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Unravelling the functional role of rhizospheric mycobiome associated with *Dactylorhiza hatagirea* (D. Don) Soo growing in the Kashmir Himalaya

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The rhizospheric mycobiome functions as a biological facilitator, providing a supportive vehicle for plant species to thrive in their habitats and significantly bolstering their chances of survival. Thus, the functional role with respect plant growth-promoting and enzymatic activities of rhizospheric fungi associated with *Dactylorhiza hatagirea,* need to be explored in detail. The isolated fungal species were evaluated and estimated for a range of activities like enhancement in plant growth, IAA synthesis, phosphate solubilization, and ammonia production. Among the isolated fungal isolates, ~70% of the isolates possessed one or more of these plant growth-promoting capabilities. In addition, the fungal isolates were screened for different enzymatic activities such as amylase, cellulase and pectinase activity and ~80% isolates depicted one or the other enzymatic activity. These fungi produced and released enzymes which contributed to changing nutrient levels in the rhizosphere there by playing a major role in breaking down carbon substrates and organic nutrient forms such as Nitrogen, Phosphorus and Sulphur. The results revealed that rhizospheric fungal species likely play a key role for their survival, fitness and establishment of *D.hatagirea*(a medicinally important orchid) in habitats away from their natural habitat and pave for its conservation in the Kashmir Himalaya.

Keywords : Enzymatic activity, fungi, plant growth promoting activity, rhizosphere

INTRODUCTION

The rhizosphere is defined as the “bio-influenced zone,” signifying the spatial interface between a plant, microorganisms and their surrounding environment. This designation highlights the dynamic interplay of biological factors in this region. Described as a “hot spot of activity,” the rhizosphere functions as a pivotal relationship for diverse biogeochemical processes operating within soil matrices. Its significance lies in its role as a critical bottleneck regulating the supply of essential elements necessary for the maintenance of ecosystem productivity and integrity (Hinsinger *et al.* 2008).The rhizospheric fungal community serves various crucial functions in the lifecycle of a host plant species, impacting processes such as seed germination (Moussa *et al.* 2023) and promoting plant growth (Qu *et al.,* 2020) by modulating by the host’s nutrient status(Cai *et al.* 2017) and physiology (Ali *et al.* 2017).

The mycobiome within the rhizosphere additionally enhances the health and ability of plants to withstand environmental pressures by regulating the synthesis of hormones and biologically active secondary compounds. (Pozo *et al.* 2021; Afrid *et al.*2022).

Considering the extensively documented significance of fungal communities in the soil surrounding plant roots for plant growth and development. The rhizospheric mycobiome may be crucial in the establishment and plant fitness of *Dactylorhiza hatagirea.* In this context, we investigated the diversity of culturable rhizospheric fungal species associated with the *Dactylorhiza hatagirea*. *Dactylorhiza hatagirea*,

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also known as Himalayan Marsh Orchid or "Salem Panja," is a perennial herb with an upright stem that can grow up to 40 cm tall. It is a member of the Orchidaceae family and is commonly found in marshy areas in the Himalayan region. The nickname "Himalayan Marsh Orchid" accurately reflects its favourite living spot (Kumari *et al.* 2022; Sharma *et al.* 2023) However, there remains a significant gap in understanding the role and function of fungal isolates within the rhizosphere of *Dactylorhiza hatagirea*. To address this gap, culturable fungal species associated with *Dactylorhiza hatagirea* across different sites in the Kashmir Himalaya region were documented and subjected to analysis for different functions that enhance plant growth like synthesizing IAA (Indole-3-acetic acid), solubilizing phosphate, and assimilating ammonia. In addition, the fungal isolates were screened for their enzymatic activity.

MATERIALS AND METHODS

Collection of rhizospheric soil samples

Soil samples were obtained from the rhizosphere zone of *Dactylorhiza hatagirea*. growing at different sites in Kashmir Himalaya, specifically Pahalgam, Gulmarg, and Sonamarg. The *Dactylorhiza hatagirea* plants were carefully uprooted to collect the soil adhering to their roots, and the sampling depth was 0-40cm. To ensure the samples' integrity, composite soil samples were created by combining the collected soil from each site. These composite samples were then stored in sterilized polythene bags and taken to the Plant Pathology and Mycology Laboratory, Department of Botany, University of Kashmir. To maintain the samples freshness and prevent any potential contamination, the soil-filled polythene bags were stored in a refrigerator at a temperature of 4±1°C until they were processed for further observations and analysis.

