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Seasonal changes in the storage compounds and enzymatic activities in three Indian leafy liverworts

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ABSTRACT

Present study is aimed to investigate the effect of seasonal changes on the storage compounds i.e. free amino acids, RNA, proteins, carbohydrates alongwith enzymatic activities in three species of leafy liverworts, namely *Chiloscyphus gollani* Steph., *Solenostoma crenulata* (Sm.) Steph. and *Fossombronia himalayensis* Kash. Activities of enzymes α-amylase, β-amylase and invertase were found to be reducing towards the end of the growing season i.e. January-March. This is correlated with the accumulation of carbohydrates in the plant body in this season. The content of free amino acids coupled with the highest activity of enzyme protease was found on peak in the rainy season i.e. July-September. The maximum value of proteins was observed in the winter season i.e. October-December and the content of protein is found to be inversely proportional to the content of free amino acids.

Key Words: Seasonal changes, *Chiloscyphus gollani*, *Solenostoma crenulata*, *Fossombronia himalayensis*, inverse relation.

INTRODUCTION

Liverworts are comprised of two forms, thalloid and leafy. In thalloid liverworts, main plant body is flat and lacks leaves and stems. Leafy liverworts have leaves that are arranged in two or three rows dorsiventrally. *Chiloscyphus gollani* is a dorsiventral plant with leaves having apices broader than the base, in *Fossombronia himalayensis* leaves are oblong, wavy and overlapping to about one-third of the plant length, whereas in *Solenostoma crenulata* leaves are bordered with swollen cells and size of marginal cells two times larger than middle leaf cells. The studies conducted to better understand the seasonal variation in the activity of enzymes and various storage compounds are limited and scattered in bryophytes as compared to higher plants.

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Lewis (1970, 1971) reported a considerable amount of free sugars including acyclic sugar alcohols in the leafy liverworts *Plagiochila* and *Jamesoniella*. Melick and Seppelt (1994) reported negligible seasonal variations in the soluble carbohydrates of Antarctic bryophytes. Mosses growing in hydric habitat contain lower amount of carbohydrates but higher content of proteins than the mosses from the xeric and mesic habitats (Davey, 1999).

Oil bodies found in thalloid liverworts contain various organic compounds responsible for various biological activities viz. antifungal, antibacterial, plant growth regulatory, insecticidal, piscicidal (Asakawa 1999, 2004) and also allelopathic effects against weeds (Thakur and Kapila, 2015).

Preliminary biochemical study on Indian bryophytes was done by Kapila and Dhawan (2000). Later, Kaur *et al.* (2010 a, 2010 b) quantitative biochemical analysis in some west Himalayan bryophytes. Kapila *et al.* (2014) studied seasonal variations in carbohydrates, proteins, free amino acids and enzyme activities in *Marchantia palmata*, *M. nepalensis* and *Dumortiera hirsuta.* Recently, Thakur and Kapila (2016) observed higher content of proteins, amino acids and carbohydrates along with enhanced activity of invertase in archegoniophores as compared to the vegetative thallus.

Materials and Methods

Sample collection:

Each of the presently studied species was collected from different areas of Himachal Pradesh in different seasons. Locality from where each species was collected and the substratum on which plants were growing along with the respective herbarium reference number are given in Table-1. Plants were washed with distilled water to remove soil particles and contaminant parts of other plants and then blotted to dry. Extracts were prepared from entire plant body. For the estimation of carbohydrates, proteins and enzymes, the extract was made in distilled water. The extract for the amino acids was prepared in 80% ethanol.

Biological assays:

The concentration of RNA was estimated by following the method of Mezbaum (1939). The content of proteins was measured by the method of Lowry *et al.* (1951). Total water soluble carbohydrates were determined by the method of Yemm and Willis (1954). Method of Lee and Takahashi (1966) was followed for the determination of free amino acids content. The activity of enzyme protease was measured according to the method of Basha and Beevers (1975). The activities of enzymes α-amylase, β-amylase and invertase were assayed according to Muentz (1977) and Bernfeld (1951) and Sumner (1935).

Statistical Analysis:

Values were performed in triplicates and represented as mean±SE (standard error). Statistical analysis was performed using Friedman test as our sample size was small. Differences at p> 0.05 were considered statistically significant.

RESULTS

Effects of various seasonal changes such as fluctuations in temperature, precipitation and associated variations of relative humidity on the content of storage

Table-1. The name of species, period of collection, locality, altitude, substratum and herbarium reference number.

