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Propagation of Some Wild Edible Ferns for Environmental Sustainability and Nutritional Security in Sikkim Himalaya

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ABSTRACT

Wild edible ferns are considered as high value nutritive indigenous crops in Sikkim Himalayan region. These edible ferns are directly harvested from the wild and sold in local markets. Foraging is an age old tradition in the Sikkim Himalayas which has adverse effects. Wild edible ferns are given least attention on their conservation as compared to higher plants and indiscriminate collections and foraging endangered the very existence of wild edible ferns. Therefore, the present study aimed at propagating three edible fern species *viz. Dryopteris cochleata, Diplazium esculentum* and *Tectaria coadunata* through rhizomes in hydroponics and soil and through spores in soil has been attempted as part of our continued efforts to conserve these species through cultivation. Growth parameters like germination percentage, time taken for germination, time taken for harvest, length and weight of the crozier, leaf per rhizome were recorded during the growth period. This study can be useful for the propagation and conservation of wild edible ferns.

Key words: *Wild edible fern, Hydroponics, Nutritional security, Conservation, Propagation*

Introduction

In India, most of the ethnic communities in the rural areas depend on wild resources to meet their food requirements (Medak and Singha, 2017). A large section of the population in Sikkim Himalaya, particularly in remote areas, depends upon a variety of traditional plants for their survival. The wild edible plants form an important constituent of traditional diets in the Himalaya (Sundriyal and Sundriyal, 2004). In addition, their consumption adds variety and flavour to the diet by diversifying daily food intake. Wild edible plants refer to those plants which are neither cultivated nor domesticated but are collected from their wild habitat for consumption. In the Sikkim Himalaya, a total of 190 species have been screened as edible species out of which nearly 47 species come to the market (Sundriyal and Sundriyal, 2001). The nutritional values and mineral content of the wild edible plants were richer than that of commercial vegetables and could be used for nutritional purpose (Seal, 2012). The edible ferns are nutritionally important and are traditionally used as supplements to the staple grain-dominated diets and agricultural crops that are only seasonally available. It can thus raise rural people's nutritional intake by providing a year-round supply of food. As per earlier findings of Chettri *et al* (2018) on nutritional status of six wild edible ferns, *Tectaria coadunata* is a good protein supplement with 12.10

% and *Diplazium maximum* is a source of vitamin C with 25.38mg/100g apart from other nutrients.

Fern species are considered to be the most important wild vegetable for human consumption but further research is needed to culture this species. It has been predicted that successful domestication of such potential wild edible plants could uplift the economic condition of poor farmers in rural localities (Barucha and Pretty, 2010) in addition to helping the conservation of biodiversity and ensure nutritional security as these ferns are rich in â-carotene, ascorbic acid, iron, zinc, folate and dietary fibre (Chettri *et al.*, 2018). The wild plants especially edible ferns are dependent on the microclimatic conditions of the region for their successful survival in the region. Any disturbance can hinder the evolutionary process leading to their population decline. Factors such as climate change, industrialization, encroachment of forest lands, over exploitation of natural resources, large scale collection of ferns from the forests by visitors and local people for ornamental, culinary and medicinal purpose pose a major threat to the survival of these groups of plants. Due to increased demand, several species of edible ferns are becoming rare in nature. The rules of sustainable utilization are not followed during collection of fern fiddleheads (Kholia, 2010). It was recorded that plant dwellers have open access for the collection of these plant resources, which often leads to their over exploitation (Sundriyal and Sundriyal, 2004). Sooner or later, the rhizome of such plants from where all croziers are plucked becomes weak and ultimately die due to starvation. Therefore, adoption of these species in traditional agroforestry system, according to local desire, will be a welcome step for harvesting these species for the benefit of the community (Sundriyal and Sundriyal, 2003). Conservation approaches like propagation can play a vital and complementary role in guaranteeing the survival of these species by providing genetic resources from wild populations (Marimuthu and Manickam, 2011). As a first step in this direction, commercial propagation method could be standardized along with package of practices for its domestication. In the past, numerous authors have described the medicinal uses, ornamental value, *in-vitro* cultivation of various species of ferns, while some have reported the heavy metal accumulation ability of these plants. However, no considerable research has been conducted on cultivation and its conservation via hydroponics. The pertinent research questions of the study are : (i) how edible ferns can contribute for environmental sustainability through enhanced propagation methods? (ii) Is hydroponics a feasible method for growing ferns also? (iii) What is the effect of different media (soil and hydroponics) on the growth of ferns?

