KAVAKA 61(1): 34-39 (2025) DOI: 10.36460/Kavaka/61/1/2025/34-39

Aspergillus insuetus: A New Addition to Indian Funga

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(Submitted on January 17, 2025; Accepted on January 29, 2025)

ABSTRACT

Extreme habitats provide unique conditions that foster the evolution of novel and specialized microbes including fungi. These microbes either in association or free-living exhibit a wide range of adaptations enabling them to survive in such challenging and harsh conditions. A study was conducted to explore the fungal diversity in the rhizospheric soil of Elaeagnus angustifolia L. growing wild in Kargil (Ladakh), India. Of the recovered fungal species, we hereby report a microfungi (*Aspergillus insuetus*) of rare distribution which was identified based on the cultural, micro-morphological and molecular characterization (nrITS). The current investigation represents the first attempt to isolate fungal species from the rhizospheric soil of E. angustifolia L. To the best of our knowledge, A. insuetus constitutes the first report to the Indian mycoflora showing a colonization frequency 6.6%..

Keywords: Rhizosphere, Taxonomy, Cold desert, High-altitude

INTRODUCTION

The soil ecosystem especially in extreme habitats constitutes a highly diverse environment and provides a rich habitat for a wide range of microbes including microalgae, bacteria, actinomycetes, fungi and protozoa (Rao, 1994; Adawiah, 2016). These microbes are vital to the soil ecosystem particularly those in the rhizosphere which is a zone of intense microbial activity and offers a unique niche for microbial colonization. Moreover, it is a complex ecosystem and acts as an interface for plant-microbe communication (Bukhat *et al.,* 2020). The high microbial growth and proliferation in this area is due to the abundance of resources available including amino acids and sugars which act as sources of nitrogen and carbon. These nutrients are provided by sloughed-off root cells and mucilage root exudates (Shrivastava, 2015).

Of the diverse organisms inhabiting the rhizosphere, fungi exhibit a close association with the roots of most plants and numerous microfungi have been isolated from various extreme habitats, such as, deep-sea basins, arid rocks, hypersaline water, low pH water, and in hot deserts (Sonjak *et al.,* 2006). Similarly, various cold-tolerant fungal species have been reported from extremely cold habitats, such as sub-arctic habitats, snow-covered tundra, permafrost, and a few from arctic regions (Babjeva and Reshetova 1998; Tosil *et al.,* 2002; Sonjak *et al.,* 2006). There are many reports of various species of *Aspergillus* that have been commonly reported from cold habitats (Cantrell *et al.,* 2011; Nonzom and Sumbali, 2015, 2021a,b).

Ladakh, an interesting place with rugged topography, bordered by Karakoram Mountain ranges in the North and the Himalayas in the South is popularly known as the land of passes. The temperature of Ladakh ranges between 27°C in summer to –45°C in winter. These climatic conditions and its geography make it an interesting place to study the diversity of microbes occupying the rhizospheric soil of the native plants growing in such cold conditions. As discussed, there are few reports on the exploration of fungal diversity in this area (Deshmukh *et al.,* 2010; Sing, 2013; Nonzom and Sumbali, 2015; Hussain *et al.,* 2024). However, no studies on the microbial entities inhabiting the rhizospheric soil of the plants growing in the study area have been carried out so far. Therefore, the present study was undertaken to explore the fungal diversity prevailing in the rhizosphere of *E. angustifolia* L.

MATERIALS AND METHODS

Study area and collection of samples

The rhizospheric soil samples were collected from the Kargil district (34°22'08" N and 76°24'31" E; 3,497 masl) of Ladakh (UT), India. The samples were collected from up to 15 cm of rhizospheric soil of the selected plant, *Elaeagnus angustifolia* L. with the help of a sterilized spatula. After

collection, the rhizospheric soil samples were kept in pre-sterilized polythene bags and brought to the laboratory for further mycological examinations.

Isolation of fungi

The collected soil samples were firstly sieved and serial dilution along with pour plate technique was used for the isolation of the fungal species. In this method, modified Czapek's Dox agar (CDA) medium supplemented with streptomycin (250 mg/L) and Rose Bengal (0.1 mg/100 ml) was used. After this, the Petri plates were kept in the incubator for 2-5 days at 28 ± 2°C to examine the growth of different fungal colonies on the growth medium. The individual colonies were purified by streaking them on potato dextrose agar-plated Petri plates. These purified fungal cultures were then transferred to the PDA slants and stored at 4˚C for future use.