Name of Taxon	Locality	Substratum	Herbarium Reference No.
Chiloscyphus gollani	August, Shimla December, Shimla February, Shimla	On wet soil	PAN 6176
Solenostoma crenulata	September, Mandi November, Mandi February, Mandi	On wet soil near water stream, shady habitat	PAN 6177
Fossombronia himalayensis	August, Kasauli October, Kasauli January, Kasauli	On wet soil	PAN 6178

Table-2. The localities with range of temperature and total precipitation during the three seasons.

Period of collection	Rainy season (July-September)	Winter season (October-December)	End of growing season (January-March)
Kasauli (Altitude 1927 m) Mean Air Temp. (°C) Total precipitation (mm.)	29.27 195-70	19.34 12-24	15.67 43-53
Shimla (Altitude 2200 m) Mean Air Temp. (°C) Total precipitation (mm.)	20.6-19.4 424-160	17.2-10.6 33-28	8.3-13.9 60-61
Mandi (Altitude 1044 m) Mean Air Temp. (°C) Total precipitation (mm.)	25.5-25.3 240-130	23.1-17.4 25-10	16.8-21 30-22

compounds and enzymatic activities were studied by collecting the plant material for biochemical analysis during three different periods i.e. July-September (rainy season), October-December (winter season) and January-March (end of the growing season). The amount of total precipitation, range of temperature and altitude in three areas of collection during the three seasons are given in Table-2.

The results obtained from the present study are given in Figs. 1-8. The rainy season i.e. July-September is the active period for the growth of bryophytes. The plants are in their juvenile growing stage as water is abundantly present in the surrounding environment for their active growth. The winter season i.e. October-December is the most suitable period for the growth of the bryophytes. In this season, they can flourish luxuriantly as the environmental conditions are fully favorable for their growth. End of the growing season i.e. January-March is the dormant period for the growth of the bryophytes.

The present study with the use of Friedman test revealed that the content of free amino acids, carbohydrates and the activity of α-amylase as well as β- amylase showed significant ($p < 0.05$) seasonal variation while others showed insignificant ($p > 0.05$).

Friedman test revealed that the content of RNA showed insignificant seasonal variation with $p > 0.05$. The amount of RNA was noticed maximum (Fig-1) in the winter season (17.35±0.44 mg g^{-1} fresh weight (fw) in *Chiloscyphus gollani*, 6.04±0.03 mg g^{-1} fw in *Solenostoma crenulata*, 7.60±0.26 mg g^{-1} fw in *Fossombronia himalayensis*) and minimum in the rainy season i.e. July- September (7.04±0.16 mg g^{-1} fw in *C. gollani*, 2.88±0.20 mg g^{-1} fw in *S.crenulata*, 3.90±0.02 mg g^{-1} fw in *F. himalayensis*.

The amount of proteins (Fig-2) showed statistically insignificant seasonal variation ($p > 0.05$). The content of proteins was observed highest in the winter season i.e. October-December (48.23±0.19 mg g^{-1} fw in *C. gollani*, 27.99±0.04 mg g^{-1} fw in *S.crenulata*, 24.45±0.17 mg g^{-1} fw in *F. himalayensis*), whereas the lowest amount was noticed in the rainy season (12.92±0.17 in *S. crenulata*, 14.26±0.07 in *F. himalayensis*) except *C. gollani* which showed lowest value in the end of the growing season. Among the three species of leafy liverworts, *C. gollani* showed highest content of proteins as compared to *S. crenulata* and *F. himalayensis* in all the seasons.

Protease is involved in the proteolysis of proteins into free amino acids and showed insignificant ($p > 0.05$)

Figure-1. Content of RNA in three periods of collection. Results are mean of triplates±standard error (SE).

