versicolor*, *Aspergillus wentii*, *Aspergillus violaceofuscus* has very least frequency.

Table 2: Observation table for colony diameter in cm (PDA)

Sr. No.	Name of species	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	<i>Aspergillus awamori</i>	0.2	0.4	1.0	2.4	3.6	4.8	5.8
2	<i>Aspergillus candidus</i>	0.4	0.8	2	2.6	3.6	4.2	5.6
3	<i>Aspergillus flavus</i>	0.4	1.2	2.4	2.8	4.6	5.8	6.2
4	<i>Aspergillus japonicus</i>	0.4	1.6	2.6	3.6	4.4	5.2	5.6
5	<i>Aspergillus niger</i>	0.4	1.8	3.6	4.2	5.2	6	6.8
6	<i>Aspergillus ochraceus</i>	0.4	1.6	2.8	3.8	5.2	6.2	7.2
7	<i>Aspergillus oryzae</i>	0.4	1.2	2.6	4	5	6.2	7
8	<i>Aspergillus parasiticus</i>	0.4	1.4	2.6	3.6	4.4	5	5.8
9	<i>Aspergillus phoenicis</i>	0.2	0.8	2.4	3.6	4.8	5.8	6.2
10	<i>Aspergillus sulphureus</i>	0.2	0.6	0.8	1.2	1.6	1.8	2
11	<i>Aspergillus versicolor</i>	0.2	0.4	0.8	1.2	1.6	2	2.6
12	<i>Aspergillus violaceofuscus</i>	0.2	0.4	0.8	1.2	1.6	2.4	3.4
13	<i>Aspergillus wentii</i>	0.4	1.2	2.4	3.4	4.4	5.6	6.2

Table 3: Observation table for colony diameter in cm (MEA)

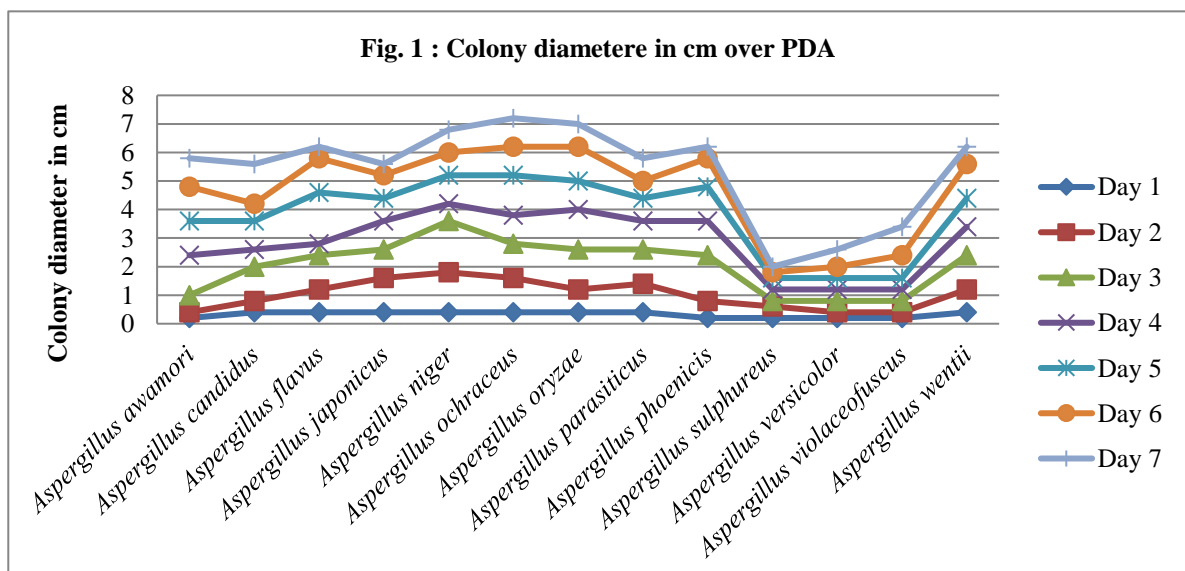
Sr. No.	Name of species	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	<i>Aspergillus awamori</i>	0.2	0.6	1.2	3	3.8	4.6	5.8
2	<i>Aspergillus candidus</i>	0.4	0.8	1.4	2.4	3.6	4.8	5.8
3	<i>Aspergillus flavus</i>	0.4	0.8	2.4	3	4.1	4.8	5.6
4	<i>Aspergillus japonicus</i>	0.4	0.8	2	2.8	3.8	4.4	5
5	<i>Aspergillus niger</i>	0.2	1.6	2.4	3.2	4.4	5.2	5.8
6	<i>Aspergillus ochraceus</i>	0.4	0.8	2	2.4	3.6	4.4	5.2
7	<i>Aspergillus oryzae</i>	0.2	1.2	2.4	2.8	4.4	5.6	6.4
8	<i>Aspergillus Parasiticus</i>	0.4	1.6	2	3.2	4	4.4	5.6
9	<i>Aspergillus phoenicis</i>	0.2	0.4	0.8	1.2	3	3.8	5.2
10	<i>Aspergillus sulphureus</i>	0.2	0.4	1	1.4	1.8	2.2	2.6
11	<i>Aspergillus versicolor</i>	0.2	0.4	0.6	0.8	1	1.2	1.4
12	<i>Aspergillus violaceofuscus</i>	0.2	0.4	2.4	3	4	5.2	6.2
13	<i>Aspergillus wentii</i>	0.4	1.2	2.6	3.8	4.4	5.8	7

