



## COLLECTION OF *TAXUS* FROM TIRTHAN VALLEY, HIMACHAL PRADESH, INDIA FOR CONSERVATION IN NON NATURAL ENVIRONMENT

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### Abstract

Taxol is the prime component of Himalayan yew (*Taxus baccata*) and it has led to its over-exploitation. Himalayan Yew has slow growing nature and even the survival percentage remains low due to longer seed dormancy. The study aimed at conserving Himalayan yew (*Taxus baccata*) in its non-native conditions and it became evident to collect it from documented natural reserves and then carry out conservation strategies. The lower Himalayan range has enormous *Taxus* plant but the approachability and feasibility of bring plants from such location always remains questionable due to safety concerns. Tirthan valley near Manali, Himachal Pradesh, India was found as an approachable and appropriate place for collecting Yew plants and was executed. The plants were growing in some protected area and with the help of local guides and support extended by the forest department the workers were able to collect the plants as a part of research material in sufficient quantities.

**Keywords:** *Taxus*; Yew; Bio conservation; Soil-less culture; Taxol; Toxoids.

### Introduction

Yew (*Taxus baccata*) is found in lower Himalayan range and is found to be distributed in the states like Arunachal Pradesh, Manipur, Assam, Uttarakhand, Sikkim, Meghalaya, Jammu and Kashmir around an altitude of above 2000 asl (Paul *et al.*, 2013). Since, the Himalayan range is present in some other countries also hence the presence of *Taxus* is also witnessed in Pakistan, Tibet, Nepal, Bhutan and China (Sharma and Garg 2015; Rathore *et al.*, 2019). *Taxus* leaves have many different types of medicinal properties which helps in treating diseases like epilepsy, lung disorders, hysteria, nervousness, malaria etc. (Juyal *et al.*, 2014). Other than leaves, bark of *Taxus* is known to have medicinal importance and has been traditionally been the centre of local therapeutic drugs for years (Nadeem *et al.*, 2002). The other local applications of Yew includes oil extraction, making of local beverages using leaf extract, fuelwood, timber, namkeen chai etc. (Pant and Samant, 2008). However, the most important application of this species remains the production of toxoids which are one of the prime ingredients of any anti-cancerous drugs (Fatima *et al.*, 2016; Kundu *et al.*, 2017 and Vadivel *et al.*, 2018). The local population when realized the importance of this plant has used extensively this plant as a source of their livelihood which has worsened the situation of this plant and pushed its entry into the Red data book as endangered species (Saha *et al.*, 2015; Joshi *et al.*, 2017).

The difference between the demand and supply ratio of *Taxus* has widened the gap which is actually the prime problem for its inclusion into the Red data book. Moreover, the Taxol demand is increasing day by day which has forced the official to protect the plant from unauthorized access and save from extinction but still the rate of its disappearance is very high (Lanker *et al.*, 2010; Adhikari and Pandey, 2018 and Puri *et al.*, 2018). Biotechnologists have tried for the production of secondary metabolite from the past using different strategies and were able to produce alkaloids (Barrios *et al.*, 2009; Tapia *et al.*, 2013; Milutinović *et al.*, 2015 and Hashemi and Naghavi, 2016). However, all these efforts were not successful for providing solution for large

commercial requirements of Taxol. It was thought that whatever strategy we need to use but it will only sustain if we are able to carry out biological conservation of this plant. If the plant is not conserved it will become impossible even to locate this plant and extract Taxol in near future. Procedures like yew cuttings, hydroponics, seed dormancy breaking, in vitro embryo culture, etc. (Wickremesinhe and Arteca 1994; Wickremesinhe and Arteca 1996; Rajewski *et al.*, 2000; Pandey *et al.*, 2002; Aloni *et al.*, 2006; Muller and Leyser, 2011; Singh *et al.*, 2011; Sinha *et al.*, 2018; ) would be required for conserving these plants. If this plant is conserved and saved from extinction, it would be one of the most critical steps for making the availability of anti-cancerous drug easier. Our work in the manuscript attempted at collection of *Taxus* from approachable Himalayan region and then implement conservation strategies.