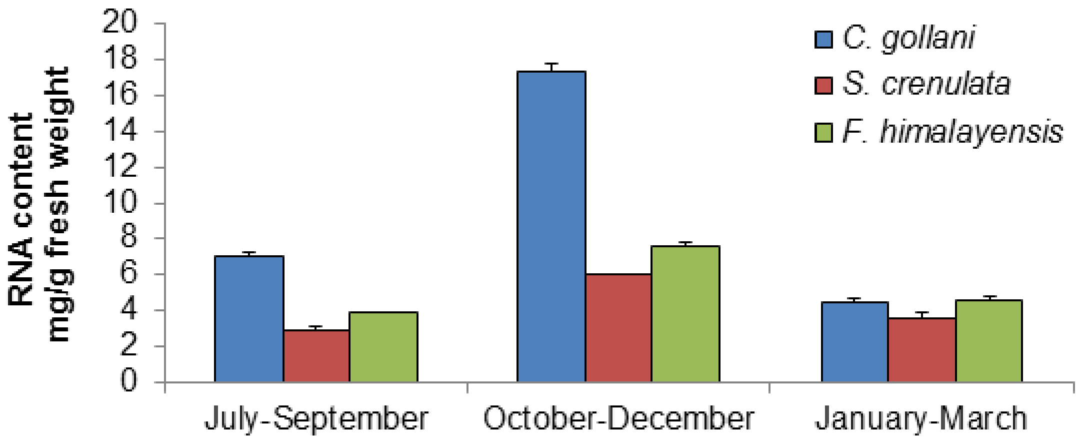
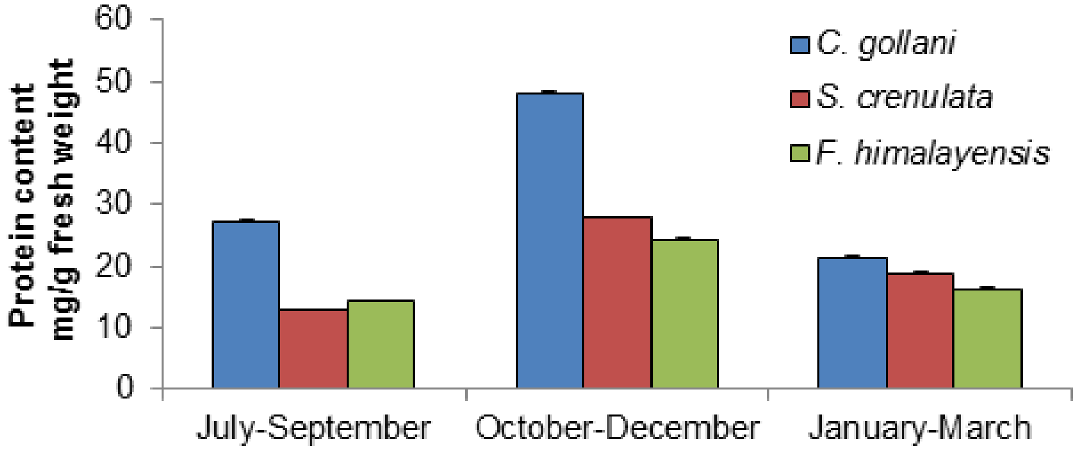


Figure-2. Content of proteins in three periods of collection. Results are mean of triplates±SE.



values on the application of Friedman test in different seasons. The specific activity of protease was at the peak (Fig-3) in the rainy season (22.4±0.97 µg hr^{-1} mg^{-1} protein in *C. gollani*, 32.28±0.30 µg hr^{-1} mg^{-1} protein in *S. crenulata*, 18.86±0.21 µg hr^{-1} mg^{-1} protein in *F. himalayensis*) and minimum in the winter season i.e. October-December and then again an increase in activity was observed during January-March i.e. at the end of growing season.

The value of free amino acids was significantly higher ($p < 0.05$) during July-September (Fig-4) and the lowest in the winter season (30.17±2.12 mg g^{-1} fw in *C. gollani*, 18.39±0.05 mg g^{-1} fw in *S. crenulata*, 8.98±0.26 mg g^{-1} fw in *F. himalayensis*).

The amount of carbohydrates after the Friedman test indicated significant ($p < 0.05$) seasonal changes during three seasons selected for the collection. The carbohydrate content in all the three leafy liverworts (Fig-5) was found to be maximum at the end of growing season i.e. January-March (97.05±3.54 mg g^{-1} fw in *C. gollani*, 85.17±2.44 mg g^{-1} fw in *S. crenulata* 55.34±1.18 mg g^{-1} fw in *F. himalayensis*,) and minimum in the rainy season i.e. July-September (43.99±4.85 mg g^{-1} fw in *C. gollani*, 22.11±0.43 mg g^{-1} fw in *S. crenulata*, 9.45±0.37 mg g^{-1} fw in *F. himalayensis*).

