# ISOLATION AND CHARACTERIZATION OF DOMINANT PATHOGENIC FUNGUS *ASPERGILLUS FLAVUS* FROM STORAGE SEEDS OF GROUNDNUT

[1]Rishabh Chitranshi, [2*]Naveen Kumar Arora
[1]Department of Environmental Microbiology, School for Environmental Sciences Babasaheb Bhimrao Ambedkar Central University, Lucknow, Utter Pradesh, India 226025
[2*] Professor in Department of Environmental Sciences, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar Central University, Lucknow, Utter Pradesh, India 226025

***Abstract:*** *Aspergillus flavus* is one of the well-known storage fungal pathogen. It causes very harmful effect for agricultural crops. Infection spreads dangerously, especially in the oilseeds having excessive moisture and nutrients. Keeping in view of the availability of fungus and its pathogenicity, traces of dominant fungal strain RF-03 have been taken from the infected groundnut seeds. These seeds were collected form warehouse of a local farmer of district Manpuri. The isolated strain dominantly covers the petri plates and also affects the development and quality of seed. Pure culture of RF-03 was analyzed firstly through electron microscope for morphological characterization. Further, the fungal strain was identified for genus and species level and resulted as *A. flavus* by 18S rRNA sequencing, the obtained sequence was then submitted in NCBI under the accession number MF120213.1.

***IndexTerms - Aspergillus flavus, storage oil seeds, fungal pathogens, SEM analysis.***

## I. INTRODUCTION

In concern of oil seeds, Groundnut (*Arachis hypogaea L*) is one of the most important oil seed crop worldwide and Indian is second largest producer of groundnut after china Pandey et al, (2012).It covers more than 82% of total oil seed production in India. Groundnut typically contains ample amount of protein and oil in it. Moreover, it is a rich source of multiple nutrients including Vitamin E and magnesium. Shalini et al. (2016). According to Adeyeye and Ajewole (1992); Sadasivam and Manickam (2008) the quality parameters for oil and products of groundnut are directly influenced by fatty acid profile along with growing conditions. That's why the crop is more susceptible to a variety of plant pathogens. Li et al. (2013).

*Aspergillus flavus* is one of the predominant pathetic fungi for oilseed crops also known as opportunistic pathogenesis in plants. A. flavus is one of the major factor for inhibit good quality seeds. Considerable economic loss takes place during international trade is only because of lower quality seed production. The seeds can be contaminated by Aspergillus spp. during harvest, pre and post-harvest, in storage or during transport. Shekhar et.al (2018); Vankayalapati (2018). The aflatoxin levels increases due to improper harvesting. The attacks of pathogens resulting yield losses and lower quality of the produce. Romani (2004). A yield loss due to occurrence of the diseases in groundnut is higher than 50%. Oilseeds may moderated there chemical composition due to the production of the potent carcinogen aflatoxin by fungal pathogens. Aflatoxins are toxic, mutagenic, and carcinogenic compounds Chen et al. (2013). These toxins occur naturally and have been found in a wide range of commodities, including groundnuts used for animal and human consumption. Williams et al. (2004)

The presence of pathetic fungi in storage area is a major threat of quality seeds production and fulfills the yield gap between supplies and demand. For sustainable agriculture and healthy food production it is very important to identify the dominant pathogens of storage area. Hence, the present study is formulated to identify and characterize dominant fungal pathogen from actual environmental conditions found in warehouse located within the groundnut producing area.