Isolation of rhizospheric soil fungi

The soil dilution plate method, was employed to isolate fungi from soil samples. The process involved creating soil dilutions by suspending 1g of soil from each sample in 10ml of sterile distilled water. From this, other dilutions ranging from 10^{-1} to 10^{-6} were prepared. To carry out the isolation, 1ml of each diluted soil suspension was added to separate sterile Petri dishes that contained Potato Dextrose Agar medium, which had been sterilized and supplemented with a 1% streptomycin solution. The Petri dishes were then incubated at a constant temperature of 25±2°C for a period of 4-7 days. To ensure accuracy and reliability, each treatment was replicated three times. The fungi present in the soil samples were successfully isolated and identified. As a result of the dilutions, surface colonies of fungi were formed and well dispersed, particularly at the higher dilution levels. This facilitated the identification and study of individual fungal species without overcrowding, allowing for a more effective analysis of the fungal population in the soil samples.

Screening for Plant Growth Promoting activities

All the isolated fungi were screened for different plant growth promoting activities as follows:

IAA production

The production of IAA was assessed following the procedures outlined by Patten and Glick (1996). Fungi were cultured in PD broth supplemented with L-tryptophan (100 ìg ml^{-1}) for four days. After incubation, the cultures were centrifuged at 10,000g for 10 minutes, and supernatants were collected. One millilitre of the culture filtrate was mixed with 2 ml of Salkowsky's reagent, which consists of 1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$ and incubated at 28 ± 2 °C for 30 minutes. The presence of IAA was determined by the development of a pink colour. Absorbance was measured at 530 nm using a SkanIt multimode reader for quantification of IAA. The experiment was repeated three times, and the values were expressed as the mean. To measure the concentration of IAA (ìg ml^{-1}) in the culture filtrate, a standard curve was created. This involved measuring the optical density of standard IAA (Sigma-Aldrich, United States) with known concentrations ranging from 10 to 100 µg/ml.

Phosphate solubilisation

Phosphate solubilization by fungi was evaluated following the method outlined by Pikovskaya

(1948) using Pikovskaya agar medium. Fungal colonies at the logarithmic phase (5 days old) were centrally inoculated on culture plates and then incubated at 28°C for 7 days. The presence of a distinct clear zone surrounding the colonies indicates phosphate solubilization. Each fungal strain was tested twice. The positive microbial cultures showing phosphate solubilization were further quantitatively assessed. The isolates were cultured in Pikovskaya's broth (HiMedia, India) and incubated at 28°C in a shaker incubator for 7 days. After centrifugation at 13,000 g for 10 minutes, 100 µl of the supernatant was added in triplicate to 96-well microplates. Un-inoculated sterile media were used as the negative control. Barton's reagent, composed of 2.5% ammonium molybdate, 0.125% ammonium metavanadate, and 250 ml concentrated HNO3, was added to the supernatant at a 5:1 ratio (Pande *et al.,* 2017). After 10 minutes, the optical density was measured at 430 nm using a SkanIt multimode reader, and the ability to solubilize phosphate was determined from a standard curve. This curve was constructed by measuring the optical density of solutions with known concentrations of $KH2PO_4$ (ranging from 10 to 100 µg ml^{-1}) at 430 nm and plotting it against the concentration. The experiments were performed in triplicate, and the results were reported as mean values.

Ammonia production

The assessment of Ammonia production followed the methodology described by Singh *et al.* (2014). Each fungal isolate was cultured in 5 ml of peptone water for 72 hours at 30°C with agitation in a shaking incubator (150 rpm). After 7 days of incubation at 28°C, the cultures were centrifuged at 10,000 rpm for 10 minutes (Singh *et al.,* 2014). To the resulting supernatant, Nessler's reagent (composed of 7% KI, 10% HgI_2, and a 50% aqueous solution of NaOH at 32%) was added in a 1:2 ratio, resulting in the development of a yellow to brown colour. The intensity of the colour reflected the concentration of ammonia present. The optical density (OD) was then measured at 450 nm. To determine the ammonia production by each isolate, a standard curve was created using a known concentration range of ammonium chloride solution (0.5 to 4 mmol/ml) from Thermo Fisher Scientific, United States.

