Mycobiology

Research Article

Mycoherbicidal Potential of *Phaeoacremonium italicum*, A New Pathogen of *Eichhornia crassipes* Infesting Harike Wetland, India

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Abstract Mycoherbicides are exclusive biotechnology products which offer a non-chemical solution to control noxious weeds on the land as well as aquatic in systems, viz a viz saving environment from hazardous impact of synthetic chemicals. The present paper highlights the mycobiota associated with *Eichhornia crassipes* infesting Harike wetland area of Punjab and evaluation of their pathogenic potential for futuristic application as a mycoherbicide. Of the 20 isolates tested by leaf detached assay and whole plant bioassays, only one isolate (#8 BJSSL) caused 100% damage to *E. crassipes*. Further, the culture filtrate of this isolate also exhibited a similar damage to the leaves in an *in vitro* detached leaf assay. The potential isolate was identified as *Phaeoacremonium italicum* using classical and modern molecular methods. This is the first report of *P. italicum* as a pathogen of *E. crassipes* and of its potential use as a biological control agent for the management of water hyacinth.

Keywords β-Tubulin, Fungi, Mycoherbicides, Plant pathogen, Ramasar site, Weeds

Weeds are attacked by fungi, bacteria and viruses. Fungi, predominantly are responsible for an array of weed diseases and thus possess potential to be developed as mycoherbicides/mycoweedicides based on their pathogenic potential. The main advantage of using mycoherbicides/mycoweedicides is reduction in use of chemical weedicides which pose a severe threat to human health and environment [1]. Fungi attacking weeds are diverse assemblage of species which markedly differ in their morphology, physiology, and pathogenicity.

Water hyacinth [*Eichhornia crassipes* Mart. Solms (Pontederiaceae)] is an invasive and noxious aquatic weed posing serious economic, social and environmental problems in India and other tropical and subtropical regions of the world [2]. *E. crassipes* currently holds the status of one of the world's worst aquatic weed [3, 4]. Water hyacinth continues to be a serious problem in Harike wetland, a Ramsar site created in 1953 by construction of a barrage at the confluence of rivers Sutlej and Beas in Punjab, India. Harike wetland is a premium water source for irrigation and drinking through its feeder canals in the states of Punjab and Rajasthan, India. Apart from this, it is also inhabited by a rare flora and fauna of which many have been designated as threatened species. It has been declared as a bird sanctuary since a huge concentration of migratory birds apart from 400 species of avifauna exists in the wetland area. Approximately, 40% of Harike is infested by water hyacinth, which leads to water loss due to excessive evapotranspiration and causing water borne diseases downstream. Hence, there is an urgent need to rejuvenate this water body by suppressing the growth of water hyacinth. Since, this surface water is not only a major drinking water source but also used for irrigation. The chemical methods of controlling water hyacinth are not recommended as they cause adverse effects and often lead to residual toxicity. Mechanical operations have been tried and are successful for short duration as resurgence of water hyacinth occurs.

Biological control using plant pathogens in recent years has proven to be a cost effective, environmentally safe solution to control weeds. Of the several fungal pathogens reported to attack water hyacinth, some prominent ones are *Acremonium zonatum*, *Cercospora piaropi*, *Myrothecium roridum*, *Rhizoctonia solani*, *Uredo eichhorniae*, *Helminthosporium/Bipolaris* spp.,

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and *Alternaria eichhorniae*. Controlled studies have indicated that *C. piaropi*, *Alternaria eichhorniae*, *Alternaria alternata*, and *Acremonium zonatum* induce biomass reduction during biocontrol of water hyacinth [5-8]. Thus, it is proven that these fungal pathogens could be successfully used as classical or innundative biological control agents for water hyacinth.

There exists very limited information on mycobiota associated with *E. crassipes* for prospective use as a mycoherbicide from India. The first report of *Alternaria eichhorniae* causing blight of water hyacinth was given by Nag Raj and Ponnappa [9], followed by *Alternaria alternata* (Fr.) Keissler from Haryana [10], *Alternaria alternata* from Tamil Nadu and Kerala [11], *Fusarium pallidoroseum* and *Myrothecium advena* Sacc. from Kerala [12].

As innundative biological control advocates the isolation and selection of natural enemy from the same geographical area where the weed is infesting, the present investigation was undertaken to explore the pathogenic mycoflora associated with *E. crassipes* infesting in Harike wetland for their possible use as mycoherbicides.