Hence, a sincere effort was made to propagate the edible ferns namely *Dryopteris cochleata, Diplazium esculentum* and *Tectaria coadunata* using rhizomes under hydroponics and in natural soil medium and spores using soil medium alone.

Materials and Methods

The present investigation was carried out in the polyhouse, Department of Horticulture, Sikkim University, 6th mile Samdur, Tadong, Gangtok, East Sikkim at an altitude of 1610m and with latitude as 27.30827 (N 27°18′29.73744") and longitude 88.58812 (E 88°35′17.26044") during the years of 2018 and 2019.

Experimental materials: Healthy rhizomes of *Dryopteris cochleata, Diplazium esculentum* and *Tectaria coadunata* were collected from Khamdong, Relli from Kalimpong, West Bengal during the months of June and July. Soils were collected from the growing sites of the ferns. The plant specimen was identified by consulting available taxonomic literature, such as flora, manual, monograph, as well as research papers published on ferns and confirmed through expert service from the Botanical Survey of India (BSI).

Pot culture: Immediately after collection, soil samples were air dried, crushed, and sieved through a 2-mm nylon sieve. The sieved soil and leaf compost was mixed thoroughly and filled with equal proportion (1:1) in each pot sized 10×10cm. The rhizome samples were washed with running water to remove all the soil and soil sediments. The rhizomes were weighed before planting. For each species three replications with three biological replicates were planted. The pots were placed in the open environment.

Hydroponics: A simple hydroponics system was established by using utility trays (40cm×26cm×8cm) and for supporting the rhizome hard plastic sheet were cut and placed on top of the trays. Each tray was filled with 4 litres of hydroponic solution. Aquarium air pumps (SB-548A) were used for aeration. These trays were placed in the greenhouse until the time of harvest. Here again for each species three

replications with three biological replicates were planted.

Preparation of hydroponic stock solution (Hoagland's nutrient): Fern rhizomes were grown in solution culture with their roots suspended directly in a nutrient solution for varying period of time. The nutrient solutions used were changed after every tenth day after close monitoring of the condition of roots and solutions used. Stock solution was prepared according to Hoagland and Arnon (1950) with minor modification (Table 1). The micro nutrients except the Fe EDTA were dissolved in 500 ml distilled water. Finally volume was made up 1litre. Fe EDTA was always prepared fresh as and when required. Nutrient solution was prepared every time freshly by drawing the recommended quantity of stock solution each time. The pH was maintained at 5.5-6.0. For adjusting pH 0.1N NaOH and 0.1N HCl was used.

Table 1. Relative constitution of Hoagland's solution

Component	Stock solution	ml of stock solution/1 litre nutrient solution
Macro nutrients		
1 M NH_4H_2PO4	115.0257 g L^{-1}	1ml
1M KNO_3	101.1032 g L^{-1}	6ml
1 M $Ca(NO_3)2.4H_2O$	164.0878 g L^{-1}	4ml
1 M $MgSO_4.7H_2O$	120.3676 g L^{-1}	2ml
Micro nutrients		
$H3BO_3$	2.86 g L^{-1}	1ml
$MnCl_2.4H_2O$	1.81 g L^{-1}	
$ZnSO_4.7H_2O$	0.22 g L^{-1}	
$CuSO_4.5H_2O$	0.08 g L^{-1}	
$H_2MoO_4.H_2O$	0.02 g L^{-1}	
Fe EDTA		0.25ml

Observations Recorded

Time taken for germination: The time taken for 1st and 2nd germination of crozier was noted down for all the plants of each replication and their average was worked out and expressed in percentage.

Germination % (GP): Germination % was calculated by the formula given below:

GP = Total number of germinated rhizomes × 100

Time taken for first harvest and second harvest: Time taken for first and second harvest was recorded for all the replications and the average was worked out and expressed in days.

Weight of the croziers: Weight of the croziers after harvest was measured and their average was calculated and expressed in grams.

Length of the croziers: Length of the croziers after harvest was measured and their length calculated and expressed in centimetre.

Leaf per rhizome: Leaf per rhizome was counted for each treatment and the average was calculated.