The activity of α-amylase was found significantly ($p < 0.05$) highest (Fig-6) in the rainy season (3.06±0.002 µg min^{-1} mg^{-1} protein in *C. gollani*, 6.42±0.10 µg min^{-1} mg^{-1} protein in *S. crenulata*, 8.37±0.06 µg min^{-1} mg^{-1} protein in *F. himalayensis*) and lowest in the end of the growing season i.e. January-March.

Similarly, β- amylase also showed statistically significant ($p < 0.05$) highest activity in the winter season and lowest (Fig-7) in the end of the growth period (0.72±0.14 µg min^{-1} mg^{-1} protein in *C. gollani*, 0.28±0.08 µg min^{-1} mg^{-1} protein in *S. crenulata*, 1.76±0.30 µg min^{-1} mg^{-1} protein in *F. himalayensis*).

The activity of invertase in *S. crenulata* (Fig-8) was noticed higher (4.55±0.21 µg min^{-1} mg^{-1} protein in rainy season, 1.60±0.31 µg min^{-1} mg^{-1} protein in winter season, 0.71±0.13 µg min^{-1} mg^{-1} protein in end of the growing season) as compared to the other leafy liverworts i.e. *C. gollani* and *F. himalayensis*.

The activities of α-amylase, β- amylase and invertase were observed lowest in the end of the growing season resulting into the synthesis and accumulation of carbohydrates during this period. But the activity of invertase showed statistically insignificant ($p > 0.05$) seasonal variations.

Figure-3. Specific activity of protease in three periods of collection. Results are means of triplates±SE.

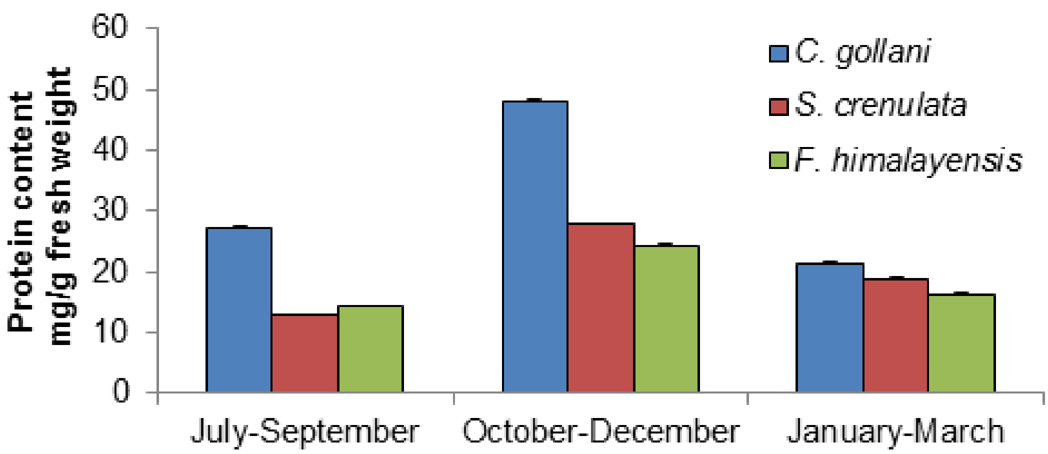


Figure-4. Content of amino acids in three peri¬ods of collection. Results are mean of triplates±SE.

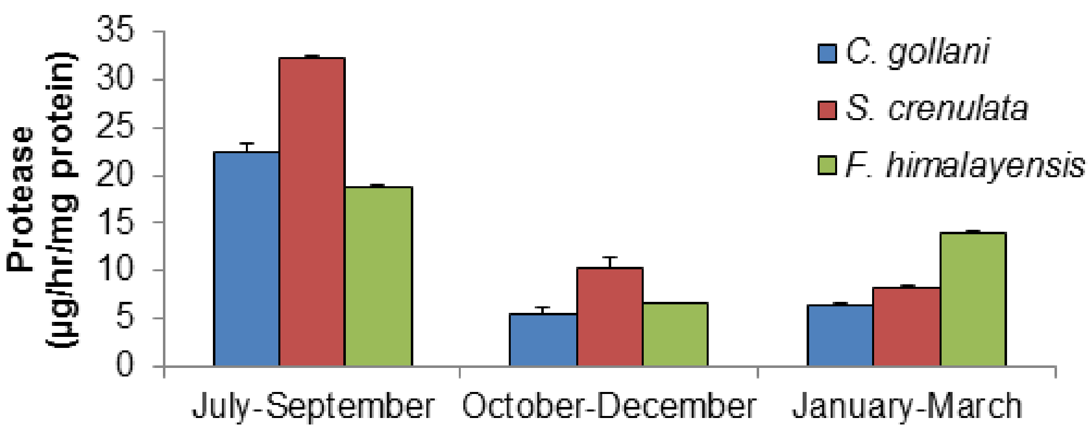


Figure-5. Content of carbohydrates in three peri¬ods ofcollection. Results are mean of triplates±SE.