MATERIALS AND METHODS

Sample collection and isolation of pathogenic fungi. Infected plant parts (stolons and leaves) with necrotic spots were collected from the Harike wetland area, Taran Taran Sahib, Punjab, India in June 2011. The plant parts were kept in sterile bags, brought to the laboratory, and processed within 24 hr. Plant parts were washed under running tap water for 15 min to remove the surface debris. The infected portions were then dissected into 1 × 1 cm segments using a sterile blade. Infected plant parts were subsequently washed with 1% sodium hypochlorite for 1~2 min followed 30% ethanol for 1 min and then finally rinsed with sterile water thrice and air dried in laminar air flow. These surface sterilized plant segments were further cut into 2~4 mm segments and inoculated on potato dextrose agar (PDA) medium (initial pH 5.5; HiMedia, Mumbai, India) supplemented with streptomycin (1 mg/mL; HiMedia). Maximum of eight segments were inoculated per Petri dish and incubated at 26 ± 1°C for 7 days. The obtained fungal isolates were then sub-cultured over PDA plate as pure cultures and subsequently stored on PDA slants containing 15% glycerol [13]. These were tentatively identified using standard mycological keys given by Barnett and Hunter [14].

Frequency of fungal isolates. The fungal isolates obtained from the diseased water hyacinth located at different sites in Harike wetland were counted based on their frequency of occurrence. The percentage of occurrence was expressed using the formula:

Frequency (%) = Number of isolates of a genus × 100/Total number of isolates.

Inoculum production and pathogenicity test on detached leaves. For each of twenty fungal isolates, spores were harvested by flooding the 7-day-old colonies with sterile distilled water grown on PDA at 28 ± 2°C. Thereafter spore concentration was adjusted to 1×10^6 spores/mL using a hemocytometer and then mixed with 0.05% of Tween 20 (Sigma Aldrich, St. Louis, MO, USA). These were then sprayed over healthy *Eichhornia* leaves using a hand atomizer (Borosil, Mumbai, India) which were then placed in an aseptic moist chamber prepared in 90-mm Petri dishes (Tarsons, Kolkata, India) and incubated at 28 ± 2°C for 7 days under 12 hr of photoperiod (7350 lx). Leaves were visually examined every 24 hr and percentage of symptomatic area of the leaves were recorded. All the tests were performed in triplicates [15]. The disease progression in the *in vitro* detached leaf bioassay was established by determining the area under disease progressive curve (AUDPC) value of each isolate using the formula

$$AUDPC = \Sigma_i^{n-1}[(y_i + y_{i+2})_2/(t_{i+1} - t_i)]$$

, where 'y_i' is the assessment of the disease (percentage, proportion, ordinal score, etc.) at the ith observation, 't_i' is time (in days, hours, etc.) at the ith observation, and 'n' is the total number of observations. The isolate exhibiting maximum pathogenecity in detached leaf assay was selected for testing.

Pathogenicity tests on the whole plants. Four-to six-week old plants having 4~8 leaves were grown in tubs with illumination of 12 hr daily for 1 wk for acclimatization under laboratory conditions. These were then sprayed with fungal inoculum of a spore concentration to 1×10^6 spores/mL. Ten replicates were used in the experimental as well as in the control set. The control set received only sterile distilled water. Plants were observed daily for the disease severity according to Chiang *et al.* [16] until all plants died. The experiment was repeated thrice. The disease progression was determined by calculating the AUDPC values as mentioned in the previous section.

Production of the culture filtrate. The isolate, #8 BJSSL which exhibited highest pathogenicity in the *in vitro* detached leaf bioassay was grown in liquid culture to assess the phytotoxic potential of the culture filtrate. A 5-mm mycelial plug of the 7-day-old fungal culture was inoculated into 100 mL of pre-sterilized Richard's broth (HiMedia) in 250 mL Erlenmeyer flasks (Schott Duran, Mainz, Germany). These were incubated in an orbital shaker (Eppendrof, Chennai, India) at 28 ± 2°C, 120 rpm for 15 days. The mycelium was separated from the liquid medium initially with a sterile muslin cloth followed by Whatmann No. 4 filter paper and finally through 0.22 µm nitrocellulose membrane (Whatmann, GE health care and Life Sciences, Piscataway, NJ, USA) to make it cell free [17].

Detached leaf bioassay of the culture filtrates. Healthy leaves of water hyacinth was sprayed with cell free culture

filtrate (1 mL) of #8 BJSSL and incubated at 28 ± 2°C for 7 days with 12 hr of light and dark conditions as used previously for testing the pathogenic potential using spores. The phytotoxicity was recorded as percentage of leaf area damaged. All the tests were performed in triplicate [15].