Spore collection: The mature fronds were washed in running tap water followed by distilled water for a few minutes. The fronds were cut into small pieces and dried over absorbent paper at room temperature (25^0C). After drying the fronds at room temperature for 24 hour, the extracted spores were passed through 40 mm nylon mesh to remove the sporangial wall materials and the cleaned spores were collected and stored in a refrigerator at 4^0C until further use.

Spores sterilization and treatment: Spores (1g) were suspended and wetted with 4% (v/v) Tween solution for 5 min in a 1.5 ml centrifuge tube. Suspended spores were collected through a filter funnel (made by fast filter paper), which was placed on a proper conical flask. The tube was washed three times with fresh water and the water was poured into the funnel to collect the residual spores of the tube. Spores of ferns were surface sterilized with 0.1 % $HgCl_2$ solution for 5 min and washed with sterile distilled water for 10 min. Then the spores were subjected to following treatments

a) 100 ppm IBA
b) 100 ppm NAA
c) 100 ppm GA
d) 100 ppm BAP
e) Hot Water
f) Cold water
g) Thiourea 1%
h) Control (without any treatment)

Media preparation for spore planting: Disinfecting of the soil was done by using boiling water. The soil was placed into a clay pot with drain holes and boiling water was poured over the soil until it drains out. After letting the soil in the pot drain the next portion of water was poured. The disinfected soil was covered, allowed for cooling and was spooned into the sowing containers (Planton).

Sowing of spores: One gram of spores were mixed with distilled water and by using a dropper, the spores were sown into the disinfected prepared media consisting of forest soil. The spores were sown into a planton of size 10×11 cm for germina-

tion. Later after the spores germinate into clumps of gametophyte, they were transplanted into a protray, the second transplanting was done into another tray when the gametophyte produces leaves.

Statistical analysis

The treatments were subjected to ANOVA using Factorial Completely Randomized Design (CRD) for the present study. In case of rhizome germplasm and media were two factors with 03 replications and 03 biological replicates. In case of spores the germplasm and treatments were two factors with three replications. The average data of two years were analyzed as per the statistical procedure suggested by Gomez and Gomez (1984). The critical difference at 5% level was used for testing the significant differences.

Results and Discussion

Rhizome as Explants

Hydroponics was significantly superior as it took 11 days for germination against 16.91 days in soil medium. Among the species *D. esculentum* required minimum days (7.61 days) for first germination followed by *D. cochleata* (12.28 days) and they were at par with each other but significantly superior than *T. coadunata*. Interaction effect between germplasm and media was found to be non significant. Neither the media nor the germplasm had any effect on the germination percentage (Table 2).

Shortest time for the first harvest was recorded in *D. esculentum* (24.52 days) which was at par with *D. cochleata* (31.17 days) and significantly shorter to *T. coadunata*. Here again the hydroponics (27.27 days) was significantly superior to soil medium (37.74 days). The interaction effect was non-significant. However, there was no significant difference in weight of crozier at first harvest as it varied from 2.52 g to 3 g amongst species. The media too couldn't make any difference in weight of the crozier. Similarly, there was not much variation in the length of the crozier between species during the first harvest as it ranged from 27.54 cm to 30.61 cm. There was no potential variation amongst the media for length of the crozier.

It was observed that *D. esculentum* with 18.45 days had the quickest second germination (Table 3) and it was at par with *D. cochleata.* Longest time was taken by *T. coadunata* (29.25 days) which was significantly longer than other two species studied. Minimum days to second harvest were observed in *D. esculentum* (34.70 days) and it was par with *D. cochleata* (39.39 days), where as it was significantly faster growth than *T. coadunata*. However, *T. coadunata* and *D. cochleata* were at par.

Weight of the crozier during second harvest was significantly high in *D. cochleata* (3.11 g) followed by *T. coadunata* (2.56 g) and the least weight were observed *in D. esculentum* (1.93 g). Effect of media could not produce any perceptible variation. Length of the crozier at second harvest did not show any significant variation amongst germplasm as well as between media.

The maximum numbers of leaves were recorded in *D. esculentum* (4.06) which was superior to other germplasm and the least was observed in *T. coadunata* (Table 4). There was no significant difference between hydroponics and soil as far as leaf per rhizome was concerned.

