J. Biol. Chem. Chron. **2018, 4***(2),* 41-47
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Variations in Physicochemical Parameters and Distribution of Mycoflora in Rhizospheric Soil due to Charcoal Rot of Jowar (*Sorghum bicolor* L.)

M. T. Gavali[1], Mane S. R.[2], Kumbhar V. R.[3], Birajdar G. M.[4] and U. N. Bhale[1*]

[1, 2, 3 & 4]*Research Laboratory, Department of Botany, Arts, Science and commerce college Naldurg, Tq-Tuljapur, Dist-Osmanabad.-413602 (M.S), INDIA*

[*] *Correspondence: E-mail: unbhale2007@rediffmail.com*

(Received 09 Aug, 2018; Accepted 28 Aug, 2018; Published 01 Sept, 2018)

ABSTRACT: Jowar (*Sorghum bicolor* L.) plant belongs to family poaceae is cultivated in warm climates worldwide and it is inhabitant to the tropics and subtropics region. The present investigation was studied physicochemical characterization and rhizosphere soil mycoflora in the field of jowar charcoal rot caused by *Macrophomina phaseolina.* Mycoflora of infected field of jowar, need to improve the knowledge of diversity of fungi associated with infected rhizosphere soil. Physico-chemical analyses of infected rhizosphere soil were studied by standard soil testing methods and fungal population dynamics by using soil dilution technique. Sixteen physicochemical parameters were analyzed. It was found that, soil has alkaline pH but EC, Ca, Na, S and Mo contents were found least, whereas OC, P and K were high in infected soil as compared to standard range. Due to rots infection, chemical contents of soil also changed. Eleven samples of soil were analyzed during January-March, 2015 and 2016. In all tested soil samples, 13 genera and 20 species of fungi were detected. *Aspergillus, Mucor, Rhizopus, Penicillium, Fusarium* species were found dominant in all tested study sites followed by *Trichoderma, Monilia, Pythium, Phytophthora, Humicola* etc. Total number of fungal species colony was found dominant in Sangola (S) and Mohal (Moh) sites. Percentage of frequency and % of contribution was found more in *Aspergillus niger* and *Fusarium oxysporum*.

Keywords: Mycoflora; Rhizosphere; physicochemical parameters; Jowar and Charcoal rot.

INTRODUCTION: Jowar (*Sorghum bicolor* L.) plant belongs to family poaceae is cultivated in Rabi and Kharif warm climates worldwide. Sorghum is a type of grass which is rich in antioxidants and high in fat and B vitamins. Most of the varieties are drought and heat tolerant and are especially important in arid regions, where the grain is one of the staple foods for poor and rural people. It is an important food crop in Africa, Central America, and South Asia, and is the fifth-most important cereal crop grown in the world. Fungal diseases are widely prevalent throughout the Jowar producing countries including India. As per the survey of literature revealed that, the charcoal rot caused by *Macrophomina phaseolina* causes huge losses in grain yield of sorghum.[1, 2 & 3] This can be a major disease in the drier regions of the world during late season as maturity of plants. It appears destructive on high yielding cultivars. Fungi play a vital role in nutrient cycling by regulating soil biological activity[4] and the rate organic matter is decomposed by the microbes is consistent to the chemical composition of the substrate and climatic conditions. Therefore the present investigation was made to studies on variations in physicochemical parameters and distribution of mycoflora in rhizospheric soil due to charcoal rot of jowar (*Sorghum bicolor* L.).