Mycological Examination

The fungal isolates were identified based on cultural, morphological, and molecular characterization using nrITS sequencing.

1. **Cultural Characteristics:** To study the cultural details, the fungal isolate (RZS-208) was grown on two different agar media, Potato dextrose agar (PDA) and Malt extract agar (MEA). The colony characteristics, such as size, texture, diameter, growth pattern, pigments above and reverse, and exudates, if any, were examined.
2. **Micro-morphological Characteristics:** The detailed micro-morphological characteristics, such as hyphae-pigmented/hyaline, conidiophore-types, characteristics, number of metulae, phialides, etc. and conidia-size, ornamentations if any, were studied under the compound microscope. The morphological analysis was performed with the help of two compound light microscopes, Magnus, India and Leica DM2000 LED, Germany. Camera lucida drawings were drawn with the help of camera lucida attached near the eyepiece of the compound microscope and rotary pens (Artline) with line thickness varying from 0.05-0.5mm.
3. **Molecular characterization or ITS sequencing:** It includes DNA extraction, PCR amplification, sequencing and phylogeny analysis. The nuclear DNA was isolated by using the standard chloroform/phenol extraction method given by Sambrook *et al.* (1989) followed by PCR (polymerase chain reaction) amplification of the ITS regions using two universal primers, ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The resultant PCR product was further purified by PEG-NaCl precipitation and sequencing was done on ABI® 3730XL robotic DNA sequencer (Applied Biosystems, Inc., USA) following manufacturer's orders. The sequencing was performed from both ends to ensure reading each position twice. The assembly was carried out by using Lasergene package followed by NCBI blast. The phylogenetic trees were constructed using the maximum-likelihood method and Tamura–Nei model (Tamura *et al.,* 2021) in the MEGA XI software **(Figure 1).** The consensus sequences of the identified species were deposited at the National Centre for Microbial Resource (NCMR), with GenBank accession number PP626205 (*Aspergillus insuetus,* RZS-208).

RESULTS AND DISCUSSION

Colonization frequency and colony forming units

Out of total 50 isolation plates of rhizospheric soil fungi, 420 fungal colonies were recovered. Of these, only single isolate of RZS-208 with a colonization frequency 6.6% was retrieved. In addition, RZS-208 showed 0.1×10^5 colony forming units. Based on cultural, morphological and molecular characterization, the fungal species RZS-208 was identified as *Aspergillus insuetus* (Bainier) Thom & Church.

Taxonomy

Aspergillus insuetus (Bainier) Thom & Church

Habitat- rhizospheric soil of *E. angustifolia*

Distribution- Canada, Portugal, Italy, New York, USA, South Africa, China, Israel, Spain, South Korea.

Morphological Characterization

Cultural Characteristics- On PDA, purplish grey to black upon sporulation, margin floccose, reverse olive yellow, brown exudates present, diameter 30-

35mm after 5 days of incubation at 28˚C **(Figure 2a).** On MEA, greyish at the centre, with off white periphery, floccose margin, reverse yellowish brown, no exudates, 28-30mm diameter after 5 days of incubation at 28˚C **(Figure 2b).**