Morphotaxonomy of the selected fungal pathogen. The selected fungal isolate (#8 BJSSL) was grown over PDA, malt extract agar (MEA), and corn meal agar (CMA) medium (HiMedia) [18, 19]. The plates were incubated at 28 ± 2°C for 7~10 days with alternate cycle of light and dark for 12 hr. Colony morphology, growth pattern and appearance were noted. For studying microscopic characters, the mycelial mass was picked from the fine tip of the pre-sterilized needle and teased over the glass slide. The fungal mass was mounted using lacto phenol cotton blue dye (HiMedia). The microscopic characters were then observed using Nikon Stereozoom microscope (SMZ 745T; Nikon, Gurgaon, India) coupled with NIS element D 3.2 software and Nikon Eclipse Compound microscope (E100). Microscopic measurements were done using stage and ocular scale and confirmed by Image J software with at least 30 observations per structure.

Phylogenetic analysis of the selected fungal pathogen. Fungal genomic DNA isolation was carried out by using Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). For genomic DNA isolation, 0.5 g of the mycelial mass was scrapped from the 10-day-old culture and crushed into very fine powder in mortar and pestle using liquid nitrogen. The powder was transferred into micro-centrifuge tube and DNA isolation was further carried out by using Wizard Genomic DNA purification kit (Promega). The β-tubulin gene sequence was amplified using bena-T1 (5′ AACATGCGTGAGATTGTAAGT 3′), bena-T22 (5′ TCT-GGATGTTGTTGGGAATCC 3′) primer pair. Amplification was performed in a 25 μL reaction mixture volume consisting of 25 ng of extracted genomic DNA, 0.8 μM of each primer pair, 2.5 mM of dNTPs, 1.5 mM $MgCl_2$, 1.5 U of *Taq* DNA polymerase. The thermal cycling parameters was 96°C for 5 min followed by 35 cycles of 95°C for 1 min, 58°C for 1.30 min, 72°C for 1.30 min followed by final extension at 72°C for 7 min [18, 19]. The amplified product (≈ 800 bp) was purified using Wizard SV gel and PCR clean up system kit (Promega) and the purified products were sequenced at Xcleris Labs (Ahmadabad, Gujarat, India).

Sequence assembly, alignment, and phylogenetic analysis. The chromatograms obtained following sequencing were manually edited and checked for its purity using Sequencher ver. 5 (http://www.genecodes.com). The final consensus sequence was submitted in GenBank under accession number KP696755. The final sequence of #8 BJSSL was then subjected for BLAST similarity search in the NCBI database to ascertain the homology with closely related organisms. The sequences showing highest similarity for each locus were selected and aligned with the respective sequences obtained in the present work by using CLUSTALW.

Table 1. Fungal isolates inhabiting *Eichhornia crassipes* and their pathogenicity in *in vitro* detached leaf bioassay

Sample No.	Culture code	Tentative identification	% Leaf area damage[a] after different hours post inoculation (hpi)[b]						
			0	24	48	72	96	120	144
1	#3 BJSSL	*Fusarium* sp.	0	0	0	11 ± 1	16 ± 2	24 ± 1.7	26.7 ± 0.6
2	#4 BJSSL	*Fusarium* sp.	0	0	0	6 ± 1	11 ± 1	11 ± 1.2	12 ± 2
3	#5 BJSSL	*Alternaria* sp.	0	0	23 ± 3	52 ± 3	73 ± 3	83 ± 2.9	90 ± 2.9
4	#6 BJSSL	Zygomycetes	0	0	7 ± 3	22 ± 6	38 ± 10	47 ± 2.9	50 ± 2.9
5	#7 BJSSL	*Botryosphaeria* sp.	0	0	22 ± 3	42 ± 3	85 ± 9	92 ± 2.9	95.3 ± 0.6
6	#8 BJSSL	*Phaeoacremonim* sp.	0	17 ± 3	28 ± 5	57 ± 6	87 ± 3	96 ± 1.7	100 ± 0
7	#9 BJSSL	*Fusarium* sp.	0	0	12 ± 3	33 ± 3	68 ± 3	70 ± 0	71.7 ± 2.9
8	#10 BJSSL	*Fusarium* sp.	0	0	37 ± 6	46 ± 1	57 ± 3	72 ± 2.9	83.3 ± 2.9
9	#11 BJSSL	*Acremonium* sp.	0	0	12 ± 3	32 ± 3	52 ± 3	73 ± 2.9	81.7 ± 2.9
10	#12 BJSSL	*Nigrospora* sp.	0	0	0	0	5 ± 0	5 ± 0	6.67 ± 2.9
11	#14 BJSSL	*Alternaria* sp.	0	0	23 ± 3	37 ± 3	50 ± 5	57 ± 2.9	83.3 ± 2.9
12	#15 BJSSL	*Penicillium* sp.	0	0	7 ± 3	18 ± 3	33 ± 3	42 ± 2.9	48.3 ± 2.9
13	#17 BJSSL	*Penicillium* sp.	0	0	32 ± 3	44 ± 3	60 ± 3	72 ± 2.5	81.7 ± 2.9
14	#20 BJSSL	*Alternaria* sp.	0	0	7 ± 3	22 ± 3	38 ± 3	43 ± 2.9	45.3 ± 0.6
15	#34 BJSSL	*Curvularia* sp.	0	0	0	0	0	0	0
16	#54 BJSSL	*Trichoderma* sp.	0	0	0	0	0	0	0
17	#57 BJSSL	*Aspergillus* sp.	0	0	0	0	0	0	0
18	#72 BJSSL	Zygomycetes	0	0	8 ± 3	17 ± 3	23 ± 3	32 ± 2.9	33.3 ± 2.9
19	#8 BJSSS	Zygomycetes	0	0	0	0	0	0	0
20	#14 BJSSS	*Aspergillus* sp.	0	0	0	0	0	0	0
21	Control	No organism	0	0	0	0	0	0	0