MATERIAL AND METHODS:

Physicochemical Analyses: Physicochemical analysis of infected rhizosphere soil were collected from study area and used for physicochemical characterization. Soil were spread out on a tray for air drying and sieved over a 150 mm and used for characterization. Each sample is weighed using digital balance. The samples were then oven-dried at a temperature of 110ºC for 24 hours and reweighed. Electrical conductivity and pH of compost were measured.[5] Nitrogen content was determined by the Kjeldahl method.[6] Organic Carbon was evaluated[7] method by oxidizing organic carbon with potassium dichromate and sulphuric acid. Phosphorus in soil was determined by Olsens method by using spectrophotometer.[8 & 9] Water soluble and exchangeable Potassium was calculated by Ammonium acetate method[10] using Flame photometer. Sodium, Calcium and Magnesium cations were

estimated by EDTA titration.[11] Analysis of Ferrous, Mangenese, Copper, Boron, Sulphur, Zinc and Molybdenum were done by acid digestion of soil.[12]

Isolation, Identification and quantification of Soil Mycoflora:

Collection of soil samples: The infected rhizospheric soil of charcoal rots of jowar incited by *Marophomina phaseolina* were collected during January-March, 2015 and 2016. The soil samples were collected from eleven different locations of Solapur districts viz, Mangalwedha, Mohol, North Solapur, Akkalkot, South Solapur, Barshi, Madha, Karmala, Pandharpur and Sangola. Three different replications of rhizospheric soil mycoflora were collected were determined from each site.

Dilution Plating Method: For the isolation of mycoflora, dilution plate method was employed.[13] Ten grams of sample were transferred to a flask containing 100 ml sterile water. The contents were crushed and shaken on a mechanical centrifuge for 15 min and then serially diluted to obtain 10^{-3} and of 0.5 ml of each was transferred to sterile petri plates containing potato dextrose agar (PDA) medium. The pH of medium was adjusted by adding 0.1N HCl or 0.1N NaOH. Then it was supplemented with1% streptomycin to prevent bacterial growth. The inoculated plates were incubated in an inverted in BOD incubator at 27±2ºC for 7 days.

Observation and Identification of fungi: The colonies growing on PDA plates wide edge of the colony was picked up with the help of a paw of needles and cotton blue stain. The slide was gently heated and if any, the excess stain was removed with the help of tissue paper and then the cover slip was sealed with transparent nail polish. The slide was observed under a compound microscope. Colony color and morphology was noted besides hyphal structure, spore size, shapes and spores bearing structures.[14] They were compared with the standard manual.[15, 16 & 17] The percentage of frequency and contribution were calculated by employing the following formulae:

$$\% \text{ Frequency} = \frac{No\ of\ observations\ in\ which\ species\ appeared}{Total\ no\ of\ observations} \times 100$$

$$\% \text{ Contribution} = \frac{Total\ no\ of\ CFU * of\ an\ individual\ species}{Total\ no\ of\ CFU\ of\ all\ species} \times 100$$

**CFU- Colony Forming Unit*

Statistical Analysis: The number of colonies per plate in 1 g of soil was calculated and the percent frequency and contribution of each isolated fungi were determined.[18] Physicochemical data were statistically analyzed and the significance of differences was determined by using book.[19]

RESULTS AND DISCUSSION:

Physico-chemical analyses: Infected rhizosphere soil was collected from charcoal rot of jowar and sixteen physicochemical parameters were analyzed. Sixteen physicochemical parameters were analyzed. It founds alkaline pH but EC, Ca, Na, S and Mo contents were found least whereas OC, P and K, high in infected soil as compared to standard range. Due to rots infection chemical contents of soil is also changed. Among 16 characterization, Organic Carbon (3.12±1.13%), Phosphorus (149±11.27kg/ha) and Potassium (1008±14.11kg/ha) contents were found very high as compared to standards. In case of Nitrogen, Magnesium, Zinc, Ferrous, Copper and Boron were found balanced in infected soil (Table 1).

Isolation, Identification and quantification of infected Soil Mycoflora: Total eleven samples of infected rhizosphere soil (surface of 0-5cm deep) from different localities were collected during charcoal rot infection of jowar and carried out for isolation, quantification and identification of microflora by dilution plate technique and data were analyzed (Table 2; fig 1 & 2). Overall 13 genera and 20 species viz. *Aspergillus niger, Aspergillus flavus, Aspergillus repens, Aspergillus terrus, Rhizopus microspores, Rhizophus nigricans, Mucor racemosus, Penicillium* sp. *Pennicillim* sp. *(unidentified), Penicillium brefelldianum, Penicillium claviformae, Fusarium oxysporum, Trichoderma harzianum, Monilia sitophila, Pythium aphanidermatum. Phytophthora infestans, Humicola insolens,Curvularia spicifera, Gleiocladium virens* and *Rhizoctonia solani. Aspergillus, Mucor, Rhizopus, Penicillium, Fusarium* species were found dominant in all tested study sites followed by *Trichoderma, Monilia, Pythium, Phytophthora, Humicola* etc.