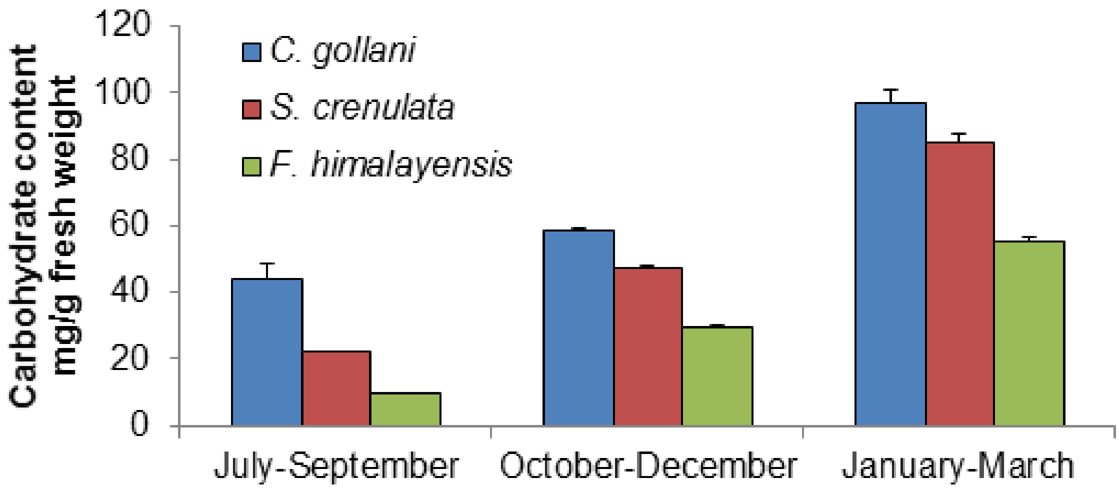


Figure-6. Specific activity of α-amylase in three periods of collection. Results are means of triplates±SE..

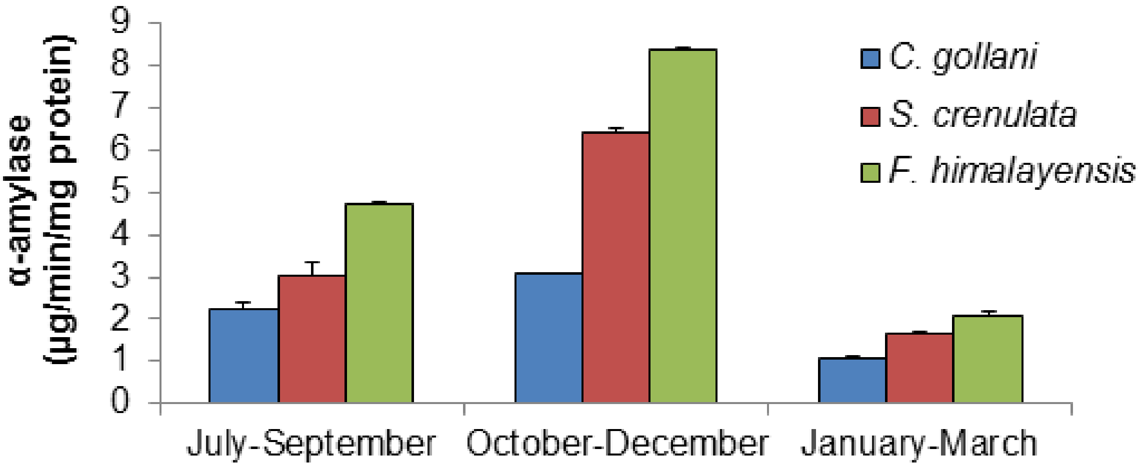


Figure-7. Specific activity of β-amylase in three periods of collection. Results are means of triplates±SE.