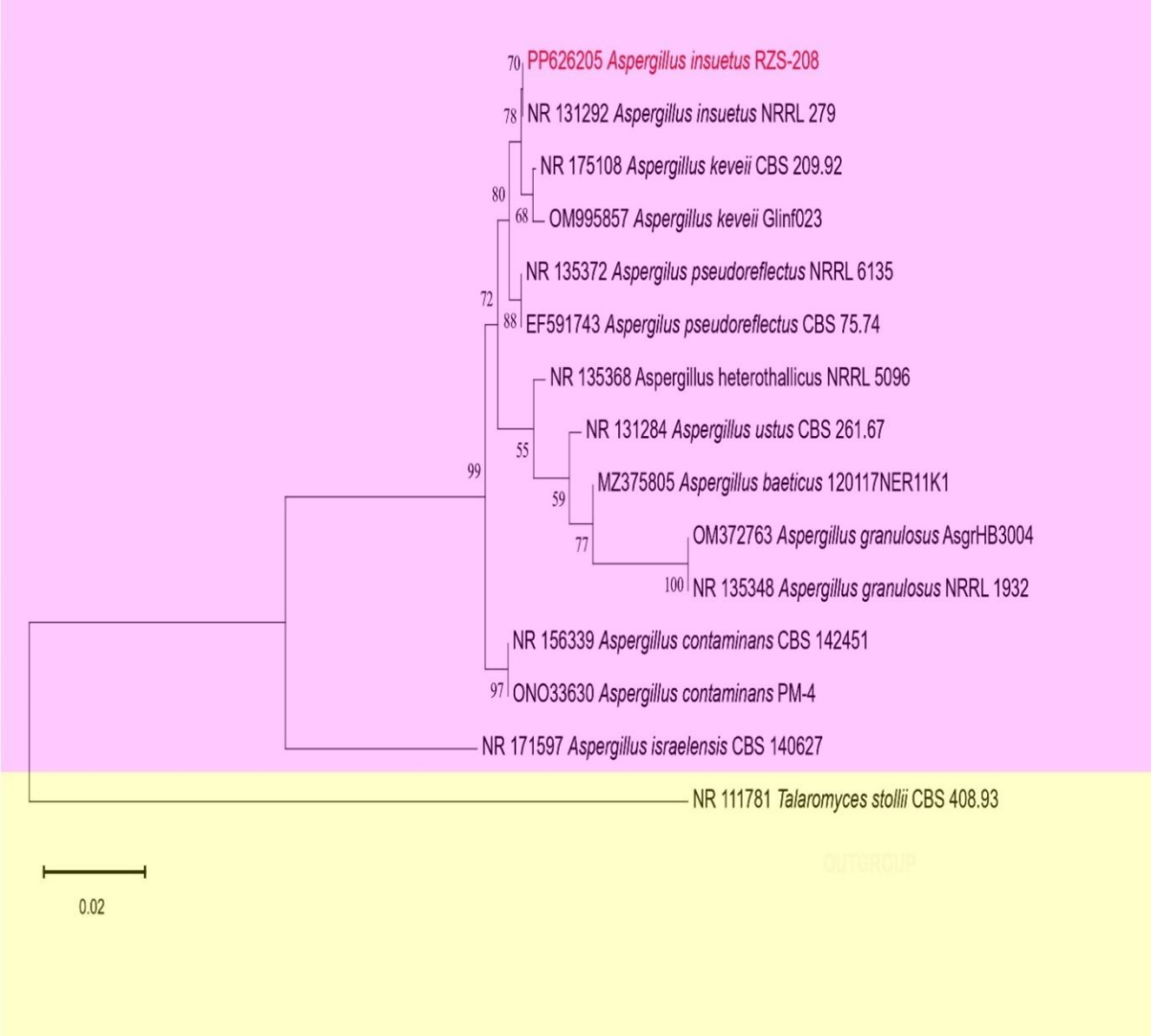

Figure 1: Phylogenetic tree inferred from maximum likelihood analysis based on ITS sequences from nrDNA of RZS-208.

Micromorphology

Mycelium; septate, branched, hyaline, with smooth-walled hyphae. Conidial head-radiate to hemispherical and biseriate; Conidiophore-smooth, pigmented, aseptate, 200-416×6.4-8µm in size; vesicle-globose to sub-globose, 8-11.2µm in diameter; metulae-hyaline, 3.2-4×1.6-3.2 µm in size; phialides-smooth, ampulliform, 6.4-8×1.6µm in size **(Figure 2c-d)**; conidia-pigmented, rough, globose, yellowish-brown **(Figure 2e)**. Hulle cells, not formed.

Aspergillus insuetus was first described as *Sterigmatocystis insueta* Bainieris (basionym) in the year 1908 and later was rediscovered as *A. insuetus* (Thom and Chuch, 1926). After some years, based on some morphological characters, it was considered as a synonym of *A. ustus* (Raper and Fennell, 1965). However, in 2007, based on various phenotypic characters, *A. insuetus* was again revived and studied in detail by Houbraken who marked it as a separate valid species (Houbraken *et al.,* 2007).

The current isolate *A. insuetus* RZS-208 recovered from the rhizospheric soil, has earlier been found to be associated with various media, such as, air (Canada), marine sponge *Petrosia ficiformis* (Spain), a Mediterranean sponge *Psammocinia* (Israel), epiphytic algae of a marine plant (Italy), sap beetles (New York), rhizospheric soil (South

Korea), grass (South Africa), deep sea sediments (China) and soil (Portugal) (Slack *et al.,* 2009; Lopez-Gresa *et al.,* 2009; Cohen *et al.,* 2011; DiGirolomo *et al.,* 2020; Visagie and Houbraken, 2020; Chi *et al.,* 2021; Lee *et al.,* 2021; Fernandes *et al.,* 2024).