Total Fungal colony was found dominant in the sites of Mohol i.e. (09) and Sangola i.e. (09), which was followed by Madha i.e. (08) and Malshirus i.e. (08) while least in Karmala i.e. (04). The percentage (%) of fungal frequency and contribution was found dominant in all study sites. Maximum % frequency and contribution was found in *Fusarium oxysporum* (100%;15.50%) and *Aspergillus niger* (100%;15.50%) which was followed by *Rhizopus microspores*

(90.90%;14.9%) and *Mucor racemosus* (63.63;09.09%) respectively while minimum in *Trichoderma harzianum*, *Pythium aphanidermatum. Phytophthora infestans* and *Curvularia spicifera.*

Charcoal rot disease of sorghum has managed a critical position while the previous literature contains the reports. It was reported that the *Macrophophomina phasolina* grew and produce abundant Sclerotia at pH7 (neutral) and 30°C temperature.[20] The pH of soil is equally important for charcoal rot. The results of our e experiment have clearly established that always a neutral pH (7.5) is more favorable for disease development.[21] It was reported fungi particularly *Fusarium moniliforme* and *Aspergillus niger* species were more prevalent in the sampled area.[22] It was reported the Soil pH, organic content and water are the important features affecting the fungal population and diversity.[23] The organic carbon, nitrogen, phosphorus, potassium are important for fungi, if absence of these the growth and sporulation of moulds fungi and other microorganisms are susceptible more.[24] It was observed the most predominant reported fungi infecting seed germplasm were *Aspergillus* and *Fusarium* species of maize kernels.[25] It was reported the most common fungi *viz; Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Penicillium chrysogenum, Penicillium frequentans, Penicillium funiculosum, Trichoderma viride, Trichoderma harzianum, Fusarium oxysporum, Fusarium solani, Curvularia clavata, Curvularia lunata and Rhizopus stolanifer* were isolated and characterized on crop of Paddy, Corn, Ragi, Red gram, Cotton and Sugarcane.[26] It was reported the occurrence of mold fungi at different grain development stages in sorghum.[27]

Recently, it was reported a total of 10 fungal species were identified from the genera *Aspergillus, Fusarium, Trichoderma, Mucor, Penicillium* and *Verticillum. Trichoderma* was the most frequent genus among the fungi species identified from water hyacinth (*Eichhornia crassipes*) compost.[28] It was reported that A total 37 species of fungi were isolated and the number of fungi was found to be maximum in rhizosphere region than the non-rhizosphere region, maximum number of fungal species were found in *Abutilon indicum* (11) followed by *Aloe vera* (9), *Achyranthus aspera* (9),*Amaranthus polygamus* (8) and *Argemone maxicana* (7) from rhizosphere and rhizoplane of some Indian herbs.[29]

Table1: Physico-chemical characters of infected soil of charcoal rot of jowar caused by *Marophomina phaseolina.*

Sr. No.	Parameters	Standard Range	Infected soil (±SE)
1	pH	6.5 to 7.5	7.56±0.12
2	Ele. Conductivity³ mS ´	<1.0	0.52±0.11
3	Organic carbon %	0.41-0.60	3.12±1.13
4	Nitrogen ³kg/ ha´	161-320	156.8±12.33
5	Phosphorus ³ kg/ha´	31 to 50	149±11.27
6	Potassium ³ kg/ha´	181- 240	1008±14.11
7	Calcium (mg/kg)	65-80	4.19±1.01
8	Magnesium (mg/kg.)	10 - 15	11±2.33
9	Sodium (mg/kg)	5 - 15	0.87±0.03
10	Zinc (ppm)	1.0 - 5.0	4.47±1.11
11	Ferrous (ppm)	2.5 - 5.0	4.79±1.04
12	Manganese (ppm)	2.0 - 5.0	3.71±1.32
13	Copper (ppm)	0.2 - 0.5	0.5±0.04
14	Boron (mg/gm)	30 - 100	87±5.12
15	Sulphur (mg/kg)	10 - 20	7.56±2.33
16	Molybdenum(mg/kg)	0.8-3.3	0.52±0.14

