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Growth response of four dominant species of *Trentepohlia* (Trentepohliales, Chlorophyta) grown at different relative humidity

Diana Kharkongor*, Papiya Ramanujam
Algal Ecology Laboratory, Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong, Meghalaya, India

Abstract
Growth and development of four dominant species of *Trentepohlia* was analysed by inoculating each species of *Trentepohlia* on a solidified BBM (Modified) with 2 % agar and keeping under different relative humidity (33%, 55%, 75%, 85% and 98%). It was clearly observed that growth of all *Trentepohlia* species were optimum at 98 % relative humidity. With increase in relative humidity the length and width of the filament cells, apical cells and sporangia increased with incubation period from first to fifth weeks. At 98 % RH growth and survival of all four species studied was high, whereas at lower relative humidity retarded growth could be observed. Comparatively, *T. diffracta and T. abietina* showed better growth in different relative humidity. Significant increase in cells width and sporangia width was observed after three weeks of inoculation and in the 5th week growth of new filaments could be observed.

Keywords: *Trentepohlia*, subaerial algae, relative humidity, growth, cell length, cell width, sporangia, filaments

Introduction

Aeroterrestrial microalgae typically colonize in biofilms the interface between all kinds of hard substrata and the atmosphere. The surface can be of natural origin, e.g., tree barks, soil, and rocks, or of artificial origin, e.g., roof tiles, concrete, or building facades in urban areas. Additionally, some taxa are known as photobionts of lichens (Ettl and Gartner, 1995) [1]. Algae colonizing subaerial biofilms are much more exposed to harsh and rapidly changing environmental conditions such as radiation, temperature, nutrient concentrations, and in particular desiccation stress as compared to aquatic habitat (Karsten *et al*, 2007)[2]. Regardless of their desiccation tolerance, all terrestrial green algae require liquid water, or an atmosphere saturated with humidity, to be metabolically active (Bartoli *et al*, 2019) [3]. Islam (1960)[4] had explained that very heavy rainfall and prevailing humidity provided ideal conditions for growth of subaerial algae the year around. Water and high relative humidity are the prerequisite for optimum growth and photosynthesis in subaerial microalgae. When dried, these microorganisms become inactive but can recover quickly if water is suddenly available, e.g., after rainfall. These physiological capabilities explain well the ecological success of aeroterrestrial microalgae in occupying many man-made substrata such as building facades and roof tiles in urban areas (Haubner *et al*, 2006) [5].

The genus Trentepohlia Martius, a subaerial or terrestrial alga, is the largest genus in the family Trentepohliaceae, order Trentepohliales, class Ulvophyceae and division Chlorophyta (Allali, 2011[6]; Guiry and Guiry, 2015[7]; John, 2002[8]; López-Bautista *et al*, 2002[9]; Rindi, 2007[10]; Rindi *et al*, 2009[11]). This genus of subaerial green algae is widespread in regions with humid climates (Chapman 1984[12]; Rindi *et al*. 2005[13]) and they are also reported to thrive well in areas with high relative humidity and rainfall (Rindi and Guiri 2002)[14] and in areas with adequate light intensity (Neustupa and Skaloud 2008) [15]. In general, like all other microalgae, moisture, light and temperature are important factors for the growth and development of *Trentepohlia*. Growth under high air humidities has been previously reported for aeroterrestrial algae such as *Prasiola crispa* and *Trebouxia* sp (Fraymouth 1928)[16], *Apatococcus lobatus* (Bertsch 1966) [17], *Trentepohlia odorata* (Lee *et al* 1990[18]; Ong *et al* 1992[19]) and *Stichococcus* sp (Haubner *et al* 2006) [5]. Lee *et al* (1990) [18], demonstrated in culture, the effect of different humidity, and pH on morphological variations in *Trentepohlia odorata*. They reported a change in morphology and a significant increase in growth with increase in relative humidity.

Subaerial algae mainly members of Trentepohliales grow luxuriantly in Meghalaya and their presence impart yellowish or reddish orange colour to most of the tree barks, walls, and rocks. Meghalaya experiences high rainfall and high humidity which provides ideal condition for the growth of subaerial algal communities the whole year around (Kharkongor and Ramanujam, 2014[20]). Besides optimum temperature and photon irradiance, relative humidity is another crucial factor which determines the growth and development of subaerial algae. The present work aimed at evaluating the influences of relative humidity on the growth pattern of four dominant species of *Trentepohlia* under laboratory conditions.