In general, the hydroponics medium was proved to be better than the soil medium as far as initial germination and time taken for first and second harvest of croziers. In hydroponics the absorption and

Table 2. Effect of media on wild edible fern species till first harvest of the croziers

Germplasm	Time taken for 1st germination (days)			Time taken for 1st harvest (days)			Weight of crozier at 1st harvest (g)			Length of crozier 1st harvest (cm)		
	HP	SOIL	MEAN	HP	SOIL	MEAN	HP	SOIL	MEAN	HP	SOIL	MEAN
Dryopteris cochleata	10.39	14.17	12.28	24.61	37.72	31.17	2.38	2.66	2.52	27.28	33.67	30.47
Diplazium esculentum	5.67	9.56	7.61	20.76	28.28	24.52	3.84	2.15	3.00	35.69	25.53	30.61
Tectaria coadunata	16.94	27.00	21.97	36.44	47.22	41.83	3.12	2.83	2.98	27.44	27.63	27.54
Mean B	11.00	16.91		27.27	37.74		3.11	2.55		30.14	28.94	
Factors	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)
Factor(A)	4.97	2.26	1.60	6.90	3.13	2.22	N/A	0.43	0.30	N/A	2.63	1.86
Factor(B)	4.06	1.84	1.30	5.64	2.56	1.81	N/A	0.35	0.25	N/A	2.15	1.52
Factor(A X B)	N/A	3.19	2.26	N/A	4.43	3.13	N/A	0.60	0.43	8.20	3.72	2.63

*HP-Hydroponics

uptake of nutrients would have been unhindered as the medium has sufficient amount of nutrients in the liquid form which is readily absorbable and pH of the medium is maintained at optimum level so that all the nutrients are in available form for uptake. Hydroponically grown plants showed a more luxuriant and faster growth rate of above ground parts than the soil grown plants (Gurung and Gurung, 2019). Arcand and Ranker (2008) earlier reported that the success and application of new concepts like hydroponics for fern conservation requires multiple efforts of awareness and dissemination of scientific knowledge and skill in order to have a complete and balanced view of studies of pteridophyte for *ex situ* conservation and recovery programs.

Among the species studied, *D. esculentum* was quicker,*T. coadunata* took longer time and *D. cochleata* was in germination to harvest suggesting *D. esculentum* was a quick growing fern and attained maturity early apart from the weight of the crozier also was high especially at second harvest whereas, *D. cochleata* was taking average time for maturity process and its weight per crozier drastically reduced at second harvest. The difference was according to the genetic makeup of the species. Singh, *et al.* (2022) has also mentioned in his paper the importance of conservation of wild edible plants utilized by local indigenous communities for sustainable utilization and forest management as it can encourage multifunctional forestry.

Spores as Explants

The study revealed that among the germplasm *T. coadunata* took significantly longer time for germination (44.20 days). Minimum days to germination were recorded in *D. esculentum* 33.65 days which was followed by *D. cochleata* 35.92 days (Table 5). On the contrary, the time taken for sporophyte development was least in *Tectaria coadunata* followed by *D. esculentum.* Longer duration was observed in *D. cochleata.* Highest number of transplantable sporophytes per gram of spore was produced by *D. esculentum* followed by *D. cochleata* and least by *Tectaria coadunata* (Table 6).

As far as treatments were concerned IBA, BAP, NAA and GA each separately @ 100 ppm were

Table 3. Effect of media on wild edible fern species till second harvest of the croziers

Germplasm	Time taken for 2nd germination (Days)			Time taken for 2nd harvest (Days)			Weight of crozier at 2nd harvest (g)			Length of crozier at 2nd harvest (cm)		
	HP	Soil	Mean	HP	Soil	Mean	HP	Soil	Mean	HP	Soil	Mean
Dryopteris cochleata	21.28	16.50	18.89	35.06	43.72	39.39	1.91	1.95	1.93	27.05	34.25	30.65
Diplazium esculentum	14.50	22.39	18.45	30.11	39.28	34.70	3.32	2.90	3.11	32.98	26.94	29.96
Tectaria coadunata	24.50	34.00	29.25	42.39	54.78	48.59	2.90	2.23	2.56	25.11	25.48	25.30
Mean B	20.09	24.30		35.85	45.93		2.71	2.36		28.38	28.89	
Factors	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)
Factor(A)	6.59	2.99	2.11	9.66	4.39	3.10	0.53	0.24	0.17	N/A	2.61	1.85
Factor(B)	N/A	2.44	1.73	7.89	3.58	2.53	N/A	0.20	0.14	N/A	2.13	1.51
Factor(A X B)	N/A	4.23	2.99	N/A	6.20	4.39	N/A	0.34	0.24	N/A	3.69	2.61