Table 2: Mycoflora of infected rhizosphere soil of charcoal rot of jowar caused by *Marophomina phaseolina.*

Sr. No	Name of fungus												T	% F	% C
		Man	Moh	Ns	A	Ss	B	Mad	K	P	Mal	S			
1	*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+	11	100	15.50
2	*Aspergillus flavus*	-	+	-	+	+	+	-	-	-	-	-	04	36.36	5.64
3	*Aspergillus repens*	-	-	-	-	-	-	-	-	-	-	+	01	09.09	1.40
4	*Aspergillus terrus*	-	-	-	-	-	-	+	-	-	-	-	01	09.09	1.40
5	*Rhizopus microsporus*	+	+	+	+	+	+	-	+	+	+	+	10	90.90	14.9
6	*Rhizophus nigricans*	-	-	-	-	-	-	-	-	+	-	-	01	09.09	1.40
7	*Mucor racemosus*	+	-	+	-	+	+	-	-	+	+	+	07	63.63	9.85
8	*Penicillium* sp.	-	+	+	+	+	+	+	-	-	-	-	06	54.54	8.45
9	*Pennicillim* sp. *(Yel-low)*	-	-	-	-	-	-	+	+	-	-	-	02	18.18	2.82
10	*Penicillium brefelldianum*	-	-	-	-	-	-	-	-	-	+	-	01	09.09	1.40
11	*Penicillium claviformae*	-	-	-	-	-	-	-	-	-	+	-	01	09.09	1.40
12	*Fusarium oxysporum*	+	+	+	+	+	+	+	+	+	+	+	11	100	15.50
13	*Trichoderma harzianum*	-	-	-	-	-	-	+	-	-	-	-	01	09.09	1.40
14	*Monilia sitophila*	+	+	-	+	-	-	-	-	+	+	+	06	54.54	8.45
15	*Pythium aphanidermatum.*	-	+	-	-	-	-	-	-	-	-	-	01	09.09	1.40
16	*Phytophthora infestans*	-	+	-	-	-	-	-	-	-	-	-	01	09.09	1.40
17	*Humicola insolens*	-	-	-	-	+	-	+	-	+	-	-	03	27.27	4.23
18	*Curvularia spicifera*	-	-	-	-	-	-	-	-	-	-	+	01	09.09	1.40
19	*Gleiocladium virens*	-	+	-	-	-	-	-	-	-	-	-	01	09.09	1.40
20	*Rhizoctonia solani*	-	-	-	-	-	-	+	-	-	-	-	01	09.09	1.40
Total Fungal colony		**06**	**09**	**07**	**07**	**07**	**06**	**08**	**04**	**07**	**08**	**09**	**71**		

Legands: *Man-Mangalwedha, MOH-Mohol, NS-North Solapur, A-Akkalkot, SS-South Solapur, B-Barshi,Mad-Madha,K-Karmala, P-Pandharpur, MAL-Malshirus, S-Sangola, T- Total, %F-Percent Frequency, %C-Percent Contribution.*