Materials and Methods

Study area and collection of Algal samples

Four dominant species of *Trentepohlia* were collected in Shillong, which lies approximately between latitudes 25°07' to 25°41' N and longitude 91°21' to 92°09' E from four different substrata *viz*. *Trentepohlia diffracta* (Krempelhuber) Hariot from cemented wall, *Trentepohlia arborum* (C.Agardh) Hariot from rock surface, *Trentepohlia umbrina* (Kutzing) Bornet from iron electric pole (since it was difficult to segregate *T. umbrina* from other groups of algae growing along with it on bark of trees, hence it was collected from an electric pole where the growth of other groups were negligible) and *Trentepohlia abietina* (Flotow) Hansgirg was collected from bark of *Eucalyptus* tree.

Growth of selected *Trentepohlia* species under different relative air humidities

To achieve the required relative air humidities (33%, 55%, 75%, 85% and 98%), saturated salt solution of MgCl2, Ca(NO3)2, NaCl, KCl, KH_2PO_4 were prepared respectively following the methods of Winston and Bates (1960) [21]. Each saturated salt solution was poured in separate container which provided an atmosphere with respective relative air humidities.

Each species of *Trentepohlia* was homogenized and inoculated on a modified Bold Basal Medium (Abe *et al* 2008) [22]) with 2 % agar. The petriplates with inoculated algal sample were then kept inside the sealed containers with specific relative humidities of 33%, 55%, 75%, 85% and 98%. In order to raise the level of petriplates with algal material a few centimetres above the fluid level glass slides and stands were used as support. These sealed containers were then placed in the culture rack at 40 μmol photons m^{-2} sec^{-1}, at photoperiod of 16:8 Light: Dark and at 25°C temperature. The experiment was carried out with three replicates for each relative humidity.

Measurement of algal growth

Morphological features like length and width of filament cells, apical cells and sporangia were used as parameters to measure the growth of *Trentepohlia* exposed to different relative humidity. At the end of every week i.e. from the 1st week till the 5th week, each sample kept at different relative humidity were scraped and observed under a light microscope measured and photographed. From each treatment fifty filaments were measured.

Results

It was observed that with increase in relative humidity from 33 % to 98 %, the length and width of the filament cells, apical cells and sporangia increased with incubation period from first to fifth weeks in all the four species of *Trentepohlia* i.e. *T. abietina*, *T. arborum*, *T. diffracta* and *T. umbrina*. At 98% relative humidity all species of *Trentepohlia* showed better growth compared to that kept at 33%, 55%, and 75% relative humidity. At lower relative humidity (33% and 55 %) retarded growth could be observed in all four species of *Trentepohlia*, where samples did not show any signs of growth and in fact most of the filaments shrank and died after third week. At 98 % RH, the changes in cells and sporangia size in the four species were observed in the third week of incubation which then continued growing till the fifth week. Increase in width of cells of the filaments in these three species was clearly observed, especially in *T. diffracta* and *T. abietina*, whereby, the filaments were swollen and looked like beads on string. In the fifth week growth of new filaments could be observed in three species except in *T. arborum*. In *T. arborum* fragmentation of the filament was seen after five weeks of inoculation, which then grew into new filaments in 98 % RH. The response of *T. arborum* was quite poor compared to the other three species even at 98 % RH, where 65 % of the inoculated filaments became pale and died after the third week. The best response was exhibited by *T. diffracta*, followed by *T. abietina and T. umbrina*.

Cell length

Comparing amongst the four species of *Trentepohlia*, mean increase in length of cells was maximum in *T. diffracta* in all relative humidity, followed by *T. umbrina* and least was observed in *T. abietina*. It was observed that length of cells increased significantly with increase in relative humidity from 33% to 98 % and was maximum at 98% in all the four species of *Trentepohlia*. At 33% relative humidity, there was absolutely no increase in the length of cells, in all the four species of *Trentepohlia* till the fifth week of incubation. However, slight increase was observed at 55 %. In *T. diffracta* at 98% RH, mean increase of 4.8 µm in length of cells was observed (Figure 1). The changes in length of cells in the four species were observed in the third week of incubation which then continues growing till the fifth week.

