INTRODUCTION

Endophytic fungi colonize plants internally without causing any disease occur symbiotically and produce many compounds which can inhibit pathogens[1] and improves fertility and nutrient retention[2]. Endophytes have a unique ecological niche and play an important role on distribution, ecology, physiology and biochemistry of plants[3]. These fungi promote plant growth as they produce secondary metabolites which are required for plant defence mechanisms[4]. They have also been reported to produce enormous number of antimicrobial, antioxidant and anti-cancer compounds[5,6].

The aquatic hyphomycetes which are frequently known to occur on submerged leaf litter in running freshwater bodies[7] also now recovered as endophyte by Waid[8], Fisher and Petrini[9], Marvanova *et al.*[10], Sridhar and Barlocher[11], Sati and Belwal[12] and Sati *et al.*[13]. Occurrence of these fungi as root endophytes in healthy plants indicates that they are biologically important and may have some beneficial role in plant health[14,15].

The bioprospection of fungi for their natural products are continuing, but information on aquatic hyphomycetous fungi for their role as plant growth promoter is quite meagre. In the present investigation, the role of isolated root endophytic aquatic hyphomycetes as plant growth promoter using pot experiments under controlled glass house conditions was studied.

MATERIALS AND METHODS

Study area: Root samples were collected from different localities of Nainital, Kumaun Himalaya, India, in the month of January, 2018 and the experiment was carried out during June-September, 2018 at the Department of Botany, D.S.B. Campus, Kumaun University, Nainital, India.

Isolation of Root Endophytic Aquatic Hyphomycetes (REAH): The collected root samples were processed for the isolation of root endophytic aquatic hyphomycetes[12]. The isolates were identified with the help of relevant monographs and papers[16]. Cultures have been submitted in Kumaun University, Mycological Slide (KUMS) collection of the Department of Botany, Kumaun University, Nainital, India.

Preparation of inoculum: Small agar blocks (5 mm) were cut from the apical margin of pure culture of isolated REAH and transferred into conical flasks containing 150 mL of sterilized Malt extract broth medium. These flasks were incubated in BOD incubator at 20±2°C. After 15 days, broths were filtered through Whatman No.1 filter paper into a sterile beaker and filtrate was used as inoculum for the pot experiments. For control experiments, un-inoculated flasks were prepared in same manner and kept under the same conditions.

Preparation of pots and seed sowing: Seeds of chilli, *Capsicum annuum* L. (Solanaceae) were grown and taken as test plant. The seeds were surface sterilized by soaking them in 1% sodium hypochlorite solution for 1-5 min and then rinsed with sterilized distilled water. These seeds were dipped in fungal and medium or control broths for test and control experiments, respectively and kept overnight. The seeds were sown to respective pots having sterilized nutrient deficient soil by mixing sand (1:3; soil:sand) prepared for pot experiments and allowed to grow under the glass house conditions (Temperature 28±2°C and relative humidity 40%).

Inoculation to the test plants: The pot experiments were conducted by sowing the treated seeds of chilli into prepared pots. Twenty seeds of chilli were sown to each pot containing about 5 kg of mixed soil. After 10 days of germination, 50 mL of fungal broth (inoculum) and medium broths were supplied to the test and controlled plants, respectively and next successive treatments were applied after an interval of 7 days till the finishing of experiment (8 weeks) in glass house conditions (Average temp. 28±2°C, relative humidity 40%). All pots were watered with sterilized distilled water in alternate days. The experimental plants were harvested after 8 weeks of growth (Fig. 1). The growth parameters used were; (i) Shoot and root length of test plant, (ii) Shoot and root diameter of test plant, (iii) Fresh mass of shoot and root, (iv) Dry mass of shoot and root and (v) Number of leaves.

The dry mass of plants was measured after drying the samples at 70°C for 48 h in an oven[17].

Statistical analysis: The data were recorded for each set of test and control plant separately in triplicate. Analysis of variance (ANOVA) was determined among fungal treated and non-treated plants.

RESULTS

On the basis of morphological characters the isolates were identified as *Campylospora parvula* Kuzuha and *Tetracladium setigerum* (Grove) Ingold recovered from *Pilea scripta* and *Barberries vulgaris*, respectively (Fig. 1a-b). These two isolates were evaluated for their effect on plant growth by using chilli as the test plant.