[a]Mean values along with their standard error (±) are given in the table.
[b]Spore concentration = 1×10^6 spores/mL.

Evolutionary relationship of the selected isolate based on β-tubulin gene was inferred using the maximum parsimony (MP) method. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (20 replicates). The tree was drawn to scale, with branch lengths calculated using the average pathway method and were in the units of the number of changes over the whole sequence. The confidence interval of internal nodes was assessed by employing bootstrap analysis (1,000 replicates). Gaps and missing characters were excluded from the study [20-22].

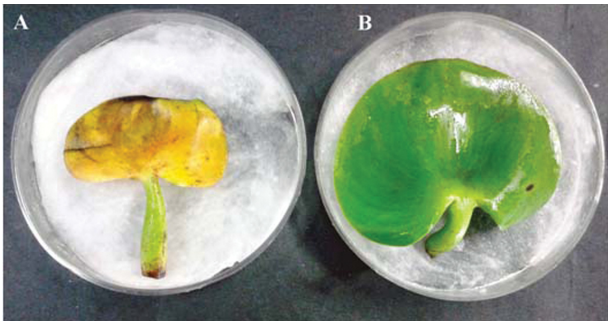

Fig. 1. *In vitro* detached leaf assay of spores of *Phaeoacremonium italicum* (#8 BJSSL) after 144 hpi. A, Test leaf showing damage; B, Control leaf.

RESULTS

Isolation of pathogenic fungi. Twenty fungal isolates were obtained from diseased leaves and stolons of *Eichhornia crassipes* collected from Harike wetland. These isolates comprised of 11 different genera (Table 1). The frequency of occurrence of *Fusarium* is 20% (04), followed by *Alternaria* with 15% (03) and *Penicillium* and *Aspergillus* species with 10% (02 isolates each). The other fungi which have been isolated are *Botryosphaeria* sp., *Pheoacremonium* sp., *Acremonium* sp., *Nigrospora* sp., *Curvularia* and *Trichoderma* sp.

Pathogenicity test on detached leaves. All the fungi recovered from diseased samples of *E. crassipes* were tested using *in vitro* detached leaf bioassay. Out of 20 isolates tested, only *Phaeoacremonium* species (#8 BJSSL) exhibited 100% kill after 144 hr post inoculation (hpi). The development of symptoms started after 24 hr and became prominent after 48 hr. The symptoms included chlorosis of the leaves and appearance of black and brown spots in due course of time due to necrosis (Fig. 1). The other isolates exhibiting pathogenic potential were *Botryosphaeria* species (#7 BJSSL) and *Alternaria* species (#5 BJSSL) which were exhibiting 95.3% and 90% killing of the leaves after 6 days (Table 1). Further higher AUDPC also indicated #8 BJSSL to be the most pathogenic isolate followed by #7 BJSSL and #5 BJSSL (Figs. 1 and 2). To date, *Phaeoacremonium* species has yet not been reported as a pathogen or a possible biological control agent on *Eichhornia crassipes*. Hence, *Phaeoacremonium* species #8 BJSSL was further selected to test its potential as an innundative biocontrol agent using *in vitro* whole plant bioassay.