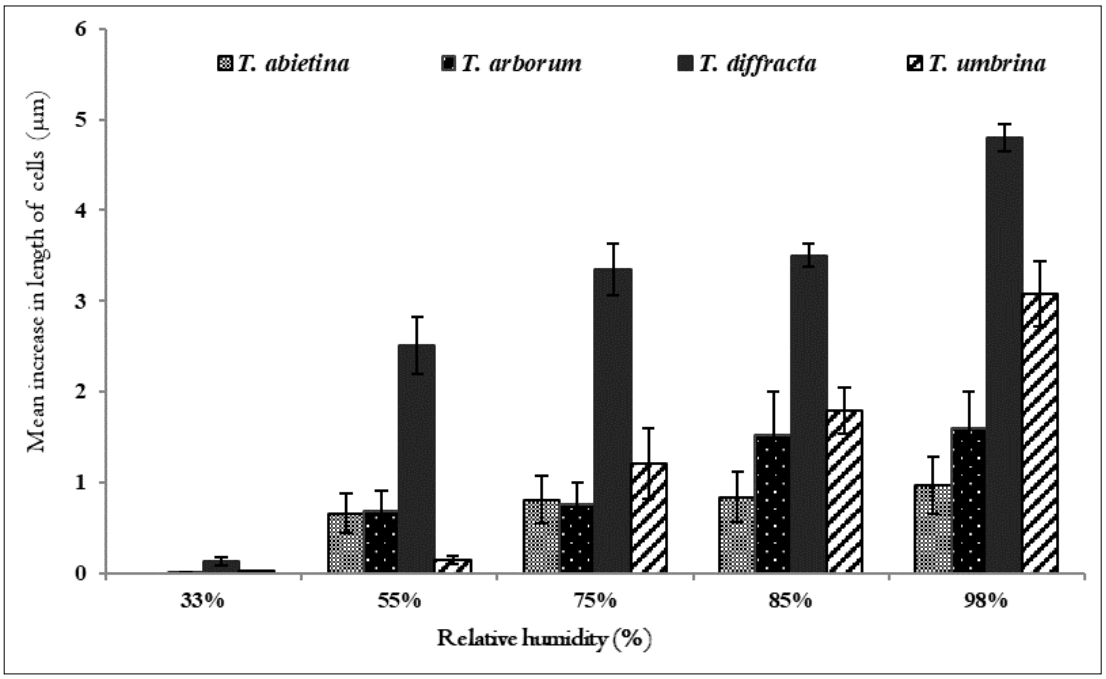

Fig 1: Effect of relative humidity on the length of cells in four species of *Trentepohlia*

Cells width

Mean width of cells showed a remarkable increase with increase in relative humidity. At 33 % RH, there was no increase in width but from 55% to 98 % the mean increase in width of the cells was observed. Comparing amongst four species of *Trentepohlia*, maximum increase in width was in *T. diffracta* followed by *T. abietina* and *T. umbrina.* No marked response was observed in *T. arborum* at different relative humidity treatments (Figure 2). Increase in width of cells in both *T. diffracta* and *T. abietina* was very prominent, and in the third week at 98 % RH the cells of the filament increased significantly in size and became globular in shape. At 98 % RH in the third week, *T. diffracta* cells grew profusely and could not be differentiated into erect and prostrate filament, width of the cells increased and 70% of filaments became globular which consequently gave the filaments the beaded shape. The increase in width of cells continued till the fourth and fifth week. Similarly in *T. abietina* and *T. umbrina* increased in width of cells continued till the fifth week and almost 45% of filaments became globular, however in *T arborum* there was no significant increase in width of the cells till the fifth week. In the third week at 98% RH, many of the cells which had attained maximum increase in width started dispersing the content of the cells in the medium.