### Materials and Methods

#### Literature survey

Review of literature was extensively done and places were identified which could be targeted for collection of the plant. Many places near Himalayan ranges were identified but Tirthan valley was found more appropriate due to its close vicinity with Manali, a well-known tourist spot. It was thought that the place offers advantages like availability of tourist vehicles in reasonable rates, local guides and support from the locals as they are dependent upon tourist as a means of livelihood. Moreover, the language being spoken in and around Tirthan valley is also mostly Hindi which was also supposed to be one of the prime factors for understanding the plant, plant attributes and other problems associated with growing these plants better.

#### Collection of *Taxus*

Most of the places from Manali to Tirthan valley was covered using a private taxi and a local guide for identification of plants and carrying for safety purpose was also hired. There exist almost a small forest of *Taxus* at that place (Figure 1 and Figure 2) and few small plants were uprooted, collected in nursery bags with some soil attached to

them. It was taken care that being a plant of high altitude, it may not be possible for the plant to survive in its not native conditions and soil from the place was also collected. The soil, *Taxus* plant and few shed leaves lying on the ground were collected. After the collection of the plant, the researchers rushed towards the working place named Department of Biotechnology, Sharda University, Greater Noida, India and were maintained under artificial 900-1200 lux, 60% RH, 25±1 °C and 16/8 h photoperiod in the tissue culture room.

### Result and Discussion

The ecological habitat of *Taxus* has been disturbed due to human interference for reasons like livelihood, profitability and survival of humans but measures like ex-situ and in-situ conservations are required for keeping this plant alive for future (Mohamed and Vidaver 1990; De Klerk *et al.*, 1997; Larsen and Olsen 2007; Nimachow *et al.*, 2010; Aslam 2016). The plant was successfully collected from the terrains of Tirthan valley but the success rate of growing

gymnosperms of Himalayan regions is always a challenging task. Previously workers have attempted rhizogenesis, tissue culture, auxin supplementation etc for growing these plants in lab like conditions (Pilet and Saugy, 1987; Kevers *et al.*, 1997; Fogaca and Fett-Neto, 2005; Chauhan *et al.*, 2014; Shah *et al.*, 2008; Kumar *et al.*, 2017).

The plants brought in the nursery polybags were transferred to earthen pots and initially the soil brought from their native place was used for planting them. This actually helped the plants to thrive a little better as the soil of Greater Noida will not have offered the kind of nutrition required by these plants for initial survival. The study has targeted a difficult task of conserving *Taxus* in its non-native conditions with limited resources. The plant was collected successfully and it thrived well initially in the provided artificial conditions similar to what the plant usually witness in Himalayas. This was necessary to ensure for the survival of the plant otherwise the plant could die and the efforts taken for bringing it from difficult terrain will go in vain.



**Fig. 1:** *Taxus* species growing in wild protected areas in Tirthan valley, Himachal Pradesh, India.



**Fig. 2:** Collection of *Taxus baccata* from reserved region of Great Himalayan National Park, Tirthan Valley, Manali, Himachal Pradesh India.

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## Authors contribution

PS collected the material and drafted the manuscript; VBS did literature survey, designed the work and finalized the manuscript for publication.

## Conflict of interest:

Authors declare none.