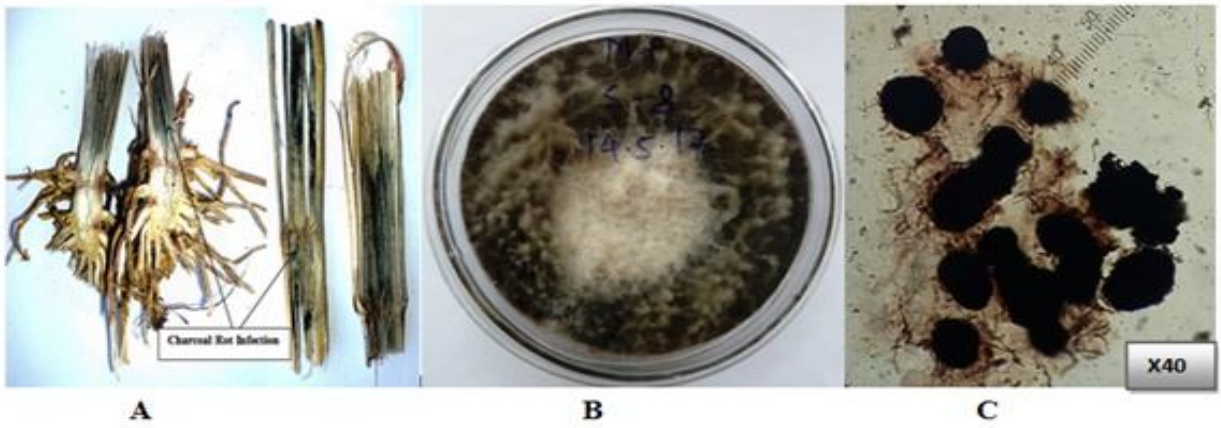

Figure 1: Charcoal rots of Jowar caused by *Macrophomina phaseolina*, A-Infected root and stem, B-Culture colony, C-Sclerotial morphology.