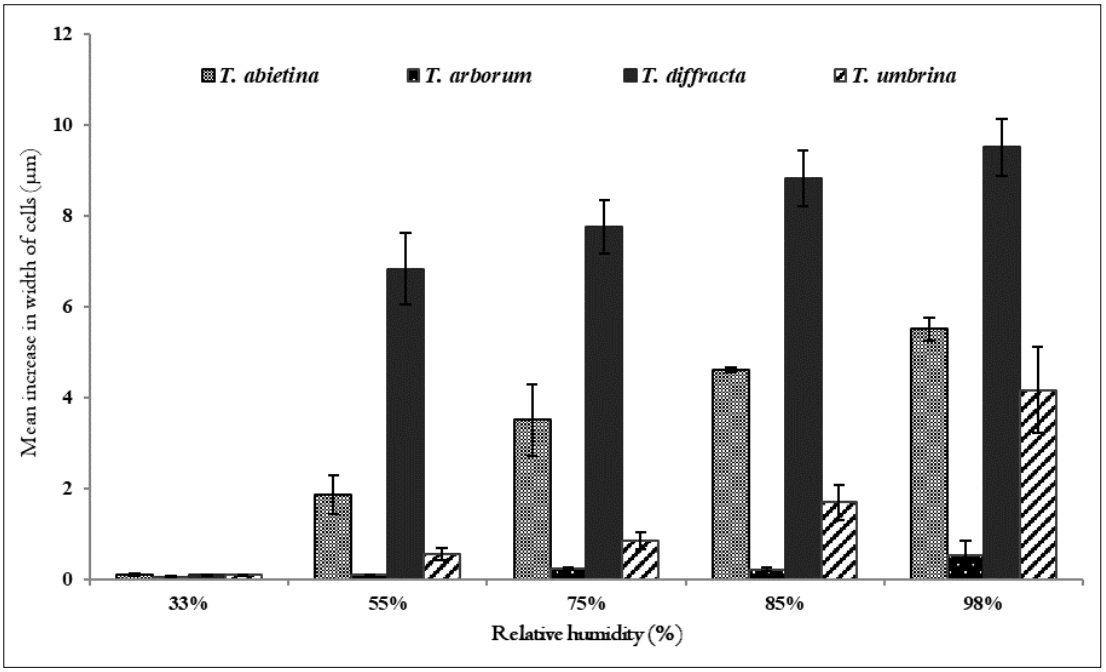

Fig 2: Effect of relative humidity on the width of the cells in four species of *Trentepohlia*

Apical cells length and width

length and width of apical cells also increased with increase in relative humidity. Maximum growth was observed at 98 % RH and least was at 33 %. Maximum increase in length of apical cells was observed in *T. abietina* followed by that in *T. umbrina* and *T. diffracta*. Maximum mean increase in width of apical cells was observed at 98 % RH in *T. diffracta* followed by that in *T. abietina* and *T. umbrina* (Figure 3 & Figure 4).