## References

- Adhikari, P. and Pandey, A. (2018). Diversity of Endophytic Fungi Associated with Himalayan Yew (*Taxus wallichiana* Zucc.) Roots. Proceedings of Himalayan Researchers Consortium, 1, 1.
- Aloni, R.; Aloni, E.; Langhans, M. and Ullrich, C.I. (2006). Role of auxin in regulating Arabidopsis flower development. *Planta*, 223(2): 315-328.
- Aslam, M. (2016). Himalayan Yew, *Taxus wallichiana*: An Exciting Conifer with an Uncertain Future. New Delhi, India: Accepted by Science Reporter, CSIR-NISCAIR.
- Barrios, H.; Zhang, Y.L.; Sandoval, C. and Xiao, Z.A. (2009). Increase of taxol production in *Taxus globosa* Shoot callus by Chlorocholine Chloride. *The Open Natural Products Journal*, 2: 33-37.
- Chauhan, R.S.; Tiwari, D.; Bisht, A.S. and Shukla, A. (2014). Ex situ conservation of medicinal and aromatic plants in Bharsar, Uttarakhand, India. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 6(4): 282-92.
- DeKlerk, G.J.; Ter Brugge, J. and Marinova, S. (1997). Effectiveness of indoleacetic acid, indolebutyric acid and naphthaleneacetic acid during adventitious root formation *in vitro* in Malus 'Jork 9'. *Plant cell, tissue and organ culture*, 49(1), 39-44.
- Fatima, N.; Tamara, P.K.; Eun-Jung, P.; Laura, E.M.; Muniba, J.; Muneer, A.Q.; Hira, M.M. *et al.* (2016). Endophytic fungi associated with *Taxus fuana* (West Himalayan Yew) of Pakistan: potential bio-resources for cancer chemopreventive agents. *Pharmaceutical biology*, 54(11): 2547-2554
- Fogaça, C.M. and Fett-Neto, A.G. (2005). Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. *Plant Growth Regulation*, 45(1): 1-10.
- Hashemi, S.M. and Naghavi, M.R. (2016). Production and gene expression of morphinan alkaloids in hairy root culture of *Papaver orientale* L. using abiotic elicitors. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 125(1): 31-41.
- Joshi, N.; Dhakal, K.S. and Saud, D.S. (2017). Checklist of CITES Listed Flora of Nepal.
- Juyal, D.; Thawani, V.; Thaledi, S. and Joshi, M. (2014). Ethnomedical properties of *Taxus wallichiana* zucc. (Himalayan yew). *Journal of traditional and complementary medicine*, 4(3): 159-161.
- Kevers, C.; Hausman, J.F.; Faivre-Rampant, O.; Evers, D. and Gaspar, T. (1997). Hormonal control of adventitious rooting: progress and questions. *Journal of Applied Botany*, 71(3-4): 71-79.
- Kundu, S.; Jha, S. and Ghosh, B. (2017). Metabolic Engineering for Improving Production of Taxol. *Transgenesis and Secondary Metabolism*, 463-484.
- Kumar, P.; Kulwinder, K.; Ram, S.P.; Madan, M. and Burma, P.K. (2017). Development of male sterile transgenic lines in rice by tapetum specific expression of barnase gene. *Journal of Plant Biotechnology*, 44(4): 364-371.
- Lanker, U.; Malik, A.R.; Gupta, N.K. and Butola, J.S. (2010). Natural regeneration status of the endangered medicinal plant, *Taxus baccata* Hook. F. syn. *T. wallichiana*, in northwest Himalaya. *International Journal of Biodiversity Science, Ecosystem Services & Management*, 6(1-2): 20-27.
- Larsen, H.O. and Carsten, S.O. (2007). Unsustainable collection and unfair trade? Uncovering and assessing assumptions regarding Central Himalayan medicinal plant conservation. *Biodiversity and Conservation*, 16(6): 1679-1697.
- Milutinović, M.G.; Stanković, M.S.; Cvetković, D.M.; Topuzović, M.D.; Mihailović, V.B. and Marković, S.D. (2015). Antioxidant and anticancer properties of leaves and seed cones from European yew (*Taxus baccata* L.). *Archives of Biological Sciences*, 67: 525-534.
- Mohammed, G.H. and Vidaver, W.E. (1990). The influence of acclimatization treatment and plantlet morphology on early greenhouse-performance of tissue-cultured Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco]. *Plant Cell, Tissue and Organ Culture*, 21(2): 111-117.
- Müller, D. and Leyser, O. (2011). Auxin, cytokinin and the control of shoot branching. *Annals of Botany*, 107(7): 1203-1212.
- Nadeem, M.; Rikhari, H.C.; Kumar, A.; Palni, L.M.S. and Nandi, S.K. (2002). Taxol content in the bark of Himalayan Yew in relation to tree age and sex. *Phytochemistry*, 60(6): 627-631.
- Nimachow, G.; Rawat, J.S. and Dai, O. (2010). Status of Himalayan yews in West Kameng district of Arunachal Pradesh. *Current science*, 98(11): 1434-1437.
- Pandey, A.; Nadeem, M. and Palni, L.M.S. (2002). Improvement in seed germination of Himalayan yew through simple soil treatments. *Indian Journal of Forestry*, 25(2): 109-113.
- Pant, S. and Samant, S.S. (2008). Population ecology of the endangered Himalayan Yew in Khokhan Wildlife Sanctuary of North Western Himalaya for conservation management. *Journal of Mountain Science*, 5(3): 257-264.
- Paul, A.; Bharali, S.; Khan, M.L. and Tripathi, O.P. (2013). Anthropogenic disturbances led to risk of extinction of *Taxus wallichiana* Zuccarini, an endangered medicinal tree in Arunachal Himalaya. *Natural areas journal*, 33(4): 447-455.
- Pilet, P.E. and Saugy, M. (1987). Effect on root growth of endogenous and applied IAA and ABA: a critical reexamination. *Plant Physiology*, 83(1): 33-38.
- Puri, S.K.; Habbu, P.V.; Kulkarni, P.V. and Kulkarni, V.H. (2018). Nitrogen Containing Secondary Metabolites from Endophytes of Medicinal Plants and their Biological/Pharmacological Activities-A Review. *Systematic Reviews in Pharmacy*, 9(1): 22-30.
- Rajewski, M.; Lange, S. and Hattemer, H.H. (2000). Problems of reproduction in the genetic conservation of rare tree species: the example of comglon yew (*Taxus*