## II. RESEARCH METHODOLOGY

### II.1 Sample Collection

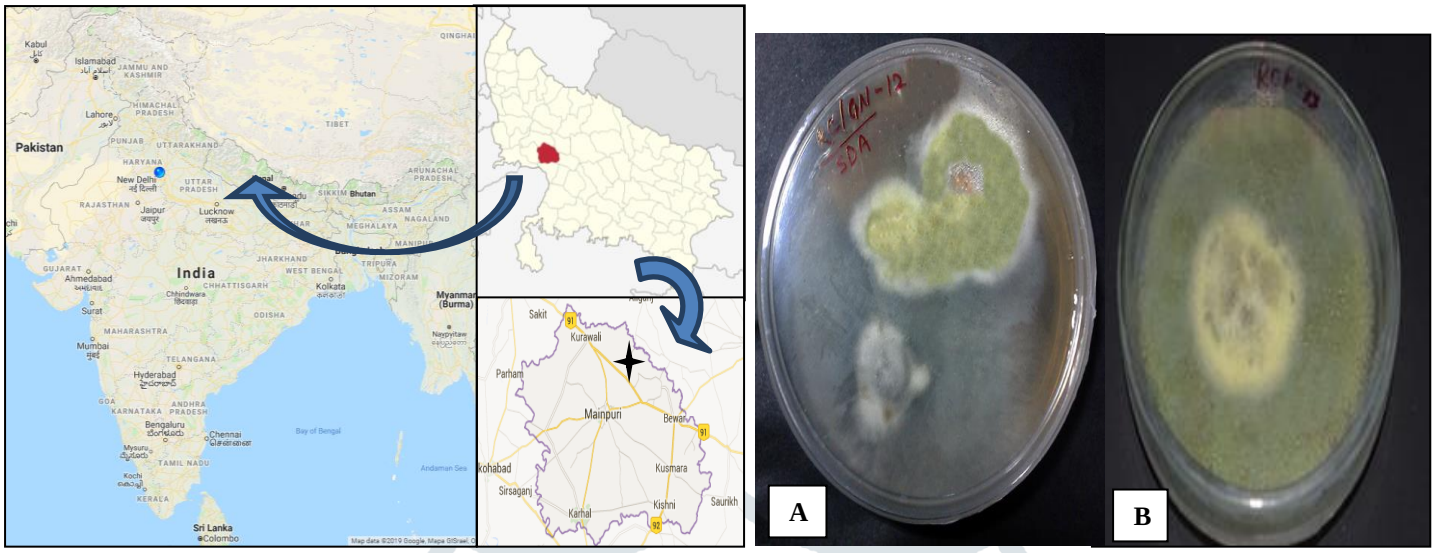
For the isolation of fungal pathogens, one year aged groundnut seeds were collected from the natural agriculture environment at village Alipurkheda district Manipuri, Uttar Pradesh (**Figure-1**). Infected seeds were collected in polybags and stored at room temperature in laboratory conditions.

### II.2 Isolation of pathogen

Fungal pathogens were isolated from infected seeds and shells of groundnut. All the infected groundnuts were going through the surface sterilization with 1% of sodium hypochlorite for 30 seconds, followed by three thorough rinses with sterilized distilled water. Air dried infected seeds were separated from shells, and then placed on potato dextrose agar (Hi media) plates. After incubation on 28°C for 5-7 days fungal colonies appeared on seeds and shells were picked up and purified further on PDA plates. Among all RF-03 was found in both shells and seeds were separated for further study (**Figure-2**)

**Fig-1 Site map of sampling In District Manpuri UP** **Fig-2 (A) isolate *Aspergillus flavus* (B) RF-03 *Aspergillus flavus***

**plates of isolated fungal deteriorating agents of Groundnut.**




**II.3 SEM Analysis**

Purified fungal strain RF-03 was analyzed through surface electron microscope , fresh fungal samples were prepared and fixed by using 2% glutaraldehyde in 0.2 M phosphate buffer (pH 6.8) at room temperature for 4 to 6 h. further, the fixed samples were carefully rinsed with 0.2 M phosphate buffer (pH 6.8) for 1–2 h, and then dehydrated in a graded acetone series (30, 50, 70, 80, 90, and 100%), each grade for 30 min and three times for 100% acetone. Fully dehydrated samples were completely dried and mounted on stubs for examination under SEM (**Figure-3**).

**Fig-3 SEM micrographs of isolated fungal deteriorating agents of Groundnut.**

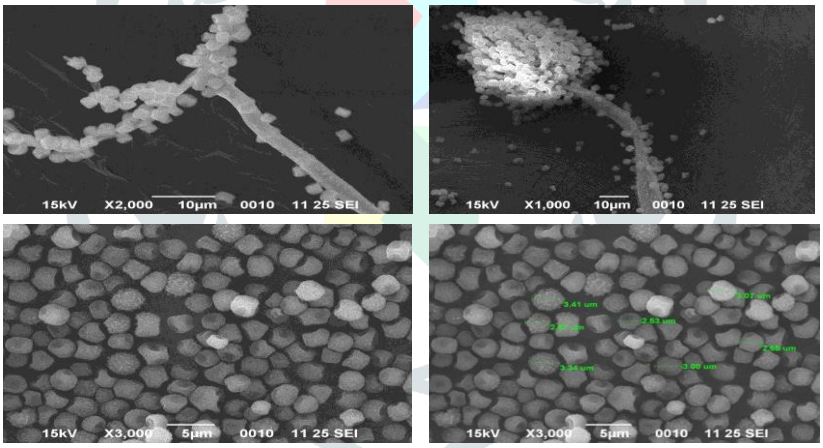
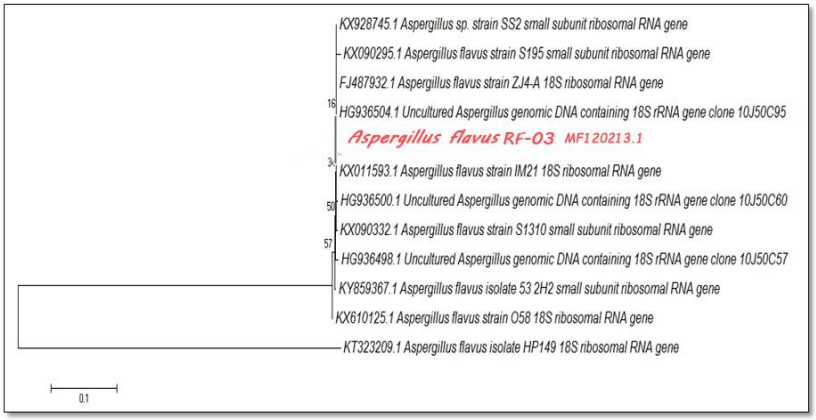