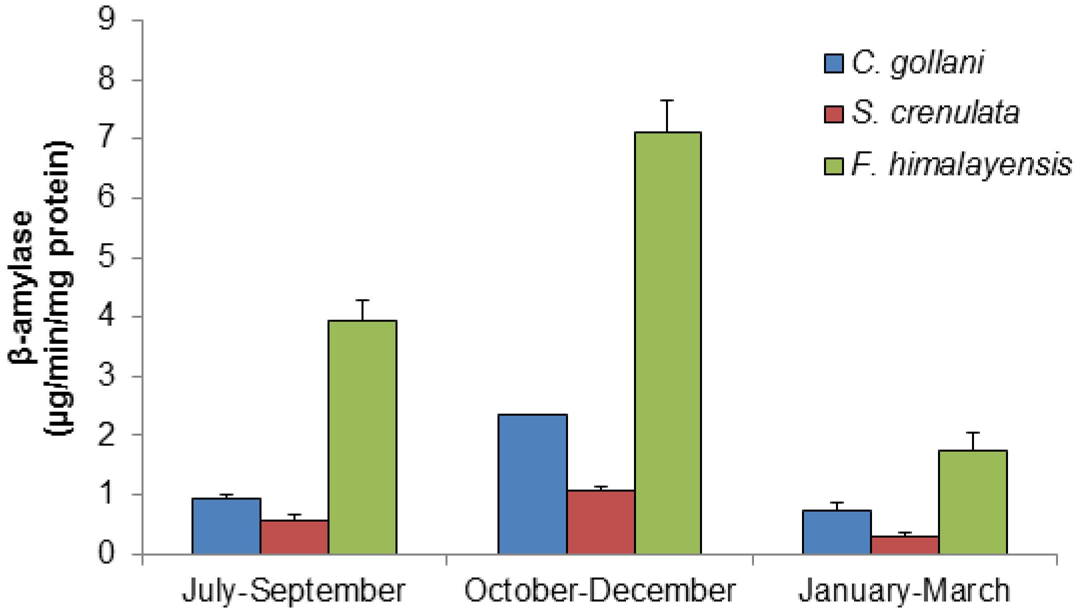
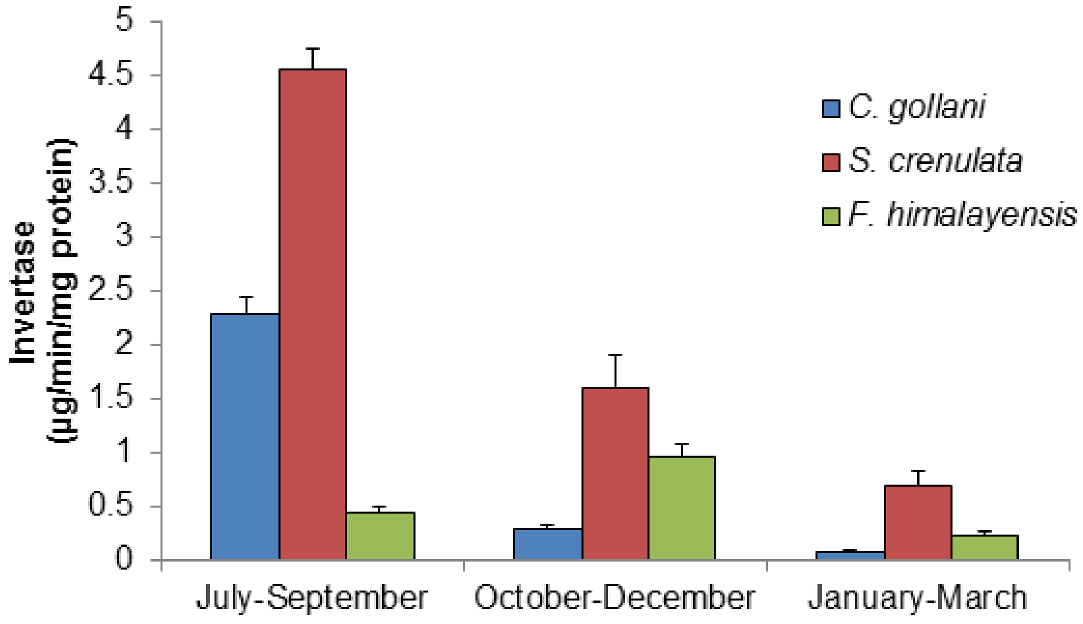


Figure-8. Specific activity of invertase in three periods of collection. Results are means of triplates ± SE.