- baccata* L.). Forest Snow and Landscape Research, 75(1/2): 251-266.
- Rathore, P.; Roy, A. and Karnatak, H. (2019). Modelling the vulnerability of *Taxus wallichiana* to climate change scenarios in South East Asia. Ecological Indicators, 102: 199-207.
- Saha, D.; Ved, D.; Ravikumar, K. and Haridasan, K. (2015). *Illicium griffithii*. The IUCN Red List of Threatened Species 2015: e.T50126617A50131370. Accessed on 03 April, 2017
- Shah, P.; Singh, N.K.; Neeraj, K.; Meenal, R.; Anandhan, S.; Arif, M.; Singh, R.K.; Das, S.C.; Ahmed, Z. and Kumar, N. (2008). Agrobacterium mediated genetic transformation of summer squash (*Cucurbita pepo* L. cv. Australian green) with *cbf-1* using a two vector system. Plant cell, tissue and organ culture, 95(3): 363-371.
- Singh, S.; Meenal, R.; Danswring, G.; Singh, R.K.; Sivalingam, A.; Dinesh, K.S.; and Zakwan, A. (2011). Induced ectopic expression of *At-CBF1* in marker-free transgenic tomatoes confers enhanced chilling tolerance. Plant cell reports, 30(6): 1019-1028.
- Sharma, H. and Garg, M. (2015). A review of traditional use, phytoconstituents and biological activities of Himalayan yew, *Taxus wallichiana*. Journal of integrative medicine, 13(2): 80-90.
- Sinha, V.B.; Atul, G.; Patade, VY. and Veena, P. (2018). Salt and osmotic stress response of tobacco plants over expressing *Lepidium latifolium* L. *Ran* GTPase gene. Indian Journal of Plant Physiology, 23(3): 494-498.
- Tafreshi, S.A.H.; Shariati, M.; Mofid, M.R. and Nekui, M.K. (2011). Rapid germination and development of *Taxus baccata* L. by *in vitro* embryo culture and hydroponic growth of seedlings. In Vitro Cellular & Developmental Biology-Plant, 47(5): 561-568.
- Tapia, N.; Zamilpa, A.; Bonfill, M.; Ventura, E.; Cruz-Vega, D.; Del Villar, A.; Sosa-Cruz, F. and Osuna, L. (2013). Effect of the culture medium and biotic stimulation on taxane production in *Taxus globosa* Schltld *in vitro* cultures. Acta physiologiae plantarum, 35(12): 3447-3455.
- Vadivel, V.; Anand, P.; Manijkumar, S.; Rajalakshmi, P. and Brindha, P. (2018). Chemical Fingerprints of an India Traditional Herbal Drug *Talisapatra (Abies webbiana)* and Comparison with English yew (*Taxus baccata*). Int J PharmacogPhytochem Res, 10(2): 84-91.
- Wickremesinhe, E.R. and Arteca, R.N. (1994). Roots of hydroponically grown *Taxus* plants as a source of taxol and related taxanes. Plant Science, 101(2): 125-135.
- Wickremesinhe, E.R.M. and Richard, N.A. (1996). Effects of plant growth regulators applied to the roots of hydroponically grown *Taxus* media plants on the production of taxol and related taxanes. Plant Science, 121(1): 29-38.