*HP- Hydroponics

Table 4. Effect of media on wild edible fern species till second harvest of the croziers

Germplasm	No. of Leaves per rhizome			Germination (%)		
	HP	Soil	Mean	HP	Soil	Mean
Dryopteris cochleata	3.26	2.51	2.89	77.78	83.33	80.55
Diplazium esculentum	3.99	4.13	4.06	83.33	72.22	77.78
Tectaria coadunata	2.23	1.68	1.96	94.44	88.89	91.67
Mean B	3.16	2.78		85.18	81.48	
Factors	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)
Factor(A)	0.53	0.24	0.17	N/A	7.17	5.07
Factor(B)	N/A	0.20	0.14	N/A	5.86	4.14
Factor(A X B)	N/A	0.34	0.24	N/A	10.14	7.17

*HP- Hydroponics

found to be better in making early germination compared to hot water and thiourea 1% treatments. However, *D. cochleata* could not develop in to sporophyte in GA 100 ppm and thiourea 1% treatments. In the same way sporophyte development could not be observed in *Tectaria coadunata* under hot water and thiourea treatments. GA 100 ppm treatment did not give any sporophyte in *D. esculentum.* This finding indicated that NAA 100 ppm was significantly superior in sporophyte production followed by IBA 100 ppm. Similarly, time taken for sporophyte development was quicker in NAA 100 ppm followed by IBA 100 ppm treatments indicating that auxins are playing major role in sporophyte development (Table 5,6). Auxins in general and NAA in particular was shown to produce better sex ratio in gametophyte (Hickok and Kiriluk, 1984) which had contributed to sporophyte development time and number of sporophyte. In addition, BAP was shown to favour formation of sporophyte (Somer *et al*., 2010).

Thus, it was evident from the experiment that though germination could take place in all the treatments, only IBA, NAA, BAP and GA were contributing to quicker germination. However, in sporophyte formation and time taken for sporophyte formation NAA @100 ppm was found superior followed by IBA @100 ppm.

Wild edible plants like edible ferns provide a wealth of essential nutrients and bioactive compounds. There are dietary, food-security, health, and medicinal benefits by using these edible ferns. Therefore, the documentation, conservation, characterisation and evaluation of these neglected edible plants by propagation and domestication helps in the conservation of wild edible plants commonly utilized by local indigenous communities for sustainable utilization and forest management as it can also encourage multifunctional forestry. Overall this study on ferns will help in the conservation of ferns by propagation and will also benefit farmers and other stakeholders involved in the production economically.

Table 5. Effect of treatments on the sporophyte development of wild edible fern species

	Time Taken for Germination (Days)							Time Taken for Sporophyte Development (Days)						
	IBA 100 ppm	BAP 100 ppm	NAA 100 ppm	GA 100 ppm	Hot water	Thio urea	Mean A	IBA 100 ppm	BAP 100 ppm	NAA 100 ppm	GA 100 ppm	Hot water	Thio urea	Mean A
Dryopteris cochleata	37.72	34.85	36.17	33.94	36.07	36.79	35.92	160	157	150	ND	168	ND	159
Diplazium esculentum	31.74	33.03	33.27	34.00	34.79	35.06	33.65	97	98	100	ND	130	129	111
Tectaria coadunata	43.20	45.08	43.24	43.93	45.84	43.92	44.20	100	103	95	115	ND	ND	103
Mean B	37.56	37.65	37.56	37.29	38.90	38.59		119	119	115	115	149	129	
Factors	C.D.	SE(d)	SE(m)					C.D.	SE(d)	SE(m)				
Factor(A)	0.53	0.26	0.19					2.15	1.06	0.75				
Factor(B)	0.75	0.37	0.26					3.04	1.49	1.06				
Factor(A X B)	1.30	0.64	0.45					5.26	2.59	1.83				

ND- None developed

Table 6. Effect of treatments on the number of sporophyte development of wild edible fern species