Table 1: Effect of fungal treatment on the growth of chilli plants in pot experiments after 60 days of sowing

	Treatments		
Parameters	*Campylospora parvula*	*Tetracladium setigerum*	Control
Shoot length (mm)	480.00 (±7.7)	510.00 (±6.8)	340.00 (±6.8)
Shoot diameter (mm)	22.90 (±0.4)	23.87 (±0.5)	15.23 (±0.5)
Root length (mm)	240.00 (±1.7)	260.00 (±1.5)	160.00 (±1.2)
Root diameter (mm)	14.30 (±0.3)	15.05 (±0.3)	9.62 (±0.1)
Fresh weight of shoot (g/plant)	12.41 (±0.5)	12.95 (±0.7)	8.30 (±0.7)
Fresh weight of root (g/plant)	7.94 (±0.4)	8.50 (±0.6)	4.64 (±0.8)
Dry weight of shoot (g/plant)	3.48 (±0.2)	3.86 (±0.3)	2.27 (±0.1)
Dry weight of root (g/plant)	1.60 (±0.01)	1.69 (±0.03)	0.87 (±0.01)
No. of leaves	17-21	18-25	11-15

±Standard error of mean of three replicates

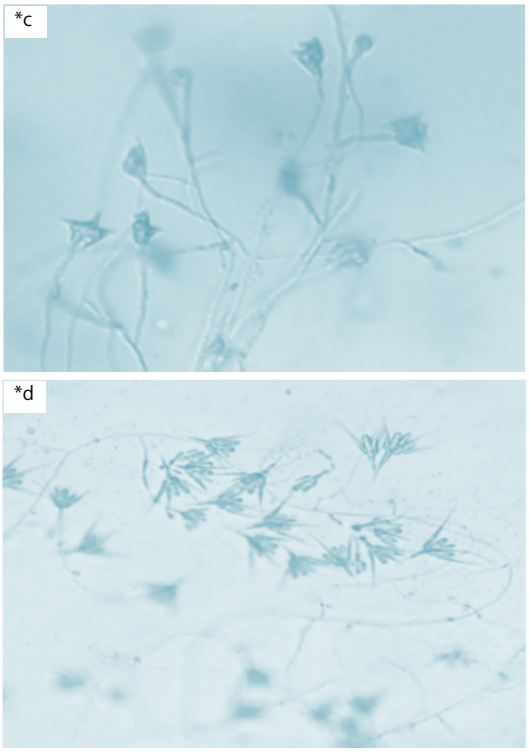

Fig. 1(a-b): Conidia of root endophytic aquatic hyphomycetes of (a) *Campylospora parvula* and (b) *Tetracladium setigerum*

The effect of both REAH (*C. parvula* and *T. setigerum*) on the growth of test plant (Chilli) is presented in Table 1. As evident from the Table 1, plants treated with fungal broth showed an increment in all parameters such as; shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and number of leaves compared to the control pot plants (Fig. 2a-c, Fig. 3a-c, Table 1).

One way ANOVA conducted between the treated and control plants showed a noticeable difference for all the used parameters and both the REAH had significant potentiality to enhance the plant growth (Table 2). It is interesting to note

Fig. 2(a-c): Effect of fungal treatment on shoot length of chilli plant, (a) Control, (b) Treated with *C. parvula* and (c) Treated with *T. setigerum*

Fig. 3(a-c): Effect of fungal treatment on roots of chilli, (a) Control, (b) Treated with *C. parvula* and (c) Treated with *T. setigerum*

Table 2: Results of ANOVA for test plants in different parameters

Parameters	Sum of squares	df	Mean square	F-value	p-value
Shoot length	45088.889	2	22544.444	49.488	0.000*
Root length	5488.889	2	2744.444	29.939	0.000*
Shoot diameter	117.787	2	58.893	33.464	0.000*
Root diameter	52.212	2	26.106	78.031	0.000*
Fresh weight of shoot	32.162	2	16.081	14.909	0.005
Dry weight of shoot	4.197	2	2.099	32.349	0.000*
Fresh weight of root	16.299	2	8.150	1.047	0.407
Dry weight of root	1.372	2	0.686	36.100	0.000*
Number of leaves	69.556	2	34.778	13.042	0.007

*Significant (<.005)