Screening for enzymatic activities

Amylase activity

The test of Amylase production was carried by following the protocol of (Sunita *et al.,* 2012). The fungal culture, cultivated on Potato Dextrose Agar (PDA), underwent a meticulous process wherein it was sectioned into 5 mm discs using a borer. These discs were then strategically positioned on Petri plates filled with auto claved glucose yeast extract peptone agar (GYP) medium, comprising specified quantities of glucose, yeast extract, peptone, agar, and distilled water, enriched further with 0.2% soluble starch and maintained at a pH of 6.0. Following an incubation period, during which the fungal culture thrived under conducive conditions, the assessment of amylase activity was conducted. To achieve this, the Petri plates were inundated with a solution containing 1% iodine and 2% Potassium iodide. The subsequent reaction with starch led to the formation of a dark blue complex, facilitating the measurement of a distinct zone of clearance encircling the fungal colony. This zone's size served as a quantitative indicator of the amylase activity, allowing for a comprehensive evaluation of the fungal culture's enzymatic prowess in starch hydrolysis. The precision of this method ensures a detailed characterization of the amylolytic potential of the endophytic fungal strain, providing valuable insights into its enzymatic capabilities.

Cellulase activity

The study evaluated the cellulase-producing capabilities of a purified fungi isolate by cultivating it in Carboxymethyl Cellulose (CMC) media (Wisdawati *et al.* 2021). The composition of the CMC media agar included 2 g of $NaNO_3$, 1 g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.5 g of KCl, 2 grams of CMC, 0.2 g of peptone, and 17 g of agar. The fungi isolates were allowed to grow on this medium for a period of 3 days. After the incubation period, an iodine solution was applied to the media. The iodine solution was made by dissolving 2.0 grams of KI and 1.0 gram of iodine in 300 ml of aquadest. The media was treated with the iodine solution and left undisturbed for a duration of 3-5 minutes. The ability to produce

cellulase enzyme was determined by observing the presence of a distinct clear zone surrounding the colony (Kasana *et al.* 2012). The cellulolytic index, which quantifies cellulolytic activity, was calculated by comparing the size of the clear zone with the diameter of the fungal colony. This calculation was conducted after 72 hours of incubation. In essence, this screening process aimed to identify and measure the fungi isolate's potential for cellulase production based on the clear zone formation around the colonies.

Pectinase activity

The assessment of pectinase activity of the isolates was conducted using the plate assay method as described by Kabir and Tasmin(2019). The organisms were inoculated on a modified MS medium (Gerhardt *et al.*1994), which consisted of 0.3% KH^2PO^4, 0.2% NH4Cl,0.6% Na_2HPO_4,0.01% $MgSO_4$, 0.5% NaCl, $7H_2O$, 1.5% agar, and 0.2% pectin. The pectin containing MS medium was then incubated at 30ÚC for 48 hours. To determine pectin utilization, the culture plates were flooded with a freshly prepared Iodine-Potassium Iodide solution (Hanking *et al.* 1971). The presence of a translucent halo zone indicated pectin depolymerization. We observed pectinase activity (known as the Zone of pectinolysis) as a clear zone around the bacterial growth. To quantify the enzyme activity, we measured the diameter of the clear zone in mm around each colony. We carefully recorded these clear zone measurements for each isolate for further analysis. This plate assay method was used to screen and measure the pectinase activity of the isolates based on the formation of clear zones around their colonies after incubation.

RESULTS

In this study, 17 fungal species were isolated from the rhizosphere of *Dactylorhiza hatagirea* to and their ability to promote plant growth and enzymatic characteristics were determined which include the production of indole-3-acetic acid (IAA), ammonia production, phosphate solubilization, amylase activity, cellulase activity and pectinase activity.

Indole acetic acid production

Out of the 17 fungal species tested for indole-3-acetic acid (IAA) production, 13 fungal species (76%) exhibited positive activity. These 13 fungal species produced IAA in varying quantities, extending from 17.4 to 42.51 ìg ml^{-1} Among them, *Aspergillus flavus* displayed the highest activity, producing 42.51 µg ml^{-1}, which was significantly different from all other cultures. Followed by *Fusarium solani* and *Penicillium verrucosum*, producing 36.5 µg ml^{-1} and 36.34 µg ml^{-1} respectively. Conversely, *Trichothecium roseum* showed the lowest concentration of auxin production at 17.47 µg $ml^{"1}$ (Fig. 1, Table. 1).