Whole plant bioassay. In the whole plant bioassay, the disease onset began after 48 hpi. Fifty percent damage of

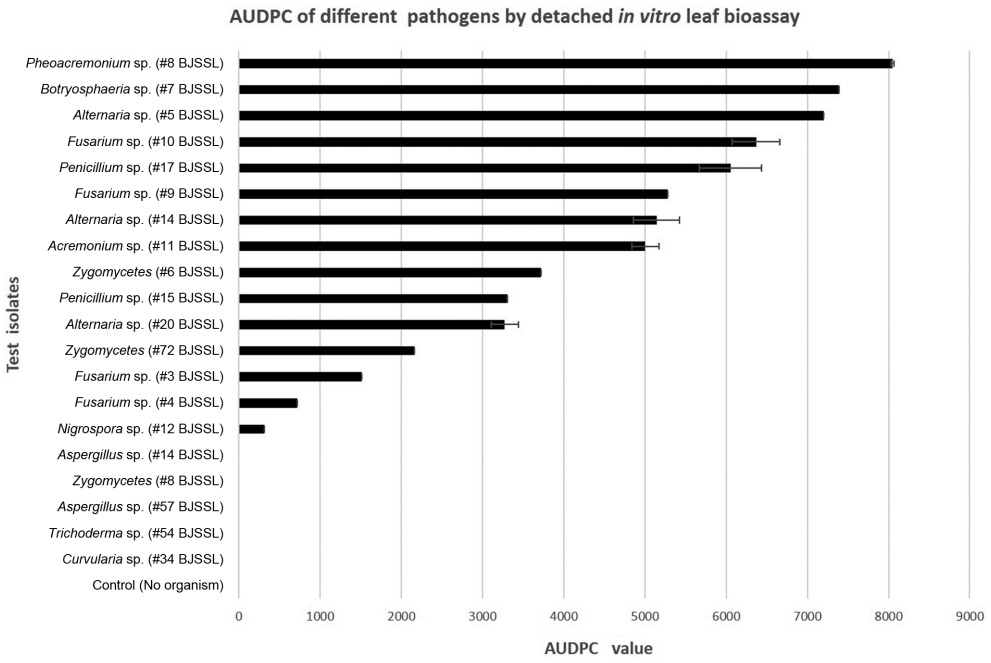

Fig. 2. Area under disease progressive curve (AUDPC) of different test pathogens by *in vitro* detached leaf bioassay.

the whole plants was observed at around 84 hpi and above 90% death by 144 hpi (Figs. 3 and 4). A significant difference was observed in AUDPC between the *in vitro* leaf assay and whole plant assay with values of 8,049.6 ± 14.6 and 7,842 ± 411.8, respectively. To further potentiate the pathogenic efficacy and process of disease development phytotoxic activity of the secondary metabolites of the test, isolate #8 BJSSL was again tested by *in vitro* detached leaf bioassay.

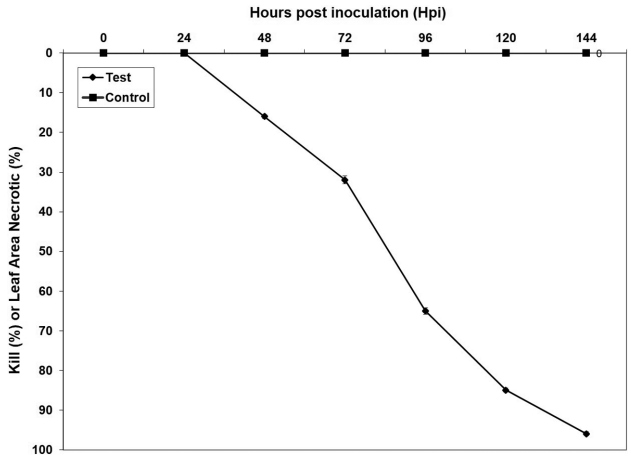

Fig. 3. Disease progress caused by spores spray (1×10^6 spores/mL) of *Phaeoacremonium italicum* (#8 BJSSL) during whole plant bioassay.

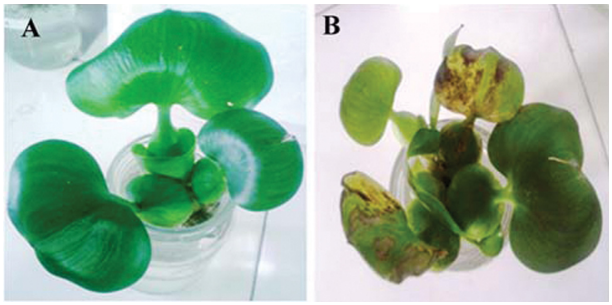

Fig. 4. Whole plant bioassay exhibiting the kill caused by spore suspension (1×10^6 spores/mL) of *Phaeoacremonium italicum* (#8 BJSSL). A, Control plant with no pathogenic symptoms; B, Test plant showing pathogenic symptoms developed post-inoculation.