Table 3: Percentage of effectiveness of applied treatments on the growth of chilli plant

Parameters	CP against C (%)	TS against C (%)
Shoot length (mm)	41.1	50.0
Shoot diameter (mm)	50.3	56.7
Root length (mm)	50.0	62.5
Root diameter (mm)	48.6	56.4
Fresh weight of shoot (g/plant)	49.5	56.2
Fresh weight of root (g/plant)	71.1	83.1
Dry weight of shoot (g/plant)	53.3	70.0
Dry weight of root (g/plant)	89.9	94.2

CP: *Campylospora parvula*, TS: *Tetracladium setigerum*, C: Control

that the yield of biomass from the test plants inoculated with *C. parvula* and *T. setigerum* was also found greater than the control plants (Table 1).

A comparative effect of the used endophytic fungi on test plants was calculated to determine the potentiality of used fungi. The effect of *T. setigerum* against control plants were found to be maximum with 94.2% for dry weight of root and minimum 50% for shoot length (Table 3).

DISCUSSION

Since the pioneering work of Ingold[7] on the aquatic hyphomycetes, a large number of reports are available on the occurrence and distribution of these fungi from the various regions of the world[18]. However, knowledge on these fungi as endophyte is quite meagre. The fungal endophytes live asymptomatically on host plant[19,20]. In the present investigation, the chilli as test plants grown in pots directly from the seeds were inoculated with two endophytic aquatic hyphomycetes such as; *C. parvula* and *T. setigerum* recovered from the roots of *Pilea scripta* and *Barberries vulgaris*, respectively. It is noteworthy that the chilli plants which were used as test plant inoculated with REAH grew better than un-inoculated test plants (Fig. 2, Table 1).

The test plant inoculated with the root endophytic aquatic hyphomycetes showed no symptoms of disease. Sridhar and Raviraja[3] also suggested that the host plants might have coped with the changing environment to maintain fungi in endophytic state rather than pathogenic state. In present investigation, the applied REAH were found responsible to enhance the growth of test plant chilli (Table 1). This suggested that some metabolites or growth promoting chemicals might be produced in fungal broths which influenced the plant growth of test plants. Since, the metabolite production also depends upon the characteristics of habitat and species. The used REAH were recovered from a unique habitat (freshwater habitat) might be the good resource of some novel compounds.

The role of aquatic hyphomycetes in plant growth has also been studied earlier and reported a considerable increment in the growth of test plants[21]. It was also noticeable that the lengths of roots were found greater in treated plants compared with control plant (Fig. 3) and supported the findings of earlier workers. *Tatracladium setigerum* was more effective for the growth increment compared to *C. parvula* (Table 1). It is well known that endophytic fungi produce a large number of secondary metabolites[22] which are used as plant growth promoters[23,24]. It is interesting to note that this is the first report on plant growth promoting capacity of these species of aquatic hyphomycetes.

The occurrence of these fungi as root endophyte and produce potent antimicrobial compounds has been suggested by Gulis and Stephanovich[25], Sati and Arya[26], Arya and Sati[27], Sati and Singh[28] and Singh and Sati[29]. Recently these fungi have also been reported as plant growth promoting fungi[21] as well as phosphate solubilizers[30]. The present findings also strongly supported the importance of REAH for plant growth. Thus, these endophytic fungi would be the good source of new secondary metabolites required for bio-controlling agents as well as bio-fertilizers.

CONCLUSION

In the microbial world, large numbers of species are yet to be explored and the bio-prospection is the first approach to search new useful products within the endophytic fungi. The

fungal endophytes may facilitate their host plants to survive under any stress condition by secreting favorable secondary metabolites. Hence, the discovery of plant growth promoting fungal metabolites may lead to isolate some novel growth promoting chemical compounds. The present findings strongly support the utility of root endophytic aquatic hyphomycetes for the growth of plants. In future, these strains of REAH may be useful to enhance the growth as well as yields of plants in eco-friendly way.

SIGNIFICANCE STATEMENT

This study discovered the role of root endophytic aquatic hyphomycetes as plant growth promoter that can be beneficial in the field of agriculture for better plant productivity. This study will also help the new researchers to uncover the critical areas of applied aquatic mycology which has not well been explored by earlier workers. Thus, it would be a new theory on endophytic aquatic hyphomycetes as growth promoter.

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