Phosphate solubilisation

Out of all the isolates examined, phosphate solubilization activity was observed in 12 isolates, accounting for 70.5% of the total. The quantitative analysis showed that P solubilisation activity was significantly different from one another and ranged from 5.5-12.69 µg ml^{-1} (Table 1). Among the fungal species, *Aspergillus flavus* showed the highest activity (12.69 µg ml^{-1}*), Alternaria alternata*(10.8 µg ml^{-1}), *Aspergillus candidus*(9.5 µg ml^{-1}*), Fusarium chlamodosporum*(5.5 µg ml^{-1}) showed the lowest activity followed by *Talaromyces falvus* (5.6 µg ml^{-1}) (Fig.2, Table 1).

Ammonia Production

Out of the 17 fungal species, 11 (64.7%) exhibited ammonia production activity, ranging from 1.49-2.96 mM ml^{-1}. Among the tested cultures, *Aspergillus flavus* showed highest ammonia production (3.9 mM ml^{-1}) followed by *Penicillium oxalicum*(2.67 mM ml^{-1}), *Fusarium solani*(2.6 mM ml^{-1}) and *Trichoderma asperellum*(2.5 mM ml^{-1}). The activity was lowest in *Trichothec-ium roseum*(1.49 mM ml^{-1}) and *Trichoderma harzianum*(1.65 mM ml^{-1}) (Fig. 3, Table2).

Amylase activity

Out of the 17 fungal cultures that were screened for amylolytic activity on solid media, 15 (88.2%) of them yielded positive results. These isolates were then categorized into three groups based on their amylolytic index. The isolates with amylolytic index of greater than 3 were categorised as strong amylase producers, while isolates with amylolytic index between 2 and 3, and less than 2 are categorised as medium and

Table. 1: *invitro* plant growth promotion and enzymatic activities shown by fungal isolates

Isolates	IAA production	Phosphate solubilisation	Ammonia production	Amylase activity	Cellulase activity	Pectinase activity
Alternaria alternata	31.94± 0.68	10.82±0.2	2.54±0.03	1.38±0.04	1.74±0.02	9.3±0.07
Aspergillus candidus	-	9.52±0.38	-	1.64±0.03	1.88±0.18	12.3±0.1
Aspergillus flavus	42.51± 0.46	12.69±0.41	2.96±0.03	2.1±0.1	1.34±0.15	14.4±0.057
Aspergillus niger	32.49± 0.23	6.66±0.28	1.96±0.0	2.67±0.11	1.95±0.075	8.7±0.057
Aspergillus sydowii	-	7.77±0.18	-	2.58±0.032	1.36±0.082	11.2±0.15
Cephalosporium acremonium	25.51± 0.27	9.47±0.39	1.88±0.01	1.98±0.03	2.2±0.1	6.76±0.05
Circinella muscae	25.57±0.42	-	-	2.9±0.1	1.32±0.03	5.77±0.07
Curvularia pallescens	34.64± 0.39	6.6±0.34	2.04±0.02	-	-	-
Fusarium chlamodosporum	-	5.52±0.33	-	2.87±0.03	1.04±0.13	15.3±0.05
Fusarium solani	36.5±0.27	-	2.6±0.1	2.25±0.13	1.4±0.02	10.7±0.1
Penicillium brevicompactum	28.46±0.4	9.43±0.11	2.5±0.036	3.73±0.01	-	12.3±0.10
Penicillium oxalicum	19.81± 0.18	-	2.67±0.02	3.28±0.06	1.49±0.045	5.53±0.05
Penicillium verrucosum	36.34± 0.15	6.86±0.32	-	3.72±0.05	1.67±0.01	-
Talaromyces falvus	-	5.62±0.14	-	-	-	11.4±0.05
Trichoderma asperellum	19.29± 0.12	-	2.5±0.025	2.92±0.12	-	7.22±0.1
Trichoderma harzianum	32.54± 0.31	5.64±0.22	1.65±0.02	3.18±0.07	1.27±0.06	3.22±0.02
Trichothecium roseum	17.47± 0.36	-	1.49±0.1	4.25±0.05	1.87±0.05	6.25±0.05

Data is the mean of three replicates (±sd).

weak amylase producers respectively. Among these, 5 isolates show strong amylase activity, 7 isolates show medium and 3 isolates show weak amylase activity. Overall, *Trichothecium roseum* with the amylolytic index of 4.25, showed highest amylolytic activity, followed by *Penicillium brevicompactum, Penicillium verrucosum*and *Penicillium oxalicum*with the amylolytic index of 3.73, 3.71, 3.28 respectively. However, *Alternaria alternata* and *Aspergillus candidus* demonstrated the lowest levels of amylolytic activity, with amylolytic indices of 1.37 and 1.64 respectively (Fig. 4, Table 1).