DISCUSSION

RNA plays a vital role in the biological reactions within the cell such as cell signaling and regulation of gene expression, or sensing and communicating responses to cellular signals. The concentration of RNA was maximum in the winter season and minimum in the rainy season, since the high temperature limits the rate of RNA synthesis (Farewell and Neidhardt, 1998). As RNA is helpful in the synthesis of peptide bond, the content of RNA is directly linked to the amount of proteins.

Highest content of proteins observed during winter season as compared to that in the other two periods of collection might be due to the low temperature conditions. The low temperature induces the synthesis of antifreeze proteins (Fowler *et al.,* 2001; Yeh *et al*., 2000; Galiba *et al*., 2009). The highest temperature during rainy season is responsible for the lower content of proteins as the enzymes and other factors involved in the synthesis of proteins are also heat labile. Davey (1999) and Kapila *et al.* (2014) suggested that the protein content was higher in the hydric mosses as well as in the liverworts growing along the water streams than the ones which are growing in the shaded areas in humid conditions. But the present study is not consistent with these reports since *S. crenulata* growing along the water streams showed lower values of proteins as compared to the other leafy liverworts i.e. *C. gollani* and *F. himalayensis* growing on wet soil except in the winter season when *S. crenulata* showed higher protein content than *F. himalayensis*. However, all the three leafy liverworts showed higher protein content ((12.92-48.23 mg g^{-1} fw) as compared to *Marchantia palmata*, *M. nepalensis* and *Dumortiera hirsuta* (11.22-26.97 mg g^{-1} fw) studied by Kapila *et al.* (2014).

Enzyme protease helps in the degradation of peptide bonds in proteins, gene expression, cell cycle, protein sorting and their quality control (Lin *et al.*, 2011; Hsiao *et al.*, 2014). In the present study, the activity of protease was at peak in rainy season which is responsible for the proteolysis of proteins into amino acids for the production of new proteins. This conforms to earlier studies (Karmous *et al.*, 2012) on the proteolytic activities in *Phaseolus vulgaris* cotyledons under stress conditions and on the seasonal variation in the activity of protease (Kapila *et al.*, 2014).

The high content of amino acids and lower content of protein in the rainy season indicate that the content of protein is inversely proportional to the content of amino acids. Higher content of free amino acids observed during this period of collection is also in agreement with an earlier observation on the three members of Marchantiaceace (Kapila *et al.*, 2014). Amino acids released from the degradation of proteins by the proteolytic activity of enzyme protease play a vital role in nitrification and mineralization (Jones and Kielland, 2002; Gallet-Budynek *et al.,* 2009). The content of free amino acids in all the studied leafy liverworts is observed to be higher than the other liverworts studied by Kapila *et al.* (2014).

In the present study, highest content of carbohydrates was recorded during dormant period of growth i.e. January-March. Kapila and Dhawan (2000) also reported in *Dumortiera hirsuta* and *Conocephalum conicum* that carbohydrates are stored in their thalli towards the end of their growth season. Kapila *et al.* (2014) recorded the same observation in *Marchantia palmata*, *M. nepalensis* and *Dumortiera hirsuta.*

Carbohydrates are the reserve food material which supplies energy for various metabolic activities. Carbohydrate hydrolyzing enzymes i.e. α-amylase, β-amylases and invertase showed lower activity towards the end of their growth season leading to the accumulation and storage of carbohydrates in this season. Among the three studied liverworts *F. himalayensis* showed highest activity of α-amylase as well as β-amylases in all the three seasons under study. Marschall *et al.* (1998) reported a decrease in the activity of enzyme invertase during rehydration in the leafy liverwort *Porella platyphylla*. Presently, the activity of invertase was observed higher in *S. crenulata* than in *C. gollani* leading to lower amount of carbohydrates in the former species.

Conclusion

It is concluded from the present study that variations in the temperature and relative humidity due to the changes in seasons have a pronounced effect on the content of various storage compounds and enzymatic activities. The changes in temperature, rainfall, relative humidity, light quality and intensity, photoperiod, duration of exposure to sunshine and the conditions prevalent in their microhabitats and the growing stage of plant at the time of collection have tremendous effect on the storage compound accumulation and on the activities of various enzymes leading to great fluctuations during different seasons.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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