**Fig-4 Phylogenetic analysis of isolate RF-03**



**II.4 18S rRNA gene sequencing**

Fungal pathogen was further identified at genus and species level by using the 18S rRNA sequencing technique. Genomic DNA was isolated as described by Smit et al. in (1999). Moreover, amplification and purification of PCR product was completed as per the instruction of primer manufactures on the kit (Sigma Aldrich). 18S rRNA ITS Region universal primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC), PCR product was sequenced using the ITS1/ITS4 primers. Result sequence was further submitted to NCBI.

## III. RESULTS AND DISCUSSION

**III.1 Identification of pathogen**

Among all isolate the dominant fungal pathogen RF-03 was resulted as Aspergillus flavus after 18S rRNA sequencing. The FASTA format of sequence was submitted to NCBI under the accession number MF120213.1.(**Figure-4)** *A. flavus* highly contagious and covered full petri dish during isolation process further, the fungi is responsible for foodborne disease along with aflatoxin production in seeds under storage conditions. Chen et al, (2002) ; Gupta and Chauhan (1970) isolated A. flavus from groundnut seeds. Similarly, Lalithakumari et al. (1971) observed presence of A. flavus as dominant fungi on groundnut seeds. Bhattcharya and Raha (2002) reported that in groundnut seeds Aspergillus niger, A. rubber and A. flavus were initially very abundantly found in storage condition.

**III.2 Surface morphology of pathogen**

Scanning Electron Microscopy is done for revealing the surface morphology of isolated fungi. SEM creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. Conidia of *A. flavus* have relatively thin walls which are finely to moderately rough ened. Their shape can vary from spherical to elliptical. Scanning Electron Micoroscopy (SEM) micrographs clearly show these ornamentation differences Furthermore, once SEM micrographs have been studied and compared, then with practice these differences become apparent using light microscopy. Fungal species have been detected and also identified through SEM.

The present study thus reports fungal strain RF-03 as *A. flavus* which cause savior infections in groundnut seeds during storage. Biological methods are seems to be very useful and environmental friendly alternative to combat such fungal pathogens so as to prevent the use of pesticides/fungicides (Arora et al. 2018; Mishra et al. 2016). Many biocontrol agents are known which should be exploited and used for control of such seed deteriorating and harmful fungi (Verma et al. 2019; Mishra and Arora 2017;Tiwari and Arora 2018)

## IV. CONCLUSION

At the end of this study, the authors concluded that *A. flavus* is a very infectious storage fungi, which effectively causes infections in nutrient rich seeds during long term storage. Because of these lethal infections, groundnut seeds can't be properly developed. Moreover, their ability to wear oil becomes very less. However, prevention of groundnut seeds from mycobial contamination environmental friendly subways i.e. microbial metabolites should be used for better storage of groundnut seeds. Microbial metabolites are naturally according substances with no harmful effect to humans and animals as well. The use of metabolites for cure and control of fungal pathogens during storage seems to be very cheap and best alternatives of chemical based fungicides. Quality research is needed for proper storage treatments of oil seeds worldwide.

## V. ACKNOWLEDGEMENT

The authors of this paper are very thankful to Babasaheb Bhimrao Ambedkar, Central University, Lucknow for providing such research facility in the Department of Environmental Microbiology and Dr. Mukesh Kumar form USIC; moreover we are also very thankful to the university grant commission UGC for providing us financial support.