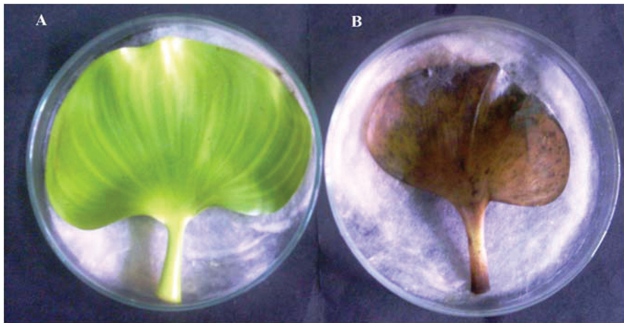

Fig. 5. *In vitro* detached leaf assay of culture filtrate of *Phaeoacremonium italicum* (#8 BJSSL) after 144 hpi. A,Test leaf showing damage; B, Control leaf.

***In vitro* detached leaf assay of culture filtrate of #8 BJSSL.** The *in vitro* leaf assay using cell free culture filtrate of #8 BJSSL also exhibited prominent phytotoxic effect. The symptoms appeared after 48 hpi and rapidly damaged the leaf causing chlorosis and necrosis at 96 hpi and complete death of leaves by 144 hpi (Fig. 5). The AUDPC values of *in vitro* leaf assay of spores and culture filtrate were quite similar viz. 8,049.6 ± 14.6 and 8,780 ± 320 suggesting that they could be mixed together to develop a potential formulation.

Morphotaxomony of #8 BJSSL. Colonies over PDA medium were moderately growing reaching a radius of

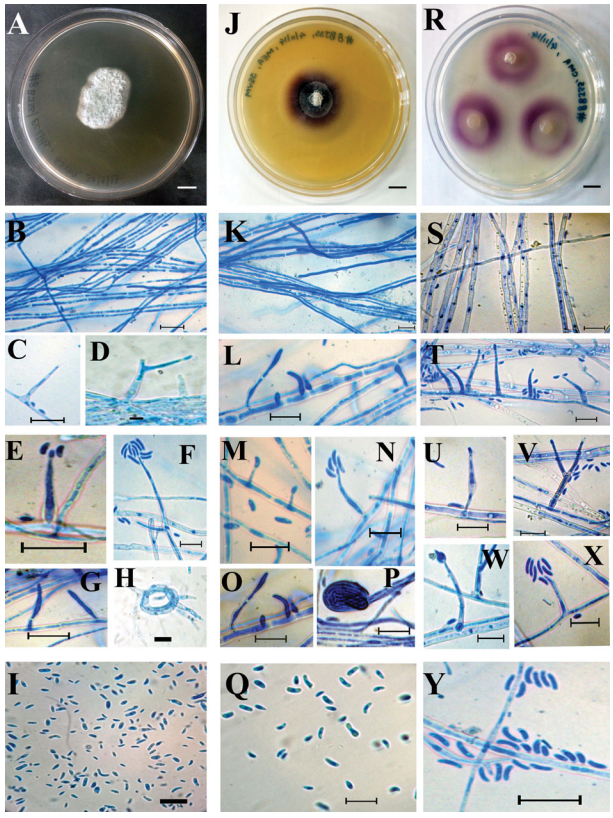

Fig. 6. Morphological and microscopic structures of *Phaeoacremonium italicum* (#8 BJSSL). A, J, R: Fourteen-day-old colonies at 28°C on potato dextrose agar (PDA) (A), malt extract agar (MEA) (J), and corn meal agar (CMA) (R) (scale bar = 10 mm). B~I, Structures on the surface of PDA: B, Hyphae over PDA medium; C, G, Unbranched conidiophore; D, Branched conidiophore; E, F, Adelophialides with conidia; H, Hyphal coils; I, Conidia; K~Q, Microscopic characters on produced over MEA; K, Hyphae; L, O, Unbranched conidiophores; M, N, Adelophialides with conidia; P, Hyphal coil; Q, Conidia; S~Y, Features produced over CMA; S, Hyphae; T, U, Unbranched conidia; V, Branched conidiophore; W~X, Adelophialides with conidia; Y, Conidia (scale bars = 10 µm).

12 ± 1.2 mm in 14 days at 28°C. Colonies were white in color, reverse vinaceous, flat, cottony at the centre and powdery with felty margins (Fig. 6A). Hyphae was found singly or in bundle of 6~7 (Fig. 6B). Conidiophores borne on the aerial mycelium were usually single and unbranched having an inflated bottom and tapering towards the edge (Fig. 6C~6G). Certain hyphal coils were also observed (Fig. 6H). The dimension of the conidia were (3.78) 6.22 (10.15) × (1.52) 2.03 (3.23) µm and were allantiod to obovate in shape (Fig. 6I).