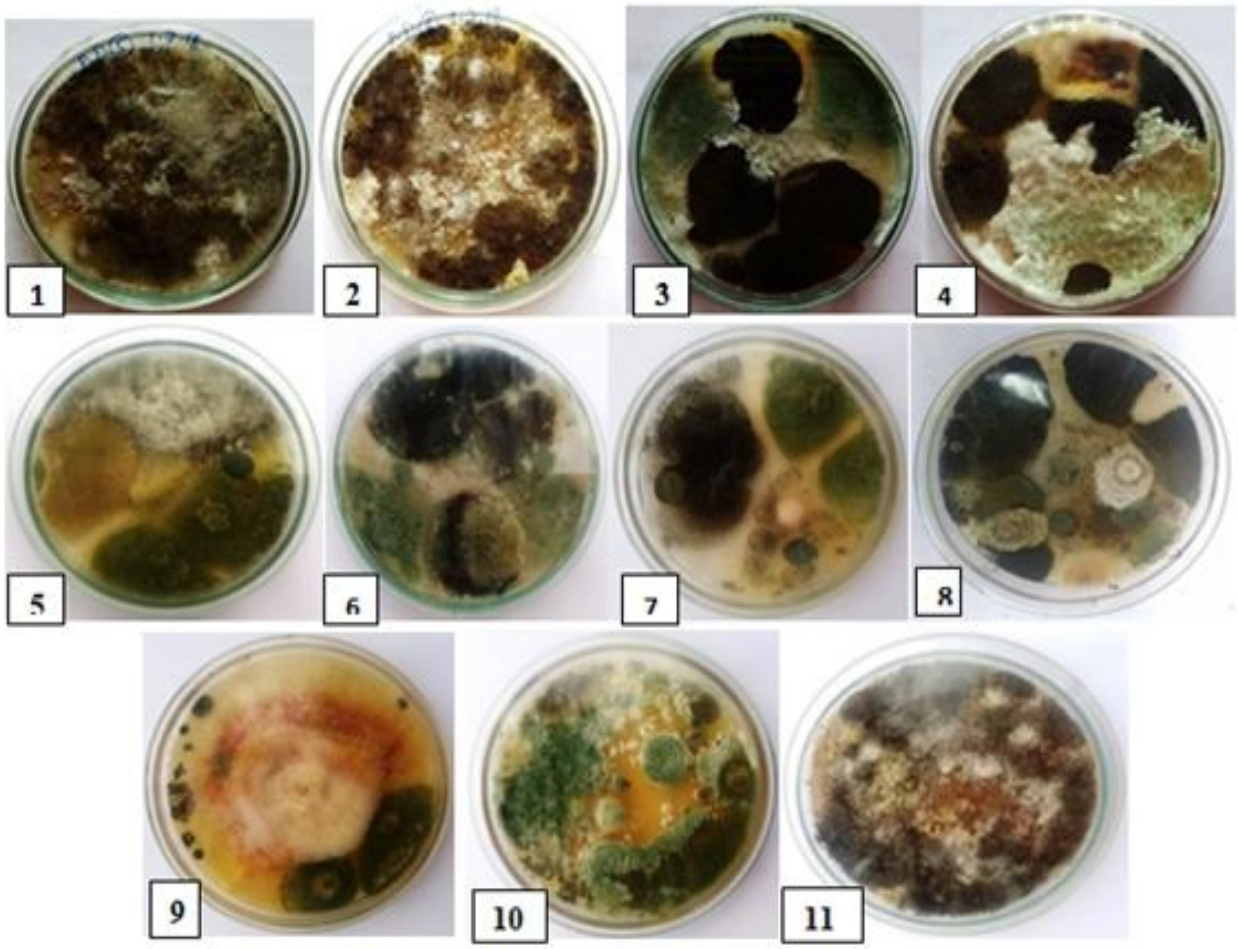

Figure 2: Fungal colonies on different charcoal rot infected rhizosphere soil of jowar (1-Pandharpur, 2-Mohol, 3-North Solapur, 4-Mangalwedha, 5-Akkalkot, 6-South Solapur, 7-Barshi, 8-Sangola, 9-Karmala, 10-Madha and 11-Malshirus).

CONCLUSION: These studies concluded that, selection hybrid, planting date and environmental conditions that favor disease development and dissemination at crop maturity appear to influence disease severity. Study showed a positive correlation between disease rating and percentage loss in the grain yield due to fungal population in infected jowar. Fungi secrets different kinds of toxins enzymes and it may prevent the growth of other fungal species. The frequency of mycoflora in jowar fields were found to be

regulated by many factors like temperature, pH, organic and inorganic elements, soil type and texture etc. *Aspergillus, Mucor, Rhizopus, Penicillium, Fusarium species* were found dominant. It was observed from finding that when more population of fungi shows less infection by *Marophomina phaseolina*. Our finding determines the differences in fungal species composition of jowar infected soils and management practices have greater prospective to influence the soil fungal community correlation.

REFERENCES:

1. Pedgaonkar, S. M. and Mayee, C. D. (1990) (Department of plant Pathology, Marathwada Agricultural Papaviza G. C. and N. G. Klag, 1975). Isolation and quantitative determination of *M phaseolina* from soil, *Pathology*, 65, 182-187.
2. Hiremath, R. V. and Palakshappa, M. G. (1994) (AICRP (Sorghum), University of Agricultural Sciences, Dharwad 580 05, Karnataka, India). Severe incidences of charcoal rot of Sorghum at Dharwad. (En). Current Research- University of Agricultural Sciences (Bangalore), 23(3/4), 38.
3. Narayana Rao, J.; T. B. Garud; S. Pande, P. Mohan Rao and Deshmukh, R.N. (1998) Charcoal rot of Survey of diseases of Sorghum in Maharashtra during 1995 rainy season, *International Sorghum & Millet Newsletter No*. 38, 61-62.
4. Arunachalam, K., Arunachalam, R. S., Tripathi and Pandey, H. N. (1997) Dynamics of microbial population during the aggradation phase of a selectively logged subtropical humid forest in north east India, *Trop. Ecol*, 38, 333-341.
5. Subbiah, B. V. and Asija, G. L. (1956) A rapid procedure for determination of available nitrogen in soils, *Curr. Sci*, 259-260.
6. Sahilemedhin, S and Bekele, T. (2000) Procedures for soil and plant analysis. National soil research centre Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia.
7. Walkely, A. J., and Black, I. A. (1934) Estimation of soil organic carbon by the chromic acid titration method, *Soil Sci.*, 37, 29-38.
8. Olsen, S. R., C. V. Cole, F. S. Watanabe and Dean, L. A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No., 939.
9. Bray, R. H. and Kurtz, L. T. (1945) Determination of total, organic and available forms of phosphorus in soils, *Soil Sci.*, 59, 30-45.
10. Hanway, J. J. and Heidel, H. (1952) Soil analysis methods as used in Iowa state college soil testing laboratory, *Iowa Agri*, 57, 1-31.
11. GOI. (2011) Methods Manual Soil Testing in India. Ministry of Agriculture Government of India, 1-215.
12. Jackson, M. L. (1967) Soil chemical analysis. Prentice Hall of India Pvt. Ltd. New Delhi, 36-82.
13. Warcup, J. H. (1950) The soil plate methods for isolation of fungi from soil, *Nature*, 117 –118.
14. Aneja, K. R. (2001) Experiments in Microbiology, Pant pathology and Biotechnology, *Newage International Publishers*, 4, 157-162.
15. Nagamani, A., I. K., Kunwar and Manoharachary, C. (2006) Hand book of soil fungi, I. K. International Pvt. Ltd.
16. Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S. (1973) The fungi – An advanced treatise: A taxonomic review with keys. In: Ascomycetes and fungi Imperfecti, *New York; Academic Press*, 4 (A), 621.
17. Ellis, M. B. (1976) *More dematiaceous Hyphomycetes*. Common wealth mycological institute, Pub., Kew, Survey, England.
18. Girisham, S. (1986) Studies on mycotoxin producing fungi associated with pearl millet.(*Pennisetum americanum* L.). Ph.D. thesis, Kakatiya University, Warangal.
19. Mungikar, A. M. (1997) An Introduction to Biometry. Saraswati Printing Press, Aurangabad, pp., 57-63.
20. Singh, K.; V. P. Agnihotri; S. N. Srivastava and Misra, S. K. (1974) Factors affecting growth and production of sclerotia by *Rhizoctonia bataticola*, *Indian Phytopath*., 3, 194-195.
21. Arora, M. K. (2016) Effect of enviornmental factors on charcoal rot of *Sorghum bicolor* caused by *Macrophomina phaseolina* (Tassi) Goid, *Life Sciences International Research Journal*, 3(2), 136-140.
22. Orisi, R. B., B. Correa, C. R. Possi, E. A. Schammass, J. R. Nogueira, S. M. Dias and Malozzi, M. A. (2000) Mycoflora and occurrence of fumonisins in freshly harvested and stored hybrid maize, *J. Stor. Prod. Res*., 36, 75-87.
23. Yu., C. D. G. Lv., S. J. Qin., Liu, G. C. (2007) Diversity analysis of soil dematiaceous hyphomycetes from the Yellow River source area, *J. Appl. Ecol*., 18(10), 2277-2281.
24. Saksena, S. B. (1955) Ecological factors governing distribution of soil microfungi in some forest soils of Saugar, *J. Indian Bot. Soc*, 34, 262-298.
25. Askun, T. (2006) Investigation of fungal species diversity of maize kernels, *J. of Biol. Sci.*, 6(2), 275-281.
26. Gaddeyya, G. P. Shiny Niharika, P. Bharathi and Ratna Kumar, P. K. (2012) Isolation and identifi-

cation of soil mycoflora in different crop fields at Salur Mandal, *Adv. Appl. Sci. Res*., 3(4), 2020-2026.

27. Magar, S. J. (2003) Occurrence of mold flora at different grain development stages in sorghum. M.Sc. (Agri.) dissertation submitted to Marathwada Agriculture University, Parbhani (M.S.) pp-85.

28. Jha, S. K. and Thapa, L. B. (2018) Microfungi isolated from Water hyacinth (*Eichhornia crassipes*) compost. *Bio Bulletin,* 4(1), 43-47.

29. Srivastava, V. and Kumar, A. (2013) Biodiversity of Mycoflora in Rhizosphere and Rhizoplane of Some Indian Herbs, B*iological Forum – An International Journal,* 5(2), 123-125.