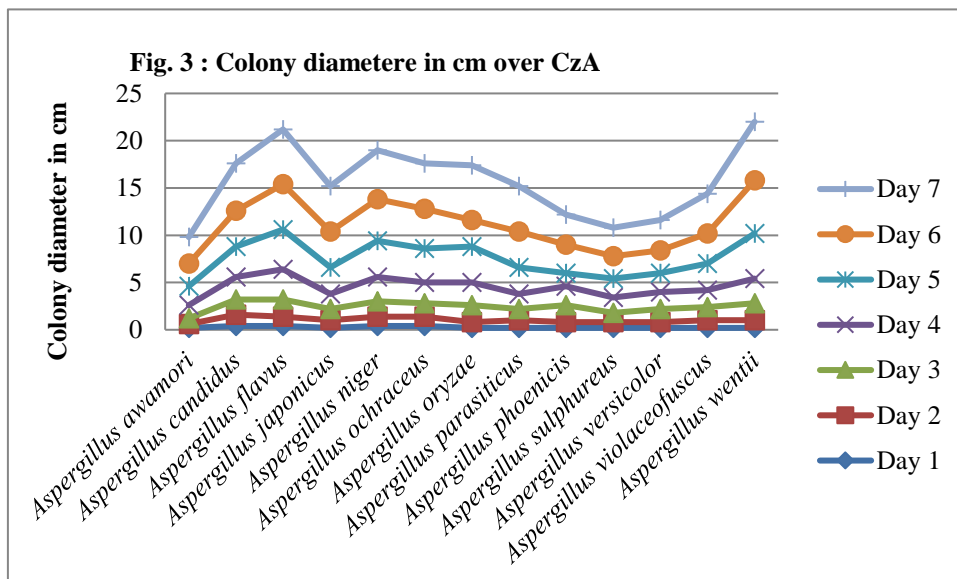
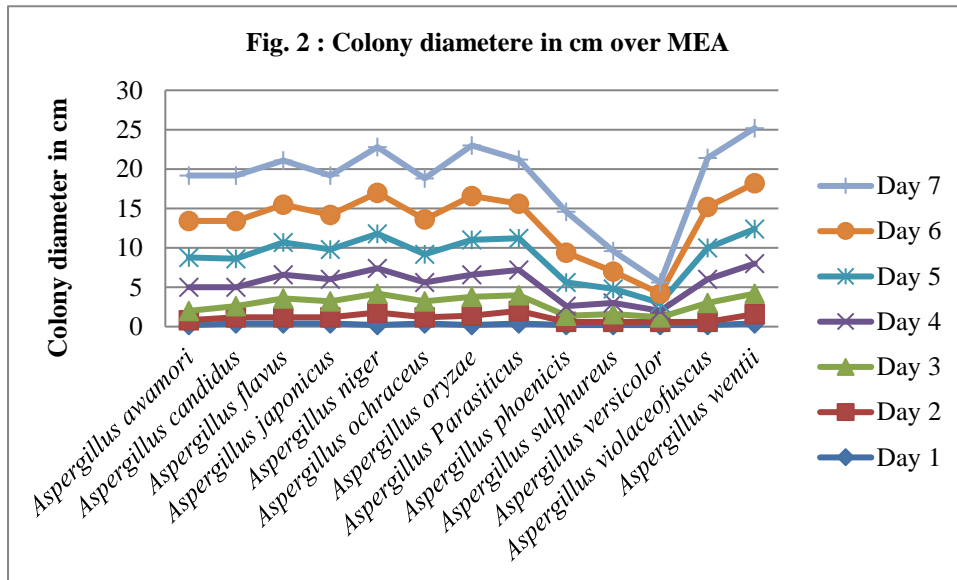
Table 4: Observation table for colony diameter in cm (CzA)