Cellulase activity

The assessment for cellulose production in fungal isolates involved cultivating them on a 1% Carboxy Methyl Cellulose (CMC) media. CMC, which is a cellulose derivative dissolved in water, was used as a substrate to stimulate cellulase enzyme production. The formation of a clear zone around the colony after adding iodine solution was used to identify cellulose-producing fungi. Among the 17 isolates tested, 13(76.4%) exhibited positive results, indicating their capability to produce cellulase enzymes, with a cellulolytic index (CI) ranging from 1.04 to 2.2. The isolates were divided into three categories based on their cellulolytic index. The isolates with CI of greater than 2 were categorised as strong cellulose producers, while isolates with CI between 1 and 2, and less than 1 are categorised as medium and weak cellulose producers respectively. Only 1 isolate *Cephalosporium acremonium* was found to be strong cellulose producer while the remaining isolates were medium cellulose producers (Fig. 5, Table. 1).

Pectinase activity

The ability of all 17 isolates to degrade pectin was examined using the plate assay method. A modified MS medium with 0.2% pectin as the sole carbon source was used. The clear zones on the plates were measured and recorded. Based on the extent of pectinolysis, the isolates were categorized as follows: very good pectinase

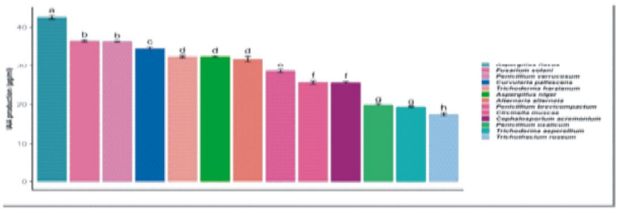

Fig. 1: Range of IAA production by different fungal isolates

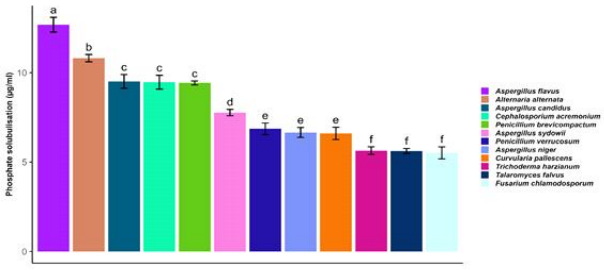

Fig 2: Range of phosphate solubilisation by different fungal isolates.

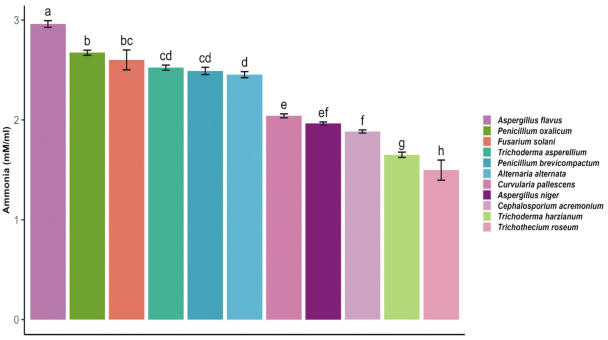

Fig. 3: Ammonia solubilisation by different fungal isolates

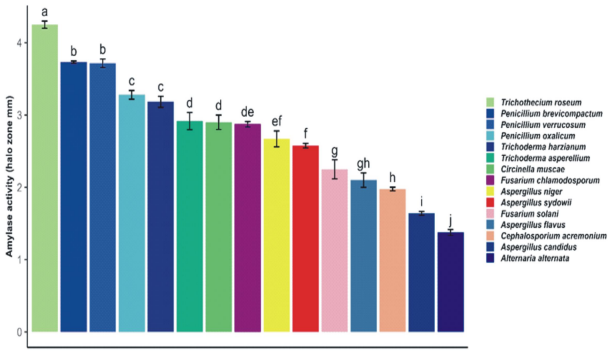

Fig. 4: Amylolytic activity by different isolated fungi.