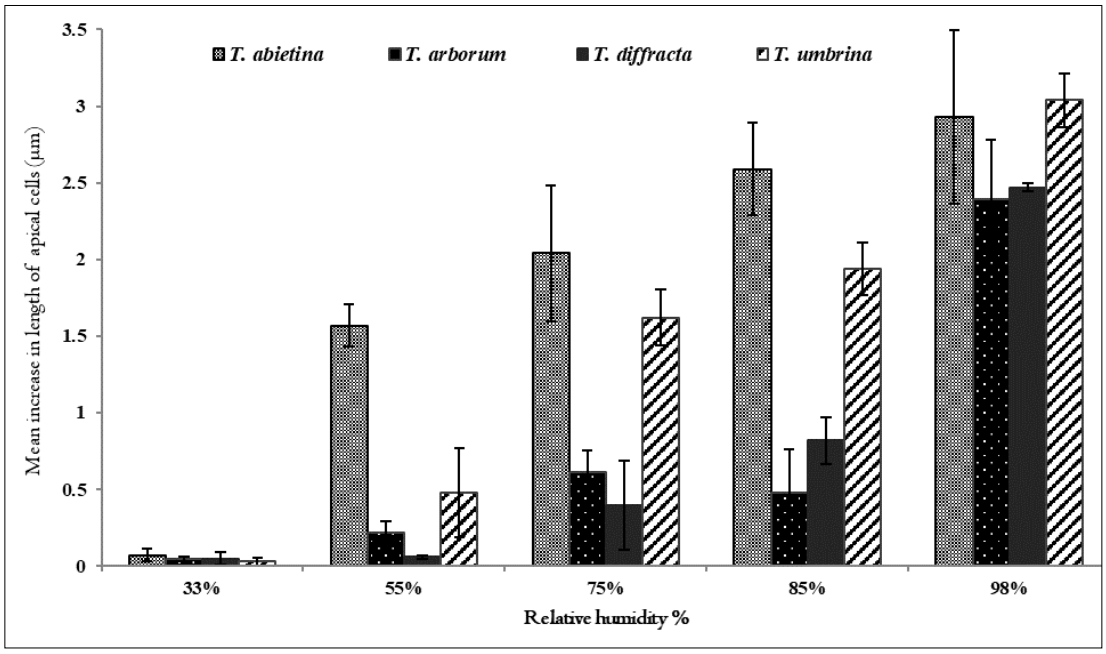

Fig 3: Effect of relative humidity on the length of apical cells in four species of *Trentepohlia*

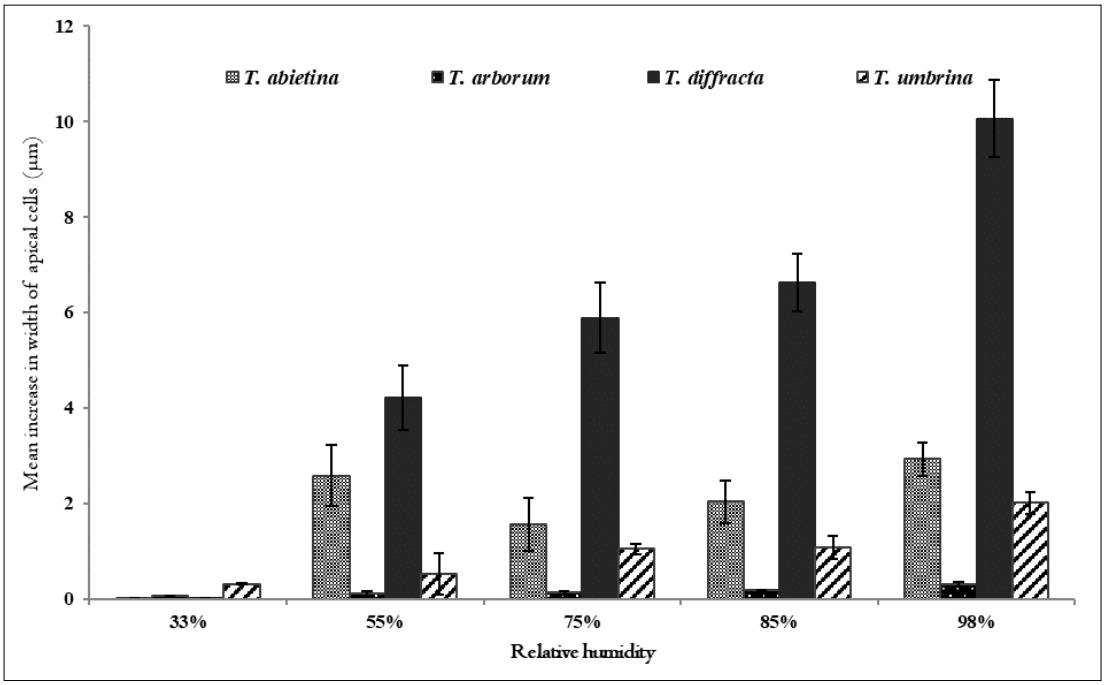

Fig 4: Effect of relative humidity on the width of apical cells in four species of *Trentepohlia*

Sporangia length and width

Sporangia length and width also increased significantly at higher relative humidity. In *T. diffracta* and *T. abietina* sporangia size increased 4-5 times than that at the initial stage of inoculation and was maximum at 98 % RH was observed (Figure 5 & Figure 6). After reaching their maximum growth the sporangia disintegrated and dispersed the spores in the vicinity of the medium. In the fifth week after inoculation some new filaments had started growing in the three species of *Trentepohlia* except in *T. arborum*.