Sr. No.	Name of species	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	<i>Aspergillus awamori</i>	0.2	0.4	0.6	1.4	2	2.4	2.8
2	<i>Aspergillus candidus</i>	0.4	1.2	1.6	2.4	3.2	3.8	5
3	<i>Aspergillus flavus</i>	0.4	1	1.8	3.2	4.2	4.8	5.8
4	<i>Aspergillus japonicus</i>	0.2	0.8	1.2	1.6	2.8	3.8	4.8
5	<i>Aspergillus niger</i>	0.4	1	1.6	2.6	3.8	4.4	5.2
6	<i>Aspergillus ochraceus</i>	0.4	1	1.4	2.2	3.6	4.2	4.8
7	<i>Aspergillus oryzae</i>	0.2	0.6	1.8	2.4	3.8	2.8	5.8
8	<i>Aspergillus parasiticus</i>	0.2	0.8	1.2	1.6	2.8	3.8	4.8
9	<i>Aspergillus phoenicis</i>	0.2	0.6	1.8	2	1.4	3	3.2
10	<i>Aspergillus sulphureus</i>	0.2	0.6	1	1.6	2	2.4	3
11	<i>Aspergillus versicolor</i>	0.2	0.6	1.4	1.8	2	2.4	3.2
12	<i>Aspergillus violaceofuscus</i>	0.2	0.8	1.4	1.8	2.8	3.2	4.2
13	<i>Aspergillus wentii</i>	0.2	0.8	1.8	2.6	4.8	5.6	6.2

Table 1: *Aspergillus* species isolated from study area

Sr.No	Fungal taxa	Air		Soil		Leaf litter		Total colonies	Total Frequency
		colony count	Frequency	colony count	Frequency	colony count	Frequency		
1	<i>Aspergillus awamori</i>	2	0.16	49	3.95	0	0.00	51	4.11
2	<i>Aspergillus candidus</i>	12	0.97	0	0.00	14	1.13	26	2.09
3	<i>Aspergillus flavus</i>	75	6.04	82	6.60	70	5.64	227	18.28
4	<i>Aspergillus japonicus</i>	39	3.14	0	0.00	64	5.15	103	8.29
5	<i>Aspergillus niger</i>	142	11.43	105	8.45	132	10.63	379	30.52
6	<i>Aspergillus ochraceus</i>	0	0.00	52	4.19	0	0.00	52	4.19
7	<i>Aspergillus oryzae</i>	0	0.00	6	0.48	2	0.16	8	0.64
8	<i>Aspergillus parasiticus</i>	0	0.00	33	2.66	11	0.89	44	3.54
9	<i>Aspergillus phoenicis</i>	63	5.07	46	3.70	74	5.96	183	14.73
10	<i>Aspergillus sulphureus</i>	34	2.74	29	2.33	37	2.98	100	8.05
11	<i>Aspergillus sydowi</i>	12	0.97	0	0.00	0	0.00	12	0.97
12	<i>Aspergillus versicolor</i>	11	0.89	3	0.24	0	0.00	14	1.13
13	<i>Aspergillus violaceofuscus</i>	2	0.16	0	0.00	0	0.00	2	0.16
14	<i>Aspergillus wentii</i>	0	0.00	0	0.00	21	1.69	21	1.69
	Total	392	31.56	425	34.22	425	34.22	1242	100





REFERENCES

- [1]. Bignell E, "Aspergillus: molecular biology and genomics". *Caister Academic Press* 2010
- [2]. Osmani, S. A, Goldman, G. H., "The Aspergilli: Genomics, medical aspects, biotechnology, and research methods". Boca Raton: CRC Press 2008.
- [3]. Samson, R.A., and Vargas, J., "Aspergillus Systematics in the Genomic Era veterinary mycology" 44(1):133-148, 2008.
- [4]. Samson RA, Visagie C, Houbraken J, Hong S, Hubka V, Klaassen C, Perrone G, Seifert A, Susca A, Tanney J, Varga J, Kocsub S, Szigeti G, Yaguchi T, Frisvad J., "Phylogeny identification and nomenclature of the genus *Aspergillus*". *Studies in mycology* 78: 141–173, 2008.
- [5]. Raper KB and Fennell DI, "The genus *Aspergillus*". Williams and Wilkins company, 1965.
- [6]. Wikipedia, <https://en.wikipedia.org/wiki/Nagpur>, 2020.
- [7]. Warcup JH. "The soil plate method for isolation of fungi from soil". *Nature* 166: 116-118, 1950
- [8]. Waksman SA and Fred EB, "A tentative outline of the plate method for determining the number of micro-organisms in the Soil". *J. Soil Sci.* 14(1):27-28, 1922.
- [9]. Aneja KR. "Experiments on Microbiology, Plant Pathology and Biotechnology". 4th ed., New Delhi: New Age International Pvt.Ltd. 2004.
- [10]. Kayarkar A and Bhajbhuj MN, "Comparative studies on indoor Aeromycoflora from the laboratories". *International Journal of Life Sciences*, Vol. 2(4): 318-324, 2014.
- [11]. Kayarkar A & Dongarwar N, "Phylloplane of *Habenaria foliosa*- the threatened terrestrial orchid". *International journal for Inno. Res. in multidisciplinary field* Vol 5(7): 99-103, 2019.
- [12]. Thom C. and Raper KB. "A manual of Aspergilli". The Williams and Wilkins Company, Baltimore, 1945.
- [13]. Nagamani A, Kunwar IK, Manocharachary C., "Handbook of Soil Fungi". I K International Pvt. Ltd. 2006