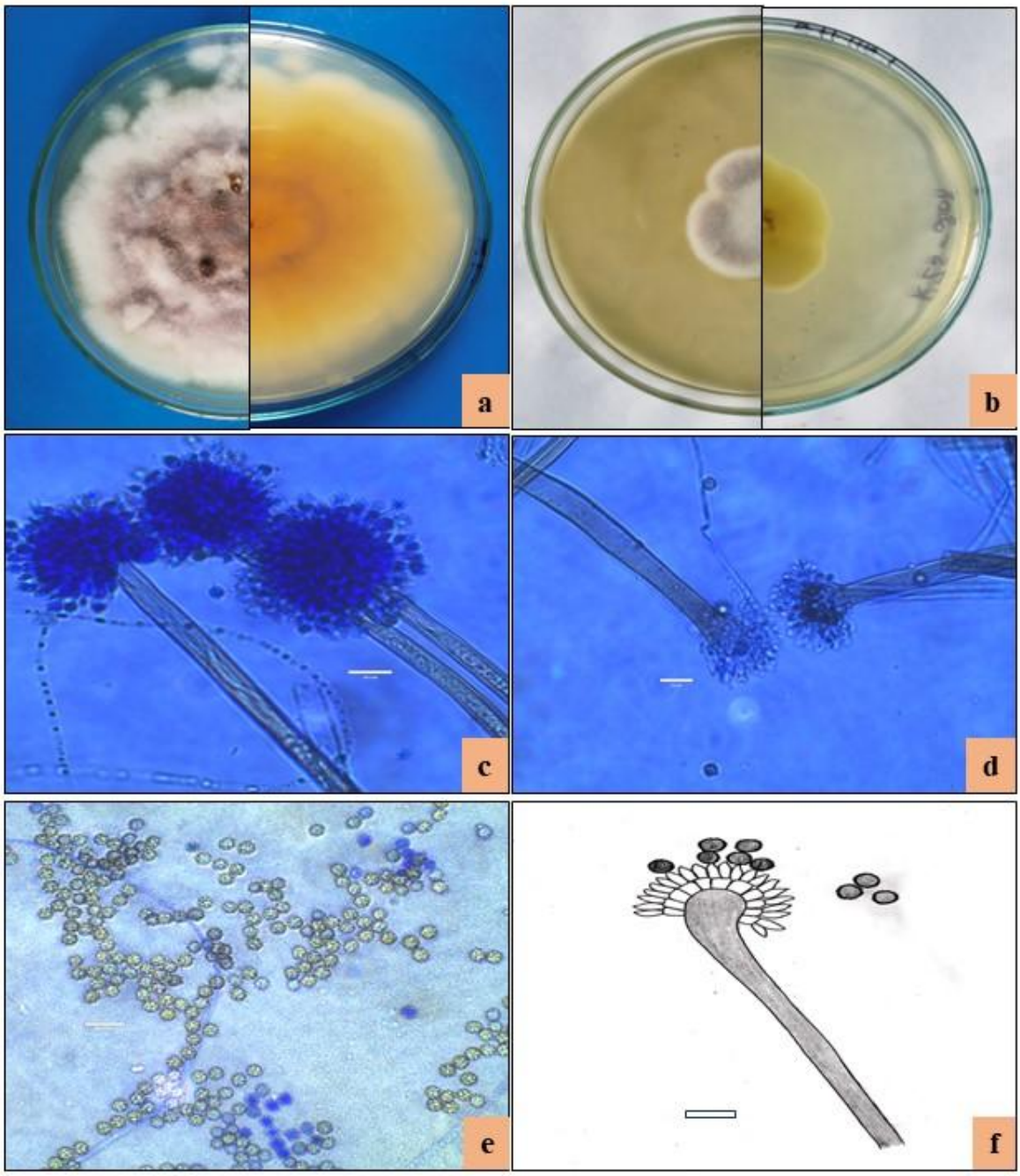

Figure 2: *Aspergillus insuetus* a, Colonies on PDA (front; reverse); b, Colony on MEA (front-reverse); c-d, Conidiophores with attached phialides and conidia; e, pigmented conidia; f, Camera lucida drawings; (Bars: c-f-10µm).