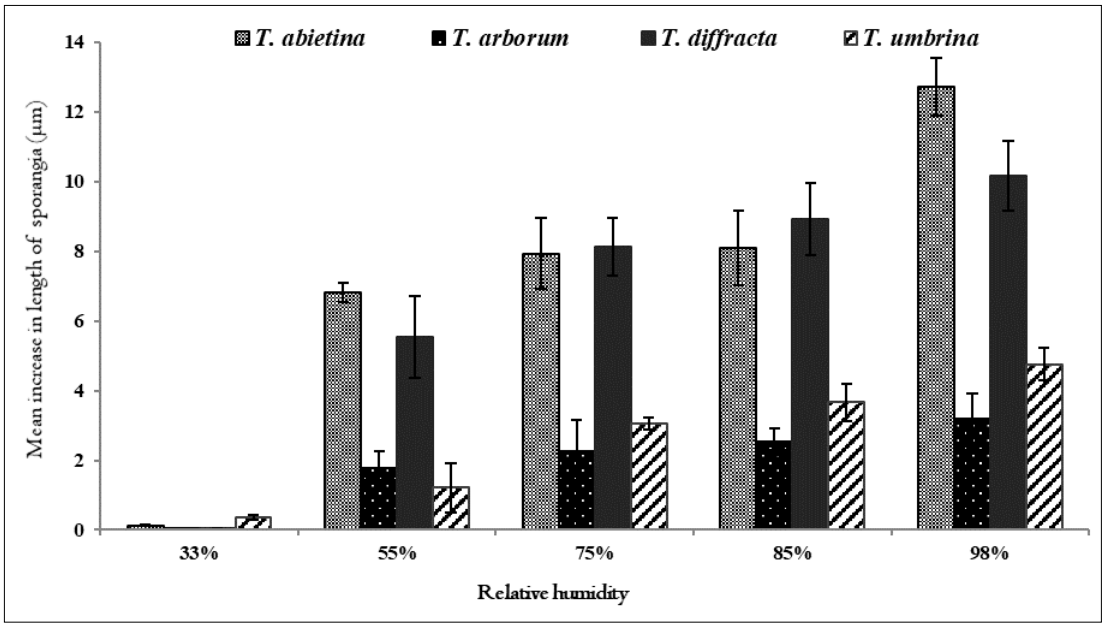

Fig 5: Effect of relative humidity on the length of sporangia in four species of *Trentepohlia*