Over MEA medium colonies reach a radius of 39.67 ± 1.15 mm after 14 days at 28°C. Colonies were olive grey to vinaceous colored, flat and felty margined (Fig. 6J). Mycelium consisting of branched septate hyphae measuring (0.99) 1.64 ± 0.37 (2.64) µm that occur singly or as bundle of 6~8 (Fig. 6K). The dimensions of conidiophore was [(3.35) 7.43 ± 2.48 (12.08)] × [(1) 1.54 ± 0.42 (2.89)] µm which were borne on the aerial mycelium and were usually single, unbranched, cylindrical, slightly inflated with narrow collarate (Fig. 6L~6O). Adelophialides were terminal, monophialadic, smooth and collarate (Fig. 6M and 6N). Several hyphal coils were also observed (Fig. G). Conidia [(2.48) 3.72 ± 0.69 (5.09)] × [(0.93) 1.14 ± 0.73 (1.87)] were single celled, allantoid to oblong-ellipsoidal in shape (Fig. 6Q).

Colonies on CMA medium were moderately growing (41.33 ± 2.08 mm), vinaceous colored, flat, with a felty margin (Fig. 6R). Mycelium consisting of long cylindrical and septate hyphae measuring (1.2) 2.22 ± 0.43 (2.96) µm that occured singly or as synmeta of 5~6 (Fig. 6S). The conidiophore measured [(6.96) 17.43 ± 4.67 (27.83)] × [(1.03) 1.75 ± 0.41 (2.17)] µm which arised from the aerial mycelium or the submerged hyphae, usually single, long, branched, slightly inflated at the bottom and collarate at the edge (Fig. 6T~6V). Adelophialides were terminal and collarate (Fig. 6W and 6X) conidia. The conidia measured [(2.29) 3.8 ± 0.91 (6.94)] × [(0.76) 1.11 ± 0.22 (1.52)] µm and were single celled and allantoid in shape (Fig. 6Y). Thus based on the above features, the isolate was identified as *Pheoacremonium italicum* sp.

Molecular identification of #8 BJSSL. The MP tree based on β-tubulin region resolved into 4 different clades of *Pheoacremonium* sp. Clade I comprised of eight strains of *P. italicum* and #8 BJSSL. Clade II grouped three strains of *P. alvesii*, Clade III grouped *P. ruberigenum* species and Clade IV clustered three strains of *P. scolyti*. The tree was rooted with *Lasiodiplodia gonubiensis*. Based on the phylogenetic tree of β-tubulin the correct speciation of the selected isolated #8 BJSS was confirmed as *P. italicum* (Fig. 7).

DISCUSSION

Over the years, several management strategies such as chemical, physical, and biological, etc. have been adopted to control water hyacinth. However, strategies like chemical treatment and physical removal have failed because of their hazardous environment effects and large-scale implementation problem. This calls for an alternative organic approach to clean up the water bodies. The biological method including usage of microorganism or their metabolites has attracted