Few mycological studies that have been carried have reported the dominance of *Aspergillus* and *Penicillium* in these cold arid regions (Deshmukh *et al.,* 2010; Kotwal and Sumbali 2014; Nonzom and Sumbali, 2015, 2021 a,b). This dominance could be attributed to the fact that in these genera, conidia are produced in large numbers and possess a greater ability to survive for several decades in cold and dry environments (Robinson, 2001). Additionally, the genus *Aspergillus* is cosmopolitan in distribution and is frequently found growing on refrigerated food items, demonstrating its

exceptional tolerance to low temperatures. For instance, a study conducted at a high-altitude cold arid pass (4000 msl) reported the rare occurrence of another *Aspergillus* species (*A. aeneus*), highlighting its capacity to thrive in stressed habitats (Nonzom and Sumbali, 2021a). In the present investigation, *A. insuetus* RZS-208 was isolated from the rhizospheric soil of *Elaeagnus angustifolia* L., suggesting its potential role in plant-soil interactions. This isolate may exhibit diverse adaptive mechanisms, such as the production of secondary metabolites, exudates, and enzymes with enhanced catalytic activities, which enable it to flourish in such extreme environments.

CONCLUSION

This study represents the first report of *Aspergillus insuetus* isolated from the rhizospheric soil of *Elaeagnus angustifolia* in the cold arid region of Kargil, Ladakh, India. The identification of this species in such an extreme habitat highlights its ecological adaptability and potential functional role in plant-soil interactions. The ability of *A. insuetus* to survive and thrive under harsh environmental conditions may be attributed to its production of secondary metabolites, exudates, and enzymes with significant catalytic activity. This finding not only contributes to the expanding knowledge of fungal diversity in cold arid regions but also provides a foundation for exploring the ecological, agricultural, and biotechnological potential of the fungal isolates in stress-prone ecosystems.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Head, Department of Botany, University of Jammu, for providing laboratory facilities. The first author is thankful to the University Grants Commission (UGC), India for providing fellowship (SRF) to the first author.

REFERENCES

Adawiah, P.R. 2016. Isolasi dan Identifikasi Cendawan Indigenous Rhizosfer Tanaman Kentang (*Solanum tuberosum* L.) di Buluballea Kelurahan Pattappang Kecamatan Tinggi Moncong, Kabupaten Gowa. [Thesis]. UIN Alauddin, Makassar [Indonesian].

Babjeva, I. and Reshetova, I. 1998. Yeast resources in natural habitats at polar circle latitude. *Food Technology and Biotechnology*, 36:1–5.

Bukhat, S., Imran, A., Javaid, S., *et al.,* 2020. Communication of plants with microbial world: Exploring the regulatory networks for PGPR mediated defense signaling. *Microbiological Research,* 238:126486; doi: 10.1016/j.micres.2020.126486.

Cantrell, S.A., Dianese, J.C., Fell, J., *et al.,* 2011. Unusual fungal niches. *Mycologia,* 103:1161-1174; doi: 10.3852/11-108.

Chi, L.P., Li, X.M., Wan, Y.P., *et al.,* 2021. Two new phenol derivatives from the cold seep-derived fungus *Aspergillus insuetus* SD-512. *Chemistry & Biodiversity,* 18:e2100512; doi: 0.1002/cbdv.202100512.

Cohen, E., Koch, L., Thu, K.M., *et al.,* 2011. Novel terpenoids of the fungus *Aspergillus insuetus* isolated from the Mediterranean sponge *Psammocinia* sp. collected along the coast of Israel. *Bioorganic & Medicinal Chemistry,* 19:6587-93. doi: 10.1016/j.bmc.2011 .05.045.

Deshmukh, S. K., Verekar, S. A., and Shrivastav, A. 2010. The occurrence of keratinophilic fungi in selected soils of Ladakh (India). *Natural Science*, 2:1247; doi: 10.4236/ns.2010.211151.

DiGirolomo, M.F., Munck, I.A., Dodds, K.J., *et al.,* 2020. Sap beetles (Coleoptera: Nitidulidae) in oak forests of two northeastern states: A comparison of trapping methods and monitoring for phoretic fungi. *Journal of Economic Entomology,* 113:2758-71; doi: 10.1093/jee/toaa195.

Fernandes, M., Salvador A.F. and Vicente, A.A. 2024. Biodegradation of PHB/PBAT films and isolation of novel PBAT biodegraders from soil microbiomes. *Chemosphere,* 362:142696; doi: 10.1016/j.chemosphere. 2024.142696.