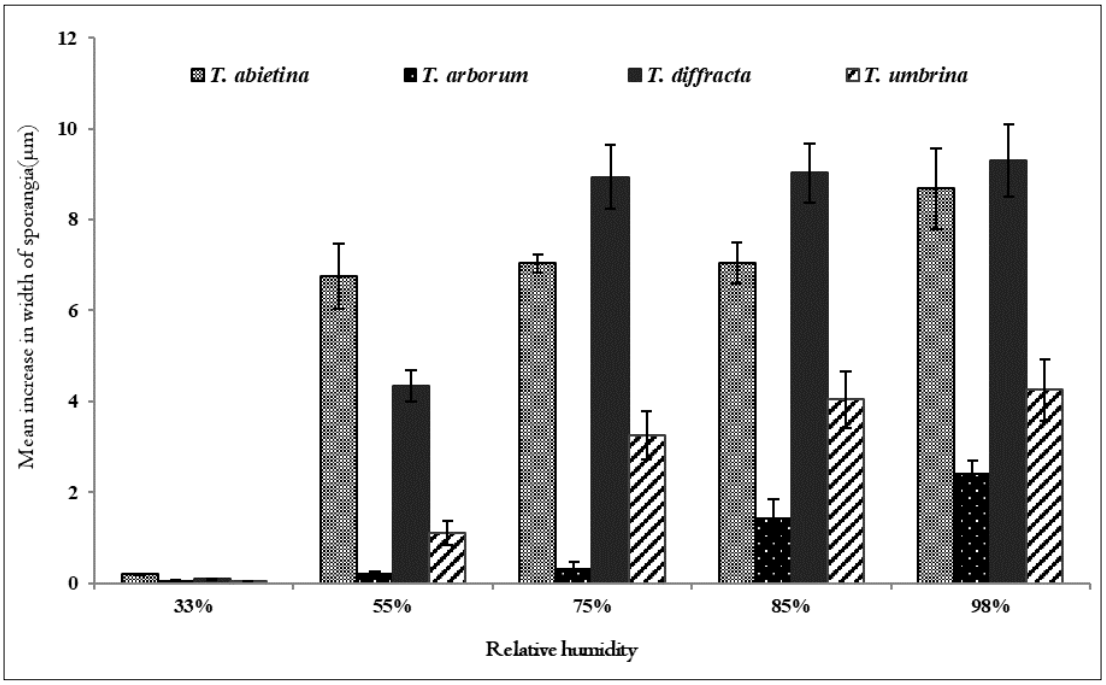

Fig 6: Effect of relative humidity on the width of sporangia in four species of *Trentepohlia*

Discussion

Growth and development of four species of *Trentepohlia* at different relative humidity demonstrated that the optimum relative humidity for growth of *Trentepohlia* was 98 %. At low relative humidity retarded growth could be seen in all four species of *Trentepohlia*. With increase in relative humidity growth and survival of *Trentepohlia* also increased and it was evident, since culture of all four species kept at 98% RH was growing and surviving even after one year, whereas those kept under lower RH could not survive. als. Increase in the mean length and width of the filament cells, apical cells and sporangia was strongly observed with increase in relative humidities in all the four species of *Trentepohlia*. This clearly explained that high relative humidity was essential for growth and development of *Trentepohlia* just like in the case of other microalgae. Seraphol *et al* (2019) [23] reported the occurrence of *T. monilia* in areas with a high relative humidity of 74.50% to 83.93%. Enhanced growths of all the four dominant species observed in this experiment also were completely dependent on the increase in air humidities within the cultured apparatus. Similarly Bartoli *et al* 2019 [3] explained the probable requirement of water being derived from atmospheric humidity and dew formation in the case of *T. umbrina*, since the need for substrate moisture was relatively low, considering that it occurs almost entirely on vertical, lithic surfaces. Haubner *et al* (2006) [5] demonstrated that 100% air relative humidity were the prerequisite for optimum photosynthesis and growth of aeroterrestrial microalga *Stichococcus* sp and at air humidities below 93%, both processes were strongly inhibited. Lee *et al* (1990) [10] reported a change in morphology of

Trentepohlia odorata with changes in humidity and growth of colony increased significantly with increase in relative humidity and also they demonstrated that in cultures where moisture and nutrients are not limiting, cells are narrow and elongated, with the filaments radiating from a central mass, and the sporangia are of the sessile and pedicellate types.

Among the four species studied *T. diffracta and T. abietina* showed better growth rate at different humidities. It was previously reported by Kharkongor and Ramanujam (2021) [24] the better growth rate were exhibited by *T. diffracta and T. abietina* at irradiance of 40 μmol photons m^{-2} sec^{-1}, and at 25°C temperature. Similarly *T. diffracta* accumulated the highest amount of carotenoids (Kharkongor and Ramanujam, 2021)[25] and *T. abietina* extract contained the highest amount of total phenolic compound and flavonoids content (Kharkongor and Ramanujam, 2017)[26]. The best result showed by these two species signifies the better response of some species over another species.

Conclusion

The growth response of the four species of *Trentepohlia* i.e. *T. diffracta* (Krempelhuber), *T. arborum* (C.Agardh) Hariot, *T. umbrina* (Kutzing) and *T.abietina* (Flotow) Hansgirg grown at different relative humidity in this particular study strongly supports the importance of air humidities on the growth and development of *Trentepohlia* a dominant genus of subaerial algae. Hence besides maintaining the optimum temperature and light intensity increasing the air humidity at 98% or 100% may greatly enhance the growth of different species of *Trentepohlia in vitro*. Comparatively among the four species better growth rate in culture was exhibited by *T. diffracta and T. abietina* which highly denoted that these two species have better potential for mass cultivation and utilization as natural source of carotenoids and antioxidants.

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