producers (clear zone e" 15 mm), good pectinase producers (clear zone e" 10 mm), weak pectinase producers (clear zone e" 5 mm), and poor pectinase producers (clear zone d" 5 mm). Out of the 17 fungi isolates investigated, 15 (88%) were found to produce pectinase

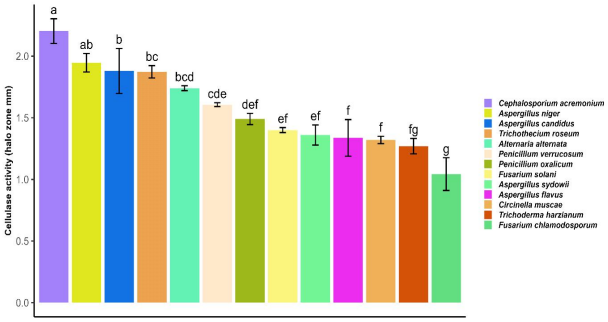

Fig. 5: Cellulolytic index by different isolated fungi

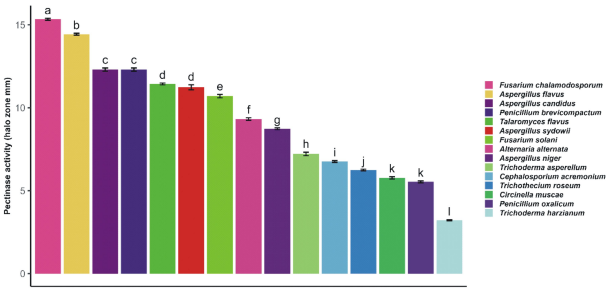

Fig. 6 : Pectinolytic activity (mm) produced by fungal isolates

enzymes. One isolate was classified as a very good pectinase producer, with a zone of pectinolysis measuring 15.33 mm. Six isolates were categorized as good producers, including *Fusarium solan, Aspergillus sydowii, Talaromyces flavus, Aspergillus candidus, Penicillium brevicompactum,* and *Aspergillus flavus.* Seven isolates were classified as weak pectinase producers, and 1 isolate was a poor pectinase producer (Fig. 6, Table1).

DISCUSSION

Rhizosphere fungi may also promote plant growth directly (Adedayo *et al.*2023). Fungal species have been reported to influence the phenotypic plasticity in certain plant traits and bolster their plant resilience to drought, heat, and salinity (Singh *et al.* 2011). Additionally, some fungi have been shown to affect plant pathogens (Busby *et al.* 2016a). This capacity to alter host plant traits, coupled with their widespread presence, diverse host range, and non-pathogenic characteristics of many fungal species, highlights their potential for mitigating stress in plants and enhance growth, while the precise mechanism through which fungal species enhance abiotic stress tolerance has been reported by (Tibpromma *et al.* 2017)

due to the production specific metabolites by many fungi like *Leucosporidium aff. golubevii, Cystobasidium laryngis, Sporidiobolus ruineniae, Rhodotorula mucilaginosa,* and *Cryptococcus victoriae* which boost plant (hosts) ability to withstand stressful conditions. similarly *Piriformospora indica* has been shown to enhance the salt tolerance of salt-sensitive barley cultivars by increasing the levels of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX). These enzymes are known to be associated with plant salt tolerance (Baltruschat *et al.,* 2008). Another study has demonstrated that fungal species can improve the drought and salinity tolerance of rice by adjusting the levels of abscisic acid, jasmonic acid, and salicylic acid (Tiwari *et al.* 2012). Fungal species have also been found to regulate the levels of salicylic acid, jasmonate, indole-3-acetate, and ABA in the shoot tissues of *Colobanthus quitensis*, thereby increasing its tolerance to UV-B radiation (Barrera *et al.,* 2020). These investigations confirm the significant role of fungal species in promoting plant growth and enhancing enzymatic activities.