Houbraken, J., Due, M., Varga, J., *et al.,* 2007. Polyphasic taxonomy of *Aspergillus* section Usti. *Studies in Mycology,* 59:107-28. doi: 10.3114/sim.2007.59.12.

Hussain, M., Kiran, S., Sayed, I., *et al.,* 2024. Altitude-dependent influence of *Artemisia brevifolia* on its rhizosphere microbiome in Ladakh region of the Western Himalayas. *Rhizosphere*, 100918. doi: 10.1016/j.rhisph.2024.100918.

Kotwal, S. and Sumbali, G. 2014. Comparative analysis of keratinophilic fungi from the soils of Khardung and Khardung La (Ladakh), India. *Biolife,* 2:1326-1331; doi:10.5281/zenodo.7238636.

Lee, J.W., Kim, S.H., You, Y.H., *et al.,* 2021. Four unrecorded *Aspergillus* species from the rhizosphere soil in South Korea. *Mycobiology,* 49:346-354; doi: 10.1080/12298093.2021.1944461.

Lopez-Gresa, M.P., Cabedo, N., González-Mas, M.C., *et al.,* 2009. Terretonins E and F, inhibitors of the mitochondrial respiratory chain from the marine-derived fungus *Aspergillus insuetus*. *Journal of Natural Products,* 72:1348-51; doi: 10.1021/np900085n.

Nonzom, S. and Sumbali, G. 2015. Incidence and diversity spectrum of mitosporic fungi from the cold arid base soil of Moonland landscape (Ladakh), India. *International Journal of Pharma and Bio Sciences,* 6:B252-B261.

Nonzom, S. and Sumbali, G. 2021a. *Aspergillus aeneus* rediscovered in India from an extreme habitat. *Current Science,* 120:514-518; doi: 10.18520/cs/v120/i3/514-518.

Nonzom, S. and Sumbali, G. 2021b. Dominance of monoverticillate penicilli in the cold arid soils of Drass valley, India. *Studies in Fungi*, *6*:378-390; doi: 10.5943/sif/6/1/28.

Rao, N.S. 1994. Mikroorganism Tanah dan Pertumbuhan Tanaman. Edisikedua. Jakarta: Penerbit Universitas Indonesia.

Raper, K.B. and Fennell, D.I. 1965. *The genus Aspergillus.* Baltimore*:* Williams & Wilkins.

Robinson, C.H. 2001. Cold adaptation in Arctic and Antarctic fungi. *New Phytologist*, 151:341-353; doi:10.1046/j.1469-8137.2001.00177.x.

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular cloning. A laboratory manual. – Cold Spring Harbor Laboratory Press, New York.

Shrivastava, U.P. 2015. Plant microbe interaction in rhizosphere. *International Journal of Graduate Research and Review,* 1:10-24.

Sing, H.V. 2013. Soil carbon sequestration and rhizospheric microbial population in apricot orchards following plastic film mulching under cold arid region. *International Journal of Horticulture*, 3:35-41; doi: 10.5376/ijh. 2013.03.0008.

Slack, G.J., Puniani, E., Frisvad, J.C., *et al.,* 2009. Secondary metabolites from *Eurotium* species, *Aspergillus calidoustus* and *A. insuetus* common in Canadian homes with a review of their chemistry and biological activities. *Mycological Research,* 113:480-90; doi: 10.1016/j.mycres.2008.12.002.

Sonjak, S., Frisvad, J.C. and Gunde-Cimerman, N. 2006. *Penicillium* mycobiota in Arctic subglacial ice. *Microbial Ecology*, 52:207-216; doi: 10.1007/s00248-006-9086-0.

Tamura, K., Stecher, G. and Kumar, S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. – *Molecular Biology and Evolution*, 38:3022–3027; doi: https://doi.org/10.1093/molbev/msab120.

Thom, C. and Church, M.B. 1926. The *Aspergilli*. Baltimore: Williams and Wilkins.

Tosil, S., Casado, B., Gerdol, R., *et al.,* 2002. Fungi isolated from Antarctic mosses. Polar Biology, 25:262–268; doi: 10.1007/s00300-001-0337-8.

Visagie, C.M. and Houbraken, J. 2020. Updating the taxonomy of *Aspergillus* in South Africa. *Studies in Mycology,* 95:253-92; doi: 10.1016/j.simyco.2020.02.003..