## VI. REFERENCES

[1] Pandey, M. K., Gautami, B., Jayakumar, T., Sriswathi, M., Upadhyaya, H. D., Gowda, M. V. C., & Varshney, R. K. (2012). Highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (Arachis hypogaea). Plant breeding, 131(1), 139-147.

[2] Arya, S. S., Salve, A. R., & Chauhan, S. (2016). Peanuts as functional food: a review. Journal of food science and technology, 53(1), 31-41.

[3] Adeyeye, A., & Ajewole, K. (1992). Chemical composition and fatty acid profiles of cereals in Nigeria. Food Chemistry, 44(1), 41-44.

[4] Sivananth, C., Amutha, S., Hemalatha, G., Mini, M. L., & Karthikeyan, G. (2017). Bioactive Components (Polyphenols, Flavonoids, Total Carotenoids, Antioxidant Activity) and Proximate Chemical Composition of Selected Food Processing Industry By-Products. Int. J. Curr. Microbiol. App. Sci, 6(7), 4126-4130.

[5] Li, R., Afsheen, S., Xin, Z., Han, X., & Lou, Y. (2013). OsNPR1 negatively regulates herbivore-induced JA and ethylene signaling and plant resistance to a chewing herbivore in rice. Physiologia plantarum, 147(3), 340-351.

[6] M. Shekhar, N. Singh, S. Bisht, V. Singh, A. Kumar, (2018)Biological Control of Aflatoxin in Maize Grain at Ambient Storage Conditions. Int.J.Curr.Microbiol. *App.Sci*; 7(4): 3031-3037.

[7] V. K. Vankayalapati (2018), Aflatoxins: Properties, Toxicity and Detoxification. Nutri Food Sci Int J.; 6(5): 555-696.

[8] Y. C. Chen, C. D. Liao, H. Y. Lin, L. C. Chiueh, and D. Y. C. Shih, "Survey of aflatoxin contamination in peanut proucts in Taiwan from 1997 to 2011," Journal of Food and Drug Analysis, vol. 21, no. 3, pp. 247–252, 2013.
[9] J. H. Williams, T. D. Phillips, P. Jolly, J. K. Styles, C. M. Jolly, and D. Aggarwal,(2004) "Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions," American Journal of Clinical Nutrition, vol. 80, no. 5, pp. 1106–1122.
[10] Romani L. (2004). Immunity to fungal infections. Nat. Rev. Immunol. 4 1–23. 10.1038/nri1255
[11] Smit, E., Leeflang, P., Glandorf, B., van Elsas, J. D., & Wernars, K. (1999). Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. Appl. Environ. Microbiol., 65(6), 2614-2621.
[12] Chen, R. S., Tsay, J. G., Huang, Y. F., & CHIOU, R. Y. Y. (2002). Polymerase chain reaction–mediated characterization of molds belonging to the Aspergillus flavus group and detection of Aspergillus parasiticus in Peanut Kernels by a multiplex polymerase chain reaction. Journal of Food Protection, 65(5), 840-844.
[13] Gupta, Y. N., & Chauhan, P. N. S. (1970). Effect of ultraviolet radiation on essential oils. Indian Perfumer, 14, 63-66.
[14] Lalithakumari, D., Govindaswamy, C. V., & Vidhyasekaran, P. (1971). Effect of seed-borne fungi on the physico-chemical properties of groundnut oil. Indian phytopathology.
[15] Bhattacharya, K., & Raha, S. (2002). Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. Mycopathologia, 155(3), 135-141.
[16] NK Arora, T. Fatina, I. Mishra, M. Verma, J. Mishra, V. Mishra, Environmental sustainability: challenges and viable solutions. Environmental Sustainability.1(4); 309-340.
[17] J Mishra, J Prakash, NK Arora,(2016) Role of Beneficial Soil Microbes in Sustainable Agriculture and Environmental Management. Climate Change and Environmental Sustainability.; 4(2):137-49.
[18] M. Verma, J. Mishra, N. Arora, (2019) Plant Growth-Promoting Rhizobacteria: Diversity and Applications. Book: Environmental Biotechnology: For Sustainable Future (Eds: Sobti RC, Arora NK, Kothari R.), 129-173.
[19] J. Mishra, N. Arora, (2017). Secondary metabolites of fluorescent pseudomonads in biocontrol of phytopathogens for sustainable agriculture. Mishra, J., Applied Soil Ecol, https://doi.org/10.1016/j.apsoil.2017.12.004
[20] S. Tiwari, N Arora, (2018) Role of salicylic acid from Pseudomonas aeruginosa PF23EPS+ in growth promotion of sunflower in saline soils infested with phytopathogen Macrophomina phaseolina. Environmental Sustainability.1(1): 49-59.