Our study demonstrated that each of the culturable fungal species exhibited various plant growth-promoting and enzymatic activities. Thirteen fungal species showed auxin-producing capabilities, with indole-3-acetic acid (IAA) concentrations ranging from 17.4 to 42.51.2 µg ml^{-1}.Our findings are consistent with previous studies, although the IAA concentrations observed in our study exceeded those reported in other taxa (Radu *et al.,* 2021). Indole-3-acetic acid (IAA) is well-known for its effects on root development, lateral root growth, and root hair formation, which can enhance water and nutrient uptake, thereby promoting plant growth (Fouda *et al.* 2015**).** Recent investigations have highlighted the significant role of indole-3-acetic acid (IAA) in plant-pathogen interactions, including aspects of pathogenesis and defense mechanisms (Kumla *et al.* 2020). Therefore, it is likely that these fungal species may contribute to the fitness of *Dactylorhiza hatagirea* in the Kashmir Himalayan region. Additionally, 12 fungal species were found to exhibit phosphate solubilization activity. Quantitative analysis revealed that the solubilization activity varied significantly among the species, ranging from 5.5 to 12.69 µg ml^{-1}.Mycobial phosphate solubilization is a beneficial process for plant health, involving the conversion of insoluble inorganic phosphate, which is typically found in soil as crystalline calcium, aluminium, and iron phosphates, into a soluble form. Microorganisms achieve this by secreting organic acids, which lower the pH of the environment and facilitate the exchange of cations from insoluble phosphate salts to potassium or sodium, resulting in the formation of soluble phosphate salts. These soluble salts can then be easily absorbed by plants for growth and development (Raghavendra *et al.* 2015). Numerous studies have demonstrated that fungal species are capable of solubilizing inorganic phosphate within a similar range, enabling plants to access the soluble phosphate easily (Kumar *et al.* 2015). Furthermore, inoculating plants with phosphate-solubilizing fungal species has been shown to enhance the efficiency of phosphate uptake by plants and significantly improve their growth (Rawat *et al.* 2021). In the current study, another noteworthy plant-promoting function identified was ammonia production. It was observed that 11 fungal species exhibited ammonia production activity. This group of fungal species is recognized for augmenting the nitrogen supply to host plants through the hydrolysis of urea. However, the recorded values for ammonia production in this study were lower compared to those reported in certain earlier studies (Pertile *et al.* 2021). It is plausible that these ammonia-producing fungal species facilitate access to nitrogen for germinating plants, thereby promoting seedling growth. We also screened enzymatic activity of fungal species. The prevalent positive results for amylase activity,15among the screened fungal species highlight a widespread enzymatic capability, indicating a proclivity for starch hydrolysis. This suggests the presence of fungi equipped with the metabolic machinery to efficiently participate in starch degradation processes. Similarly, the majority of isolates exhibiting positive cellulase activity; 13 among the screened fungal species signified collective proficiency in cellulose degradation. This enzymatic attribute is biologically relevant in cellulose-rich environments, such as those containing plant cell walls, emphasizing the fungi's potential role in biomass degradation. Moreover,

the widespread demonstration of pectinase activity, 15 among the screened fungal species highlights the collective ability of these fungi to degrade pectin, a complex polysaccharide found in plant cell walls. This enzymatic competence is crucial for plant-associated interactions, indicating a shared enzymatic adaptation that enhances the biological versatility of these fungi.

CONCLUSION

The rhizospheric mycobiome of *Dactylorhiza hatagirea* in the Kashmir Himalaya was found to be diverse and about 17 different species of culturable fungi. These fungi were systematically evaluated and found to have significant plant growth-promoting activities and enzymatic properties. It is worth noting that 76% of the isolated fungal species showed positive production of the plant hormone indole acetic acid (IAA), with *Aspergillus flavus* demonstrating the highest IAA production. Furthermore, 70.5% of the isolates displayed phosphate solubilization activity, with *Aspergillus flavus* again featuring prominently in this regard. In terms of ammonia production, 64.7% of the fungal isolates exhibited activity, with *Aspergillus flavus* demonstrating the highest ammonia production. Enzymatic analyses revealed that 88.2% of the isolates exhibited positive amylase activity, and *Trichothec-iumroseum* emerged as the most potent producer. Additionally, 76.4% of the isolates demonstrated cellulase enzyme production, with *Cephalosporium acremonium* identified as a strong producer. Pectinase enzyme production was observed in 88% of the isolates, showcasing varied levels of activity. Overall, these findings highlight the multifaceted role of the rhizospheric mycobiome in facilitating plant growth through hormone production, nutrient solubilization, and enzymatic activities, providing comprehensive insights into their functional contributions in plant-microbe interactions and nutrient cycling. It is also imperative to document and study the role the non-culturable fungal diversity associated with rhizosphere of *Dactylorhiza hatagirea*.

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DECLARATION

Conflict of Interest. The authors declare no conflict of interest .

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