Germplasm	Number of sporophyte						
	IBA 100 ppm	BAP 100 ppm	NAA 100 ppm	GA 100 ppm	Hot Water	Thiourea	Mean A
Dryopteris cochleata	16.00	15.00	16.00	0.00	12.00	0.00	9.83
Diplazium esculentum	22.00	21.00	25.00	0.00	9.00	9.00	14.33
Tectaria coadunata	10.00	11.00	13.00	10.00	0.00	0.00	7.33
Mean B	16.00	15.67	18.00	3.33	7.00	3.00	
Factors	C.D.	SE(d)	SE(m)				
Factor(A)	0.18	0.09	0.06				
Factor(B)	0.25	0.12	0.09				
Factor(A X B)	0.44	0.21	0.15				

Conclusion

From the above mentioned observation and based on the statistical analysis, it was concluded that comparison between growing conditions had revealed that hydroponics was significantly superior regarding time taken for germination, time taken for the first harvest and second harvest, weight of the croziers, leaf per rhizome and germination percentage than the soil medium. Comparison of these three species revealed that rhizome along with spore of *D. esculentum* took lesser days for germination in hydroponics as well as pot and reached maturity fast as compared to other species. Treating spore with NAA 100 ppm could make the spores germinate faster, and produce more number of sporophytes in short period of time. The results of the present study can be the ready source of information for the propagation of three wild ferns which is not commonly practiced. Standardization of cultivation practice will reduce the growing pressure on these species through on field conservation and management practices.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

References

Arcand, N.N. and Ranker, T.A. 2008. Conservation Biology. p. 257 – 283. In: *Biology and Evolution of Ferns and Lycophytes*, T.A. Ranker and C.H. Haufler (eds.). Cambridge University Press, Cambridge, UK.

Barucha, Z. and Pretty, J. 2010. The roles and values of wild foods in agricultural systems. *Biol. Sci*. 365: 2913-2926.

Chettri, S., Manivannan, S. and Muddarsu, V. 2018. Nutrient and Elemental Composition of Wild Edible Ferns of the Himalaya. *Amer. Fern J.* 108(3): 95-106.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research.* 2nd Edition, John Wiley and Sons, New York, 680 p.

Gurung, C. and Gurung, A. 2019. *Ex situ* conservation of three Himalayan ferns through hydroponic culture under Greenhouse conditions. *Pleione*. 13(2): 216 - 225.

Hickok, L.G. and Kiriluk, R.M. 1984. Effects of Auxins on Gametophyte Development and Sexual Differentiation in the fern *Ceratopteris thalictroides* (L.) Brongn.. *Bot. Gaz.* 145(1): 37-42.

Hoagland, D.R. and Arnon, D.I. 1950. The Water-Culture Method for Growing Plants without Soil. California Agricultural Experiment Station, Circular-347.

Kholia, B. S. 2010. *Ferns and fern-allies of Sikkim*. State Biodiversity Board and Botanical Survey of India.

Marimuthu, J. and Manickam, V.S. 2011. *Ex situ* conservation of two threatened ferns of the Western Ghats through *in vitro* spore culture. *J. Threat. Taxa* 3(7): 1919–1928.

Medak, B. and Singha, L. 2017. Nutritional Contribution by Wild Plants as Novel Food to the Ethnic Tribes of Arunachal Himalaya, India. *IOSR J. of Pharm. Biol. Sciences.* 12: 73-79.

Seal,T. 2012. Evaluation of nutritional potential of wild edible plants, traditionally used by the tribal people of Meghalaya State in India. *Amer. J. Plant Nutr. Fert. Technol.* 2(1): 19-26.

Singh, N., Pandey, R., Chandraker, S.K., Pandey, S. and Malik, S., Patel, 2022. Use of Wild Edible Plants Can Meet the Needs of Future Generation. p. 341-366. In: *Agro-biodiversity and Agri-ecosystem Management*. P. Kumar.,R.S. Tomar., J.A. Bhat., M. Dobriyal., M.Rani., (eds). Springer, Singapore.

Somer, M., Arbesu , R., V. Menendez. V., Revilla, M. A., and Fernandez, H. 2010. Sporophyte induction studies in ferns *in vitro*. *Euphytica*, 171(2): 203-210.

Sundriyal, M. and Sundriyal, R.C. 2001. Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. *Eco. Bot.* 55: 377-390.

Sundriyal, M. and Sundriyal, R.C. 2003. Underutilized edible plants of the Sikkim Himalaya: Need for domestication. *Current Sci.* 85(6): 731-793.

Sundriyal, M. and Sundriyal, R.C. 2004. Wild Edible Plants of the Sikkim Himalaya: Nutritive Values of Selected Species. *Econ. Bot.* 55: 377-390.