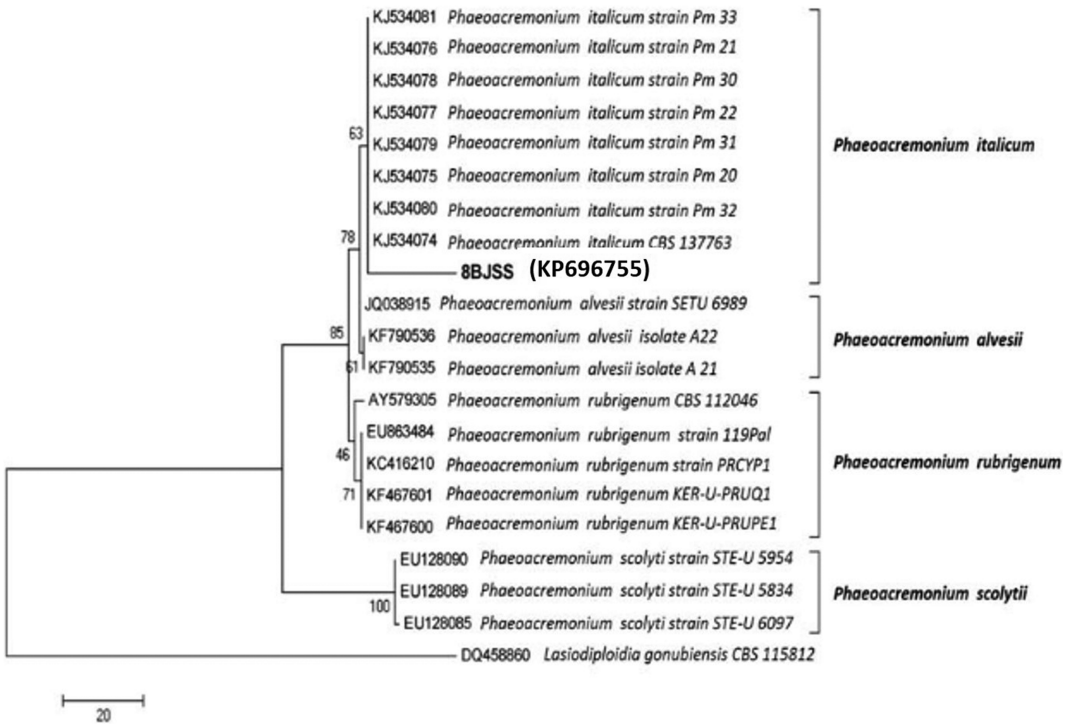

Fig. 7. Phylogenetic placement of #8 BJSSL as *Phaeoacremonium italicum*.

researchers to exploit them as a novel bio-resource for controlling this obnoxious weed [23]. The current study focuses on isolation and exploration of pathogenic mycobiota of water hyacinth to control them. During the study, 20 pathogenic fungal isolates belonging to 11 different genera were isolated. *Fusarium* was the most dominant fungal colonizer followed by *Aspergillus* and *Alternaria* sp. which are amongst the most common isolated plant pathogens. Fungal pathogens like *Acremonium*, *Alternaria*, *Fusarium*, *Aspergillus*, and *Penicillium* sp. have been previously reported from water hyacinth [8, 13]. Praveena and Naseema [12] documented isolation of 21 fungal isolates occurring on water hyacinth in Kerala which includes *Alternaria* sp., *Aspergillus* sp., *Curvularia* sp., *Fusarium* sp., *Nigrospora* sp. Shaker [24] isolated 20 fungal species from water hyacinth inhabiting in middle and south of Iraq of which *Alternaria alternata*, *Acremonium* sp., *Cladsporium herbarum*, and *Fusarium* sp. were the most frequently isolated species. During the study, fungal species like *Botryosphaeria* and *Phaeoacremonim* sp. have been recovered which were never reported earlier from water hyacinth to best of our knowledge. In the screening studies through *in vitro* detached leaf assay, we have found that spore suspension of *Phaeoacremonim* sp. (#8 BJSSL) exhibited maximum leaf damaging property causing 100% killing in 144 hpi. Similarly, *Botryosphaeria* sp. (#7 BJSSL) and *Alternaria* sp. (#5 BJSSL) also caused 96% and 90% killing of the leaves after 144 hpi. Further, spore suspension of #8 BJSSL also exhibited similar activity in whole plant bioassay. El-Morsy [13] screened 22 fungal isolates for their ability to infect water hyacinth out of which *Alternaria alternata*, *Drechslera hawaiiensis*, and *Ulocladium atrum* showed 79%, 78%, and 70% tissue death after four weeks post inoculation. Fungal species like *Myrothecium advena* and *Fusarium pallidoroseum* caused over 50% infection of the weed [12]. Shaker [24] found that *Alternaria alternata*, *Acremonium* sp., and *Phoma eupyrena* were the most aggressive fungal isolate to water hyacinth causing 91.66%, 83%, and 75% damage in pathogenicity test. During our study, the development of pathogenic symptom was assessed using AUDPC. The AUDPC analysis provides the rate of disease severity during a time course as a single unit and therefore is considered as an appropriate measure in studying the disease development in the plants. When tested on whole plants of water hyacinth, *P. italicum* (#8 BJSSL) was causing dieback symptoms leading to the death of the plant. A significant difference in the AUDPC values of the *in vitro* leaf assay and the whole plant assay indicated the role of inherent plant mechanisms which resist the process of disease development. This could probably be due to development of physiological or genetic resistance mechanisms which delays the onset of disease development. Production of phytoalexins in response to pathogens is also a mechanism of resisting disease development [25-27]. In an attempt to understand the role of phytotoxins produced by *P. italicum*, the culture filtrate of #8 BJSSL was also tested. This could be used in development of suitable mycoherbicidal formulations. Further, based on classical and molecular tools, the isolate #8 BJSSL has been identified as *P. italicum*. Similar morphological structures were produced by *Phaeoacremonium* species associated with olive wilt [19]. This is the first record of its occurrence as a pathogen on *Eichhornia crassipes* from Harike wetland, Punjab, India as well as a possible biocontrol agent. Considering the importance of water hyacinth as a problematic weed, *P. italicum* (#8 BJSSL) may have potential benefits also for biological control, when its host specificity and pathogenicity has been tested at physiological as well as genetic levels in plant and animal systems.

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